Dissertation zur Erlangung des Doktorgrades

der Fakultät für Chemie und Pharmazie

der Ludwig-Maximilians-Universität München

Metaproteomics of the gut microbiota in mouse models of Parkinson's disease and anxiety disorders to delineate molecular biosignatures and pathways

von

Jinqiu Xiao

aus

Ganzhou, Jiangxi, China

2024

Erklärung

Diese Dissertation wurde im Sinne von § 7 der Promotionsordnung vom 28. November 2011 von Herrn Prof. Dr. Christoph Turck betreut.

Eidesstattliche Versicherung

Diese Dissertation wurde eigenständig und ohne unerlaubte Hilfe erarbeitet.

München, ...23.01.2024.....

.....

Jinqiu Xiao

Dissertation eingereicht am 24.01.2024

1. Gutachter: Apl.-Prof. Dr. Christoph Turck

2. Gutachter: PD Dr. Hermann Heumann

Mündliche Prüfung am 07.05.2024

SUMMARY

The human microbiota plays a critical role in health and homeostasis. Recent evidence suggests that the human microbiota is also involved in the pathogenesis of brain disorders including neurodegenerative and psychiatric diseases. For the analysis of the molecular constituents of the microbiota residing in the gut, fecal specimens are frequently used as a proxy. As part of my thesis projects, I have used mouse models for Parkinson's disease (PD) and anxiety disorders and established an experimental workflow to simultaneously analyze the metaproteome and metabolome from one single mouse fecal pellet. The specimen extracts were analyzed by proteomics and metabolomics mass spectrometry and the acquired -omics data subjected to extensive bioinformatic analyses. The workflow was applied to mouse models of PD and anxiety disorders to investigate the possible impact of the gut microbiota on disease pathogenesis.

PD, a neurodegenerative disorder that primarily affects the elderly, places a significant burden on healthcare systems worldwide. PD primarily affects motor function and can lead to cognitive problems. Anxiety disorders are often caused by chronic social stress such as daily challenges or trauma. It has lasting physical and psychological effects, weakens the immune system and can lead to various psychiatric disorders. Despite extensive research, a complete understanding of the causes of PD and anxiety disorders remains elusive.

My study provides new insights into specific taxa and biological pathways involved in the pathobiology of PD. The results of my study highlight the role of pathogen-induced gut inflammation and reduced protection due to decreased SCFA-producing bacteria, coupled with decreased dopamine production activated by the phosphotransferase system (PTS), as potential contributors to the development of PD.

Metaproteomic analysis of the anxiety disorder mouse model revealed distinct gut microbial compositions of the DBA/2NCrl and C57BL/6NCrl mouse strains whose microbiota molecular constituents were investigated. The genetic background differences between the two mouse strains turned out to have a stronger effect on the microbiota composition than the chronic social defeat stress (CSDS) paradigm that was used to induce the anxiety phenotype. In both mouse

strains that differ in their susceptibility to CSDS, oxidative stress-related pathways were the major pathways affected by CSDS.

Metaproteomics is an emerging field and provides many challenges due to the great and still incompletely characterized great variety of microorganisms in the microbiota. The results of my projects provide important molecular insights into the gut microbiota of PD and anxiety disorder mouse models that may be relevant for disease pathogenesis. I was successful in detecting protein expression level changes even when only subtle alterations in microbial composition are observed. Furthermore, the results of my projects suggest that fecal pellet samples hold great promise to serve as a proxy for the gut microbiota and the identification of disease biomarkers. Future studies have to verify that this approach can also be translated to the clinic for early diagnosis and disease interventions in patients afflicted with PD and anxiety disorders.

Table of Contents

SUMMARY I

Table of Contents III

Abbreviations VI

1 Introduction 1

1.1 Neurodegenerative disorders 1

1.2 Parkinson's disease 2

1.2.1 Rotenone-treated mouse model 3

1.3 Neuropsychiatric disorders 5

1.3.1 Chronic stress 5

1.3.2 Chronic social defeat stress (CSDS) mouse model 6

1.4 Microbiota 7

1.4.1 Microbial diversity 8

1.4.2 Challenges in studying microbiota 8

1.4.3 Human microbiota 9

1.4.4 Gut microbiota 11

1.4.5 Microbiota-gut-brain axis 13

1.5 Parkinson's disease and microbiota-gut-brain axis 14

1.6 Chronic stress and microbiota-gut-brain-axis 16

1.7 Omics techniques for the study of microbiota 16

1.7.1 16S rRNA sequencing and shotgun metagenomics 16

1.7.2 Metatranscriptomics 17

1.7.3 Proteomics 18

1.7.4 Metabolomics 24

2 Research aims 26
3 Materials and methods 27
3.1 Reagents, equipment and software 27
3.2 Study design 29
3.3 Parkinson's disease mouse model 31
3.3.1 Animals and rotenone infusion 31
3.3.2 Motor function assessment 31
3.3.3 Intestinal transit measurement 32
3.3.4 Tissue preparation and immunohistochemistry 32
3.3.5 Image analysis 32
3.4 Anxiety disorders mouse model 33
3.4.1 Animals 33
3.4.2 CD-1 aggressor screening 33
3.4.3 CSDS exposure 33
3.4.4 Social avoidance (SA) test and fecal pellets collection 34
3.5 Sample processing 35
3.5.1 Metabolite extraction 35
3.5.2 Metaproteome extraction and digestion 35
3.5.3 TMT labeling and fractionation 36
3.6 Mass spectrometry and data analysis 37
3.6.1 Metabolomics analysis 37
3.6.2 Metabolomics data processing and statistical analysis 38
3.6.3 Proteomics analysis 39
3.6.4 Metaproteomics data analysis 39
3.6.5 Molecular pathway analysis 40
4 Results 42

4.1 Parkinson's disease mouse model 42
4.1.1 Rotenone treatment generates PD phenotypes in mice 42
4.1.2 16S rRNA based microbial taxonomy profile 43
4.1.3 TMT6plexed quantitative analysis of fecal metaproteome 46
4.1.4 Protein expression comparison between rotenone and vehicle group 47
4.1.5 Metaproteome based microbial taxonomy profile 49
4.1.6 Pathway enrichment analysis 51
4.1.7 Significantly changed proteins associated with PD indicators 52
4.1.8 Significantly changed metabolites between rotenone- and vehicle-treated groups 53
4.2 Anxiety disorders mouse model 54
4.2.1 Genetic background's impact on behavior response to CSDS 54
4.2.2 TMT10plexed quantitative analysis of fecal metaproteome 56
4.2.3 Comparison of protein expression levels between different groups 58
4.2.4 Metaproteome based microbial taxonomy profile 62
4.2.5 Pathway enrichment analysis 65
4.2.6 Significantly changed metabolite levels between different groups 70
5 Discussion 72
5.1 Parkinson's disease mouse model 72
5.2 Anxiety disorders mouse model 75
5.3 Challenges in metaproteomics and metabolomics analysis 79
6 Summary and Outlook 80
References 82
Appendices 97
Acknowledgement 171

Abbreviations

Abbreviation	Full name
AA	Amino acid
AD	Alzheimer's disease
B6	C57BL/6NCrl
BC	B6 control group
BR	B6 stress-resilient group
BS	B6 stress-susceptible group
CD-1	Clr-CD1
CNS	Central nervous system
COG	Clusters of Orthologous Genes
CSDS	Chronic social defeat stress
CTF	Contrast transfer function
D2	DBA/2NCrl
DC	D2 control group
DDA	Data dependent acquisition
DIA	Data independent acquisition
DMSO	Dimethyl sulfoxide
DR	D2 stress-resilient group
DS	D2 stress-susceptible group
EggNOG	Evolutionary genealogy of genes: Non-supervised Orthologous Groups
EIC	Extracted ion chromatogram
ENS	Enteric nervous system
ESI	Electrospray ionization
F/B ratio	*Firmicutes*/*Bacteroidetes* ratio
FC	Fold change
FDR	False discovery rate
FMT	Fecal microbiota transplantation
GC-MS	Gas chromatography–mass spectrometry

Abbreviation	Full name
GFAP	Glial fibrillary acidic protein
GI	Gastrointestinal
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High performance liquid chromatography
IBD	Inflammatory bowel disease
IRS	Internal reference scaling
iTRAQ	Isotope-labeled relative and absolute quantification
IZ	Interaction zone
KEGG	Kyoto Encyclopedia of Genes and Genomes
LCA	Lowest common ancestor
LC-MS	Liquid chromatography–mass spectrometry
MDS	Multidimensional scaling
MPIP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NEC	Normalized collision energy
PD	Parkinson’s disease
PEP	Phosphoenolpyruvate
PTM	Post-translational modification
PTS	Phosphotransferase system
QC	Quality control
RNA-seq	RNA sequencing
RP	Reserve phase
SA	Social avoidance
SAGE	Serial analysis of gene expression
SCFA	Short chain fatty acid
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SI	Social interaction

Abbreviation	Full name
SN	Substantia nigra
SNO	S-nitrosylation
SNRIs	Serotonin-norepinephrine reuptake inhibitors
SOH	S-sulfenylation
SSRIs	Selective serotonin reuptake inhibitors
TMT	Tandem mass tag
TNF	Tumor necrosis factor
UHPLC	Ultra high performance liquid chromatography

1 Introduction

1.1 Neurodegenerative disorders

Neurodegenerative disorders are caused by the progressive loss of function or structure of neurons in the brain and spinal cord[1]. During childhood, neural stem cells produce the majority of neurons by cell proliferation, and the number of neural stem cells is significantly reduced in adulthood[2, 3]. Neurons are not immortal[4], the progressive loss of neurons and neural structures is called neurodegeneration[5]. Neurodegeneration is associated with a dysfunction of the neural network and has been associated with many brain disorders. Since the brain affects different parts and aspects of the body, neurodegeneration can further affect a range of basic functions such as speech, movement and balance, as well as complicated tasks such as bowel functions and cognitive abilities[6, 7].

The most common neurodegenerative diseases, Parkinson's disease (PD) and Alzheimer's disease (AD), affect millions of people and involve a significant loss of nerve cells (Figure 1.1)[8]. They place an enormous burden on families and healthcare systems[9]. The causes of these diseases are still unknown. They have been linked to both genetic and environmental factors[10, 11]. LRRK2 and APP mutations are the most common known genetic contributors to familial PD and AD, respectively[12, 13]. However, only about 10-15% of PD and 1-2% of AD can be explained by family history[14, 15],

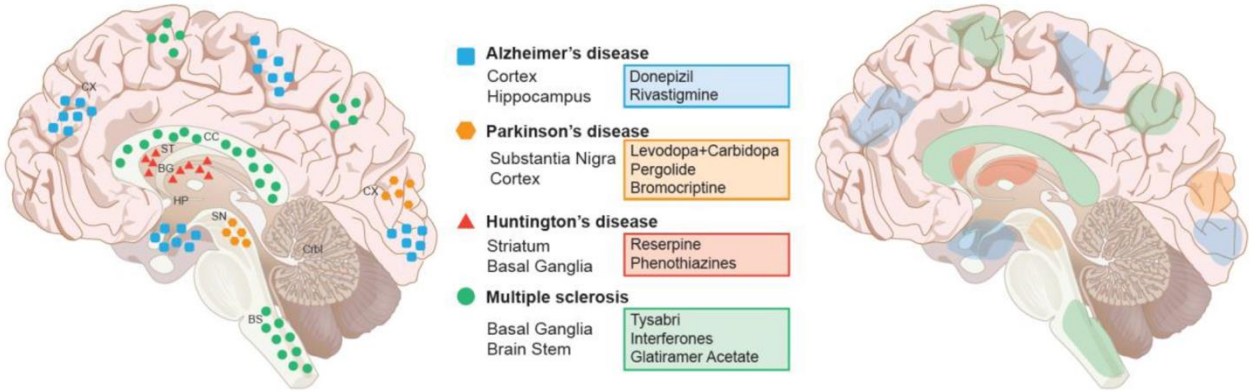


Figure 1.1. Major neurodegenerative diseases, their associated regions, and current therapeutic interventions[8].

In addition to genetic factors, environmental risk factors including lifestyle, stress, sleep and smoking, also contribute to the genesis and development of neurodegenerative disorders [16-19].

More recently, microbiota constituents have also been associated with neurodegenerative diseases[20]. Several studies have shown that the composition of the gut microbiota varies between AD patients and healthy controls, with reduced microbial diversity and relative abundance of butyrate-producing bacteria (*Faecalibacterium*) observed in AD patients compared to controls[21], while the relative abundance of lactate-producing bacteria (*Bifidobacterium*) is significantly increased in AD patients[22]. Fecal microbiota transplantation (FMT) from healthy donors to an AD mouse model was able to alleviate cognitive deficits and reduce the brain deposition of amyloid-β[23].

1.2 Parkinson's disease

PD is a long-term neurodegenerative disease that typically affects people older than 60 years of age[24]. It was first described in 1817 by English surgeon James Parkinson in *An Essay on the Shaking Palsy* and later named after him[25]. PD primarily affects motor function and causes cognitive problems. The incidence of PD worldwide has increased from 2.5 million in 1996 to 6.1 million in 2016 and represents a huge burden on healthcare systems[26]. A complete understanding of the pathobiology of PD remains elusive[27].

Only a small percentage of PD cases can be explained by genetic factors. Several genes have been linked to the development of PD by molecular genetics studies. Non-synonymous mutations such as G2019S in LRRK2 result in an increase of kinase activity[28], which likely contributes to the development of PD. In this regard, several studies have shown that LRRK2 kinase inhibitors can prevent brain pathology in rodent models of PD[29, 30]. Mutations in SNAC, the gene that encodes alpha-synuclein, lead to the protein's accumulation in the brain[31]. Around 5 to 20% of PD patients carry mutations in the GBA1 gene[32], with mutations such as E326K resulting in a decrease of enzymatic activity, thereby reducing alpha-synuclein degradation in lysosomes[33]. Research implicates several environmental factors in

PD, including infections, metabolic disorders, and exposure to certain toxins, but the overall risk is rather low[34-36].

The main pathological feature of PD is the death of dopaminergic neurons. Aggregates of specific substances within brain cells called Lewy bodies, are always observed in PD patients and are a microscopic marker of PD[37, 38]. The major component within Lewy bodies is misfolded alpha-synuclein protein. Currently, the clinical diagnosis of PD is mainly based on motor symptoms that occur in the late stages of the disease as a result of cell death in the brain[39]. As with any other diseases, early diagnosis of PD is very important to allow intervention to improve brain function and prevent worsening of PD symptoms.

The impaired motor function of PD is due to cell death in the *substantia nigra*, resulting in a dopamine deficit[40]. Since dopamine cannot cross the blood-brain barrier, it cannot be used as a drug to increase the depleted dopamine levels in the brain. However, one of the precursors of dopamine, levodopa, can get into the brain where it is readily converted to dopamine, and when administered, can temporarily relieve the motor symptoms in PD patients. Levodopa administration has been the most widely used treatment for PD for more than 40 years[41-43].

1.2.1 Rotenone-treated mouse model

Rotenone is a colorless, odorless, crystalline isoflavone compound (Figure 1.2). It can be naturally produced by the seeds, stems, and roots of various leguminous plants and vines. It is the major active insecticidal component in the root of *Derris trifoliata*. Rotenone became one of the most widely used pesticides with a broad spectrum and transient environmental side effects[44].

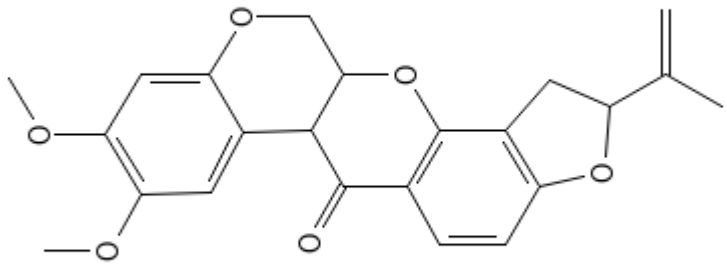


Figure 1.2. Molecular structure of rotenone.

While studying the general toxicity of rotenone, researchers found that injecting rats with rotenone could induce PD-like symptoms.[45] Although this does not directly suggest that rotenone can trigger PD, it has contributed to the belief that exposure to environmental toxins may increase the risk of developing PD. Gao et.al. found that the addition of low-dose rotenone to primary cultures of rat neurons and microglia can induce oxidative damage to dopaminergic cells, which is similar to the cell death process observed in PD patients[46]. Studies in mice have shown that not only infusion of rotenone into the brain, but also chronic intragastric ingestion of low doses of rotenone can induce PD-like symptoms[47].

Both acute and prolonged rotenone exposure can increase the risk of developing PD in humans. In 2008, Dhillon et al. found that household use of rotenone was associated with an increased risk of developing PD[48]. A 2011 case-control study of 84,000 farmers showed a strong association between rotenone exposure and PD[49]. De Miranda's 2019 study also found an association between rotenone exposure and PD, with males having a higher risk developing the disease compared to females[50]. Several epidemiologic studies also support the link between rotenone exposure and the development of PD[49].

Rotenone inhibits the electron transport chain in mitochondrial complex I and deficiency of the mitochondrial respiratory chain has been linked to PD[51]. Rotenone, unlike other mitochondrial complex I inhibitors such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(MPIP), can penetrate the cellular membrane due to lipophilic structure. This allows it allows to get into brain cells where it binds to mitochondrial complex I[52]. Rotenone not only targets dopaminergic cells, but also non-neuronal and non-dopaminergic cells[53]. Studies in humans have shown that complex I inhibition occurs systemically in patients. In addition to oxidative damage, rotenone is also able to induce dopaminergic neuron death, alpha-synuclein accumulation, and GI symptoms in rodents[54-56]. It is therefore frequently used to generate animal models of PD[57].

1.3 Neuropsychiatric disorders

Neuropsychiatric disorders are characterized by a range of symptoms that affect a person's thoughts, mood and behavior[58]. One specific neuropsychiatric disorder is panic disorder, where an individual is experiencing recurrent and unexpected panic attacks[59]. A panic attack is a sudden onset of intense fear or anxiety accompanied by physical symptoms such as heart palpitations, sweating, shaking, shortness of breath, and chest pain[60].

In addition to the panic attack itself, people with panic disorder may have persistent worries or concerns about future panic attacks and may avoid situations or places that they believe may trigger a panic attack. Panic disorder can seriously affect a person's quality of life, making it difficult to perform daily activities and participate in social situations[61]. It can also lead to other mental health problems, such as depression and chronic stress[62].

1.3.1 Chronic stress

Chronic stress is the physical and psychological response to prolonged exposure to stress[63]. The stress can result from various aspects of daily life such as work, family, finances and physical health, or it can be ongoing stress caused by traumatic events, chronic illness, domestic violence, etc[64]. Unlike acute stress, chronic stress tends to persist over time and can have negative physical and psychological effects[65].

Physically, chronic stress can lead to compromised immune system function, susceptibility to disease, and physical ailments such as headaches, stomach aches, and muscle aches[66-69]. In

addition, chronic stress affects the cardiovascular system, leading to an increased risk of cardiovascular diseases such as high blood pressure, coronary heart disease, and stroke[70-72]. Chronic stress also affects the endocrine system, causing hormonal imbalances that affect the body's metabolism, reproduction, and growth and development[73, 74]. Psychologically, chronic stress can lead to emotional instability, anxiety, depression and other psychological problems[75-77]. Chronic stress can also affect an individual's social skills, ability to work, and quality of life[78, 79].

1.3.2 Chronic social defeat stress (CSDS) mouse model

In humans, social defeat is characterized by feelings of being relegated to a subordinate or undesirable position. In animals such as rodents, social defeat is induced by introducing a male into the home cage of an older and more aggressive male[80]. Although this defeat experience appears to be a simple physical stressor, it can cause psychological stress and, through a chronic process, trigger a series of molecular and behavioral changes[81].

The CSDS model is a widely accepted method for studying psychosocial stress-related disorders in mice[82]. This model has construct, face, discriminant, and predictive validity. Different mouse strains like C57BL/6NCrl (B6) and DBA/2NCrl (D2) differ in their susceptibility to stress[83]. The CSDS model involves 10 days of daily brief confrontation between two male mice, the resident aggressor (CD-1) and the intruder (Figure 1.3). The intruder responds with defensive, flight or submissive behavior, and social avoidance is measured to assess stress-related symptoms. While all defeated mice are exposed to stressful stimuli, only some display stress-related symptoms, making this model suitable for studying mechanisms associated with resilience and vulnerability towards stress[80]. The behavioral outcome of CSDS is strongly influenced by genetic factors, and inbred mouse strains differ in their stress coping behaviors and in the proportion of susceptible and resilient animals[82].

Accumulating evidence suggests that the gut-microbiota-brain axis may contribute to whether rodents exhibit resilience or susceptibility after repeated stress[84]. Studies have reported higher levels of *Bifidobacterium* in resilient mice compared to susceptible mice after CSDS, and oral supplementation with *Bifidobacterium* has been shown to induce resilience in mice exposed to CSDS[85]. In addition, repeated oral administration of betaine was found to promote

stress resilience in mice after CSDS through the gut-microbiota-brain axis[86]. Taken together, these findings suggest that the gut-microbiota-brain axis may play a critical role in determining whether rodents exhibit resilience or vulnerability in response to stress.

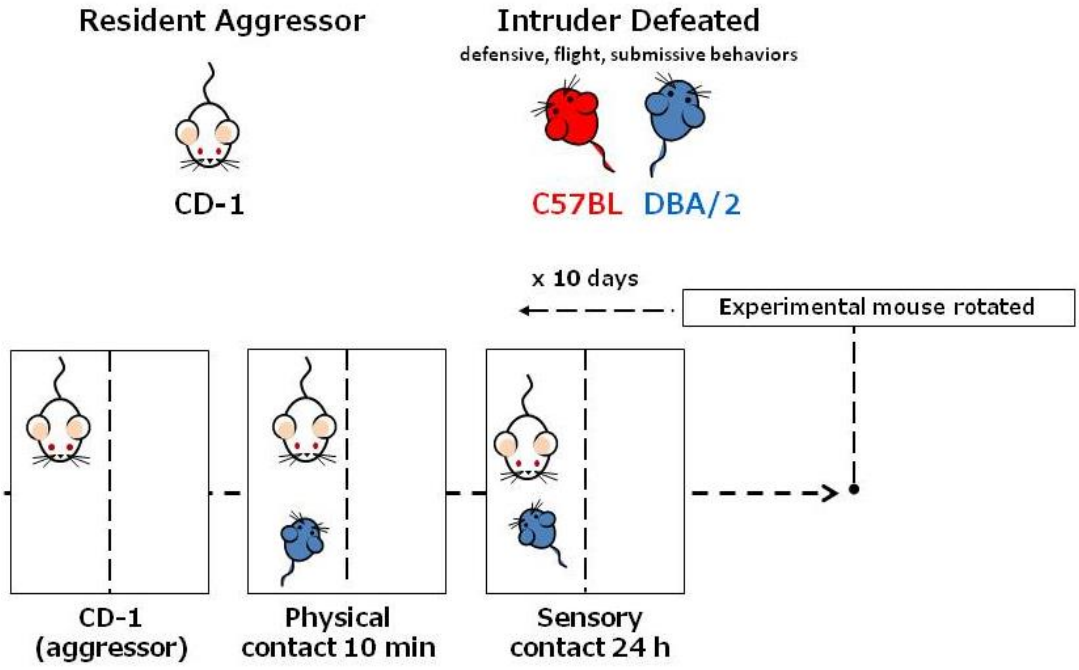


Figure 1.3. Schematic illustration of the chronic social defeat stress (CSDS) mouse model. C57BL/6NCrl or DBA/2NCrl mice are defeated by CD-1 mice. The protocol involves a daily confrontation of 10 min of two conspecific mice, followed by sensory contact for 24 hours. The defeated mice meet a new aggressor every day during the 10 days of CSDS[77].

1.4 Microbiota

With an estimated number of 10^{12}, microorganisms are the most abundant organisms on Earth[87]. They can withstand extreme temperatures, pH and nutrient conditions and can live in very extreme environments such as deserts and hydrothermal vents[88, 89]. Microorganisms can also live on and in other organisms. They can absorb molecules that the host cannot digest as nutrients and in return produce nutrients that can be used by the host[90]. They can also inhibit the growth of pathogens by competing for nutrients or secreting molecules that can act as growth inhibitors[91, 92].

The microbiota is the collection of microorganisms that live in and on multicellular organisms or environments is in a dynamic state of equilibrium and contributes to the homeostasis of the host[93, 94]. Microorganisms interact with each other within the microbiota and with the host or environment as a whole[95]. They are characterized by a very high diversity within and between environments[96].

1.4.1 Microbial diversity

Alpha diversity, also known as within-habitat diversity, refers to the diversity within a given region or ecosystem[97]. The alpha diversity index reflects the number of species and their relative abundance in the community and is the result of competition or symbiosis between species in the community using the same habitat. Comparing the alpha diversity indices can reveal differences in the diversity of samples. Alpha diversity can be calculated as one or more diversity indices such as species richness, Simpson's index or Shannon's index[98, 99].

Beta diversity, also known as between-habitat diversity, on the other hand refers to the dissimilarity of species composition between different habitat communities along an environmental gradient, or the replacement rate of species along an environmental gradient[97]. The fewer species are shared between different communities or different points on an environmental gradient, the higher the beta index diversity. It can be calculated as absolute species turnover, Whittaker's beta diversity, Whittaker's species turnover or proportional species turnover[98, 100].

1.4.2 Challenges in studying microbiota

The sampling strategy is critical in microbiota studies. Due to its high diversity, the microbiota can be very different between individuals and environments of the same group[101]. Due to its dynamic nature, microbiota samples collected at different times can vary in their constituents even if they were collected from the same individual or environment[102],[103]. All this making the collection of representative samples for microbiota studies very challenging[104].

Another key issue is sample processing. Since there are frequently hundreds to thousands of species in one sample, it is impossible to isolate and culture each microorganism individually[105]. In addition, microorganism will behave differently in lab-cultured environments than in their natural environment due to many interactions within the microbiota and between the microbiota and the host or environment[106, 107]. Because of the difficult isolation and culture of microbes, many of them have not been sequenced yet and are referred to as the "dark matter" in microbiota studies[108].

Because of the complex composition of microbiota, the data analysis is much more complex than in single organism studies[109]. With regard to omics studies, one needs to consider taxonomic composition as another layer of information[110]. Also, interactions occur not only within the same level, but also between different taxonomic levels, which needs to be taken into accout during data analysis and interpretation.

Most of microbiota research involves associative studies which makes it very difficult to distinguish between correlation and causation[111]. Because of the complexity of the microbiota, it is currently very difficult to manipulate specific species without changing the others[112]. This makes it especially challenging in human studies due to the many factors that can affect the human microbiota that are difficult to control and account for[113].

1.4.3 Human microbiota

Humans are colonized by a wide variety of microorganisms on or in tissues and biofluids, including the gastrointestinal tract, skin, mouth, nose, vagina and glands (Figure 1.4[114]). The number of bacteria that make up the human microbiota is approximately 1.3 times as many as the number of human cells[115]. The human microbiota is therefore sometimes referred to as our "second self".

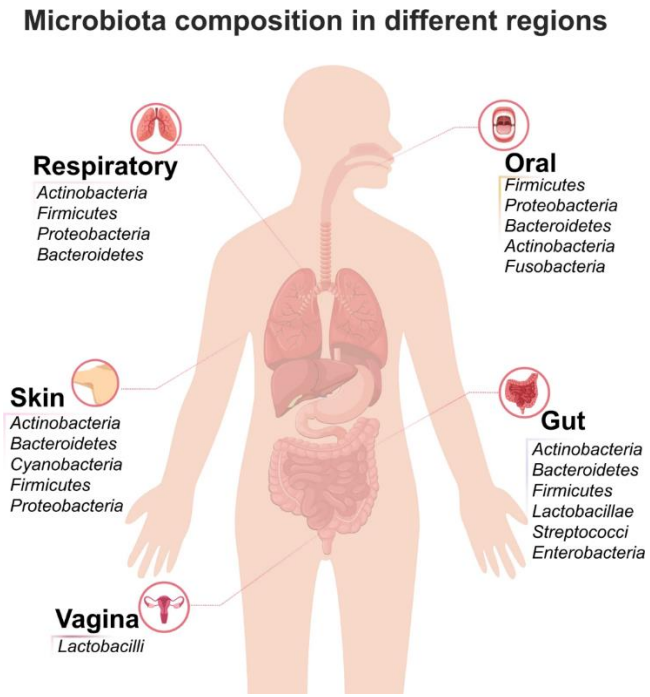


Figure 1.4. Human microbiota composition in different locations[114].

The human microbiota plays a critical role in health and homeostasis[114, 116]. It participates in human energy metabolism by absorbing and storing energy from human cells or carbohydrates that humans cannot digest[117]. The microbiota also produces a variety of molecules, such as short-chain fatty acids (SCFAs), that influence host metabolism[118] and influences the immune system by activating the immune response and promoting the development of immune cells[119, 120].

Microorganisms colonize all body surfaces in contact with the environment. The human skin is colonized by a variety of microbial species that, together with the epidermis, act as a barrier against the invasion of microbial pathogens by secreting chemicals such as antimicrobial peptides and competing for nutrients[121, 122]. In return, the glands provide water and nutrients to beneficial bacteria. The majority of human microbiota reside in the gastrointestinal (GI) tract and are referred to as the gut microbiota. The microbial density of gut contents in the colon can reach up to 10^{12} cells per gram, and in feces up to 60% of the dry mass is bacteria[123, 124]. Thus, feces are frequently used as a proxy to study the gut microbiota in a non-invasive manner[125].

1.4.4 Gut microbiota

The fetal GI tract is germ-free and is colonized by microbiota after birth[126]. In neonates, the initial composition of the gut microbiota is strongly influenced by the mode of delivery. It is similar to the vaginal microbiota of the mother for vaginal delivery, whereas it is similar to the hospital environment for cesarean delivery[127]. The different composition of gut microbiota resulting from different modes of delivery affects the development of the neonatal immune system and has been linked to a variety of autoimmune diseases and metabolic disorders[128, 129].

During the first year after birth, the composition of the gut microbiota is relatively simple and fluctuates over time[130]. It is influenced by many factors such as food, breast milk and the environment to which the infant is exposed[131-133]. Then the gut microbiota becomes more diverse with the introduction of solid foods, exposure to a more complex diet and the environment[134]. At around 2.5 years of age, it gradually converges to a composition that is stable throughout adulthood[135, 136].

Once the gut microbiota is established, its composition remains relatively stable, with less variation between individuals[137]. However, it can be altered by long-term changes in diet, exercise, environment, or short-term changes due to drug treatment (especially antibiotics) and bacterial infections[138] (Figure 1.5[139]). In older people, the gut microbiota becomes less stable and shows greater variation between individuals[140]. It is also characterized by a significant decrease in beneficial bacteria and overall microbial diversity[141]. Dysbiosis, the disruption of the microbiota resulting in an imbalance, is also more common in the elderly[142].

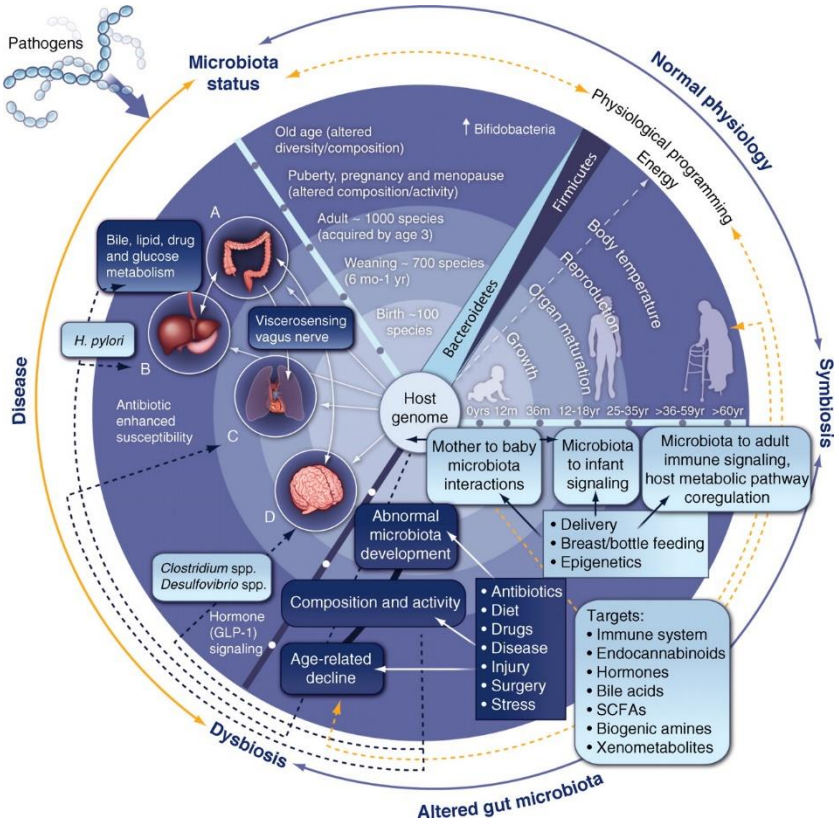


Figure 1.5. The gut microbiota in development and disease[139].

Under normal physiological conditions, the gut microbiota maintains a dynamic balance in the GI tract, with microorganisms being mutually restricted and interdependent[143]. However, when the internal or external environment changes, it can lead to decreased microbial diversity and dysbiosis, which can lead to the onset and development of disease[144]. In the human gut microbiota, the five most abundant phyla are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria*, representing >90% of the known components of the human gut microbiota[101]. The *Firmicutes* and *Bacteroidetes* are two of the most dominant phyla in the human gut microbiota. Their ratio is widely believed to be important in maintaining normal intestinal homeostasis, and an altered ratio causing the development of some diseases[145].

It is conceivable that perturbations in the composition and function of the gut microbiota are associated with intestinal disease. Inflammatory bowel disease (IBD) is a group of chronic intestinal disorders that cause intestinal inflammation in the absence of structural and biochemical abnormalities of the GI tract[146]. Recent research suggests that gut microbiota

dysbiosis may contribute to the development of IBD by disrupting host physiology[147]. IBD patients exposed to FMT treatment often show significant improvement in their symptoms[148].

Since many organs are connected to and frequently interact with the GI tract, the gut microbiota can also indirectly affect distal organs[149]. For example, the liver is connected to the GI tract through the portal vein, and molecules produced by the gut microbiota that penetrate the intestinal epithelium and mucosa can affect liver function[150]. Research shows that excessive growth of Gram-negative bacteria can promote insulin resistance and non-alcoholic liver disease. In addition and of relevance for my thesis projects, the gut microbiota is thought to affect brain function[151].

1.4.5 Microbiota-gut-brain axis

The first observation of the connection between the brain and the gut dates back more than 200 years. The French anatomist Xavier Bichat discovered that the gut had its own nerve, which is not part of the central nervous system (CNS), later identified as the vagus nerve. From 1825 to 1831, William Beaumont conducted a series of experiments on digestion with his patient and later collaborator Alexis St. Martin, who was shot and left with a fistula in his stomach. In 1838 Beaumont published the record of his experiments with Alexis St. Martin as *Experiments and Observations on the Gastric Juice, and the Physiology of Digestion*[152]. He noticed that mood could affect the speed of digestion. When Alexis St. Martin was angry or anxious, his digestion slowed down. After that, the notion of the gut-brain axis was gradually established as the bidirectional signal communication between the GI tract and the CNS.

As the importance of the gut microbiota in health and disease has become more evident, research has intensified to further investigate the role of the gut microbiota in the gut-brain axis[153]. Studies in germ-free mouse models have shown that brain development is strongly influenced by the gut microbiota and that FMT can reverse changes in the CNS[154].

One mechanism is when microbial-associated molecules are sensed by immune cells and enteric glial cells, which then secrete neuro-modulatory molecules that affect the CNS[155]. The gut microbiota can also induce an inflammatory environment and alter the permeability of the GI tract, affecting the brain by secreting molecules such as SCFAs that can penetrate the leaky

gut[156] (Figure 1.6[153]). The microbiota-gut-brain axis has been linked to a variety of diseases, including psychiatric disorders, stroke, and neurodegenerative disease[153].

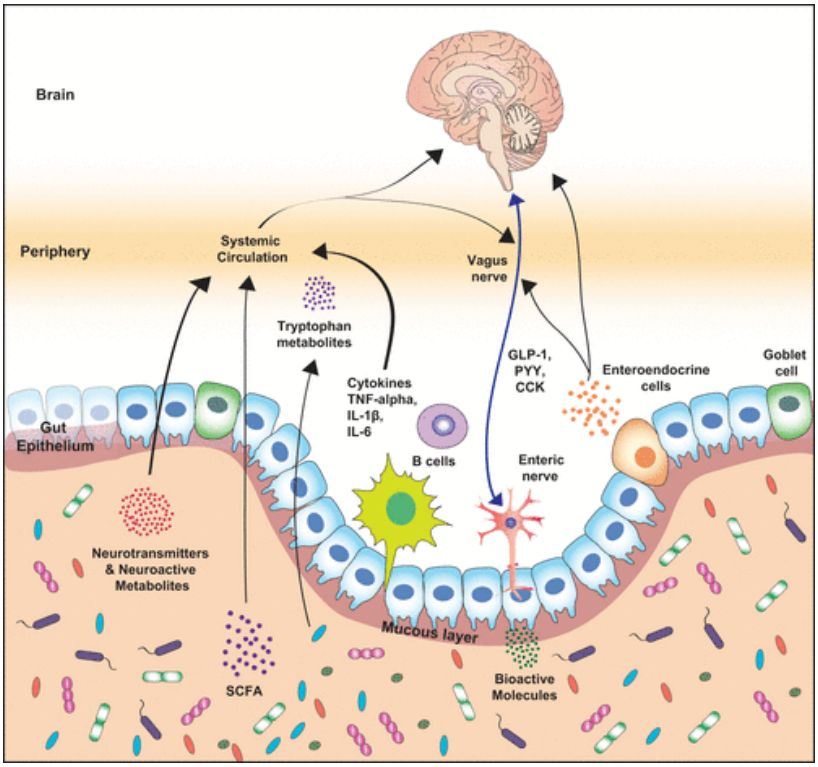


Figure 1.6. Schematic outline of the various known bidirectional pathways of communication between the gut-microbiota and the brain[153].

1.5 Parkinson's disease and microbiota-gut-brain axis

The main symptoms of Parkinson's disease are unstable movements, including tremor, rigidity, and slowness of movement[157]. However, in James Parkinson's first observations of PD patients, he reported that some patients experienced constipation, and treatment of the gastrointestinal problems in one of the six patients showed relief of PD-associated motor symptoms[25]. Since then, clinicians have found that about half of Parkinson's patients develop gastrointestinal symptoms such as constipation, making it one of the most common symptoms after motor dysfunction. These symptoms may even precede motor symptoms by many years[158]. In addition, the occurrence of gastrointestinal symptoms in healthy individuals is

associated with a higher risk of developing Parkinson's disease[159]. This suggests that the microbiota-gut-brain axis is involved in PD pathology.

The enteric nervous system (ENS) plays an important role in the transport of information in the microbiota-gut-brain axis[160]. It consists of neurons that control the function of the GI tract in which it is embedded. It is also able to communicate with the CNS through the parasympathetic and sympathetic nervous systems[161].

Research suggests that intestinal inflammation can contribute to the development of PD. Peter et.al. reported that the probability of developing PD is 28% higher among inflammatory bowel disease patients than healthy controls, and the probability of developing neurodegenerative disease dropped by 78% in patients treated with tumor necrosis factor (TNF) inhibitors,[162]. The comorbidity of two diseases suggests that PD is primarily mediated by inflammatory processes in the gut[163].

Intestinal inflammation may contribute to the development of PD through several pathways. During the intestinal inflammation, the elevation of cytokines and other molecules could further promote the inflammation[164] can lead to an increase the expression and accumulation of alpha-synuclein[165] Subsequently, inflammation can increase the permeability of the intestine, allowing alpha-synuclein to travel to other parts of the body[166].

Studies have found accumulated alpha-synuclein in the ENS along with brain pathology in early untreated PD patients[167]. In 2007, Hawkes et al. proposed a "dual-hit hypothesis" for PD, suggesting that PD may originate in the ENS and then spread to the brain[168]. In 2016, Sampson et al. found that gut microbiota is a key factor in triggering motor deficits and alpha-synuclein pathology in a mouse model overexpressing alpha-synuclein[169]. So far, most investigations on the role of gut microbiota in PD were based on genetic studies. In order to get a better understanding of the active biological processes of gut microbiota relevant for PD pathology, proteomics studies are needed which enticed me to carry out the current thesis project.

1.6 Chronic stress and microbiota-gut-brain-axis

The community structure and function of the gut microbiota are closely related to chronic stress[170]. When we are in a state of chronic stress, the gut microbiota can be affected, which in turn affects our physical and mental health[171].

Specifically, chronic stress can lead to an imbalance in the gut microbial community, known as dysbiosis, which is characterized by a decrease in certain beneficial microorganisms and an increase in certain harmful microorganisms, leading to gut inflammation and immune system dysregulation[144]. This microecological imbalance can further lead to impaired intestinal barrier function and increased entry of foreign substances, harmful bacteria and toxins into the circulation, which can trigger or exacerbate a number of diseases such as enteritis, irritable bowel syndrome, autoimmune diseases, cardiovascular diseases and obesity[172-177].

In addition, the permeability of the intestinal barrier can be increased by stress and depression, leading to a condition known as "leaky gut". As a result, bacteria can escape into the bloodstream and trigger an inflammatory response[178]. Chronic stress can also affect the metabolic function of the gut microbiota, for example, causing changes in the way the gut microbiome absorbs and metabolizes certain nutrients, which can further affect systemic metabolism and thus physical health[179, 180].

1.7 Omics techniques for the study of microbiota

1.7.1 16S rRNA sequencing and shotgun metagenomics

16S rRNA is a component of the prokaryotic small ribosomal subunit and represents an evolutionary marker due to the slow rate of evolution of this gene. The 16S rRNA gene sequence is about 1500 base pairs long. It contains both highly conserved regions, which could be used as primer binding sequences, and hypervariable regions as species-specific signature sequences. It has been widely used to differentiate prokaryotic species and to characterize the composition of microbial communities[181].

In contrast to 16S rRNA sequencing, shotgun metagenomics does not target specific sequences, but analyzes the entire genetic material of microbial communities[182]. Extracted DNA is amplified and sequenced, quality control is performed, and reads are assembled into contigs, which are then assembled into genomes. Microbial genes can be predicted and annotated directly from the reads or assembled genomes. In addition to microbial composition, metagenomics also provides functional information based on predicted gene sequences[183].

16S rRNA sequencing and shotgun metagenomics allow the study of microbial communities in a culture-independent and high-throughput manner. Microorganisms that cannot be cultured in the laboratory can be identified, and bias toward specific microbial species can be avoided because DNA is extracted from all microorganisms in a single sample[184].

1.7.2 Metatranscriptomics

Metatranscriptomics, also known as environmental transcriptomics, refers to the study of the total gene transcription and transcriptional regulation of a specific environment and time period to reveal the gene expression profile of microorganisms under different environmental stresses and the mechanisms of environment-microorganism interactions[185]. It has applications in human microecology, environment, industry, agriculture, and other fields[186-188]. Like metagenomics, metatranscriptomics takes all RNAs in the ecological environment as a research object without facing the difficulty of isolating and cultivating microorganisms.

After obtaining total RNA from the microbiota and removing rRNA, the metatranscriptome is reverse transcribed into cDNA, and a library of insert fragments of appropriate length is constructed, and these libraries are subjected to high-throughput DNA sequencing so that the entire gene expression profile of the microbiota can be accurately quantified. The composition of active species and the expression level of corresponding functions can be used to find the key functional pathways in the microbiota and clarify their biological significance[185].

1.7.3 Proteomics

The term ‘proteomics’ was coined by Marc Wilkins by combining ‘protein’ and ‘genomics’[189]. Proteomics focuses on the entire set of proteins in a system by studying the structure, activity, interaction, post-translational modifications (PTMs), and expression of proteins[190-193]. Proteins perform a variety of functions and are involved in a wide range of biological activities. The complexity of the proteome is much greater than that of the genome and transcriptome. From DNA to mRNA to protein, there are three levels of regulation: transcriptional regulation, translational regulation and post-translational regulation. Compared to the genome, the proteome is less stable and its expression varies in different environments[194]. At the mRNA level, it actually only includes the regulation of the transcription level and cannot fully represent the level of protein expression. Experiments also proved that the correlation between mRNA abundance and protein abundance is not good, especially for low-abundance proteins, the correlation is even worse[195]. Prediction of protein expression levels based on DNA and RNA expression is limited. More importantly, complex post-translational modifications of proteins, protein-protein interactions and subcellular localization can hardly be interpreted from the mRNA or DNA level[191, 192, 196].

The study of protein structure and function directly clarifies the mechanism of biological state changes under physiological or pathological conditions. The existence form and activity patterns of the protein itself, such as post-translational modification, protein-protein interaction and protein conformation, still rely on direct protein research to solve[197]. Although the special properties of proteins, such as variability and diversity, make protein research techniques far more complex and difficult than techniques for nucleic acid research, it is these properties that participate in and affect the entire process of life. In summary, proteomics is a critical tool for assessing biological function and change in response to different conditions. Proteomics has been widely applied in fields such as drug discovery and biomarker identification[198, 199].

1.7.3.1 Mass spectrometry-based proteomics

Mass spectrometry is a technique that identifies and quantifies proteins, peptides and other biomolecules based on their mass-to-charge ratio. Mass spectrometry-based proteomics is a high-throughput technique that allows quantitative profiling of a large numbers of proteins. In bottom-up proteomics, proteins are digested into smaller peptides and then analyzed by mass spectrometry[200].

In many applications, peptides are first fractionated by high performance liquid chromatography (HPLC) coupled to the mass spectrometer[201, 202]. HPLC is an analytical chemistry technique used to separate each component of a mixture. There are two phases in HPLC: the mobile phase and the stationary phase. For proteomics studies, the C18 column is commonly used as the stationary phase for peptide separation. Peptides typically have hydrophobic regions that can interact with the hydrophobic C18 column through van der Waals forces; peptides that are highly polar or charged tend to have less interaction with a C18 column, allowing them to move faster than non-polar peptides. The hydrophobic interactions between peptides and the C18 phase can be adjusted by changing the composition of the mobile phase, increasing the ratio of organic solvent will decrease the interaction between the peptides and the C18 column, that's how peptides are separated by HPLC based on their chemical properties[203].

Then the fractionated peptides are nebulized by electrospray ionization (ESI) and introduced into the mass spectrometer[204]. The peptides that are present in a stable nebulized flow are ionized and converted to the gas phase under high voltage (typically 2-4 kV). Intact peptide ions are then separated by their mass-to-charge ratio (m/z) and the intact ions are detected as precursors. The precursor ions can be further fragmented and detected as MS2 spectra for peptide sequence determination[200].

Data dependent acquisition (DDA) and Data independent acquisition (DIA) are two commonly used data acquisition modes in mass spectrometry[205, 206]. DDA mode is the most basic and simplest data acquisition mode. After the first stage of mass spectrometry analysis, the precursor ions are screened according to the set screening conditions for the second stage of mass spectrometry analysis. This method uses narrow windows that change from circle to circle for screening target ions, which can reduce the existence of interfering ions to a certain extent, and it often uses ions with high peak intensity as target ions for further fragmentation in MS2

analysis. DDA mode may contain more sample-to-sample variability and bias due to the fragment selection procedure[207, 208].

The DIA mode is an extension and development of the DDA mode. In contrast to the DDA mode, it uses wide windows that are the same in all precursor groups. All precursor ions are analyzed in the second stage of mass spectrometry analysis, so fragmentation in MS2 analysis occurs in parallel across peptides without selection. The DIA mode provides greater reproducibility, but the size of the data generated tends to be larger than in the DDA mode[209, 210].

Mass spectrometry features are extracted from output files containing both MS and MS2 spectra using software programs. These features are matched to peptide sequences generated by *in silico* digestion of the corresponding protein sequence database. The matched peptides are assembled into proteins and quantified[211].

1.7.3.2 Quantitative proteomics

Quantitative proteomics is a method for identifying and quantifying all proteins expressed by a genome or complex system[212]. Dynamic changes in proteome abundance have important implications for various biological processes. For example, the onset and development of many diseases are often associated with abnormal expression of certain proteins[213, 214].

Label-free quantification has been an important and classic MS quantitative method for many years[215]. The quantitative change of protein in samples from different sources is analyzed by comparing the number of spectra or peak intensity[216, 217]. Label-free quantification only needs to analyze the mass spectrometry data generated from MS measurement to compare the signal intensities of corresponding peptides in different samples to perform relative quantification of proteins corresponding to peptides[218].

There is no need to label the protein or peptides with expensive isotope labels, and the sample preparation procedure is simple. Thus, the experimental cost is relatively low and the sample preparation time is shorter. It is also not limited by the number of samples and the throughput is relatively high. However, the reproducibility of label-free quantification is relatively low.

The stability of the instrument, the operation of the experimenter, and other factors that may cause system errors result in low accuracy of quantitative results[219].

Chemical labeling technology uses chemical reactions to introduce isotope groups onto proteins or peptides to achieve sample labeling. It is the fastest growing type of in vitro quantitative labeling technology and is currently widely used in quantitative proteomics research. There are many types of such technologies according to different labeling groups and detection methods[220].

Tandem mass spectrometry-based chemical labeling technology realizes quantitative analysis by comparing the peak intensities of quantitative reporter ions of different samples in the secondary or tertiary mass spectrograms, and is currently the mainstream of quantitative proteomic labeling technology. Isobaric isotope-labeled relative and absolute quantification (iTRAQ) is an in vitro isotope-labeled relative quantification technique developed by Ross et al[221]. It has the advantages of high throughput, strong stability and wide application range, and can compare the protein expression of up to eight groups of samples simultaneously[222]. The tandem mass tag (TMT) reagent is similar to the principle of iTRAQ reagent, which consists of three parts: reporter group, balance group, and reactive group[223, 224]. Commonly used TMT kits include 6-plex, 10-plex and up to18-plex[225]. The 6-plex label 6 groups of samples. 10-plex can use a neutron label with a reporter ion mass/charge ratio difference of 6mDa. It labels 10 groups of samples. Currently, TMT reagents are widely used in proteomics research. This type of quantitative technology can be combined with other labeling technologies and high-resolution mass spectrometry to achieve higher throughput quantitative analysis of proteomics. For example, Dephoure et al. combined TMT technology with SILAC to realize 18-fold (18-plex) labeling, and combined with a high-resolution mass spectrometer, accurate quantitative analysis of various proteins can be performed[226]. In Wojdyla et al. study, combinatorial analysis of cysteine oxidation and its reverse reaction using TMT labeling and antibody enrichment methods, which replaces existing cysteine oxidation assays, facilitates understanding of cysteine nitrosylation (The relationship between S-nitrosylation, SNO) and sulfenylation (S-sulfenylation, SOH)[227].

Enrichment of modified peptides is performed simultaneously after each group is labeled and mixed. Because multiple samples are measured simultaneously, it is less affected by instrument

conditions. The reproducibility of the data is relatively good, the sensitivity is high, the sample amount requirement is low, and the quantitative results are more accurate. However, due to the need to use isotope labels, the experimental cost is high. The throughput is lower than label-free quantification because of the limited number of labels used in a set. If the sample volume is large, it is necessary to add internal reference samples each time the instrument is used, which further increases the cost[228].

1.7.3.3 Proteomics data analysis

The data obtained from the mass spectrometer is a collection of spectra data recorded over time. It is necessary to perform the database search to identify and quantify proteins. Database search refers to matching the spectra obtained by experiments with the theoretical spectra generated by *in silico* digestion and prediction from the database to obtain possible peptide sequences to identify proteins[211]. The procedure of database search is to input the spectra obtained from the mass spectrometer into the database searching software. Commonly used database search software includes MaxQuant, MSFragger and ProteomeDiscoverer[229, 230]. The main steps of database search software include: selecting peptides with molecular weights equal/similar to the input from the database, forming theoretical fragments and further generating theoretical spectra, then matching experimental spectra with theoretical spectra, scoring matches and sorting the scores, and through statistical analysis, the best matching results are determined and exported[231].

The selection of the database is very important in protein identification, the finally identified protein sequences are all derived from the selected database, so the proteins that are not included in the database cannot be identified[232]. If it is an organism that has been sequenced, a protein database directly selected from corresponding species from public databases such as UniProtKB or constructed from the transcriptome/genome of the same batch of samples can be used for database search[233]. If it is an unknown organism, only the large proteome database most relevant to the tested sample or constructed from the transcriptome/genome of the same batch of samples can be used for database search[234].

The false discovery rate (FDR) control is always performed to check the quality of the identification. Perform 1% FDR filtering at the spectrum/peptide level to obtain a list of dominantly identified spectra and peptides. Based on the parsimony principle, peptides are used for protein assembly and protein groups are also generated based on similar rules. Filter again at the protein level with FDR 1% to control the false positive of the protein. Then the list of surviving proteins after FDR control can be used for further analysis[235].

1.7.3.4 Metaproteomics

Metaproteomics refers to the application of quantitative proteomics to the analysis of microbiota[236]. As mentioned above, the DNA sequence only provides information about the potential function of an organism. To assess the expression levels of the molecules that carry out the function, the proteins, a comprehensive analysis of the proteome is required. In addition, metaproteomic data provide information about the microbial composition[237]. Typically, both microbial and host proteins are analyzed from the same sample, allowing the simultaneous assessment of the biological function of the microbiota and the host and the interaction between them[238, 239].

Unlike traditional proteomics studies that focus on a single organism, metaproteomics analyzes proteins from hundreds to thousands of different microbial species[240]. The complexity of the metaproteome is much greater than for traditional proteomics studies, with the mouse gut microbiota consisting of about 1500 microbial species and the human gut microbiota consisting of about 2000 microbial species. As a consequence, the protein sequence database is vastly more complex and includes all potential microbial sequences to detect all potential proteins present in microbial samples. Databases containing protein sequences for the gut microbiota of model organisms such as humans and mice have been assembled, combining sequences from each host to detect proteins. The size of the database used for a metaproteomics study is much larger than those used for mouse or human proteomics projects[241]. The mouse protein database consists of about 50,000 proteins, while the mouse gut microbiota currently consists of about 2.6 million proteins[242, 243].

Although the protein database for metaproteomics study is huge, it's still incomplete due to the lack of sequence information of some unknown microorganisms that cannot be isolated and cultured in the laboratory at present. Without the corresponding protein sequences, the proteins from unknown microbes cannot be identified in the metaproteomics study. It is estimated that around 70% of the microbial species have not been isolated and cultured from human gut microbiota, and around 40% of the proteins do not have functional annotations[244]. This is even worse for environmental samples, such as microbiota from the ocean, where an estimated 91% of species have not been identified[245].

For known microbes where sequence information is available, it is still difficult to determine which species an identified protein is derived from, since many protein sequences are very similar or identical between different microorganisms within the same taxon. By applying the lowest common ancestor (LCA) algorithm, every protein/peptide sequence is allocated to a specific taxonomic rank shared by all matched organisms[246]. If the protein/peptide is very specific to a single taxon, then it is assigned to that taxon. The less specific a protein/peptide matches a taxa, the higher it will be placed in the taxonomy. The lower the taxonomic level, the fewer annotations will be obtained[247].

1.7.4 Metabolomics

Many biological processes in cells occur at the metabolic level, so changes in metabolites are a more direct reflection of the environment in which cells live, such as nutritional status, drug effects, and environmental influences. The metabolic capacity of the host involves complex interactions between the gut microbiota and host cells[248]. Small molecules such as vitamins, fatty acids, amino acids (AAs), and bile acids modulate host-gut metabolic homeostasis through the involvement of host biological processes[249]. The gut microbiota facilitates the development of the host immune system, and the immune system in turn shapes the composition of the microbiota[250].

Metabolomics is a high-throughput technique that analyzes molecules smaller than 1.5 kDa. Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) are used[251-253] and NMR are used for metabolite analysis[254]. Metabolite levels

change very rapidly compared to protein levels, requiring time series experiments. Measuring the small molecules involved in the microbiota-host interaction by metabolomics technique will provide complementary data resulting in an improved understanding of the interaction as a whole[255].

Unlike DNA, RNA and proteins, small molecules are not part of the central dogma, so there's no sequence information from which to infer origin. Some molecules can be produced by both the host and the microbiota[256]. Currently, there are some bioinformatics tools to infer the origin of small molecules based on the coexistence and abundance of small molecules and microbial species[257]. However, in microbial studies, it's still very difficult to distinguish whether a small molecule is originating from the microbiota or the host[258].

2 Research aims

The main goal of the thesis project is to investigate the gut microbiota in mouse models of Parkinson's disease and anxiety disorders by delineating changes in microbial composition and biological pathways using metaproteomics and metabolomics analyses.

(1) Establishment of an experimental workflow to simultaneously interrogate the metaproteome and metabolome from one single mouse fecal pellet.

(2) Development of a data analysis workflow including data normalization, statistical analysis and data visualization for metaproteomics studies in R and Python scripts.

(3) Identification of microbial composition alterations in PD and anxiety disorders mouse models.

(4) Identification of affected biological pathways involved in PD and anxiety disorders mouse models.

3 Materials and methods

3.1 Reagents, equipment and software

Table1. Chemicals

Reagent	Company
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Sigma
Acetonitrile	Merck
BCA protein assay	Thermo Fisher
Dithiothreitol (DTT)	Merck
Formic acid	Thermo Fisher
HEPES	Thermo Fisher
Hydroxylamine 50%	Thermo Fisher
Isopropanol	Merck
Methanol	Merck
Sodium chloride (NaCl)	Sigma
TMT10plex Isobaric Label Reagent Set	Thermo Fisher
TMT6plex Isobaric Label Reagent Set	Thermo Fisher
Tris-HCl	Thermo Fisher
Trypsin	Serva
Urea	Merck
Water (mass spec grade)	Honeywell

Table 2: Consumables

Consumable	Company
C18 precolumn	Thermo Fisher
C18 ReproSil particles 1.9µm	Dr. Maisch GmbH
Capillary analytical column	New Objective Inc

epT.I.P.S. Reloads	Eppendorf
Kinetex C18 column (50 x 2.1mm, 1.7 µm)	Phenomenex
Microcon Centrifugal Filters 30K	Merck
Pierce C18 tips	Thermo Fisher
Pierce™ High pH Reversed-Phase Peptide Fractionation Kit	Thermo Fisher
Safe-Lock Tubes	Eppendorf
SecurityGuard ULTRA cartridge	Waters
SecurityGuard ULTRA holder	Waters
Serological pipette	Greiner bio-one
VanGuard Pre-column	Waters
XBridge Amide column (100 x 2.1mm, 2.5 µm)	Waters

Table 3: Equipment

Instrument	**Company**
Bullet Blender	SERVA GmbH
Centrifuge 5804 R	Eppendorf
Galaxy mini centrifuge	VWR
Elute UHPLC system	Bruker
Impact II TOF mass spectrometer	Bruker
Mechanical Pipette	Eppendorf
Q-Exactive Plus mass spectrometer	Thermo Fisher
Sonifier 250	Branson
SpeedVac Plus SC210A	Thermo Scientific
Thermomixer 5436	Eppendorf
Ultimate 3000 UHPLC system	Thermo Fisher
Centrifuge 5804 R	Eppendorf
Galaxy mini centrifuge	VWR

Table 4: Software and tools

Software/Tools	Source
BioRender	https://www.biorender.com/
Proteome Discoverer 2.4	Thermo Fisher
Hystar 5.0	Bruker
KEGG ORTHOLOGY Database	https://www.genome.jp/kegg/ko.html
MetaboScape 4.0	Bruker
Perseus 1.6.14.10	https://maxquant.net/perseus/
Python 3.8.0	https://www.python.org/
R 3.6.1	https://cran.r-project.org/
Xcalibur 4.2.47	Thermo Fisher

3.2 Study design

For the PD project, fecal pellets were collected from 10 rotenone- and vehicle-treated mice and proteins and metabolites were extracted from one fecal pellet from each mouse. RNA was extracted from another fecal pellet from each mouse. For metaproteome analysis, fecal proteins were digested and labeled with TMT6 plex reagent (channel 126 from each set was used as master control for data normalization) and analyzed using LC-MS/MS in DDA mode. The fecal metabolome was analyzed using untargeted LC-MS/MS. Fecal RNA was processed and used for 16S rRNA sequencing (Figure 3.1 A).

For the anxiety disorders project, fecal pellets were collected from 4 C57BL/6NCrl control (BC) mice, 2 C57BL/6NCrl stress-susceptible (BS) mice, 3 C57BL/6NCrl stress-resilient (BR) mice, 4 DBA/2NCrl control (DC) mice, 6 DBA/2NCrl stress-susceptible (DS) mice and 3

DBA/2NCrl stress-resilient (DR) mice. For metaproteome and metabolome analyses, fecal proteins and metabolies were processed as outlined above for the PD project (Figure 3.1 B).

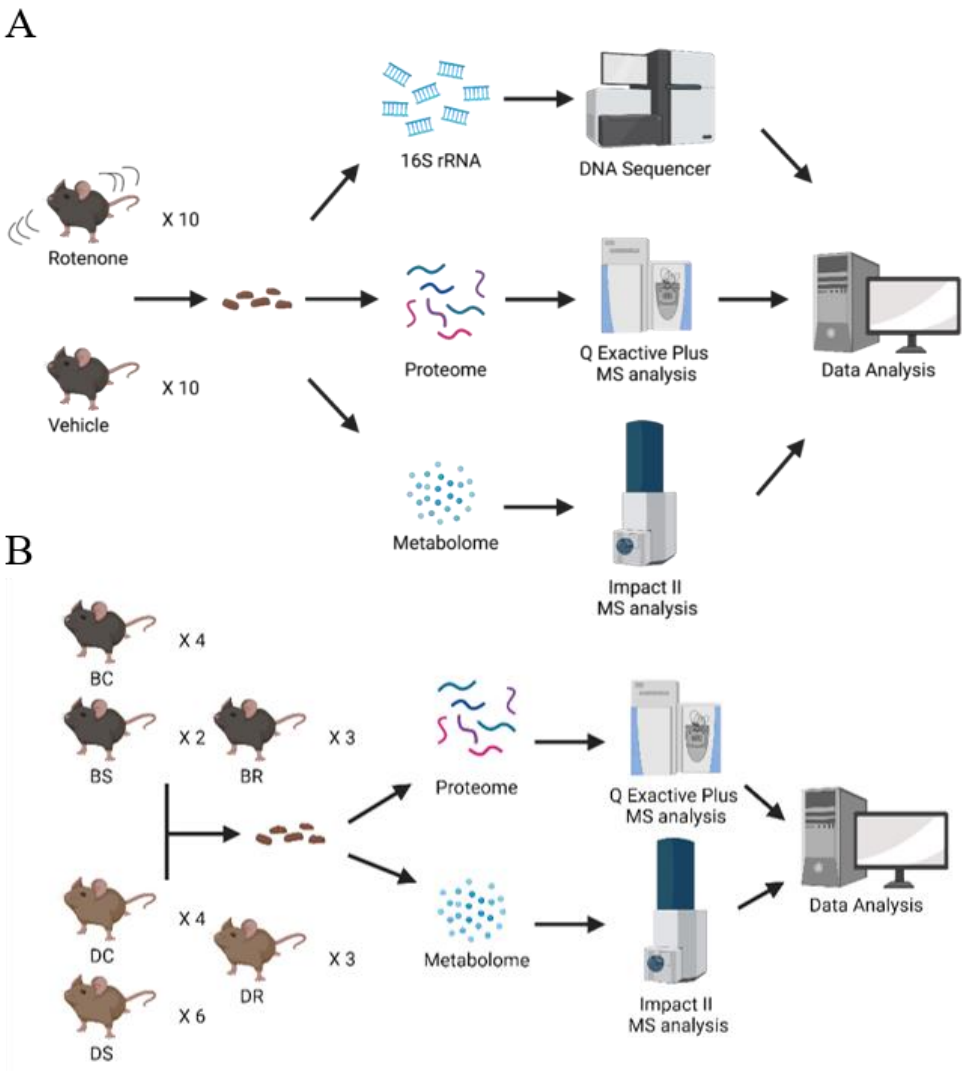


Figure 3.1. Study design of (A) Parkinson's disease project and (B) anxiety disorders project. BC (C57BL/6NCrl control mice), BS (C57BL/6NCrl stress-susceptible mice), BR (C57BL/6NCrl stress-resilient mice), DC (DBA/2NCrl control mice), DS (DBA/2NCrl stress-susceptible mice), DR (DBA/2NCrl stress-resilient mice), MS (mass spectrometry).

3.3 Parkinson's disease mouse model

3.3.1 Animals and rotenone infusion

The PD mouse model was generated, analyzed and fecal pellets provided by Dr. Paula Perez Pardo's group at Utrecht University[47]. Eight-week-old male C57BL/6J male mice (Charles River Laboratories, the Netherlands) were housed under 12 hours light/dark cycle for the rotenone and vehicle infusion (n=10 for each group), food and water were provided *ad libitum*. The protocols were approved by the Ethical Committee of Animal Research of Utrecht University, Utrecht, the Netherlands.

Stereotactic surgery was performed under isoflurane anesthesia. A cannula was inserted into the right striatum through the hole drilled in the skull of mice, and 5.4 μg of freshly prepared rotenone dissolved in 2 μL dimethyl sulfoxide (DMSO) was infused. This dose was tested as the lowest effective dose without increasing mortality in the pilot experiment. The stereotaxic coordinates were set as follows: AP +0.4, ML -2.0 from the bregma and DV -3.3 below the dura. Control animals received vehicle injections. Six weeks after surgery, fecal pellets were collected from the cage floor in the morning between 8 am and 9 am, and mice were euthanized by decapitation.

3.3.2 Motor function assessment

Six weeks after the surgery, the motor function of mice was assessed by the rotarod test. The rotarod test is a performance test based on mice placed on a motor-driven rotating rod. The riding time on spinning rod can be used as a measure of the motor function, as impaired motor function is one of the most significant symptoms in PD patients.

Mice were placed on an accelerating rotating cylinder (speed start with 2 rpm and gradually increases to 20 rpm). The riding time that shows the latency to fall was recorded with a maximum of 310 s. Mice were tested at baseline and every 5 days until day 40.

3.3.3 Intestinal transit measurement

Since many PD patients develop gastrointestinal symptoms like constipation, intestinal transit was assessed in all mice after the euthanasia. Thirty minutes before the euthanasia, 0.3 mL 2.5% Evans blue in 1.5% methylcellulose was administered intragastrically for each mouse. After euthanasia, intestinal transit was measured as the distance that the blue dye traveled (cm) from the pylorus to the most distal position of migration.

3.3.4 Tissue preparation and immunohistochemistry

The colon of the animals was embedded in paraffin. Tissue sections (15 µm) were incubated with 0.3% H_2O_2 for 30 min, rehydrated and incubated with citrate buffer. Sections of colon were then incubated overnight with the primary antibodies (rabbit anti-alpha-synuclein (1: 1000, Millipore, USA), rabbit anti-ZO-1 (1: 500, Abcam, UK), and rabbit anti-CD3 (1: 1000, Abcam, UK)).

3.3.5 Image analysis

For immunostained sections, images were captured with an Olympus BX50 microscope (Olympus, Japan) equipped with a Leica DFC320 digital camera (Leica, Germany). TH-immunopositive neurons were quantified stereologically on regular spaced brain sections throughout the SN.37. One of the main pathological characteristics in PD patients is the accumulation of alpha-synuclein in the brain as well as ENS in colon. To measure alpha-synuclein expression in colon, the optical density (OD) in the area of interest was measured and corrected for non-specific background. Alpha-synuclein OD was analyzed in at least 100 crypts (with similar orientation) per animal and is expressed per 5 crypts. Stereology was performed to quantify the number of CD3- positive cells on regular spaced sections throughout the colon. 38 immunofluorescence images of ZO-1 staining were made using a Keyence BZ-9000 microscope.

3.4 Anxiety disorders mouse model

3.4.1 Animals

The mouse model of chronic social defeat stress was generated, behavior analyzed and fecal pellets provided by Prof. Iiris Hovatta's group at the University of Helsinki[77]. Five-week-old male B6, D2 and 13-26-week-old Clr-CD1 (CD-1) mice (Charles River Laboratories, Sulzfeld, Germany) were housed under 12 hours light/dark in a pathogen-free, humidity-(50 ± 15%) and temperature-controlled (22 ± 2˚C) animal facility, food and water were provided *ad libitum*. The protocols were approved by the Regional State Administration Agency for Southern Finland (ESAVI-3801-041003-2011 and ESAVI/2766/04.10.07/2014) and carried out in accordance with directive 2010/63/EU of the European Parliament and of the Council, and the Finnish Act on the Protection of Animals Used for Science or Educational Purposes (497/2013).

Before CSDS, groups of 4-6 B6 and D2 mice were housed in Makrolon Type III cages and given 10 days to acclimate. Meanwhile, CD-1 mice were individually housed in Tecniplast IVC cages and acclimatized for one week before undergoing screening as aggressors.

3.4.2 CD-1 aggressor screening

Prior to the CSDS test, all CD-1 mice were screened for appropriate levels of aggression. CD1 mice with appropriate levels of aggression were selected through a 3-day screening process prior to conducting any social defeat experiments. The selected aggressors had to meet certain criteria, including attacking in at least two consecutive sessions, having a latency to attack of less than 90 seconds, and refraining from attacking for 1-5 seconds during each session.

3.4.3 CSDS exposure

Male B6 and D2 mice were exposed to a 10-day version of the CSDS protocol with minor modifications[77]. Briefly, this involved daily confrontations of up to ten minutes between two mice of the same species: the resident (CD-1), also known as the aggressor, and the intruder (B6 or D2). To prevent habituation to the aggressors, the intruder mice were rotated every day for the duration of the CSDS protocol. The average duration of contact with an aggressor was

6 minutes and 5 seconds for B6 mice and 3 minutes and 15 seconds for D2 mice. Throughout the 10-day protocol, control mice of both strains were housed in identical cages with a clear Plexiglas divider separating them, with one mouse per side. Like the defeated mice, every 24 hours the control mice were placed in a cage with a new unfamiliar neighbor mouse. However, unlike the defeated mice, the control mice were always separated from their neighbors by a perforated Plexiglas wall and never had any physical contact with them.

3.4.4 Social avoidance (SA) test and fecal pellets collection

Twenty-four hours after the last day of CSDS, mice underwent a social avoidance test consisting of two 150-second trials. In the first trial (no target), the defeated or control mouse was placed in the center of an open arena with an empty perforated plexiglass cylinder next to one of the walls. In the second trial (target), the empty cylinder was replaced with a new one containing an unfamiliar social target CD-1 mouse. The movements of the mice were recorded with a camera connected to a computer with EthoVision XT 10 software, and the time spent in the interaction zone (IZ), a semicircle area around the cylinder, was recorded. Based on the social interaction (SI) ratio, this test allowed a categorization of the defeated mice as either stress-susceptible or stress-resilient. To obtain normal distribution and remove outliers, the data from both groups were log-transformed, and mice with SI ratios greater than 3 interquartile ranges from the median were excluded. The SI ratio border value for B6 was 76.49, and for D2 105.99. The defeated and control mice used for fecal pellet derived microbiota gene expression and proteomic profiling experiments were collected across six cohorts, with each cohort equally divided between the experiments and SI ratios being equally distributed within each phenotypic group. A subset of defeated and control animals were selected for sequencing and proteomics analyses based on their SI ratios, with mice representing the phenotypic extremes selected for the resilient and susceptible groups and mice with SI ratios closest to the mean selected for the control groups. The sample size was estimated based on successful prior sequencing experiments using the CSDS model, although availability of some groups, particularly D2 resilient mice, was limited by the number of defeated animals that could be obtained.

Seven days after the SA test, fecal pellets were collected from the cage floor between 8:00 to 11:00 am in the morning, and the bedding was changed within 24 hours. The fecal pellets were then stored at -80°C for further use.

3.5 Sample processing

3.5.1 Metabolite extraction

One fecal pellet from each mouse was used for metabolome and metaproteome extraction. One steel bead (5mm) and 300 µL freshly prepared 50% methanol were added to each sample, subjected to bead beating for 3 minutes at speed 4 in the Bullet Blender (SERVA GmbH, Germany). Then the mixtures were centrifuged at 14,000 G for 20 minutes at 4°C. 200 µL supernatant was collected for each sample in two aliquots and dried by SpeedVac (Thermo Scientific, USA) without heating, and immediately stored in -80°C for further use.

3.5.2 Metaproteome extraction and digestion

The protein pellet obtained following metabolite extraction was dried in a SpeedVac without heating. Then 120 µL pre-heated (95°C) extraction buffer (2% SDS, 100 mM DTT, 20mM Tris-HCl, pH = 8.8) was added to the dried pellet from each sample, briefly vortexed and spun for a few seconds to remove dispersed sample fragments. Then the mixtures were incubated at 95°C for 20 minutes with agitation (500 rpm) in a Thermomixer Comfort (Eppendorf, Germany), and incubated at -80°C for 10 minutes. Then the samples were subjected to bead beating for 1 minute at speed 4 in the Bullet Blender, incubated at -80°C for 10 minutes and at 95 °C for 10 minutes. Afterwards the samples were subjected to bead beating for 1 minute at speed 4 in the Bullet Blender, centrifuged at 20,000 G for 10 minutes, and the supernatant representing the protein extract was collected.

A Microcon filter (YM-30, Ultracel-30 Membrane, 30 kDa) was first rinsed with 400 µL UA solution (8 M urea, 100 mM Tris-HCl, pH = 8.8) and centrifuged at 14,000 G for 10 minutes and the eluate discarded. For protein digestion, 20 µL protein extract from the previous step was diluted to 400 µL with UA solution. Then the diluted protein extract was loaded onto the

filter and centrifuged at 14,000 G for 15 minutes with the eluate discarded. Then 200 µL UA solution was added to the filter and centrifuged at 14,000 G for 15 minutes with the eluate discarded. Protein extract on the filter was reduced with 100 µL 50 mM iodoacetamide in UA solution to the filter, mixed at 600 rpm in Thermomixer for 1 minute, incubated at 20°C for 20 minutes in the dark, then centrifuged at 14,000 G for 15 minutes with the eluate discarded. Afterwards 100 µL UA solution was added and centrifuged at 14,000 G for 15 minutes and this step repeated[259]. After adding 100 µL 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH = 8.5) solution to the filter and centrifuging at 14,000 G for 10 minutes the eluate was discarded. This step was repeated twice. 110 µL HEPES was added, mixed and 10 µL used for BCA assay with the Pierce™ BCA Protein Assay Kit (Thermo Scientific, USA) according to manufacturer's instructions to determine the protein concentration.

Trypsin was added (1:100 protein-to-trypsin ratio) to the filter, mixed at 600 rpm in Thermomixer for 1 minute and incubated at 37°C for 17 hours. Afterwards the filter unit was transferred to a new collection tube, centrifuged at 14,000 G for 10 minutes and the eluate was collected as peptide extract. After adding 100 µL 25 mM HEPES and centrifuging at 14,000 G for 10 minutes the eluate was collected. The peptide extract was then dried in a SpeedVac without heating and immediately stored at -80°C for further use.

3.5.3 TMT labeling and fractionation

The peptides were reconstituted with 25 mM HEPES solution to 1 µg/ µL, then 30 µL from each sample was taken and labeled with TMT Mass Tag Labeling Kit (Thermo Scientific, USA) according to manufacturer's instructions. For PD project, 5 µL from each sample was taken and mixed as a master control for 4 TMT6 set. For social stress project, 3 µL from each sample was taken and mixed as a master control for 3 TMT10 sets. For each set the master control was labeled with either TMT^{6}-126 or TMT^{10}-126, each sample was randomly assigned to different channels.

After TMT labeling, for each TMT set, 2 µL was taken from each labeled sample, and each aliquot was quenched with 1 µL 5% hydroxylamine, combined as a quality control (QC) sample

and dried in a SpeedVac without heating, then reconstituted with 20 µL 0.1% formic acid and desalted with Pierce™ C18 Tips (10 µL bed, Thermo Scientific, USA). QC samples were measured with a Q-Exactive Plus mass spectrometer with the same gradient as sample measurement. The mixing ratio of each channel and labeling efficiency were calculated according to the measurement. For each set the labeled peptides were quenched with 2 µL 5% hydroxylamine and mixed according to the mixing ratio from the measurement of QC samples in each set.

The labeled peptide mixture from each set was then fractionated with Pierce™ High pH Reversed-Phase Peptide Fractionation Kit (Thermo Scientific, USA) according to manufacturer's instructions. In-gradient elution was used to elute peptides, for each set 8 fractions of eluted peptides were collected, then dried in a SpeedVac without heating and stored immediately at -20 °C.

3.6 Mass spectrometry and data analysis

3.6.1 Metabolomics analysis

Two aliquots of metabolite extracts were reconstituted with 120 µL 50% methanol and 120 µL 75% acetonitrile, then centrifuged at 14000 G for 10 min at 4°C. The supernatant was divided into two aliquots for two separate measurements using reversed-phase (RP) column and hydrophilic interaction liquid chromatography (HILIC) column in both ion modes. For detecting instrument variations within each experiment, a quality control (QC) sample comprised of a mixture of 5 µL of each sample was measured. The QC samples were measured at the beginning and after every tenth injection.

Samples were analyzed using a Bruker Elute UHPLC system coupled with a Bruker Impact II TOF MS system (Bruker Daltonik GmbH, Bremen, Germany) controlled by Bruker Hystar 5.0 Software.

For RP chromatographic separation a Kinetex C18 column (50 x 2.1mm, 1.7 µm) with a SecurityGuard ULTRA holder and a SecurityGuard ULTRA cartridge (Phenomenex, USA) at 20°C in the column oven with a flow rate of 0.3 mL/min was used. Samples were kept cooled

in the autosampler at 4 °C until injection of 5 µL into the 20 µL injection loop of the HPLC. The mobile phase composition was as follows: eluent A containing 10mM ammonium formate, 0.1% formic acid, 5% acetonitrile in water and eluent B containing 10mM ammonium formate, 0.1% formic acid, 90% acetonitrile in water. The elution gradient was: 0.0 to 1.0 min, 5% B; 1.0 to 7.0 min, 5% to 95% B; 7.0 to 8.0 min, 95% B; 8.0 to 8.2 min, 95% to 5% B and 8.2 to 10 min, 5% B.

For HILIC chromatographic separation a XBridge Amide column (100 x 2.1mm, 2.5 µm) with a VanGuard Pre-column (Waters, MA, USA) at 20°C in the column oven with a flow rate of 0.4 mL/min was used. Samples were kept cooled in the autosampler at 4 °C until injection of 5 µl into the 20 µl injection loop of the HPLC. The mobile phase composition was as follows: eluent A containing 10mM ammonium formate, 0.1% formic acid, 5% acetonitrile in water and eluent B containing 10mM ammonium formate, 0.1% formic acid, 90% acetonitrile in water. The elution gradient was: 0.0 to 2.0 min, 99% B; 2.0 to 22.0 min, 99% to 50% B; 22.0 to 25.0 min, 50% B; 25.0 to 25.2 min, 50% to 99% B and 25.2 to 30 min, 99% B.

Samples flowed splitless into the ESI source. Compounds were ionized with an end plate offset of 500 V, a capillary voltage of 4500 V in positive and 3500 V in negative mode. The nebulizer nitrogen gas flow was 10 L/min with a pressure of 2.5 bar heated to 240 °C. Profile data were acquired with Bruker Compass 3.0 with a spectra rate of 1 Hz in full scan and a mass range from 50-1300 m/z. MS/MS spectra data were generated from sample QCs of the 3 most intense ions selected for fragmentation within a mass range of ±4-6 Da and fragmented. Collision energies were 16 eV for ions with m/z of 100 to 25 eV for ions with m/z of 500 and 40 eV for ions with m/z 1000 and larger, with a linear energy interpolation and a spectra rate of 5 Hz.

3.6.2 Metabolomics data processing and statistical analysis

The calibration, feature extraction and annotation were performed with MetaboScape 4.0 (Bruker, USA). The metabolite features were extracted with the following parameters: intensity threshold 1500, minimum peak length 10, minimum peak length 8 (recursive), minimum feature for extraction were 70% of all samples, 30 min retention time, mass range 50-1300 m/z, 0.8 EIC correlation. Features were annotated with an in-house metabolite library generated from

measuring purified compounds under the same experimental conditions. Default settings for tolerances and scoring were applied for metabolite annotation.

Annotated bucket tables generated by MetaboScape were processed with Perseus 1.6.14.0[260]. Raw intensities for each metabolite features were median normalized and log2 transformed. A two-sample Welch's T-test was conducted with p-value of 0.05 as cut-off.

3.6.3 Proteomics analysis

Samples were analyzed using a Thermo UltiMate 3000 UHPLC coupled with a Q-Exactive Plus Mass Spectrometer (Thermo Scientific, USA) under the control of Xcalibur v4.2.47 (Thermo Scientific, USA). For chromatographic separation, an in-house packed C18 column (150 mm x 2.1mm, particle size 1.9 μm) with a flow rate of 300 μL/min was used. Samples were kept cooled in the autosampler at 4 °C until injection of 2 μL into the HPLC. The mobile phase composition was as follows: eluent A containing 0.1% formic acid in water and eluent B containing 10mM ammonium formate, 0.1% formic acid, 90% acetonitrile in water. The elution gradient was: 0 to 15 min, 5% B; 15 to 125 min, 5% to 30% B; 125 to 145 min, 30% to 60% B; 145 to 146 min, 60% to 99% B; 146 to 151min, 98% B; 151 to 153min, 98% to 4% B and 153 to 170 min, 4% B.

The mass spectrometer was operated in the data-dependent acquisition (DDA) MS/MS mode. The spray voltage and temperature of ion transfer capillary were set to 1.85 kV and 250°C. The full mass scans were acquired with the range of 375 to 1400 m/z and a resolution of 70,000 in profile mode. For each full scan the 10 most intense precursor ions were selected and fragmented with 32% value for normalized collision energy (NEC), and the dynamic exclusion time was set to 30 s. The MS/MS scans were acquired with a resolution of 35,000 in centroid mode.

3.6.4 Metaproteomics data analysis

Target-decoy database search was carried out using Proteome Discoverer 2.4 (Thermo Scientific, USA) with the Sequest HT search engine against a combined database consisting of

the mouse gut microbiota metagenomic database and all mouse (*Mus musculus*) proteins from Swiss-Prot (October on 2020)[233, 243, 261]. Parameters for database search were set as follows: precursor mass tolerance of 10 ppm; fragment mass tolerance of 0.02 Da; cysteine carbamidomethylation, TMT 6-plex/TMT 10-plex tags on lysine and peptide N-terminus as static modifications; oxidation of methionine as dynamic modification; trypsin as protease and a maximum 2 missed cleavages. Percolator was used for peptide validation, false discovery rate (FDR) of 0.01 was applied for protein and peptide identification.

TMT reporter ion intensities with sample loading normalization from MS2 spectra were used for quantitative analysis, intensities were normalized between sets with internal reference scaling (IRS) method. Briefly, a scaling factor for each TMT set was calculated as intensities of master control divided by the average intensities of all master controls. Then each channel was divided by the scaling factor from corresponding TMT set. Proteins that were identified in more than 70% of samples were kept for further analysis.

The Welch's T-test was conducted for all identified proteins, the p-value of 0.05 and the pi-score of 1.3 (pi-score = -log10p-value * log2FoldChange) were applied as the cutoff.

3.6.5 Molecular pathway analysis

Identified proteins from the mouse gut microbiota metagenomic database were annotated with KEGG Orthology (KO) id using the criteria of identity >95% and overlap >90%[243]. All altered microbial proteins that annotated with KO id were mapped to KEGG pathway for enrichment analysis[262]. The Fisher's exact test was conducted and enrichment ratio was also calculated for each pathway. Enrichment ratio = (# of significant proteins in pathway(k) / total # of detected proteins in pathway(m)) / (total # of significant proteins(n) / total # of detected proteins(N))[263]. An enrichment ratio greater than 1 indicates that the pathway contains more significantly changed proteins compared to the overall study. Pathways with more than 1 protein hit, an enrichment ratio greater than 1 and p-value less than 0.05 were considered significant.

The workflow for metaproteomics data analysis is shown in Figure 3.2. The R and Python scripts are attached in Appendices 1-3. The Python scripts in Appendix 2 are modified based on the scripts from Mills, Robert H., et al.[264].

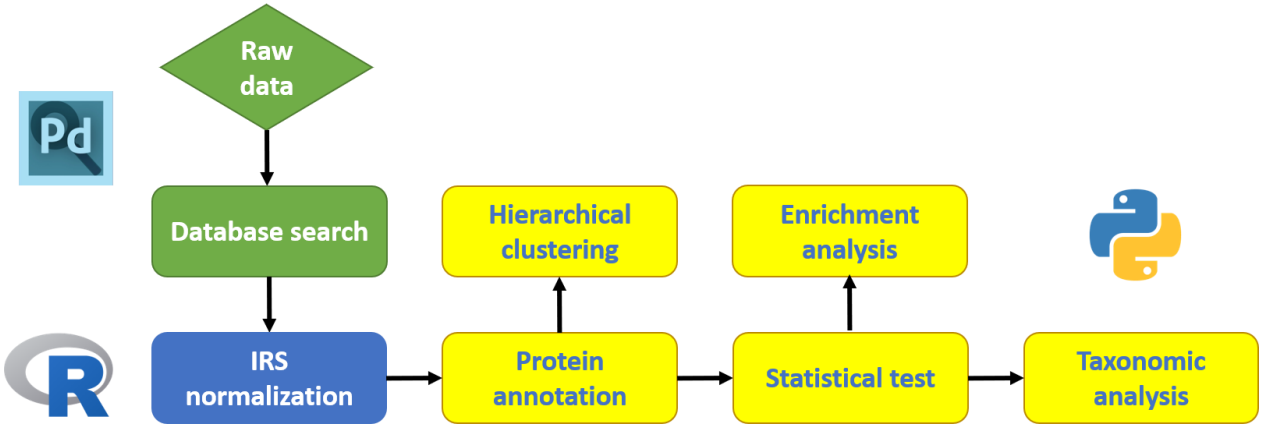


Figure 3.2. Metaproteomics data analysis workflow including Proteome Discoverer software (green), R scripts (blue) and Python scripts (yellow). IRS (Internal Reference Scaling).

4 Results

4.1 Parkinson's disease mouse model

4.1.1 Rotenone treatment generates PD phenotype in mice

The mouse experiments were carried at Utrecht University by Dr. Paula Perez Pardo's group. To assess whether rotenone treatment induces a PD-like phenotype, three indicators of PD, motor function, intestinal transit and alpha-synuclein expression in ENS were measured.

In the rotarod test, all rotenone-treated mice fell before the end of the test (310s), while all vehicle-treated mice stayed on the rotating cylinder until the end. The rotenone-treated mice showed significant motor deficits in terms of the latency to fall compared with vehicle-treated mice (p-value < 0.01, Figure 4.1 A).

The distance that the blue dye traveled in GI tract was measured as intestinal transit time to assess the effects of rotenone in GI function. Rotenone-treated mice showed a significant decreased intestinal transit (p-value < 0.01, Figure 4.1 B), which represented the delayed intestinal transit.

The alpha-synuclein expression in the ENS was also measured since an abnormal accumulation of alpha-synuclein is always observed in PD patients. The expression level of alpha-synuclein in the rotenone-treated group was significantly higher than in vehicle-treated mice (p-value < 0.01, Figure 4.1 C). Taken together the above experiments suggest that the rotenone treatment induced a PD-like phenotype in the mice.

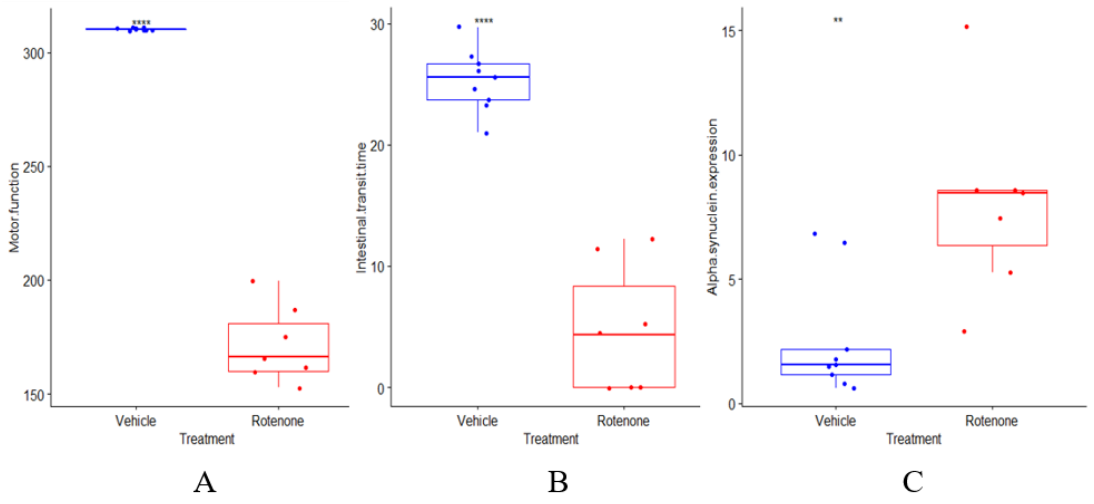


Figure 4.1. Effects of rotenone treatment on (A) motor function (B) intestine transit time and (C) alpha-synuclein expression in enteric nervous system.

4.1.2 16S rRNA based microbial taxonomy profile

The 16S rRNA sequencing was carried out at the Micalis Institute by the group of Dr. Sylvie Rabot. The fecal microbial composition was assessed in rotenone-treated and vehicle-treated mice by 16S rRNA sequencing. The microbial structure was not affected by rotenone treatment. According to the Shannon index, which is a quantitative measure of alpha diversity representing the richness of the microbial community, there was no significant difference in richness between the fecal microbial community in rotenone- and vehicle-treated mice (adjusted p-value = 0.13) (Figure 4.2 A).

The beta diversity, which represents the diversity between the rotenone- and vehicle-treated group, was assessed by calculating the Bray-Curtis diversity. It also did not reveal a significant difference between rotenone and vehicle treated groups, and no separation between two groups according to the ordinal plot (Figure 4.2 B).

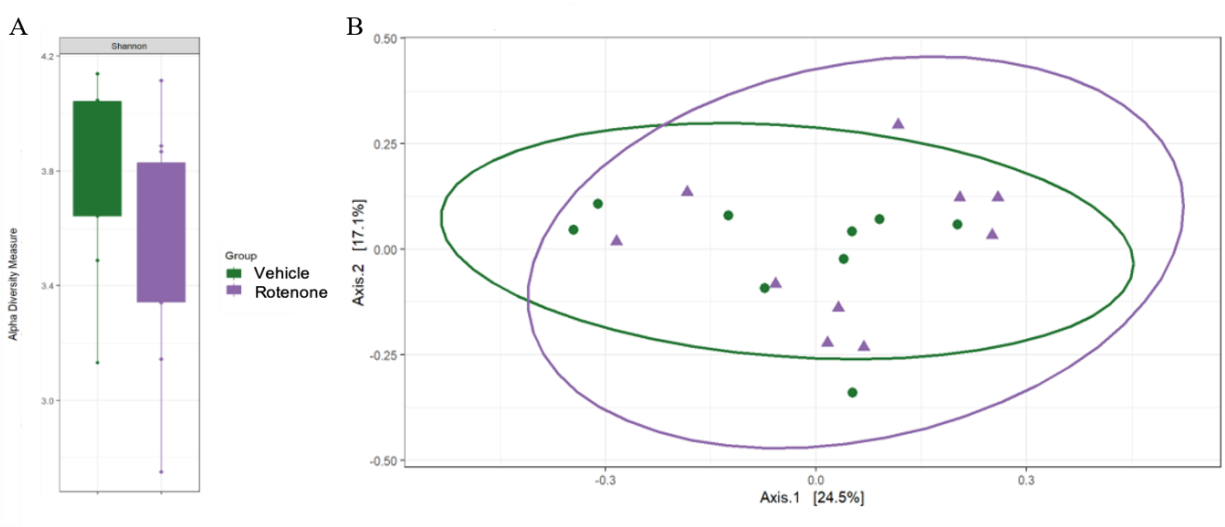


Figure 4.2. (A) Alpha diversity comparison between rotenone- and vehicle-treated mice in Shannon index. (B) Ordinal plot (Multidimensional scaling plot based on Bray-Curtis matrix) of microbial community structure at family level.

I next performed Welch's T-test at different taxonomic levels (phylum, family, order and genus) shown in Table 4.1 (Figure 4.3). At the phylum level there was no significant difference, and the difference in the *Firmicutes/Bacteroidetes* (F/B) ratio between the two groups was also not significant. At the order level, the rotenone-treated group showed a significant decrease in the relative abundance of *Peptococcales* (p-value = 0.0157) and *Peptostreptococcales-Tissierellales* (p-value = 0.0093). At the family level, the rotenone-treated group showed a significant decrease in the relative abundance of *Peptococcaceae* (p-value = 0.0157), *Peptostreptococcaceae* (p-value = 0.0073) and a significant increase in *[Eubacterium] coprostanoligenes* group (p-value = 0.0257). At the genus level, the rotenone-treated group showed a significant decrease in the relative abundance of *Peptococcus* (p-value = 0.0157).

Table 4.1. Significantly changed taxon at order, family and genus levels based on 16S rRNA.

	Name	**Vehicle**	**Rotenone**	**P-value**
Order	*Peptococcales*	0.0846	0.0450	0.0157

	Peptostreptococcales-Tissierellales	1.3913	0.4504	0.0093
Family	*Peptococcaceae*	0.0846	0.0450	0.0157
	Peptostreptococcaceae	1.3193	0.3563	0.0073
	[Eubacterium] coprostanoligenes group	0.6941	2.1806	0.0257
Genus	*Peptococcus*	0.0846	0.0450	0.0157

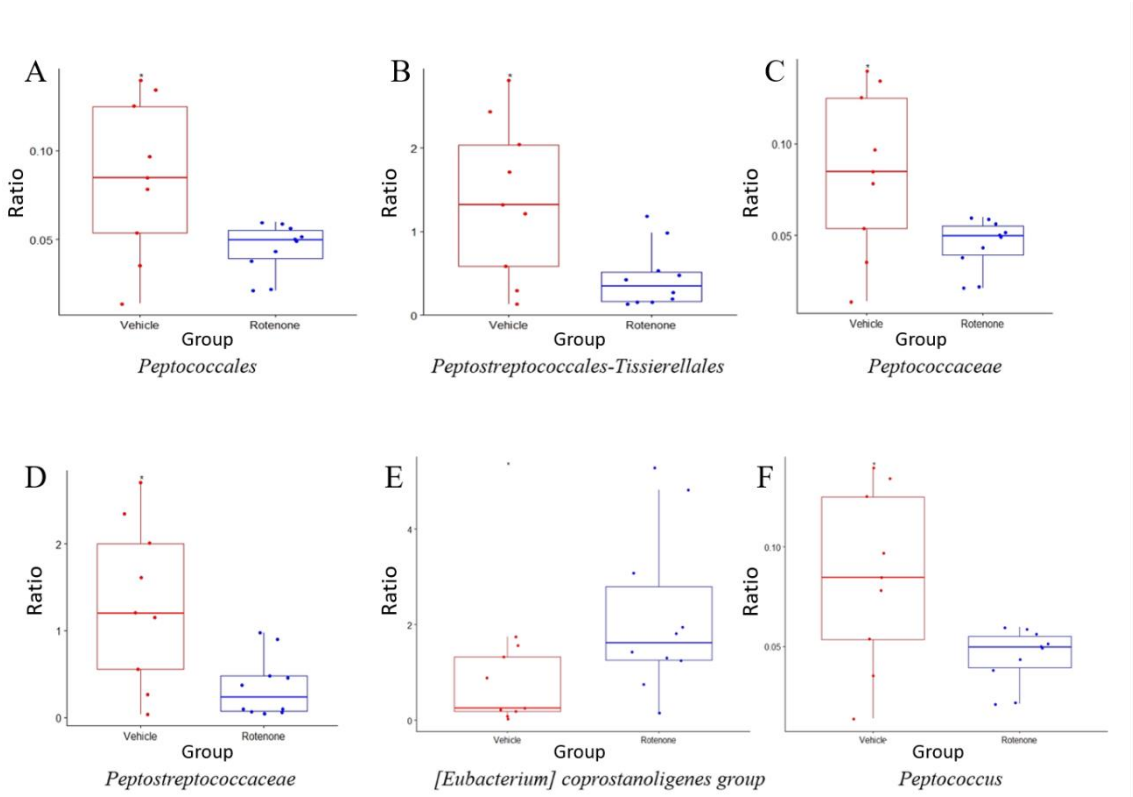


Figure 4.3. Significantly changed taxon at (A-B) order, (C-E) family and (F)genus levels based on 16S rRNA.

4.1.3 TMT6plexed quantitative analysis of fecal metaproteome

To investigate the fecal metaproteome profile, we performed mass spectrometry with TMT6plexed labeling and peptide fractionation to achieve a comprehensive coverage of protein identification with high quantitative accuracy.

Internal reference scaling (IRS) normalization was applied by using the scaling factor calculated with the master control within each set[265]. Prior to the IRS normalization there were significant batch effects due to different TMT sets (126 channel), and samples within the same TMT set were grouped together despite different treatment (Figure 4.4 A). After the IRS normalization the batch effect introduced by different TMT sets was corrected and samples were grouped by biological treatment rather than TMT set (Figure 4.4 B). Sample 4.4 from rotenone treated group was removed for further analysis as an outlier.

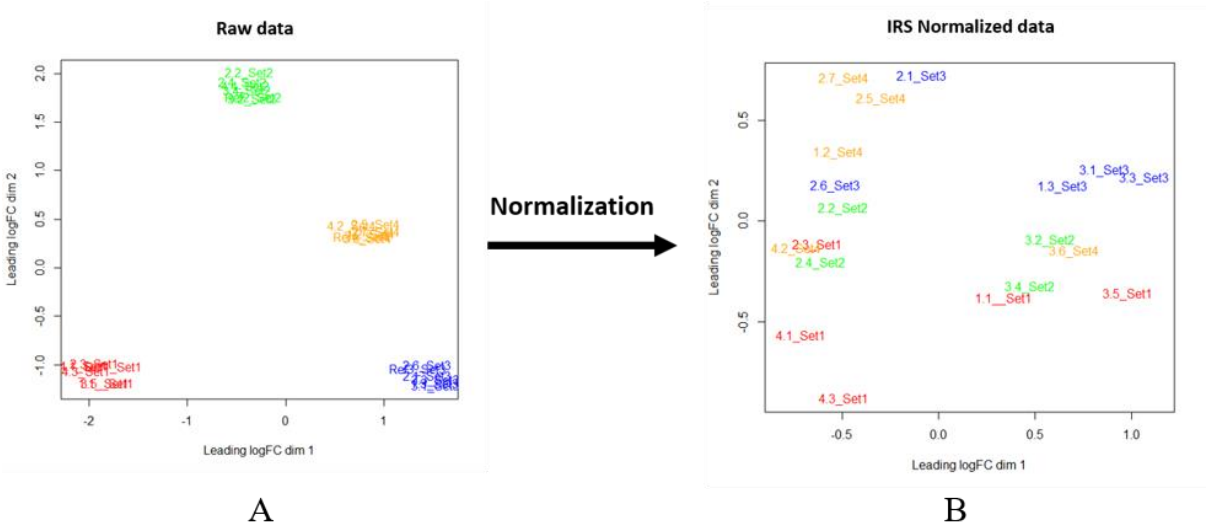


Figure 4.4. Multidimensional scaling plot of four TMT sets before (A) and after (B) internal reference scaling normalization.

A total of 13,396 unique proteins were identified and quantified in 4 TMT sets (Figure). Among them, 7,246 unique proteins were identified in TMT set1, 6,349 unique proteins in TMT set2, 6,470 unique proteins in TMT set3 and 6,523 unique proteins in TMT set4. 4,688 unique proteins were identified across all samples and 6,327 unique proteins were identified in more

than 70% of the samples, including 5,279 microbial proteins and 1,048 mouse proteins, which were used for further quantitative analysis (Figure 4.5). All identified mouse proteins were annotated with the Swiss-Prot database (October to 2020), and all microbial proteins were annotated with the annotation tables provided by the public metagenomic database we used for database search. Each microbial protein was annotated with taxonomic information, COG ID, EggNOG ID, KEGG Orthogoly ID and function description.

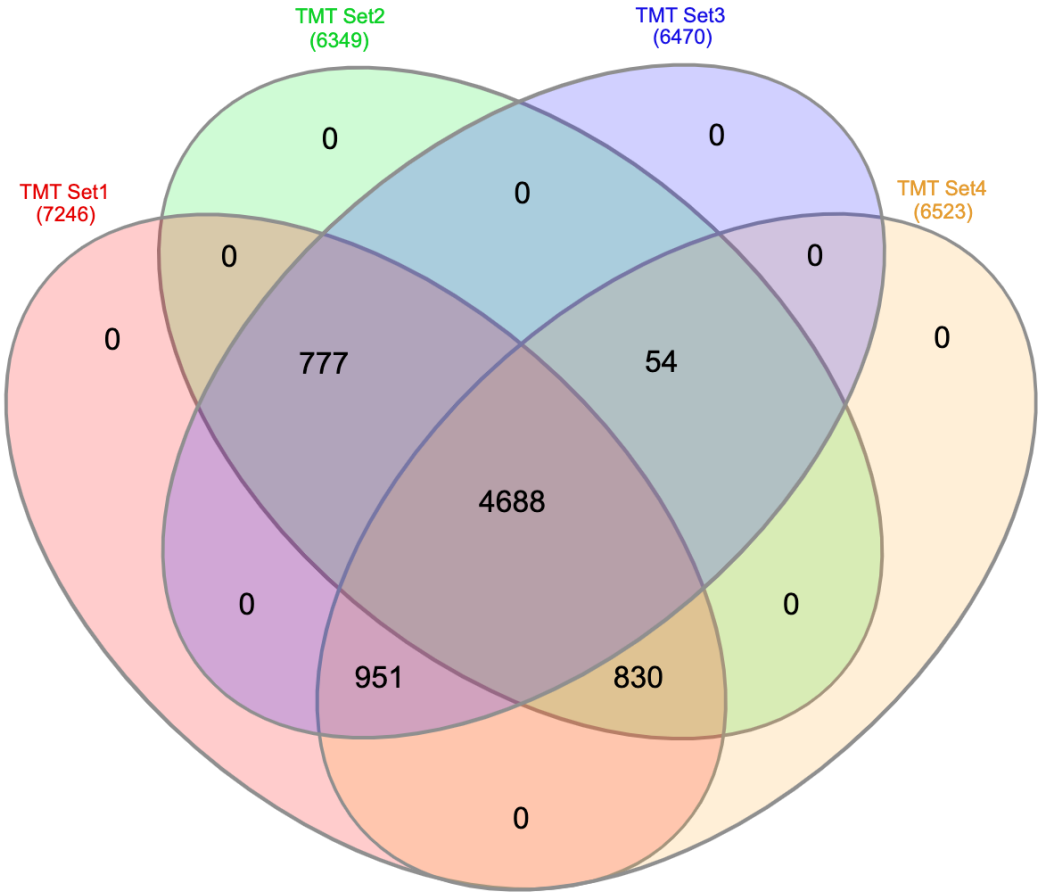


Figure 4.5. Proteins identified and quantified in all TMT datasets.

4.1.4 Protein expression comparison between rotenone and vehicle group

To get an overview of mouse and microbial protein expression, we performed hierarchical clustering and created separate heatmaps. The heatmap of all mouse proteins did not result in

two major groups corresponding to rotenone- and vehicle-treated mice (Figure 4.6 A). In contrast, the heatmap of all microbial proteins resulted in two major groups that approximately correspond to rotenone- and vehicle-treated mice (Figure 4.6 B).

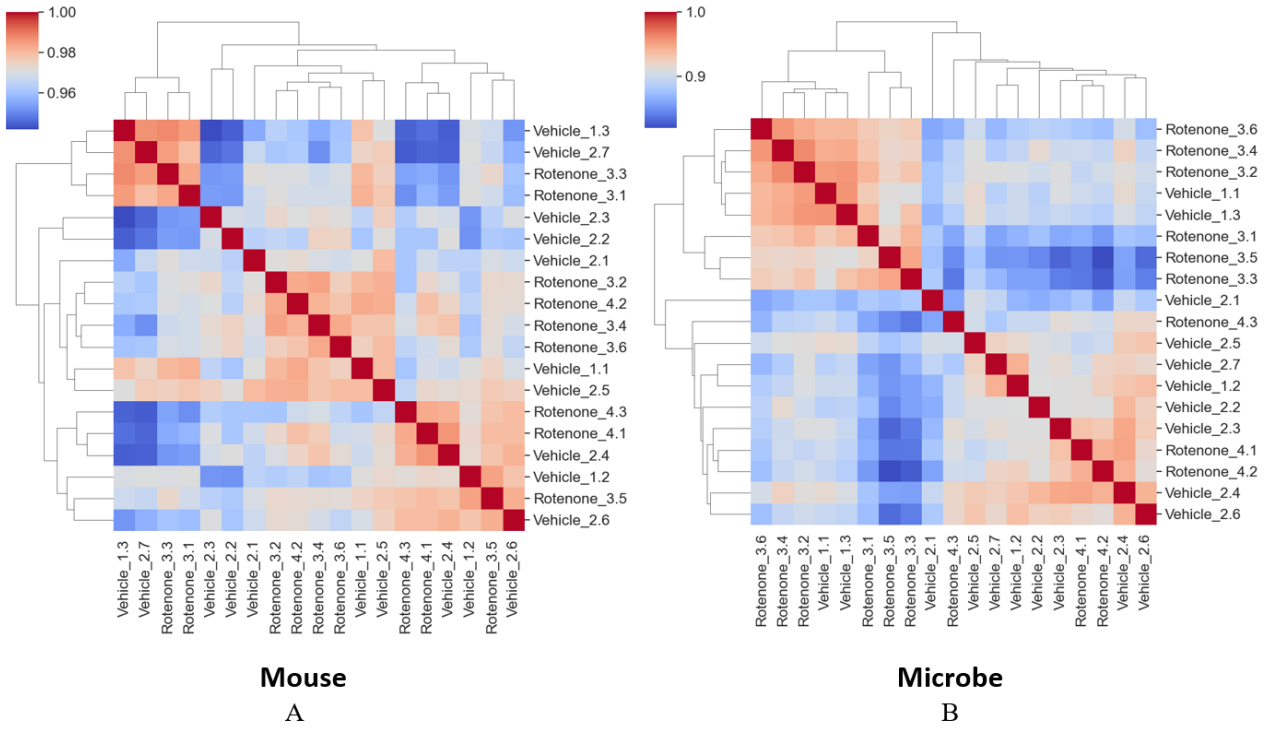


Figure 4.6. Hierarchically-clustered heatmap of mouse proteins and microbial proteins based on Spearman's correlation.

Quantitative analysis of protein expression was performed by comparing rotenone and vehicle-treated groups. Welch's T-test was performed for all proteins quantified in the two groups, using a p-value of 0.05 and a pi-score of 1.3 (pi-score = -Log10p-value * Log2FC, pi-score is equal to 1.3 when FC = 2 and p-value = 0.05) as the cutoff. No mouse proteins showed significant changes (Figure 4.7 A). However, 178 microbial proteins were significantly changed between the two groups, with 110 proteins upregulated and 68 proteins downregulated compared to the vehicle-treated group (Figure 4.7 B, Appendix 1).

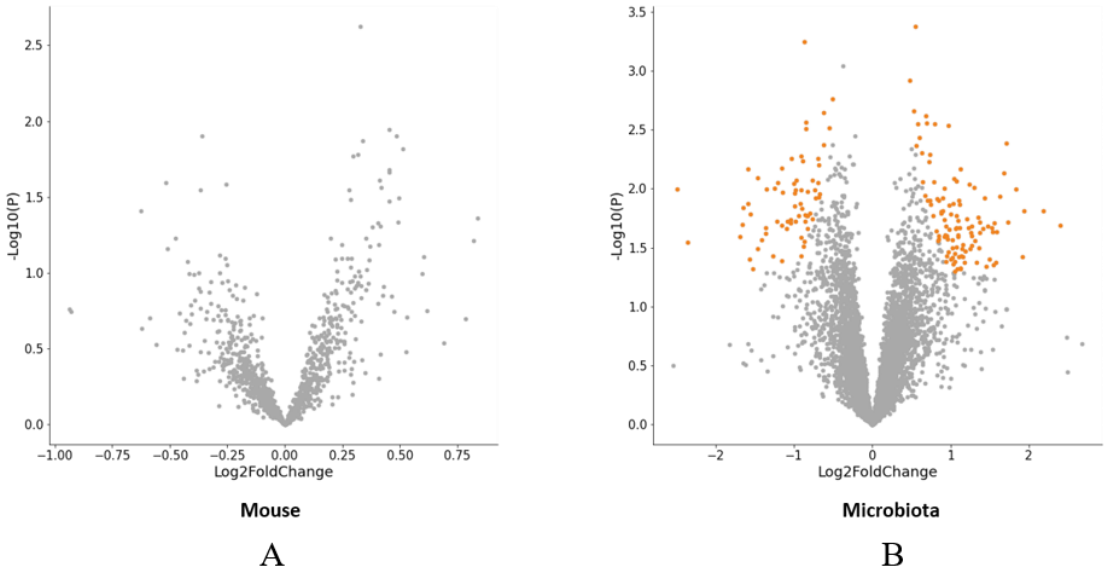


Figure 4.7. Volcano plot of quantified (A) mouse proteins and (B) microbial proteins.

4.1.5 Metaproteome based microbial taxonomy profile

I next evaluated the fecal microbial profile in rotenone- and vehicle-treated mice using metaproteomics data to check whether there was a significant difference in protein expression of certain taxa and also whether certain taxa were significantly enriched at the corresponding taxonomic level.

I performed Welch's T-test at different taxonomic levels (phylum, family, order and genus) as shown in Table 4.2 (Figure 4.8). At the phylum level, the rotenone-treated group showed a significant decrease in the relative abundance of *Fibrobacteres* (p-value = 0.0241). The difference in the *Firmicutes*/*Bacteroidetes* (F/B) ratio between the two groups was not significant. At the order level, there was no significant difference. At the family level, the rotenone-treated group showed a significant increase in the relative abundance of *Peptostreptococcaceae* (p-value = 0.0290). At the genus level, the rotenone-treated group showed a significant decrease in the relative abundance of *Dorea* (p-value = 0.0365) and *Porphyromonas* (p-value = 0.0480).

Table 4.2. Significant changed taxon at order, family and genus level based on microbial proteins

	Name	**Vehicle**	**Rotenone**	**P-value**
Phylum	*Fibrobacteres*	4.16E-4	2.76E-4	0.0241
Family	*Peptostreptococcaceae*	4.00E-3	8.30E-3	0.0290
Genus	*Dorea*	4.44E-3	3.08E-3	0.0365
	Porphyromonas	9.40E-4	7.89E-4	0.0480

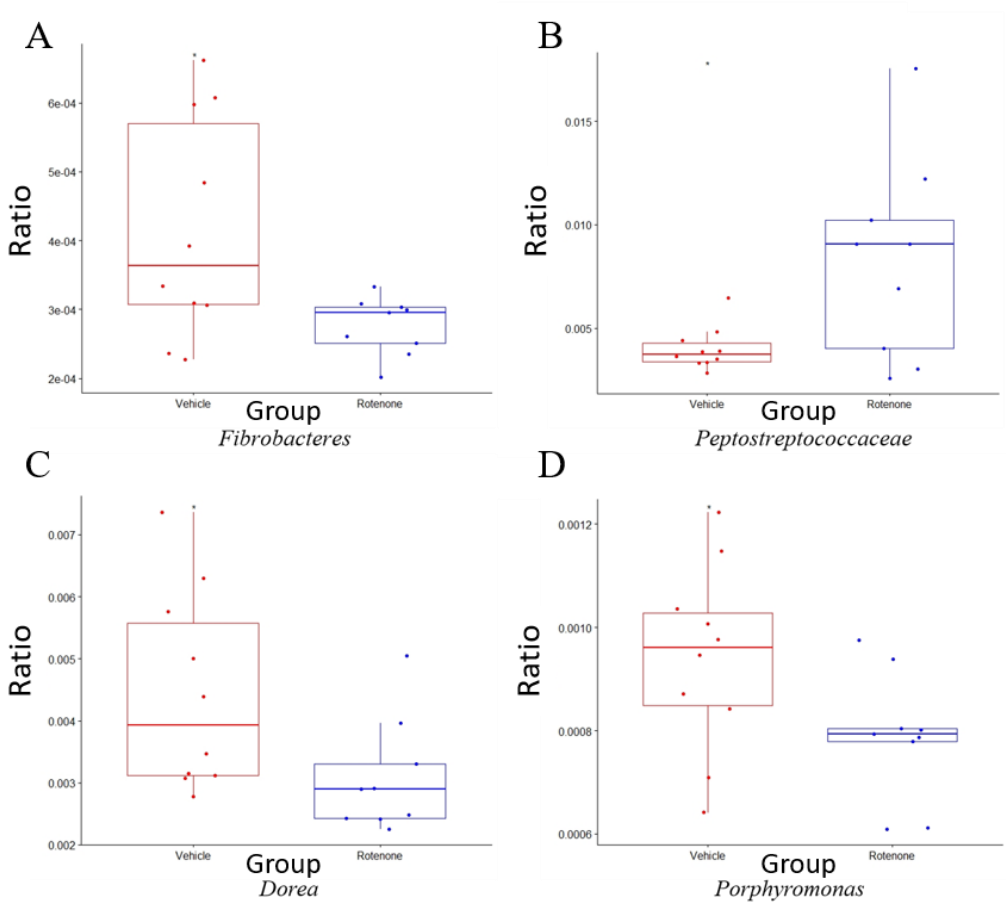


Figure 4.8. Significant changed taxon at (A-B) order, (C-E) family and (F) genus levels based on microbial proteins.

Enrichment analysis was performed to determine whether differentially expressed proteins were enriched in specific taxa. Using the protein hit of 1, enrichment ratio of 1 and a p-value of 0.05 as cutoff, we found several taxa that were enriched at different taxonomic levels. At the phylum level, *Firmicutes* was significant with an enrichment ratio of 1.75 and a p-value of 1.2E-6. At the order level, *Clostridialesa* was significantly enriched with an enrichment ratio of 1.77 and a p-value of 7.34E-6. At the family level, *Lachnospiraceae* was significant with an enrichment ratio of 2.85 and p-value of 2.21E-5. At the genus level, *Butyrivibrio* was significant with an enrichment ratio of 6.39 and a p-value of 5.41E-4, and *Clostridium* was significant with an enrichment ratio of 2.81 and a p-value of 0.028 (Figure 4.9).

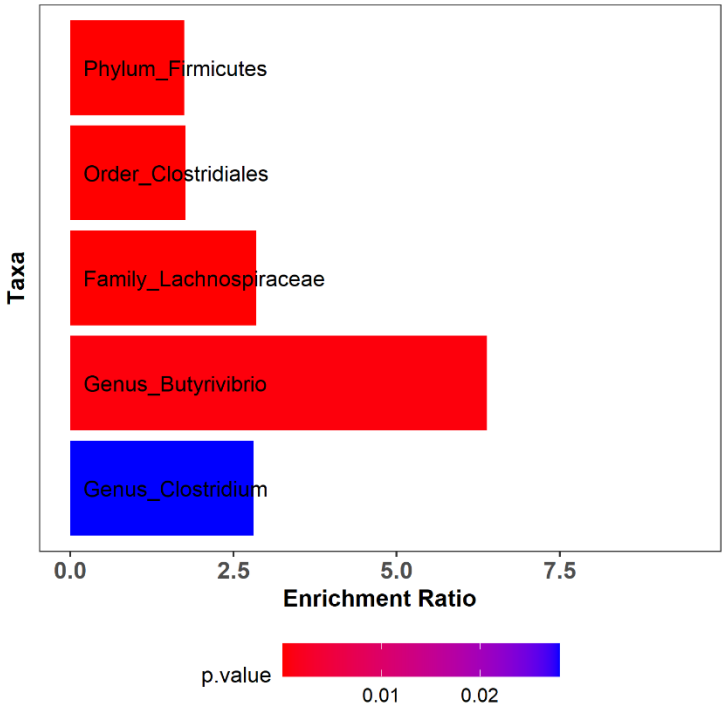


Figure 4.9. Significantly enriched taxa

4.1.6 Pathway enrichment analysis

To investigate the active microbial functions that were affected by rotenone treatment, we performed a pathway enrichment analysis with all of the identified microbial proteins with KO id annotation (Figure 4.10). First, all proteins were mapped to the KEGG pathways based on KO id. The enrichment ratios were calculated as (number of significant proteins in the

pathway/total number of detected proteins in the pathway) / (total number of significant proteins/total number of detected proteins).

With the protein count of 1, enrichment ratio of 1 and a p-value of 0.05 as cutoff, 10 pathways were enriched. Interestingly, nine pathways ("Shigellosis", "Flagellar assembly", "NOD-like receptor signaling pathway", "Plant-pathogen interaction", "Two-component system", "Legionellosis", "ABC transporters", "Salmonella infection", and "Bacterial chemotaxis") were associated with the immune response, based on sequence homolgy between the identified microbial proteins and functional orthologs of the KEGG Orthology database. The most significantly enriched pathway is "Shigellosis", while the pathway with the highest enrichment ratio is "Phosphotransferase system (PTS)".

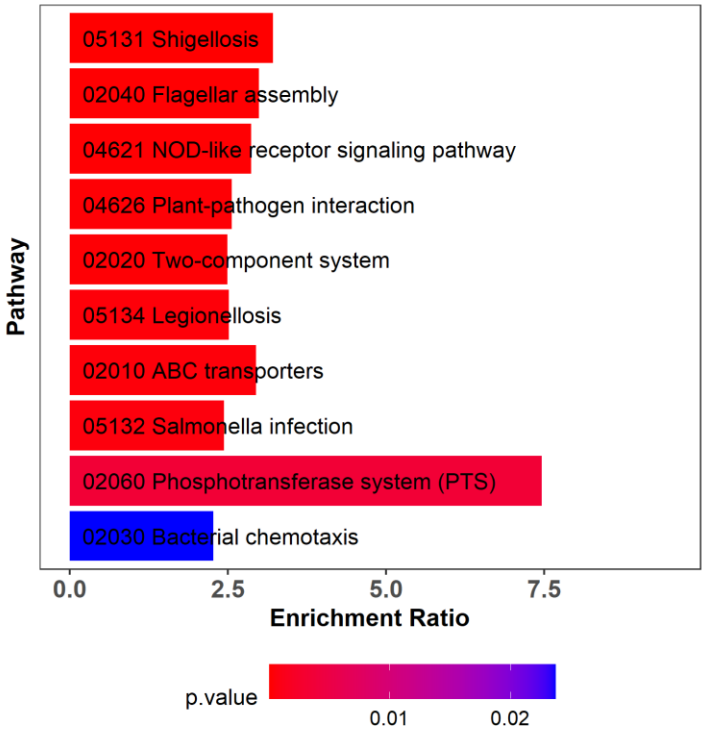


Figure 4.10. Significantly enriched pathways

4.1.7 Significantly changed proteins associated with PD indicators

The rotarod test was measured as an indicator of impaired motor function, intestinal transit time was measured as a test of intestinal function, and alpha-synuclein expression in the ENS was

measured as a test of alpha-synuclein accumulation. All three analyses showed that the rotenone-treated group had a PD-like phenotype.

To further investigate whether the microbial proteins were involved in the development of the PD phenotype in the rotenone-treated group, we calculated the Spearman's correlation coefficient between the top 10 significantly upregulated and downregulated microbial proteins with these three indicators. The results showed that most of the proteins were highly correlated with all three indicators. Specifically, they were positively correlated with motor function and intestinal transit time and negatively correlated with alpha-synuclein expression in the ENS (Figure 4.11).

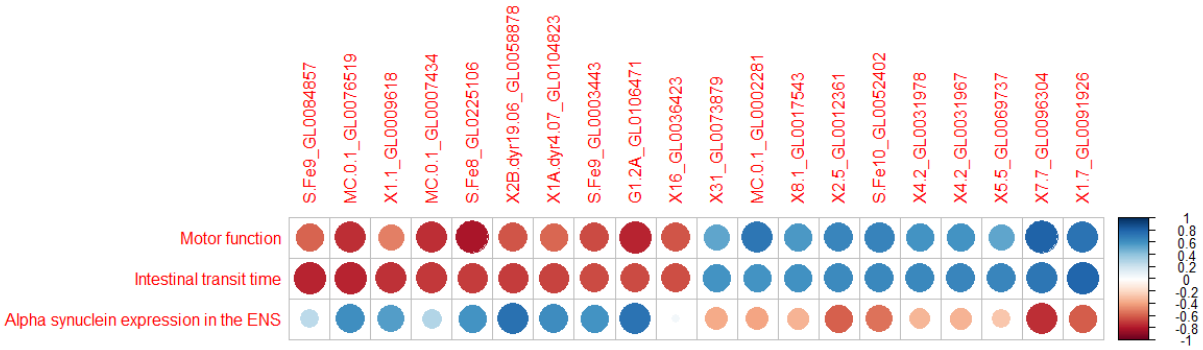


Figure 4.11. Pearson correlation between top 10 significantly upregulated and downregulated microbial proteins with motor function, intestinal transit time and alpha-synuclein expression in the enteric nervous system.

4.1.8 Significantly changed metabolites between rotenone- and vehicle-treated groups

Next I checked for altered metabolite levels in fecal pellets using untargeted metabolomics analysis by LC-MS/MS. Samples were measured using both HILIC and RP columns in positive and negative modes and annotated with our in-house metabolite database.

In total, 92 unique metabolites either from were annotated and quantified in more than 70% of the samples. We performed Welch's T-test to compare rotenone and vehicle-treated groups using a p-value of 0.05 as the cut-off. Among the quantified metabolites, four were upregulated (purine, cortisol, nutriacholic acid, and deoxycorticosterone acetate) and five were downregulated (2-hydroxybutyric acid, bis(2-ethylhexyl) phthalate, glycolic acid, succinic acid, pipecolic acid) (Figure 4.12).

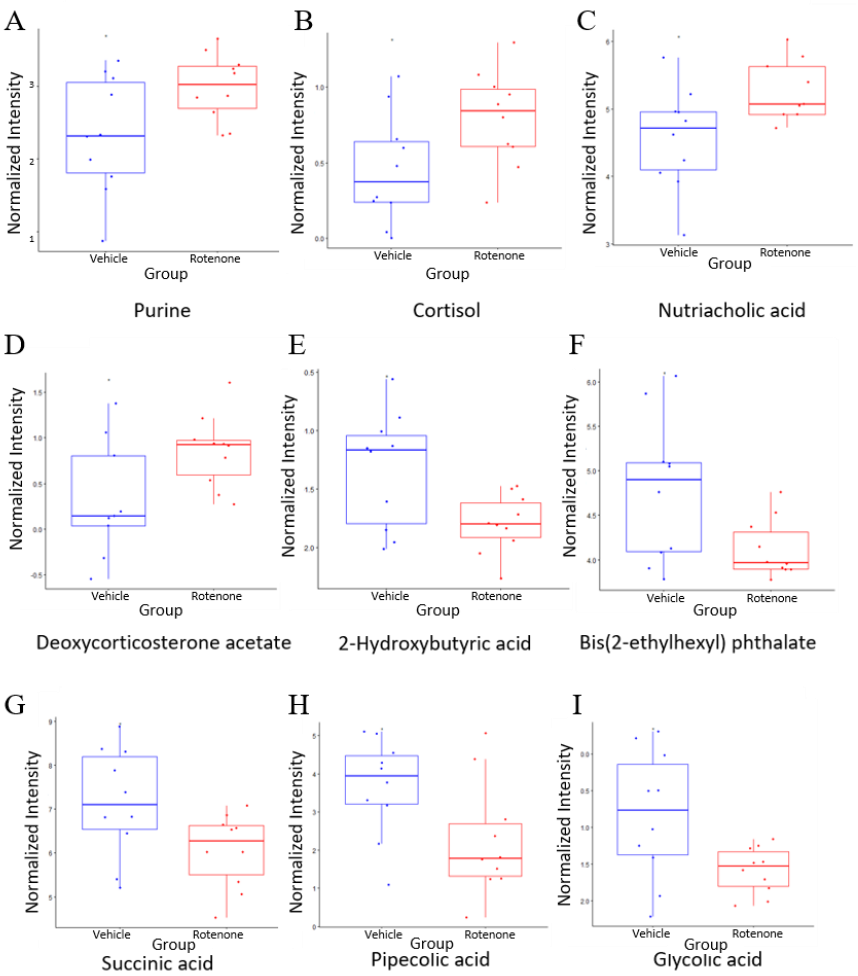


Figure 4.12. Significantly changed metabolites.

4.2 Anxiety disorders mouse model

4.2.1 Genetic background's impact on behavior response to CSDS

To assess the effect of the genetic background on the behavioral response to chronic psychosocial stress, B6 and D2 mice were exposed to a 10-day CSDS protocol and then subjected to the SA test 24 hours later. Fecal pellets were collected from the defeated mice 7 days after the SA test (Figure 4.13 A). The behavior of the defeated mice was compared with that of the same-strain controls and the defeated mice were divided into two groups based on their SI ratios (Figure 4.13 B). Stress-susceptible mice were defined as those with SI ratios less than one standard deviation from the mean SI ratio of the same-strain controls. All other

defeated mice with SI ratios above these values and similar to controls, were classified as resilient. The strains exhibited distinct responses to stress, as 71.4% of D2 mice but only 16.7% of B6 mice exhibited social avoidance behavior and were considered susceptible to chronic psychosocial stress (Pearson's chi-square, $\chi 2 = 7.80$, P = 5.23E-3) (Figure 4.13 C).

Mice were divided into 6 groups based on the strain (B6 or D2), treatment (control or defeat) and response (resistance or susceptible). For B6 strain, BC indicates control group, BS indicates stress-susceptible group, BR indicates stress-resilient group; for D2 strain, DC indicates control group, DS indicates stress-susceptible group, DR indicates stress-resilient group. Fecal pellets from 4 BC mice, 2 BS mice, 3 BR mice, 4 DC mice, 6 DS mice and 3 DR mice were used for metaproteomics and metabolomics analyses.

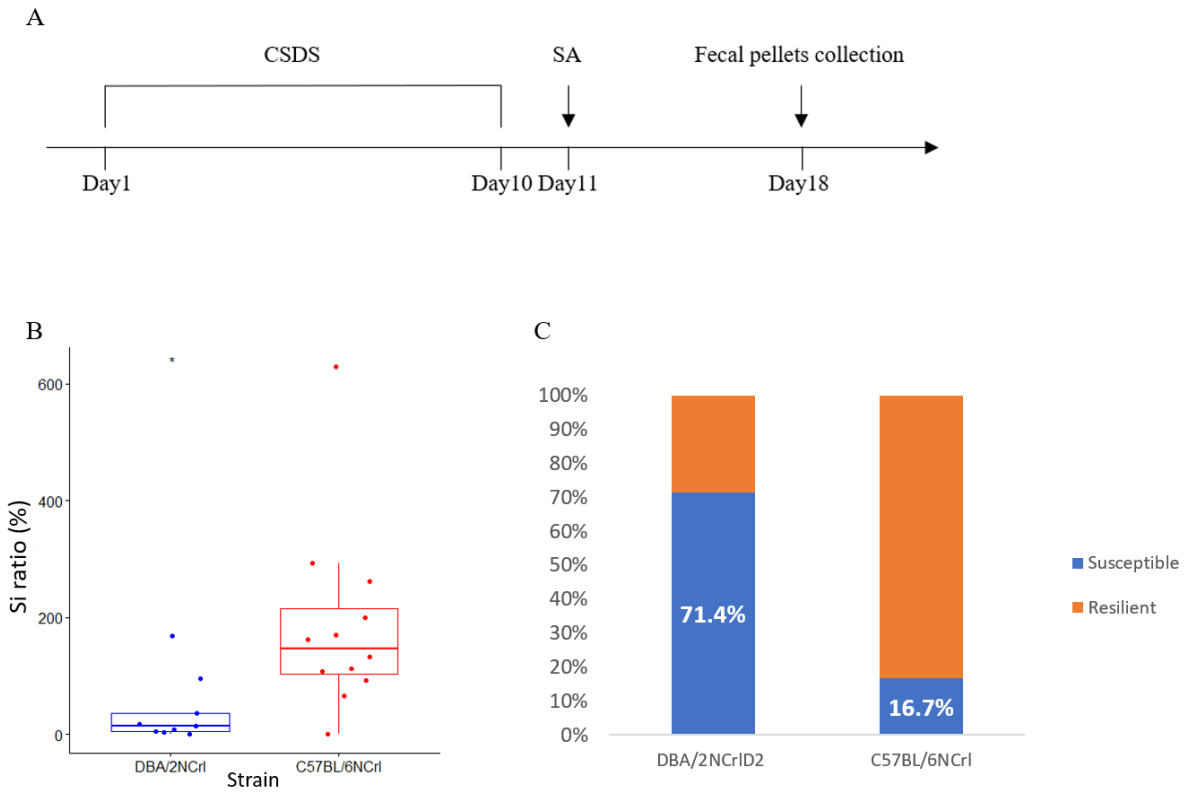


Figure 4.13. Effect of genetic background on behavioral response to chronic social defeat stress (CSDS) test. (A) Timeline of the CSDS test, social avoidance (SA) test, and fecal pellet collection. (B) Social interaction (Si) ratios

of the two strains in the SA test. (C) Percentage of mice resilient and susceptible to CSDS test. C57BL/6NCrl strain: Susceptible N = 2, Resilient N = 10; DBA/2NCrl strain: Susceptible N =10, Resilient N = 4.

4.2.2 TMT10plexed quantitative analysis of fecal metaproteome

To investigate the fecal metaproteome profile, we performed mass spectrometry with TMT10plexed labeling and peptide fractionation to achieve a comprehensive coverage of identified proteins with high quantitative accuracy.

Internal reference scaling (IRS) normalization was applied by using the scaling factor calculated with the master control within each set[265]. Before the IRS normalization there were significant batch effects due to different TMT sets, and samples within the same TMT set were grouped together despite different treatment (Figure 4.14 A). After the IRS normalization the batch effect introduced by different TMT sets was corrected and samples were grouped by biological treatment rather than by TMT set (Figure 4.14 B).

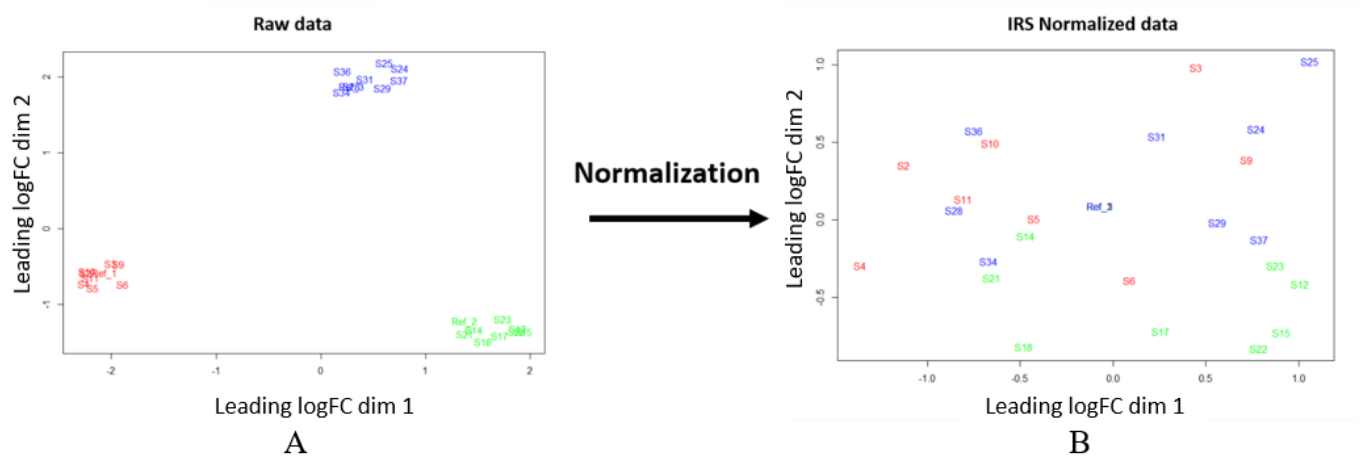


Figure 4.14. MDS plot of three TMT sets before (A) and after (B) IRS normalization.

A total of 13,396 unique proteins were identified and quantified in 3 TMT sets (Figure 4.15). Among them, 16,710 unique proteins were identified in TMT set1, 12,563 unique proteins in

TMT set2 and 10,742 unique proteins in TMT set3. 47,92 unique proteins were identified in all samples. 10,622 unique proteins were identified in more than 2 TMT sets, including 10,072 microbial proteins and 550 mouse proteins which were used for further quantitative analysis (Figure 2.15). All identified mouse proteins were annotated with the Swiss-Prot database (October 2020), and all microbial proteins were annotated with the annotation tables provided by the public metagenomic database that we used for database search. Each microbial protein was annotated with taxonomic information, COG ID, EggNOG ID, KEGG Orthogoly ID and function description.

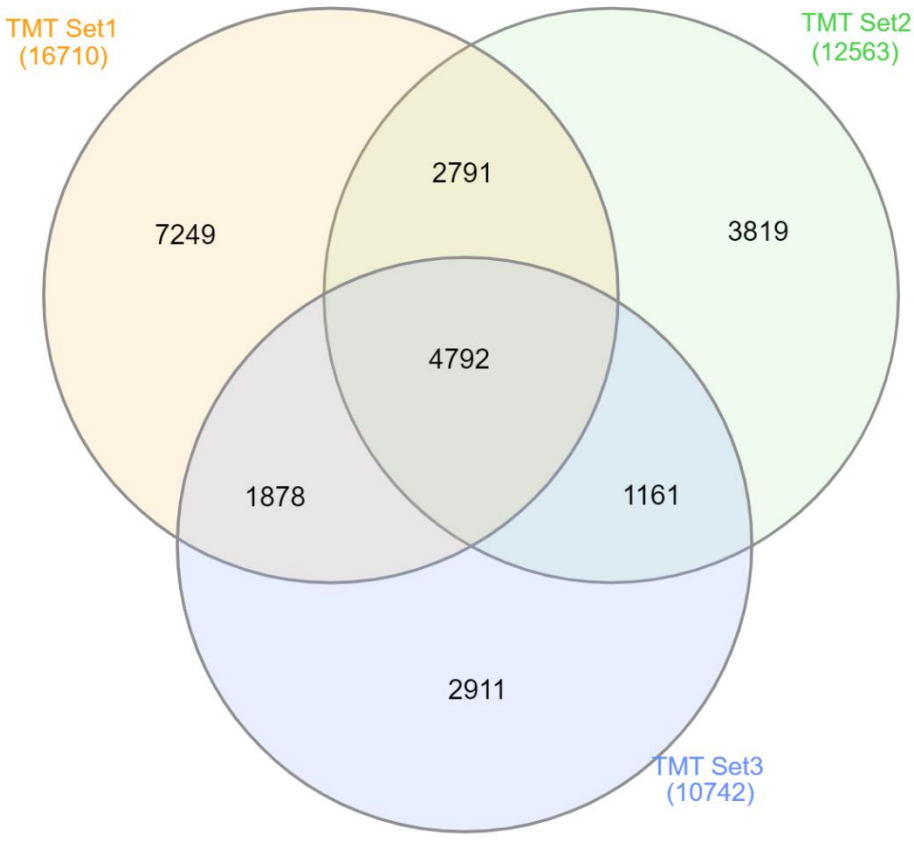


Figure 4.15. Number of fecal pellet proteins identified and quantified in TMT sets.

4.2.3 Comparison of protein expression levels between different groups

To get an overview of mouse and microbiota protein expression, we performed hierarchical clustering and created separate heatmaps. The heatmap of all mouse proteins did not show clusters of groups corresponding to the different strains (B6 or D2), treatment (control or defeat) and response (resistance or susceptible) (Figure 4.16 A). However, the heatmap of all microbial proteins resulted in two main groups that correspond exactly with B6 and D2 mouse strains (Figure 4.16 B).

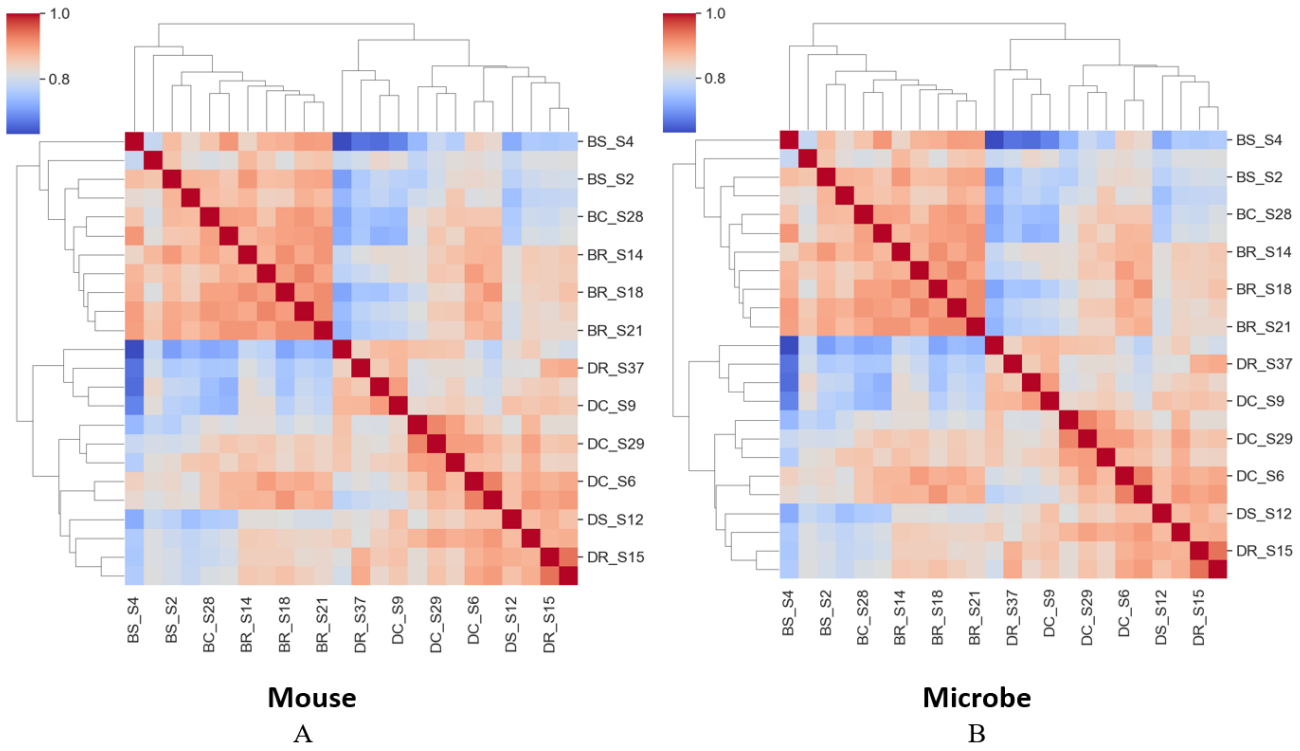
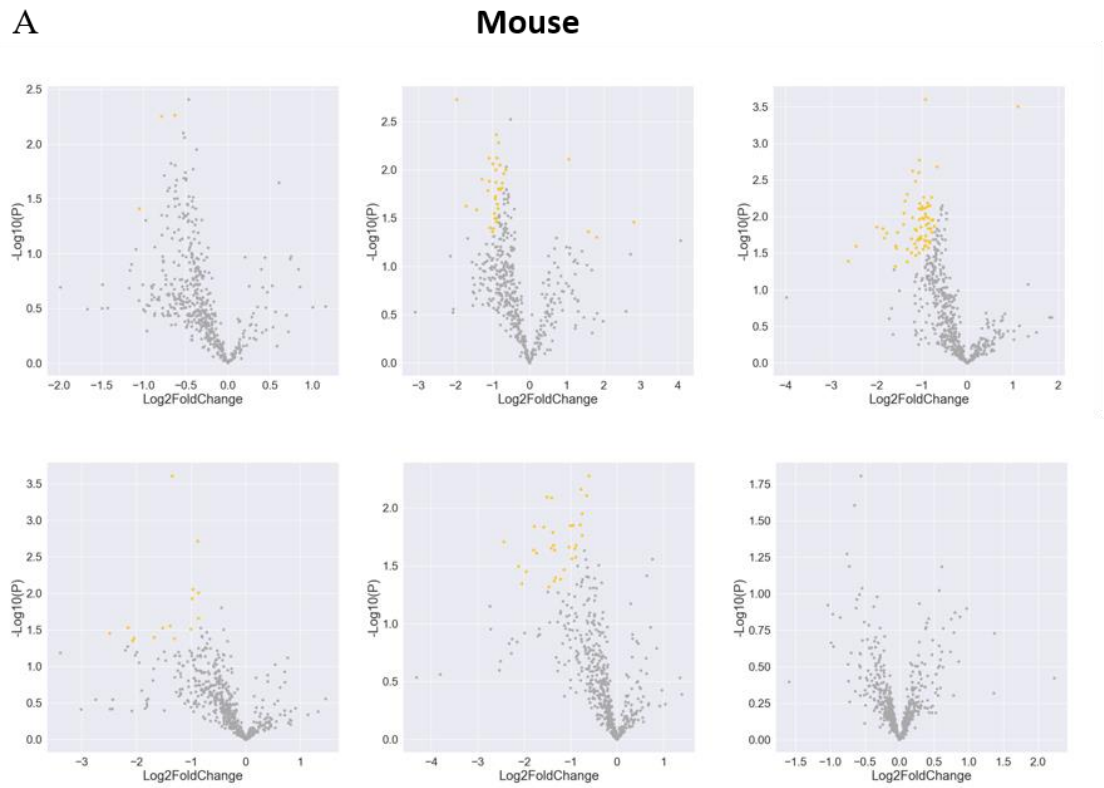


Figure 4.16. Hierarchically-clustered heatmap of fecal pellet mouse proteins (A) and microbial proteins (B) based on Spearman's correlation.

Quantitative analysis of protein expression was performed by comparing BR and BC, DC and BC, DR and BR, DR and DC, DR and DS, DS and DC. The Welch's T-test was performed for all proteins quantified in the two groups, the p-value of 0.05 and the pi-score of 1.3 (pi-score = -Log10p-value * Log2FC, pi-score is equal to 1.3 when FC = 2 and p-value = 0.05) were applied cutoffs.

Between BR and BC, 3 mouse proteins were significantly downregulated; between DC and BC, 4 mouse proteins were significantly upregulated and 32 were significantly downregulated; between DR and BR, 1 mouse protein was significantly upregulated and 70 were significantly downregulated; between DR and DC, 15 mouse proteins were significantly downregulated; between DR and DS, 32 mouse proteins were significantly downregulated; between DR and DC, there were no significantly changed mouse proteins (Figure 4.17 A, Figure 4.18 A).

Between BR and BC, 80 microbial proteins were significantly upregulated and 77 were significantly downregulated; between DC and BC, 149 microbial proteins were significantly upregulated and 514 were significantly downregulated; between DR and BR, 187 microbial proteins were significantly upregulated and 708 were significantly downregulated; between DR and DC, 143 microbial proteins were significantly upregulated and 62 were significantly downregulated; between DR and DS, 164 microbial proteins were significantly upregulated and 63 were significantly downregulated; between DS and DC, 21 microbial proteins were significantly upregulated and 34 were significantly downregulated (Figure 4.17 B, Figure 4.18 B).



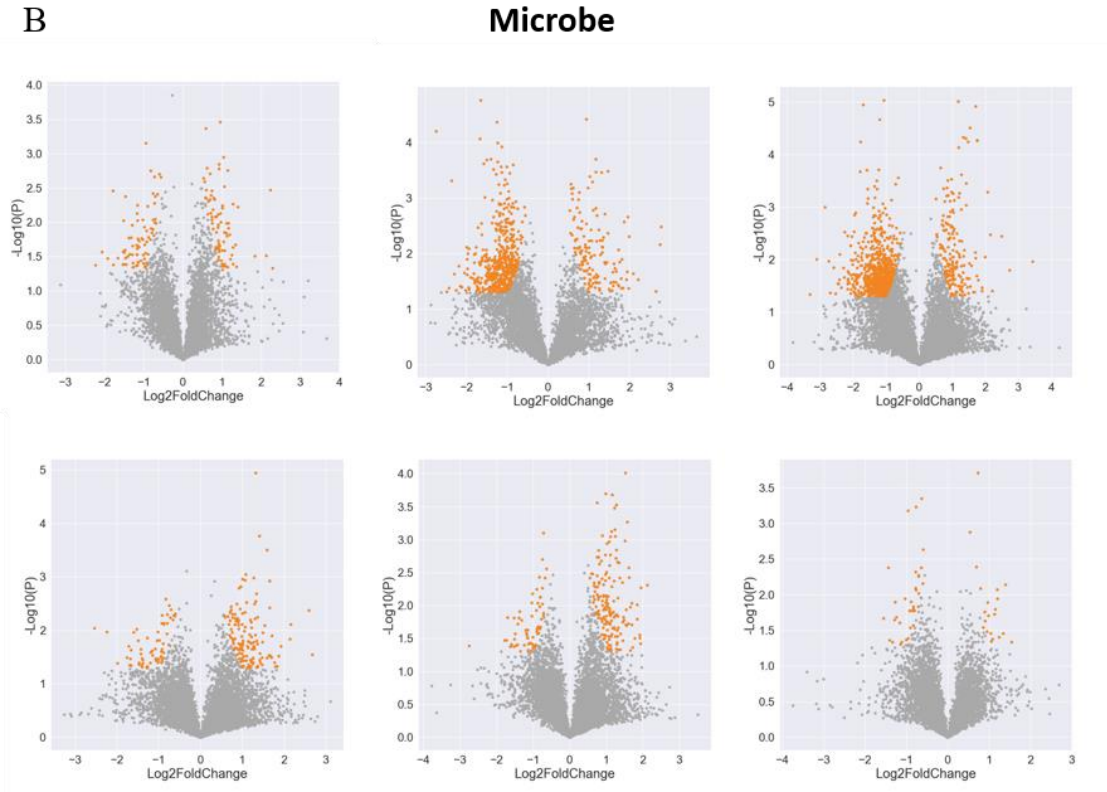


Figure 4.17. Volcano plot of differentially expressed fecal pellet mouse (A) and differentially expressed fecal pellet microbial (B) proteins.

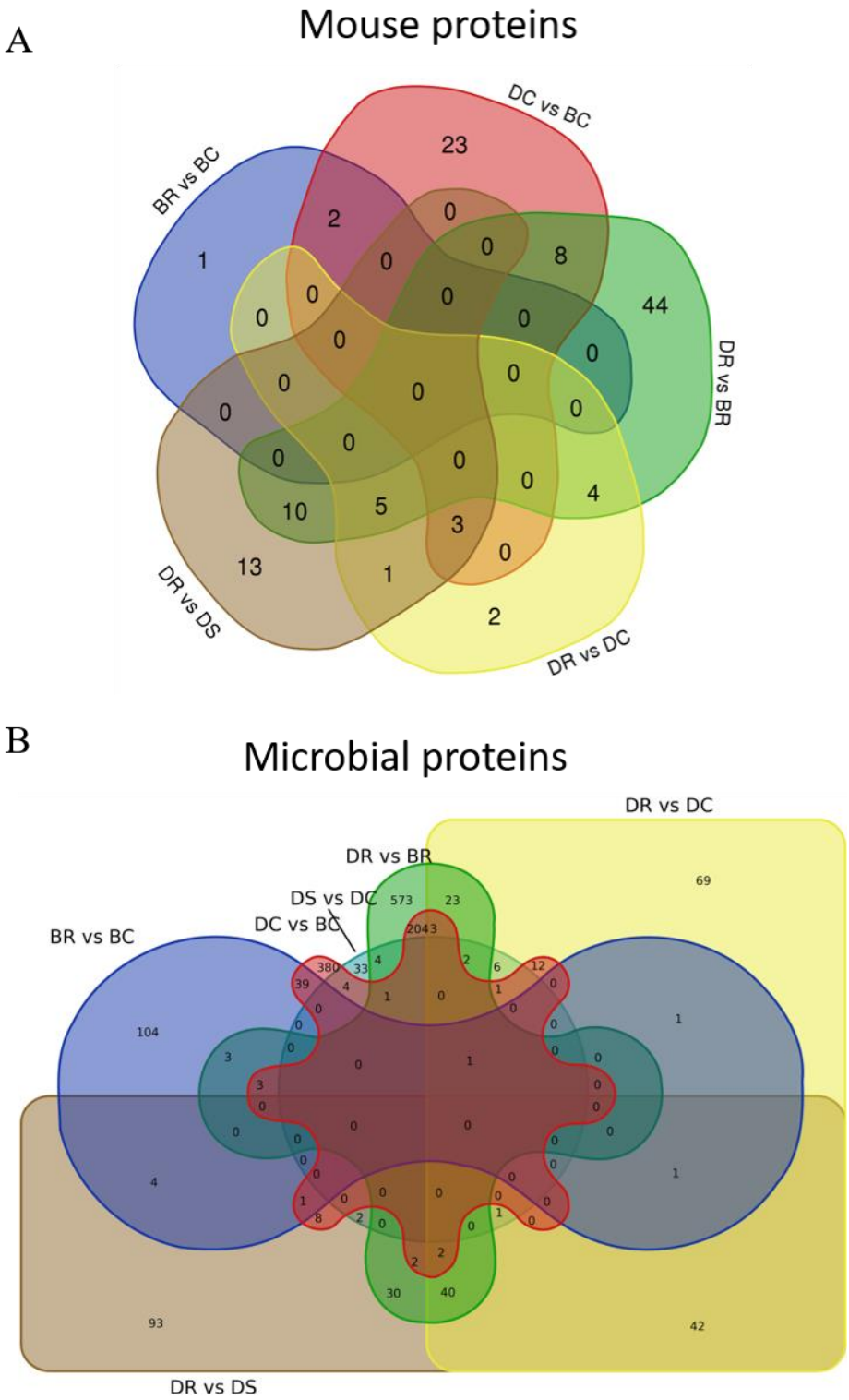


Figure 4.18. Number of significantly changed fecal pellet mouse proteins (A) and microbial proteins (B).

4.2.4 Metaproteome based microbial taxonomy profile

I next evaluated the fecal metaproteomic microbial profile of different mouse groups to check whether there was a significant difference in protein expression of certain taxa and also whether certain taxa were significantly enriched at the corresponding taxonomic level. For this purpose, I performed Welch's T-test at different taxonomic levels (phylum, family, order and genus) shown in Supplementary Table 4.3. BR vs. BC: at phylum level, BR group showed a significant increase in the relative abundance of *Spirochaetes* (p-value = 0.0214), and significant decrease in the relative abundance of *Tenericutes* (p-value = 0.0223), *Euryarchaeota* (p-value = 0.0264) and *Cyanobacteria* (p-value = 0.0280); at order level, BR group showed significant increase in the relative abundance of *Spirochaetales* (p-value = 0.0137) and *Deferribacterales* (p-value = 0.0263), and significant decrease in the relative abundance of *Methanobacteriales* (p-value = 0.0221); at family level, BR group showed significant increase in the relative abundance of *Deferribacteraceae* (p-value = 0.0255) and *Thermoanaerobacterales Family III. Incertae Sedis* (p-value = 0.0352), and significant decrease in the relative abundance of *Staphylococcaceae* (p-value = 0.0286); at genus level, BR group showed significant increase in the relative abundance of *Butyrivibrio* (p-value = 0.0103) and *Alloprevotella* (p-value = 0.0273), and significant decrease in the relative abundance of *Streptococcus* (p-value = 0.0125), *Dorea* (p-value = 0.0186) and *Odoribacter* (p-value = 0.0284).

DC vs. BC: at phylum level, DC group showed significant decrease in the relative abundance of *Tenericutes* (p-value = 0.0361); at order level, DC group showed significant increase in the relative abundance of *Bacillales* (p-value = 0.0361), and significant decrease in the relative abundance of *Cytophagales* (p-value = 0.0028), *Enterobacteriales* (p-value = 0.0221) and *Erysipelotrichales* (p-value = 0.0300); at family level, DC group showed significant increase in the relative abundance of *Leuconostocaceae* (p-value = 0.0283), and significant decrease in the relative abundance of *Prevotellaceae* (p-value = 0.0070), *Cytophagaceae* (p-value = 0.0002), *Enterobacteriaceae* (p-value = 0.0099), *Porphyromonadaceae* (p-value = 0.0123), *Erysipelotrichaceae* (p-value = 0.0463); at genus level, DC group showed significant increase in the relative abundance of *Leuconostoc* (p-value = 0.0291), and significant decrease in the relative abundance of *Prevotella* (p-value = 0.0195), *Porphyromonas* (p-value = 0.0478) and *Escherichia* (p-value = 0.0025).

DR vs. BR: at phylum level, DR group showed significant increase in the relative abundance of *Firmicutes* (p-value = 0.0355) and *Synergistetes* (p-value = 0.0117), and significant decrease in the relative abundance of *Bacteroidetes* (p-value = 0.0473), *Deferribacteres* (p-value = 0.0199) and *Elusimicrobia* (p-value = 0.0150); at order level, DR group showed significant decrease in the relative abundance of *Deferribacterales* (p-value = 0.0251) and *Campylobacterales* (p-value = 0.0261); at family level, DR group showed significant increase in the relative abundance of *Clostridiaceae* (p-value = 0.0488) Veillonellaceae (p-value = 0.0498) and *Bacillaceae* (p-value = 0.0196), and significant decrease in the relative abundance of *Helicobacteraceae* (p-value = 0.0439), *Enterococcaceae* (p-value = 0.0206), *Deferribacteraceae* (p-value = 0.0274), *Peptostreptococcaceae* (p-value = 0.0439), *Porphyromonadaceae* (p-value = 0.0262) and *Prevotellaceae* (p-value = 0.0063); at genus level, DR group showed significant increase in the relative abundance of *Clostridium* (p-value = 0.0277), *Bilophila* (p-value = 0.0470) and *Geobacillus* (p-value = 0.0023), and significant decrease in the relative abundance of *Enterococcus* (p-value = 0.0199), *Porphyromonas* (p-value = 0.0152), *Prevotella* (p-value = 0.0011), and *Parabacteroides* (p-value = 0.0318).

DR vs. DC: at family level, DR group showed significant decrease in the relative abundance of *Leuconostocaceae* (p-value = 0.0239); at genus level, DR group showed significant increase in the relative abundance of *Bilophila* (p-value = 0.0470), and significant decrease in the relative abundance of *Leuconostoc* (p-value = 0.0199). Between DR and DS group: at family level, DR group showed significant decrease in the relative abundance of *Prevotellaceae* (p-value = 0.0298) and *Leuconostocaceae* (p-value = 0.0152). Between DR and DC group: at genus level, DR group showed significant increase in the relative abundance of *Bilophila* (p-value = 0.0476) and *Escherichia* (p-value = 0.0423).

Table 4.3: Significantly changed taxa at phylum, order, family and genus levels based on microbial proteins.

BR vs. BC	**Name**	**-log10(P-value)**
Phylum	*Spirochaetes*	1.66939670853819
	Tenericutes	1.63050334614039
	Euryarchaeota	1.57876721319517
	Cyanobacteria	1.55298830719522
Order	*Spirochaetales*	1.86238259259068
	Methanobacteriales	1.65568085602977

	Deferribacterales	1.5796480837878
Family	*Deferribacteraceae*	1.59410793303456
	Staphylococcaceae	1.54355522492573
	Thermoanaerobacterales Family III. Incertae Sedis	1.45376172608271
Genus	*Butyrivibrio*	1.98858301113102
	Streptococcus	1.90179974220438
	Dorea	1.73125796837589
	Alloprevotella	1.56318263827474
	Odoribacter	1.54661711696138

DC vs. BC	**Name**	**-log10(P-value)**
Phylum	*Tenericutes*	1.44199191841896
Order	*Cytophagales*	2.54598235674246
	Enterobacteriales	1.52264204395559
	Bacillales	1.44239795871571
	Erysipelotrichales	1.32670617704444
Family	*Cytophagaceae*	3.6438394967319
	Prevotellaceae	2.15776624653536
	Enterobacteriaceae	2.00283135199126
	Porphyromonadaceae	1.91102836554369
	Leuconostocaceae	1.5479589396386
	Erysipelotrichaceae	1.33411884098962
Genus	*Escherichia*	2.59988001010858
	Prevotella	1.71018645136975
	Leuconostoc	1.53516036918476
	Porphyromonas	1.32034998359074

DR vs. BR	**Name**	**-log10(P-value)**
Phylum	*Synergistetes*	1.92996629443707
	Elusimicrobia	1.82451917888254
	Deferribacteres	1.70094489351989
	Firmicutes	1.45002928129947
	Bacteroidetes	1.32474720833404
Order	*Deferribacterales*	1.60045903032571
	Campylobacterales	1.58341668798012
Family	*Prevotellaceae*	2.20158308853095
	Bacillaceae	1.7075157167421
	Enterococcaceae	1.68705134349099
	Porphyromonadaceae	1.58167009966934
	Deferribacteraceae	1.56287358743857
	Helicobacteraceae	1.35799204630078

	Peptostreptococcaceae	1.35771522150072
	Clostridiaceae	1.31160056616006
	Veillonellaceae	1.30275610105048
Genus	*Prevotella*	2.96299095626102
	Geobacillus	2.63670038406554
	Porphyromonas	1.81799607002932
	Enterococcus	1.70104954687143
	Clostridium	1.5578103634932
	Parabacteroides	1.49744152020381
	Bilophila	1.32829543970041

DR vs. DC	**Name**	**-log10(P-value)**
Family	*Leuconostocaceae*	1.62188827840141
Genus	*Bilophila*	2.33115765946013
	Leuconostoc	1.87621868429575

DR vs. DS	**Name**	**-log10(P-value)**
Family	*Leuconostocaceae*	1.81746157127268
	Prevotellaceae	1.52511317572537

DS vs. DC	**Name**	**-log10(P-value)**
Genus	*Escherichia*	1.37290526018129
	Bilophila	1.32246789897374

4.2.5 Pathway enrichment analysis

To investigate the active microbial functions that were affected by social defeat, we performed a pathway enrichment analysis with all of the identified mouse and microbial proteins with KO id annotation. First, all mouse or microbial proteins were mapped to the KEGG pathways based on KO id. The enrichment ratios were calculated as (number of significant proteins in pathway/total number of detected proteins in pathway) / (total number of significant proteins/total number of detected proteins). The protein count of 1, enrichment ratio of 1 and p-value of 0.05 were applied as cutoffs for significantly enriched pathways.

For mouse proteins, between DC and BC group, two pathways were significantly enriched (Figure 4.19 A). The most significantly enriched pathway, also with the highest enrichment ratio is “Serotonergic synapse”, which plays important roles in physiological functions such as learning, memory and also in pathological states including stress and abnormal cognition[266-268]. Between DR and BR groups, four pathways were significantly enriched (Figure 4.19 B). The most significantly enriched pathway, also with the highest enrichment ratio is “Motor proteins”. Between DR and DC groups, six pathways were significantly enriched (Figure 4.19 C). The most significantly enriched pathway is “Pancreatic secretion”, which has been reported to be involved in the stress response[269]. The enriched pathways with the highest enrichment ratio were “Fat digestion and absorption” and “Glycerolipid metabolism”. Between DR and DS groups, five pathways were significantly enriched (Figure 4.19 D). The most significantly enriched pathway was again “Pancreatic secretion” as found for the DR and DC group comparison. The enriched pathway with the highest enrichment ratio was “Nucleocytoplasmic transport”.

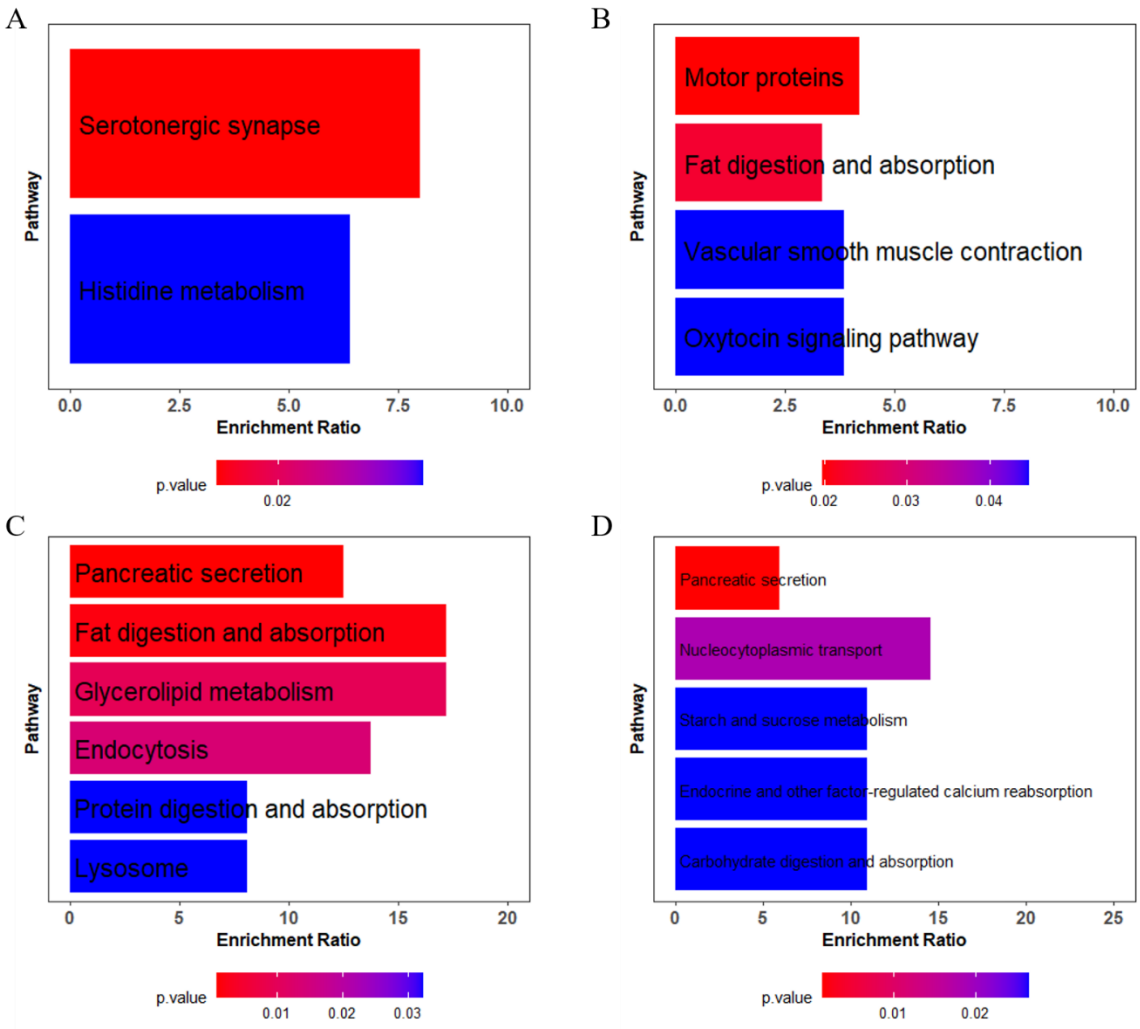


Figure 4.19. Significantly enriched pathways of mouse proteins between (A) DC and BC group, (B) DR and BR group, (C) DR and DC group, as well as (D)DR and DS group

Microbial protein differences between the BR and BC groups significantly enriched ten molecular pathways (Figure 4.20 A). Interestingly, based on sequence homolgy between the identified microbial proteins and functional orthologs of the KEGG Orthology database, the most significantly enriched pathway is “GABAergic synapse”, which plays an essential role in stress development[270], while the pathway with the highest enrichment ratio is “Histidine metabolism”. Comparing DC and BC group, “Citrate cycle (TCA cycle)” and “Ribosome” pathways were significantly enriched (Figure 4.20 B) and comparing DR and BR groups, six pathways were significantly enriched (Figure 4.20 C). The most enriched pathway is

“Ribosome”, while the pathway with the highest enrichment ratio is “Epithelial cell signaling in Helicobacter pylori infection”. Comparing DR and DC groups, seven pathways were significantly enriched (Figure 4.20 D). The most enriched pathway is “Methane metabolism”, which is related to oxidative stress, while the pathway with the highest enrichment ratio is “Nitrotoluene degradation”. Comparing DR and DS groups, twelve pathways were significantly enriched (Figure 4.20 E). The most enriched pathway is “Thiamine metabolism”, which is related to oxidative stress, while the pathway with the highest enrichment ratio is “Nitrotoluene degradation”. Comparing DS and DC groups, the “Purine metabolism” pathway is significantly enriched.

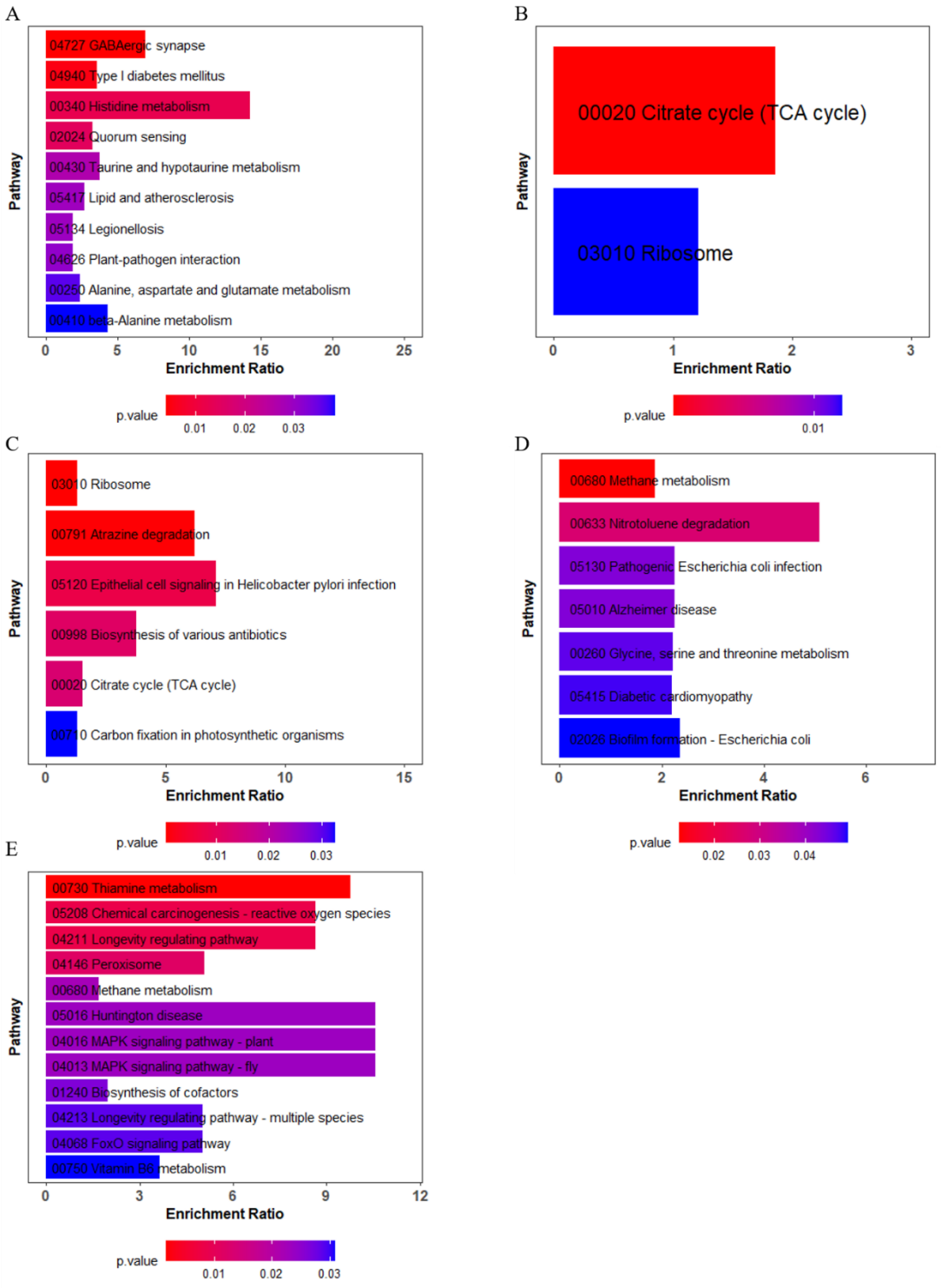


Figure 4.20. Significantly enriched pathways of microbial proteins. between (A) BR and BC group, (B) DC and BC group, (C) DR and BR group, (D)DR and DC group as well as (E) DR and DS group.

4.2.6 Significantly changed metabolite levels between different groups

To further evaluate microbiota pathways affected by CSDS, I also assessed fecal pellet metabolite levels by untargeted metabolomics analysis of fecal pellet extracts using LC-MS/MS. Samples were measured using both HILIC and RP columns in positive and negative modes and annotated with our in-house metabolite database.

In total 174 unique metabolites were annotated and quantified in more than 70% of the samples. Welch's T-test was performed to compare different groups using a p-value of 0.05 as cut-off. DC vs. BC: D-ornithine was upregulated and deoxycarnitine was downregulated in the DC group. DR vs. DC: in DR group D(+)-Cellobiose was downregulated. DR vs. DS: in DR group D(+)-Cellobiose, 3-(2-Hydroxyphenyl)propanoate and D-Xylose were downregulated, and D-Galactose was upregulated. DR vs. BR: in DR group N-Acetyl-D-galactosamine, maltose,

D(+)-cellobiose, cortisone and D-Xylose were downregulated, and D-Galactose was upregulated (Figure 4.21).

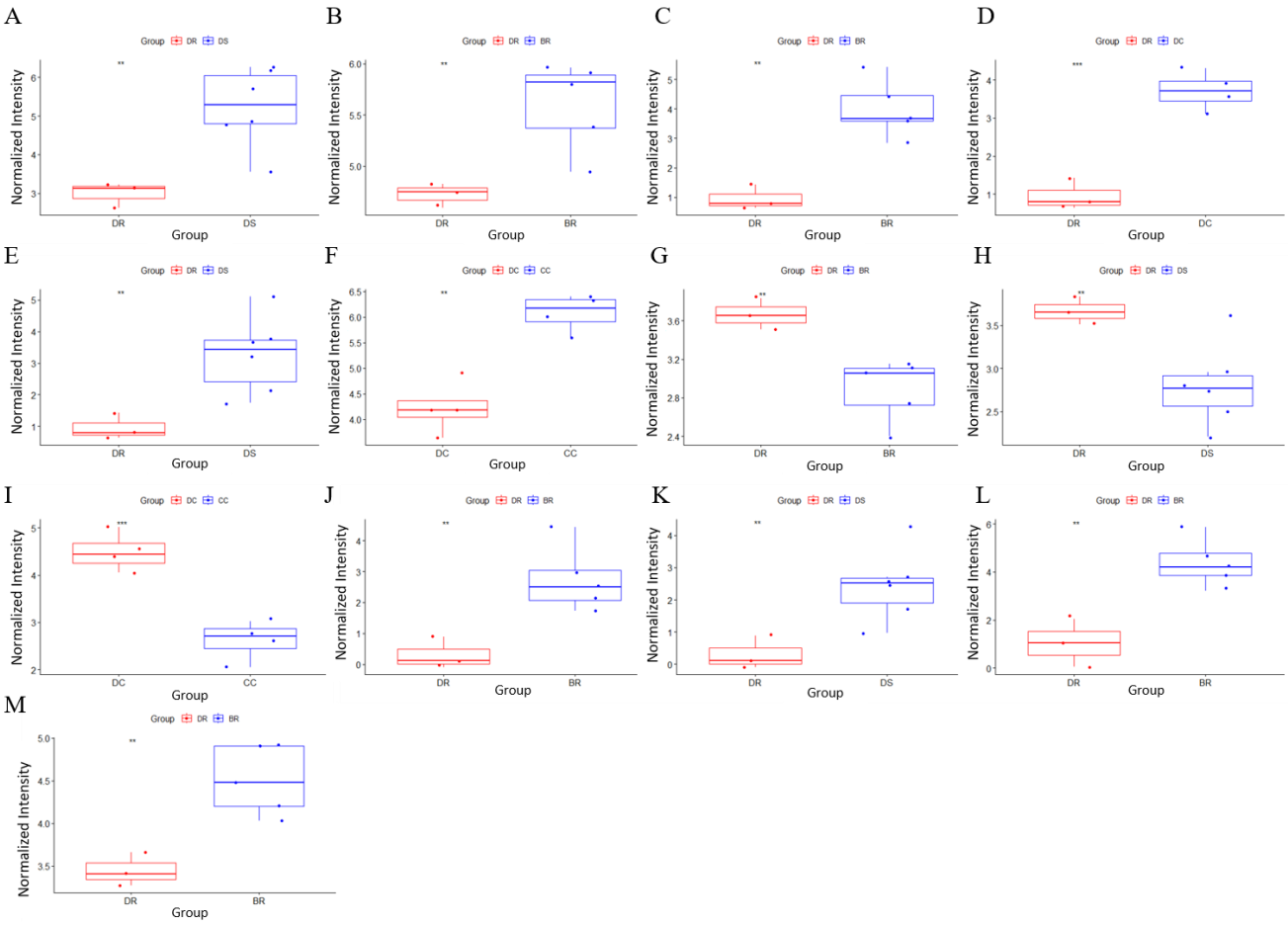


Figure 4.21. Significantly changed fecal pellet metabolite levels. (A) 3-(2-Hydroxyphenyl) propanoate between DR and DS; (B) Cortisone between DR and BR; D(+)-Cellobiose between DR and BR (C), DR and DC (D), DR and DS (E); (F) Deoxycarnitine between DC and BC; (G) D-Galactose between DR and BR; (H) D-Galactose between DR and DS; (I) D-ornithine between DC and BC; D-Xylose between DR and BR (J), DR and DS (K); (L) Maltose between DR and BR; (M) N-Acetyl-D-galactosamine between DR and BR. BC (C57BL/6NCrl control mice), BS (C57BL/6NCrl stress-susceptible mice), BR (C57BL/6NCrl stress-resilient mice), DC (DBA/2NCrl control mice), DS (DBA/2NCrl stress-susceptible mice), DR (DBA/2NCrl stress-resilient mice), MS (mass spectrometry).

5 Discussion

5.1 Parkinson's disease mouse model

Two rotenone PD mouse models are commonly used in PD studies. Rotenone is either infused or orally administered. Perez-Pardo et. al have found that both models show a similar phenotype and pathology[47]. Since our study focused on the gut microbiota in PD, we wanted to exclude a direct effect of rotenone on the GI tract and gut microbiota and used the rotenone infusion PD mouse model.

The composition of the microbiota varies between different collection sites and sample types in the GI tract. Among them, feces are always used as a proxy for gut microbiota because it can reflect different host states and it's easy to collect avoiding invasive procedures[125]. The Human Microbiome Project also used feces as a sample to study the gut microbiota[271]. Therefore, we used mouse feces as a proxy to study gut microbiota in this study instead of using caecum mucosa or caecum contents as previously reported by Perez-Pardo et.al[272]. Because fecal collection is easier and noninvasive, it is also more relevant for translating research findings to humans.

The rotenone treatment led to impaired motor function, delayed intestinal transit time and accumulation of alpha-synuclein in ENS (Figure 4.1). This is consistent with previous studies of this PD mouse model, which showed clear PD-like phenotypes[47, 55, 273].

In the current study no significant differences in alpha diversity was found between rotenone- and vehicle-treated mice (Figure 4.2 A), which was consistent with the results of previous observations on caecum mucosa and caecum content[272], suggesting that the overall microbial diversity was stable. Unlike previous studies which were carried out with caecum mucosa and caecum where a clear separation between the two groups in the MDS plot was observed, also no significant differences in beta diversity content was found in the current study (Figure 4.2 B). Again, suggesting that the microbial diversity between the two groups was stable when using fecal samples[272]. The microbial community structure was not affected according to the 16S rRNA sequencing data. The number of significantly changed taxa was much lower in feces compared to caecum mucosa and caecum, and none of the dominant taxa, including *Firmicutes* and *Bacteroidetes*, were significantly changed. The significant decrease in the order

Peptostreptococcales-Tissierellales was consistent with results from Lopetuso et. al in 2013. *Clostridia* are commensal bacteria involved in the maintenance of gut homeostasis[274], and decreased levels may affect gut homeostasis and an increased risk of PD. The in the current study observed significant decrease in the relative abundance of family *Peptostreptococcaceae* has also been reported for feces by Sampson et al. in a germ-free PD mouse model that overexpresses alpha-synuclein and from feces of normal germ-free mice subjected to PD patient fecal specimen transplantation[169].

Based on the metaproteomics data from my study, the metaproteome is significantly more affected in the rotenone PD model than the microbiota composition. This is based on the identified protein expression changes between the rotenone- and vehicle-treated groups. Apparently, rotenone has a much greater effect on microbial protein expression than on microbiota composition. Microbial protein expression showed separation of the two groups in both the MDS plot and the hierarchically clustered heatmap. In the taxonomic analysis, we found that the dominant phylum *Firmicutes* was significantly enriched and *Clostridiales* was highly enriched. At the family level, the relative abundance of *Peptostreptococcaceae* was significantly increased while it was decreased at the 16S rRNA level. This suggests that the relative abundance of this family was decreased while the protein expression is increased. As assessed by 16S rRNA sequencing, the significantly enriched family *Lachnospiraceae*, a butyrate-producing bacteria that can inhibit inflammation in the gut, has also been previously found to be significantly decreased in several studies with different PD mouse models[275-277]. At the genus level, the significantly enriched *Clostridium*, *Butyrivibrio* and significantly decreased *Dorea* were reported to be significantly decreased in several studies with different PD mouse models based on 16S rRNA sequencing data[278-281]. The genus *Clostridium* includes several pathogens, including *Clostridioides difficile*. *Clostridioides difficile* is an anaerobic bacillus and is the leading cause of nosocomial infectious diarrhea[282]. In 2020 Kang et.al found that *Clostridioides difficile* was associated with a 16% increase in short-term risk of PD in a Swedish population-based cohort study[283]. This suggests that *Clostridioides difficile* triggered infection may cause gut inflammation which is commonly believed to be associated with an increased risk of PD. The genus *Dorea* and *Butyrivibrio* are SCFA-producing bacteria that can inhibit gut inflammation in the gut and are associated with neuroprotective effects[284-286].

For the enrichment analysis, we noticed that most of the enriched pathways were related to immune response, which is consistent with previous studies showing that gut microbiota can induce an inflammatory environment in the GI tract during PD development[287, 288]. The PTS had the highest enrichment ratio. Another study showed that the activation of the PTS pathway could inhibit the dopamine production by competing for phosphoenolpyruvate (PEP)[289], which is essential for dopamine production. Activation of the PTS pathway, along with the death of dopaminergic cells, could further exacerbate PD symptoms.

The death of the dopaminergic neurons plays an important role in the development of PD. It leads to a decrease in dopamine levels, which affects the brain regions responsible for controlling movement, resulting in the commonly observed PD motor symptoms[290, 291]. Therefore, one of the most common treatments for Parkinson's disease is to increase dopamine levels to alleviate symptoms. Although dopamine therapy does not cure PD, it can help in the relieve of symptoms[43, 292].

The identified significantly changed proteins were also highly correlated with three indicators of PD, suggesting that they may be involved in the development of PD symptoms.

In addition. I found 9 significantly changed metabolites with some of them reported to be significantly altered in previous PD research studies using invasive sampling methods[293-296]. I observed elevated purine levels in the rotenone-treated group, which may indicate an inhibition of purine metabolism. This is in accordance with a previous study that showed that uric acid, which is the final product of purine metabolism has a neuroprotective effect in PD by preserving dopaminergic signaling[297]. Elevated cortisol levels found in the rotenone-treated mouse group have also been reported in a human study that suggested that the induced stress response can promote the development of PD[294]. Decreased fecal pipecolic acid levels were also reported by Gonzalez-Riano, et al. in pre-PD patients who developed PD after sample collection in a follow-up study. According to this study, pipecolic acid can act as a neurotransmitter modulating GABAergic transmission[298].

In summary, the current study confirms some previously reported findings and also provided new insights into specific taxa and biological pathways that are involved in PD pathobiology. The pathogen-induced gut inflammation and the reduced protection provided by the decreased SCFA-producing bacteria, together with the activated PTS system triggered decreased

dopamine production, may contribute to the development of PD. To our knowledge, this is the first application of metaproteomics to gut microbiota in PD. It allows to capture changes in protein expression levels even when microbial composition is only affected by minor changes. I therefore submit that fecal samples have great potential for identifying biomarkers for earlier diagnosis and intervention in PD patients.

5.2 Anxiety disorders mouse model

The chronic social defeat stress model consists of two mouse strains (B6 and D2) that differ in their susceptibility to stress induced by the aggressor mouse strain CD-1. The genetic background of the mouse strain has a strong effect on the behavioral response to chronic stress, with B6 mice showing a higher tendency to be resilient, while D2 mice more likely to be susceptible to CSDS[77].

The difference in fecal microbial composition of different mouse strains has been previously reported using DNA sequencing-based methods[299]. The current study carried out as part of my thesis projects further verified this observation at the proteome level. Using metaproteomics of fecal pellets, mouse host proteins were examined together with microbial proteins. According to hierarchical clustering, microbial proteins showed far more differences between the B6 and D2 mouse strains than mouse proteins. The contribution of the genetic background to the microbial proteome was even greater than that of social defeat. This becomes apparent by the missing clustering in the stressed animal groups. While microbial proteins were nicely clustered into two groups that corresponded to the mouse strain, no clusering was observed for social defeat vs. control mice. My results demonstrate that the host genetic contribution needs to be considered when using different mouse strains to study gut microbiota.

Only a few significantly different proteins were shared between all comparisons. In accordance with the above observation, the comparison of the same CSDS group, susceptible or resilient, between the two mouse strains also showed the greatest number of differences (DC vs BC: 663, DR vs BR: 895), while the intro strain comparisons (BR vs BC: 157, DS vs DC: 55, DR vs DC: 205, DR vs DS: 227) showed much fewer differences. Overall this indicates that the genetic background also has a major influence on the microbiota composition and microbial protein

expression. In addition, there are more significantly changed microbial than mouse proteins between the groups, which explains the observed hierarchical clustering results (Figure 4.16).

As expected, and based on the microbial protein difference data, the microbial composition also was found to have the greatest differences in the cases when the same response groups from different mouse strains were compared (DC vs. BC and DR vs. BR). On the other hand, for intra strain comparisons, like the BR vs. BC group, the protein expression of 4 phyla, 3 order, 3 families and 5 genera were significantly changed, which is comparable to the number of significantly changed taxa between the two mouse strains. The comparison between the D2 resistant and control groups (DR vs. DC), only revealed one family and two genera to be significantly changed. For other comparisons within the D2 mice (DR vs. DC and DR vs. DS), there are even more significantly differentially expressed proteins than for the BR vs. BC group, but few changes for specific taxa (for DR vs DC 1 family and 2 genera, for DR vs DS 2 families). The results suggest that for the two different mouse strains the microbial proteins changed in different ways. While for B6 mice the changes were found to be more for specific taxa, for D2 mice specific taxa did not contribute as much.

The observed significantly decreased protein expression levels from *Dorea* in BR group compared with BC group was consistent with a previous study by Bailey et. al in 2011 who found that the relative abundance of *Dorea* was decreased after the social disruption exposure and was inversely associated with IL-6 levels, a cytokine elevated in response to stress[300]. We also observed significantly increased protein expression of *Butyrivibrio* in the BR group compared to the BC group. The genus *Butyrivibrio* has SCFA-producing bacteria that can inhibit gut inflammation and have been associated with neuroprotective effects. The increased protein expression from *Butyrivibrio* might be responsible for the B6 mice resilience in response to stress[285].

The observed significantly decreased protein expression of *Odoribacter* in BR group compared to BC group was in contradiction with data from Maltz et. al. that showed an increased abundance of *Odoribacter* in stress exposed mice based on 16S rRNA gene sequencing[301]. However, as mentioned previously, RNA abundance often does not correlate well with protein expression levels[195].

We also found that in both stressed and control groups, the protein expression levels of *Prevotella* were significantly lower in D2 vs. B6 strains. Reduced abundance of *Prevotella* in acute psychological stress has been reported by Maslanik et. al in 2012[302]. According to their study, stress-induced reduction in *Prevotella* has the potential to decrease the anti-inflammatory status of the mucosal immune system and may be a contributing factor to the pro-inflammatory state induced by stress. The higher abundance of *Prevotella* proteins may therefore contribute to the stress-resilient phenotype of the B6 strain.

Pathway enrichment analysis of the identified mouse protein level alterations indicates that the "Serotonergic synapse" pathway was significantly enriched in D2 vs. B6 control mice. Serotonin is a neurotransmitter involved in many psychological functions such as mood, memory, learning and cognition[303]. It participates in the stress responses by interacting with other neurotransmitters such as cortisol, which is known as the stress hormone[304], or by influencing the activity of brain regions that are involved in regulating the stress response[305]. An imbalance in serotonin levels can affect the organism's resistance to stress. Selective Serotonin Reuptake Inhibitors (SSRIs) are a class of antidepressants used for the treatment of major depressive disorder and anxiety disorders. They work by blocking the reuptake of serotonin in the brain to increase the concentration of serotonin available for neurotransmission[306]. Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) are another class of antidepressants used to treat major depressive disorder and anxiety disorders that work by inhibiting the reuptake of serotonin and norepinephrine and have a broader effect on neurotransmitter balance[307]. Interestingly, approximately 90% of serotonin is produced in the GI tract, and several studies have reported that serotonin levels in the GI tract can affect the serotonin levels in the brain[156, 308]. The enrichment in the "Serotonergic synapse" pathway found in the current study may explain the different resistance of the B6 and D2 mouse strains to social stress due to their different ability to regulate serotonin levels.

Pathway enrichment analysis of microbial proteins also showed that both "ribosome" and "citrate cycle (TCA cycle)" pathways enriched in DC vs. BC were also enriched in DR vs. BR. This suggests that stress did not completely compensate for genetic background effects. In addition to these two pathways, several pathways related to infection were also enriched, which was consistent with previous studies showing that chronic stress can induce an inflammatory environment in the GI tract. For the D2 strain only the "purine metabolism" pathway was

enriched between DS and DC, which was less than the number of the 7 enriched pathways between DR and DC that included "methane metabolism" which is related to oxidative stress[309], and "Alzheimer's disease" related to neurodegenerative disease. "GABAergic synapse" pathway was found to be enriched for both BR vs. BC and DR vs. DC comparisons. This is of interest since previous studies have provided evidence that the "GABAergic synapse" pathway plays an essential role in the stress responses[310, 311].

The metabolomics analysis identified a total of 13 significantly changed metabolite levels. D(+)-cellobiose levels in the DR group were significantly lower than in all other groups (BR, DC and DS). Previous studies have shown that cellobiose supplementation has a positive impact on gut health especially under osmotic stress conditions[312]. Compared with BR mice, DR mice have significantly lower cortisone levels. Cortisone can be converted to cortisol, a primary stress hormone in mice, by the action of 11β-hydroxysteroid dehydrogenase type 1. Significantly lower levels of xylose, which is related to oxidative stress[313], were observed in the DR group compared to the BR and DS groups.

In summary, the acquired metaproteomics data show that the D2 and B6 mouse strains have very different gut microbial constituents, and the genetic background effect on the microbiota is significantly greater than that of CSDS. Within each strain, the stress-resilient B6 and stress-susceptible D2 mice had the fewest significantly changed proteins compared to the control group. In contrast to B6 mice, microbial protein abundance in certain taxa was not significantly altered in D2 mice. I found several pathways and levels of metabolites associated with oxidative stress that were affected by CSDS and have been previously reported in mice subjected to CSDS. Whereas previous studies were carried out using 16S rRNA or DNA data, my study is to the best of my knowledge, the first application of metaproteomics to gut microbiota in a mouse model of CSDS. Fecal pellet samples can be collected noninvasively and have great potential for identifying biomarkers in mouse models of disease. Further functional studies underlying the mechanisms of the observed differences in microbial protein levels and a more detailed investigation of specific taxa will provide much needed insight into the role of the gut microbiota in chronic stress, which is a critical step in the development of future targeted therapies for patients.

5.3 Challenges in metaproteomics and metabolomics analysis

Metaproteomics is an emerging field and provides many challenges due to the great and still incompletely characterized great variety of microorganisms present in the microbiota. For mass spectrometry data analysis, there currently does not exist a software like for standard traditional proteomics data analysis on a single organism. Annotation of the microbial proteins is also limited. In the case of microbiota, proteins identified by mass spectrometry can be derived from many different organisms. In addition to functional information an additional layer of taxonomy needs to be taken into account.

Functional analysis in metaproteomics is further challenged by the fact that most functional annotations of microbial proteins are based on sequence alignment with proteins from well-defined model organisms without experimental verification. However, high sequence homology and overlap does not necessarily imply identical function. The analysis of the microbiota as a whole and mapping all proteins to universal pathways is also different from an analysis focused on a single organism, since proteins may not interact the same way as in a single organism. Metaproteomics data can suggest an involvement of potential candidate pathways in disease pathobiology, but additional follow up experiments are required to identify the precise contribution of the microbial community to host physiology.

Unlike proteins, small molecules such as metabolites do not have sequence information from which to infer their origin. The identified metabolites could originate from any microorganism, the host, or even the environment, such as undigested food, posing a challenge for exploiting metabolomics data to clarify correlation and causation in microbiota studies.

6 Summary and Outlook

Both Parkinson's disease and social stress induced anxiety disorders severely affect the quality of life of patients and place a huge burden on the health care system. Despite extensive studies, the mechanisms of development of these two diseases are still unknown. Several epidemiological studies have shown that many patients suffering from these diseases also develop gastrointestinal dysfunction together or even before the main symptoms occur, suggesting that the microbiota-gut-brain axis might be involved in the development of both diseases.

For the Parkinson's disease project, I studied the gut microbiota using the rotenone-treated mouse model which showed clear PD-like phenotypes. Based on rRNA data, there is no significant change in microbial diversity, which shows that the microbial structure was not affected. Based on the analysis on the metaproteome, rotenone has a much greater effect on microbial than mouse protein levels. The protein expression levels of several SCFA-producing microbial taxa were significantly decreased and several inflammation pathways and the activated PTS system triggering decreased dopamine production, may contribute to the development of PD-like symptoms in the rotenone-treated mice. Several metabolites that were previously identified as significantly changed in either tissue or blood were also identified in the current study.

For the anxiety disorders project, B6 and D2 mice showed significantly different susceptibility towards chronic social stress induced by aggressor CD-1 mouse with B6 showing a higher tendency to be resilient. Microbial proteins have a much greater number of expression differences than mouse proteins for both mouse strains, and the genetic background effect on the microbiota is significantly greater than that of CSDS. Also, in the stressed groups, in contrast to D2 mice, the majority of significantly changed proteins from B6 mice were from specific taxa. Several pathways that are involved in neurodegenerative diseases and neuropsychiatric disorders such as Alzheimer's disease and GABAergic synapse were enriched for microbial protein differences, suggesting that gut microbiota might affect these biological processes. Several metabolite levels that were previously identified to be significantly changed in PD were also identified in the current study.

To the best of my knowledge, my thesis projects represent the first application of metaproteomics to the study of gut microbiota in PD and anxiety disorder mouse models. The acquired data provide information about affected biological processes of both host and microbiota at the protein level. They verify results from previous studies and at the same time provide new insights into the role of gut microbiota in PD and anxiety disorders. Future metaproteomics studies with human specimens have the potential to be of use for identifying biomarkers, facilitating early diagnosis, and advancing disease interventions.

References

1. Dugger, B.N. and D.W. Dickson, *Pathology of Neurodegenerative Diseases.* Cold Spring Harbor Perspectives in Biology, 2017. **9**(7).
2. Bittigau, D., et al., *Apoptotic neurodegeneration following trauma is markedly enhanced in the immature brain.* Annals of Neurology, 1999. **45**(6): p. 724-735.
3. Ma, D.K., et al., *Adult neural stem cells in the mammalian central nervous system.* Cell Res, 2009. **19**(6): p. 672-82.
4. Hutchins, J.B. and S.W. Barger, *Why neurons die: cell death in the nervous system.* Anat Rec, 1998. **253**(3): p. 79-90.
5. Mayeux, R., *Epidemiology of neurodegeneration.* Annu Rev Neurosci, 2003. **26**: p. 81-104.
6. Wareham, L.K., et al., *Solving neurodegeneration: common mechanisms and strategies for new treatments.* Molecular Neurodegeneration, 2022. **17**(1).
7. Lamptey, R.N.L., et al., *A Review of the Common Neurodegenerative Disorders: Current Therapeutic Approaches and the Potential Role of Nanotherapeutics.* Int J Mol Sci, 2022. **23**(3).
8. Hussain, R., et al., *Neurodegenerative Diseases: Regenerative Mechanisms and Novel Therapeutic Approaches.* Brain Sciences, 2018. **8**(9).
9. De Marchi, F., et al., *Telehealth in Neurodegenerative Diseases: Opportunities and Challenges for Patients and Physicians.* Brain Sci, 2021. **11**(2).
10. Price, D.L., S.S. Sisodia, and D.R. Borchelt, *Genetic neurodegenerative diseases: the human illness and transgenic models.* Science, 1998. **282**(5391): p. 1079-83.
11. Cannon, J.R. and J.T. Greenamyre, *The Role of Environmental Exposures in Neurodegeneration and Neurodegenerative Diseases.* Toxicological Sciences, 2011. **124**(2): p. 225-+.
12. Rui, Q., et al., *The Role of LRRK2 in Neurodegeneration of Parkinson Disease.* Curr Neuropharmacol, 2018. **16**(9): p. 1348-1357.
13. Tcw, J. and A.M. Goate, *Genetics of beta-Amyloid Precursor Protein in Alzheimer's Disease.* Cold Spring Harb Perspect Med, 2017. **7**(6).
14. Skrahina, V., et al., *The Rostock International Parkinson's Disease (ROPAD) Study: Protocol and Initial Findings.* Mov Disord, 2021. **36**(4): p. 1005-1010.
15. Long, J.M. and D.M. Holtzman, *Alzheimer Disease: An Update on Pathobiology and Treatment Strategies.* Cell, 2019. **179**(2): p. 312-339.
16. Santiago, J.A. and J.A. Potashkin, *Physical activity and lifestyle modifications in the treatment of neurodegenerative diseases.* Front Aging Neurosci, 2023. **15**: p. 1185671.
17. Song, H., et al., *Association of Stress-Related Disorders With Subsequent Neurodegenerative Diseases.* JAMA Neurol, 2020. **77**(6): p. 700-709.
18. Abbott, S.M. and A. Videnovic, *Chronic sleep disturbance and neural injury: links to neurodegenerative disease.* Nat Sci Sleep, 2016. **8**: p. 55-61.
19. Cho, H., et al., *Impact of smoking on neurodegeneration and cerebrovascular disease markers in cognitively normal men.* Eur J Neurol, 2016. **23**(1): p. 110-9.
20. Zhu, X.L., et al., *The Relationship Between the Gut Microbiome and Neurodegenerative Diseases.* Neuroscience Bulletin, 2021. **37**(10): p. 1510-1522.
21. Ling, Z., et al., *Structural and Functional Dysbiosis of Fecal Microbiota in Chinese Patients With Alzheimer's Disease.* Front Cell Dev Biol, 2020. **8**: p. 634069.
22. Vogt, N.M., et al., *Gut microbiome alterations in Alzheimer's disease.* Sci Rep, 2017. **7**(1): p. 13537.

23. Sun, J., et al., *Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice.* Transl Psychiatry, 2019. **9**(1): p. 189.
24. Willis, A.W., *Parkinson disease in the elderly adult.* Mo Med, 2013. **110**(5): p. 406-10.
25. Parkinson, J., *An essay on the shaking palsy.* Archives of Neurology and Psychiatry, 1922. **7**(6): p. 683-710.
26. Collaborators, G.B.D.P.s.D., *Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016.* Lancet Neurol, 2018. **17**(11): p. 939-953.
27. McDonald, C., et al., *200 Years of Parkinson's disease: what have we learnt from James Parkinson?* Age and Ageing, 2018. **47**(2): p. 209-214.
28. Schmidt, S.H., et al., *Conformation and dynamics of the kinase domain drive subcellular location and activation of LRRK2.* Proc Natl Acad Sci U S A, 2021. **118**(23).
29. Rocha, E.M., et al., *LRRK2 inhibition prevents endolysosomal deficits seen in human Parkinson's disease.* Neurobiol Dis, 2020. **134**: p. 104626.
30. Daher, J.P., et al., *Leucine-rich Repeat Kinase 2 (LRRK2) Pharmacological Inhibition Abates alpha-Synuclein Gene-induced Neurodegeneration.* J Biol Chem, 2015. **290**(32): p. 19433-44.
31. Polymeropoulos, M.H., et al., *Mutation in the alpha-synuclein gene identified in families with Parkinson's disease.* Science, 1997. **276**(5321): p. 2045-7.
32. Migdalska-Richards, A. and A.H.V. Schapira, *The relationship between glucocerebrosidase mutations and Parkinson disease.* Journal of Neurochemistry, 2016. **139**: p. 77-90.
33. Blauwendraat, C., et al., *Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia.* Brain, 2020. **143**(1): p. 234-248.
34. Matheoud, D., et al., *Parkinson's Disease-Related Proteins PINK1 and Parkin Repress Mitochondrial Antigen Presentation.* Cell, 2016. **166**(2): p. 314-327.
35. Soni, R. and J.G. Shah, *Deciphering Intertwined Molecular Pathways Underlying Metabolic Syndrome Leading to Parkinson's Disease.* Acs Chemical Neuroscience, 2022.
36. Salama, M. and O. Arias-Carrion, *Natural toxins implicated in the development of Parkinson's disease.* Ther Adv Neurol Disord, 2011. **4**(6): p. 361-73.
37. Meara, R.J., *Review: the pathophysiology of the motor signs in Parkinson's disease.* Age Ageing, 1994. **23**(4): p. 342-6.
38. Villar-Pique, A., T. Lopes da Fonseca, and T.F. Outeiro, *Structure, function and toxicity of alpha-synuclein: the Bermuda triangle in synucleinopathies.* J Neurochem, 2016. **139 Suppl 1**: p. 240-255.
39. Kalia, L.V. and A.E. Lang, *Parkinson's disease.* Lancet, 2015. **386**(9996): p. 896-912.
40. Jellinger, K.A., *Neuropathology of movement disorders.* Neurosurgery Clinics of North America, 1998. **9**(2): p. 237-+.
41. Dahlin, M., B. Jansson, and E. Björk, *Levels of dopamine in blood and brain following nasal administration to rats.* European Journal of Pharmaceutical Sciences, 2001. **14**(1): p. 75-80.
42. Armstrong, M.J. and M.S. Okun, *Diagnosis and Treatment of Parkinson Disease A Review.* Jama-Journal of the American Medical Association, 2020. **323**(6): p. 548-560.
43. Connolly, B.S. and A.E. Lang, *Pharmacological Treatment of Parkinson Disease A Review.* Jama-Journal of the American Medical Association, 2014. **311**(16): p. 1670-1683.
44. Turner, N., *Notes on rotenone as an insecticide.* Journal of Economic Entomology, 1932. **25**: p. 1228-1237.

45. Sherer, T.B., et al., *Mechanism of toxicity in rotenone models of Parkinson's disease.* J Neurosci, 2003. **23**(34): p. 10756-64.
46. Gao, H.M., B. Liu, and J.S. Hong, *Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons.* J Neurosci, 2003. **23**(15): p. 6181-7.
47. Perez-Pardo, P., et al., *Gut-brain and brain-gut axis in Parkinson's disease models: Effects of a uridine and fish oil diet.* Nutr Neurosci, 2018. **21**(6): p. 391-402.
48. Dhillon, A.S., et al., *Pesticide/environmental exposures and Parkinson's disease in East Texas.* J Agromedicine, 2008. **13**(1): p. 37-48.
49. Tanner, C.M., et al., *Rotenone, paraquat, and Parkinson's disease.* Environ Health Perspect, 2011. **119**(6): p. 866-72.
50. De Miranda, B.R., et al., *Sex Differences in Rotenone Sensitivity Reflect the Male-to-Female Ratio in Human Parkinson's Disease Incidence.* Toxicol Sci, 2019. **170**(1): p. 133-143.
51. Won, J.H., et al., *Rotenone-induced Impairment of Mitochondrial Electron Transport Chain Confers a Selective Priming Signal for NLRP3 Inflammasome Activation.* J Biol Chem, 2015. **290**(45): p. 27425-27437.
52. Pereira, C.S., et al., *Mechanism of rotenone binding to respiratory complex I depends on ligand flexibility.* Sci Rep, 2023. **13**(1): p. 6738.
53. Kweon, G.R., et al., *Distinct mechanisms of neurodegeneration induced by chronic complex I inhibition in dopaminergic and non-dopaminergic cells.* J Biol Chem, 2004. **279**(50): p. 51783-92.
54. Ullrich, C. and C. Humpel, *Rotenone induces cell death of cholinergic neurons in an organotypic co-culture brain slice model.* Neurochem Res, 2009. **34**(12): p. 2147-53.
55. Rocha, S.M., et al., *Rotenone induces regionally distinct alpha-synuclein protein aggregation and activation of glia prior to loss of dopaminergic neurons in C57Bl/6 mice.* Neurobiol Dis, 2022. **167**: p. 105685.
56. Johnson, M.E., A. Stringer, and L. Bobrovskaya, *Rotenone induces gastrointestinal pathology and microbiota alterations in a rat model of Parkinson's disease.* Neurotoxicology, 2018. **65**: p. 174-185.
57. Cannon, J.R., et al., *A highly reproducible rotenone model of Parkinson's disease.* Neurobiol Dis, 2009. **34**(2): p. 279-90.
58. Chou, I.H. and T. Chouard, *Neuropsychiatric disease.* Nature, 2008. **455**(7215): p. 889.
59. Ziffra, M., *Panic disorder: A review of treatment options.* Ann Clin Psychiatry, 2021. **33**(2): p. 124-133.
60. Kircanski, K., et al., *Subtypes of panic attacks: a critical review of the empirical literature.* Depress Anxiety, 2009. **26**(10): p. 878-87.
61. Potter, C.M., et al., *Situational panic attacks in social anxiety disorder.* J Affect Disord, 2014. **167**: p. 1-7.
62. Goddard, A.W., *The Neurobiology of Panic: A Chronic Stress Disorder.* Chronic Stress (Thousand Oaks), 2017. **1**: p. 2470547017736038.
63. Yaribeygi, H., et al., *The impact of stress on body function: A review.* EXCLI J, 2017. **16**: p. 1057-1072.
64. Mariotti, A., *The effects of chronic stress on health: new insights into the molecular mechanisms of brain-body communication.* Future Sci OA, 2015. **1**(3): p. FSO23.
65. Schneiderman, N., G. Ironson, and S.D. Siegel, *Stress and health: psychological, behavioral, and biological determinants.* Annu Rev Clin Psychol, 2005. **1**: p. 607-28.

66. Segerstrom, S.C. and G.E. Miller, *Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry.* Psychol Bull, 2004. **130**(4): p. 601-30.
67. Feuerstein, M., C. Bush, and R. Corbisiero, *Stress and chronic headache: a psychophysiological analysis of mechanisms.* J Psychosom Res, 1982. **26**(2): p. 167-82.
68. Bhatia, V. and R.K. Tandon, *Stress and the gastrointestinal tract.* J Gastroenterol Hepatol, 2005. **20**(3): p. 332-9.
69. Melzack, R., *Pain and the neuromatrix in the brain.* J Dent Educ, 2001. **65**(12): p. 1378-82.
70. Golbidi, S., J.C. Frisbee, and I. Laher, *Chronic stress impacts the cardiovascular system: animal models and clinical outcomes.* Am J Physiol Heart Circ Physiol, 2015. **308**(12): p. H1476-98.
71. Wirtz, P.H. and R. von Kanel, *Psychological Stress, Inflammation, and Coronary Heart Disease.* Curr Cardiol Rep, 2017. **19**(11): p. 111.
72. Everson-Rose, S.A., et al., *Chronic stress, depressive symptoms, anger, hostility, and risk of stroke and transient ischemic attack in the multi-ethnic study of atherosclerosis.* Stroke, 2014. **45**(8): p. 2318-23.
73. Tsigos, C., et al., *Stress: Endocrine Physiology and Pathophysiology*, in *Endotext*, K.R. Feingold, et al., Editors. 2000: South Dartmouth (MA).
74. Stratakis, C.A., P.W. Gold, and G.P. Chrousos, *Neuroendocrinology of stress: implications for growth and development.* Horm Res, 1995. **43**(4): p. 162-7.
75. Vyas, A. and S. Chattarji, *Modulation of different states of anxiety-like behavior by chronic stress.* Behav Neurosci, 2004. **118**(6): p. 1450-4.
76. Tafet, G.E. and J. Smolovich, *Psychoneuroendocrinological studies on chronic stress and depression.* Ann N Y Acad Sci, 2004. **1032**: p. 276-8.
77. Misiewicz, Z., et al., *Multi-omics analysis identifies mitochondrial pathways associated with anxiety-related behavior.* PLoS Genet, 2019. **15**(9): p. e1008358.
78. Braund, T.A., et al., *Increased chronic stress predicts greater emotional negativity bias and poorer social skills but not cognitive functioning in healthy adults.* Anxiety Stress Coping, 2019. **32**(4): p. 399-411.
79. Baum, A., H. Herberman, and L. Cohen, *Managing stress and managing illness: Survival and quality of life in chronic disease.* J Clin Psychol Med Settings, 1995. **2**(4): p. 309-33.
80. Golden, S.A., et al., *A standardized protocol for repeated social defeat stress in mice.* Nat Protoc, 2011. **6**(8): p. 1183-91.
81. Iniguez, S.D., et al., *Social defeat stress induces depression-like behavior and alters spine morphology in the hippocampus of adolescent male C57BL/6 mice.* Neurobiol Stress, 2016. **5**: p. 54-64.
82. Wang, W., et al., *Chronic social defeat stress mouse model: Current view on its behavioral deficits and modifications.* Behav Neurosci, 2021. **135**(3): p. 326-335.
83. Laine, M.A., et al., *Genetic Control of Myelin Plasticity after Chronic Psychosocial Stress.* eNeuro, 2018. **5**(4).
84. Wang, S.M., et al., *Antibiotic-induced microbiome depletion is associated with resilience in mice after chronic social defeat stress.* Journal of Affective Disorders, 2020. **260**: p. 448-457.
85. Yang, C., et al., *Bifidobacterium in the gut microbiota confer resilience to chronic social defeat stress in mice.* Sci Rep, 2017. **7**: p. 45942.

86. Qu, Y., et al., *Betaine supplementation is associated with the resilience in mice after chronic social defeat stress: a role of brain-gut-microbiota axis.* Journal of Affective Disorders, 2020. **272**: p. 66-76.
87. Locey, K.J. and J.T. Lennon, *Scaling laws predict global microbial diversity.* Proc Natl Acad Sci U S A, 2016. **113**(21): p. 5970-5.
88. Alsharif, W., M.M. Saad, and H. Hirt, *Desert Microbes for Boosting Sustainable Agriculture in Extreme Environments.* Front Microbiol, 2020. **11**: p. 1666.
89. Alain, K., et al., *Early steps in microbial colonization processes at deep-sea hydrothermal vents.* Environmental Microbiology, 2004. **6**(3): p. 227-241.
90. Hooper, L.V., T. Midtvedt, and J.I. Gordon, *How host-microbial interactions shape the nutrient environment of the mammalian intestine.* Annual Review of Nutrition, 2002. **22**: p. 283-307.
91. Ghoul, M. and S. Mitri, *The Ecology and Evolution of Microbial Competition.* Trends Microbiol, 2016. **24**(10): p. 833-845.
92. Kamada, N., et al., *Control of pathogens and pathobionts by the gut microbiota.* Nat Immunol, 2013. **14**(7): p. 685-90.
93. Candela, M., et al., *Dynamic efficiency of the human intestinal microbiota.* Crit Rev Microbiol, 2015. **41**(2): p. 165-71.
94. Sommer, F. and F. Backhed, *The gut microbiota--masters of host development and physiology.* Nat Rev Microbiol, 2013. **11**(4): p. 227-38.
95. Simon, J.C., et al., *Host-microbiota interactions: from holobiont theory to analysis.* Microbiome, 2019. **7**(1): p. 5.
96. Lozupone, C.A., et al., *Diversity, stability and resilience of the human gut microbiota.* Nature, 2012. **489**(7415): p. 220-30.
97. Whittaker, R.H., *Vegetation of the Siskiyou Mountains, Oregon and California.* Ecological Monographs, 1960. **30**(3): p. 280-338.
98. Lande, R., *Statistics and partitioning of species diversity, and similarity among multiple communities.* Oikos, 1996. **76**(1): p. 5-13.
99. Veech, J.A., et al., *The additive partitioning of species diversity: recent revival of an old idea.* Oikos, 2002. **99**(1): p. 3-9.
100. Tuomisto, H., *A diversity of beta diversities: straightening up a concept gone awry. Part 2. Quantifying beta diversity and related phenomena.* Ecography, 2010. **33**(1): p. 23-45.
101. Rinninella, E., et al., *What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases.* Microorganisms, 2019. **7**(1).
102. Pyke, G.H. and P.R. Ehrlich, *Biological collections and ecological/environmental research: a review, some observations and a look to the future.* Biological Reviews, 2010. **85**(2): p. 247-266.
103. Vogtmann, E., et al., *Comparison of Collection Methods for Fecal Samples in Microbiome Studies.* Am J Epidemiol, 2017. **185**(2): p. 115-123.
104. Thomas, V., J. Clark, and J. Dore, *Fecal microbiota analysis: an overview of sample collection methods and sequencing strategies.* Future Microbiol, 2015. **10**(9): p. 1485-504.
105. Lewis, W.H., et al., *Innovations to culturing the uncultured microbial majority.* Nat Rev Microbiol, 2021. **19**(4): p. 225-240.
106. Panthee, B., et al., *Environmental and Human Microbiome for Health.* Life (Basel), 2022. **12**(3).
107. Ottman, N., et al., *The function of our microbiota: who is out there and what do they do?* Frontiers in Cellular and Infection Microbiology, 2012. **2**.

108. Zhang, Y.L., et al., *The microbial dark matter and "wanted list" in worldwide wastewater treatment plants.* Microbiome, 2023. **11**(1).
109. Tsilimigras, M.C. and A.A. Fodor, *Compositional data analysis of the microbiome: fundamentals, tools, and challenges.* Ann Epidemiol, 2016. **26**(5): p. 330-5.
110. Ngom-Bru, C. and C. Barretto, *Gut microbiota: methodological aspects to describe taxonomy and functionality.* Brief Bioinform, 2012. **13**(6): p. 747-50.
111. Koh, A. and F. Backhed, *From Association to Causality: the Role of the Gut Microbiota and Its Functional Products on Host Metabolism.* Mol Cell, 2020. **78**(4): p. 584-596.
112. Ericsson, A.C. and C.L. Franklin, *Manipulating the Gut Microbiota: Methods and Challenges.* Ilar Journal, 2015. **56**(2): p. 205-217.
113. Vujkovic-Cvijin, I., et al., *Host variables confound gut microbiota studies of human disease.* Nature, 2020. **587**(7834): p. 448-454.
114. Hou, K.J., et al., *Microbiota in health and diseases.* Signal Transduction and Targeted Therapy, 2022. **7**(1).
115. Sender, R., S. Fuchs, and R. Milo, *Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans.* Cell, 2016. **164**(3): p. 337-340.
116. Wang, B.H., et al., *The Human Microbiota in Health and Disease.* Engineering, 2017. **3**(1): p. 71-82.
117. Ramakrishna, B.S., *Role of the gut microbiota in human nutrition and metabolism.* J Gastroenterol Hepatol, 2013. **28 Suppl 4**: p. 9-17.
118. Silva, Y.P., A. Bernardi, and R.L. Frozza, *The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication.* Front Endocrinol (Lausanne), 2020. **11**: p. 25.
119. Belkaid, Y. and T.W. Hand, *Role of the microbiota in immunity and inflammation.* Cell, 2014. **157**(1): p. 121-41.
120. Zheng, D.P., T. Liwinski, and E. Elinav, *Interaction between microbiota and immunity in health and disease.* Cell Research, 2020. **30**(6): p. 492-506.
121. Grice, E.A., et al., *A diversity profile of the human skin microbiota.* Genome Res, 2008. **18**(7): p. 1043-50.
122. Rademacher, F., R. Glaser, and J. Harder, *Antimicrobial peptides and proteins: Interaction with the skin microbiota.* Exp Dermatol, 2021. **30**(10): p. 1496-1508.
123. Stephen, A.M. and J.H. Cummings, *The microbial contribution to human faecal mass.* J Med Microbiol, 1980. **13**(1): p. 45-56.
124. Mackie, R.I., A. Sghir, and H.R. Gaskins, *Developmental microbial ecology of the neonatal gastrointestinal tract.* Am J Clin Nutr, 1999. **69**(5): p. 1035S-1045S.
125. Song, S.J., et al., *Comparative Analyses of Vertebrate Gut Microbiomes Reveal Convergence between Birds and Bats.* Mbio, 2020. **11**(1).
126. Tanaka, M. and J. Nakayama, *Development of the gut microbiota in infancy and its impact on health in later life.* Allergol Int, 2017. **66**(4): p. 515-522.
127. Biasucci, G., et al., *Mode of delivery affects the bacterial community in the newborn gut.* Early Hum Dev, 2010. **86 Suppl 1**: p. 13-5.
128. Reyman, M., et al., *Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life.* Nat Commun, 2019. **10**(1): p. 4997.
129. Sanz, Y., et al., *Understanding the role of gut microbiome in metabolic disease risk.* Pediatr Res, 2015. **77**(1-2): p. 236-44.
130. Fouhy, F., et al., *Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps.* Gut Microbes, 2012. **3**(3): p. 203-20.

131. Boudry, G., et al., *The Relationship Between Breast Milk Components and the Infant Gut Microbiota.* Frontiers in Nutrition, 2021. **8**.
132. Street, M.E., et al., *Current knowledge on the effects of environmental contaminants in early life nutrition.* Front Nutr, 2023. **10**: p. 1120293.
133. Suarez-Martinez, C., et al., *Infant gut microbiota colonization: influence of prenatal and postnatal factors, focusing on diet.* Front Microbiol, 2023. **14**: p. 1236254.
134. Mueller, N.T., et al., *The infant microbiome development: mom matters.* Trends Mol Med, 2015. **21**(2): p. 109-17.
135. Maynard, C.L., et al., *Reciprocal interactions of the intestinal microbiota and immune system.* Nature, 2012. **489**(7415): p. 231-41.
136. Yatsunenko, T., et al., *Human gut microbiome viewed across age and geography.* Nature, 2012. **486**(7402): p. 222-7.
137. Rodriguez, J.M., et al., *The composition of the gut microbiota throughout life, with an emphasis on early life.* Microb Ecol Health Dis, 2015. **26**: p. 26050.
138. Hasan, N. and H. Yang, *Factors affecting the composition of the gut microbiota, and its modulation.* PeerJ, 2019. **7**: p. e7502.
139. Nicholson, J.K., et al., *Host-Gut Microbiota Metabolic Interactions.* Science, 2012. **336**(6086): p. 1262-1267.
140. Kim, S. and S.M. Jazwinski, *The Gut Microbiota and Healthy Aging: A Mini-Review.* Gerontology, 2018. **64**(6): p. 513-520.
141. Salazar, N., et al., *Nutrition and the gut microbiome in the elderly.* Gut Microbes, 2017. **8**(2): p. 82-97.
142. Haran, J.P. and B.A. McCormick, *Aging, Frailty, and the Microbiome-How Dysbiosis Influences Human Aging and Disease.* Gastroenterology, 2021. **160**(2): p. 507-523.
143. Thursby, E. and N. Juge, *Introduction to the human gut microbiota.* Biochem J, 2017. **474**(11): p. 1823-1836.
144. Petersen, C. and J.L. Round, *Defining dysbiosis and its influence on host immunity and disease.* Cell Microbiol, 2014. **16**(7): p. 1024-33.
145. Magne, F., et al., *The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients?* Nutrients, 2020. **12**(5).
146. Seyedian, S.S., F. Nokhostin, and M.D. Malamir, *A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease.* J Med Life, 2019. **12**(2): p. 113-122.
147. Shan, Y., M. Lee, and E.B. Chang, *The Gut Microbiome and Inflammatory Bowel Diseases.* Annu Rev Med, 2022. **73**: p. 455-468.
148. Stojek, M., A. Jablonska, and K. Adrych, *The Role of Fecal Microbiota Transplantation in the Treatment of Inflammatory Bowel Disease.* J Clin Med, 2021. **10**(18).
149. Jandhyala, S.M., et al., *Role of the normal gut microbiota.* World J Gastroenterol, 2015. **21**(29): p. 8787-803.
150. Hsu, C.L. and B. Schnabl, *The gut-liver axis and gut microbiota in health and liver disease.* Nat Rev Microbiol, 2023. **21**(11): p. 719-733.
151. Jasirwan, C.O.M., et al., *The role of gut microbiota in non-alcoholic fatty liver disease: pathways of mechanisms.* Biosci Microbiota Food Health, 2019. **38**(3): p. 81-88.
152. *Experiments and Observations on the Gastric-Juice and the Physiology of Digestion - Beaumont,W.* Psychiatric Quarterly, 1960. **34**(4): p. 742-742.
153. Cryan, J.F., et al., *The Microbiota-Gut-Brain Axis.* Physiological Reviews, 2019. **99**(4): p. 1877-2013.

154. Manca, C., et al., *Germ-free mice exhibit profound gut microbiota-dependent alterations of intestinal endocannabinoidome signaling.* J Lipid Res, 2020. **61**(1): p. 70-85.
155. Yoo, B.B. and S.K. Mazmanian, *The Enteric Network: Interactions between the Immune and Nervous Systems of the Gut.* Immunity, 2017. **46**(6): p. 910-926.
156. Appleton, J., *The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health.* Integr Med (Encinitas), 2018. **17**(4): p. 28-32.
157. Poewe, W., et al., *Parkinson disease.* Nat Rev Dis Primers, 2017. **3**: p. 17013.
158. Warnecke, T., et al., *Gastrointestinal involvement in Parkinson's disease: pathophysiology, diagnosis, and management.* NPJ Parkinsons Dis, 2022. **8**(1): p. 31.
159. Weimers, P., et al., *Inflammatory Bowel Disease and Parkinson's Disease: A Nationwide Swedish Cohort Study.* Inflamm Bowel Dis, 2019. **25**(1): p. 111-123.
160. Geng, Z.H., *Enteric Nervous System: The Bridge Between the Gut Microbiota and Neurological Disorders.* Frontiers in Aging Neuroscience, 2022. **14**.
161. Rao, M. and M.D. Gershon, *The bowel and beyond: the enteric nervous system in neurological disorders.* Nat Rev Gastroenterol Hepatol, 2016. **13**(9): p. 517-28.
162. Peter, I., et al., *Anti-Tumor Necrosis Factor Therapy and Incidence of Parkinson Disease Among Patients With Inflammatory Bowel Disease.* Jama Neurology, 2018. **75**(8): p. 939-946.
163. Li, Y., et al., *Intestinal Inflammation and Parkinson's Disease.* Aging Dis, 2021. **12**(8): p. 2052-2068.
164. Strober, W. and I.J. Fuss, *Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases.* Gastroenterology, 2011. **140**(6): p. 1756-1767.
165. Allen Reish, H.E. and D.G. Standaert, *Role of alpha-synuclein in inducing innate and adaptive immunity in Parkinson disease.* J Parkinsons Dis, 2015. **5**(1): p. 1-19.
166. Kelly, L.P., et al., *Progression of intestinal permeability changes and alpha-synuclein expression in a mouse model of Parkinson's disease.* Mov Disord, 2014. **29**(8): p. 999-1009.
167. Sprenger, F.S., et al., *Enteric nervous system alpha-synuclein immunoreactivity in idiopathic REM sleep behavior disorder.* Neurology, 2015. **85**(20): p. 1761-8.
168. Hawkes, C.H., K. Del Tredici, and H. Braak, *Parkinson's disease: a dual-hit hypothesis.* Neuropathol Appl Neurobiol, 2007. **33**(6): p. 599-614.
169. Sampson, T.R., et al., *Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease.* Cell, 2016. **167**(6): p. 1469-1480 e12.
170. Foster, J.A., L. Rinaman, and J.F. Cryan, *Stress & the gut-brain axis: Regulation by the microbiome.* Neurobiol Stress, 2017. **7**: p. 124-136.
171. Clapp, M., et al., *Gut microbiota's effect on mental health: The gut-brain axis.* Clin Pract, 2017. **7**(4): p. 987.
172. Di Vincenzo, F., et al., *Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review.* Internal and Emergency Medicine, 2023.
173. Mizutani, T., et al., *Gut microbiota signature of pathogen-dependent dysbiosis in viral gastroenteritis.* Sci Rep, 2021. **11**(1): p. 13945.
174. Wang, L., et al., *Gut Microbial Dysbiosis in the Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis of Case-Control Studies.* J Acad Nutr Diet, 2020. **120**(4): p. 565-586.
175. Mousa, W.K., F. Chehadeh, and S. Husband, *Microbial dysbiosis in the gut drives systemic autoimmune diseases.* Front Immunol, 2022. **13**: p. 906258.
176. Wang, L., et al., *The role of the gut microbiota in health and cardiovascular diseases.* Mol Biomed, 2022. **3**(1): p. 30.

177. Geng, J.F., et al., *The links between gut microbiota and obesity and obesity related diseases.* Biomedicine & Pharmacotherapy, 2022. **147**.
178. Camilleri, M., *Leaky gut: mechanisms, measurement and clinical implications in humans.* Gut, 2019. **68**(8): p. 1516-1526.
179. Deng, Y.Y., et al., *Involvement of the microbiota-gut-brain axis in chronic restraint stress: disturbances of the kynurenine metabolic pathway in both the gut and brain.* Gut Microbes, 2021. **13**(1).
180. Kelly, J.R., et al., *Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders.* Front Cell Neurosci, 2015. **9**: p. 392.
181. Woese, C.R., O. Kandler, and M.L. Wheelis, *Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya.* Proc Natl Acad Sci U S A, 1990. **87**(12): p. 4576-9.
182. Handelsman, J., et al., *Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products.* Chem Biol, 1998. **5**(10): p. R245-9.
183. Quince, C., et al., *Shotgun metagenomics, from sampling to analysis.* Nat Biotechnol, 2017. **35**(9): p. 833-844.
184. Forbes, J.D., et al., *Metagenomics: The Next Culture-Independent Game Changer*. Front Microbiol, 2017. **8**: p. 1069.
185. Shakya, M., C.C. Lo, and P.S.G. Chain, *Advances and Challenges in Metatranscriptomic Analysis.* Front Genet, 2019. **10**: p. 904.
186. Ojala, T., E. Kankuri, and M. Kankainen, *Understanding human health through metatranscriptomics.* Trends in Molecular Medicine, 2023. **29**(5): p. 376-389.
187. Mukherjee, A. and M.S. Reddy, *Metatranscriptomics: an approach for retrieving novel eukaryotic genes from polluted and related environments.* 3 Biotech, 2020. **10**(2): p. 71.
188. Sharuddin, S.S., et al., *Advancement of Metatranscriptomics towards Productive Agriculture and Sustainable Environment: A Review.* Int J Mol Sci, 2022. **23**(7).
189. Wasinger, V.C., et al., *Progress with Gene-Product Mapping of the Mollicutes - Mycoplasma-Genitalium.* Electrophoresis, 1995. **16**(7): p. 1090-1094.
190. Fiser, A., *Protein structure modeling in the proteomics era.* Expert Rev Proteomics, 2004. **1**(1): p. 97-110.
191. Richards, A.L., M. Eckhardt, and N.J. Krogan, *Mass spectrometry-based protein-protein interaction networks for the study of human diseases.* Mol Syst Biol, 2021. **17**(1): p. e8792.
192. Zhao, Y. and O.N. Jensen, *Modification-specific proteomics: strategies for characterization of post-translational modifications using enrichment techniques.* Proteomics, 2009. **9**(20): p. 4632-41.
193. Boersema, P.J., A. Kahraman, and P. Picotti, *Proteomics beyond large-scale protein expression analysis.* Curr Opin Biotechnol, 2015. **34**: p. 162-70.
194. Yates, J.R. and A.L. Osterman, *Introduction: Advances in genomics and proteomics.* Chemical Reviews, 2007. **107**(8): p. 3363-3366.
195. Sinitcyn, P., J.D. Rudolph, and J. Cox, *Computational Methods for Understanding Mass Spectrometry-Based Shotgun Proteomics Data.* Annual Review of Biomedical Data Science, Vol 1, 2018. **1**: p. 207-234.
196. Christopher, J.A., et al., *Subcellular proteomics.* Nat Rev Methods Primers, 2021. **1**.
197. Kuhlman, B. and P. Bradley, *Advances in protein structure prediction and design.* Nat Rev Mol Cell Biol, 2019. **20**(11): p. 681-697.
198. Meissner, F., et al., *The emerging role of mass spectrometry-based proteomics in drug discovery.* Nat Rev Drug Discov, 2022. **21**(9): p. 637-654.

199. Alharbi, R.A., *Proteomics approach and techniques in identification of reliable biomarkers for diseases.* Saudi J Biol Sci, 2020. **27**(3): p. 968-974.
200. Aebersold, R. and M. Mann, *Mass spectrometry-based proteomics.* Nature, 2003. **422**(6928): p. 198-207.
201. Horvath, C.G. and S.R. Lipsky, *Use of liquid ion exchange chromatography for the separation of organic compounds.* Nature, 1966. **211**(5050): p. 748-9.
202. Zotou, A., *An overview of recent advances in HPLC instrumentation.* Central European Journal of Chemistry, 2012. **10**(3): p. 554-569.
203. Cruz, E., et al., *Chromatographic classification of commercially available reverse-phase HPLC columns.* Chromatographia, 1997. **44**(3-4): p. 151-161.
204. Fenn, J.B., et al., *Electrospray ionization for mass spectrometry of large biomolecules.* Science, 1989. **246**(4926): p. 64-71.
205. Law, K.P. and Y.P. Lim, *Recent advances in mass spectrometry: data independent analysis and hyper reaction monitoring.* Expert Rev Proteomics, 2013. **10**(6): p. 551-66.
206. Cody, R.B. and B.S. Freiser, *Collision-Induced Dissociation in a Fourier-Transform Mass-Spectrometer.* International Journal of Mass Spectrometry and Ion Processes, 1982. **41**(3): p. 199-204.
207. Davies, V., et al., *Rapid Development of Improved Data-Dependent Acquisition Strategies.* Anal Chem, 2021. **93**(14): p. 5676-5683.
208. Rozanova, S., et al., *Quantitative Mass Spectrometry-Based Proteomics: An Overview.* Methods Mol Biol, 2021. **2228**: p. 85-116.
209. Krasny, L. and P.H. Huang, *Data-independent acquisition mass spectrometry (DIA-MS) for proteomic applications in oncology.* Mol Omics, 2021. **17**(1): p. 29-42.
210. Hu, A., W.S. Noble, and A. Wolf-Yadlin, *Technical advances in proteomics: new developments in data-independent acquisition.* F1000Res, 2016. **5**.
211. Yates, J.R., 3rd, *Database searching using mass spectrometry data.* Electrophoresis, 1998. **19**(6): p. 893-900.
212. Schubert, O.T., et al., *Quantitative proteomics: challenges and opportunities in basic and applied research.* Nat Protoc, 2017. **12**(7): p. 1289-1294.
213. Jungblut, P.R., et al., *Proteomics in human disease: cancer, heart and infectious diseases.* Electrophoresis, 1999. **20**(10): p. 2100-10.
214. Thongboonkerd, V., J. LaBaer, and G.B. Domont, *Recent advances of proteomics applied to human diseases.* J Proteome Res, 2014. **13**(11): p. 4493-6.
215. Bantscheff, M., et al., *Quantitative mass spectrometry in proteomics: a critical review.* Anal Bioanal Chem, 2007. **389**(4): p. 1017-31.
216. Arike, L. and L. Peil, *Spectral counting label-free proteomics.* Methods Mol Biol, 2014. **1156**: p. 213-22.
217. Zhu, W., J.W. Smith, and C.M. Huang, *Mass spectrometry-based label-free quantitative proteomics.* J Biomed Biotechnol, 2010. **2010**: p. 840518.
218. Bubis, J.A., et al., *Comparative evaluation of label-free quantification methods for shotgun proteomics.* Rapid Commun Mass Spectrom, 2017. **31**(7): p. 606-612.
219. Sandin, M., A. Chawade, and F. Levander, *Is label-free LC-MS/MS ready for biomarker discovery?* Proteomics Clinical Applications, 2015. **9**(3-4): p. 289-294.
220. Adam, G.C., E.J. Sorensen, and B.F. Cravatt, *Chemical strategies for functional proteomics.* Mol Cell Proteomics, 2002. **1**(10): p. 781-90.
221. Ross, P.L., et al., *Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents.* Mol Cell Proteomics, 2004. **3**(12): p. 1154-69.

222. Aggarwal, K., L.H. Choe, and K.H. Lee, *Shotgun proteomics using the iTRAQ isobaric tags.* Brief Funct Genomic Proteomic, 2006. **5**(2): p. 112-20.
223. Gygi, S.P., et al., *Quantitative analysis of complex protein mixtures using isotope-coded affinity tags.* Nat Biotechnol, 1999. **17**(10): p. 994-9.
224. Thompson, A., et al., *Tandem mass tags:: A novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS.* Analytical Chemistry, 2003. **75**(8): p. 1895-1904.
225. Li, J., et al., *TMTpro-18plex: The Expanded and Complete Set of TMTpro Reagents for Sample Multiplexing.* J Proteome Res, 2021. **20**(5): p. 2964-2972.
226. Dephoure, N. and S.P. Gygi, *Hyperplexing: a method for higher-order multiplexed quantitative proteomics provides a map of the dynamic response to rapamycin in yeast.* Sci Signal, 2012. **5**(217): p. rs2.
227. Wojdyla, K., et al., *The SNO/SOH TMT strategy for combinatorial analysis of reversible cysteine oxidations.* J Proteomics, 2015. **113**: p. 415-34.
228. Sivanich, M.K., et al., *Recent advances in isobaric labeling and applications in quantitative proteomics.* Proteomics, 2022. **22**(19-20): p. e2100256.
229. Tyanova, S., T. Temu, and J. Cox, *The MaxQuant computational platform for mass spectrometry-based shotgun proteomics.* Nat Protoc, 2016. **11**(12): p. 2301-2319.
230. Kong, A.T., et al., *MSFragger: ultrafast and comprehensive peptide identification in mass spectrometry-based proteomics.* Nat Methods, 2017. **14**(5): p. 513-520.
231. Cox, J., et al., *Andromeda: A Peptide Search Engine Integrated into the MaxQuant Environment.* Journal of Proteome Research, 2011. **10**(4): p. 1794-1805.
232. Vizcaino, J.A., et al., *A guide to the Proteomics Identifications Database proteomics data repository.* Proteomics, 2009. **9**(18): p. 4276-83.
233. Boutet, E., et al., *UniProtKB/Swiss-Prot, the Manually Annotated Section of the UniProt KnowledgeBase: How to Use the Entry View.* Plant Bioinformatics: Methods and Protocols, 2nd Edition, 2016. **1374**: p. 23-54.
234. Tanca, A., et al., *The impact of sequence database choice on metaproteomic results in gut microbiota studies.* Microbiome, 2016. **4**(1): p. 51.
235. Aggarwal, S. and A.K. Yadav, *False Discovery Rate Estimation in Proteomics.* Methods Mol Biol, 2016. **1362**: p. 119-28.
236. Wilmes, P. and P.L. Bond, *Metaproteomics: studying functional gene expression in microbial ecosystems.* Trends Microbiol, 2006. **14**(2): p. 92-7.
237. Wilmes, P., A. Heintz-Buschart, and P.L. Bond, *A decade of metaproteomics: Where we stand and what the future holds.* Proteomics, 2015. **15**(20): p. 3409-3417.
238. Tanca, A., et al., *Enrichment or depletion? The impact of stool pretreatment on metaproteomic characterization of the human gut microbiota.* Proteomics, 2015. **15**(20): p. 3474-85.
239. Starr, A.E., et al., *Proteomic and Metaproteomic Approaches to Understand Host-Microbe Interactions.* Anal Chem, 2018. **90**(1): p. 86-109.
240. Heyer, R., et al., *Challenges and perspectives of metaproteomic data analysis.* J Biotechnol, 2017. **261**: p. 24-36.
241. Blakeley-Ruiz, J.A. and M. Kleiner, *Considerations for constructing a protein sequence database for metaproteomics.* Comput Struct Biotechnol J, 2022. **20**: p. 937-952.
242. Giansanti, P., et al., *Mass spectrometry-based draft of the mouse proteome.* Nat Methods, 2022. **19**(7): p. 803-811.

243. Xiao, L., et al., *A catalog of the mouse gut metagenome.* Nat Biotechnol, 2015. **33**(10): p. 1103-8.
244. Almeida, A., et al., *A unified catalog of 204,938 reference genomes from the human gut microbiome.* Nat Biotechnol, 2021. **39**(1): p. 105-114.
245. Mora, C., et al., *How Many Species Are There on Earth and in the Ocean?* Plos Biology, 2011. **9**(8).
246. Glansdorff, N., Y. Xu, and B. Labedan, *The last universal common ancestor: emergence, constitution and genetic legacy of an elusive forerunner.* Biol Direct, 2008. **3**: p. 29.
247. Mesuere, B., et al., *Unipept web services for metaproteomics analysis.* Bioinformatics, 2016. **32**(11): p. 1746-8.
248. Zamboni, N., A. Saghatelian, and G.J. Patti, *Defining the metabolome: size, flux, and regulation.* Mol Cell, 2015. **58**(4): p. 699-706.
249. Marcobal, A., et al., *A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice.* Isme Journal, 2013. **7**(10): p. 1933-1943.
250. Barbara, G., et al., *Gut microbiota, metabolome and immune signatures in patients with uncomplicated diverticular disease.* Gut, 2017. **66**(7): p. 1252-1261.
251. Beale, D.J., et al., *Review of recent developments in GC-MS approaches to metabolomics-based research.* Metabolomics, 2018. **14**(11): p. 152.
252. Zhou, B., et al., *LC-MS-based metabolomics.* Mol Biosyst, 2012. **8**(2): p. 470-81.
253. Dettmer, K., P.A. Aronov, and B.D. Hammock, *Mass spectrometry-based metabolomics.* Mass Spectrom Rev, 2007. **26**(1): p. 51-78.
254. Emwas, A.H., et al., *NMR Spectroscopy for Metabolomics Research.* Metabolites, 2019. **9**(7).
255. Bauermeister, A., et al., *Mass spectrometry-based metabolomics in microbiome investigations.* Nat Rev Microbiol, 2022. **20**(3): p. 143-160.
256. Lamichhane, S., et al., *Gut metabolome meets microbiome: A methodological perspective to understand the relationship between host and microbe.* Methods, 2018. **149**: p. 3-12.
257. Shaffer, M., et al., *AMON: annotation of metabolite origins via networks to integrate microbiome and metabolome data.* BMC Bioinformatics, 2019. **20**(1): p. 614.
258. Yin, X., et al., *A Comparative Evaluation of Tools to Predict Metabolite Profiles From Microbiome Sequencing Data.* Front Microbiol, 2020. **11**: p. 595910.
259. Wisniewski, J.R., et al., *Universal sample preparation method for proteome analysis.* Nat Methods, 2009. **6**(5): p. 359-62.
260. Tyanova, S., et al., *The Perseus computational platform for comprehensive analysis of (prote)omics data.* Nat Methods, 2016. **13**(9): p. 731-40.
261. Eng, J.K., A.L. McCormack, and J.R. Yates, *An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database.* J Am Soc Mass Spectrom, 1994. **5**(11): p. 976-89.
262. Mao, X.Z., et al., *Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary.* Bioinformatics, 2005. **21**(19): p. 3787-3793.
263. Crestani, E., et al., *Untargeted metabolomic profiling identifies disease-specific signatures in food allergy and asthma.* J Allergy Clin Immunol, 2020. **145**(3): p. 897-906.
264. Mills, R.H., et al., *Evaluating Metagenomic Prediction of the Metaproteome in a 4.5-Year Study of a Patient with Crohn's Disease.* mSystems, 2019. **4**(1).

265. Plubell, D.L., et al., *Extended Multiplexing of Tandem Mass Tags (TMT) Labeling Reveals Age and High Fat Diet Specific Proteome Changes in Mouse Epididymal Adipose Tissue.* Mol Cell Proteomics, 2017. **16**(5): p. 873-890.
266. Cano-Colino, M., R. Almeida, and A. Compte, *Serotonergic modulation of spatial working memory: predictions from a computational network model.* Front Integr Neurosci, 2013. **7**: p. 71.
267. Albert, P.R., F. Vahid-Ansari, and C. Luckhart, *Serotonin-prefrontal cortical circuitry in anxiety and depression phenotypes: pivotal role of pre- and post-synaptic 5-HT1A receptor expression.* Front Behav Neurosci, 2014. **8**: p. 199.
268. Svob Strac, D., N. Pivac, and D. Muck-Seler, *The serotonergic system and cognitive function.* Transl Neurosci, 2016. **7**(1): p. 35-49.
269. Wang, H.M., et al., *A stress response pathway in mice upregulates somatostatin level and transcription in pancreatic delta cells through Gs and β-arrestin 1.* Diabetologia, 2014. **57**(9): p. 1899-1910.
270. Hou, X., et al., *GABAergic System in Stress: Implications of GABAergic Neuron Subpopulations and the Gut-Vagus-Brain Pathway.* Neural Plast, 2020. **2020**: p. 8858415.
271. Turnbaugh, P.J., et al., *The human microbiome project.* Nature, 2007. **449**(7164): p. 804-10.
272. Perez-Pardo, P., et al., *Gut bacterial composition in a mouse model of Parkinson's disease.* Benef Microbes, 2018. **9**(5): p. 799-814.
273. Miyazaki, I., et al., *Chronic Systemic Exposure to Low-Dose Rotenone Induced Central and Peripheral Neuropathology and Motor Deficits in Mice: Reproducible Animal Model of Parkinson's Disease.* Int J Mol Sci, 2020. **21**(9).
274. Lopetuso, L.R., et al., *Commensal Clostridia: leading players in the maintenance of gut homeostasis.* Gut Pathog, 2013. **5**(1): p. 23.
275. Romano, S., et al., *Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation.* NPJ Parkinsons Dis, 2021. **7**(1): p. 27.
276. Vacca, M., et al., *The Controversial Role of Human Gut Lachnospiraceae.* Microorganisms, 2020. **8**(4).
277. Claus, S.P., H. Guillou, and S. Ellero-Simatos, *The gut microbiota: a major player in the toxicity of environmental pollutants?* NPJ Biofilms Microbiomes, 2016. **2**: p. 16003.
278. Vascellari, S., et al., *Gut Microbiota and Metabolome Alterations Associated with Parkinson's Disease.* mSystems, 2020. **5**(5).
279. Keshavarzian, A., et al., *Colonic bacterial composition in Parkinson's disease.* Mov Disord, 2015. **30**(10): p. 1351-60.
280. Tan, A.H., et al., *Gut Microbial Ecosystem in Parkinson Disease: New Clinicobiological Insights from Multi-Omics.* Ann Neurol, 2021. **89**(3): p. 546-559.
281. Petrov, V.A., et al., *Analysis of Gut Microbiota in Patients with Parkinson's Disease.* Bull Exp Biol Med, 2017. **162**(6): p. 734-737.
282. Czepiel, J., et al., *Clostridium difficile infection: review.* Eur J Clin Microbiol Infect Dis, 2019. **38**(7): p. 1211-1221.
283. Kang, X., et al., *Clostridium difficile infection and risk of Parkinson's disease: a Swedish population-based cohort study.* Eur J Neurol, 2020. **27**(11): p. 2134-2141.
284. Schirmer, M., et al., *Linking the Human Gut Microbiome to Inflammatory Cytokine Production Capacity.* Cell, 2016. **167**(4): p. 1125-1136 e8.
285. Markowiak-Kopec, P. and K. Slizewska, *The Effect of Probiotics on the Production of Short-Chain Fatty Acids by Human Intestinal Microbiome.* Nutrients, 2020. **12**(4).

286. Singh, V., et al., *Butyrate producers, "The Sentinel of Gut": Their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics.* Frontiers in Microbiology, 2023. **13**.
287. Yang, D., et al., *The Role of the Gut Microbiota in the Pathogenesis of Parkinson's Disease.* Front Neurol, 2019. **10**: p. 1155.
288. Wang, Q., et al., *The role of gut dysbiosis in Parkinson's disease: mechanistic insights and therapeutic options.* Brain, 2021. **144**(9): p. 2571-2593.
289. Munoz, A.J., et al., *Metabolic engineering of Escherichia coli for improving L-3,4-dihydroxyphenylalanine (L-DOPA) synthesis from glucose.* J Ind Microbiol Biotechnol, 2011. **38**(11): p. 1845-52.
290. Naoi, M. and W. Maruyama, *Cell death of dopamine neurons in aging and Parkinson's disease.* Mech Ageing Dev, 1999. **111**(2-3): p. 175-88.
291. Surmeier, D.J., *Determinants of dopaminergic neuron loss in Parkinson's disease.* FEBS J, 2018. **285**(19): p. 3657-3668.
292. Armstrong, M.J. and M.S. Okun, *Diagnosis and Treatment of Parkinson Disease: A Review.* JAMA, 2020. **323**(6): p. 548-560.
293. Von Seggern, M., et al., *Purine molecules in Parkinson's disease: Analytical techniques and clinical implications.* Neurochem Int, 2020. **139**: p. 104793.
294. Herrero, M.T., et al., *Inflammation in Parkinson's disease: role of glucocorticoids.* Front Neuroanat, 2015. **9**: p. 32.
295. Voigt, R.M., et al., *Gut microbial metabolites in Parkinson's disease: Association with lifestyle, disease characteristics, and treatment status.* Neurobiol Dis, 2022. **170**: p. 105780.
296. Toyoda, Y., et al., *Products of the Parkinson's disease-related glyoxalase DJ-1, D-lactate and glycolate, support mitochondrial membrane potential and neuronal survival.* Biol Open, 2014. **3**(8): p. 777-84.
297. Yu, Z., et al., *The significance of uric acid in the diagnosis and treatment of Parkinson disease: An updated systemic review.* Medicine (Baltimore), 2017. **96**(45): p. e8502.
298. Gonzalez-Riano, C., et al., *Prognostic biomarkers of Parkinson's disease in the Spanish EPIC cohort: a multiplatform metabolomics approach.* NPJ Parkinsons Dis, 2021. **7**(1): p. 73.
299. McKnite, A.M., et al., *Murine Gut Microbiota Is Defined by Host Genetics and Modulates Variation of Metabolic Traits.* Plos One, 2012. **7**(6).
300. Bailey, M.T., et al., *Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation.* Brain Behav Immun, 2011. **25**(3): p. 397-407.
301. Maltz, R.M., et al., *Social Stress Affects Colonic Inflammation, the Gut Microbiome, and Short-chain Fatty Acid Levels and Receptors.* J Pediatr Gastroenterol Nutr, 2019. **68**(4): p. 533-540.
302. Maslanik, T., et al., *Commensal bacteria and MAMPs are necessary for stress-induced increases in IL-1beta and IL-18 but not IL-6, IL-10 or MCP-1.* PLoS One, 2012. **7**(12): p. e50636.
303. Moncrieff, J., et al., *The serotonin theory of depression: a systematic umbrella review of the evidence.* Mol Psychiatry, 2022.
304. Reimold, M., et al., *Central serotonin transporter levels are associated with stress hormone response and anxiety.* Psychopharmacology (Berl), 2011. **213**(2-3): p. 563-72.
305. Carhart-Harris, R.L. and D.J. Nutt, *Serotonin and brain function: a tale of two receptors.* J Psychopharmacol, 2017. **31**(9): p. 1091-1120.

306. Edinoff, A.N., et al., *Selective Serotonin Reuptake Inhibitors and Adverse Effects: A Narrative Review.* Neurol Int, 2021. **13**(3): p. 387-401.
307. Locher, C., et al., *Efficacy and Safety of Selective Serotonin Reuptake Inhibitors, Serotonin-Norepinephrine Reuptake Inhibitors, and Placebo for Common Psychiatric Disorders Among Children and Adolescents A Systematic Review and Meta-analysis.* Jama Psychiatry, 2017. **74**(10): p. 1011-1020.
308. Barandouzi, Z.A., et al., *Associations of neurotransmitters and the gut microbiome with emotional distress in mixed type of irritable bowel syndrome.* Sci Rep, 2022. **12**(1): p. 1648.
309. Boros, M. and F. Keppler, *Methane Production and Bioactivity-A Link to Oxido-Reductive Stress.* Front Physiol, 2019. **10**: p. 1244.
310. Heshmati, M., et al., *Depression and Social Defeat Stress Are Associated with Inhibitory Synaptic Changes in the Nucleus Accumbens.* J Neurosci, 2020. **40**(32): p. 6228-6233.
311. Lalive, A.L., et al., *Mild stress accumulation limits GABAergic synaptic plasticity in the lateral habenula.* Eur J Neurosci, 2022. **55**(2): p. 377-387.
312. Heinritz, S.N., et al., *Effect of cellobiose supplementation on in vitro fermentation activity and bacterial numbers of porcine inocula.* J Anim Physiol Anim Nutr (Berl), 2018. **102**(2): p. 474-482.
313. Kilgour, A.H., et al., *11beta-Hydroxysteroid dehydrogenase activity in the brain does not contribute to systemic interconversion of cortisol and cortisone in healthy men.* J Clin Endocrinol Metab, 2015. **100**(2): p. 483-9.

Appendices

Appendix 1. R scripts for IRS normalization.

```
# Set working directory
setwd("directory for the analysis")

# Load libraries
library(tidyverse)
library(limma)
library(edgeR)
library(sva)

# Read the protein intensities
data_raw <- read_csv("proteins.csv") ["column indexes for the intensities"]

# Fix the column headers
col_headers <- colnames(data_raw)

# Separate the TMT data by experiment (set)
exp1_raw <- data_raw[c(col_index_exp1)]
exp2_raw <- data_raw[c(col_index_exp2)]
exp3_raw <- data_raw[c(col_index_exp3)]

# Create new data frame with row sums from each frame
irs <- tibble(rowSums(exp1_raw), rowSums(exp2_raw), rowSums(exp3_raw))
colnames(irs) <- c("sum1", "sum2", 'sum3')

# Calculate the geometric average intensity for each protein
irs$average <- apply(irs, 1, function(x) exp(mean(log(x),na.rm = TRUE)))

# Compute the scaling factor vectors based on the reference channel
irs$fac1 <- irs$average / exp1_raw$Ref_1
irs$fac2 <- irs$average / exp2_raw$Ref_2
irs$fac3 <- irs$average / exp3_raw$Ref_3

# Create new data frame with IRS normalized data
data_irs <- exp1_raw * irs$fac1
data_irs <- cbind(data_irs, exp2_raw * irs$fac2)
data_irs <- cbind(data_irs, exp3_raw * irs$fac3)

# Create MDS plot
plotMDS(log2(data_irs), col = rep(c("red", "green",'blue'), each = 9), main = "IRS clusters")
```

Appendix 2. Python scripts for protein annotation, hierarchical clustering, statistical test and taxonomic analysis.

```
#!/usr/bin/env python
# coding: utf-8

# # Social stress mouse model (Metaproteomic data analysis, p_value 0.05, pi-score 1.3, <30% NA)
# ### Load dependencies

import pandas as pd
import matplotlib.pyplot as plt
import numpy as np
from scipy import stats
import scipy
import math
import seaborn as sns
from scipy.stats import ttest_ind
import re
get_ipython().run_line_magic('pylab', 'inline')

# Makes plots inline.
get_ipython().run_line_magic('matplotlib', 'inline')
# Sets the seaborn plot style. More info: http://seaborn.pydata.org/tutorial/aesthetics.html
sns.set(style= 'white', color_codes=True)
# ### User-defined functions
ttests=[]
def ttest(df,ttdf1,ttdf2):
from scipy.stats import ttest_ind
ttdf1 = ttdf1.transpose()
ttdf2 = ttdf2.transpose()
ttests = ttest_ind(ttdf1,ttdf2,equal_var=False,nan_policy='omit')
ttests = ttests.pvalue.transpose()
df['ttest_pvalue']=ttests

def volcano(df, significantdf, foldchange, pvalue, savename):
plt.figure(figsize=(10, 10))
plt.scatter(df['%s' % (foldchange)], df['%s' % (pvalue)], c='#A9A9A9',s=20)
# #FFC61E for mouse and '#F28522'for microbiota
```

```
plt.scatter(significantdf['%s' % (foldchange)], significantdf['%s' % (pvalue)], c='#FFC61E', s=20)
plt.ylabel('-Log10(P)', fontsize=18)
plt.xlabel('Log2FoldChange', fontsize=18)

plt.xticks(fontsize=15)
plt.yticks(fontsize=15)

for pos in ['right', 'top']:
plt.gca().spines[pos].set_visible(False)

   plt.savefig('%s' % (savename))

def fold(df, sub1, sub2, sub1name, sub2name):
df['%s Mean' % (sub1name)]=sub1.mean(axis=1,skipna=True)
df['%s Mean' % (sub2name)]=sub2.mean(axis=1,skipna=True)
# Fold change is sub1/sub2
df['Fold Change(%s/%s)' % (sub1name,sub2name)]=df['%s Mean' % (sub1name)]/df['%s Mean' % (sub2name)]
   print("Fold Change Column Name = 'Fold Change(%s/%s)'" %(sub1name,sub2name))

def piscore(df,sub1name,sub2name):
df['Log2(Fold Change)'] = log2(df['Fold Change(%s/%s)'% (sub1name,sub2name)])
df['-Log10(P-value)'] = -np.log10(df['ttest_pvalue'])
df['%s/%s pi score' % (sub1name,sub2name)] = df['-Log10(P-value)']*df['Log2(Fold Change)']

def TwoCategoryMaster(dfmain,dfsub1,dfsub2,sub1name,sub2name):
ttest(dfmain,dfsub1,dfsub2)
fold(dfmain,dfsub1,dfsub2,sub1name,sub2name)
   piscore(dfmain,sub1name,sub2name)

# Expand the taxonomic information with specific taxon (like ['phylum', 'order', 'genus','species']), only run this
function after taxonomic annotation
def expand_taxa(df,list_of_taxa):
taxa = df['All Taxa'].str.split('|').tolist()
indexes = df['Unknown2'].str.split('|').tolist()
for taxon in list_of_taxa:
new_col = []
for n in range(len(taxa)):
if taxon in str(indexes[n]):
```

```
new_col.append(taxa[n][indexes[n].index(taxon)])
else:
new_col.append('')
df[taxon] = new_col
# ### Load the data
df_all = pd.read_csv("IRS_normalized_data.csv")

# Build up annotation files
taxa = pd.read_csv("/184_gene_catalog/184sample.uniq_gene.NR.anno.merge.txt", sep = "\t")
KO = pd.read_csv("/184_gene_catalog/184sample.uniq_gene.KAAS.anno.txt", sep = "\t")
eggNOG = pd.read_csv("/184_gene_catalog/184sample.uniq_gene.eggNOG3.anno.txt", sep = "\t")

# Annotate COG Categories
COG = pd.read_csv('COG/COG.description.csv')
COG.columns = ['COG', 'COG Protein Name']
COG_cats = pd.read_csv('COG/COG.funccat.csv')
COG_cats.columns = ['COG', 'eggNOG_Code']
Cat_key = pd.read_csv('COG/eggNOG_FunCats.csv')

# ### Annotate the normalized data

# Merge the annotation information to the normalized data
df_anno_bac = df_all.merge(taxa, left_on = "Accession", right_on = "Protein", how = 'left')
df_anno_bac =  df_anno_bac.merge(KO, left_on = "Accession", right_on = "Protein", how = 'left')
df_anno_bac = df_anno_bac.merge(eggNOG, left_on = "Accession", right_on = "Protein")

# Expand the taxa at phylum, order, family, genus, species level
expand_taxa(df_anno_bac, ['phylum', 'class', 'order', 'family', 'genus','species'])

# Add eggNOG annotations
df_anno_bac = df_anno_bac.merge(COG, left_on = 'eggNOG3', right_on = 'COG', how = 'left')
df_anno_bac = df_anno_bac.merge(COG_cats, left_on = 'COG', right_on = 'COG', how = 'left')

df_anno_bac = df_anno_bac.merge(Cat_key, left_on = 'eggNOG_Code', right_on = 'eggNOG Functional
Assignment', how = 'left')

# Export the annotated bacterial proteins
df_anno_bac.to_csv("/export/tables/SS_Bac_func.csv")
```

```
# Split the normalized data and export the annotated mouse proteins
HostAnn = pd.read_csv('/184_gene_catalog/mouse_swiss_prot.csv', index_col = 'Entry')
protein_ls = list(df_anno_bac['Accession'])
df_mouse = df_all[~df_all['Accession'].isin(protein_ls)]
print(len(df_mouse))
df_mouse = df_mouse.merge(HostAnn, left_on = 'Accession', right_index = True)
#df_mouse.to_csv('/SS_Mouse_proteins.csv')



# ### Biological Replicate Cluster Map
# #### Bacterial proteins

# Create a correlation matrix for bacterial proteins using spearman as the method
df_anno_bac = pd.read_csv("/export/tables/SS_Bac_func.csv", index_col=0, usecols=range(1, 26))

corr_bac = df_anno_bac.corr(method = "spearman")

# Create a seaborn clustermap
sns.clustermap(corr_bac, cmap='coolwarm').fig.suptitle('Spearman correlation of Microbiobial proteins')
savefig("/export/figures/SS_Correlation_Plot_Microbes.png")
# #### Mouse proteins
# Create a correlation matrix for mouse proteins using spearman as the method
df_mouse = pd.read_csv("/export/tables/Mouse_proteins.csv",index_col=0, usecols=range(1, 26))
#df_mouse['Length'] = df_mouse['Length'].astype(str)
corr_mouse = df_mouse.corr(method = "spearman")

# Create a seaborn clustermap
sns.clustermap(corr_mouse, cmap='coolwarm').fig.suptitle('Spearman Correlation of Mouse Proteins')
savefig("/export/figures/Correlation_Plot_Mouse.png")

# ### Taxonomic analysis
samples = ["Sample_1",...,"Sample_N"]

# Load the data
df_anno_bac = pd.read_csv("/export/tables/Bac_func.csv")

# Define a taxon to be analyzed
```

```
taxon = 'Taxa'

df_anno_bac_taxa = df_anno_bac[samples].groupby([df_anno_bac[taxon.lower()]]).agg(['sum'])
#df_anno_bac_func = df_anno_bac[samples].groupby([df_anno_bac.Description]).agg(['sum'])
df_anno_bac_taxa.columns = df_anno_bac_taxa.columns.droplevel(1)
df_anno_bac_taxa['Sum'] = df_anno_bac_taxa.sum(axis=1)

for i in samples:
df_anno_bac_taxa[i] = df_anno_bac_taxa[i]/df_anno_bac_taxa[i].sum()

# Transpose the plot to make it fit the code structure for the stacked bar chart

# Remove index name
df_anno_bac_taxa.index.name = None
# Transpose
df_anno_bac_taxa = df_anno_bac_taxa.transpose()
# Get the dates in the correct order
df_anno_bac_taxa = df_anno_bac_taxa.reindex(samples)
# Get taxon into an order which depends on the abundance of each phyla for easier interpretation
df_anno_bac_taxa = df_anno_bac_taxa[df_anno_bac_taxa.sum().sort_values(ascending = False).index]
df_anno_bac_taxa.head()

# export the result
trans_df_anno_bac_taxa = df_anno_bac_taxa.transpose()
trans_df_anno_bac_taxa.to_csv('/export/tables/taxonomy/ratio_' + taxon + '_level.csv')

# ### Determining Proteins of Interest
# #### Bacterial proteins_Function

# Load the data
df_anno_bac = pd.read_csv("/export/tables/Bac_func.csv")
df_anno_bac.head()

# Assign bacterial subgroups
Group1 = df_anno_bac[["Group1_Sample_1",...,"Group1_Sample_N"]]

# Save host protein significance dataframe
df_anno_bac.to_csv('/export/tables/Bac_piscores_Group1_Group2.csv')
```

```
# Determine significant proteins, setting significance to piscore > 1.3
df_pi_bac= df_anno_bac[abs(df_anno_bac['Group1/Group2 pi score']) > 1.3]
df_sign_bac= df_pi_bac[(df_anno_bac['ttest_pvalue']) < 0.05]

df_sign_bac.to_csv('/export/tables/t_test/Bac_sig_Group1_Group2.csv')

df_Group1_B = df_sign_bac[df_sign_bac['Group1/Group2 pi score'] > 0] # Upregulated in Group1
df_Group2_B = df_sign_bac[df_sign_bac['Group1/Group2 pi score'] < 0] # Downregulated in Group2

# #### Mouse proteins
# Load the data
df_anno_mouse = pd.read_csv("/Mouse_proteins.csv")

# Assign mouse subgroups
Group1_M = df_anno_mouse[["Group1_Sample_1",...,"Group1_Sample_N"]]
Group2_M = df_anno_mouse[["Group1_Sample_2",...,"Group2_Sample_N"]]

# Using TwoCategoryMaster function, we can perform a ttest as well as several other statistical tests commonly
used when comparing two categories in proteomic datasets
TwoCategoryMaster(df_anno_mouse,Group1_M,Group2_M,'Group1','Group2')

# Determine significant proteins, setting significance to piscore > 1.3, pvalue < 0.05
df_pi_mouse= df_anno_mouse[abs(df_anno_mouse['Rotenone/Vehicle pi score']) > 1.3]
df_sign_mouse= df_pi_mouse[(df_anno_mouse['ttest_pvalue']) < 0.05]

# Save host protein significance dataframe
df_sign_mouse.to_csv('/Mouse_sig_proteins.csv')

# ### Comparing significant proteins from different groups
# #### Bacterial proteins
# Load the data
df_anno_bac = pd.read_csv("/export/tables/Bac_func.csv")
df_anno_bac.head()

df_anno_bac = df_anno_bac.drop(Group1, axis=1)
df_anno_bac = df_anno_bac.drop(Group2, axis=1)
df_anno_bac.head()
```

```
# Using TwoCategoryMaster function, we can perform a ttest as well as several other statistical tests commonly used when comparing two categories in proteomic datasets
TwoCategoryMaster(df_anno_bac,Group1,Group2,'Group1','Group2')

# Get the Fold Change STD and subset out the significant hits (Top Responders)
#Fold_Change_STD = df['Log2(Fold Change)'].std()

df_pi_bac= df_anno_bac[abs(df_anno_bac['Group1/Group2 pi score']) > 1.3]
df_sign_bac= df_pi_bac[(df_anno_bac['ttest_pvalue']) < 0.05]

df_sign_mouse.to_csv('./final_new_cutoff/Mouse_sig_proteins.csv')
df_sign_mouse.head()

# Volcano plot
volcano(df_anno_bac, df_sign_bac,'Log2(FoldChange)','-Log10(P-value)',
'/export/figures/Bac_Group1_Group2_Volcano.png')
```

Appendix 3. Python scripts for enrichment analysis.

```
# ### Extract the results from the website of KEGG pathway mapping
# First you have to reconstruct pathway through https://www.genome.jp/kegg/tool/map_pathway.html and download the result page as html

import re

# Construct the dictionary for significantly different mapping result
file_name_sig = input('Enter the file name for significantly different mapping result:')
f_sig = open(file_name_sig, 'r').read()

# Extract pathways
pathway = r'ce" target="_blank">(.*?)</dl>'
pathways = re.findall(pathway,f_sig, re.DOTALL)

# Extract all pathway names
p_name = r'ce" target="_blank">(.*?) '
p_names = re.findall(p_name,f_sig, re.DOTALL)
```

```
# Create a new file to store the updated information
new = open('enrichment.txt','w')
protein = r'dd(.*?)</dd>'

for i in range(len(pathways)):
  proteins = re.findall(protein,pathways[i], re.DOTALL)
  count_proteins = len(proteins) + ''.join(proteins).count(',')
  new.write(' '.join(p_names[i].split('</a>')) + ' (' +str(count_proteins) + ')' +'\n')

new.close()


# ### Calculate the enrichment values and p-value for each pathway
import scipy.stats as stats

# Construct the dicrary for significantly different mapping result
file_name_sig = input('Enter the file name for significantly different mapping result:')
f_sig = open(file_name_sig, 'r')
pathways_sig = [line.strip() for line in f_sig if line.strip()]

sig_dic = dict()
for pathway in pathways_sig:
  sig_dic[' '.join(pathway.split()[:-1])] = int(pathway.split()[-1][1:-1])

n_sig = sum(sig_dic.values())
print("There're in total " + str(n_sig) + " hits in" + file_name_sig + '.')


# Construct the dicrary for all mapping result
file_name_all = input('Enter the file name for all mapping result:')
f_all = open(file_name_all, 'r')
pathways_all = [line.strip() for line in f_all if line.strip()]

all_dic = dict()
for pathway in pathways_all:
  all_dic[' '.join(pathway.split()[:-1])] = int(pathway.split()[-1][1:-1])
```

```
n_all = sum(all_dic.values())
print("There're in total " + str(n_all) + " hits in" + file_name_all + '.')

# Calculate the enrichment value and p-value (one-tail Fisher's exact test) for each pathway and write it down in
a new txt file
new_output = open('enrichment_values.txt', 'w')
enrich_dic = dict()
p_value_dic = dict()

for key in sig_dic.keys():
  enrich_dic[key] = (sig_dic[key]/all_dic[key])/(n_sig/n_all)
  p_value_dic[key] = stats.fisher_exact([[sig_dic[key], n_sig],[all_dic[key],n_all]],'greater')[1]
  new_output.write(str(key) + ';' + str(enrich_dic[key]) + ';' + str(p_value_dic[key]) +'\n')

f_sig.close()
f_all.close()
new_output.close()
```

Appendix 4. List of significantly changed microbial proteins between rotenone- and vehicle-treated groups.

Accession	**KO**	**Phylum**	**Family**	**Genus**	**Pi score**
S-Fe1_GL0044311		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.20449
3-1_GL0054518	K00656	*Firmicutes*			-1.47812
1-1_GL0092522	K02358	*Firmicutes*	*Lachnospiraceae*		1.315015
2-5_GL0003928	K04077	*Firmicutes*			-1.46462
25_GL0017537	K02406				-2.81521
1-1_GL0130011	K04077	*Firmicutes*			-1.4054
2-5_GL0023856	K02358	*Firmicutes*			-1.94067
5-3_GL0006268	K02406				-2.09517
34_GL0071997	K01006	*Firmicutes*			1.78918
8-1_GL0017543	K02406	*Firmicutes*			-1.57228
34_GL0009955	K00134	*Firmicutes*			1.442946
2-5_GL0024453	K04043	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-2.27293
S-Fe7_GL0098036	K02406	*Firmicutes*			-1.3612
2-1_GL0045974	K10112	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	2.189115
38_GL0059412	K10200	*Firmicutes*			3.15259
34_GL0027199		*Firmicutes*	*Peptostreptococcaceae*		2.476524
Group2-6A_GL0079618	K00134	*Firmicutes*			-1.64368

1-7_GL0050848	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.723802
G1-1A_GL0034793	K01689				-1.51777
MH-6-4_GL0094837	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.645431
S-Fe10_GL0170141	K01689				-1.39128
S-Fe9_GL0084857	K02406	*Firmicutes*			2.593749
Group2-3A_GL0096287	K02406	*Firmicutes*			-1.60544
34_GL0068749	K03521	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.62601
G1-5A_GL0096260	K02406	*Firmicutes*	*Lachnospiraceae*		-1.72384
Group2-4A_GL0073267	K00248	*Firmicutes*			-1.65313
Group2-3A_GL0215737	K00248	*Firmicutes*			1.470024
1A-dyr4-07_GL0104823	K02406	*Firmicutes*			1.629129
54_GL0074441		*Firmicutes*			1.608213
7-5_GL0135604	K02406	*Firmicutes*	*Lachnospiraceae*		-2.492
34_GL0061818	K01624	*Firmicutes*	*Lachnospiraceae*		1.571372
Group2-6A_GL0142076	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.4708
34_GL0043650	K07335	*Firmicutes*			2.671218
S-Fe9_GL0003443	K02358	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.3389
34_GL0027649		*Firmicutes*			2.041676
1-1_GL0072116	K10439	*Firmicutes*			2.611631
35_GL0070575	K02406	*Firmicutes*			-2.1795
7-5_GL0112530					2.051347
34_GL0014287	K10540	*Firmicutes*			2.133826
34_GL0061311	K02945	*Firmicutes*	*Lachnospiraceae*		2.083029
3-3_GL0060758	K02035	*Firmicutes*			1.694707
S-Fe16_GL0056988	K02406	*Firmicutes*			-3.651
5-6_GL0021409	K10540	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-3.05788
3-2_GL0056345	K02027	*Firmicutes*			1.415052
3-1_GL0063415		*Firmicutes*	*Lachnospiraceae*		-2.32121
34_GL0014951	K04072	*Firmicutes*			2.747579
1-1_GL0005593	K01571	*Firmicutes*			1.771263
34_GL0005923	K01966	*Firmicutes*			1.367885
38_GL0007538		*Firmicutes*	*Ruminococcaceae*		3.596354
34_GL0031188	K00975	*Firmicutes*			1.504888
2-1_GL0050294	K02027	*Firmicutes*			1.948716
34_GL0056148	K02082	*Firmicutes*			1.739845
54_GL0025231	K03521				1.399544
2-8_GL0045976					1.866121
Group2-8A_GL0129562	K10112	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	2.216345
Group2-8A_GL0047867		*Firmicutes*			2.443175

3-1_GL0097688	K02406	*Firmicutes*	*Lachnospiraceae*		-1.80192
S-Fe11_GL0055974	K02406	*Firmicutes*			-1.30029
7-5_GL0010739		*Firmicutes*	*Lachnospiraceae*		-3.01337
2-1_GL0087635	K02988	*Firmicutes*	*Lachnospiraceae*		-2.69088
6-1_GL0103473	K02406	*Firmicutes*			1.841338
MH-6-5_GL0159066		*Firmicutes*			1.327388
34_GL0037102	K00074	*Firmicutes*	*Eubacteriaceae*		2.355412
1-1_GL0026953	K00703	*Firmicutes*	*Lachnospiraceae*		1.942279
1-1_GL0017259	K02564	*Firmicutes*			1.364464
Group2-8A_GL0148965		*Firmicutes*			-1.32922
25_GL0022833	K02864	*Firmicutes*			-1.80086
4-4_GL0006579		*Firmicutes*			-1.80504
34_GL0055592	K00929	*Firmicutes*			1.604678
8-2_GL0013510		*Firmicutes*			2.052386
34_GL0042757	K02986	*Firmicutes*			2.379328
34_GL0005795	K00789	*Firmicutes*			3.516236
G1-1A_GL0028032	K02965	*Firmicutes*			-3.42185
5-5_GL0047625	K02357	*Firmicutes*	*Lachnospiraceae*		-1.94855
34_GL0056147	K01443	*Firmicutes*			1.914045
1-1_GL0111826	K02996	*Firmicutes*			4.094119
S-Fe3_GL0173811	K02406				3.658901
1-1_GL0008130	K01784	*Firmicutes*			1.491556
34_GL0040220	K08483	*Firmicutes*	*Ruminococcaceae*		1.525136
1-1_GL0046100	K00634	*Firmicutes*			1.324597
1-1_GL0115225	K01628	*Firmicutes*			1.617616
MC-0-1_GL0002281	K02956	*Bacteroidetes*			-1.37276
1-3_GL0115555	K02747	*Firmicutes*	*Ruminococcaceae*		2.550378
MH-6-5_GL0056132	K10112	*Firmicutes*			1.783299
38_GL0077092	K03415	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.467205
7-5_GL0044432	K02926	*Firmicutes*			1.752732
38_GL0037917	K02745	*Firmicutes*			2.032203
5-2_GL0067967	K02926	*Firmicutes*			-1.33727
38_GL0050490	K03406				1.621024
17_GL0011034	K02950	*Firmicutes*	*Lachnospiraceae*	*Dorea*	-2.01338
4-2_GL0031967	K00197	*Firmicutes*			-1.63735
MC-6-2_GL0128713	K10540	*Firmicutes*			-1.83114
10_GL0006935	K02904	*Firmicutes*			-1.32922
2-5_GL0059022		*Firmicutes*	*Lachnospiraceae*		-1.43587
7-7_GL0096304	K02945	*Firmicutes*	*Lachnospiraceae*		-2.82714
6-1_GL0072263	K02904	*Firmicutes*			1.404781
34_GL0067726		*Firmicutes*			2.111587
34_GL0026513	K00849	*Firmicutes*	*Lachnospiraceae*		1.933431

38_GL0079365	K02051	*Firmicutes*			2.042848
1A-dyr3-07_GL0040272	K02895	*Firmicutes*			1.70514
1-7_GL0050864	K00179	*Firmicutes*			2.027255
16_GL0036423					1.414124
6-2_GL0078653	K02968	*Firmicutes*	*Lachnospiraceae*	*Blautia*	-2.69354
MH-6-2_GL0086648	K00059	*Firmicutes*	*Ruminococcaceae*		1.398274
S-Fe10_GL0052402					-1.39959
5-5_GL0069737	K03111	*Firmicutes*			-1.56499
34_GL0016130	K11189	*Firmicutes*			2.229458
34_GL0008174	K10117	*Firmicutes*			1.30688
1-1_GL0111801	K02994	*Firmicutes*			1.835772
17_GL0137077	K05349	*Firmicutes*	*Lachnospiraceae*		1.780644
34_GL0065943	K01999	*Firmicutes*			1.347787
34_GL0020020		*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.582933
7-6_GL0000835	K10540	*Firmicutes*	*Lachnospiraceae*		-2.20784
Group2-4A_GL0088601	K05795	*Firmicutes*	*Lachnospiraceae*		-2.51712
2-5_GL0068001		*Firmicutes*			-2.7728
1-1_GL0068022	K02959	*Firmicutes*			1.305657
1-1_GL0101550					1.752347
38_GL0077822		*Firmicutes*			1.386009
MH-6-5_GL0181064					1.456052
6-1_GL0062496	K02878	*Firmicutes*			-1.86686
34_GL0017609		*Firmicutes*			2.106055
2B-dyr19-06_GL0058878	K02881	*Firmicutes*			1.389156
MC-0-1_GL0076519					1.575702
4-2_GL0081601		*Firmicutes*	*Lachnospiraceae*		-2.96985
34_GL0075952	K01624	*Firmicutes*	*Lachnospiraceae*		1.415098
34_GL0031643	K02431	*Firmicutes*			1.597651
1-7_GL0091926	K02996	*Firmicutes*			-2.179
54_GL0132741		*Firmicutes*	*Lachnospiraceae*		2.167045
2-1_GL0094558	K02904	*Firmicutes*			1.448473
S-Fe10_GL0021215		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.42159
34_GL0013858	K03569	*Firmicutes*			1.772873
6-5_GL0027977		*Firmicutes*			-2.23998
8-2_GL0113497		*Firmicutes*			2.105071
35_GL0152677	K02959	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-2.03841
1-1_GL0040809	K07814				1.627333
34_GL0065270	K02952	*Firmicutes*			1.507937
6-2_GL0068302	K01138	*Firmicutes*			1.504203
34_GL0009469	K02660	*Firmicutes*			1.839462
17_GL0044420	K07080				2.509893

7-5_GL0013357	K02109	*Firmicutes*			1.368053
11_GL0009410	K02926	*Firmicutes*			-1.49828
3-7_GL0058829	K02895	*Firmicutes*			1.450046
34_GL0067731	K02012	*Firmicutes*			1.631557
4-2_GL0031978	K03615	*Firmicutes*	*Lachnospiraceae*		-2.05954
S-Fe2_GL0112540	K02864	*Firmicutes*			-1.32337
2-5_GL0012361	K00059	*Firmicutes*			-1.94159
2-5_GL0040573		*Firmicutes*			-1.98256
S-Fe8_GL0225106	K01689	*Proteobacteria*			1.877118
10_GL0056353	K02952	*Bacteroidetes*			-1.31033
13_GL0023553		*Firmicutes*			2.478236
34_GL0072662	K07138	*Firmicutes*			1.88701
38_GL0122669		*Firmicutes*			4.06655
2_GL0074166	K02895	*Firmicutes*			1.614618
23_GL0077908	K02113	*Firmicutes*			1.335587
1A-dyr1-07_GL0044210	K03778	*Firmicutes*			1.585304
5_GL0080052	K02434	*Firmicutes*			-1.47496
MC-0-1_GL0007434	K02895	*Bacteroidetes*			1.617089
1-1_GL0009618	K12960				1.524574
MH-0-5_GL0013844	K10108	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	2.739633
1A-dyr3-06_GL0041826		*Bacteroidetes*	*Porphyromonadaceae*		-1.67584
2-5_GL0065661	K03545	*Firmicutes*			-1.81575
1-1_GL0117867	K02049	*Firmicutes*			1.535036
4-2_GL0094202	K08483	*Firmicutes*			-1.45816
1-1_GL0094905		*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	3.957426
2-5_GL0008595	K05795	*Firmicutes*			-2.47579
1B-dyr12-07_GL0019010	K01808	*Firmicutes*			2.346461
2-5_GL0028038		*Firmicutes*			-2.01384
34_GL0078218	K02073	*Firmicutes*			1.798974
34_GL0071999		*Firmicutes*			2.523362
G1-2A_GL0106471		*Bacteroidetes*			1.488662
4-8_GL0074033		*Arthropoda*			-4.96396
MC-0-1_GL0017448	K03217				-2.12555
38_GL0079370		*Firmicutes*	*Lachnospiraceae*		2.108765
14_GL0054944					-2.193
5-3_GL0090848		*Firmicutes*			-1.7843
38_GL0147849	K03210	*Firmicutes*			2.975674
2B-dyr19-06_GL0028374	K03413	*Firmicutes*			1.864165
3-1_GL0089388	K01940	*Firmicutes*			-1.63354

Appendix 5. List of significantly changed mouse proteins between BR and BC groups.

Accession	Entry name	Pi score
P29391	FRIL1_MOUSE	-1.48477
P47740	AL3A2_MOUSE	-1.42599
Q9Z0G9	CLD3_MOUSE	-1.77754

Appendix 6. List of significantly changed mouse proteins between DC and BC groups.

Accession	Entry name	Pi score
Q91XA9	CHIA_MOUSE	4.100462
P97429	ANXA4_MOUSE	-2.27025
Q6Q473	CLA4A_MOUSE	-2.00118
Q62468	VILI_MOUSE	-1.37664
Q9CQ52	CEL3B_MOUSE	2.359558
P05208	CEL2A_MOUSE	2.156149
Q00898	A1AT5_MOUSE	-5.36943
O88310	ITL1A_MOUSE	-1.86909
Q64133	AOFA_MOUSE	-1.42881
P09103	PDIA1_MOUSE	-1.48108
Q8R1M8	MPTX_MOUSE	-2.32315
Q64521	GPDM_MOUSE	-1.36271
Q9D816	CP255_MOUSE	-1.59122
P27773	PDIA3_MOUSE	-1.50611
Q8BHH8	CLRN3_MOUSE	-1.9179
P47740	AL3A2_MOUSE	-1.37234
P07743	BPIA2_MOUSE	-1.41363
Q8K0C9	GMDS_MOUSE	-1.30512
P01665	KV3AD_MOUSE	2.239011
Q9Z0G9	CLD3_MOUSE	-1.63404
Q9CR68	UCRI_MOUSE	-1.36114
P17665	COX7C_MOUSE	-1.57878
Q91VR2	ATPG_MOUSE	-1.35708
Q9DAS9	GBG12_MOUSE	-1.82682
Q9R1P3	PSB2_MOUSE	-1.43046
O09131	GSTO1_MOUSE	-1.32639
P43137	LIT1_MOUSE	-2.14238
Q9R1P4	PSA1_MOUSE	-1.52039
Q76MZ3	2AAA_MOUSE	-2.05468
Q3TMQ6	ANG4_MOUSE	-2.03644

P51879	ONCO_MOUSE	-2.44905
Q9DD20	MET7B_MOUSE	-1.30113
Q3UZZ6	ST1D1_MOUSE	-1.70503
O09061	PSB1_MOUSE	-1.37059
Q9JLJ2	AL9A1_MOUSE	-2.78728
P14211	CALR_MOUSE	-1.49713

Appendix 7. List of significantly changed mouse proteins between DR and BR groups.

Accession	Entry name	Pi score
Q6P8U6	LIPP_MOUSE	-3.15364
P17892	LIPR2_MOUSE	-2.11091
Q62468	VILI_MOUSE	-1.99221
P60710	ACTB_MOUSE	-2.92781
P00687	AMY1_MOUSE	-2.85217
Q8C6C9	LEG1H_MOUSE	-3.69816
Q00898	A1AT5_MOUSE	-2.65323
P68134	ACTS_MOUSE	-3.33449
O88329	MYO1A_MOUSE	-1.79399
P10126	EF1A1_MOUSE	-2.82686
P09103	PDIA1_MOUSE	-2.28642
Q9Z0L8	GGH_MOUSE	-1.58895
P62806	H4_MOUSE	-1.8833
P40142	TKT_MOUSE	-1.35711
P26040	EZRI_MOUSE	-2.7845
P0DP27	CALM2_MOUSE	-1.88759
P51881	ADT2_MOUSE	-1.82544
Q9D312	K1C20_MOUSE	-1.78016
Q3TIW9	ENPP7_MOUSE	-2.05402
Q6T3U4	NPCL1_MOUSE	-1.91094
Q6URW6	MYH14_MOUSE	-1.83194
Q64475	H2B1B_MOUSE	-1.98838
P00405	COX2_MOUSE	-1.58686
Q9Z0Y2	PA21B_MOUSE	-2.09772
P07743	BPIA2_MOUSE	-2.02914
Q60605	MYL6_MOUSE	-1.85149
Q61207	SAP_MOUSE	-1.49228
P35700	PRDX1_MOUSE	-1.35014
Q64444	CAH4_MOUSE	-2.07511
P47856	GFPT1_MOUSE	-1.48756

Q8K1H9	OBP2A_MOUSE	-2.52303
P26231	CTNA1_MOUSE	-1.73382
P01807	HVM37_MOUSE	3.907394
Q68FD5	CLH1_MOUSE	-1.88839
Q99JW5	EPCAM_MOUSE	-2.14216
Q91WR8	GPX6_MOUSE	-3.17196
Q9DCX2	ATP5H_MOUSE	-2.2523
P58252	EF2_MOUSE	-3.04889
Q99JZ0	SDCB2_MOUSE	-1.7116
Q8C3K6	SC5A1_MOUSE	-2.39868
Q9Z1Q5	CLIC1_MOUSE	-1.35168
P11499	HS90B_MOUSE	-1.6405
P62908	RS3_MOUSE	-3.03021
Q6P069	SORCN_MOUSE	-1.39338
P17665	COX7C_MOUSE	-1.79125
Q91VC3	IF4A3_MOUSE	-3.41519
Q9DCD0	6PGD_MOUSE	-1.88489
P49183	DNAS1_MOUSE	-2.75148
O08601	MTP_MOUSE	-1.83607
P43137	LIT1_MOUSE	-1.70264
Q60597	ODO1_MOUSE	-1.76989
O08992	SDCB1_MOUSE	-2.18991
O54990	PROM1_MOUSE	-1.50099
Q60604	ADSV_MOUSE	-1.7636
Q76MZ3	2AAA_MOUSE	-1.48539
P55050	FABPI_MOUSE	-1.91016
Q3TMQ6	ANG4_MOUSE	-2.45677
Q99P86	RETNB_MOUSE	-3.64625
P11983	TCPA_MOUSE	-1.53189
P62264	RS14_MOUSE	-1.85118
Q9D855	QCR7_MOUSE	-1.49888
Q99LC5	ETFA_MOUSE	-1.78849
Q61171	PRDX2_MOUSE	-1.37236
Q9JI02	S2B20_MOUSE	-1.68642
Q8BH70	FBXL4_MOUSE	-1.57769
Q9QY30	ABCBB_MOUSE	-1.60923
Q6PZE0	MUC19_MOUSE	-1.51042
P14131	RS16_MOUSE	-3.11805
Q9WVA4	TAGL2_MOUSE	-2.0977
P06328	HVM49_MOUSE	-3.8983
P51150	RAB7A_MOUSE	-2.11014

Appendix 8. List of significantly changed mouse proteins between DR and DC groups.

Accession	Entry name	Pi score
Q6P8U6	LIPP_MOUSE	-2.16195
Q91XA9	CHIA_MOUSE	-3.61875
P17892	LIPR2_MOUSE	-2.32398
Q9CQ52	CEL3B_MOUSE	-2.83067
P05208	CEL2A_MOUSE	-3.30359
Q91X79	CELA1_MOUSE	-2.80183
Q61207	SAP_MOUSE	-1.99987
Q68FD5	CLH1_MOUSE	-2.39403
Q91VC3	IF4A3_MOUSE	-2.34204
P18524	HVM53_MOUSE	-4.87866
O08601	MTP_MOUSE	-1.44163
Q99P86	RETNB_MOUSE	-1.90469
P62264	RS14_MOUSE	-1.80548
P46638	RB11B_MOUSE	-1.74486
Q6PZE0	MUC19_MOUSE	-1.51825

Appendix 9. List of significantly changed mouse proteins between DR and DS groups.

Accession	Entry name	Pi score
Q80Z19	MUC2_MOUSE	-1.7225
P00688	AMYP_MOUSE	-4.1594
P18761	CAH6_MOUSE	-3.1508
Q6P8U6	LIPP_MOUSE	-2.9414
Q91XA9	CHIA_MOUSE	-2.7596
P17892	LIPR2_MOUSE	-2.2954
Q9R100	CAD17_MOUSE	-1.4902
Q9CQ52	CEL3B_MOUSE	-3.1809
P00687	AMY1_MOUSE	-2.4862
P05208	CEL2A_MOUSE	-2.8494
Q8C6C9	LEG1H_MOUSE	-3.2908
Q9JLT2	TREA_MOUSE	-1.5264
Q91X79	CELA1_MOUSE	-2.9457
Q9CR35	CTRB1_MOUSE	-2.201
P10126	EF1A1_MOUSE	-1.4084

Q3SYP2	CTRC_MOUSE	-2.7896
Q9Z0L8	GGH_MOUSE	-1.6847
P0DP27	CALM2_MOUSE	-1.7631
P15947	KLK1_MOUSE	-1.8574
Q62471	VNS1_MOUSE	-1.3374
Q8K1H9	OBP2A_MOUSE	-2.3484
Q68FD5	CLH1_MOUSE	-1.4668
Q8BK48	EST2E_MOUSE	-1.381
Q8C3K6	SC5A1_MOUSE	-1.859
P62908	RS3_MOUSE	-1.4974
P01631	KV2A7_MOUSE	-1.9323
P01635	KV5A3_MOUSE	-1.8662
Q91VC3	IF4A3_MOUSE	-2.9047
O08992	SDCB1_MOUSE	-1.3938
P11983	TCPA_MOUSE	-1.6798
Q6PZE0	MUC19_MOUSE	-1.6849
P62242	RS8_MOUSE	-1.4752

Appendix 10. List of significantly changed microbial proteins between BR and BC groups.

Accession	**KO**	**Phylum**	**Family**	**Genus**	**Pi score**
2-1_GL0080403	K01610	*Firmicutes*			1.514137
1-1_GL0083048	K02406	*Firmicutes*			-2.0795
1B-dyr12-07_GL0023314	K02358	*Bacteroidetes*			-1.78872
25_GL0017537	K02406				1.853189
11_GL0143116	K02406	*Firmicutes*	*Lachnospiraceae*		1.589568
S-Fe20_GL0101053	K02406	*Firmicutes*	*Clostridiaceae*		-1.32587
S-Fe12_GL0081748	K02406	*Firmicutes*			5.511695
G1-1A_GL0100813	K01580	*Bacteroidetes*			-1.55978
MC-6-3_GL0031266	K01610				-2.61088
6-2_GL0022431	K00257	*Firmicutes*	*Clostridiaceae*		2.256528
MC-6-1_GL0036584		*Firmicutes*			3.123945
S-Fe10_GL0122713	K04077				-1.93878
10_GL0087391	K04043	*Firmicutes*			-1.79523
4-1_GL0075275		*Firmicutes*			1.862293
11_GL0139961	K02406	*Firmicutes*	*Lachnospiraceae*		1.555153
4-1_GL0082414	K03522	*Firmicutes*			1.888896
8-1_GL0053268	K01624	*Firmicutes*			1.344018

6-2_GL0004627	K01915	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.377399
23_GL0023755	K03737	*Firmicutes*			-1.54671
4-6_GL0053572	K01610	*Firmicutes*			1.337036
1-1_GL0073107	K00262	*Firmicutes*			1.497275
3-3_GL0068589	K10112	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.402315
S-Fe11_GL0067219	K01580	*Bacteroidetes*			-2.09783
8-5_GL0024395		*Firmicutes*			1.686296
G1-1A_GL0010516	K01580	*Bacteroidetes*			-2.44295
G1-2A_GL0000922	K07749				-1.36541
2-2_GL0104799	K02027	*Firmicutes*	*Ruminococcaceae*		2.179639
4-1_GL0001158	K03040	*Firmicutes*			1.954137
10_GL0043144		*Bacteroidetes*			-1.4648
6-4_GL0065486	K03521				1.494827
MH-6-2_GL0027157	K01847	*Bacteroidetes*	*Rikenellaceae*		-1.62111
MC-6-3_GL0020434	K09758	*Bacteroidetes*			-1.61316
4-1_GL0005108	K10188	*Firmicutes*			1.903879
MH-6-3_GL0099998	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.8941
1-6_GL0056681	K00134	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	1.782928
8-2_GL0073909	K02992	*Firmicutes*			3.050306
3-5_GL0115359	K02035	*Firmicutes*			1.494602
6-4_GL0062275	K01870	*Firmicutes*			1.364513
1A-dyr4-07_GL0111745	K02935	*Firmicutes*			1.688676
6-3_GL0120092	K03522				1.441212
2-1_GL0005243		*Firmicutes*			1.715086
4-1_GL0082411	K00074	*Firmicutes*	*Lachnospiraceae*		1.716182
2-3_GL0012648	K00939	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	2.225458
MC-6-2_GL0154047		*Firmicutes*			-2.17353
1A-dyr4-07_GL0027597	K01571	*Firmicutes*			1.513538
Group2-8A_GL0004149	K02946	*Firmicutes*	*Lachnospiraceae*	*Roseburia*	1.335652
16_GL0057650	K04079	*Firmicutes*			-1.65525
4-7_GL0002310	K02931	*Firmicutes*			-1.30901
2-1_GL0055226	K02988	*Firmicutes*			-2.03114
2-5_GL0030947	K10546	*Firmicutes*	*Lachnospiraceae*		1.452689
13_GL0013959	K02355	*Bacteroidetes*	*Rikenellaceae*		-1.79567
4-1_GL0036739		*Firmicutes*			-1.54997
4-8_GL0033438	K02904	*Firmicutes*			1.967062
2-3_GL0082942					-1.97574
1-1_GL0060070		*Firmicutes*			1.469812
52_GL0096773	K03522				1.788818
2-1_GL0001188		*Firmicutes*			-3.48625
17_GL0014693	K02357	*Firmicutes*	*Lachnospiraceae*	*Dorea*	-1.58554

MH-0-3_GL0026841	K01810	*Firmicutes*			-2.45116
3-3_GL0019886	K02967	*Firmicutes*			2.023095
54_GL0089633	K02950	*Firmicutes*			1.769534
G1-5A_GL0200419	K02887	*Firmicutes*			2.31161
10_GL0036243	K03969				-1.71877
S-Fe19_GL0061295	K03286	*Bacteroidetes*			-1.57014
S-Fe10_GL0113327	K03386	*Bacteroidetes*			-2.123
11_GL0037479		*Firmicutes*			1.308271
7-8_GL0119582	K02895	*Firmicutes*			1.540273
8-6_GL0066292	K02904				-1.34725
4-1_GL0029484		*Firmicutes*			-1.63664
G1-2A_GL0031495	K02986	*Firmicutes*			-1.80757
S-Fe6_GL0231981	K10439	*Firmicutes*			1.618433
Group2-5A_GL0000249	K02996	*Firmicutes*			1.508009
S-Fe10_GL0121845	K02355	*Bacteroidetes*			-1.44818
MC-6-2_GL0059817					-2.4645
6-4_GL0001701	K01999	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.233973
2-2_GL0037306	K01870	*Firmicutes*			1.824375
1-4_GL0097472	K02990				1.396651
4-1_GL0014462	K09766	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.951783
S-Fe10_GL0024170		*Bacteroidetes*			-1.73462
10_GL0066542	K02355	*Firmicutes*	*Erysipelotrichaceae*		-2.53181
4-1_GL0069165	K02892	*Firmicutes*			1.538656
16_GL0041024	K01193				-2.33905
S-Fe12_GL0185247	K03561	*Bacteroidetes*			-3.00259
6-5_GL0006089	K02864	*Firmicutes*			1.46599
1-6_GL0097787	K04077	*Firmicutes*	*Streptococcaceae*	*Lactococcus*	2.083721
6-2_GL0064228	K02956	*Firmicutes*			1.87875
10_GL0039541					-1.63185
G1-5A_GL0140442	K01914	*Firmicutes*	*Lachnospiraceae*		1.713189
S-Fe6_GL0251004	K02907	*Firmicutes*			-1.85289
6-5_GL0050439	K00615	*Firmicutes*			2.631409
MC-6-3_GL0022447		*Proteobacteria*			-1.896
10_GL0056520	K03531	*Firmicutes*	*Erysipelotrichaceae*		-1.83747
4-1_GL0058230					-1.63947
1-7_GL0037676	K00850	*Firmicutes*			-1.86157
4-1_GL0050292	K03612	*Firmicutes*			-2.01796
10_GL0093910	K02356	*Firmicutes*	*Erysipelotrichaceae*		-3.23805
MH-6-5_GL0156039	K10559	*Firmicutes*			1.476261
Group2-7A_GL0080944	K03530	*Firmicutes*			-2.34054
1A-dyr2-07_GL0044881	K03530				2.249637

17_GL0132312	K01039				1.430159
20_GL0152599		*Firmicutes*			-1.49206
20_GL0133608	K03406	*Firmicutes*	*Lachnospiraceae*		1.577084
1A-dyr1-07_GL0034261	K00925	*Firmicutes*	*Erysipelotrichaceae*		-1.41138
4-1_GL0032705					1.323783
S-Fe10_GL0129234	K01710	*Firmicutes*			-1.60261
MC-6-5_GL0091603	K02863	*Firmicutes*			-1.37163
2-1_GL0062630	K03530	*Firmicutes*			-4.3951
8-1_GL0125338	K02965	*Firmicutes*			1.481883
8-5_GL0088175		*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.477421
S-Fe12_GL0009091	K02358				-2.13397
10_GL0066543	K06867	*Firmicutes*	*Erysipelotrichaceae*		-2.82986
10_GL0002908		*Firmicutes*	*Erysipelotrichaceae*		-1.30505
11_GL0111350	K04567	*Firmicutes*	*Lachnospiraceae*		-2.00544
10_GL0077219	K01814	*Firmicutes*			-1.98169
S-Fe10_GL0003799	K04564	*Bacteroidetes*			-1.45137
10_GL0063070	K02073	*Firmicutes*			-2.40913
11_GL0126003		*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-1.54846
2-1_GL0047226	K02864	*Firmicutes*			-1.30201
S-Fe10_GL0151814	K02897	*Bacteroidetes*	*Porphyromonadaceae*	*Odoribacter*	-1.70986
G1-3A_GL0108906	K02890	*Firmicutes*			-2.34906
2-1_GL0021912		*Firmicutes*	*Erysipelotrichaceae*		-2.0837
S-Fe9_GL0167560	K02904	*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	-3.06374
G1-1A_GL0036828		*Bacteroidetes*			-1.45149
8-2_GL0124976		*Firmicutes*			3.038551
4-6_GL0026072	K00600	*Firmicutes*			-1.55343
MH-0-5_GL0025079	K02890	*Bacteroidetes*			-2.49244
13_GL0004688		*Firmicutes*	*Erysipelotrichaceae*		-2.1277
MC-0-3_GL0033598	K02902	*Bacteroidetes*	*Rikenellaceae*		-1.90875
S-Fe9_GL0062172		*Firmicutes*			-3.06299
2A-dyr16-07_GL0027252		*Firmicutes*	*Streptococcaceae*	*Streptococcus*	-1.45974
43_GL0097625					1.526397
8-1_GL0013528	K01006	*Firmicutes*			1.369064
1-5_GL0050281	K04077	*Firmicutes*			1.772203
1A-dyr4-07_GL0023556	K04077	*Firmicutes*			-2.35075
G1-1A_GL0078250	K01624	*Firmicutes*	*Lachnospiraceae*		1.411164
23_GL0078573	K02358	*Firmicutes*			3.146863
7_GL0033364	K02406	*Firmicutes*			3.221668
6-4_GL0014587	K02931	*Firmicutes*			1.721686
1A-dyr4-07_GL0088827	K01803	*Firmicutes*			1.390247

Group2-7A_GL0080998	K02406	*Firmicutes*			-1.9432
2_GL0089318	K02035	*Firmicutes*	*Lachnospiraceae*		2.044769
G1-5A_GL0189719	K02967	*Firmicutes*			1.389563
S-Fe11_GL0032826	K02959	*Bacteroidetes*			-1.42745
6-4_GL0088559	K00688	*Firmicutes*			2.662388
1-1_GL0117776	K00688	*Firmicutes*			2.901693
1-1_GL0091378	K02988	*Firmicutes*			2.765801
1A-dyr4-07_GL0032844	K02926	*Firmicutes*	*Lachnospiraceae*		-1.67586
1-1_GL0022563	K02899	*Firmicutes*			3.283971
1-1_GL0081834	K01686	*Firmicutes*			-1.48387
S-Fe9_GL0035903	K02926	*Firmicutes*			1.551488
2-2_GL0064882	K00013	*Firmicutes*			2.548087
5-8_GL0146490	K01491	*Firmicutes*			-2.27169
4-2_GL0042457	K03407	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.78536
1-1_GL0035426		*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.206028
S-Fe2_GL0038629	K01591	*Firmicutes*			1.8721
1-1_GL0075236		*Firmicutes*	*Lachnospiraceae*		2.031914
1A-dyr1-07_GL0002799		*Firmicutes*			1.386725

Appendix 11. List of significantly changed microbial proteins between DC and BC groups.

Accession	**KO**	**Phylum**	**Family**	**Genus**	**Pi score**
1-1_GL0083048	K02406	*Firmicutes*			-1.42466
4-1_GL0079284	K01181	*Firmicutes*			-2.31252
4-1_GL0079285	K01181	*Firmicutes*			-2.25273
G1-1A_GL0075950	K01006				-2.47719
19_GL0073930	K01006				-1.36913
11_GL0014868		*Firmicutes*			3.092764
G1-7A_GL0141747	K01006				-1.84784
G1-1A_GL0058894	K04043	*Bacteroidetes*			-1.53452
S-Fe10_GL0170141	K01689				-2.99306
G1-1A_GL0096667	K01689				-2.00171
G1-1A_GL0093263	K01610				-1.74235
S-Fe7_GL0098036	K02406	*Firmicutes*			-1.88248
MC-6-3_GL0041016	K02358	*Bacteroidetes*			-1.75554
Group2-2A_GL0145990	K02358	*Bacteroidetes*			-1.65742
S-Fe11_GL0081407	K01006				-1.67013
1-1_GL0078299	K02406				2.94229

S-Fe10_GL0005531	K01006				-2.2241
S-Fe14_GL0044680	K02406				2.543043
11_GL0002095	K02358	*Bacteroidetes*			-1.80659
Group2-8A_GL0216363	K02406	*Firmicutes*			1.738933
MC-6-1_GL0032534	K01610				-1.62659
2-1_GL0057488	K00262	*Firmicutes*			1.353258
S-Fe10_GL0073221	K01689				-3.46349
S-Fe10_GL0163207	K01805				-2.2373
G1-1A_GL0024937	K00134	*Bacteroidetes*			-2.75555
31_GL0011831	K01610				-1.72174
G1-1A_GL0024446	K01805	*Bacteroidetes*			-1.89487
8-5_GL0107730	K03737	*Firmicutes*			-2.31524
S-Fe10_GL0065079	K02358				-2.04165
G1-1A_GL0096517	K03737	*Bacteroidetes*			-2.85887
S-Fe10_GL0031497	K00134				-2.57352
MH-6-4_GL0119314	K02358	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-2.03959
1-2_GL0002789	K02406				2.466289
4-8_GL0028110	K00262	*Firmicutes*			1.89729
MH-0-4_GL0088579	K02355	*Bacteroidetes*	*Porphyromonadaceae*		-2.40041
2-1_GL0033349	K00262	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	2.128834
S-Fe20_GL0101053	K02406	*Firmicutes*	*Clostridiaceae*		-2.19565
S-Fe10_GL0064044	K01610				-5.6947
G1-3A_GL0097093	K03737	*Bacteroidetes*			-1.45481
G1-5A_GL0169212		*Firmicutes*			2.651597
Group2-4A_GL0039847		*Firmicutes*			1.375786
MH-6-5_GL0025727	K02406	*Firmicutes*			2.373998
S-Fe10_GL0164975	K01610	*Firmicutes*			-2.17956
S-Fe10_GL0123281	K04043				-3.9088
MH-6-2_GL0050923	K02358	*Firmicutes*			-1.3905
G1-1A_GL0104448	K04077				-1.3946
MC-0-1_GL0060587	K02355	*Bacteroidetes*	*Porphyromonadaceae*		-2.17807
G1-1A_GL0100813	K01580	*Bacteroidetes*			-1.36491
MC-6-3_GL0031266	K01610				-1.37243
G1-1A_GL0063304	K04077	*Bacteroidetes*			-1.71444
S-Fe10_GL0056937	K02358	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.32167
S-Fe10_GL0122713	K04077				-2.92851
S-Fe1_GL0079912		*Bacteroidetes*	*Rikenellaceae*		-1.97278
Group2-6A_GL0005221	K02406	*Firmicutes*			5.926889
G1-1A_GL0096460		*Bacteroidetes*			-2.12858
MH-6-4_GL0000793	K02358	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	-1.43657
5-8_GL0074657	K01610	*Firmicutes*			-1.37553

G1-1A_GL0022223	K02112	*Firmicutes*			-1.66201
G1-1A_GL0096553	K00615				-2.18909
6-3_GL0000806	K02406	*Firmicutes*			2.269454
G1-1A_GL0002589	K03046	*Bacteroidetes*			-2.53132
S-Fe10_GL0153182	K03737	*Bacteroidetes*			-2.1957
4-1_GL0041723	K04077	*Bacteroidetes*			-1.63718
11_GL0051937	K01610	*Bacteroidetes*			-1.91766
11_GL0138839	K02355	*Bacteroidetes*			-1.49741
S-Fe10_GL0048913	K03737	*Bacteroidetes*			-1.41308
MC-0-3_GL0041514	K02355	*Bacteroidetes*			-2.17949
10_GL0087391	K04043	*Firmicutes*			-2.30929
10_GL0038120	K00248	*Firmicutes*			-1.89769
MH-6-5_GL0039873	K02406	*Firmicutes*			-1.59081
G1-1A_GL0002609		*Bacteroidetes*			-5.48047
10_GL0070094	K02945				-2.16823
G1-5A_GL0096260	K02406	*Firmicutes*	*Lachnospiraceae*		2.663152
S-Fe1_GL0119537		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.25331
G1-1A_GL0002606		*Bacteroidetes*			-4.10686
1-1_GL0078624	K10112	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.68278
2B-dyr23-07_GL0049983	K02406				2.709077
49_GL0015734	K00134	*Bacteroidetes*			-2.04038
S-Fe12_GL0026793	K03695	*Bacteroidetes*			-1.53897
S-Fe10_GL0044720	K00688				-2.85518
2-2_GL0070064		*Firmicutes*			1.303437
29_GL0023124		*Firmicutes*			1.626973
S-Fe11_GL0060366	K02945	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.10992
G1-1A_GL0075913	K00262	*Bacteroidetes*			-2.19108
1-1_GL0012052					3.777181
S-Fe10_GL0055365		*Bacteroidetes*			-2.42586
20_GL0047623	K00134	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.35217
S-Fe1_GL0132619	K07263	*Bacteroidetes*			-1.8055
MC-6-2_GL0121460	K00895	*Bacteroidetes*			-2.53496
G1-1A_GL0045828	K00927	*Bacteroidetes*			-2.83796
S-Fe10_GL0065072	K03043	*Bacteroidetes*			-3.00096
G1-1A_GL0110280	K01810				-2.19671
S-Fe10_GL0168640	K02355	*Bacteroidetes*			-2.21727
7-4_GL0056601		*Firmicutes*			2.266026
S-Fe10_GL0048936		*Proteobacteria*			-2.32973
G1-1A_GL0021808		*Bacteroidetes*			-2.45685
S-Fe10_GL0021215		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.82482
S-Fe10_GL0122689	K02945	*Bacteroidetes*			-2.09628
17_GL0005632	K00626				-2.08858

G1-1A_GL0045900	K01428	*Bacteroidetes*			-2.87066
S-Fe10_GL0080771	K01805	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.07829
S-Fe20_GL0104847	K02406	*Firmicutes*			-1.70081
MC-0-3_GL0016796	K02355	*Bacteroidetes*			-1.4564
G1-1A_GL0024115	K00262	*Bacteroidetes*			-1.6217
MC-6-3_GL0008086	K01624				-5.53791
G1-1A_GL0002607		*Bacteroidetes*			-3.47314
11_GL0082733	K00688				-1.35558
4-1_GL0069044	K02406	*Firmicutes*			2.472525
S-Fe10_GL0170082		*Bacteroidetes*			-2.45387
11_GL0152071	K12257	*Bacteroidetes*			-4.51937
S-Fe10_GL0056454	K01006	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.95887
S-Fe10_GL0008866	K02967	*Bacteroidetes*			-1.60035
26_GL0025975	K02992	*Firmicutes*			1.796494
S-Fe10_GL0140534	K03043	*Bacteroidetes*			-3.02433
S-Fe10_GL0074773	K00239	*Bacteroidetes*			-1.81859
20_GL0146153	K02935	*Bacteroidetes*			-2.41512
10_GL0012604	K01624				-1.3365
S-Fe10_GL0031147	K00927	*Bacteroidetes*			-1.5531
G1-1A_GL0100771	K00831	*Bacteroidetes*	*Porphyromonadaceae*		-1.90532
G1-1A_GL0116809	K03286	*Bacteroidetes*	*Porphyromonadaceae*		-1.43901
G1-1A_GL0110315	K01804	*Bacteroidetes*			-3.51169
S-Fe10_GL0169925		*Bacteroidetes*			-2.22629
6-2_GL0084706	K02982	*Firmicutes*			1.992022
Group2-3A_GL0117738	K00927	*Firmicutes*	*Lachnospiraceae*		1.355865
1-7_GL0118751	K02897	*Bacteroidetes*	*Porphyromonadaceae*		-2.09424
G1-1A_GL0025052	K03772				-1.79688
S-Fe10_GL0029119	K02967	*Bacteroidetes*			-1.67028
1-1_GL0085766	K02863				-1.92331
10_GL0022078	K00927	*Bacteroidetes*			-3.6122
10_GL0062514		*Bacteroidetes*	*Rikenellaceae*		-2.77861
11_GL0051934	K01006				-2.55361
MC-0-3_GL0008155	K03070				-2.22964
11_GL0014548	K02406	*Firmicutes*			-1.71973
S-Fe10_GL0035419					-2.07515
26_GL0024979	K10439	*Firmicutes*	*Lachnospiraceae*		3.453524
MC-6-1_GL0052341	K02986	*Bacteroidetes*			-3.23504
G1-1A_GL0096461		*Bacteroidetes*			-1.88276
10_GL0009190	K10112	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-2.48689
G1-1A_GL0063364					-2.54362
S-Fe10_GL0160603	K00927	*Bacteroidetes*			-1.69471
G1-1A_GL0030006		*Bacteroidetes*			-1.99013

S-Fe10_GL0059024		*Bacteroidetes*			-1.43342
G1-1A_GL0117677		*Bacteroidetes*			-2.88016
G1-1A_GL0026584	K00239				-1.56831
S-Fe10_GL0013550		*Bacteroidetes*			-1.67738
8-1_GL0072988	K02931	*Firmicutes*			1.482161
G1-1A_GL0093277	K01858	*Bacteroidetes*			-3.49164
S-Fe10_GL0073084	K00615	*Bacteroidetes*			-1.89625
S-Fe10_GL0065073	K02935	*Bacteroidetes*			-2.79234
S-Fe10_GL0056933	K02864	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.0214
MC-0-1_GL0042903	K02112	*Bacteroidetes*			-1.62245
8_GL0153264	K02967	*Bacteroidetes*			-1.49394
S-Fe10_GL0141758	K00262	*Bacteroidetes*			-2.28824
S-Fe10_GL0044469	K01624	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.44221
2-1_GL0023506	K02931	*Firmicutes*			-7.83101
7-2_GL0015190	K02931	*Firmicutes*			1.351604
1A-dyr2-07_GL0039124	K03332				-1.88355
G1-1A_GL0048786	K00024	*Bacteroidetes*			-1.56564
G1-1A_GL0021777	K00895	*Bacteroidetes*			-2.12614
S-Fe1_GL0146005	K02967	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.0108
S-Fe10_GL0145991		*Bacteroidetes*			-1.69073
G1-1A_GL0045858		*Bacteroidetes*			-2.89401
G1-1A_GL0096610	K01676	*Bacteroidetes*	*Porphyromonadaceae*		-2.08437
4-4_GL0060409	K01915	*Firmicutes*			-1.47474
G1-1A_GL0096567	K02967	*Bacteroidetes*			-3.55922
10_GL0081640	K02355	*Firmicutes*			-1.8235
S-Fe10_GL0027246	K00262	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.08554
S-Fe10_GL0141770	K03286	*Bacteroidetes*			-2.33099
MC-0-1_GL0010166	K00962	*Bacteroidetes*			-2.10161
1-7_GL0013213	K02992	*Bacteroidetes*			-1.55171
G1-1A_GL0045950	K01847				-2.43124
S-Fe10_GL0127874	K07749				-2.60064
1-8_GL0095236	K02988	*Firmicutes*			1.6038
MC-0-1_GL0078736	K00262	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	1.824242
G1-1A_GL0010516	K01580	*Bacteroidetes*			-2.7378
S-Fe10_GL0110876	K00262	*Bacteroidetes*			-1.58661
S-Fe10_GL0039362	K00927	*Bacteroidetes*	*Prevotellaceae*		-2.21882
G1-5A_GL0052549		*Firmicutes*			1.608031
2-1_GL0113853	K02035	*Firmicutes*	*Lachnospiraceae*		1.301496
MC-6-3_GL0091839	K02863				-1.41009
MC-0-3_GL0030050	K02897	*Bacteroidetes*	*Porphyromonadaceae*		-1.35913
10_GL0070110	K03772				-1.38734
S-Fe9_GL0075827	K02906	*Firmicutes*			2.021443

S-Fe11_GL0075845	K00927	*Bacteroidetes*			-2.32947
8-1_GL0089359	K02035	*Firmicutes*	*Lachnospiraceae*		1.491799
MH-0-5_GL0104986	K01960	*Bacteroidetes*			-2.1425
3-1_GL0015551	K02935	*Bacteroidetes*			-2.11626
1-7_GL0094016	K01624	*Firmicutes*			-1.61599
10_GL0055487	K02935	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	-1.38094
S-Fe10_GL0045789	K04077	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.74394
S-Fe10_GL0047359		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.07593
G1-1A_GL0045137		*Bacteroidetes*			-1.53518
S-Fe10_GL0072597	K02992	*Bacteroidetes*	*Porphyromonadaceae*		-2.75584
S-Fe10_GL0176063		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.89665
10_GL0007379	K02355	*Bacteroidetes*			-2.33396
G1-1A_GL0096551	K00239	*Bacteroidetes*			-1.72493
S-Fe10_GL0021247		*Proteobacteria*			-4.71078
4-1_GL0040689		*Firmicutes*			-2.44246
10_GL0056350	K03040				-1.40547
S-Fe10_GL0087175	K01624				-2.66722
2-2_GL0005704	K00194	*Firmicutes*			-1.38671
6-4_GL0096205		*Firmicutes*			1.370289
13_GL0071234	K00134	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-2.68096
6-2_GL0039188	K02906	*Firmicutes*			1.455447
G1-1A_GL0034849	K00024	*Bacteroidetes*			-1.42909
5-2_GL0032209	K02986	*Firmicutes*			1.66882
MC-6-3_GL0020434	K09758	*Bacteroidetes*			-2.32884
S-Fe10_GL0056932	K02935	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.11471
MH-6-4_GL0067781		*Bacteroidetes*			-2.01438
S-Fe10_GL0110880	K03286	*Bacteroidetes*			-3.10647
1-1_GL0093801	K01596	*Firmicutes*			-2.07752
MH-6-3_GL0099998	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	3.495845
G1-1A_GL0100781	K07405	*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	-1.89157
S-Fe1_GL0132724		*Bacteroidetes*			-3.05021
10_GL0065644	K02986	*Bacteroidetes*			-1.90326
G1-5A_GL0017879	K02926	*Firmicutes*			1.524364
1-7_GL0088542	K02982	*Bacteroidetes*			-1.44081
11_GL0156513		*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	-1.54748
G1-1A_GL0036599	K04079				-1.37003
S-Fe10_GL0019271	K01840				-2.13766
10_GL0040485	K02939	*Bacteroidetes*			-1.86943
5-3_GL0063901	K02926	*Firmicutes*			3.584227
7-4_GL0052881	K01692	*Firmicutes*	*Eubacteriaceae*		1.586671
G1-1A_GL0063272	K03561	*Bacteroidetes*			-7.86086
MC-6-2_GL0131450					-2.9903
MC-0-1_GL0041841					-1.40804

10_GL0077662		*Bacteroidetes*			-2.05579
S-Fe10_GL0115270	K02355	*Bacteroidetes*	*Prevotellaceae*		-3.73129
S-Fe10_GL0037919	K00239	*Bacteroidetes*			-2.014
6-6_GL0089075	K00634	*Firmicutes*			-1.84592
G1-1A_GL0002591	K00024	*Bacteroidetes*			-2.4502
4-1_GL0061743	K02390	*Firmicutes*			2.381715
23_GL0148044	K03521				-1.6489
2-1_GL0005243		*Firmicutes*			2.812222
S-Fe10_GL0019265	K03545				-1.34255
10_GL0081639	K02992	*Firmicutes*	*Erysipelotrichaceae*		-1.80243
10_GL0048174	K00849	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.45056
G1-2A_GL0094277	K01960	*Bacteroidetes*			-1.95246
G1-5A_GL0010513	K02864	*Firmicutes*			1.857534
S-Fe10_GL0115243	K02986	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.36806
2-6_GL0057011	K10546	*Firmicutes*			1.620825
S-Fe10_GL0141735	K02897	*Bacteroidetes*			-3.14252
10_GL0076221	K01999	*Firmicutes*	*Erysipelotrichaceae*		-2.54476
G1-1A_GL0117380	K00053	*Bacteroidetes*			-1.66318
MC-6-2_GL0154047		*Firmicutes*			-3.0948
MC-0-3_GL0061849	K01270	*Bacteroidetes*			-1.85367
G1-1A_GL0002605		*Bacteroidetes*			-3.47662
1-1_GL0011264		*Firmicutes*			1.699679
G1-1A_GL0045772					-2.53418
G1-1A_GL0024866		*Bacteroidetes*			-3.02587
MC-6-2_GL0059497	K03040	*Bacteroidetes*			-4.04656
2-4_GL0011825	K02926	*Firmicutes*			2.068105
MC-6-1_GL0010304	K01868	*Bacteroidetes*			-1.94845
1-7_GL0103248	K02867	*Bacteroidetes*			-3.07331
Group2-8A_GL0004149	K02946	*Firmicutes*	*Lachnospiraceae*	*Roseburia*	1.533192
G1-5A_GL0017878	K02906	*Firmicutes*			2.543734
26_GL0113988	K02864	*Firmicutes*	*Lachnospiraceae*		2.979271
S-Fe10_GL0040204	K00962	*Bacteroidetes*			-1.89468
16_GL0057650	K04079	*Firmicutes*			-2.29055
Group2-5A_GL0126015	K00615	*Firmicutes*			-2.12805
G1-1A_GL0002549		*Bacteroidetes*			-1.81218
13_GL0003408	K01692	*Firmicutes*	*Erysipelotrichaceae*		-1.90295
G1-1A_GL0052804					-1.39328
G1-5A_GL0072934	K02994	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.972877
G1-1A_GL0096658	K02876	*Bacteroidetes*			-3.9128
8-1_GL0114127	K02931	*Firmicutes*			1.702373
10_GL0072244	K00975	*Firmicutes*	*Erysipelotrichaceae*		-1.4869
S-Fe10_GL0134063	K02935	*Bacteroidetes*			-1.50534

4-8_GL0047587	K02879	*Firmicutes*	*Lachnospiraceae*		1.767613
S-Fe10_GL0141664	K00831	*Bacteroidetes*	*Porphyromonadaceae*		-3.17408
3-5_GL0113614	K02952	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-1.60901
S-Fe10_GL0017083	K00024	*Bacteroidetes*			-2.19108
S-Fe10_GL0014799	K03286	*Bacteroidetes*			-1.85996
11_GL0061635	K01187				-1.32992
G1-1A_GL0045861	K01803	*Bacteroidetes*			-1.58373
11_GL0051301	K01676	*Bacteroidetes*			-1.70877
G1-2A_GL0087373	K01847	*Bacteroidetes*			-2.29747
1A-dyr2-07_GL0059448	K02906	*Firmicutes*	*Clostridiaceae*		1.729697
G1-4A_GL0020479	K06178	*Bacteroidetes*			-2.16451
G1-1A_GL0063273					-2.64993
MH-6-2_GL0008607					-3.59697
S-Fe5_GL0013685	K02874	*Firmicutes*			4.3513
2-3_GL0082942					-2.45771
1-3_GL0114313		*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	2.382794
10_GL0008437	K03521	*Firmicutes*	*Erysipelotrichaceae*		-1.98575
G1-5A_GL0152602	K02988	*Firmicutes*			2.552728
40_GL0031405	K01676	*Bacteroidetes*			-2.69886
MC-6-4_GL0004291		*Firmicutes*			1.365501
MC-6-2_GL0136446	K04072	*Firmicutes*			-1.71077
1-7_GL0120192	K01430	*Bacteroidetes*			-1.46325
S-Fe10_GL0099383		*Bacteroidetes*			-1.36968
G1-1A_GL0063130	K02939	*Bacteroidetes*			-2.60614
2-1_GL0001188		*Firmicutes*			-3.01492
MC-0-1_GL0006784		*Bacteroidetes*			2.027617
3-5_GL0078596	K01687	*Firmicutes*	*Lachnospiraceae*		-2.66318
S-Fe10_GL0010674	K04043	*Bacteroidetes*			-2.4321
S-Fe10_GL0002669	K02357	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.24658
5-6_GL0021409	K10540	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-2.19083
2-7_GL0101884	K02994	*Firmicutes*			2.166865
G1-1A_GL0045911	K03545				-1.89284
MC-0-3_GL0082686	K01270	*Bacteroidetes*			-2.36008
S-Fe1_GL0161902	K01966	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.60098
G1-1A_GL0006484		*Bacteroidetes*			-1.57835
4-1_GL0031938	K01834	*Firmicutes*			-1.86512
4-1_GL0001167	K02996	*Firmicutes*			1.922024
10_GL0100029	K02988	*Firmicutes*	*Erysipelotrichaceae*		-1.45616
10_GL0026071		*Bacteroidetes*			-1.65965
G1-1A_GL0052858	K02982	*Bacteroidetes*			-1.73799
S-Fe10_GL0153354	K00615	*Bacteroidetes*			-1.38845
8-5_GL0098490	K02992	*Firmicutes*			1.752953

10_GL0100036	K02879	*Firmicutes*	*Erysipelotrichaceae*		-2.19644
S-Fe10_GL0162733	K02939				-2.19479
1-2_GL0106703	K02994	*Firmicutes*			3.308722
S-Fe10_GL0103907	K14155				-1.44694
G1-1A_GL0096638	K02946	*Bacteroidetes*			-2.10027
1-7_GL0133815					-3.32975
10_GL0100024	K02931	*Firmicutes*	*Erysipelotrichaceae*		-1.89558
MC-6-1_GL0029232	K00174	*Bacteroidetes*			-1.36245
10_GL0059947	K01810	*Bacteroidetes*			-1.74524
G1-1A_GL0100772	K00058	*Bacteroidetes*			-1.83743
1-6_GL0082350	K02904	*Firmicutes*			2.457542
MC-0-3_GL0078316	K01960	*Bacteroidetes*			-3.48754
S-Fe10_GL0111933	K00600	*Bacteroidetes*			-2.33514
G1-1A_GL0115485	K01834	*Bacteroidetes*			-1.35549
50_GL0016700	K02871	*Firmicutes*			2.032558
1-7_GL0049412	K03671	*Bacteroidetes*			-1.71566
6-1_GL0032139	K02904	*Firmicutes*			2.089761
25_GL0005234	K02946	*Firmicutes*	*Lachnospiraceae*		1.628638
MH-6-1_GL0083500	K02982	*Firmicutes*			-2.56388
S-Fe10_GL0132208	K02078	*Bacteroidetes*			-2.18242
MC-0-1_GL0004951	K02939	*Bacteroidetes*			-1.41282
54_GL0089633	K02950	*Firmicutes*			3.228389
G1-5A_GL0200419	K02887	*Firmicutes*			4.785038
19_GL0036352	K00351	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.42785
S-Fe10_GL0072600	K02906	*Bacteroidetes*			-4.21898
MC-6-4_GL0029728	K03545				-2.07625
1-1_GL0029130	K02878	*Firmicutes*			1.788988
S-Fe11_GL0104098	K00239	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	1.62776
4-7_GL0055421	K10188	*Firmicutes*	*Lachnospiraceae*		-2.08526
1-1_GL0126894	K02111	*Bacteroidetes*	*Porphyromonadaceae*		-2.60573
S-Fe10_GL0140493	K02931	*Bacteroidetes*			-2.05418
11_GL0125895		*Bacteroidetes*			-1.48895
Group2-3A_GL0053830	K00616	*Firmicutes*			1.381255
1-4_GL0093240		*Firmicutes*			1.315237
MH-0-3_GL0066986	K02884	*Bacteroidetes*			-1.69731
MH-6-3_GL0058794	K02935	*Firmicutes*	*Ruminococcaceae*		-1.5191
MC-0-1_GL0010197					-1.65194
S-Fe10_GL0074772	K00240	*Bacteroidetes*			-1.33075
MC-6-1_GL0036083	K00645	*Bacteroidetes*			-1.7303
S-Fe10_GL0056930	K03046	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.60914
7-6_GL0077633	K02073	*Firmicutes*			4.145107
G1-1A_GL0045901	K01429	*Bacteroidetes*			-2.02272

Group2-8A_GL0208700		*Firmicutes*			2.799564
S-Fe10_GL0131407	K04564				-2.23564
MH-6-4_GL0059762		*Firmicutes*	*Clostridiaceae*		-1.63699
G1-1A_GL0068195	K02879	*Firmicutes*			1.859258
4-1_GL0026767	K02390	*Firmicutes*			-3.11701
S-Fe11_GL0076125	K03561	*Bacteroidetes*			-1.93624
4-1_GL0069176	K02933	*Firmicutes*			3.10505
S-Fe10_GL0056935	K02867	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.45507
10_GL0098134	K00024				-1.7458
MC-0-3_GL0097780	K00831	*Bacteroidetes*	*Porphyromonadaceae*		-1.60218
G1-1A_GL0048782		*Bacteroidetes*			-1.37301
7-8_GL0119582	K02895	*Firmicutes*			3.378165
3-1_GL0088265	K02935	*Firmicutes*			-4.66569
G1-1A_GL0063275	K03832	*Bacteroidetes*			-1.6218
G1-1A_GL0063132	K02990	*Bacteroidetes*	*Porphyromonadaceae*		-1.71828
G1-1A_GL0063367	K01881	*Bacteroidetes*			-1.88583
4-1_GL0029484		*Firmicutes*			-1.92703
10_GL0051759	K02954	*Bacteroidetes*			-1.92892
1-1_GL0018193	K02073	*Firmicutes*	*Lachnospiraceae*		2.653447
G1-2A_GL0031495	K02986	*Firmicutes*			-2.02661
S-Fe10_GL0105581		*Bacteroidetes*			-2.68925
10_GL0054688	K00634				-1.32973
MC-6-1_GL0046539	K02939	*Bacteroidetes*			-1.56415
G1-1A_GL0048809	K01955	*Bacteroidetes*			-1.7292
8-7_GL0069101	K02904	*Firmicutes*			2.22118
G1-1A_GL0116773	K02902	*Bacteroidetes*			-3.25915
6-2_GL0078653	K02968	*Firmicutes*	*Lachnospiraceae*	*Blautia*	3.254008
S-Fe10_GL0111950	K02959				-1.75712
G1-1A_GL0070524	K02954	*Bacteroidetes*			-1.52339
S-Fe10_GL0115249	K02876	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.35485
17_GL0034252	K02906	*Firmicutes*			1.822551
MC-6-2_GL0057904	K01989	*Firmicutes*	*Lachnospiraceae*		-2.14612
S-Fe10_GL0063313	K02959	*Bacteroidetes*	*Prevotellaceae*		-2.03772
MC-6-1_GL0108164	K02895	*Firmicutes*			1.715654
S-Fe10_GL0121845	K02355	*Bacteroidetes*			-2.74199
20_GL0076544	K02887	*Firmicutes*			1.519311
MC-6-3_GL0010464	K02950	*Bacteroidetes*			-2.26918
MC-6-2_GL0059817					-1.84871
S-Fe10_GL0007537	K01847	*Bacteroidetes*			-2.74557
S-Fe10_GL0037648	K02897	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.75001
Group2-3A_GL0016107		*Firmicutes*			1.726261
4-1_GL0069177	K02881	*Firmicutes*			1.484212

4-1_GL0023022	K02926	*Firmicutes*			-2.30326
1-4_GL0097472	K02990				5.200536
G1-1A_GL0045080		*Bacteroidetes*			-3.29656
2B-dyr24-06_GL0035335		*Bacteroidetes*			-2.81207
G1-1A_GL0063372	K00059	*Bacteroidetes*			-2.39216
4-1_GL0014462	K09766	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.663788
8-2_GL0059642	K02950	*Firmicutes*	*Lachnospiraceae*	*Coprococcus*	4.539434
MC-6-3_GL0102001	K02357	*Bacteroidetes*			-1.37577
10_GL0015146	K02926	*Firmicutes*			-2.09934
S-Fe10_GL0172458	K00024	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.12137
MH-6-2_GL0082800	K03522				-2.29833
G1-7A_GL0056105		*Firmicutes*			-2.97648
2-8_GL0057512	K04027	*Firmicutes*			-2.26826
35_GL0020817	K01999	*Firmicutes*			1.592618
10_GL0075363		*Bacteroidetes*			-2.09399
1-1_GL0147319	K02946	*Bacteroidetes*			-2.17265
Group2-3A_GL0189253	K02994	*Firmicutes*			2.237684
S-Fe10_GL0047040	K01810				-1.83966
10_GL0015145	K02906	*Firmicutes*			-1.94748
MC-0-1_GL0007440	K02881	*Bacteroidetes*			-2.30484
10_GL0066542	K02355	*Firmicutes*	*Erysipelotrichaceae*		-2.1053
S-Fe20_GL0041474	K00548	*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	-1.52931
4-1_GL0001159	K02879	*Firmicutes*			1.498791
S-Fe10_GL0027399		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.04231
2-4_GL0014223	K02887	*Firmicutes*			2.008272
S-Fe10_GL0177393	K03561	*Bacteroidetes*	*Prevotellaceae*		-1.63662
S-Fe10_GL0052542		*Bacteroidetes*			-1.30339
10_GL0051261	K00016	*Firmicutes*	*Erysipelotrichaceae*		-1.56618
4-1_GL0069165	K02892	*Firmicutes*			4.066519
S-Fe10_GL0072030	K00046	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.63681
G1-1A_GL0002586	K02864	*Bacteroidetes*			-2.20246
S-Fe10_GL0084623		*Bacteroidetes*			-1.68951
11_GL0019947	K02338	*Bacteroidetes*			-2.83652
16_GL0041024	K01193				-2.34185
10_GL0072245	K00975	*Firmicutes*	*Erysipelotrichaceae*		-1.54021
S-Fe10_GL0052490	K02990	*Bacteroidetes*			-4.05044
S-Fe12_GL0185247	K03561	*Bacteroidetes*			-11.5235
4-2_GL0012239	K02968	*Firmicutes*			-3.58106
G1-1A_GL0105421	K02372	*Bacteroidetes*			-1.48392
G1-1A_GL0105421	K02535	*Bacteroidetes*			-1.48392
S-Fe10_GL0115262	K02982	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.92975
S-Fe10_GL0123401					-4.34952

S-Fe10_GL0027400	K03561	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.67586
2B-dyr23-07_GL0015305	K01847				-1.67184
S-Fe10_GL0119226	K03545	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.00234
2-1_GL0042756	K02881	*Firmicutes*			1.631934
G1-2A_GL0100549		*Bacteroidetes*			-2.41711
2-1_GL0115705	K02990	*Firmicutes*			1.575593
S-Fe11_GL0079172	K00174	*Bacteroidetes*			-1.66628
3-5_GL0116071	K02884	*Firmicutes*			-1.38337
1A-dyr2-07_GL0012594	K09013	*Bacteroidetes*			-2.07952
S-Fe10_GL0115241	K02879	*Bacteroidetes*	*Prevotellaceae*		-2.64144
MC-6-2_GL0100409	K01939	*Bacteroidetes*			-1.74394
Group2-3A_GL0058594	K01023	*Firmicutes*			1.60725
1-7_GL0107205	K02954	*Bacteroidetes*	*Porphyromonadaceae*	*Porphyromonas*	-1.68409
S-Fe10_GL0044731		*Bacteroidetes*			-1.58294
S-Fe10_GL0113031	K01810	*Bacteroidetes*			-1.48607
10_GL0087733	K01923	*Bacteroidetes*	*Porphyromonadaceae*		-2.74687
MC-6-2_GL0096264	K02890	*Firmicutes*			1.594184
G1-1A_GL0052865	K02954	*Bacteroidetes*			-2.49255
6-2_GL0064228	K02956	*Firmicutes*			2.550064
46_GL0027227		*Bacteroidetes*			-3.02108
MC-0-1_GL0076876	K00831	*Bacteroidetes*	*Porphyromonadaceae*		-1.72141
MH-6-4_GL0060480	K02030	*Firmicutes*	*Ruminococcaceae*		2.127606
10_GL0084320	K00873	*Firmicutes*	*Erysipelotrichaceae*		-1.71018
G1-1A_GL0045905	K00257	*Bacteroidetes*			-2.3555
10_GL0053647	K08289				-1.66375
11_GL0078831		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.39438
S-Fe10_GL0067434		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-4.22264
1-7_GL0082305	K05808	*Bacteroidetes*			-2.85959
16_GL0086249	K03654	*Bacteroidetes*			-1.42405
10_GL0065600	K02906	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	1.720226
1-1_GL0144185		*Firmicutes*			-1.59752
S-Fe10_GL0099387					-1.77479
S-Fe10_GL0111945		*Bacteroidetes*			-2.4753
10_GL0036914	K03545	*Bacteroidetes*			-1.89827
10_GL0064646	K02990	*Firmicutes*			1.367366
MC-6-3_GL0022447		*Proteobacteria*			-1.91177
10_GL0005128		*Bacteroidetes*			-2.48761
G1-5A_GL0200745	K02030				3.106416
1-7_GL0129239	K02996	*Bacteroidetes*			-1.69917
4-8_GL0106017	K02959	*Firmicutes*			1.776019
MC-6-3_GL0060183	K02939	*Bacteroidetes*			-2.11458

S-Fe10_GL0133370	K02897	*Bacteroidetes*			-4.45879
Group2-8A_GL0119573	K01915	*Firmicutes*			-3.87463
S-Fe10_GL0176924		*Bacteroidetes*			-1.32529
10_GL0056520	K03531	*Firmicutes*	*Erysipelotrichaceae*		-2.03564
4-1_GL0058230					-1.43653
S-Fe8_GL0110898	K02027	*Firmicutes*	*Ruminococcaceae*		-2.12134
S-Fe10_GL0067698	K05606				-1.81548
4-1_GL0050292	K03612	*Firmicutes*			-1.77392
S-Fe10_GL0037907	K02078	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.76396
Group2-7A_GL0111332	K02356	*Firmicutes*			-2.35662
1-1_GL0060460		*Bacteroidetes*			-1.40528
10_GL0093910	K02356	*Firmicutes*	*Erysipelotrichaceae*		-2.70141
6-4_GL0077822	K09807	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.642456
2-3_GL0044613	K01689	*Firmicutes*			-2.00272
S-Fe10_GL0072615	K02933	*Bacteroidetes*			-1.66143
S-Fe10_GL0052371	K03781				-3.05871
10_GL0052822		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.63551
10_GL0067629	K01258				-2.6355
S-Fe10_GL0131019	K00024	*Bacteroidetes*			-3.10686
1A-dyr2-07_GL0044881	K03530				3.432277
5-2_GL0041388	K02968	*Firmicutes*	*Lachnospiraceae*	*Blautia*	1.45773
S-Fe6_GL0224554	K03640	*Proteobacteria*	*Desulfovibrionaceae*		1.560198
5-6_GL0042529	K10439	*Firmicutes*	*Lachnospiraceae*		-1.72595
MH-0-4_GL0030997	K00024	*Bacteroidetes*			-1.30498
S-Fe10_GL0072601	K02926				-1.44302
6-4_GL0028060	K02029	*Firmicutes*			2.846784
6-4_GL0028060	K02030	*Firmicutes*			2.846784
20_GL0061686	K02939	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	-1.89763
7-5_GL0129618	K01181	*Firmicutes*			3.03298
2A-dyr14-07_GL0014020	K03737	*Firmicutes*			-2.16392
S-Fe10_GL0168644	K02892	*Bacteroidetes*			-1.7343
10_GL0058370	K06142	*Bacteroidetes*			-1.3754
S-Fe10_GL0135010		*Bacteroidetes*			-3.044
29_GL0036836	K02892	*Firmicutes*			4.589856
7-1_GL0047873	K02990	*Firmicutes*			2.935681
1-1_GL0002356	K01786	*Firmicutes*	*Lachnospiraceae*		3.5639
MH-6-1_GL0035960	K01999	*Firmicutes*			-1.37275
10_GL0019366	K00625	*Firmicutes*	*Erysipelotrichaceae*		-3.23152
16_GL0085831	K00927	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	-1.75339
MC-6-5_GL0091603	K02863	*Firmicutes*			-2.89679
3-3_GL0045971	K02887	*Firmicutes*			2.371878

S-Fe2_GL0138212	K02933	*Firmicutes*			1.481238
S-Fe10_GL0037967		*Bacteroidetes*			-1.8309
2-1_GL0062630	K03530	*Firmicutes*			-6.7709
S-Fe10_GL0005474		*Bacteroidetes*			-1.87886
3-5_GL0006184	K01808	*Firmicutes*			-1.38815
G1-1A_GL0104455	K00705	*Bacteroidetes*			-4.91277
S-Fe1_GL0019189	K01625	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.32004
S-Fe11_GL0118695		*Bacteroidetes*			-1.76217
10_GL0100020	K02904	*Firmicutes*			-3.25723
S-Fe10_GL0175198		*Bacteroidetes*			-3.04744
20_GL0144360	K05878	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.55256
10_GL0018184		*Bacteroidetes*			-2.41511
MH-6-2_GL0054493	K01809	*Bacteroidetes*			-2.05669
S-Fe10_GL0121839	K02939	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.65629
G1-1A_GL0096641	K02892	*Bacteroidetes*			-1.84865
2-2_GL0125121	K02895	*Firmicutes*			-2.75499
10_GL0044214	K02884	*Firmicutes*			-2.62114
10_GL0029189		*Bacteroidetes*			-2.92523
S-Fe12_GL0009091	K02358				-2.61252
2-1_GL0091421	K10189	*Firmicutes*			1.998795
S-Fe10_GL0006965		*Bacteroidetes*			-3.23994
G1-1A_GL0036616	K00845	*Bacteroidetes*			-1.47948
4-3_GL0040585	K02887	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.984859
10_GL0066543	K06867	*Firmicutes*	*Erysipelotrichaceae*		-1.88801
Group2-3A_GL0221462	K02030	*Firmicutes*	*Lachnospiraceae*		2.682661
Group2-3A_GL0104109	K07567	*Firmicutes*			3.433609
16_GL0025420	K03072	*Bacteroidetes*			-1.84751
4-1_GL0015208	K02888	*Firmicutes*			1.853391
7-8_GL0007091	K02946	*Firmicutes*	*Ruminococcaceae*		2.132998
16_GL0069107		*Bacteroidetes*			-1.47675
S-Fe10_GL0029121	K02871	*Bacteroidetes*			-3.12703
S-Fe10_GL0100074	K03773	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.27508
S-Fe10_GL0072031	K03530	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.46146
10_GL0063070	K02073	*Firmicutes*			-1.55626
S-Fe10_GL0023187	K01676	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.99675
MC-0-1_GL0055007		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.30484
13_GL0093821	K01915				-1.65475
16_GL0065987	K02950	*Bacteroidetes*			-2.64039
S-Fe10_GL0115253	K02933	*Bacteroidetes*			-3.22817
MC-6-4_GL0092805		*Bacteroidetes*			-1.41856
1-1_GL0033955	K07567	*Firmicutes*			1.787713
2-8_GL0055633	K06412	*Firmicutes*			-1.38343

1-8_GL0021952	K02968	*Firmicutes*			2.22954
1-2_GL0051974	K02879	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	3.25422
1-1_GL0049767	K01264	*Firmicutes*			5.102039
S-Fe10_GL0091456	K02871	*Bacteroidetes*	*Rikenellaceae*		2.466423
G1-1A_GL0096550	K00241	*Bacteroidetes*			-1.84726
MC-6-2_GL0099681	K05606				-1.92019
2-3_GL0033567	K02876	*Firmicutes*			2.556287
G1-6A_GL0076191	K02887	*Firmicutes*			1.954811
23_GL0065500	K03568				2.834512
S-Fe10_GL0148991		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.01685
7-7_GL0027934		*Firmicutes*			1.920026
2-2_GL0023527	K02968	*Firmicutes*			2.035682
MC-6-2_GL0167139	K03210	*Bacteroidetes*			-1.60108
S-Fe10_GL0013552	K09704	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.68681
S-Fe10_GL0045711		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.33719
S-Fe10_GL0129314					-3.02457
MC-6-3_GL0007858		*Bacteroidetes*			-3.36014
MC-0-1_GL0010195	K03832	*Bacteroidetes*			-2.53364
S-Fe10_GL0029120	K02996	*Bacteroidetes*			-2.35704
MH-0-5_GL0034634	K01960				-2.18928
S-Fe1_GL0124371	K07568	*Bacteroidetes*			-2.69859
MC-6-1_GL0056899					-1.45047
11_GL0007781	K02970	*Firmicutes*	*Erysipelotrichaceae*		-1.87621
S-Fe10_GL0044726	K03671	*Bacteroidetes*			-3.05907
G1-5A_GL0109919	K02959	*Firmicutes*			1.933695
19_GL0028970		*Bacteroidetes*			-2.2475
S-Fe15_GL0069002		*Bacteroidetes*			1.587806
10_GL0025599	K07335	*Firmicutes*			-1.61072
7-5_GL0044016	K02887	*Firmicutes*			1.317258
G1-1A_GL0036828		*Bacteroidetes*			-5.18178
S-Fe11_GL0026047	K02970	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.56978
S-Fe10_GL0164970	K02968	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.94861
MH-6-2_GL0007875		*Bacteroidetes*			-3.74993
10_GL0000279	K03832	*Bacteroidetes*			-2.72995
Group2-3A_GL0139093	K02406	*Firmicutes*			-1.49161
S-Fe10_GL0052380		*Bacteroidetes*			-3.8013
S-Fe10_GL0157546	K03149	*Bacteroidetes*			-2.51684
6-8_GL0030038	K01673				1.67363
S-Fe9_GL0062172		*Firmicutes*			-2.71287
MC-0-1_GL0051482	K02939	*Bacteroidetes*			-2.5911
S-Fe13_GL0060889		*Firmicutes*			-1.34504
Group2-7A_GL0148101	K00645	*Firmicutes*			6.873356

1A-dyr1-07_GL0049019	K04077	*Firmicutes*			-1.35481
1A-dyr4-07_GL0023556	K04077	*Firmicutes*			-1.55086
1-1_GL0026923	K00134	*Firmicutes*			-1.84233
MH-6-4_GL0066872		*Firmicutes*			1.484922
3-2_GL0036211		*Firmicutes*			2.779875
2-1_GL0122108	K00257	*Firmicutes*			-1.8809
MC-0-3_GL0067315	K02945	*Bacteroidetes*			-1.97106
S-Fe8_GL0238271	K01624	*Firmicutes*	*Lachnospiraceae*		-2.15549
20_GL0057733	K02355	*Bacteroidetes*	*Rikenellaceae*		-1.314
MC-6-2_GL0082232		*Firmicutes*			-2.35985
G1-3A_GL0068368	K01624				-1.32387
S-Fe10_GL0111947	K00927	*Bacteroidetes*			-1.80746
11_GL0156489	K01624				-2.39002
4-3_GL0071645	K02871	*Firmicutes*	*Lachnospiraceae*		1.500465
1-1_GL0041609	K01209	*Firmicutes*			1.534417
MH-6-1_GL0107655	K04043	*Firmicutes*			-1.71622
16_GL0037009	K00059	*Bacteroidetes*			-1.78529
MC-0-1_GL0040326	K01803	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	1.790229
11_GL0135114	K01840	*Bacteroidetes*			-2.15347
MH-0-5_GL0091066	K00927	*Bacteroidetes*			-3.92844
S-Fe9_GL0100099	K02884	*Bacteroidetes*			-1.43701
S-Fe11_GL0142616		*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.802851
7-5_GL0112795	K03040	*Firmicutes*			-2.53523
4-2_GL0042526	K02996	*Firmicutes*			1.733698
2-3_GL0100459	K06001	*Firmicutes*			-2.44477
MC-6-4_GL0029726	K03544				-2.16231
1-7_GL0060745	K02357	*Firmicutes*			-2.29837
S-Fe7_GL0237870	K02945	*Firmicutes*			-1.73871
2-2_GL0006657	K03530	*Firmicutes*			-3.21226
5-3_GL0020715	K06942	*Firmicutes*			-2.10118
G1-1A_GL0096649	K02874	*Bacteroidetes*			-2.86167
S-Fe8_GL0274868	K02909				-1.4597
1-1_GL0112731	K03522				-1.83193
14_GL0042288	K02986	*Firmicutes*			1.38925
S-Fe9_GL0035903	K02926	*Firmicutes*			1.97226
2-1_GL0086540	K03695	*Firmicutes*			-1.51598
S-Fe11_GL0057852	K01840				-1.30799
S-Fe10_GL0057287	K00053	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.24696
S-Fe11_GL0039770	K07405	*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	-2.279
10_GL0075253		*Bacteroidetes*			-1.68262
G1-1A_GL0045114	K03530	*Bacteroidetes*	*Porphyromonadaceae*		-3.41022
6-5_GL0069771	K00600	*Firmicutes*			-2.30384

4-1_GL0010452	K06215				-1.73333
G1-2A_GL0077791		*Bacteroidetes*			-2.54344
7-5_GL0044018	K02520	*Firmicutes*			-2.23244
8-8_GL0036271	K01937	*Firmicutes*			-1.8062
2-2_GL0024478	K02988				2.850875
G1-1A_GL0036607					-1.68813
MC-6-3_GL0017680	K02056	*Firmicutes*	*Lachnospiraceae*		-1.7276
S-Fe10_GL0043422					-2.02619
S-Fe9_GL0212131	K02884	*Bacteroidetes*			-4.14634
MH-0-3_GL0009473	K09903	*Bacteroidetes*			-2.00302
S-Fe3_GL0148328	K02879	*Firmicutes*			3.487765
MC-0-1_GL0020539	K02907	*Bacteroidetes*			-1.59317
S-Fe10_GL0035436	K02437	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.41454
1-1_GL0073973	K02879	*Firmicutes*			1.712657
S-Fe5_GL0061544	K02109	*Bacteroidetes*	*Porphyromonadaceae*		-1.46488
1-1_GL0130106	K03595	*Bacteroidetes*			-2.11583
8-3_GL0029113	K02956	*Firmicutes*			-1.89663
S-Fe11_GL0017742		*Firmicutes*			1.775364
4-1_GL0029227	K02601	*Firmicutes*			-1.41524

Appendix 12. List of significantly changed microbial proteins between DR and BR groups.

Accession	**KO**	**Phylum**	**Family**	**Genus**	**Pi score**
MC-6-3_GL0050426		*Bacteroidetes*			-2.23787
MC-0-3_GL0028301	K03737	*Bacteroidetes*			-1.57307
7-4_GL0117967	K01006	*Firmicutes*			-1.31222
4-7_GL0142597	K01006	*Firmicutes*			2.428611
G1-1A_GL0075950	K01006				-2.16871
1-1_GL0135104	K01610	*Firmicutes*			-1.7923
19_GL0073930	K01006				-2.13116
1-4_GL0025198	K04077	*Firmicutes*			2.345535
1-6_GL0039171	K02355	*Firmicutes*			-2.1563
G1-1A_GL0058894	K04043	*Bacteroidetes*			-2.13279
MC-0-1_GL0026950	K01689	*Bacteroidetes*			-2.10577
S-Fe10_GL0170141	K01689				-2.75066
G1-1A_GL0096667	K01689				-3.09267
G1-1A_GL0093263	K01610				-2.87123
MC-6-3_GL0041016	K02358	*Bacteroidetes*			-1.74094
MC-0-1_GL0020527	K01689	*Bacteroidetes*			-1.45945

Group2-2A_GL0145990	K02358	*Bacteroidetes*			-3.54974
MC-0-1_GL0058727	K01610				-2.05677
13_GL0002115	K03737	*Bacteroidetes*			-1.98487
S-Fe10_GL0005531	K01006				-3.18854
10_GL0039950	K02358	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.63558
MC-0-3_GL0015481	K01006				-2.12998
8-1_GL0017543	K02406	*Firmicutes*			-1.77306
11_GL0002095	K02358	*Bacteroidetes*			-1.46092
13_GL0016888	K01689				-2.37078
10_GL0038582	K01689				-2.10075
S-Fe10_GL0163207	K01805				-2.5732
G1-1A_GL0024937	K00134	*Bacteroidetes*			-4.22946
13_GL0061039	K04043	*Bacteroidetes*			-3.73202
23_GL0060768	K02406	*Firmicutes*	*Clostridiaceae*		3.309541
G1-1A_GL0005544	K00134				-1.87037
Group2-3A_GL0096287	K02406	*Firmicutes*			-3.85495
G1-1A_GL0024446	K01805	*Bacteroidetes*			-3.30133
S-Fe10_GL0065079	K02358				-2.66025
G1-1A_GL0096517	K03737	*Bacteroidetes*			-2.24531
S-Fe10_GL0031497	K00134				-1.80975
MH-0-4_GL0088579	K02355	*Bacteroidetes*	*Porphyromonadaceae*		-1.9578
S-Fe10_GL0040650	K01610				-1.4488
MC-0-1_GL0109909	K01805	*Bacteroidetes*			-1.84629
S-Fe10_GL0064044	K01610				-2.46143
S-Fe10_GL0164975	K01610	*Firmicutes*			-2.79284
G1-1A_GL0036619	K04043	*Bacteroidetes*			-1.72667
S-Fe13_GL0047507	K01610				-2.35407
MC-6-1_GL0018608	K00688	*Bacteroidetes*			-2.20466
10_GL0009545	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.574065
G1-1A_GL0063304	K04077	*Bacteroidetes*			-5.86194
S-Fe10_GL0056937	K02358	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-8.39444
MC-0-1_GL0054811	K00688	*Bacteroidetes*			-1.6967
G1-1A_GL0096460		*Bacteroidetes*			-2.53024
S-Fe10_GL0072598	K02355	*Bacteroidetes*	*Porphyromonadaceae*		-1.48493
1A-dyr3-06_GL0038646	K00134	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.63306
G1-1A_GL0096553	K00615				-2.35465
6-3_GL0000806	K02406	*Firmicutes*			8.401043
S-Fe10_GL0153182	K03737	*Bacteroidetes*			-5.55209
11_GL0051937	K01610	*Bacteroidetes*			-1.66907
11_GL0138839	K02355	*Bacteroidetes*			-1.35352
S-Fe10_GL0048913	K03737	*Bacteroidetes*			-1.71919

MC-6-2_GL0053309	K00053	*Firmicutes*			-1.31293
MC-6-1_GL0101142	K02945	*Bacteroidetes*			-2.86253
MH-6-5_GL0039873	K02406	*Firmicutes*			-2.11444
G1-1A_GL0002609		*Bacteroidetes*			-1.76997
10_GL0000502	K01610				-3.2578
S-Fe10_GL0145465					-2.35361
G1-1A_GL0002606		*Bacteroidetes*			-2.02193
G1-1A_GL0021750	K02945	*Bacteroidetes*			-3.33373
5-4_GL0124135		*Firmicutes*	*Lachnospiraceae*		-4.83715
Group2-8A_GL0235603	K03737	*Firmicutes*			1.578097
2-1_GL0065293	K02406	*Firmicutes*			5.935898
49_GL0015734	K00134	*Bacteroidetes*			-1.79833
S-Fe10_GL0071308	K04077	*Bacteroidetes*			-3.96914
10_GL0015619	K02406				-2.4056
S-Fe10_GL0011383		*Bacteroidetes*			-2.08918
1A-dyr2-07_GL0049869	K04077	*Deferribacteres*			-1.62561
S-Fe12_GL0026793	K03695	*Bacteroidetes*			-2.40855
S-Fe10_GL0044720	K00688				-2.02261
G1-7A_GL0076892	K01006	*Firmicutes*			-2.27138
S-Fe11_GL0060366	K02945	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.14996
G1-1A_GL0075913	K00262	*Bacteroidetes*			-2.56148
1-1_GL0012052					1.813601
S-Fe10_GL0055365		*Bacteroidetes*			-2.31459
MC-6-2_GL0031878	K00927	*Bacteroidetes*			-2.32218
14_GL0022104	K01938	*Firmicutes*			1.638627
S-Fe10_GL0045753	K00134	*Bacteroidetes*			-7.56273
3-1_GL0094445	K02117	*Firmicutes*			1.925155
S-Fe10_GL0165683		*Bacteroidetes*			-1.90523
1-5_GL0011625		*Firmicutes*			2.091514
20_GL0047623	K00134	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-2.41702
2-2_GL0040883	K00197	*Firmicutes*			2.305698
MC-6-2_GL0081187	K01624				-1.55424
S-Fe1_GL0132619	K07263	*Bacteroidetes*			-1.4059
MC-6-2_GL0121460	K00895	*Bacteroidetes*			-1.78581
G1-1A_GL0045828	K00927	*Bacteroidetes*			-2.92807
32_GL0079060	K01610	*Firmicutes*			-1.45947
MC-0-1_GL0026114		*Bacteroidetes*			-2.23956
MC-6-1_GL0049904	K00615	*Bacteroidetes*			-1.56416
G1-1A_GL0110280	K01810				-2.19519
S-Fe10_GL0048936		*Proteobacteria*			-2.13534
G1-1A_GL0021808		*Bacteroidetes*			-3.84036
MC-0-3_GL0021340	K07749				-1.43539

5-6_GL0053399	K00626	*Firmicutes*			2.37411
G1-1A_GL0052880	K02945	*Bacteroidetes*			-2.16034
S-Fe10_GL0021215		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.76642
MC-6-1_GL0019122	K01810				-1.34845
G1-1A_GL0045900	K01428	*Bacteroidetes*			-3.70971
S-Fe10_GL0080771	K01805	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.65219
2-2_GL0069397	K00257	*Firmicutes*			3.334838
MH-0-4_GL0042921	K00197	*Firmicutes*			2.113207
G1-1A_GL0002607		*Bacteroidetes*			-2.9265
1-1_GL0095178	K02358	*Firmicutes*			-1.30736
S-Fe10_GL0170082		*Bacteroidetes*			-2.94921
MC-0-3_GL0036686	K02935	*Bacteroidetes*			-2.00666
14_GL0016880	K02406	*Firmicutes*			2.651154
19_GL0076428	K04043	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.42972
1-1_GL0134047		*Firmicutes*			1.612984
Group2-3A_GL0115130		*Firmicutes*	*Lachnospiraceae*		1.95095
S-Fe10_GL0076983	K00024	*Bacteroidetes*			-2.37207
47_GL0091423	K00198	*Firmicutes*			1.322693
MC-0-1_GL0058729	K00024	*Bacteroidetes*			-2.11275
13_GL0007779					-2.52476
S-Fe10_GL0056454	K01006	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.86815
G1-7A_GL0064510	K02358	*Firmicutes*			-1.7403
S-Fe10_GL0018625	K01804	*Bacteroidetes*			-1.56293
20_GL0146153	K02935	*Bacteroidetes*			-3.04027
G1-1A_GL0021765	K01624				-2.03606
G1-1A_GL0100771	K00831	*Bacteroidetes*	*Porphyromonadaceae*		-1.66939
G1-1A_GL0006267		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.86919
1-1_GL0110149	K04077	*Bacteroidetes*			-1.44303
G1-5A_GL0170455	K10112	*Firmicutes*			-3.62965
G1-1A_GL0110315	K01804	*Bacteroidetes*			-3.24566
MC-6-5_GL0110495		*Bacteroidetes*			-1.87971
1-1_GL0060052	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.941057
S-Fe10_GL0052143	K02358	*Firmicutes*			-1.32975
MC-0-1_GL0021435	K02863				-1.61744
S-Fe8_GL0098677	K00197	*Firmicutes*			1.794158
2A-dyr14-07_GL0037894	K00123	*Proteobacteria*			-1.67215
S-Fe11_GL0087929	K04043	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.45952
1-7_GL0118751	K02897	*Bacteroidetes*	*Porphyromonadaceae*		-2.19809
G1-1A_GL0025052	K03772				-2.19611
1-1_GL0085766	K02863				-2.27005
1-3_GL0011727		*Firmicutes*			3.462633
17_GL0058712		*Firmicutes*	*Lachnospiraceae*		2.134231

10_GL0007818	K02863				-2.65491
1A-dyr2-07_GL0013677	K00134	*Deferribacteres*			-2.15221
MC-0-3_GL0008155	K03070				-2.23879
13_GL0049230	K00895	*Bacteroidetes*			-1.60808
5-1_GL0048961					1.758879
S-Fe10_GL0035419					-2.20204
G1-8A_GL0119389	K00927	*Firmicutes*			-1.41738
10_GL0100485	K04077	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.52418
MC-0-1_GL0066909		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.32759
S-Fe10_GL0104485	K02986	*Bacteroidetes*			-2.08876
MH-0-3_GL0084717	K02967	*Bacteroidetes*			-1.5091
32_GL0097271	K02406	*Firmicutes*			4.084952
MC-6-1_GL0052341	K02986	*Bacteroidetes*			-1.71274
Group2-8A_GL0169991		*Firmicutes*			2.098992
Group2-8A_GL0130987		*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.759478
G1-1A_GL0096461		*Bacteroidetes*			-2.42625
10_GL0009190	K10112	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-1.75767
1-5_GL0006008	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.967344
G1-1A_GL0063364					-3.24583
10_GL0055437	K00239	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.5937
S-Fe10_GL0160603	K00927	*Bacteroidetes*			-1.98747
2-1_GL0071086	K03521	*Firmicutes*	*Clostridiaceae*		1.760599
MC-6-1_GL0003793	K00831	*Bacteroidetes*	*Porphyromonadaceae*		-1.45823
S-Fe10_GL0059024		*Bacteroidetes*			-3.12668
1-1_GL0010867	K02967	*Bacteroidetes*			-1.80004
G1-1A_GL0117677		*Bacteroidetes*			-3.24486
20_GL0109361	K02406	*Firmicutes*			-1.43367
MC-0-1_GL0068998	K02112	*Bacteroidetes*			-1.57972
5-6_GL0099235	K00194	*Firmicutes*			-1.6194
MC-0-1_GL0060608	K02876	*Bacteroidetes*			-2.3008
MH-6-2_GL0099282	K01624	*Bacteroidetes*			-1.95834
S-Fe10_GL0020332		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.42665
G1-1A_GL0093277	K01858	*Bacteroidetes*			-2.91994
S-Fe11_GL0122500	K00262	*Bacteroidetes*			-1.40933
S-Fe10_GL0073084	K00615	*Bacteroidetes*			-1.80111
MC-0-1_GL0066912		*Bacteroidetes*			-1.65335
S-Fe10_GL0056933	K02864	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.605
10_GL0098958		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.38901
S-Fe10_GL0044469	K01624	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.2876
MC-6-2_GL0179977		*Bacteroidetes*			-1.87605
G1-1A_GL0048786	K00024	*Bacteroidetes*			-2.82846

G1-1A_GL0021777	K00895	*Bacteroidetes*			-3.82733
S-Fe1_GL0146005	K02967	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.03129
S-Fe10_GL0145991		*Bacteroidetes*			-2.16023
G1-1A_GL0045858		*Bacteroidetes*			-2.68564
G1-1A_GL0096610	K01676	*Bacteroidetes*	*Porphyromonadaceae*		-2.2877
G1-1A_GL0096567	K02967	*Bacteroidetes*			-2.74731
1B-dyr12-07_GL0026739	K02931	*Bacteroidetes*			-1.45237
1A-dyr2-07_GL0080061					-1.84877
S-Fe10_GL0141770	K03286	*Bacteroidetes*			-2.84241
7-1_GL0083574	K03046	*Bacteroidetes*			-3.37449
1A-dyr4-07_GL0079487	K01915	*Firmicutes*	*Lachnospiraceae*	*Blautia*	-1.46211
MC-6-1_GL0007984	K02886	*Bacteroidetes*			-2.49757
1-7_GL0013213	K02992	*Bacteroidetes*			-1.62152
17_GL0026920	K00024	*Bacteroidetes*			-1.53668
G1-1A_GL0034771	K02864	*Bacteroidetes*			-1.62136
G1-1A_GL0045950	K01847				-1.91412
S-Fe10_GL0127874	K07749				-1.54044
MC-0-1_GL0101102	K01610				-3.33256
MC-6-3_GL0079252	K04077	*Bacteroidetes*	*Rikenellaceae*		-2.63795
1-1_GL0139225	K01966	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-2.0727
S-Fe10_GL0039362	K00927	*Bacteroidetes*	*Prevotellaceae*		-4.55375
MC-6-3_GL0091839	K02863				-2.78629
MC-0-3_GL0030050	K02897	*Bacteroidetes*	*Porphyromonadaceae*		-1.69389
11_GL0131163		*Bacteroidetes*			-1.4718
S-Fe10_GL0024129	K00927	*Bacteroidetes*			-1.68101
S-Fe2_GL0013443	K02355	*Firmicutes*			1.55467
S-Fe6_GL0098161	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.58909
G1-7A_GL0120537		*Firmicutes*			-2.02009
S-Fe11_GL0075845	K00927	*Bacteroidetes*			-4.27993
S-Fe10_GL0029731	K02945	*Bacteroidetes*			-1.833
3-1_GL0015551	K02935	*Bacteroidetes*			-3.0857
S-Fe15_GL0056677	K06871	*Bacteroidetes*			-1.64925
7-2_GL0064396	K02906	*Firmicutes*			-1.81067
S-Fe10_GL0067617	K02945	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.60894
S-Fe10_GL0045789	K04077	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.39749
S-Fe10_GL0047359		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.14601
S-Fe10_GL0123288	K01966				-1.51282
S-Fe10_GL0038718	K02967	*Bacteroidetes*	*Prevotellaceae*		-3.42377
S-Fe3_GL0182184	K02406	*Firmicutes*			-3.77715
G1-1A_GL0045137		*Bacteroidetes*			-2.49509
S-Fe10_GL0072597	K02992	*Bacteroidetes*	*Porphyromonadaceae*		-1.5238

S-Fe10_GL0176063		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.37823
35_GL0101043		*Firmicutes*			2.301074
10_GL0039955	K02935	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.60472
G1-1A_GL0096551	K00239	*Bacteroidetes*			-2.0328
MC-0-3_GL0006425					-1.74856
S-Fe10_GL0119255		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.39131
S-Fe10_GL0021247		*Proteobacteria*			-2.10986
S-Fe8_GL0281318	K02358	*Proteobacteria*	*Desulfovibrionaceae*		2.114147
37_GL0062309	K02406				-2.3443
10_GL0056350	K03040				-1.63197
S-Fe10_GL0087175	K01624				-3.58151
1-1_GL0116983	K01692	*Firmicutes*	*Eubacteriaceae*		1.844766
Group2-2A_GL0084879	K02863	*Firmicutes*			1.543154
G1-5A_GL0056962		*Firmicutes*			-1.32401
1-8_GL0029631	K02988	*Firmicutes*			1.602558
1-1_GL0083995	K02863	*Firmicutes*			1.490748
1-1_GL0067006	K01858	*Bacteroidetes*			-2.26713
10_GL0056471	K00850	*Bacteroidetes*			-1.33932
7-5_GL0112994	K00831	*Firmicutes*			1.624485
1A-dyr1-07_GL0010312	K02935	*Firmicutes*			1.830072
10_GL0067957	K00346	*Bacteroidetes*			-1.41489
S-Fe10_GL0056932	K02935	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-4.90358
MC-0-1_GL0034575	K02864	*Bacteroidetes*			-2.16822
S-Fe10_GL0115271	K02992	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.77846
10_GL0019534	K02931	*Bacteroidetes*			-1.54651
S-Fe10_GL0110880	K03286	*Bacteroidetes*			-1.81342
MC-0-3_GL0067921	K03545				-1.95622
S-Fe10_GL0135266		*Bacteroidetes*			-1.46961
MC-0-1_GL0050046	K01610	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	1.350398
S-Fe10_GL0161840	K04043	*Bacteroidetes*			-2.35021
MH-6-3_GL0099998	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	6.737121
G1-4A_GL0052229	K02982	*Bacteroidetes*			-1.45509
G1-1A_GL0100781	K07405	*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	-2.20931
MC-6-5_GL0002297	K02935	*Bacteroidetes*			-2.00423
MH-6-2_GL0008675	K02931	*Firmicutes*			1.694622
1-7_GL0088542	K02982	*Bacteroidetes*			-2.56887
1-1_GL0137633	K02027				2.496851
16_GL0002435		*Bacteroidetes*			-1.52164
G1-7A_GL0098845	K02886	*Firmicutes*	*Eubacteriaceae*		-2.6343
5-3_GL0063901	K02926	*Firmicutes*			1.33083
G1-1A_GL0063272	K03561	*Bacteroidetes*			-5.0483
5-8_GL0086146	K10670	*Firmicutes*			1.668949

G1-1A_GL0096665	K03040	*Bacteroidetes*			-3.05966
S-Fe10_GL0115270	K02355	*Bacteroidetes*	*Prevotellaceae*		-5.21499
S-Fe10_GL0068604					-1.55086
S-Fe10_GL0037919	K00239	*Bacteroidetes*			-1.83481
G1-1A_GL0105385		*Bacteroidetes*			-1.55457
1-1_GL0017917	K02959	*Bacteroidetes*			-2.25657
1-1_GL0140275	K02994	*Firmicutes*			-2.6377
G1-1A_GL0002591	K00024	*Bacteroidetes*			-3.38243
MC-6-4_GL0117996	K02867	*Bacteroidetes*			-1.80991
6-6_GL0125564	K02945	*Firmicutes*	*Lachnospiraceae*		1.630381
S-Fe10_GL0019265	K03545				-2.54607
13_GL0070994	K01840				-2.42864
S-Fe1_GL0132960	K01676				-2.3848
MC-6-4_GL0004321	K03406				1.942357
1A-dyr1-07_GL0007965	K03040				-1.78317
S-Fe10_GL0130323	K01858	*Bacteroidetes*			-1.3276
S-Fe10_GL0115243	K02986	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.05881
31_GL0069938	K02992	*Bacteroidetes*			-2.55912
S-Fe10_GL0072603	K02886				-2.16998
10_GL0077832	K07749				-1.47028
S-Fe10_GL0141735	K02897	*Bacteroidetes*			-2.21626
7-4_GL0080804	K01818	*Firmicutes*	*Lachnospiraceae*	*Blautia*	-1.55887
6-5_GL0056514	K01999	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.48049
13_GL0071923					-2.09477
MC-0-1_GL0071083		*Bacteroidetes*			-2.32767
MC-0-3_GL0061849	K01270	*Bacteroidetes*			-1.76547
MH-0-5_GL0078202	K01847	*Bacteroidetes*			-2.01559
G1-1A_GL0002605		*Bacteroidetes*			-2.24468
G1-1A_GL0045772					-2.10182
G1-1A_GL0024866		*Bacteroidetes*			-2.92806
Group2-8A_GL0148965		*Firmicutes*			1.347767
S-Fe10_GL0123268	K01428	*Bacteroidetes*			-2.36953
MC-6-2_GL0006785	K01803	*Bacteroidetes*			-1.36101
1-7_GL0103248	K02867	*Bacteroidetes*			-1.91852
1-2_GL0077661	K02357	*Firmicutes*	*Lachnospiraceae*		2.515348
MC-6-1_GL0091518	K02078	*Bacteroidetes*			-2.12366
G1-1A_GL0083453	K02906	*Bacteroidetes*			-2.30689
1A-dyr2-07_GL0017615	K01915	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.92201
G1-1A_GL0052871	K02876	*Bacteroidetes*			-1.77214
MC-0-3_GL0032795	K03561	*Bacteroidetes*			-3.58751
2-5_GL0018996	K04078	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.5881

MC-0-1_GL0098597		*Bacteroidetes*			-1.94289
G1-1A_GL0002549		*Bacteroidetes*			-2.51006
MC-6-1_GL0050844	K02519	*Bacteroidetes*			-2.08505
23_GL0056999	K00850	*Firmicutes*			2.910818
8_GL0113737	K01803	*Bacteroidetes*			-1.50467
G1-1A_GL0096658	K02876	*Bacteroidetes*			-2.02801
1A-dyr5-06_GL0043635					-1.77559
MC-0-1_GL0036393	K02939	*Bacteroidetes*			-1.75784
S-Fe10_GL0134063	K02935	*Bacteroidetes*			-2.57545
1-1_GL0065399	K02035	*Firmicutes*	*Lachnospiraceae*		-1.93152
S-Fe10_GL0014799	K03286	*Bacteroidetes*			-3.05177
G1-1A_GL0116705		*Bacteroidetes*			-3.07465
10_GL0075656	K02863	*Bacteroidetes*			-1.72917
S-Fe10_GL0131128		*Bacteroidetes*			-1.93868
10_GL0075657	K02864	*Bacteroidetes*			-2.31811
G1-1A_GL0045861	K01803	*Bacteroidetes*			-2.81349
S-Fe11_GL0075741		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.87772
G1-7A_GL0008452	K10112	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-2.05649
S-Fe20_GL0194632		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.68088
1-1_GL0077245	K00927	*Bacteroidetes*			-1.95336
G1-4A_GL0020479	K06178	*Bacteroidetes*			-1.33103
32_GL0041261	K07405				-1.84525
10_GL0075655	K02867	*Bacteroidetes*			-1.69807
G1-1A_GL0063273					-2.89857
S-Fe10_GL0118350		*Bacteroidetes*	*Prevotellaceae*		-1.52889
13_GL0078707	K02939	*Bacteroidetes*			-2.68266
MH-6-2_GL0008607					-1.69695
8-4_GL0082154	K02406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-3.3641
1-1_GL0064345	K10540	*Firmicutes*			1.824186
S-Fe10_GL0139765	K01624	*Bacteroidetes*			-1.66969
MC-0-1_GL0036391	K02990	*Bacteroidetes*	*Porphyromonadaceae*		-1.79021
G1-1A_GL0096566	K02357	*Bacteroidetes*			-1.62106
S-Fe10_GL0111946	K03561	*Bacteroidetes*			-5.39035
MC-0-3_GL0013098	K03773				-1.76684
1-7_GL0120192	K01430	*Bacteroidetes*			-2.49722
MH-6-2_GL0041983	K02935				2.969379
G1-1A_GL0063130	K02939	*Bacteroidetes*			-3.46341
G1-1A_GL0063296	K09458	*Bacteroidetes*			-2.92866
G1-1A_GL0063322	K00600	*Bacteroidetes*			-3.11874
11_GL0117532	K10200	*Firmicutes*			2.366271
S-Fe10_GL0134060	K02867	*Bacteroidetes*	*Prevotellaceae*		-2.25999
S-Fe10_GL0010674	K04043	*Bacteroidetes*			-1.31594

S-Fe10_GL0002669	K02357	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.56625
MC-6-2_GL0138472	K03521				-1.36083
MC-6-1_GL0006660	K02994	*Bacteroidetes*			-1.72203
S-Fe10_GL0061189					-1.75047
11_GL0019990	K02990	*Bacteroidetes*			-1.6812
G1-1A_GL0045911	K03545				-1.87352
MC-0-3_GL0082686	K01270	*Bacteroidetes*			-3.17593
S-Fe10_GL0072618	K02876	*Bacteroidetes*			-2.19572
3-5_GL0104202	K02357	*Firmicutes*	*Lachnospiraceae*		2.437884
S-Fe1_GL0161902	K01966	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.10772
G1-1A_GL0006484		*Bacteroidetes*			-1.51491
G1-5A_GL0138819	K03737	*Firmicutes*	*Eubacteriaceae*		-1.48718
10_GL0045677	K02965				-1.70794
10_GL0056359	K02988	*Bacteroidetes*			-1.6427
1A-dyr2-07_GL0054586	K06281				-1.6332
10_GL0068970	K02959	*Bacteroidetes*			-1.71051
S-Fe10_GL0076972	K03924				-2.74364
1-7_GL0129013	K06142	*Bacteroidetes*	*Porphyromonadaceae*		-2.84479
MC-0-1_GL0054818		*Bacteroidetes*			-2.04969
8-2_GL0130536	K02967	*Firmicutes*	*Lachnospiraceae*		-1.62015
G1-1A_GL0034838	K03924				-1.66853
32_GL0126562	K01269	*Firmicutes*			4.303498
10_GL0019531	K02933	*Bacteroidetes*			-1.59481
S-Fe2_GL0180691		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.50592
1-1_GL0029153	K02660	*Firmicutes*			1.308005
G1-1A_GL0096638	K02946	*Bacteroidetes*			-2.50298
1-7_GL0133815					-1.92806
32_GL0003549	K02406				2.1094
S-Fe10_GL0178963	K00895	*Bacteroidetes*			-3.36786
MH-6-2_GL0092596	K02897	*Bacteroidetes*			-2.11786
1A-dyr3-06_GL0039063	K00024	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.66642
MH-6-5_GL0025218	K02897	*Bacteroidetes*			-1.63831
MC-0-1_GL0066911		*Bacteroidetes*			-1.85659
16_GL0045029	K04027	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	1.811984
G1-1A_GL0100772	K00058	*Bacteroidetes*			-2.10088
MC-0-1_GL0027179	K01269	*Synergistetes*			4.908258
S-Fe2_GL0191348	K02058	*Firmicutes*	*Lachnospiraceae*	*Coprococcus*	-1.63029
10_GL0052803	K02357				-1.3734
1-3_GL0117244	K02078	*Bacteroidetes*			-1.94452
1-7_GL0049412	K03671	*Bacteroidetes*			-1.54447
MH-0-3_GL0091096	K00041	*Bacteroidetes*			-1.35341
S-Fe20_GL0067591		*Firmicutes*	*Lachnospiraceae*		2.84206

7-5_GL0009089	K01897	*Firmicutes*	*Lachnospiraceae*		2.372831
S-Fe3_GL0062242					-1.55608
S-Fe2_GL0005285	K02990	*Firmicutes*			2.723505
S-Fe10_GL0132208	K02078	*Bacteroidetes*			-2.90401
G1-1A_GL0024897		*Bacteroidetes*			-2.23632
13_GL0071922					-2.73642
MC-6-2_GL0157885		*Bacteroidetes*			-1.94885
Group2-6A_GL0064249	K02027	*Firmicutes*			2.077133
S-Fe10_GL0115267	K02926	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.4879
MC-0-1_GL0079955	K02876	*Bacteroidetes*	*Rikenellaceae*		1.869515
2-4_GL0028300	K02886	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-3.22281
S-Fe10_GL0072600	K02906	*Bacteroidetes*			-3.68137
S-Fe10_GL0174604	K03773	*Bacteroidetes*			-2.39987
10_GL0095440	K01810	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.09811
10_GL0081216	K01270	*Bacteroidetes*			-2.00591
10_GL0081451					-2.12955
20_GL0137120	K10670	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.80551
S-Fe10_GL0011145	K01803	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.07516
16_GL0018914	K00259	*Proteobacteria*	*Desulfovibrionaceae*		1.816468
G1-1A_GL0096640	K02926	*Bacteroidetes*			-2.13811
11_GL0125895		*Bacteroidetes*			-1.4143
S-Fe10_GL0024382	K00602	*Bacteroidetes*			-2.53824
13_GL0051706	K01847	*Bacteroidetes*			-3.02303
MH-0-3_GL0066986	K02884	*Bacteroidetes*			-1.7087
S-Fe10_GL0074772	K00240	*Bacteroidetes*			-2.72338
26_GL0121905	K01689	*Firmicutes*	*Enterococcaceae*	*Enterococcus*	-3.90409
3-5_GL0115917	K02892	*Firmicutes*			3.959369
10_GL0041385	K02992	*Bacteroidetes*	*Rikenellaceae*		-1.41722
6-5_GL0035181	K01269	*Firmicutes*	*Lachnospiraceae*		1.461705
S-Fe10_GL0056930	K03046	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.63012
G1-1A_GL0045901	K01429	*Bacteroidetes*			-2.72141
S-Fe10_GL0131407	K04564				-1.7298
MC-0-3_GL0012433	K01813	*Bacteroidetes*			-1.44809
10_GL0056357	K02876	*Bacteroidetes*			-1.50805
MH-6-4_GL0059762		*Firmicutes*	*Clostridiaceae*		-1.80798
Group2-5A_GL0107794		*Firmicutes*			2.222537
S-Fe8_GL0211955					-2.16015
7-1_GL0020329	K01746				1.817316
MC-6-3_GL0124037	K04077	*Bacteroidetes*	*Prevotellaceae*		-2.59173
G1-1A_GL0024447	K00854	*Bacteroidetes*			-2.17719
G1-1A_GL0110295	K00029	*Bacteroidetes*			-3.14956

1A-dyr2-07_GL0013684	K02112				-2.39196
S-Fe2_GL0215316		*Firmicutes*	*Ruminococcaceae*		2.067115
11_GL0146410	K02994	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.0169
S-Fe10_GL0056935	K02867	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.97196
10_GL0098134	K00024				-1.55648
G1-1A_GL0048782		*Bacteroidetes*			-1.75746
2-5_GL0035732	K02904	*Firmicutes*			1.46101
G1-1A_GL0063275	K03832	*Bacteroidetes*			-1.30392
G1-1A_GL0063132	K02990	*Bacteroidetes*	*Porphyromonadaceae*		-2.62953
MH-6-5_GL0105709		*Firmicutes*			1.304678
Group2-5A_GL0033504	K02904	*Firmicutes*			3.209535
43_GL0028121		*Firmicutes*	*Lachnospiraceae*		6.286236
10_GL0070783		*Bacteroidetes*			-1.88054
MH-6-5_GL0023498	K02904	*Firmicutes*			2.770038
S-Fe14_GL0007498		*Firmicutes*			2.993336
G1-1A_GL0063367	K01881	*Bacteroidetes*			-1.54713
MH-6-2_GL0116248	K02945	*Bacteroidetes*	*Porphyromonadaceae*	*Odoribacter*	-2.25037
10_GL0051759	K02954	*Bacteroidetes*			-1.79099
26_GL0064285		*Bacteroidetes*			-2.11951
S-Fe10_GL0105581		*Bacteroidetes*			-3.92569
10_GL0054688	K00634				-1.71979
S-Fe3_GL0232219	K02933	*Bacteroidetes*			-1.34996
1A-dyr2-07_GL0066020	K01428	*Bacteroidetes*			-1.58141
10_GL0007819	K02867	*Bacteroidetes*			-2.28138
MH-6-5_GL0065301	K02897	*Bacteroidetes*	*Prevotellaceae*		-2.02536
5-1_GL0066427	K02926	*Firmicutes*			1.501086
G1-1A_GL0116773	K02902	*Bacteroidetes*			-2.28895
S-Fe10_GL0111950	K02959				-2.28257
MC-0-1_GL0018755	K02888	*Bacteroidetes*			-2.04073
G1-1A_GL0024923		*Bacteroidetes*			-2.26559
S-Fe10_GL0115249	K02876	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.99892
S-Fe10_GL0063313	K02959	*Bacteroidetes*	*Prevotellaceae*		-1.54329
S-Fe3_GL0078754	K02888	*Bacteroidetes*			-1.48945
S-Fe10_GL0072606	K02982	*Bacteroidetes*			-2.25786
S-Fe10_GL0028722	K02952	*Bacteroidetes*			-1.52056
MC-6-3_GL0010464	K02950	*Bacteroidetes*			-1.64658
5-1_GL0066415	K02994	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.318228
MH-6-3_GL0043163		*Firmicutes*	*Lachnospiraceae*		2.523963
MC-0-1_GL0027531	K01923	*Bacteroidetes*			-1.5188
S-Fe10_GL0145062	K02878	*Bacteroidetes*			-1.6572
13_GL0089750	K09458	*Bacteroidetes*			-2.36087

1-1_GL0079450	K04072	*Firmicutes*			-1.96258
7-8_GL0141016	K00688	*Firmicutes*			2.023477
11_GL0131190	K02887	*Bacteroidetes*			-1.88918
11_GL0016746	K02881	*Bacteroidetes*			-1.58528
MC-0-1_GL0054816		*Bacteroidetes*			-2.11349
10_GL0098100	K02078	*Bacteroidetes*			-2.03192
MC-0-1_GL0060604	K02933	*Bacteroidetes*			-2.34892
S-Fe10_GL0037648	K02897	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.03081
4-1_GL0077349	K01923	*Firmicutes*			-1.80054
S-Fe6_GL0215897		*Proteobacteria*			-1.76154
MC-0-3_GL0033683	K00198	*Firmicutes*			6.924515
MH-6-5_GL0021788		*Bacteroidetes*			-2.16322
S-Fe10_GL0002666	K02996	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-4.98661
S-Fe10_GL0165697		*Bacteroidetes*			-1.4524
1-7_GL0129238	K02871	*Bacteroidetes*			-3.4337
G1-1A_GL0052859	K02878				-1.40273
G1-1A_GL0045080		*Bacteroidetes*			-3.84878
G1-1A_GL0006520		*Bacteroidetes*			-1.97415
10_GL0097295	K03545				-3.09841
1A-dyr2-07_GL0019966	K00405	*Deferribacteres*			-1.90328
MC-0-3_GL0059925	K02078	*Bacteroidetes*	*Prevotellaceae*		-1.93063
8-1_GL0052916		*Firmicutes*	*Lachnospiraceae*	*Blautia*	1.687024
MH-6-1_GL0002334		*Firmicutes*			1.644697
S-Fe10_GL0172458	K00024	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.76588
1-1_GL0091377	K02881	*Firmicutes*			-1.40315
MH-6-5_GL0003590		*Firmicutes*			-1.5459
4-1_GL0012700	K10200	*Firmicutes*	*Ruminococcaceae*		-1.73675
5-5_GL0070744	K00197	*Firmicutes*			-1.90326
1A-dyr2-07_GL0016988	K02939	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.34173
1-1_GL0147319	K02946	*Bacteroidetes*			-2.25773
1A-dyr2-07_GL0037152	K01966				-1.42744
10_GL0098115	K03530	*Bacteroidetes*			-2.03533
MC-6-5_GL0110497		*Bacteroidetes*			-2.237
2B-dyr19-06_GL0048336	K02906	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.69529
10_GL0075218		*Bacteroidetes*			-1.87915
1A-dyr2-07_GL0001117	K03781				-1.33674
Group2-4A_GL0185217	K02945	*Firmicutes*			2.659489
MC-0-1_GL0007440	K02881	*Bacteroidetes*			-2.90927
4-1_GL0003650	K00975	*Firmicutes*			1.559121
1-1_GL0121867	K03406				-1.69023

Group2-3A_GL0065519	K02986	*Firmicutes*			6.808831
MC-0-1_GL0066913		*Bacteroidetes*			-1.63833
S-Fe20_GL0041474	K00548	*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	-1.77104
S-Fe10_GL0027399		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.1795
4-4_GL0035702					1.786035
10_GL0095399	K03561	*Bacteroidetes*			-2.10923
2-6_GL0086238	K02952	*Firmicutes*			1.357586
2-2_GL0146624	K02027	*Firmicutes*			2.524335
G1-1A_GL0021730		*Bacteroidetes*			-1.82189
S-Fe3_GL0204094	K01803	*Bacteroidetes*			-2.42588
G1-1A_GL0002586	K02864	*Bacteroidetes*			-2.71638
4-1_GL0070108	K03406	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.566226
10_GL0001367	K03773				-2.9601
10_GL0019532	K02994	*Bacteroidetes*			-1.8703
11_GL0019947	K02338	*Bacteroidetes*			-1.34904
Group2-5A_GL0131239	K01596	*Firmicutes*			-2.22217
S-Fe10_GL0115256	K02931	*Bacteroidetes*			-4.01837
S-Fe12_GL0185247	K03561	*Bacteroidetes*			-4.98243
S-Fe8_GL0064916	K01596	*Firmicutes*	*Ruminococcaceae*		-1.63534
1-1_GL0139144	K03530	*Firmicutes*			-1.46447
Group2-6A_GL0086781	K00703	*Firmicutes*			2.414003
S-Fe10_GL0115262	K02982	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.04185
1A-dyr2-07_GL0033906	K02073				-2.01322
10_GL0062975	K02946				-1.48635
G1-1A_GL0036632	K05606				-1.72465
S-Fe10_GL0027400	K03561	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.45766
S-Fe10_GL0018656	K06142	*Bacteroidetes*			-2.46419
S-Fe10_GL0119226	K03545	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.18718
S-Fe10_GL0130208		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.07179
10_GL0037536	K02909	*Bacteroidetes*			-1.72519
16_GL0056429					-1.45397
11_GL0013191	K12373				-1.43273
14_GL0072415	K02933	*Firmicutes*			-1.68791
MC-0-3_GL0039121	K02874	*Bacteroidetes*			-3.30133
7-5_GL0143426	K00700	*Firmicutes*			2.424613
S-Fe10_GL0110482	K02909	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.91368
1-1_GL0039242	K01119	*Firmicutes*			2.018049
MC-0-3_GL0033335	K00600	*Bacteroidetes*			-1.54244
S-Fe10_GL0115241	K02879	*Bacteroidetes*	*Prevotellaceae*		-1.76758
7-5_GL0136243	K02968	*Firmicutes*			3.620784
4-1_GL0078425	K02990	*Firmicutes*			2.104401

1-7_GL0107205	K02954	*Bacteroidetes*	*Porphyromonadaceae*	*Porphyromonas*	-3.98096
1-1_GL0117261	K01999	*Firmicutes*			-2.33661
G1-1A_GL0052628		*Firmicutes*			-2.26389
MC-0-1_GL0010770		*Bacteroidetes*			-2.29169
10_GL0064382	K02217	*Bacteroidetes*			-2.44521
7-5_GL0112817	K02890	*Firmicutes*			2.233091
1-1_GL0130236	K00975	*Firmicutes*			-2.0687
G1-1A_GL0052863	K02895	*Bacteroidetes*			-3.10709
4-1_GL0029491	K02956	*Firmicutes*			1.446476
7-1_GL0053812	K02600	*Bacteroidetes*			-1.78112
17_GL0006600	K02124	*Firmicutes*			-1.43327
S-Fe11_GL0134259					-2.66565
2-1_GL0012242	K02986	*Firmicutes*			-1.84927
1-1_GL0108874	K00703	*Firmicutes*	*Lachnospiraceae*		-1.82989
10_GL0036184	K01875	*Bacteroidetes*			-1.49839
1-3_GL0023139	K02888	*Firmicutes*			6.093524
4-3_GL0064431	K02994	*Firmicutes*			-2.28062
G1-1A_GL0025051	K03773	*Bacteroidetes*			-2.02626
G1-5A_GL0190133	K00074				1.323118
S-Fe10_GL0067434		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-4.25891
7-8_GL0147205	K11189	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.469875
1-7_GL0082305	K05808	*Bacteroidetes*			-2.05061
6-5_GL0071490	K10547				2.98534
5-1_GL0064999	K02888	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.90269
G1-1A_GL0045155	K03106	*Bacteroidetes*			-1.95739
S-Fe10_GL0005478		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.93548
22_GL0032377	K11189	*Firmicutes*			1.370278
S-Fe10_GL0111943	K03832				-2.14811
2-5_GL0028745	K02887	*Firmicutes*			1.433573
G1-1A_GL0037375		*Proteobacteria*			-1.67459
1-1_GL0119832		*Firmicutes*			3.09891
S-Fe10_GL0099387					-1.63367
MC-6-4_GL0056320	K06142	*Bacteroidetes*			-1.77912
10_GL0062672	K02956	*Bacteroidetes*			-2.15924
S-Fe10_GL0165682	K03340	*Bacteroidetes*			-1.69796
S-Fe10_GL0121847	K02887	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.00415
10_GL0036185	K02899	*Bacteroidetes*			-2.60351
10_GL0005128		*Bacteroidetes*			-1.3681
S-Fe10_GL0099385		*Bacteroidetes*			-1.69986
S-Fe10_GL0115261	K02878	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.39106
G1-5A_GL0200745	K02030				2.726571
1-7_GL0129239	K02996	*Bacteroidetes*			-3.24322
MH-6-1_GL0087263	K00625				-1.37585

10_GL0052775	K02897	*Bacteroidetes*			-2.56294
MC-6-3_GL0060183	K02939	*Bacteroidetes*			-1.54026
MC-6-1_GL0037118	K00088				-1.38947
2-1_GL0022321	K02967				-1.88283
S-Fe9_GL0169128	K02994	*Firmicutes*			-1.43444
1A-dyr2-07_GL0003701	K00059	*Bacteroidetes*			-1.50784
6-6_GL0119787	K00634	*Firmicutes*			2.484258
MC-6-1_GL0028751	K02879	*Bacteroidetes*			-1.76004
34_GL0013858	K03569	*Firmicutes*			-1.35466
5-1_GL0066421	K02878	*Firmicutes*			2.019742
MC-0-1_GL0041223		*Bacteroidetes*			-4.04447
S-Fe11_GL0026221					-2.76627
G1-1A_GL0045814		*Bacteroidetes*			-3.20178
S-Fe10_GL0077078	K01866	*Bacteroidetes*			-2.30134
2-1_GL0005474		*Firmicutes*			-1.89462
10_GL0097167		*Bacteroidetes*			-2.80925
S-Fe10_GL0037907	K02078	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-5.59266
2-3_GL0034898	K02996	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-5.30377
1A-dyr4-07_GL0036960	K02863	*Firmicutes*			1.596188
5-5_GL0042056	K02888	*Firmicutes*			4.619668
10_GL0022402	K00602	*Bacteroidetes*			-1.30432
MC-6-3_GL0082271	K02931	*Bacteroidetes*			-1.56429
5-1_GL0042804		*Firmicutes*			2.569528
S-Fe10_GL0072615	K02933	*Bacteroidetes*			-3.01129
MC-6-1_GL0050592	K04078	*Bacteroidetes*			-3.59653
2-1_GL0047570	K02988	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-3.75446
13_GL0007465	K02871	*Bacteroidetes*			-2.90841
11_GL0082736					-1.4672
1-1_GL0040377	K03111	*Firmicutes*	*Lachnospiraceae*	*Roseburia*	1.962229
G1-1A_GL0036615	K02888	*Bacteroidetes*			-1.44278
3-1_GL0047060		*Firmicutes*			1.41048
10_GL0067629	K01258				-1.33138
S-Fe10_GL0029721		*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.36664
S-Fe10_GL0131019	K00024	*Bacteroidetes*			-2.24988
1A-dyr2-07_GL0044881	K03530				-3.35184
26_GL0002628	K01304	*Firmicutes*	*Lachnospiraceae*		2.168991
MH-6-2_GL0037061	K01881	*Bacteroidetes*			-1.96078
10_GL0012679	K02939	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.36346
10_GL0045676	K02890	*Bacteroidetes*			-1.62161
6-7_GL0010769	K02876	*Firmicutes*			1.742706
10_GL0036186	K02888	*Bacteroidetes*			-1.5042

MC-0-3_GL0028081	K02887	*Bacteroidetes*			-2.36166
MC-6-4_GL0084128	K03977	*Bacteroidetes*			-3.52517
3-2_GL0003611	K03408				1.973315
S-Fe9_GL0017498	K10112	*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	-2.1085
4-8_GL0055590	K03406	*Firmicutes*	*Lachnospiraceae*		2.059146
G1-3A_GL0069765	K01689	*Firmicutes*			-1.55007
1A-dyr2-07_GL0041521	K01689	*Deferribacteres*			-1.84788
G1-7A_GL0039039	K01999	*Firmicutes*			-2.81025
2A-dyr14-07_GL0014020	K03737	*Firmicutes*			-1.77655
10_GL0056352	K02948	*Bacteroidetes*			-1.66225
3-6_GL0044331					-1.32039
MC-6-5_GL0061061	K03530	*Bacteroidetes*			-1.46728
6-8_GL0004968	K02876	*Firmicutes*			1.672593
11_GL0002114	K04567	*Bacteroidetes*			-1.60328
S-Fe10_GL0135010		*Bacteroidetes*			-4.58483
S-Fe10_GL0182480	K02926	*Bacteroidetes*	*Prevotellaceae*		-1.88907
10_GL0048573	K09117	*Bacteroidetes*			-1.77361
Group2-7A_GL0052076		*Firmicutes*			-2.39645
MC-6-5_GL0091603	K02863	*Firmicutes*			-1.91063
11_GL0104918	K02887	*Bacteroidetes*			-2.48541
1-1_GL0008933	K10206	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-2.03813
S-Fe10_GL0037967		*Bacteroidetes*			-4.4997
S-Fe6_GL0119793	K00625	*Firmicutes*			1.903023
10_GL0056353	K02952	*Bacteroidetes*			-1.87444
MH-6-1_GL0157656	K00606	*Bacteroidetes*			-2.70327
5-7_GL0002564	K00540	*Firmicutes*			-2.89615
11_GL0101749	K04078	*Bacteroidetes*			-2.63829
5-1_GL0066428	K02906	*Firmicutes*			1.468704
S-Fe6_GL0072145	K01847				-2.63645
10_GL0081632	K02867	*Firmicutes*	*Erysipelotrichaceae*		-1.89122
35_GL0013435	K06142	*Bacteroidetes*			-2.90362
6-7_GL0104938	K02895	*Firmicutes*			1.549625
1-8_GL0065946		*Bacteroidetes*			-2.4224
G1-1A_GL0111534	K03106				-1.56492
MH-0-5_GL0040474	K07164	*Bacteroidetes*			-1.98665
2-2_GL0127503	K10112	*Firmicutes*			-1.47698
34_GL0016941	K02916	*Firmicutes*			1.706669
10_GL0049422	K02904	*Bacteroidetes*			-1.80918
S-Fe10_GL0121839	K02939	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-5.55421
2-6_GL0046249	K01746	*Firmicutes*			2.341343
1-4_GL0082641	K02956	*Firmicutes*			1.768909

G1-1A_GL0096641	K02892	*Bacteroidetes*			-2.85493
10_GL0029189		*Bacteroidetes*			-1.78694
1A-dyr1-07_GL0037352	K02959	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-1.91395
1A-dyr2-07_GL0076977					-4.0095
G1-1A_GL0024835	K00812	*Bacteroidetes*			-1.67766
S-Fe10_GL0006965		*Bacteroidetes*			-1.8728
1-1_GL0073324	K03413	*Firmicutes*			2.225498
G1-1A_GL0036616	K00845	*Bacteroidetes*			-1.8797
G1-6A_GL0020587	K06972	*Firmicutes*			1.956191
1-1_GL0105382		*Firmicutes*			2.830523
11_GL0091621	K02864	*Firmicutes*			3.036109
10_GL0078960	K01892	*Firmicutes*			1.357541
MC-6-1_GL0061787		*Bacteroidetes*			-1.71039
5-8_GL0076051	K04077	*Firmicutes*			-1.4568
S-Fe10_GL0172944	K11065				-1.67917
G1-1A_GL0117727	K01689	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.82021
2-1_GL0050294	K02027	*Firmicutes*			-1.4827
G1-2A_GL0042551		*Bacteroidetes*			-1.51646
G1-7A_GL0057424	K02881	*Firmicutes*	*Ruminococcaceae*		-2.19119
S-Fe2_GL0076345		*Firmicutes*			-1.52211
S-Fe11_GL0138875	K01875	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.58186
1-1_GL0020817		*Firmicutes*			6.105638
7-8_GL0043249	K03406	*Firmicutes*			5.771742
MC-0-1_GL0057206	K02437	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.51093
S-Fe10_GL0110871		*Bacteroidetes*			-1.52033
G1-1A_GL0052870	K02907	*Bacteroidetes*			-2.26072
S-Fe10_GL0139773	K02902	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.56621
1-1_GL0000484	K00645	*Bacteroidetes*			-1.9324
G1-1A_GL0036666		*Bacteroidetes*	*Porphyromonadaceae*		-1.37613
4-1_GL0075087	K03671	*Firmicutes*			1.463146
1-2_GL0046790		*Bacteroidetes*			-1.40535
S-Fe10_GL0082903	K02907	*Bacteroidetes*			-2.51528
4-1_GL0055129		*Bacteroidetes*			-3.74899
10_GL0054342	K03168	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.54142
4-8_GL0075009	K13993	*Firmicutes*	*Clostridiaceae*	*Clostridium*	7.481079
16_GL0106289		*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.800975
S-Fe10_GL0029121	K02871	*Bacteroidetes*			-2.79988
MC-6-1_GL0101085	K06142	*Bacteroidetes*			-1.96426
1_GL0056241		*Bacteroidetes*			-2.44659
S-Fe10_GL0072031	K03530	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.48272
1-1_GL0109800		*Firmicutes*			2.972341
G1-7A_GL0076784		*Firmicutes*			-1.71736

S-Fe7_GL0015571		*Proteobacteria*	*Desulfovibrionaceae*	*Desulfovibrio*	1.545257
MC-6-2_GL0127138	K02356	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.08427
1-7_GL0008070	K07567	*Firmicutes*			1.321674
13_GL0093821	K01915				-1.87155
1A-dyr2-07_GL0049110	K02988	*Deferribacteres*	*Deferribacteraceae*		-1.67492
G1-7A_GL0109682		*Firmicutes*	*Eubacteriaceae*		-3.02719
S-Fe10_GL0115253	K02933	*Bacteroidetes*			-2.81241
7-2_GL0089302	K02113	*Firmicutes*			2.395675
MH-6-2_GL0048481	K02838	*Bacteroidetes*			-3.40018
S-Fe10_GL0038719	K02996	*Bacteroidetes*	*Prevotellaceae*		-1.7584
4-1_GL0017830	K01428	*Firmicutes*			-1.893
S-Fe10_GL0119345	K00849	*Bacteroidetes*	*Prevotellaceae*		-1.81094
10_GL0090718		*Bacteroidetes*			-2.15763
MH-6-2_GL0102522		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.3348
8_GL0009350	K00615	*Firmicutes*			-1.50567
6-5_GL0012433	K07576	*Firmicutes*			1.375066
Group2-1A_GL0094062		*Bacteroidetes*			-1.71852
1-1_GL0049767	K01264	*Firmicutes*			3.464366
4-1_GL0062284		*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	3.772896
S-Fe11_GL0075744	K09704	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-3.98225
31_GL0061245	K02982				-1.7313
G1-7A_GL0091482	K02863	*Firmicutes*	*Eubacteriaceae*		-2.24672
G1-1A_GL0096550	K00241	*Bacteroidetes*			-2.49327
S-Fe2_GL0150482		*Firmicutes*			-6.23453
MH-6-3_GL0012070	K01639				3.806797
MC-6-2_GL0001750	K02899	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-6.57022
Group2-7A_GL0036321		*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	-1.97823
40_GL0014235	K02895	*Bacteroidetes*			-3.33815
49_GL0015711	K02520	*Bacteroidetes*			-1.53867
1-1_GL0046414					3.01716
MC-0-3_GL0030308	K01591				-1.34131
S-Fe10_GL0125519	K09704	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.7873
5-1_GL0066417	K02895	*Firmicutes*			1.337867
MC-0-3_GL0011973	K00599	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.86958
S-Fe10_GL0119360	K00948	*Bacteroidetes*	*Prevotellaceae*		-2.28783
G1-5A_GL0012406	K02904	*Firmicutes*			-2.0662
MC-0-1_GL0030331	K07221				-1.3402
MC-6-2_GL0114364		*Firmicutes*	*Eubacteriaceae*		1.898179
54_GL0133220	K00014	*Bacteroidetes*			-2.14104
16_GL0104681		*Bacteroidetes*			-1.40978
MH-6-3_GL0022033	K02564	*Firmicutes*			1.980448

G1-3A_GL0108906	K02890	*Firmicutes*			1.661813
MC-0-3_GL0011446		*Bacteroidetes*			-2.41024
2-2_GL0125126	K02881	*Firmicutes*			-3.4563
1A-dyr2-07_GL0035086	K02968	*Firmicutes*	*Ruminococcaceae*		-1.8152
2-2_GL0026753	K02881	*Firmicutes*			-1.44911
S-Fe10_GL0029224		*Bacteroidetes*	*Prevotellaceae*		-4.42145
1-2_GL0048736	K02884	*Firmicutes*			2.553989
G1-1A_GL0045154	K01491	*Bacteroidetes*			-3.67495
10_GL0080398	K00904	*Bacteroidetes*			-1.95213
G1-1A_GL0107723	K01811	*Bacteroidetes*			-3.70129
S-Fe11_GL0123973	K01756	*Bacteroidetes*			-2.19271
MC-6-2_GL0019983	K01866	*Proteobacteria*	*Helicobacteraceae*	*Helicobacter*	-2.45675
MC-6-1_GL0056899					-1.41992
2-5_GL0064181	K02968	*Firmicutes*			1.377554
S-Fe10_GL0044726	K03671	*Bacteroidetes*			-3.42818
S-Fe10_GL0130360	K01619	*Bacteroidetes*			-3.63911
2A-dyr16-06_GL0043385	K02372				-2.77447
2A-dyr16-06_GL0043385	K02535				-2.77447
Group2-3A_GL0122934	K01785	*Firmicutes*			1.532685
S-Fe1_GL0168465	K05366	*Bacteroidetes*			-2.58638
G1-5A_GL0079265		*Firmicutes*	*Lachnospiraceae*		7.459403
26_GL0018559		*Firmicutes*			1.345997
MH-6-5_GL0097682	K07080				1.518123
S-Fe11_GL0026047	K02970	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-4.78144
G1-5A_GL0178131	K05808	*Firmicutes*			-1.94643
MC-6-2_GL0181022	K02961	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-8.53944
S-Fe10_GL0029632	K01190	*Bacteroidetes*			-3.46439
S-Fe10_GL0006842	K03530	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-3.21163
1-1_GL0041861	K03778				-1.98279
S-Fe10_GL0115266	K02892	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.37077
4-8_GL0007109	K08600				5.314161
2-1_GL0029950					-1.56328
MH-6-4_GL0119443		*Firmicutes*			2.882226
S-Fe10_GL0062501	K03671	*Bacteroidetes*	*Prevotellaceae*		-1.9324
6-3_GL0108296		*Firmicutes*			-4.12292
5-1_GL0033106	K13993	*Firmicutes*			2.261282
1A-dyr2-07_GL0066370	K01067				-3.52179
54_GL0029562	K00975	*Firmicutes*			2.566511
5-7_GL0020968					-3.85461
S-Fe10_GL0164970	K02968	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-5.71151

8-7_GL0051636		*Firmicutes*			1.483572
20_GL0117868	K02798				-4.688
20_GL0117868	K02799				-4.688
20_GL0117868	K02800				-4.688
MC-6-2_GL0165359	K00648	*Bacteroidetes*			-1.8946
8-2_GL0052159	K02956	*Firmicutes*			-2.58606
4-3_GL0025455	K06212	*Firmicutes*	*Lachnospiraceae*		-2.49158
MH-6-2_GL0007875		*Bacteroidetes*			-2.45791
2-1_GL0032742					-3.11712
S-Fe8_GL0084990	K11181	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	2.121085
Group2-8A_GL0154681	K02879				2.624452
5-2_GL0007959		*Firmicutes*	*Lachnospiraceae*		-1.95055
Group2-4A_GL0034417	K02876	*Firmicutes*			-1.90466
6-4_GL0010598	K13993	*Firmicutes*	*Clostridiaceae*	*Clostridium*	4.895676
S-Fe11_GL0124318	K07407	*Actinobacteria*	*Bifidobacteriaceae*	*Bifidobacterium*	-2.23534
S-Fe10_GL0151773		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.45497
4-1_GL0062999	K03530	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-2.62458
G1-1A_GL0108097	K09116	*Firmicutes*			-1.92335
MC-6-5_GL0026119	K01885	*Firmicutes*			1.466804
S-Fe10_GL0152300	K00940	*Bacteroidetes*			-2.45681
S-Fe12_GL0106106	K12941	*Synergistetes*			-2.34186
10_GL0036483	K03977				-1.45487
11_GL0082226					-3.79565
MC-0-1_GL0051482	K02939	*Bacteroidetes*			-1.51657
G1-7A_GL0137627		*Firmicutes*			-3.22513
Group2-3A_GL0065157	K06960	*Firmicutes*			1.39346
MH-0-5_GL0026764		*Firmicutes*			-2.87448
41_GL0022979	K01719	*Bacteroidetes*			-2.5174
54_GL0053952		*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	2.665572
S-Fe7_GL0171211	K03737	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-2.6278
2-1_GL0030762		*Firmicutes*			1.758442
2-4_GL0075428	K02358				-1.96477
MC-0-3_GL0067315	K02945	*Bacteroidetes*			-2.29167
16_GL0005840	K00688	*Bacteroidetes*			-1.7843
S-Fe8_GL0238271	K01624	*Firmicutes*	*Lachnospiraceae*		-1.31689
6-8_GL0090796	K00134				-3.20567
1-5_GL0013954	K01624	*Firmicutes*			1.662953
17_GL0076376	K02863	*Firmicutes*			-2.25067
S-Fe10_GL0111947	K00927	*Bacteroidetes*			-1.57616
5-1_GL0082386	K02935	*Firmicutes*			2.144204
1-3_GL0092198	K02992	*Bacteroidetes*	*Porphyromonadaceae*		-1.96768

1-4_GL0028897	K02988	*Firmicutes*			1.302826
2-4_GL0092915	K04077				1.930938
G1-6A_GL0012944	K00656	*Firmicutes*			-1.56087
1-1_GL0117776	K00688	*Firmicutes*			-2.73348
2_GL0032312	K02803	*Firmicutes*			2.101292
2_GL0032312	K02804	*Firmicutes*			2.101292
7-5_GL0055838	K00845	*Firmicutes*			1.503259
16_GL0037009	K00059	*Bacteroidetes*			-3.0327
1-1_GL0087585	K00975	*Firmicutes*	*Lachnospiraceae*		1.372992
MH-0-5_GL0091066	K00927	*Bacteroidetes*			-1.90523
7-5_GL0112795	K03040	*Firmicutes*			2.172729
1-1_GL0091378	K02988	*Firmicutes*			-3.44979
S-Fe10_GL0134065	K03046	*Bacteroidetes*	*Prevotellaceae*		-2.6859
S-Fe10_GL0112575	K03386	*Bacteroidetes*			-1.33197
Group2-2A_GL0123104	K00031	*Firmicutes*			-1.31641
2-3_GL0042566	K01810	*Firmicutes*			2.872731
S-Fe8_GL0143417	K00831				2.258326
52_GL0016116	K00849	*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	-2.54996
MC-6-2_GL0181013	K02906	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.185
13_GL0007468	K02357	*Bacteroidetes*			-1.41737
G1-1A_GL0096649	K02874	*Bacteroidetes*			-3.07444
1-2_GL0066299		*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-2.35304
G1-5A_GL0012410	K02931	*Firmicutes*			-4.25384
S-Fe8_GL0274868	K02909				-2.44213
2-4_GL0080874	K02876	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-2.25828
MC-0-1_GL0094983	K07263	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	2.811222
G1-1A_GL0095183		*Bacteroidetes*			-1.51459
28_GL0073237	K02994	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.443029
MH-6-5_GL0123182		*Firmicutes*			2.783889
S-Fe11_GL0039770	K07405	*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	-5.38642
G1-1A_GL0045114	K03530	*Bacteroidetes*	*Porphyromonadaceae*		-4.10364
G1-5A_GL0012411	K02994	*Firmicutes*	*Eubacteriaceae*		-3.0936
G1-7A_GL0098842	K02926	*Firmicutes*			-3.00626
7-5_GL0044018	K02520	*Firmicutes*			1.767648
1-8_GL0081734	K06142	*Bacteroidetes*			-1.61865
23_GL0104753	K01190	*Firmicutes*			1.650563
G1-5A_GL0170492		*Firmicutes*			-2.4606
3-1_GL0097837	K01430	*Bacteroidetes*			-2.00917
8-5_GL0051769	K02407	*Firmicutes*			4.931418
S-Fe11_GL0130456	K02520	*Bacteroidetes*			-1.47048
5-1_GL0045111		*Firmicutes*			2.482957
MH-0-3_GL0009473	K09903	*Bacteroidetes*			-1.83306

19_GL0011542		*Bacteroidetes*			-4.15368
8-2_GL0062396	K02996	*Firmicutes*			-1.5806
11_GL0148943	K10546	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-3.14787
G1-7A_GL0097613		*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	-1.37061
S-Fe10_GL0027781	K06142	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.21826
1-1_GL0014249	K02025				-2.44442
29_GL0153864	K00615	*Firmicutes*			2.238029
2-1_GL0021397	K02892	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	-1.81266
2-1_GL0068730		*Firmicutes*	*Ruminococcaceae*		1.864609
G1-4A_GL0102804		*Firmicutes*			1.465409
MC-6-2_GL0177002		*Firmicutes*			-2.63984

Appendix 13. List of significantly changed microbial proteins between DR and DC groups.

Accession	**KO**	**Phylum**	**Family**	**Genus**	**Pi score**
5-4_GL0037288	K01610	*Firmicutes*			1.558628
1-4_GL0025198	K04077	*Firmicutes*			2.163757
3-2_GL0042838	K01610	*Firmicutes*			2.143686
22_GL0041445	K01006	*Firmicutes*			1.429495
1-1_GL0078299	K02406				-2.61956
5-7_GL0068497	K02358	*Firmicutes*			2.782591
1A-dyr2-07_GL0046330	K00134				-1.42061
G1-1A_GL0024937	K00134	*Bacteroidetes*			-1.44453
1-2_GL0002789	K02406				-2.28786
20_GL0015122	K03737	*Actinobacteria*			1.747127
4-8_GL0043489	K01006	*Firmicutes*			1.604143
1-1_GL0041844	K02406				-2.46039
Group2-6A_GL0005221	K02406	*Firmicutes*			-2.36714
3-6_GL0055865	K00134	*Firmicutes*			1.324801
S-Fe10_GL0045753	K00134	*Bacteroidetes*			-1.68666
3-1_GL0094445	K02117	*Firmicutes*			1.656217
2-2_GL0040883	K00197	*Firmicutes*			2.381545
4-2_GL0031968	K00194	*Firmicutes*			1.361634
47_GL0055883	K10112				1.95203
17_GL0005632	K00626				2.206888
S-Fe8_GL0285084	K00197	*Firmicutes*			1.453783
3-5_GL0030148	K02945	*Firmicutes*			2.644205
MH-0-4_GL0042921	K00197	*Firmicutes*			2.020019

14_GL0016880	K02406	*Firmicutes*			1.39676
11_GL0010651	K00257	*Firmicutes*	*Lachnospiraceae*		-1.31509
S-Fe9_GL0013307	K00257	*Firmicutes*			1.85625
1-1_GL0060052	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.279344
4-7_GL0067539		*Firmicutes*			-2.74815
S-Fe8_GL0098677	K00197	*Firmicutes*			1.860016
4-2_GL0031971	K00198	*Firmicutes*	*Lachnospiraceae*		2.321302
S-Fe9_GL0180511	K02406	*Firmicutes*			-1.75924
1-5_GL0006008	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.206066
G1-1A_GL0063364					-1.46493
2-1_GL0071086	K03521	*Firmicutes*	*Clostridiaceae*		2.107985
G1-5A_GL0063641	K02982				2.539022
MH-6-4_GL0051258	K00194	*Firmicutes*			1.6418
G1-1A_GL0043884	K00895				-1.39465
28_GL0006324	K03737				1.415672
MC-6-2_GL0008188	K01624	*Firmicutes*			-1.79835
S-Fe20_GL0020060	K00134				1.736746
11_GL0106921	K02931	*Firmicutes*			-2.06979
S-Fe20_GL0010191	K00074				1.49793
G1-1A_GL0052877	K02986	*Bacteroidetes*			-3.14634
1-7_GL0094016	K01624	*Firmicutes*			3.219527
2-2_GL0104799	K02027	*Firmicutes*	*Ruminococcaceae*		3.125361
S-Fe8_GL0281318	K02358	*Proteobacteria*	*Desulfovibrionaceae*		3.06178
6-2_GL0039188	K02906	*Firmicutes*			-1.57265
4-1_GL0031018	K02935	*Firmicutes*			1.746256
6-1_GL0094523	K10112				2.133637
1-1_GL0140275	K02994	*Firmicutes*			-5.18482
23_GL0148044	K03521				1.802111
MC-6-4_GL0004321	K03406				1.485623
7-1_GL0015471	K04078	*Firmicutes*			1.681641
1-1_GL0011264		*Firmicutes*			-1.90841
1-2_GL0077661	K02357	*Firmicutes*	*Lachnospiraceae*		2.27796
2-5_GL0018996	K04078	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.461342
G1-1A_GL0002549		*Bacteroidetes*			-1.39665
23_GL0056999	K00850	*Firmicutes*			2.501492
7-5_GL0053376	K03413	*Firmicutes*			1.726773
11_GL0100278	K00016	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-2.23979
11_GL0104167	K00134	*Firmicutes*			1.391898
MH-6-1_GL0004764	K10670	*Firmicutes*			1.377144
S-Fe20_GL0164131	K02863	*Firmicutes*			1.452199

11_GL0062770	K02035	*Firmicutes*	*Lachnospiraceae*		-2.69915
4-3_GL0014682	K02988	*Firmicutes*			-1.8482
MH-6-2_GL0041983	K02935				4.578889
G1-1A_GL0063322	K00600	*Bacteroidetes*			-1.44024
11_GL0117532	K10200	*Firmicutes*			2.143502
3-5_GL0104202	K02357	*Firmicutes*	*Lachnospiraceae*		2.618877
8-5_GL0098490	K02992	*Firmicutes*			-1.89029
1-7_GL0129013	K06142	*Bacteroidetes*	*Porphyromonadaceae*		-1.66784
32_GL0126562	K01269	*Firmicutes*			1.850507
8_GL0052796	K01692	*Firmicutes*	*Lachnospiraceae*	*Roseburia*	1.466373
16_GL0045029	K04027	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	3.604358
Group2-7A_GL0015844	K01624	*Firmicutes*			1.816092
54_GL0074441		*Firmicutes*			-1.60706
Group2-6A_GL0064249	K02027	*Firmicutes*			3.308086
S-Fe19_GL0098956	K00134	*Proteobacteria*			1.404535
16_GL0018914	K00259	*Proteobacteria*	*Desulfovibrionaceae*		2.898429
1-4_GL0093240		*Firmicutes*			-1.30884
Group2-7A_GL0062504	K02935				3.809079
11_GL0132516	K02112	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-2.34865
28_GL0002601	K03530	*Firmicutes*			2.476553
S-Fe8_GL0232913	K02032	*Firmicutes*	*Lachnospiraceae*		1.540613
Group2-5A_GL0107794		*Firmicutes*			1.909394
7-1_GL0020329	K01746				1.564712
17_GL0044668	K01193	*Firmicutes*			1.616602
MC-0-1_GL0062709	K04078	*Firmicutes*			1.355905
S-Fe2_GL0215316		*Firmicutes*	*Ruminococcaceae*		1.512247
S-Fe2_GL0048785	K00058	*Firmicutes*			1.496674
2-1_GL0115346	K02871	*Firmicutes*			-1.79343
43_GL0028121		*Firmicutes*	*Lachnospiraceae*		5.332646
S-Fe14_GL0007498		*Firmicutes*			2.963851
17_GL0147108	K02357	*Firmicutes*			1.377371
6-2_GL0078653	K02968	*Firmicutes*	*Lachnospiraceae*	*Blautia*	-2.71704
7-8_GL0118507		*Firmicutes*			1.744579
5-6_GL0055111	K01624	*Firmicutes*			2.262377
S-Fe9_GL0017967	K00257				1.550902
7-5_GL0131882		*Firmicutes*			1.325093
MC-0-3_GL0033683	K00198	*Firmicutes*			3.223115
G1-1A_GL0100166	K02961	*Firmicutes*			-1.5241

S-Fe10_GL0002666	K02996	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-2.15384
2-8_GL0023109	K02904	*Firmicutes*			-1.56722
S-Fe8_GL0208467	K02030	*Firmicutes*			-1.84334
Group2-7A_GL0136576	K02025	*Firmicutes*	*Lachnospiraceae*		-2.00808
4-2_GL0051202	K01784	*Firmicutes*			1.360792
1-3_GL0079360		*Firmicutes*			-2.05063
MH-6-1_GL0051803	K00831				2.294879
4-1_GL0003650	K00975	*Firmicutes*			1.499183
11_GL0113084	K02109	*Firmicutes*	*Lachnospiraceae*	*Roseburia*	2.01349
26_GL0091341	K02994	*Firmicutes*			-1.81416
1-4_GL0000992	K04835	*Firmicutes*			1.321592
S-Fe20_GL0026597	K00615				2.091443
14_GL0038127	K01491				2.370942
1-1_GL0039242	K01119	*Firmicutes*			1.556949
10_GL0065601	K02946	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-2.2197
2-1_GL0012242	K02986	*Firmicutes*			-1.32782
17_GL0012715	K03522	*Firmicutes*			2.613655
6-5_GL0071490	K10547				1.675712
1-1_GL0119832		*Firmicutes*			2.499472
2A-dyr14-06_GL0003431	K00703	*Firmicutes*			1.361529
4-1_GL0068233	K00975	*Firmicutes*			1.903415
4-5_GL0030359	K01923	*Firmicutes*			1.729523
6-6_GL0119787	K00634	*Firmicutes*			1.466462
2A-dyr16-07_GL0038020	K00656	*Proteobacteria*			1.331834
2-1_GL0005474		*Firmicutes*			-1.87515
1A-dyr4-07_GL0036960	K02863	*Firmicutes*			2.575518
5-5_GL0042056	K02888	*Firmicutes*			3.361511
5-1_GL0042804		*Firmicutes*			1.85395
20_GL0108954	K00703	*Firmicutes*			1.923165
7-5_GL0133410	K03624	*Firmicutes*			2.519268
41_GL0015768	K02990	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.86489
1A-dyr2-07_GL0044881	K03530				-4.4184
5-2_GL0041388	K02968	*Firmicutes*	*Lachnospiraceae*	*Blautia*	-1.45762
17_GL0132312	K01039				2.740164
4-3_GL0048965	K02030	*Firmicutes*			-1.41835
S-Fe10_GL0097175	K02887	*Bacteroidetes*			-1.98843
4-7_GL0059157	K02026	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.614437
S-Fe8_GL0110892	K01188	*Firmicutes*			1.320671
S-Fe9_GL0247939		*Proteobacteria*			2.091334

5-7_GL0002564	K00540	*Firmicutes*			-1.39753
S-Fe7_GL0048200	K03522				1.928805
2-6_GL0046249	K01746	*Firmicutes*			4.009503
1A-dyr1-07_GL0037352	K02959	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-1.89591
11_GL0042300	K02933	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-2.00291
4-7_GL0147993		*Firmicutes*			-1.50227
10_GL0078960	K01892	*Firmicutes*			1.867951
1-1_GL0020817		*Firmicutes*			1.789176
11_GL0083640	K00003	*Firmicutes*	*Lachnospiraceae*		-2.09237
7-8_GL0043249	K03406	*Firmicutes*			6.539964
10_GL0060041	K02996	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-1.53175
4-8_GL0075009	K13993	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.621931
S-Fe20_GL0041215	K11189	*Firmicutes*			2.186241
1-1_GL0109800		*Firmicutes*			1.887038
S-Fe7_GL0015571		*Proteobacteria*	*Desulfovibrionaceae*	*Desulfovibrio*	1.858734
S-Fe7_GL0186138	K03821				5.575734
4-1_GL0017830	K01428	*Firmicutes*			-1.32603
MC-6-2_GL0094791		*Firmicutes*			1.642586
10_GL0100022	K02874	*Firmicutes*	*Erysipelotrichaceae*		-1.5464
20_GL0009341					1.955976
4-1_GL0062284		*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.757266
11_GL0091485	K02796	*Firmicutes*			-2.91423
MC-6-5_GL0054982	K02034	*Firmicutes*			1.761016
14_GL0028497	K00831	*Bacteroidetes*	*Porphyromonadaceae*	*Odoribacter*	-1.50877
S-Fe10_GL0000124	K00639	*Bacteroidetes*	*Porphyromonadaceae*	*Odoribacter*	-3.07792
4-1_GL0080567		*Firmicutes*			1.995236
1-1_GL0120653	K02417	*Firmicutes*	*Clostridiaceae*		1.83973
2-1_GL0029950					-1.69105
5-1_GL0033106	K13993	*Firmicutes*			1.827648
54_GL0029562	K00975	*Firmicutes*			2.501854
S-Fe8_GL0084990	K11181	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	3.258289
2-6_GL0025516	K02051	*Firmicutes*	*Lachnospiraceae*		3.946596
2B-dyr23-07_GL0055986	K06438	*Firmicutes*			1.47474
54_GL0053952		*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	2.233656
Group2-3A_GL0017616	K01006	*Firmicutes*			2.503826
7-5_GL0167263	K02355	*Firmicutes*			2.051527
5-1_GL0082386	K02935	*Firmicutes*			2.44049
6-5_GL0115958	K01840	*Firmicutes*			1.994264
1-4_GL0028897	K02988	*Firmicutes*			1.307514

3-7_GL0065343	K03415	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.75275
S-Fe9_GL0186565	K04027	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	1.917173
7-5_GL0075461	K01681	*Firmicutes*			2.360956
4-3_GL0071645	K02871	*Firmicutes*	*Lachnospiraceae*		-1.69359
2A-dyr14-06_GL0029501	K02355	*Firmicutes*			2.426689
2_GL0032312	K02803	*Firmicutes*			4.840457
2_GL0032312	K02804	*Firmicutes*			4.840457
7-5_GL0055838	K00845	*Firmicutes*			1.736429
7-2_GL0068786	K02996	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.664853
1-1_GL0087585	K00975	*Firmicutes*	*Lachnospiraceae*		2.921186
17_GL0137863	K02871	*Firmicutes*			-2.00412
7-5_GL0112795	K03040	*Firmicutes*			2.850941
7-5_GL0107786	K01738				1.713491
S-Fe8_GL0143417	K00831				1.480665
2-4_GL0085178	K02406				-1.66829
MC-6-4_GL0006482	K02406	*Proteobacteria*	*Desulfovibrionaceae*		-1.86505
11_GL0113828		*Firmicutes*			1.36898
7-5_GL0044018	K02520	*Firmicutes*			6.156955
8-2_GL0066109	K02879	*Firmicutes*			1.557755
1A-dyr4-07_GL0048807		*Firmicutes*			2.057643
5-1_GL0078216		*Firmicutes*			2.561886
8-5_GL0051769	K02407	*Firmicutes*			4.1466
MH-6-1_GL0006056	K01869	*Firmicutes*			1.50955
16_GL0094072	K13954	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	3.274743
10_GL0023118		*Bacteroidetes*			-1.83881
G1-1A_GL0116672					1.730864

Appendix 14. List of significantly changed microbial proteins between DR and DS groups.

Accession	**KO**	**Phylum**	**Family**	**Genus**	**Pi score**
4-1_GL0071382	K03737	*Firmicutes*	*Eubacteriaceae*	*Eubacterium*	2.157735
4-7_GL0142597	K01006	*Firmicutes*			1.994797
5-4_GL0037288	K01610	*Firmicutes*			2.638343
4-1_GL0029111	K01610	*Firmicutes*			1.894645
3-2_GL0042838	K01610	*Firmicutes*			4.405079
4-2_GL0014469	K03737	*Firmicutes*			1.629989
7-5_GL0130962	K00257	*Firmicutes*			1.38061
1-3_GL0048866	K00134	*Firmicutes*			2.076912
5-7_GL0068497	K02358	*Firmicutes*			2.692905

8-5_GL0107730	K03737	*Firmicutes*			1.454575
1-1_GL0108377	K02406				-2.04495
20_GL0015122	K03737	*Actinobacteria*			1.513737
7-7_GL0107602	K00262	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	-2.24968
4-8_GL0043489	K01006	*Firmicutes*			2.11085
46_GL0030731	K00927	*Firmicutes*	*Lachnospiraceae*		2.143904
MC-6-5_GL0022456	K00134	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	-1.72721
1A-dyr1-07_GL0039079	K02112	*Firmicutes*	*Clostridiaceae*		2.126327
Group2-3A_GL0215310	K00975	*Firmicutes*			3.378685
3-6_GL0055865	K00134	*Firmicutes*			2.282689
S-Fe10_GL0045753	K00134	*Bacteroidetes*			-1.38687
3-1_GL0094445	K02117	*Firmicutes*			2.124457
2-2_GL0040883	K00197	*Firmicutes*			2.62413
2-6_GL0022887	K02117	*Firmicutes*			2.259912
Group2-8A_GL0077485	K01610	*Firmicutes*			1.73144
4-2_GL0031968	K00194	*Firmicutes*			2.003357
1A-dyr1-07_GL0014618	K01803	*Firmicutes*			1.726347
47_GL0055883	K10112				1.42229
S-Fe8_GL0285084	K00197	*Firmicutes*			2.773542
3-5_GL0030148	K02945	*Firmicutes*			2.882513
1A-dyr1-07_GL0036249	K01624	*Firmicutes*	*Lachnospiraceae*		2.630564
1-1_GL0095178	K02358	*Firmicutes*			-1.52583
14_GL0016880	K02406	*Firmicutes*			3.640021
1A-dyr1-07_GL0022532	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.83638
29_GL0009860	K00850	*Firmicutes*	*Lachnospiraceae*		1.426464
1-1_GL0060052	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.204499
S-Fe8_GL0098677	K00197	*Firmicutes*			2.058693
4-1_GL0043245	K01966	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.675602
13_GL0047875	K01006	*Firmicutes*			-2.22481
17_GL0058712		*Firmicutes*	*Lachnospiraceae*		1.521177
6-6_GL0072083		*Firmicutes*			1.467714
4-2_GL0031971	K00198	*Firmicutes*	*Lachnospiraceae*		2.666333
7-5_GL0089824	K01915	*Firmicutes*			2.030885
Group2-5A_GL0055991	K00939	*Firmicutes*			2.139176
1-5_GL0006008	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.484142
20_GL0109361	K02406	*Firmicutes*			-1.72159
4-1_GL0016153	K03043	*Firmicutes*			2.005852
MH-6-4_GL0051258	K00194	*Firmicutes*			1.66299
54_GL0061384	K01624	*Firmicutes*			1.614034

35_GL0101043		*Firmicutes*			2.522062
S-Fe8_GL0281318	K02358	*Proteobacteria*	*Desulfovibrionaceae*		2.086078
35_GL0089792	K03522	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.794729
6-2_GL0023514	K00266	*Firmicutes*			2.421062
1-1_GL0137633	K02027				1.964444
6-1_GL0094523	K10112				1.88926
MC-6-4_GL0004321	K03406				2.406954
7-1_GL0015471	K04078	*Firmicutes*			2.155495
MC-0-3_GL0034792	K04043	*Firmicutes*			1.787962
2-6_GL0089672	K02935	*Firmicutes*			2.187787
1-2_GL0077661	K02357	*Firmicutes*	*Lachnospiraceae*		2.141703
4-7_GL0002310	K02931	*Firmicutes*			-1.77324
23_GL0056999	K00850	*Firmicutes*			3.369697
11_GL0104167	K00134	*Firmicutes*			3.453493
S-Fe20_GL0164131	K02863	*Firmicutes*			3.053191
S-Fe10_GL0139765	K01624	*Bacteroidetes*			-1.6468
1A-dyr4-07_GL0095281	K02112	*Firmicutes*			1.841624
Group2-3A_GL0215228	K03569	*Firmicutes*	*Lachnospiraceae*		1.83091
MH-6-2_GL0041983	K02935				4.900363
52_GL0096773	K03522				1.441062
11_GL0117532	K10200	*Firmicutes*			2.016613
2-1_GL0001480	K02519	*Firmicutes*			1.497211
11_GL0019953	K03781	*Bacteroidetes*			-1.96684
1-1_GL0040013	K03413	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.473906
S-Fe1_GL0047662	K02986	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.50986
32_GL0126562	K01269	*Firmicutes*			3.441734
8_GL0052796	K01692	*Firmicutes*	*Lachnospiraceae*	*Roseburia*	2.662293
MH-6-4_GL0153091		*Firmicutes*			-1.91032
16_GL0045029	K04027	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	1.473811
7-5_GL0009089	K01897	*Firmicutes*	*Lachnospiraceae*		3.273293
MH-6-1_GL0083500	K02982	*Firmicutes*			2.75055
Group2-6A_GL0064249	K02027	*Firmicutes*			3.56917
8-5_GL0111994	K00266	*Firmicutes*			3.570283
S-Fe19_GL0098956	K00134	*Proteobacteria*			1.51429
16_GL0018914	K00259	*Proteobacteria*	*Desulfovibrionaceae*		1.536794
Group2-7A_GL0062504	K02935				2.52433
6-7_GL0098750	K00975	*Firmicutes*			5.121586
S-Fe8_GL0232913	K02032	*Firmicutes*	*Lachnospiraceae*		1.869271
2-2_GL0011902		*Firmicutes*			2.093279
11_GL0106920	K02994	*Firmicutes*			-3.81695
17_GL0044668	K01193	*Firmicutes*			2.015456

S-Fe2_GL0215316		*Firmicutes*	*Ruminococcaceae*		2.094489
5-6_GL0055111	K01624	*Firmicutes*			1.933731
S-Fe10_GL0182496	K02988	*Bacteroidetes*	*Prevotellaceae*		-1.48596
29_GL0115691	K02356	*Firmicutes*			1.492972
8-5_GL0117976	K03530	*Firmicutes*			-1.75361
MC-0-3_GL0033683	K00198	*Firmicutes*			2.200866
S-Fe10_GL0002666	K02996	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.70531
S-Fe10_GL0165697		*Bacteroidetes*			-1.34787
2-8_GL0023109	K02904	*Firmicutes*			-2.51727
S-Fe8_GL0208467	K02030	*Firmicutes*			-1.87949
1-1_GL0125465	K02356	*Firmicutes*	*Lachnospiraceae*		1.541656
MH-6-1_GL0051803	K00831				1.765103
Group2-6A_GL0042305	K00826	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-1.3617
1-4_GL0000992	K04835	*Firmicutes*			2.049371
1-1_GL0039242	K01119	*Firmicutes*			2.037963
Group2-3A_GL0085939	K04564	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-2.04253
1B-dyr12-07_GL0015701	K02904	*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-2.12966
8-1_GL0117490	K01572				-1.6371
1_GL0009547		*Firmicutes*	*Lactobacillaceae*	*Lactobacillus*	-2.04613
17_GL0012715	K03522	*Firmicutes*			1.410688
6-5_GL0071490	K10547				1.714373
5_GL0155572		*Firmicutes*			-1.53695
1-1_GL0119832		*Firmicutes*			2.959841
6-5_GL0050439	K00615	*Firmicutes*			1.40132
11_GL0064375	K02422	*Firmicutes*	*Lachnospiraceae*		3.296566
Group2-3A_GL0037361	K04072				-1.62882
6-6_GL0119787	K00634	*Firmicutes*			2.241823
1A-dyr4-07_GL0036960	K02863	*Firmicutes*			1.620871
20_GL0108954	K00703	*Firmicutes*			1.872829
7-5_GL0133410	K03624	*Firmicutes*			1.866335
41_GL0015768	K02990	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.70893
17_GL0132312	K01039				1.380264
G1-3A_GL0069765	K01689	*Firmicutes*			-1.72576
4-1_GL0034752		*Firmicutes*			1.639095
Group2-8A_GL0191686	K03530	*Firmicutes*			1.881748
4-7_GL0059157	K02026	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.709493
7-5_GL0079829	K01733	*Firmicutes*			1.614499
MH-6-4_GL0059966	K01615	*Firmicutes*			1.753178
S-Fe10_GL0125487		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.78845
S-Fe6_GL0119793	K00625	*Firmicutes*			1.553336

11_GL0053412	K02871	*Firmicutes*			-2.34657
3-8_GL0030619	K02518	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.727882
MH-6-5_GL0009039	K03406				2.011721
G1-5A_GL0125560	K01588	*Firmicutes*			1.51179
S-Fe3_GL0185881	K00639				1.828456
2-6_GL0046249	K01746	*Firmicutes*			2.896563
S-Fe12_GL0009091	K02358				-2.61742
2B-dyr19-06_GL0001958	K00134	*Firmicutes*	*Leuconostocaceae*	*Leuconostoc*	-1.46419
1-2_GL0120465	K02030				-1.87662
1-1_GL0073324	K03413	*Firmicutes*			1.346041
3-5_GL0118897		*Firmicutes*			3.463
10_GL0078960	K01892	*Firmicutes*			2.893371
1-1_GL0020817		*Firmicutes*			4.155352
7-8_GL0043249	K03406	*Firmicutes*			3.779614
S-Fe10_GL0003799	K04564	*Bacteroidetes*			-2.02983
MC-6-4_GL0050486	K02417	*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.527316
4-8_GL0075009	K13993	*Firmicutes*	*Clostridiaceae*	*Clostridium*	2.765524
16_GL0069107		*Bacteroidetes*			-1.45864
1-1_GL0109800		*Firmicutes*			2.249444
4-1_GL0077373	K01881	*Firmicutes*			1.595504
2A-dyr16-06_GL0053065	K02030	*Firmicutes*			-2.76713
S-Fe7_GL0186138	K03821				6.100716
MC-6-2_GL0094791		*Firmicutes*			2.3714
S-Fe10_GL0091456	K02871	*Bacteroidetes*	*Rikenellaceae*		-1.3621
4-1_GL0062284		*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	2.97151
G1-5A_GL0124160	K01785	*Firmicutes*	*Lachnospiraceae*		1.471935
2-3_GL0033567	K02876	*Firmicutes*			-1.76396
1A-dyr2-07_GL0071552	K01077	*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.30646
MH-6-3_GL0022033	K02564	*Firmicutes*			1.822556
2-2_GL0026753	K02881	*Firmicutes*			-1.30795
S-Fe10_GL0125523		*Bacteroidetes*	*Bacteroidaceae*	*Bacteroides*	-1.82599
G1-1A_GL0107723	K01811	*Bacteroidetes*			-1.35274
S-Fe10_GL0029120	K02996	*Bacteroidetes*			-2.00806
11_GL0108887	K02440	*Firmicutes*			-2.55511
MC-6-2_GL0019983	K01866	*Proteobacteria*	*Helicobacteraceae*	*Helicobacter*	-3.08522
S-Fe10_GL0044726	K03671	*Bacteroidetes*			-1.36715
2-2_GL0127446	K00059				-2.62133
S-Fe1_GL0168465	K05366	*Bacteroidetes*			-2.11185
20_GL0018358	K02356	*Firmicutes*			1.388855
1A-dyr2-07_GL0010725	K02356	*Firmicutes*	*Erysipelotrichaceae*		2.621549
G1-5A_GL0079265		*Firmicutes*	*Lachnospiraceae*		3.618043

MC-6-2_GL0181022	K02961	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.75396
5-8_GL0087468	K00941	*Firmicutes*			-2.34357
17_GL0083038	K11189	*Firmicutes*			2.104454
4-8_GL0007109	K08600				2.991225
2-1_GL0029950					-2.21913
5-1_GL0033106	K13993	*Firmicutes*			1.799286
54_GL0029562	K00975	*Firmicutes*			3.9417
14_GL0014067	K01918	*Firmicutes*			1.443751
S-Fe10_GL0164970	K02968	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	-1.46403
5-2_GL0007959		*Firmicutes*	*Lachnospiraceae*		-2.2397
14_GL0041106	K03111	*Firmicutes*			-1.4822
2-6_GL0025516	K02051	*Firmicutes*	*Lachnospiraceae*		2.344785
11_GL0151058		*Bacteroidetes*			-1.3361
G1-1A_GL0108097	K09116	*Firmicutes*			-2.24614
2B-dyr23-07_GL0055986	K06438	*Firmicutes*			2.006054
S-Fe10_GL0152300	K00940	*Bacteroidetes*			-2.43513
MC-0-1_GL0111819	K02907	*Bacteroidetes*	*Rikenellaceae*		-1.54578
54_GL0053952		*Bacteroidetes*	*Porphyromonadaceae*	*Parabacteroides*	2.185862
7-5_GL0167263	K02355	*Firmicutes*			2.321021
2-6_GL0036140	K02355	*Firmicutes*			1.340523
6-5_GL0111362	K01938	*Firmicutes*			1.781301
2-1_GL0122108	K00257	*Firmicutes*			1.55133
7-6_GL0102970	K00194	*Firmicutes*			2.728338
1-5_GL0013954	K01624	*Firmicutes*			3.877519
2-2_GL0044879		*Firmicutes*	*Clostridiaceae*	*Clostridium*	-1.32827
6-5_GL0115958	K01840	*Firmicutes*			1.777054
S-Fe20_GL0127995	K02945	*Firmicutes*			1.550872
MH-6-5_GL0010178	K01571				1.45964
2-4_GL0092915	K04077				1.838673
7-5_GL0106362	K10206	*Firmicutes*			1.360633
7-5_GL0075461	K01681	*Firmicutes*			1.902846
16_GL0016726	K02112	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	1.882786
2_GL0032312	K02803	*Firmicutes*			4.491304
2_GL0032312	K02804	*Firmicutes*			4.491304
7-5_GL0055838	K00845	*Firmicutes*			1.65443
1-1_GL0087585	K00975	*Firmicutes*	*Lachnospiraceae*		4.234711
17_GL0137863	K02871	*Firmicutes*			-1.3457
7-5_GL0112795	K03040	*Firmicutes*			2.613761
4-2_GL0042526	K02996	*Firmicutes*			-1.59999
S-Fe8_GL0143417	K00831				2.644343
7-5_GL0041572	K01876	*Firmicutes*			1.373313
S-Fe18_GL0018249	K01006	*Firmicutes*			1.618189

MH-6-5_GL0123182		*Firmicutes*			3.698369
7-5_GL0044018	K02520	*Firmicutes*			4.489843
1-1_GL0022757	K00939	*Firmicutes*			1.899592
G1-5A_GL0170492		*Firmicutes*			-1.41001
1A-dyr4-07_GL0048807		*Firmicutes*			3.314023
5-1_GL0078216		*Firmicutes*			1.924193
MH-6-1_GL0006056	K01869	*Firmicutes*			2.125049
5-1_GL0045111		*Firmicutes*			3.193614
7-5_GL0057210		*Firmicutes*			1.374318
29_GL0153864	K00615	*Firmicutes*			2.289781
16_GL0094072	K13954	*Proteobacteria*	*Desulfovibrionaceae*	*Bilophila*	4.275893
MH-6-4_GL0089458		*Firmicutes*			-1.39677
S-Fe10_GL0133980	K00831	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	1.380847
2-1_GL0068730		*Firmicutes*	*Ruminococcaceae*		1.535137
5-1_GL0082385		*Firmicutes*			1.313515

Appendix 15. List of significantly changed microbial proteins between DR and DS groups.

Accession	**KO**	**Phylum**	**Family**	**Genus**	**Pi score**
4-2_GL0062114	K01610	*Firmicutes*			-1.784619
G1-8A_GL0100601	K02406				-1.917416
2-1_GL0057488	K00262	*Firmicutes*			-1.53435
S-Fe2_GL0192770	K10112	*Firmicutes*			-1.429447
1A-dyr1-07_GL0022531	K03521	*Firmicutes*	*Clostridiaceae*	*Clostridium*	-2.130218
1-1_GL0142354	K02357	*Firmicutes*	*Lachnospiraceae*		-1.307244
MC-0-1_GL0066912		*Bacteroidetes*			-1.535996
13_GL0034842	K01624				-1.331008
4-1_GL0004142	K01952	*Firmicutes*			-2.018621
MC-0-1_GL0032314	K02886	*Bacteroidetes*			-2.592838
1A-dyr2-07_GL0064584		*Firmicutes*	*Clostridiaceae*	*Clostridium*	1.4771095
G1-1A_GL0052877	K02986	*Bacteroidetes*			-1.808863
28_GL0073235	K02931	*Firmicutes*			1.656048
5-3_GL0039091	K02996	*Firmicutes*			-1.428884
43_GL0021698					1.5740238
1-1_GL0069134	K10540	*Firmicutes*			1.4955457
MC-6-2_GL0001187	K02355	*Firmicutes*			1.9455023
1-1_GL0140275	K02994	*Firmicutes*			-3.420879
11_GL0104167	K00134	*Firmicutes*			-1.393984

7-3_GL0062023		*Firmicutes*			-1.37799
G1-1A_GL0116823	K00088				-1.484996
54_GL0074441		*Firmicutes*			-2.482337
S-Fe10_GL0174604	K03773	*Bacteroidetes*			-1.861255
11_GL0106920	K02994	*Firmicutes*			2.0637662
1A-dyr2-07_GL0052456	K01624	*Deferribacteres*			-1.319309
43_GL0028121		*Firmicutes*	*Lachnospiraceae*		2.9916246
2-1_GL0089701	K02994	*Firmicutes*			2.3474527
11_GL0161035	K02863	*Firmicutes*			1.8215553
S-Fe20_GL0100150	K01756	*Firmicutes*			1.5520883
1-3_GL0079360		*Firmicutes*			-1.495557
2-1_GL0031575	K02926	*Firmicutes*			2.7053828
1-1_GL0117261	K01999	*Firmicutes*			-1.641877
3-5_GL0017842	K02035	*Firmicutes*			1.6583989
2-2_GL0072747		*Firmicutes*			1.9444629
1-7_GL0129239	K02996	*Bacteroidetes*			-1.634238
1A-dyr2-07_GL0044881	K03530				-2.097287
1-2_GL0121808	K06972	*Firmicutes*	*Lachnospiraceae*		2.0657748
11_GL0053412	K02871	*Firmicutes*			1.4049486
MC-6-1_GL0071357	K03839	*Bacteroidetes*			-1.531537
4-7_GL0147993		*Firmicutes*			-1.69677
11_GL0143850	K01653	*Firmicutes*			-2.408603
1A-dyr2-07_GL0068618	K00074				2.4708026
MC-0-1_GL0102269	K02863	*Bacteroidetes*	*Rikenellaceae*		1.4147174
14_GL0102998	K02906	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	1.6674397
14_GL0028497	K00831	*Bacteroidetes*	*Porphyromonadaceae*	*Odoribacter*	-1.305411
MC-0-1_GL0074303	K01945	*Proteobacteria*	*Desulfovibrionaceae*		-1.500756
G1-1A_GL0052860	K02904	*Bacteroidetes*			-2.109117
7-1_GL0078399	K03060	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.5142691
4-3_GL0071645	K02871	*Firmicutes*	*Lachnospiraceae*		-1.584703
MC-0-1_GL0040326	K01803	*Bacteroidetes*	*Rikenellaceae*	*Alistipes*	-1.580046
2-3_GL0100459	K06001	*Firmicutes*			1.7382243
1-1_GL0008541	K01881	*Bacteroidetes*			-1.59461
MC-6-4_GL0006482	K02406	*Proteobacteria*	*Desulfovibrionaceae*		-1.611959
28_GL0073237	K02994	*Firmicutes*	*Lachnospiraceae*	*Butyrivibrio*	1.3201789
S-Fe3_GL0148328	K02879	*Firmicutes*			-3.063337

Acknowledgements

First of all, I would like to thank Prof. Chris Turck for giving me the opportunity to work in his group even though I had no prior wet lab experience. I learned a lot about proteomics from him and got the chance to learn and work with mass spectrometry. I really appreciate his guidance and support. He not only offered me interesting projects, but also gave me a lot of freedom to develop my own ideas and his door was always open for spontaneous discussions. As a great scientist, he also showed me how to develop management and social skills in addition to scientific research.

For the Parkinson's disease project, I would like to thank Dr. Paula Perez Pardo's group at the Utrecht University for generating the Parkinson's disease mouse model, performing the behavior analysis, and collecting the fecal pellets; and Dr. Sylvie Rabot's group at the Micalis Institute for analyzing the 16S rRNA data. For the anxiety disorder project, I would like to thank Prof. Iiris Hovatta's group at the University of Helsinki for generating the mouse model of chronic social defeat stress, performing the behavior analysis, and collecting the fecal pellets.

I am very grateful to all my Thesis Advisory Committee members: Dr. Dietmar Martin from the Gene Center and Department of Biochemistry at LMU, Prof. Dr. Jing Li from Shanghai Jiao Tong University, Dr. Alessandro Tanca from University of Sassari and Dr. Frederik Dethloff from Max Planck Institute for Biology of Ageing. Thanks to their expertise in different fields, I was able to get valuable advice on different aspects of my thesis projects.

I would like to express my gratitude to my colleagues in the group:

Giuseppina: Thank you for teaching me how to use the mass spectrometer and the importance of lab safety! I will never forget your first lesson to keep everybody safe. Also, I was always very happy to hear you share your joy of some updates about Edward:)

Frederik: I have only learned a tiny bit of your metabolomics skills, but it has been immensely beneficial for me once I started to take over the platform you had established. What a metabolomics expert!

Bozo: You showed me everything and introduced me to everyone in the institute. And you brought a lot of positive effects to my life outside the institute as well. I can definitely say that it would have been harder and more boring without you, my friend.

Yan Yu: Lucky me! Without you, I would not have had a smooth start because you went through everything before me. As an experienced student, you taught me a lot of experimental skills, and my style of doing experiments has been greatly influenced by you. I really appreciate your help at work and outside of work.

Karin: Thank you for your help in the lab! You and Yan Yu really spent a lot of your own time maintaining the instruments and helping me with troubleshooting. Everything would have been more difficult without you.

I would like to thank Prof. Bertram Müller-Myhsok and his group for taking me on and supporting me after Chris left the institute. I would like to thank the people I met at Max Planck Institute of Psychiatry, including Christina, Yijing, Anton, Ezgi, Yeho, Julia, Cristina, Dragana, Gabriel, Michaela, Dongik, Fabian, Daniel, Alessandro, Nico, Pablo, Gordan, Emanuel, Arne and Antonietta. We have been working or chilling together, I am really happy for the opportunity to spent time with you!

I would like to express my gratitude to the Roos family! Thank you for helping me get over everything related to your apartment. I only realized how lucky I am after hearing stories about struggling with setting up internet, heating, TV etc. for the apartment. You also invited me to your family events from time to time. Right after I moved in, I combined my first name with Roos to receive deliveries until I had my family name on the mailbox and door. After years of living in your apartment, I feel a connection to the Roos family.

I also want to thank all the nice people I had the pleasure meeting over the years, sometimes your kind gesture or conversation just made my day, whether we knew each other or not. This also makes me appreciate the kindness of people and still keep the faith for the bright future of the world.

Finally, a big thank you to my beloved family. My father Fanglin, my mother Shenglin and my girlfriend Yuting. It would have been impossible to achieve what I have without your support and love. I love you all!