ABSTRACT

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Diet has been implicated in many human diseases, such as colon cancer and inflammatory bowel diseases, as well as in general health promotion depending on the major dietary components consumed. Interactions of specific dietary components with the intestinal microbiota often play major roles in detrimental and beneficial health outcomes. The impact of some dietary components, such as fiber, on the microbiota and human health have been studied in great detail, while little research has been done on other major dietary components, such as protein. Dietary protein is an essential component of the human diet, as it is consumed at every meal through various sources. The process of dietary protein digestion and absorption is efficient, however, a small amount of what is ingested by the host reaches the colon, where the majority of the intestinal microbiota resides. There are indications that the source of dietary protein matters significantly in terms of health outcomes, however, the few studies that have been conducted involving different protein sources lack consensus. We used germ-free and conventional mice fed animal or plant-based protein sources to investigate host and microbiota impact on dietary protein digestion, as well as the impact of different protein sources on microbiota composition and functioning. We used metaproteomic approaches to identify and quantify tens of thousands of proteins from microbiota samples to study dietary proteins before and after passage through the intestinal tract, as well as compositional changes of the microbiota in response to different protein sources. Ultimately, this research provides insight on dietary protein sources and associated microbiota changes, which contributes to understanding the complex relationship of diet and the intestinal microbiota.

Interactions Between Dietary Protein and the Intestinal Microbiota

by
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1.1 Abstract

Evidence has started to emerge that interactions between dietary protein and the intestinal microbiota can have critical impacts on host health. Here we review the impact of dietary protein source (plant vs animal), as well as quantity consumed on this critical microbial community. We discuss factors that potentially impact interactions between dietary protein and the microbiota and the major open questions that need to be addressed.

Key Words

Dietary Protein, Gut Microbiota, Diet-Microbiome, Microbe-Host Interactions, Microbe-Microbe Interactions, Intestinal Health, Nutrient Digestion

1.2 Introduction

The community of microorganisms living in the digestive tract, the intestinal microbiota, greatly impacts human health. Diet is known to affect this vital community and interactions between dietary components and the microbiota have been implicated in a variety of human diseases (Bäckhed et al., 2005; David et al., 2014; Patnode et al., 2019; Turnbaugh et al., 2009). To unravel the complex relationship between the diet and the intestinal microbiota, recent research has focused on the impact of specific dietary components on the composition and function of the microbiota. While the interactions between the microbiota and some dietary components, such as fiber and fat, are well-studied, the impact of other major dietary components, namely protein, are still poorly understood. However, there is mounting evidence that protein source and protein quantity consumed differentially impact the intestinal microbiota and host health (Faith et al., 2011; Holmes et al., 2017; Jantchou et al., 2010; Levine et al., 2014; Song et al., 2019; Zhu et al., 2015). In this minireview, we provide an overview of what is known about the relationship between dietary protein and the microbiota, and present questions that should be addressed in future research.

1.2.1 How much dietary protein is available to the intestinal microbiota?

Studies have shown that protein affects microbiota composition and function particularly through providing nitrogen, a limiting nutrient for the intestinal microbiota (Faith et al., 2011; Holmes et al., 2017; Reese et al., 2018). Additionally, microbial fermentation of protein in the colon has been implicated in causing intestinal diseases including inflammatory bowel disease and colorectal cancer (Beaumont et al., 2017; Jantchou et al., 2010; Jowett et al., 2004). Understanding how protein influences the intestinal microbial community depends on determining the quantity of protein available to the microbiota in the colon, which is influenced by multiple factors: (1) the amount of protein consumed, (2) the conditions of the intestinal environment and (3) the source of the protein. Investigating these factors will enable determining how much of the nitrogen that arrives in the colon is converted to microbial biomass versus resilient to microbial conversion.

The amount of protein consumed influences how much protein reaches the colon, as human capacity for protein digestion and absorption is finite. Based on the USDA's 2015-2020 Dietary Guidelines for Americans, over 50% of the population surpasses the recommended daily protein allowance of 10-35% of kcal consumed (https://health.gov/our-work/food-nutrition/2015-2020-dietary-guidelines/guidelines/). The USDA recommendation is far exceeded in high-protein diets, such as those often promoted for weight loss. The quantity of dietary protein that reaches the colon due to lack of absorption by the host is estimated to range from 3-12 grams, which is approximately equal to the amount of undigested non-starch polysaccharides (Cummings and Macfarlane, 1991; Macfarlane et al., 1992a). Furthermore, studies have shown that ileal nitrogen content increases linearly as consumption of dietary nitrogen increases, suggesting that as more nitrogen is consumed, a greater quantity remains undigested (Silvester and Cummings, 1995). However, the results of these studies are based on methods of estimating human digestion of protein that are limited in multiple ways. To avoid including the microbiota's contribution of protein breakdown in estimates of protein digestion and absorption, protein digestibility is assessed from ileal effluent. Obtaining ileal effluent samples requires that protein digestibility studies are performed in ileostomy patients, sudden death victims or animal models, each of which is limited in its representation of the digestive process. Furthermore, there is currently no established method to measure dietary protein directly; common methods used to estimate protein digestibility require differentiating between endogenous and dietary protein (Macfarlane et al., 1992b). These methods make assumptions that have been shown to lead to inaccuracies in assessing protein digestibility (de Lange et al., 1990; Silvester and Cummings, 1995). We will later discuss how novel approaches offer the opportunity to measure protein directly and more accurately determine how the amount of the protein consumed impacts the amount of protein that reaches the colon.

There are various factors of the intestinal environment that can limit the availability of dietary protein, peptides and amino acids to the microbiota in the colon. One such factor, pH, may impact protease activity and the uptake of small peptides and amino acids by bacteria. The pH of the colon is influenced by undigested dietary components and metabolites. As the production of SCFAs increases, primarily from bacterial fermentation of carbohydrates, the pH of the intestinal lumen decreases, potentially inhibiting bacterial protease activity (Macfarlane et al., 1992b). Protease activity may also be impacted by other components of the extracellular environment. For example, particular amino acids inhibit protease activity in *Clostridium sporogenes* (Allison and Macfarlane, 1990). Other dietary components, such as carbohydrates, may impact microbial utilization of dietary protein by providing a more favorable substrate. Additionally, the matrix in which the dietary protein is contained may impact the availability of the protein to the microbiota (Acton et al., 1982; Barbé et al., 2014). For example, if a protein is surrounded by fiber, the carbohydrate will need to be metabolized before bacterial proteases can access the protein. This complexity may require interactions of multiple microbes to make protein accessible for microbial breakdown. Ultimately, the complex process of protein digestion is further complicated by numerous factors of the intestinal environment that can be influenced by the host, the diet or the microbial community.

Protein source dictates the amino acid profile, accessibility, processing and anti-nutritional factors affiliated with a protein, all of which influence protein digestibility (Joye, 2019). Protein source generally refers to the species from which the protein was derived, but can also be more specific (e.g. whey vs casein vs meat, all from cows). Different types of protein can differ drastically in their abundances of hundreds of different proteins that constitute a single protein source. The protein source and type also determines the amino acid profile of a protein, which influences its ability to be hydrolyzed due to the amino acid recognition specificities of proteases. Proteins with high levels of proline have less flexibility of the protein chain, resulting in less accessibility for proteases (Joye, 2019). Accessibility can furthermore be influenced by other dietary components consumed with the protein or the matrix in which the protein resides. The processing of protein can also influence digestibility. Processing can range from simple heating, such as cooking eggs, which increases the digestibility of albumin found in egg whites, to more in depth processes of protein purification for dietary supplements. Protein purification of plant-based proteins removes the majority of non-protein components of the plant, which includes many anti-nutritional factors (e.g., protease inhibitors, tannins, phytates) that decrease the digestibility of proteins (Joye, 2019). Dietary protein sources that are more resilient to digestion may have a greater impact on the microbiota, as a higher quantity will reach the colon.

The digestibility of different protein sources and the impact that source has on the microbiota may represent a link between previously observed associations of protein source and human health. For example, in a prospective cohort study looking at the diets of over 130,000 participants, high animal protein intake was associated with cardiovascular mortality while high plant protein intake was inversely associated with all-cause and cardiovascular mortality (Song et al., 2019). Furthermore, a substitution of plant protein for various animal proteins resulted in an estimation of lower mortality. It is generally accepted that animal-based protein has higher digestibility than plant-based protein, but there have been minimal investigations comparing purified protein sources. Comparing digestion of purified animal and plant protein sources would likely minimize the anti-nutritional factors associated with plants and be more reflective of the protein sources in dietary supplements. Studies involving such carefully designed diets could address whether protein sources differ in their resilience to digestion by the host and microbiota.

In addition to dietary protein, non-dietary sources of protein need to be considered when studying protein degradation and consumption by the microbiota. Protein from the host is potentially available from the secretion of digestive enzymes, tissue sloughing throughout the digestive tract, and the mucosal layer. Although the mucins that comprise the mucosal layer consist primarily (80%) of carbohydrates, protein comprises the backbone of the mucin domains (Johansson et al., 2013). This protein core is protected by glycans which must first be cleaved by the microbiota in order for the bacterial proteases to access this protein source (Johansson et al., 2013). Another source of protein available to the microbiota is microbial protein in the form of secreted proteins or those released through cell lysis in the upper regions of the digestive tract. The diet itself can also contribute microbial protein to the protein pool in the colon, as the processing of numerous foods, such as cheese, involves microbes. It has been shown that while food derived microbes may not persist in the colon, they are detectable (and viable) in appreciable numbers in fecal material, showing they are somewhat resilient to digestion and serve as an additional source of protein (David et al., 2014).

1.2.2 How does dietary protein impact the composition and function of the microbiota?

One of the major functional interactions of intestinal microorganisms with protein that has been studied in some detail is protein fermentation. Proteins, peptides and amino acids that are not absorbed in the small intestine eventually reach the colon where they alter the environment of the intestinal lumen and act as a substrate for microbial metabolism. Undigested protein is hydrolyzed to peptides and amino acids by bacterial proteases upon reaching the large intestine and fermented to various, potentially toxic, end products (Gilbert et al., 2018). Once a peptide or amino acid has been transported into a bacterial cell, it may be incorporated into microbial protein or metabolized to numerous metabolites, depending upon the particular amino acid. Dai et al., have compiled an extensive list of amino acid fermenting intestinal bacteria (Zhao-Lai Dai, Guoyao Wu, 2011). There is *in vitro* and limited *in vivo* evidence that suggests *Bacteroides* species may be partly responsible for microbiota shifts in response to dietary protein intake as these community members comprise a major percentage of the intestinal microbiota and have protein fermentation capabilities(Macfarlane et al., 1986). There are other species that comprise smaller portions of the community that are also capable of protein fermentation, such as *Clostridium* and *Propionibacterium*. The products of bacterial protein fermentation, including branched-chain fatty acids, have been measured in greater concentrations in the lower regions compared to upper regions of the intestinal tract, illustrating the prevalence of protein fermentation by the microbiota (Macfarlane et al., 1992b). Branched-chain fatty acids have frequently been used to measure microbial protein fermentation as they are an exclusive product of branched-chain amino acid breakdown. Short-chain fatty acids (SCFAs) are also a product of microbial protein fermentation, but can originate from the metabolism of carbohydrates as well as protein. Multiple reviews discuss the products of protein fermentation in greater detail (Fan et al., 2015; Gilbert et al., 2018; Windey et al., 2012) (Fan et al., 2015; Gilbert et al., 2018; Windey et al., 2012).

Studies of proteins and their effects on the intestinal microbiota have benefited from the use of mouse models. Although human and mouse intestinal microbiotas are compositionally different, they are functionally similar; mouse models can also have defined microbial communities and highly controlled diets (Doré et al., 2015). A pilot study in mice demonstrated that a high protein diet decreased the diversity of the microbiota and decreased the ratio of Firmicutes:Bacteroidetes (Kim et al., 2016). In gnotobiotic mice with a defined 10-species microbiota, dietary protein was shown to be more influential on microbial community biomass (DNA/µg fecal material) and the abundance of all community members than fat, polysaccharide, or sugar (Faith et al., 2011). Dietary protein was found to limit the biomass of the community, but changes in species abundances were not uniform. Three of the ten community members, *E. rectale*, *D. piger*, and *M. formatexigens,* decreased in abundance with an increase in dietary protein. Overall, these studies show that the quantity of dietary protein consumed is likely to be highly influential to the intestinal microbiota and merits further investigation through human and animal studies to assess the connection with host health.

Changes in the microbiota also appear to be protein source dependent. For example, hamsters fed a plant-based, soy protein diet had higher microbial diversity than hamsters fed an animal-based, milk protein diet (Butteiger et al., 2016). Additionally, rats fed a chicken meat protein diet had higher abundances of *Lactobacillus* than rats fed soy and casein protein diets (Zhu et al., 2015). Yeast-based protein originating from *Cyberlindnera jadinii* has also been correlated to compositional changes in the microbiota in piglets (Iakhno et al., 2020). Thus, although the number of animal studies examining dietary protein source are limited, they strongly suggest that dietary protein source affects microbiota composition.

Controlled diet studies in humans have presented somewhat contradictory results regarding compositional and functional changes of the microbiota in response to an increase in dietary protein consumption. These contradictions may be due to (1) interference from other dietary components, (2) the level of taxonomy investigated or (3) differences in the protein supplement sources included in the diets.

As previously mentioned, non-protein dietary components can influence the availability of protein and offer additional substrates to the microbiota. Often when high protein diets are designed in diet-microbiota studies, carbohydrates are substituted with extra protein, resulting in high protein diets that are simultaneously low carbohydrate diets. A study conducted in overweight individuals found no significant changes in abundances of the microbiota at the phylum level when a weight loss diet, high in protein and low in carbohydrates, was consumed (Walker et al., 2011). However, when targeted qPCR was used to further explore bacteria in the Firmicutes phylum, an increased abundance of uncultured *Oscillibacter,* and decreased abundances of *Eubacterium rectale* and *Collinsella aerofaciens* relatives were found for the high protein diet. Another study investigating the effects of a high protein (and low carbohydrate) diet, found increases in microbial gene richness when overweight individuals with low microbial gene richness consumed a high protein diet for 6 weeks (Cotillard et al., 2013). Fiber is a non-protein dietary component that can interfere with conclusions about the influence of protein on the microbiota. Microbiota compositional changes were quickly evident in a study in which participants were limited to solely animal-based diets or solely plant-based diets (David et al., 2014). In this study, the source of dietary protein was restricted to either plant or animal but it was not possible to disentangle the other factors accompanying the respective diets, such as limited fiber content in the animal-based diet. In future studies it is necessary to control for other dietary components in order to investigate the exclusive influence of dietary protein on the microbiota.

Contradictions between studies may also be due to the level of taxonomy investigated. Examining compositional changes at the phylum level may not provide a clear understanding due to the differing responses of individual species within each phylum. For example, while an overall decrease in Proteobacteria was reported for mice fed a high protein diet, an increase in *Escherichia coli* was reported following a high protein diet in a separate study performed in rats (Kim et al., 2016; Mu et al., 2017). A decrease in Firmicutes abundance was found when mice were fed a high protein diet, yet another study found increased dietary protein to correlate with increased abundances of *Blautia hydrogenotrophica*, a species capable of removing fermentation end products (Faith et al., 2011; Kim et al., 2016). Similarly, in a human study, Bacteroidetes abundance was negatively correlated with amino acid-derived metabolite concentrations but two genera within the Bacteroidetes phylum, *Butyricimonas* and *Odoribacter*, displayed a positive correlation (Beaumont et al., 2017). As these studies look at different levels of taxonomy, it is difficult to compare results to assess the potential impact dietary protein has on microbiota composition.

Protein supplements offer a way to increase dietary protein without altering other dietary components. However, the few studies that have used supplements have contradictory conclusions, perhaps because the protein supplement source was not consistent. When overweight individuals consumed casein or soy dietary supplements, no significant changes to microbiota composition were observed but changes to community function were suggested by fecal and urinary metabolites produced by the microbiota (Beaumont et al., 2017). Bacteria in the Firmicutes phylum including Clostridiales, *Christensenellaceae*, *Ruminococcaceae*, and *Oscillospira* were positively correlated with increased concentrations of amino acid degradation products. Contrary to the two previously mentioned studies in overweight individuals, microbiota compositional changes were observed when athletes consumed protein supplements consisting of whey isolate and beef hydrolysate (Moreno-Pérez et al., 2018). Although changes in SCFAs were not detected with protein supplementation, decreases of some SCFA producers, including the genera *Coprococcus*, *Roseburia*, and *Blautia*, and species *Bifidobacterium longum*, were found in the protein supplemented group (Moreno-Pérez et al., 2018). Also observed was an increase in the

Bacteroidetes phylum, which includes species with proteolytic activity. In summary, animal and human studies both suggest dietary protein quantity and source greatly impact the microbiota, yet more controlled studies focused on the questions we describe below are needed to clarify contradictions and understand the impact on host health.

1.2.3 Major open questions in understanding the relationship between dietary protein, the intestinal microbiota and host health, and potential methods for addressing them.

To better understand the relationship between dietary protein and the microbiota, studies must be designed using methods that investigate the fate of dietary protein in the intestinal tract, directly link dietary protein to microbial metabolizers and differentiate effects of different protein sources and quantities (Figure 1-1).

Further study of dietary protein is required to understand how this essential nutrient impacts the microbiota. Dietary components must be studied individually (e.g., as a purified soy protein supplement) and in their original form (e.g. unprocessed soybean) in order to fully understand the complex role diet plays in influencing the microbiota. Plant protein has been associated with decreased digestibility yet increased longevity. As outlined in Figure 1-2, if a non-purified plant protein is consumed, it will likely be surrounded by a matrix (e.g. fiber), decreasing the availability of the protein for microbial fermentation. Thus, unless the host consumes a diet consisting of a large quantity of *purified* plant protein, increased protein fermentation in the colon is unlikely for plant-based protein sources. Further analysis of the purity of protein sources, amino acid composition, metabolite presence and interactions with other dietary components will be highly valuable to understanding dietary protein as a nutrient and sorting out the interplay of factors influencing the availability of dietary protein to the microbiota.

Currently our understanding of protein-microbiota interactions (and overall influence of diet on the microbiota) is primarily limited to correlation-based evidence. A recent study was able to directly link dietary components to microbial metabolizers through use of magnetic beads bound with dietary fibers. The beads acted as biosensors that were recoverable following passage through the digestive tract (Patnode et al., 2019). Furthermore, mass spectrometry-based metaproteomic analyses have also been developed to directly link substrates to microbial metabolizers using natural isotope ratios (Kleiner et al., 2018). Isotopic labeling of dietary components, host and emerging Protein-SIP methods can also be used with mass spectrometry methods to track specific components as they are metabolized and assimilated in cells. These modern approaches, along with other advanced "-omics" techniques can be applied to further our understanding of the relationship between dietary protein and the microbiota.

Nitrogen balance studies aimed at estimating protein digestibility are limited by their inability to differentiate between host and dietary protein. Protein digestibility is often estimated by subtracting the nitrogen in fecal or ileal effluent produced when the animal is fed a nitrogen-free diet (i.e., host protein) from total nitrogen when the animal is returned to a nitrogen-containing diet (i.e., host and dietary protein). This method is limited as a diet completely void of nitrogen is not representative of a typical diet and may alter host secreted nitrogen. It is also worth noting that nitrogen balance studies work under the assumption that all nitrogen is present in the form of protein. Such indirect measurements of protein digestibility can now be clarified by applying high-resolution mass spectrometry based metaproteomic methods to intestinal and stool samples to understand the fate of dietary protein. These more advanced methods allow host, dietary and microbial proteins to be distinguished, which is currently a limitation in nitrogen balance studies looking at the digestion of dietary protein. Proteins from different dietary sources can also be identified from a single sample. Additionally, quantification of a large diversity of proteins from a single protein source will determine if specific proteins are differentially digestible/degradable by the host or intestinal microbiota. Mass spectrometry based studies also allow for the examination of different host digestion proteins secreted in the digestive tract. These enzymes have been shown to vary in mice with different microbial colonization states (Lichtman et al., 2015). Bioinformatic approaches to investigate biosynthetic pathways and activity-based probe enrichment are methods that have been developed to address the gap in understanding of bacterial pathways and can be applied to bacterial protein fermentation (Mayers et al., 2017; Schneider and Balskus, 2018).

Studies in animals allow for complete control of diet and examination of dietary protein throughout the digestive tract via necropsy. While studies in humans are essential for the ultimate goal of modulating the microbiota through diet, human studies must be carefully controlled to avoid issues associated with diet intervention compliance and dietary recall inaccuracies.

Currently, the relationship between dietary protein and the intestinal microbiota is not well understood. The contribution of both host digestion and microbial fermentation adds complexity to the metabolism of dietary protein. Well controlled studies that focus exclusively on dietary protein source and quantity will aid in furthering our understanding of a dietary component that constitutes a vital portion of the human diet and likely plays an impactful role in the composition and function of the intestinal microbiota, and in turn, host health.

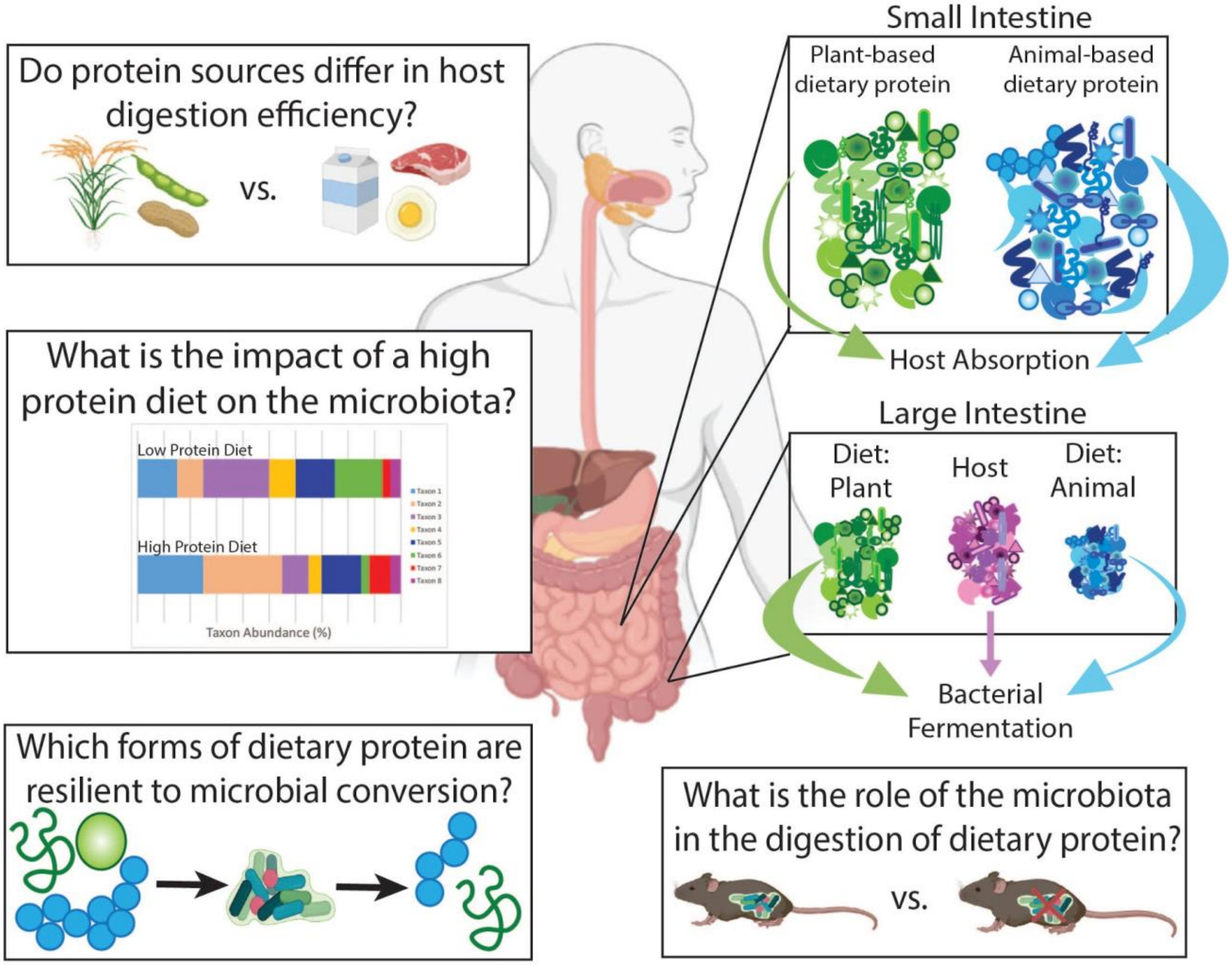

Figure 1-1. Open questions related to the relationship between dietary protein and the intestinal microbiota. Each dietary protein source consists of a unique set of hundreds to thousands of different individual proteins. These individual proteins likely have varying degrees of resiliency to digestion by both the host and microbiota. Plant-based protein is thought to be less efficiently digested by the host, compared to animal-based protein. Less host digestion would result in a greater proportion of protein reaching the colon, where it has the potential to impact the composition and function of the microbiota. In the large intestine, the microbiota ferments undigested dietary protein likely alongside other protein sources, such as host-derived protein.

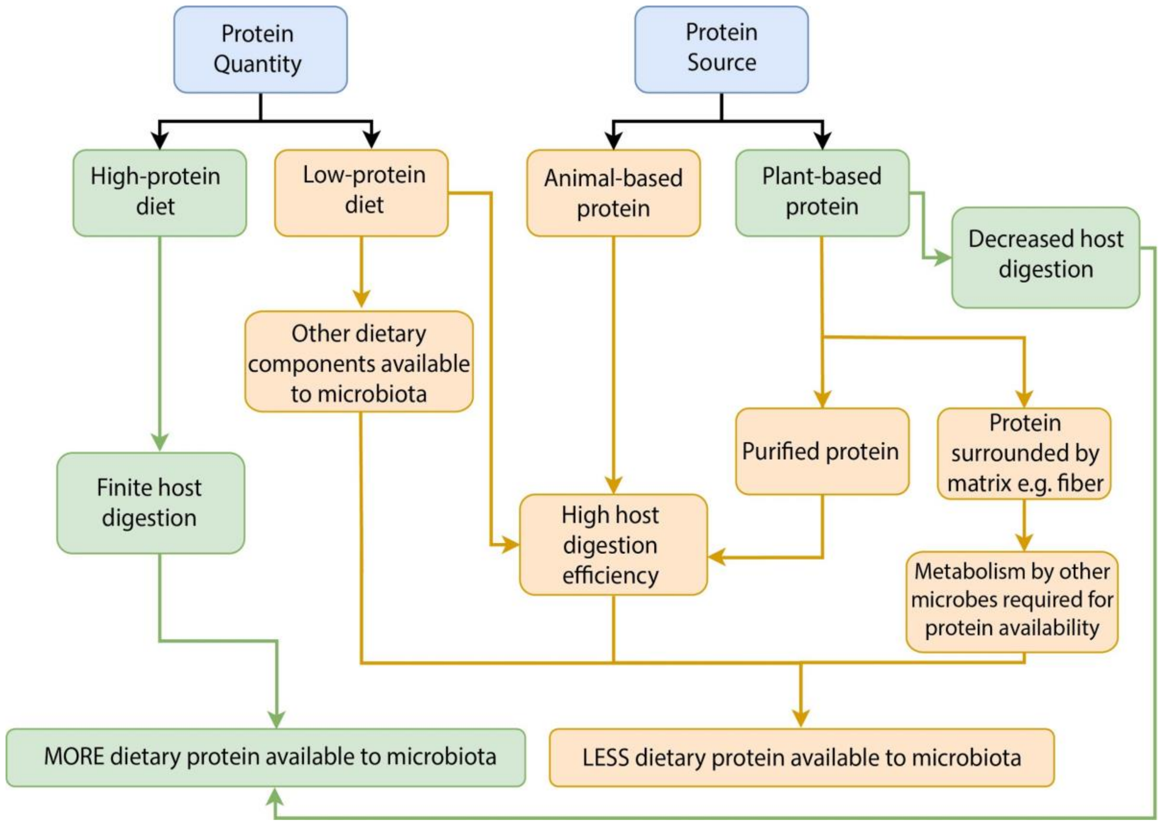

Figure 1-2. Many factors can potentially influence the availability of dietary protein for fermentation by the intestinal microbiota. Influencing factors stemming from the diet of the host (shown in blue boxes) could result in various host-microbe and microbe-microbe interactions, ultimately leading to increased or decreased protein available for fermentation in the colon. Green boxes and matching arrows illustrate factors that likely result in greater protein availability in the colon. Factors that likely result in less protein available for fermentation by the microbiota in the colon are indicated in orange boxes and arrows.

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Author Contributions

AB and MK both wrote the manuscript.

Declaration of Interests

The authors declare no competing interests.

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CHAPTER 2- METAPROTEOMICS AS A TOOL TO STUDY HOST ASSOCIATED-MICROBIOTA

2.1 Using Metaproteomics to study host-associated microbiota

The intestinal microbiota has been established to be highly influential to host health and greatly impacted by the diet of the host. Understanding the relationship between diet and the microbiota is extremely complex due to the numerous factors influencing the relationship (numerous dietary components, host digestion, etc). Metaproteomics offers a valuable advantage when approaching this challenge, as it provides the ability to identify and quantify proteins from the host, diet and microbiota in a single sample. The high throughput nature of this approach in combination with other -omics techniques is key in addressing the complex questions surrounding diet-microbiota interactions.

Proteomic analyses aim to characterize the entirety of a cell's proteome. In the field of metaproteomics, analysis extends to include all proteins in a given environmental sample. Rather than the proteome of interest containing a single cell type, a metaproteomic sample may include numerous sample types, such as a soil sample or human saliva which contains a wide array of bacterial, viral and host (plant/human) proteins. Metaproteomics is a rapidly expanding field that is complementary and extends upon other -omics technologies, such as the use of metagenomic and metatranscriptomic data in proteomic database curation. However, metaproteomics can expand on metagenomic and metatranscriptomic approaches by informing what genes are actually expressed. Furthermore, metaproteomics has the advantage over metabolomics, as proteins identified can be directly linked to the species in which they originated.

Metaproteomics is a powerful tool for studying the dynamics of a microbial community and the interactions with a host. In addition to providing valuable insight into gene expression, metaproteomics can be extended to investigate numerous questions relating to the composition and function of a microbial community. Using metaproteomics to assess taxonomic composition can provide an expanded perspective on community structure compared to traditional methods based on quantifying cell numbers (Kleiner et al., 2017). When evaluating community structure by cell numbers, a species with a small cell volume will be evaluated similarly as a species with a very large cell volume. Even if these species are present in equal cell numbers, the proportion of biomass they contribute to the community will be vastly different. Using metaproteomics to assess community structure takes into account the respective proteinaceous biomass contribution of each community member, potentially providing a more accurate picture (Kleiner et al., 2017).

Metaproteomics can also be employed to investigate the substrates of specific community members. This can involve tracking the assimilation of isotopically labeled substrates, such as ^{13}C or ^{15}N (Bryson et al., 2016). Furthermore, methods have been developed that take advantage of natural isotope ratios, avoiding the need for labeling (Kleiner et al., 2018). This method allows direct linkage of a microbe to its substrate through comparison of the natural isotope ratio of a microbe to the ratio of the available substrates. Direct linkage of bacterial species to their substrates allows for further understanding of the community metabolism and physiology.

2.2 Shotgun Metaproteomics Workflow

As a relatively new and rapidly advancing field, there are multiple approaches to metaproteomic analyses. “Top-down” metaproteomics aims to characterize intact proteins.

This approach can provide valuable insight on protein isoforms and post translational modifications, but there are currently major limitations (Shan et al., 2013). Intact proteins are more difficult to fractionate, ionize and fragment than peptides, resulting in “bottom-up” proteomics being the more widely adopted approach for metaproteomic analysis (Shan et al., 2013). Bottom-up proteomics involves the analysis of peptides to identify proteins in a sample. Hybrid approaches also exist, referred to as middle-down proteomics, which focus on larger peptides than bottom-up approaches, decreasing some of the redundancy that occurs when analyzing small peptides. When bottom-up proteomic analysis is applied to protein mixtures, such as those investigated in metaproteomic studies, the term “shotgun” proteomics is also used (Yates, 1998).

In a shotgun metaproteomics study, there is a general workflow with numerous opportunities for divergence depending upon the sample type and study design (Figure 2-1). A generalized workflow begins with extraction of proteins and digestion to peptides. The peptides are then separated by liquid chromatography (LC) and analyzed by mass spectrometry (MS). The spectra obtained are searched against a database of reference protein sequences that have undergone an *in silico* digestion to identify the peptides. This probabilistic approach requires filtering of the identified peptides (For example, by false discovery rate) to infer from which proteins the peptides originated. The identified proteins can be used to examine a wide array of biological questions.

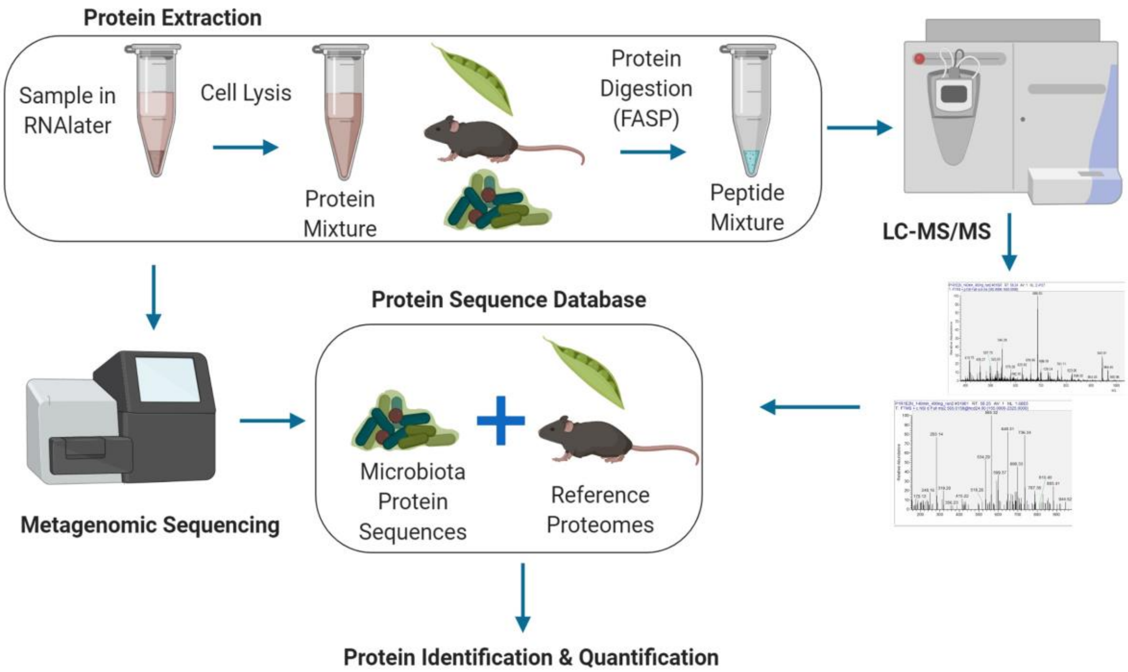

Figure 2-1. Example of shotgun metaproteomics workflow. In this example, proteins are extracted from a mouse fecal sample. Following cell lysis (by mechanical/chemical methods), the Filter Aided Sample Preparation (FASP) method is used for sample cleanup followed by tryptic digestion. Peptides are separated using liquid chromatography and analyzed by tandem mass spectrometry (LC-MS/MS). High resolution mass spectra are searched against a database containing protein sequences representative for the sample (In this example, mouse dietary and microbiota protein sequences). The ideal protein sequence database for complex samples is derived from metagenomic sequencing of the same samples for proteomic analysis. Figure created in part with biorender.com.

To begin a metaproteomic analysis, a cell lysate is generated from a sample, typically using sodium dodecyl sulfate (SDS) for protein solubilization (Wiśniewski et al., 2009; Zhang et al., 2018). Depending upon the sample, additional methods of mechanical disruption such as bead-beating or ultrasonication may be required for protein extraction, particularly for samples that include gram-positive bacteria (Zhang et al., 2018). If SDS was used, the protein mixture must be purified to remove the SDS, as it interferes with downstream enzymatic digestion and MS analysis (Wiśniewski et al., 2009). The Filter Aided Sample Preparation (FASP) protocol is frequently used for the cleanup of samples in a metaproteomics pipeline (Wiśniewski et al., 2009). Alternatively, an in-solution digestion can be performed using MS-compatible surfactants for solubilization (Choksawangkarn et al., 2012). Fractionation of proteins, through the use of 2D-PAGE or differential centrifugation, may be performed to decrease complexity of samples (Klose 1975, O'Farrell 1975). However, these methods decrease through-put, as a single sample may result in many fractions/gel slices. Liquid chromatography (LC) offers greater opportunity for separation, ultimately leading to a greater number of proteins identified in complex samples (Hinzke et al., 2019).

Following protein extraction and sample cleanup, an enzymatic digestion is performed, often using the highly specific protease trypsin or other proteases which cleave the protein at known residues, resulting in a peptide mixture for analysis (Vandermarliere et al., 2013). In bottom-up proteomics this same cleavage is performed *in silico* on a protein sequence database to produce a database of all possible peptides present in the sample. This database will later be used to search the spectra generated from MS analysis.

Separation of peptides by liquid chromatography is crucial in the detection of peptides for complex metaproteomic samples, as peptides selected for fragmentation must be more abundant than other peptides measured at the same time and also have a stronger signal than the background (Shan et al., 2013). In metaproteomics, one dimensional reversed-phase liquid chromatography is commonly used to separate peptides by hydrophobicity using a C18 column. A second degree of separation can be incorporated by prefacing the hydrophobic separation with a strong cation exchange column, providing greater sample depth (Hinzke et al., 2019).

Mass spectrometry analysis can be simplified into three major phases: (1) Generate ions, (2) Separate ions and (3) Detect ions. To generate peptide ions, nanoelectrospray and MALDI are the two main ionization methods utilized (Shan et al., 2013). Nanoelectrospray is typically used when samples are separated by reversed-phase liquid chromatography while MALDI is used for samples separated by gel electrophoresis (Han et al., 2008). Mass analyzers separate ions by their mass-to-charge ratio before they are detected qualitatively and quantitatively. Mass analyzers used in shotgun proteomic analyses include quadrupoles, ion traps, Time of Flight, Orbitrap and Ion Cyclotron Resonance, all of which offer different advantages for sensitivity, speed or accuracy (Han et al., 2008). In metaproteomics, tandem mass spectrometry is commonly used in which two mass analyzers are employed to separate and detect ions from highly complex samples. Initial precursor ions, MS1, are scanned first to determine the most abundant ions. The most abundant ions are then selected, fragmented and scanned further to produce MS2 spectra. Most commonly, collision-induced/activated dissociation is used as a method for peptide fragmentation and the majority of methods rely on proton transfer (Han et al., 2008).

For relative quantification of peptides, stable isotope labeling or label-free approaches can be used. There are numerous methods of isotopic labeling, which use isotopes as quantification standards within a sample. Quantification using the label-free approach can be performed by (1) measuring ion peak signal intensity or (2) counting peptide spectral matches (Shan et al., 2013). Measuring ion peak signal intensity, also referred to as the area under the curve quantification method, uses information acquired from the precursor ions

(MS1). Counting spectral matches uses MS2 product ion scans to correlate the number of spectra that match to all peptides from a particular protein with protein abundance. Although both quantification methods are currently used in metaproteomic studies, spectral counting has been shown to be a more robust method for protein expression in complex microbial communities (Kleiner et al., 2017).

A database search of the experimentally acquired peptide spectra against a theoretically digested protein database (based on experimental digestion) is performed for protein identification. The matched spectra are filtered based on probability often with a 5% False Discovery Rate (FDR) cutoff, indicating that 5% of the total identifications are potentially incorrect. A decoy database, composed of randomized or reversed sequences, is used to estimate the FDR. Further filtering may also be performed to increase confidence in the identified proteins. For example, one may filter identified proteins to include only those with uniquely matching peptides. Protein inference of identified peptides then results in a list of proteins from the sample (Nesvizhskii AI., 2005). Protein inference is a continuous challenge in the metaproteomics workflow as peptides can match to multiple protein sequences. The database used is highly influential to the outcome of the experiment. The best database includes all proteins potentially present in a sample with no redundancy. It has been shown that a matched metagenomic database, although more computationally and financially costly, is the best practice for metaproteomic studies (Tanca et al., 2016). Although metaproteomic analysis offers an extensive opportunity to study an uncountable number of biological questions in many fields, it is essential to note the constant changes of protein abundance and modifications mean that each sample only represents a single snapshot.

In Chapter 1, we described the many pressing questions that remain to be addressed to elucidate the relationship between dietary protein and the microbiota (Figure 1-1). Many of these questions have remained unaddressed due limitations in approaches to studying dietary protein digestion. In Chapter 3, we address these questions using mass-spectrometry based shotgun metaproteomics.

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CHAPTER 3- INTERACTIONS BETWEEN PROTEIN SOURCE AND THE INTESTINAL MICROBIOTA IMPACT THE DIGESTION OF DIETARY PROTEIN

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3.1 Abstract

Diet has been shown to be widely influential to the intestinal microbiota and in turn host health. The interactions of some dietary components, such as fiber, with the microbiota have been studied in great depth while other dietary components, namely protein, remain poorly understood. We examined the fate of dietary protein from different protein sources in both germ-free and conventional mice to a) evaluate the role of the host and microbiota in the digestion of protein and b) the impact of different protein sources on the composition and function of the microbiota. We formulated diets with a single source of dietary protein from plant (soy) and animal (casein) derived sources. Following consumption of these diets by germ-free (n=11) and conventional mice (n=11), we collected fecal samples and contents from the duodenum, ileum, cecum and colon. To perform metaproteomic analysis, we extracted and analyzed the metaproteome by LC-MS/MS. Additionally, we performed metagenomic sequencing to identify and quantify proteins using a matched metagenomic database. We identified tens of thousands of microbial, diet and mouse proteins for both germ-free and conventional mouse samples. We found dietary protein abundances in the small intestine to be similar between conventional and germ-free mice. However, in samples from the large intestine, dietary protein abundances differed by mouse type. Furthermore, we found dietary trypsin inhibitors to be enriched in the feces of germ-free mice as compared to conventional mice. This suggests that microbiota members can degrade these resilient proteins and likely play an influential role in the digestion of dietary protein. We also found significant differences in microbial community composition between mice in the different diet groups.

Key Words

Dietary Protein, Gut Microbiota, Diet-Microbiome, Microbe-Host Interactions, Microbe-Microbe Interactions, Intestinal Health, Nutrient Digestion, Lactococcus lactis, Soy Protein, Casein Protein

3.2 Introduction

Protein is a major nutrient for mammals, which can be sourced from numerous animal, plant and even microbe-based foods. A single protein source (e.g. soy) represents hundreds of individual proteins, each of which have unique properties influencing their individual digestibility (Bartlett and Kleiner, submitted, See Chapter 1). Although protein digestion and absorption by the host is efficient, a large number of these individual proteins reach the large intestine, serving as a substrate for the intestinal microbiota, the community of microorganisms within the digestive tract established to be influential to host health (Bäckhed et al., 2005; David et al., 2014; Gilbert et al., 2018; Patnode et al., 2019; Turnbaugh et al., 2008).

Some dietary components, such as fiber, have been heavily researched and shown to have major impacts on the composition and function of the intestinal microbiota with subsequent major impacts on host health (Desai et al., 2016). Other dietary components, such as protein, have remained minimally investigated. We have previously described the pressing need to understand the interactions of this essential nutrient on the microbiota (Bartlett and Kleiner submitted, See Chapter 1). Compared to fat, sucrose and polysaccharide, protein has been shown to be the most impactful dietary component in predicting the response of a 10-member microbial community in gnotobiotic mice (Faith et al., 2011). Dietary protein that reaches the colon is metabolized by a range of microbiota members, resulting in end products that may be detrimental to the host (Zhao-Lai Dai, Guoyao Wu, 2011).

There has been increasing evidence suggesting protein from different sources (animal vs plant) differentially impacts the intestinal microbiota and host health (Faith et al., 2011; Holmes et al., 2017; Jantchou et al., 2010; Levine et al., 2014; Song et al., 2019; Zhu et al., 2015). Animal protein is generally considered to have higher digestibility, resulting in less protein reaching the colon, while plant protein digestibility is generally lower due to the indigestible cell wall and anti-nutritional factors associated with plants (Gilani et al., 2012; Joye, 2019; Zhao et al., 2019). However, previously conducted studies have lacked consensus and large knowledge gaps remain in understanding the fate of dietary protein from different protein sources and the resulting impact on the intestinal microbiota (Bartlett and Kleiner submitted, See Chapter 1).

Seminal studies comparing animals with or without an intact intestinal microbiota have shown that the microbiota interacts with dietary components to affect host energy harvest (Bäckhed et al., 2004, 2007; Turnbaugh et al., 2008). Until recently, there were limitations to assess the role of the microbiota in the digestion of dietary protein due to a lack of approaches to identify and quantify large numbers of diet, host and microbiota proteins from intestinal content and stool. Here we have used mass spectrometry-based metaproteomics to compare the fate of dietary protein in conventional (C) and Germ-free (GF) mice. We have examined the role of the microbiota in the digestion of both an animal and plant-based protein source.

3.3 Results

3.3.1 Microbiota role in the digestion of dietary protein

We designed isocaloric diets with a single source of dietary protein that was either animal-based (casein) or plant-based (soy protein isolate). These 20% (by weight) protein diets were fed *ad libitum* to germ-free (n=6 soy, n=5 casein) and conventional (n=6 soy, n=5 casein) C57BL/J mice (Fig 1A). After seven days, fecal samples were collected from all mice and necropsy was performed to collect content from different regions of the intestinal tract. We used mass-spectrometry based metaproteomics to identify and quantify a total of 6,782 host proteins, 5,806 dietary (soy and cow) proteins and 28,157 microbial proteins from the intestinal content and fecal samples collected. We also included samples of the diets themselves in the metaproteomic analysis, to evaluate the fate of both an animal and plant-based dietary protein source. We identified an average of 681 soy proteins in soy diet samples and an average of 124 cow proteins in the casein diet samples, suggesting a difference in complexity of the protein sources in the two diets (Table 3-1).

Table 3-1. Average dietary proteins, bacterial proteins and bacterial PSMs for the two diets in the study.

Diet	Average Bacterial Proteins	Average Bacterial PSMs	Average Dietary Proteins
Soy	**57**	**323**	**681**
Casein	**165**	**1494**	**124**

To examine the role of the microbiota in the fate of dietary protein, we compared dietary proteins identified in GF and C mice in different regions of the digestive tract (Figure 3-1B). The overlap of dietary proteins identified in both GF and C mice is greatest in samples from the small intestine (Duodenum and Ileum samples). 83% of soy proteins and 92% of cow proteins that were identified in the duodenum were identified in at least 50% of samples in both GF and C mice. This overlap of protein identifications is lower in the cecum, colon and fecal samples. In the colon, only 50% of soy proteins and 59% of cow proteins were identified in both GF and C mice. Interestingly, a higher percentage (39%) of total soy proteins identified in the colon contents were only identified in the GF mice compared to soy proteins only identified in the C mice (11%). Similarly, 35% of all cow proteins identified in the colon contents were only identified in the GF mice, whereas only 6% were uniquely identified in the C mice.

We filtered soy dietary proteins identified in mice consuming the 20% soy-based protein diet for at least 1 protein unique peptide and 10 or more Peptide Spectral Matches (PSMs) in at least one sample per sample group (to remove low abundance proteins). When we used euclidean distance to cluster samples based on the abundances of the 136 soy proteins identified, samples separated into two major groups (Figure 3-1C). One branch encompassed samples primarily from the small intestine, including all ileum and duodenum samples in addition to two cecum samples. Within this upper intestinal tract branch, clustering by mouse type (GF vs C) was limited, illustrating similarities in the abundances of dietary proteins identified in the upper intestinal tract of both mouse groups. The second major branch was composed of cecum, colon and fecal samples. Here, we observed clustering by mouse type, showing that mouse type (GF vs C) influences the abundance of dietary proteins identified in the lower regions of the intestinal tract. We then performed this same clustering based on the abundances of the 243 cow dietary proteins identified in mice consuming the 20% casein-based protein diet and found clustering patterns to be similar to those observed for the soy dietary proteins (Figure 3-1D). These clustering patterns were further confirmed in our statistical analysis of relative abundance for individual proteins, as described below.

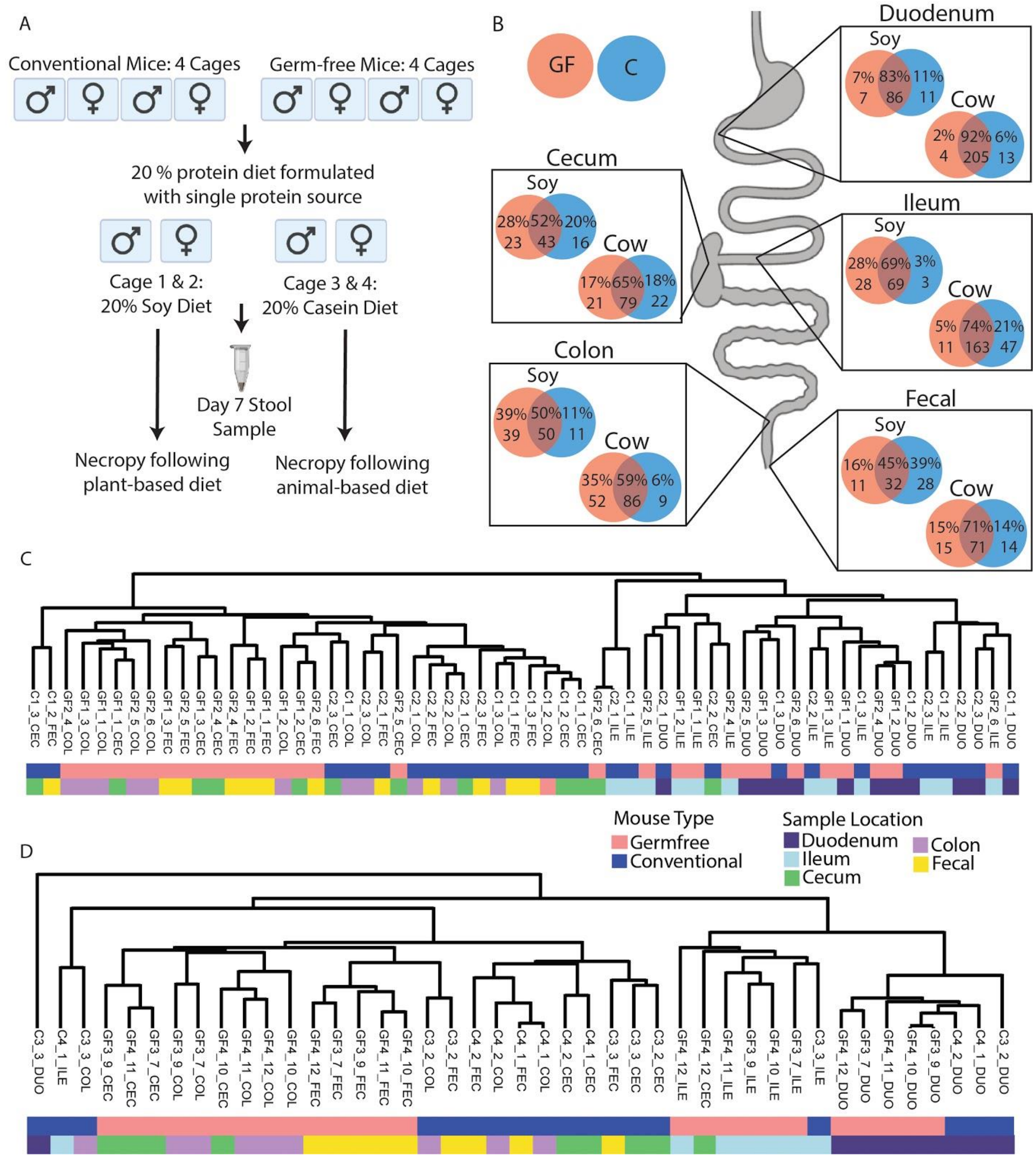

Figure 3-1. A) Experimental Design. B) Dietary soy proteins identified in different regions of the intestinal tract of germ-free and conventional mice. Soy proteins identified in only GF (blue) or C (red) mice were present in at least 50% of samples. Overlapping region represents proteins identified in at least 50% of samples for both GF and C mice. A greater percentage of overlap in soy proteins identified between germ-free and conventional mice is evident in the upper regions of the GI tract (Duodenum and Ileum samples). All percentages rounded to the nearest whole number. C) Soy and D) cow dietary proteins cluster by mouse type and sample location (Euclidean clustering, average linkage). Figure created in part with biorender.com.

Every protein source consists of many individual proteins. To examine the resiliency of dietary proteins to host and microbiota digestion, we assessed the relative abundances of each individual dietary protein in GF and C mice at different sample locations. We analyzed the %orgNSAFs of each protein in R using a linear mixed model. %orgNSAFs represent the Peptide Spectral Matches (PSMs) normalized by amino acid length and the total number of dietary PSMs (from cow or soy proteins) in each sample, expressed as a percent. We used mouse type (GF, C), location (Duo, Ile, Cec, Col, and Fec), and sex (M, F) as factors in the model and included two-factor interactions. When we analyzed the soy proteins identified in soy consuming mice, we found 37 proteins significantly different in abundance between GF and C mice ($P<0.05$, T-test, Supplementary Table 1). Furthermore, we found significant abundance differences for 50 soy proteins based on GI tract region and mouse type ($P<0.05$, T-test, Supplementary Table 1). When we analyzed the cow proteins using the linear mixed model, we found significant differences in abundance of 43 proteins between GF and C mice ($P<0.05$, T-test, Supplementary Table 1). 51 cow proteins were significantly different in abundance based on GI tract region and location ($P<0.05$, Linear Mixed Model, T-test, Supplementary Table 1).

We investigated protein abundance trends based on %orgNSAFs for the 15 most abundant soy and cow proteins identified in different locations of the GI tract in C and GF mice (Figure 3-2A & B). For most proteins, abundance trends were similar between the two groups of mice. The most abundant soy protein identified in the soy consuming mice (Q39898) is characterized as a trypsin inhibitor (Figure 3-2A). This dietary protein had a greater number of PSMs in ileum, cecum, colon and fecal samples from GF mice compared to C mice (Figure 3-2C). We identified two other trypsin inhibitors in the dataset (P25272 &

P25273) and found them both to be significantly more abundant in fecal samples compared to contents collected along the intestinal tract ($P<0.1$, T-test using Satterthwaite's method). This suggests these trypsin inhibitors are resilient to host digestion, even after the purification process used to produce the soy protein isolate in the diet.

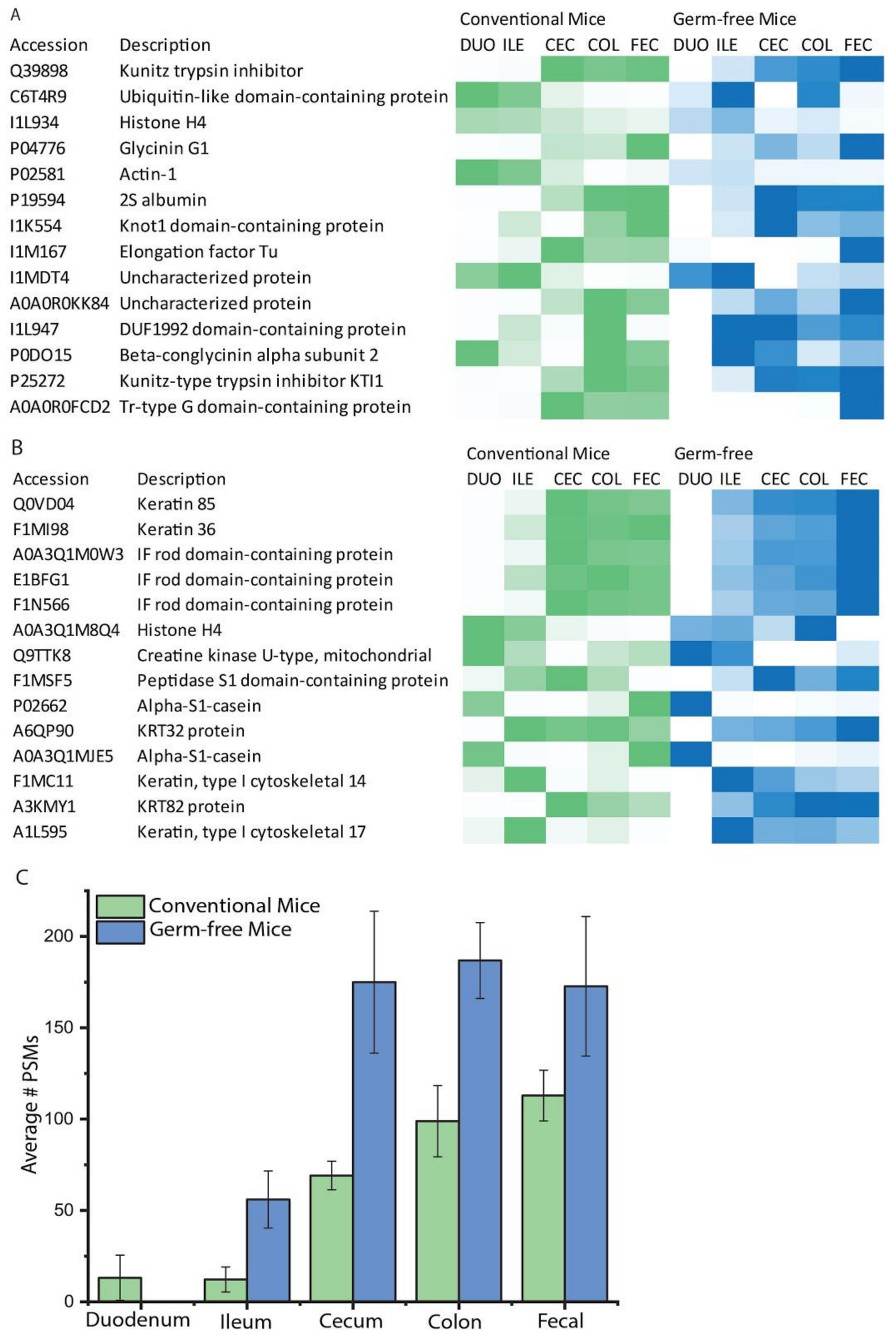

Figure 3-2. Abundance trends of the 15 most abundant A) soy proteins and B) cow proteins detected in mice consuming respective diet. Trends are indicated from light green/blue (least abundant) to dark green/blue (most abundant). Protein abundances were assessed using %NSAFs (normalized by amino acid length and total number of dietary PSMs per sample). C) Abundance of the most abundant soy protein (Q39898), a Kunitz Trypsin Inhibitor, based on PSMs detected in soy consuming mice. Only a single PSM for this protein was detected in the six Duodenum samples collected from GF mice.

3.3.2 Dietary protein source differentially impacts the intestinal microbiota

To examine the differences in microbiota composition between the soy consuming mice and casein consuming mice, we looked at the microbial proteins identified in the fecal samples from conventional mice. We performed shotgun metagenomic sequencing of a representative subset of samples to construct a matched metagenomic database for the identification of microbial proteins. Our microbial protein database was composed of predicted protein sequences from 486 Metagenome-Assembled Genomes (MAGs), each of which consists of a bin from an assembled and binned metagenome and is thought to represent a single species. We evaluated MAG quality using Quast and CheckM (Supplementary Tables 2 & 3). We used CheckM and BAT to taxonomically classify the MAGs (Supplementary Table 4). We ultimately included 326,468 protein sequences from the MAGs and 198,636 sequences from the unbinned metagenomic contigs in the final database. We identified 19,990 proteins from 248 out of the 486 MAGs in the fecal samples of the conventional mice. When we compared the percent proteinaceous biomass of murine, dietary and bacterial proteins as quantified by metaproteomics, we found that content from the upper intestinal tract was primarily made up of host proteins, while bacterial proteins represented the majority of biomass in samples from the lower intestinal tract and fecal material (Figure 3-3A).

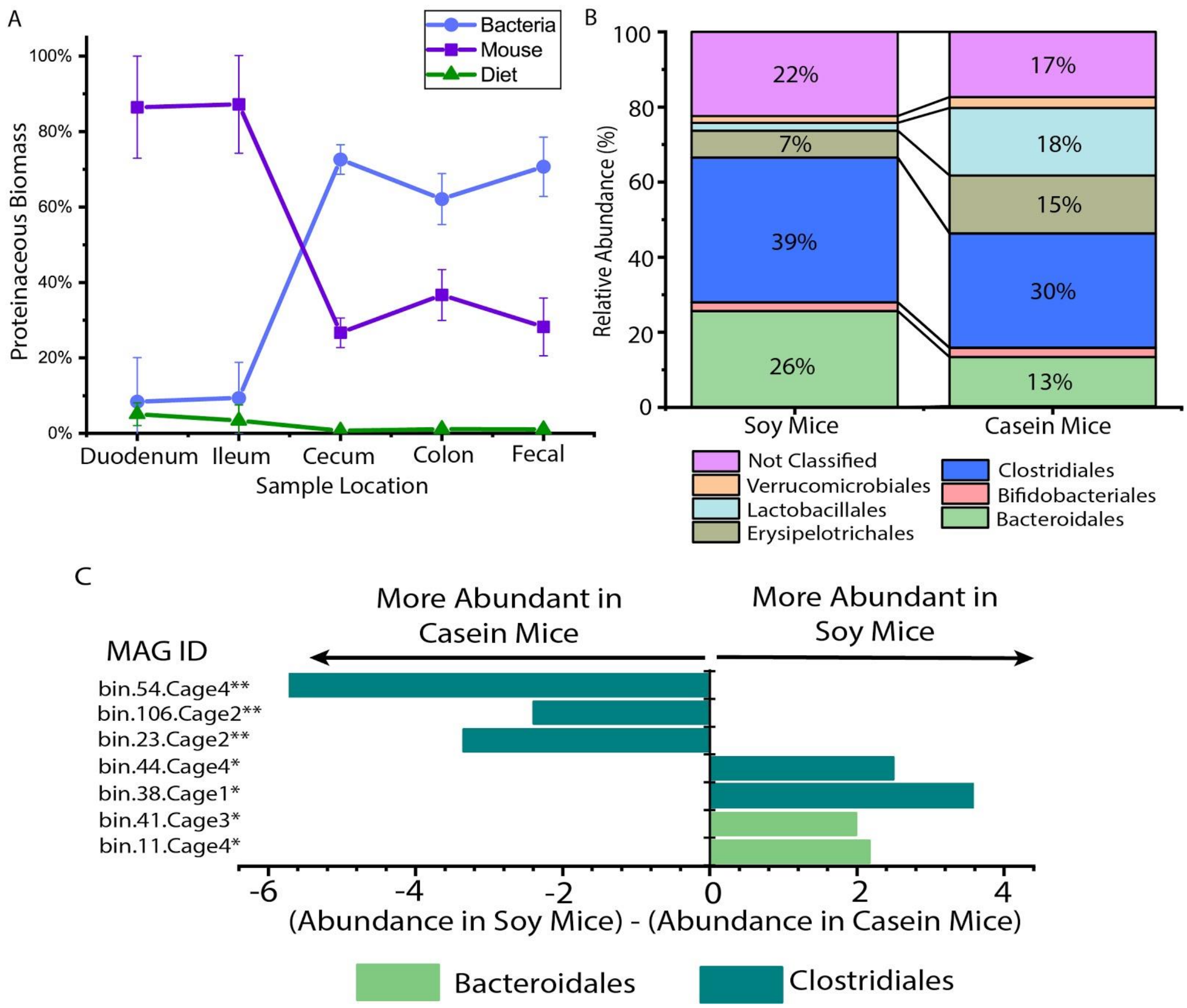

Figure 3-3. A) Proteinaceous biomass composition of GI tract contents and fecal pellets collected from conventional mice consuming a 20% protein diet. Host proteins contribute the majority of biomass in samples collected from the small intestine (Duodenum and Ileum) while bacterial proteins represent a much larger portion of biomass in the lower regions of the GI tract and fecal samples. B) Microbial proteinaceous biomass composition of fecal pellets at the order level from conventional mice consuming a soy or casein protein diet. The increase in relative abundance of Lactobacillales in the casein mice is likely due to microbial protein from the casein protein in the diet. C) MAGs found to be significantly more abundant in fecal pellets from casein or soy mice after removal of the three MAGs identified as *L. lactis* (Welch's T-test **P<0.05 *P<0.1).

We assessed microbiota composition at the order level based on the proteinaceous biomass contribution of each species and found Clostridiales members to represent the largest percentage for both soy and casein mice (Figure 3-3B). In addition to the 7 orders present in Figure 3-3B, we also detected Eggerthellales, Burkholderiales and Bacillales but these orders represented less than an average of 0.5% of the relative biomass composition in both groups of mice. Proteins making up 22% of microbial biomass in soy mice and 17% in casein mice were either predicted from MAGs that could not be taxonomically classified by BAT or matched to protein sequences that were predicted from metagenomics contigs that were not binned into a MAG. during metagenomic database creation.

We found the relative abundance of Lactobacillales to be approximately 16% greater in the casein fed mice (Fig 3B). Three MAGs within the Lactobacillales order, classified as *Lactococcus lactis,* represented 15.5% of the bacterial biomass in the casein fed mice and were not detected in the soy mice. As *L. lactis* is used in the production of milk products, we hypothesized the origin of this microbial protein contribution to be the casein protein in the diet, rather than the mouse microbiota. We tested this hypothesis by assessing the bacterial biomass of the fecal samples from germ-free mice consuming the casein diet. Microbial protein represented on average 11% of the proteinaceous biomass and the three *L. lactis* MAGs mentioned previously were responsible for 68% of the microbial biomass. Overall, the three *L. lactis* MAGs constituted 8% of the total proteinaceous biomass in the fecal samples from the casein consuming GF mice. When we assessed the proteinaceous biomass of fecal samples from GF mice consuming the soy diet, microbial biomass represented less than 1% of total biomass. We also tested the hypothesis that the *L. lactis* originated from the casein in the diet by examining the microbial biomass of the diets themselves. The casein diet averaged 1494 bacterial Peptide Spectral Matches (PSMs) while the soy diet averaged only 323 bacterial PSMs (Table 3-1). As protein source is the only difference between the soy and casein diets and we detected more than 4.5 times the amount of bacterial biomass in the casein diet compared to the soy diet, the *Lactococcus lactis* is likely from the casein protein itself. As all diets were strerilized by gamma ionizing radiation from cobalt-60 (20-50 kGy) and *L. lactis* biomass was only detected in the GF mice consuming the casein diet, this contribution of microbial protein from the diet likely increases the total microbial protein in the casein fed mice. Based on this potentially confounding factor, we assessed the proteinaceous biomass-based MAG abundances of fecal samples from conventional mice with and without the contribution of these three *L. lactis* MAGs (Figure 3-3C, Supplementary 3-2C). Covariance analysis of the MAG abundances with and without the *L. lactis* MAGs that diet is not the most distinguishing parameter, as samples clustered closest based on cage, followed by sex (Supplementary Figure 2A). Following removal of the three *L. lactis* MAGs, clustering by sex became even more pronounced, and separation based on diet was minimal (Supplementary Figure 2B). We found significant abundance differences for seven MAGs between the two diet groups, after we removed the three *L. lactis* MAGs (Figure 3-3C, $P<0.1$, Welch's T-Test). These same seven MAGs were also found to be significantly different when we included the *L. lactis* in the statistical analysis (Supplementary Figure 2C, $P<0.05$, Welch's T-Test). We found significant abundance differences for five MAGs classified as Clostridiales, three of which were more abundant in the casein mice, two in the soy mice. One of the Clostridiales found to be more abundant in soy mice (bin.44.Cage4) was further classified as belonging to the *Oscillibacter* genus. Another Clostridiales MAG (bin.38.Cage1) was not classifiable using BAT; however, CheckM classified this MAG as part of the Lachnospiraceae family. All other Clostridiales were not further classified. We also found two members of the *Bacteroides* genus to be significantly more abundant in soy fed mice.

3.4 Discussion

3.4.1 The microbiota metabolizes dietary proteins resilient to host digestion

The difference in complexity between animal-based protein and plant-based protein may contribute to differences in digestibility and the overall impact on the microbiota (Bartlett and Kleiner submitted, see Chapter 1). Our analysis of the soy and casein protein diets suggests the plant-based soy protein to be of greater complexity as we detected on average 5 times more soy proteins in the soy diet than cow proteins in the casein diet (Table 3-1). However, the number of soy protein sequences in our database (53,952) was almost twice as high as the number of cow protein sequences (28,241) due to the difference in the number of proteins encoded in the genomes of the organisms.

The 37 soy proteins and 43 cow proteins we found to have significantly different abundances between GF and C mice indicate the role of the microbiota in the digestion of dietary protein. This difference is further amplified when location in the GI tract is considered, as 50 soy proteins and 51 cow proteins were found to have significantly different abundances when we considered this two-factor interaction. The abundance pattern of the most abundant soy protein we detected, a trypsin inhibitor, suggests that the protein is resilient to digestion by the host (Figure 3-2A). This abundance pattern was similar for another trypsin inhibitor detected (P25272). Furthermore, although the process of protein purification contains steps thought to deactivate trypsin inhibitors, our findings indicate that likely some trypsin inhibitors do not lose their ability to limit host digestion (Joye, 2019).

The enrichment of both trypsin inhibitors in GF mouse fecal samples potentially alludes to the role of the microbiota in the digestion of resilient dietary proteins. Microbe-host and microbe-microbe interactions, such as concerted proteolysis, in the colon of the conventional mice may allow for these more resilient proteins to be partially metabolized by the microbiota and ultimately allow for greater energy harvest by the host. When we plotted protein abundance patterns, we found similar results whether we evaluated the raw PSM counts or the %orgNSAFs. As %orgNSAFs represent the individual contribution of PSM counts for a particular protein in a single sample, these normalized values are compositional. Similar patterns between the raw PSM counts and %orgNSAFS indicate that trends were not influenced by the compositionality of the data.

The extensive bacterial proteins present in the fecal material of the conventional mice could lead to suppression of dietary protein detection, such as the trypsin inhibitors we discuss here. However, we did not find an overall soy protein signal suppression in the conventional mice. The total number of soy protein PSMs from the fecal samples of C mice was 8,288 while the total detected in GF mice was 5,230. A greater number of total dietary PSMs in the C mice indicates that the abundance trend findings we observed are not due to suppression of dietary protein detection. Although there may be differences in the digestion of dietary protein between GF and C mice, our clustering indicates that dietary proteins identified in the upper intestinal tract, where the majority of protein digestion and absorption by the host occurs, is similar in the two groups of mice (Figure 3-1 C & D). These additional considerations provide support to our findings suggesting that the microbiota is responsible for metabolizing dietary proteins resilient to host digestion.

3.4.2 Diet can contribute microbial protein and differentially modulate the intestinal microbiota

As we have shown through the contribution of *L.lactis* from the casein diet, microbial protein in the diet has potential to influence interpretations of microbiota composition. *L. lactis* has been found to transiently colonize the human GI tract in diet-microbiota studies (David et al., 2014). Previously it has been concluded that an increase in dietary protein leads to an increase in microbial load in fecal material (Faith et al., 2011; Reese et al., 2018). Here we show this correlation may not be due to colonized microbiota community members but rather a contribution of microbial DNA/protein from the diet. In future diet-microbiota studies, it is vital that the input (diet) is carefully considered, even for sterilized diets, as the ones used in this study. This being said, our results suggest protein source to differentially impact microbiota composition, as we found 7 different MAGs to differ significantly between conventional mice consuming a soy or casein diet (Fig 3D, $P<0.1$, Welch's T-Test). Five of these MAGs were classified as Clostridiales. In previously conducted studies, relative abundances of Clostridiales members were also found to be significantly different in colon and cecum samples from hamsters fed soy and milk protein diets (Butteiger et al., 2016). Similar to the MAG abundance differences we found, Butteiger et al. found a member of the Lachnospiraceae family to be of greater relative abundance in the in cecal contents from hamsters fed soy protein compared to milk protein. However, contradictory to our findings and Butteiger et al., the relative abundance of some Lachnospiraceae members have also been found to be higher in casein fed rats compared to soy fed rats (Zhu et al., 2015). Our results also differ from those reported by Zhu et al., as we found two *Bacteroides* MAGs to be significantly more abundant in soy mice whereis they found members of this genus to be more abundant in casein rather than soy mice (Zhu et al., 2015). When we clustered samples based on MAG abundances of fecal pellets, we found samples to cluster most closely based on sex (Supplementary Figure 2A & B). The male and female mice in this study originated from different rooms at Jackson Laboratory, potentially leading to distinct microbiota distribution. Including baseline samples in the analysis of future studies may provide insight regarding the clustering pattern we found.

3.5 Materials and Methods

3.5.1 Animals, diets, sample collection

11 conventional C57BL/J6 mice (5 males, 5 females, The Jackson Laboratory) were used in this study. Mice were housed in groups of three by sex, except for one male cage with only 2. 11 C57BL/J6 germ-free mice supplied from the NCSU Gnotobiotic core, were housed in a similar fashion. One male and one female cage from each mouse type were supplied the soy diet (6 GF, 6 C) while the other two cages were supplied the casein diet (5 GF, 5 C). Mouse diets are typically supplemented with amino acids however, as this would interfere with the aims of the experiment, we used mature mice, aged 3-6 months, to avoid potential weight fluctuations due to the omission of amino acid supplements. Irradiated diets containing 20% isolated soy protein or casein as the sole protein source (Envigo Teklad Diets) were fed *ad libitum* for 7 days (Supplementary Table 4). Fecal samples were collected from all mice in the morning of day 7. Subsequently necropsy was performed to collect content from the duodenum, ileum, cecum and colon. germ-free mouse fecal samples were collected in the isolator but mice were removed from the isolator for necropsy. All animal protocols were approved by the Institutional Animal Care and Use Committee of North Carolina State University. All samples (fecal and intestinal contents) were collected directly

into NAP buffer preservation solution at a ratio of approximately 1:10 Sample Weight:Preservation Solution Volume, and roughly homogenized with a sterilized disposable pestel (Camacho-Sanchez et al., 2013; Menke et al., 2017). This preservation solution has been shown to be a valuable alternative when flash-freezing samples is not an option and we have extensively tested it in our laboratory for preservation of metaproteomic samples (Mordant et al., in preparation). This preservation solution was used instead of flash freezing to control for collection time differences between germ-free and conventional mice, as removal of samples from the germ-free isolator is time consuming.

3.5.2 Metagenomic DNA isolation

We collected fecal pellets from all 4 cages of mice (4 samples per cage) prior to introducing the soy and casein diets. We extracted DNA from the 16 representative fecal samples using the protocol described by Knudsen et al., with minor modifications. 5 mL of 1X Phosphate Buffered Saline solution (VWR) was added to samples to dilute the preservation solution in which they were collected before centrifugation (17,000 x g, 5 min) to pellet solids and bacterial cells in suspension. The bead beating procedure (3 m/s) was the only other modification as we adapted it for use with 2 mL bead beating tubes (Lysing Matrix E, MP Biomedicals) using a Bead Ruptor Elite 24 (Omni International). DNA concentration of eluates was assessed with a DS-11 FX+ Spectrophotometer (Denovix) using a Qubit™ dsDNA High Sensitivity Assay Kit (Invitrogen). Samples were extracted individually and pooled by cage with each sample contributing a total of 200 ng of DNA.

3.5.3 Metagenomic DNA sequencing

Genomic DNA (gDNA) was submitted to the North Carolina State Genomic Sciences Laboratory (Raleigh, NC, USA) for Illumina NGS library construction and sequencing to produce between 51,152,549 and 74,618,259 paired-end reads for each of the 4 samples. Prior to library preparation, the isolated DNA template was quantified by a Qubit 2.0 Fluorometer (Invitrogen, USA), with additional quality assessment using an Agilent 2200 Tapestation High Molecular Weight DNA assay (Agilent Technologies, USA). Library construction was performed using an Illumina TruSeq Nano Library kit with provided protocol. Briefly, the gDNA was fragmented using a Covaris S220 Ultrasonicator (Covaris, USA) and purified using AMPure XP beads (Beckman Coulter, USA). The fragments were then end-repaired, followed by 550 bp insert size-selection using sequential AMPure XP bead isolation steps. After adapter ligation, the library was enriched by PCR amplification. The amplified library was checked for quality and final concentration using the Agilent 2200 Tapestation (D1000) before sequencing on an Illumina NovaSeq 6000 sequencer, utilizing a divided XP lane of 150x2 bp paired end flow cell reagent kit (Illumina, USA). Raw .bcl, or base call files, were then de-multiplexed by sample into discrete .fastq files.

3.5.4 Read processing, assembly, binning and annotation

We used BBMap (Version 38.06) to decontaminate and trim raw reads. First we used the BBSplit algorithm to remove reads that matched to the Illumina control phix174 (NCBI GenBank accession CP004084.1) or mouse genome (mm10). We then performed adapter trimming on the unmapped reads using the BBDuk algorithm with the following settings: mink = 6, minlength =20. On average 60 million cleaned, trimmed paired-end reads were assembled using metaSPAdes (version 3.12.0) with error correction for each sample with k-mer lengths 33, 55, 99 (Nurk et al., 2017). We used the automated binning software MetaBAT (version 2.12.1) to bin the assembled contigs (Kang et al., 2019). The resulting Metagenome Assembled Genomes (MAGs) were evaluated for completeness and quality using CheckM (version 1.1.2) and Quast (Version 5.0.2) (Mikheenko et al., 2016; Parks et al., 2015). Prokka (version 1.13.7) was used for open reading frame prediction and gene annotation of both the MAGs and unbinned contigs (Seemann, 2014). We used CAT (version 5.0.3) with the bins parameter (BAT) and CheckM for taxonomic classification of the MAGs (von Meijenfeldt et al., 2019). There were only three instances out of the more than 450 MAGs in which the two tools provided contradictory results. We chose to use BAT information preferentially because BAT generally provided better resolved classification (Table S1).

3.5.5 Microbiota protein sequence database construction

All protein sequences from MAGs were concatenated and clustered with an identity threshold of 95% using Cd-hit (Li and Godzik, 2006). Clustering removed 52% of sequences which was expected due to redundancy of the sample types. Unbinned sequences were treated similarly, in which they were combined and clustered at 95% similarity. 2D-Cd-HIT was then used to determine unbinned sequences with less than 90% similarity to binned sequences. These novel unbinned sequences were combined with the binned sequences to produce the microbiota protein sequence database used for proteomic analysis.

3.5.6 Protein extraction, peptide preparation and determination

NAP buffer preservation solution was removed from samples by centrifugation (21,000 x g, 5 min) before solubilization of proteins with SDT lysis buffer [4% (w/v) SDS, 100 mM Tris-HCl pH 7.6, 0.1 M DTT] and bead beating in Lysing Matrix E tubes (MP Biomedicals) (5 cycles of 45s at 6.45 m/s, 1 min between cycles). Bead beating was followed by heating at 95°C for 10 min. Samples were centrifuged (21,000 x g, 5 min) prior to preparing tryptic digests (16 hour digestion) using the filter-aided sample preparation (FASP) protocol (Wiśniewski et al., 2009). Briefly, 60 µl of lysate was combined with 400 µl of UA solution (8 M urea in 0.1 M Tris/HCl pH 8.5) in 10 kDa MWCO 500 µl centrifugal filters (VWR International) and centrifuged at 14,000 *g* for 30 min. This step was repeated up to three times, depending on sample concentration, to reach filter capacity. Filters were washed with 200 µl of UA solution at 14,000 *g* for 40 min. 100 µl IAA (0.05 M iodoacetamide in UA solution) was then added to filters for a 20 min incubation followed by centrifugation at 14,000 *g* for 20 min. 100 µl of UA was added to filters and centrifuged three times. This washing was followed by a buffer change to ABC (50 mM Ammonium Bicarbonate) by adding 100 µl and centrifuging three times. 0.95 µg of MS grade trypsin (Thermo Scientific Pierce, Rockford, IL, USA) in 40 µl of ABC was added to the filters which were incubated for 16 hours in a wet chamber at 37 °C. After digestion, centrifugation at 14,000 *g* for 20 min followed by a 50 µl addition of 0.5 M NaCl and 20 min centrifugation was performed to elute peptides. Peptide concentrations were determined using the Pierce Micro BCA assay (Thermo Scientific Pierce) according to manufacturer's instructions.

3.5.7 LC-MS/MS

Tryptic peptides were analyzed with a Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) using a method similar to the one described by Kleiner et al (Species biomass paper). Samples were block-randomized as described by Oberg and Vitek, and one wash run with 100% acetonitrile was inserted between samples (Oberg and Vitek, 2009). An UltiMate™ 3000 RSLCnano Liquid Chromatograph (Thermo Fisher Scientific) was used to load 800 ng of peptides on a 5 mm, 300 µm ID C18 Acclaim® PepMap100 pre-column (Thermo Fisher Scientific) with loading solvent A (2% acetonitrile, 0.05% TFA). Following loading, peptides were eluted onto an EASY-Spray analytical column (75 cm× 75 µm) packed with PepMap RSLC C18, 2 µm material (Thermo Fisher Scientific) with eluent A (0.1% formic acid in water) and eluent B (80% acetonitrile, 0.1% formic acid). The column was heated to 60 °C via the integrated heater. Peptide separation was performed using a 260 min gradient from 5-99% B at a flow rate of 300 nl/min. Peptides were ionized with electrospray ionization using the Easy-Spray source (Thermo Fisher Scientific) and mass spectra were acquired in the Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). For MS1, a full MS scan from 380 to 1600 *m/z* was performed at a resolution of 60,000 and maximum injection time of 200 ms. Data-dependent MS2 was performed for the 15 most abundant ions at resolution of 15,000 and maximum injection time of 100 ms. Additional instrument parameters are as follows: 445.12003 lock mass, normalized collision energy equal to 24, 15 s dynamic exclusion and exclusion of ions with +1 charge state.

A complex standard consisting of pooled peptides extracted from representative samples was included for each block of samples. 2 duodenum, 2 ileum, 2 cecum, 2 colon and 4 fecal samples from a mouse consuming the soy or casein diet were pooled for the standard. Throughout the run, each standard was searched against a database containing microbiota, host and dietary protein sequences. The microbiota protein sequences were derived from the mouse gut gene catalog and clustered at a 92% similarity threshold using Cd-hit (Doré et al., 2015; Li and Godzik, 2006). The database also contained reference proteomes of Mus musculus (UP000000589, Downloaded 05Dec19), Bos taurus (UP000009136, Downloaded 05Dec19), Glycine max (UP000008827, Downloaded 05Dec19) proteomes, and the cRAP protein sequence database with common contaminants (www.thegpm.org/crap). The number of proteins and protein groups identified, MS2 spectra, and PSM:MS/MS ratio of the searched standards were monitored throughout the run, to ensure instrument stability.

3.5.8 Protein identification

MS/MS spectra were searched against a comprehensive target-decoy protein sequence database containing the matched metagenomic database for the microbiota, reference proteomes of Mus musculus (UP000000589, Downloaded 19Feb20) , Bos taurus (UP000009136, Downloaded 19Feb20) and Glycine max (UP000008827, Downloaded 19Feb20) proteomes, and the cRAP protein sequence database with common contaminants (www.thegpm.org/crap). For protein identification we searched the raw MS files against this database using the Sequest HT node in Proteome Discoverer software version 2.3 (Thermo Fisher Scientific) with the following parameters: trypsin (Full), maximum 2 missed cleavages, 10 ppm precursor mass tolerance, 0.1 Da fragment mass tolerance and maximum 3 equal dynamic modifications per peptide. We considered the following dynamic modifications: oxidation on M (+15.995 Da), carbamidomethyl on C (+57.021 Da) and acetyl on the protein N terminus (+42.011 Da). For peptide false discovery rate (FDR) calculation we used the Percolator node in Proteome Discoverer with the following parameters: maximum Delta Cn 0.05, a strict target FDR of 0.01, a relaxed target FDR of 0.05 and validation based on q-value. For protein inference, we used the Protein-FDR Validator node in Proteome Discoverer with a strict target FDR of 0.01 and a relaxed target FDR of 0.05 to restrict protein FDR to below 5%.

3.6 Data Analysis

One mouse in the casein group was excluded from all analysis that compared different regions of the GI tract due to an unusually large amount of bacterial biomass in the upper intestinal tract. Coprophagy may be a possible explanation for this outlier as the biomass composition of the ileum content for this mouse (79% bacterial) was comparable to the fecal material from other mice in this cage.

3.6.1 Proteinaceous biomass composition

To assess the proportion of biomass that is represented by host proteins, dietary proteins and bacterial proteins, the relative abundances of these three components were compared for each sample location. We filtered identified proteins for at least 2 protein unique peptides based on validation with a dataset developed specifically to assess metaproteomic biomass assessment workflows (Kleiner et al., 2017). This method was also used to assess biomass contributions of individual microbial species (MAGs) based on the microbial proteins identified in mouse fecal samples. When we assessed the proteinaceous biomass of fecal samples from GF mice consuming the soy diet, we excluded one sample, for which the microbial biomass was 55%. The microbial biomass of all other samples in this dataset were less than 1%, including mice in the same cage, suggesting a sample labeling error.

For statistical testing of MAG abundances, R (version 3.6.3) was used for imputation using the count zero multiplicative method (zCompositions R package, version 1.3.4) followed by centered log-ratio (CLR) transformation (CoDaSeq R package, version 0.99.6) of the PSMs used to assess species biomass. The CLR-transformed data was used to calculate principal components using prcomp and generate covariance biplots using the compositions

R package (version 1.40-5). A Welch's T-Test was performed on the CLR-transformed data using Perseus software version 1.6.12.0. Imputation, transformation and clustering of MAGs was performed by the NCSU bioinformatics core.

3.6.2 Dietary proteins in different GI tract regions

Dietary soy and cow proteins identified in mice consuming the respective diet were filtered for at least 1 protein unique peptide. To remove very low abundance proteins, we only included those that had at least 10 Peptide Spectral Matches (PSMs) in at least one sample per sample group (soy or casein mice). When looking at each sample location subset (e.g. GF colon samples) we filtered for proteins that were identified in at least 50% of the respective samples. Venny 2.0 was used to visualize proteins unique to GF and CV mice (Oliveros, 2007).

For statistical analysis of each dataset (soy proteins identified in soy mice and cow proteins identified in casein mice), we generated %orgNSAFs (Organism Normalized Spectral Abundance Factors) from the PSM values. %orgNSAFs represent the raw PSMs normalized by protein length and the total PSMs detected from a specific organism (in this case cow or soy) in a sample. This allows for the calculation of a relative abundance of the dietary proteins as shown below (Mueller et al., 2010).

$$\%orgNSAFs = \left(\frac{\frac{PSMs}{\#\ of\ Amino\ Acids}}{Total\ Dietary\ PSMs\ Per\ Sample} \right) * 100$$

%orgNSAFs were analyzed in R Studio (version 1.2.5019) with R (Version 4.0.0) using a linear mixed model (lme4 version 1.1-23, lmerTest version 3.1-2). The factors included were mouse type (GF, C), location (Duo, Ile, Cec, Col, and Fec), and Sex (M, F).

Two-factor interactions were included and this effect was modeled as a subject-specific intercept. Statistical modeling of %orgNSAFs was performed by the NCSU statistical consulting core.

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Author Contributions

AB and MK conceptualized the study. AB, MK and CMT designed the experiment. Sample collection and necropsy performed by AB and AJR. Metaproteomic analysis conducted by AB, TR and MK. AB wrote the manuscript.

Declaration of Interests

The authors declare no competing interests

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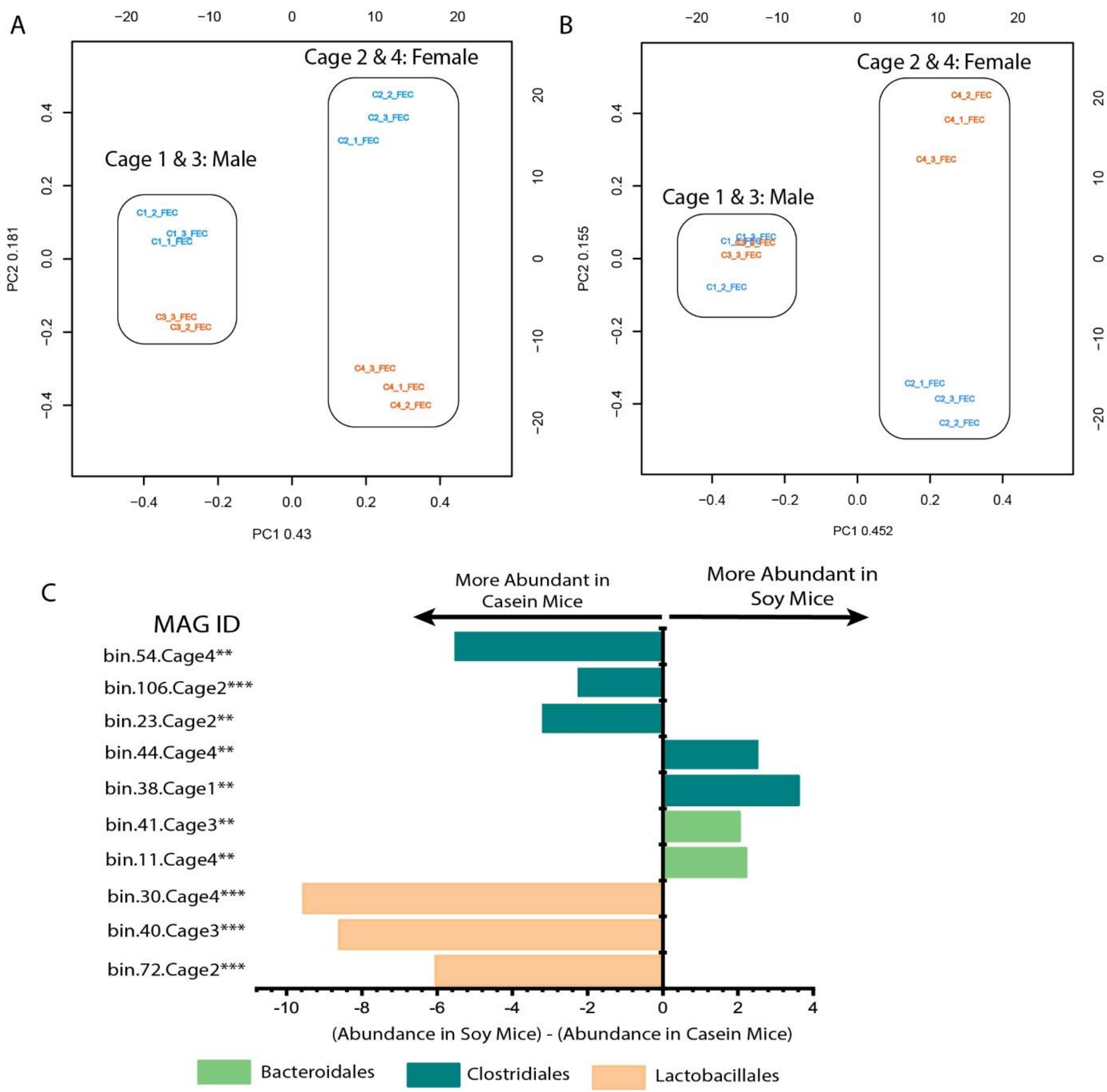
APPENDICES

Appendix 1 – Chapter 3 Supplementary Tables and Figures



Supplementary Figure 1. Principal Component Analysis of MAG abundances based on proteinaceous biomass of conventional mice fecal pellets A) prior to removal of 3 *L. lactis* MAGs and B) following removal of 3 *L. lactis* MAGs. Samples in blue are from mice on a soy-based diet while samples in orange are from mice on a casein-based diet. Samples cluster closed based on cage, then by sex and then by diet. C) Significant differences in MAG abundances based on proteinaceous biomass of conventional mice fecal pellets prior to removal of 3 *L. lactis* MAGs (Welch's T-test ***P<0.01,**P<0.05)

Supplementary Table 1. P-values of dietary proteins analyzed with linear mixed model.

Protein	Mouse_Type	Location	Sex	Type_Location	Type_Sex	Location_Sex
Soy_A0A0R0EJ63_A0A0R0EJ63_SOYBN	0.13013476	0.065157	0.379073	0.769708194	0.331015	0.802795862
Soy_A0A0R0EPH6_A0A0R0EPH6_SOYBN	0.03653459	2.78E-07	0.604728	0.000407908	0.498412	0.871241958
Soy_A0A0R0EV63_A0A0R0EV63_SOYBN	0.01381688	4.17E-10	0.022858	0.85665142	0.245943	0.577477655
Soy_A0A0R0FCD2_A0A0R0FCD2_SOYBN	5.92E-13	1.97E-08	0.223474	9.06E-07	0.716367	0.129225598
Soy_A0A0R0FY49_A0A0R0FY49_SOYBN	0.08924775	1.90E-05	0.564792	0.486898949	0.791727	0.908429337
Soy_A0A0R0FZZ9_A0A0R0FZZ9_SOYBN	0.43955825	0.063988	0.734947	0.686038322	0.600889	0.524684711
Soy_A0A0R0G6T3_A0A0R0G6T3_SOYBN	0.0553939	2.74E-07	0.511697	0.002239974	0.396226	0.748711901
Soy_A0A0R0GAG6_A0A0R0GAG6_SOYBN	0.4607049	0.038056	0.919258	0.228855299	0.0115	0.407915322
Soy_A0A0R0GAK0_A0A0R0GAK0_SOYBN	0.7212861	4.20E-05	0.963708	0.037243563	0.161777	0.798508229
Soy_A0A0R0GHB3_A0A0R0GHB3_SOYBN	0.00755291	1.55E-08	0.77821	3.06E-07	0.550154	0.387001014
Soy_A0A0R0GRY6_A0A0R0GRY6_SOYBN	0.11112133	3.28E-09	0.159396	0.042720748	0.331315	0.520911356
Soy_A0A0R0GXN6_A0A0R0GXN6_SOYBN	0.04711827	0.00328	0.730197	0.089105009	0.901346	0.035509149
Soy_A0A0R0H9T5_A0A0R0H9T5_SOYBN	0.21432641	7.52E-05	0.551158	0.533407024	0.529151	0.654818514
Soy_A0A0R0HAM0_A0A0R0HAM0_SOYBN	0.41973672	3.73E-12	0.268611	0.1554658	0.416087	0.228903263
Soy_A0A0R0HSV1_A0A0R0HSV1_SOYBN	0.15332203	1.87E-07	0.557524	0.649804353	0.684125	0.96804768
Soy_A0A0R0HVK1_A0A0R0HVK1_SOYBN	0.03932124	5.65E-06	0.310654	0.150705672	0.417675	0.94397483
Soy_A0A0R0HYM3_A0A0R0HYM3_SOYBN	0.8849973	0.366898	0.040639	0.083317329	0.884997	0.36689838
Soy_A0A0R0I2A1_A0A0R0I2A1_SOYBN	0.71171599	0.003026	0.845253	0.351513435	0.219528	0.797269177
Soy_A0A0R0I6G3_A0A0R0I6G3_SOYBN	0.91370606	0.33946	0.064708	0.057922707	0.773649	0.419675961
Soy_A0A0R0I960_A0A0R0I960_SOYBN	0.07173587	0.072297	0.760728	0.244422653	0.405155	0.986435429
Soy_A0A0R0IXL0_A0A0R0IXL0_SOYBN	0.32277848	0.417782	0.322778	0.417781622	0.322778	0.417781622
Soy_A0A0R0IYE6_A0A0R0IYE6_SOYBN	0.5862715	0.236716	0.732885	0.06166204	0.85467	0.998387237
Soy_A0A0R0J965_A0A0R0J965_SOYBN	0.44872491	0.238406	0.672686	0.01999422	0.52355	0.859397192
Soy_A0A0R0JEI9_A0A0R0JEI9_SOYBN	0.12466218	2.51E-09	0.175036	0.042092708	0.390309	0.610883559
Soy_A0A0R0JYY8_A0A0R0JYY8_SOYBN	0.9000979	2.93E-07	0.029931	0.019474025	0.135183	0.64612813
Soy_A0A0R0KA84_A0A0R0KA84_SOYBN	0.05255849	0.033464	0.864174	0.012697227	0.408217	0.960502282
Soy_A0A0R0KBI3_A0A0R0KBI3_SOYBN	0.21432641	7.52E-05	0.551158	0.533407024	0.529151	0.654818514
Soy_A0A0R0KHH6_A0A0R0KHH6_SOYBN	0.51240202	6.00E-08	0.783347	0.693208646	0.063169	0.009339779
Soy_A0A0R0KK84_A0A0R0KK84_SOYBN	0.75602842	1.73E-07	0.748714	0.039148293	0.879645	0.154717571
Soy_A0A0R0L6Q8_A0A0R0L6Q8_SOYBN	0.00981816	5.46E-10	0.297029	0.042004975	0.481084	0.034313338

Soy_A0A0R4J3S4_A0A0R4J3S4_SOYBN	0.05789225	9.20E-06	0.117477	0.114779027	0.762112	0.221829859
Soy_A0A0R4J467_A0A0R4J467_SOYBN	0.43401188	0.002455	0.226827	0.345277006	0.076136	0.656930462
Soy_A0A0R4J4C3_A0A0R4J4C3_SOYBN	0.07080351	3.66E-08	0.18741	0.69969624	0.149828	0.664317944
Soy_A0A0R4J4D6_A0A0R4J4D6_SOYBN	0.31535467	7.25E-11	0.209516	0.391149425	0.172578	0.888287408
Soy_A0A0R4J4U4_A0A0R4J4U4_SOYBN	0.44027081	0.360887	0.842762	0.295362693	0.51164	0.529738915
Soy_A0A0R4J5A7_A0A0R4J5A7_SOYBN	0.17531355	9.12E-07	0.837359	0.430981464	0.409906	0.999093229
Soy_A0A0R4J629_A0A0R4J629_SOYBN	0.11357694	0.108912	0.488218	0.245772059	0.573002	0.863914739
Soy_A0A0R4J654_A0A0R4J654_SOYBN	0.01171151	1.91E-11	0.29058	0.002920613	0.606222	0.562013128
Soy_A0A0R4J681_A0A0R4J681_SOYBN	0.52735136	1.47E-06	0.515839	0.711486213	0.266245	0.029033899
Soy_A0A368UHG1_A0A368UHG1_SOYBN	0.05503634	2.36E-07	0.50912	0.002273217	0.381412	0.746502973
Soy_A0A368UHL7_A0A368UHL7_SOYBN	0.00011279	3.33E-11	0.722352	0.013927732	0.066461	0.062738971
Soy_C6SYX2_C6SYX2_SOYBN	0.88209609	1.09E-05	0.049358	0.124367575	0.48352	0.115589786
Soy_C6T4R9_C6T4R9_SOYBN	0.00010955	9.41E-05	0.779787	0.00848483	0.39603	0.440295936
Soy_C6T871_C6T871_SOYBN	0.51062567	9.16E-12	0.349386	0.152266255	0.50262	0.213187743
Soy_C6T977_C6T977_SOYBN	0.01228095	0.040215	0.901803	0.450361517	0.970793	0.022703314
Soy_C6TKQ3_C6TKQ3_SOYBN	0.00256729	1.68E-09	0.463815	0.030088212	0.229015	0.978100957
Soy_C6TN36_C6TN36_SOYBN	0.00091771	0.000589	0.882455	0.000822258	0.093308	0.924220641
Soy_D6C4Z9_D6C4Z9_SOYBN	0.00298299	1.03E-10	0.90144	0.005251267	0.362517	0.557842971
Soy_E9KNA3_E9KNA3_SOYBN	0.07582656	1.33E-05	0.146293	0.117714409	0.822796	0.210004169
Soy_E9KNA7_E9KNA7_SOYBN	0.05008546	9.62E-06	0.133091	0.102289874	0.813562	0.295688999
Soy_F7J077_GLCB2_SOYBN	0.19113673	0.277819	0.416481	0.016039316	0.85593	0.820956567
Soy_H8Y6Q3_H8Y6Q3_SOYBN	0.54312743	2.29E-06	0.351416	0.110376529	0.255871	0.792433387
Soy_I1JEL6_I1JEL6_SOYBN	0.77723946	6.76E-06	0.667548	0.079985732	0.447162	0.736310789
Soy_I1JF86_I1JF86_SOYBN	0.62382913	5.35E-06	0.912439	0.209784838	0.368138	0.264688687
Soy_I1JGR5_I1JGR5_SOYBN	0.13562562	7.60E-12	0.0162	0.506763983	0.015314	0.983862749
Soy_I1JJL6_I1JJL6_SOYBN	0.00298299	1.03E-10	0.90144	0.005251267	0.362517	0.557842971
Soy_I1JNR3_I1JNR3_SOYBN	5.87E-13	1.94E-08	0.220921	8.93E-07	0.712313	0.128931369
Soy_I1JPW5_I1JPW5_SOYBN	0.46264979	5.14E-08	0.064167	0.051823281	0.292183	0.902041694
Soy_I1JPX8_I1JPX8_SOYBN	0.04346574	0.059095	0.029667	0.342120215	0.000949	0.810308707
Soy_I1JSP6_I1JSP6_SOYBN	0.39049095	0.000401	0.866617	0.512134614	0.843404	0.500646128
Soy_I1JXC1_I1JXC1_SOYBN	5.92E-13	1.94E-08	0.215294	8.72E-07	0.704533	0.129904594
Soy_I1K3S4_I1K3S4_SOYBN	0.03494455	2.76E-07	0.925941	0.189232532	0.343937	0.909122949
Soy_I1K554_I1K554_SOYBN	0.85439298	0.000217	0.006661	0.032155514	0.309442	0.164979417
Soy_I1K671_I1K671_SOYBN	0.33399176	0.001055	0.086759	0.881234064	0.238827	0.98375784
Soy_I1KBN9_I1KBN9_SOYBN	0.46286169	4.05E-05	0.753308	0.511615159	0.93835	0.279298698
Soy_I1KC65_I1KC65_SOYBN	0.39739642	0.215544	0.803494	0.219076178	0.466249	0.33802911
Soy_I1KCD7_I1KCD7_SOYBN	0.07087622	3.27E-10	0.080842	0.682141697	0.143917	0.190423254

Soy_I1KGB8_I1KGB8_SOYBN	0.6661019	0.078637	0.513785	0.378222306	0.22779	0.944823198
Soy_I1KPN5_I1KPN5_SOYBN	0.34587978	0.001061	0.085528	0.883201982	0.236124	0.98726279
Soy_I1KSD0_I1KSD0_SOYBN	0.00012638	1.32E-12	0.874363	0.003712604	0.059037	0.106303103
Soy_I1KU21_I1KU21_SOYBN	0.8412029	1.56E-10	0.50662	0.424596134	0.461718	0.723761074
Soy_I1KUQ2_I1KUQ2_SOYBN	0.90839473	0.09286	0.600171	0.066915092	0.844645	0.973717677
Soy_I1KUR0_I1KUR0_SOYBN	0.27820893	5.93E-06	0.910418	0.422393025	0.349473	0.999759275
Soy_I1L068_I1L068_SOYBN	0.28272402	2.12E-10	0.682023	0.437142093	0.934122	0.994934088
Soy_I1L314_I1L314_SOYBN	0.00101599	2.59E-11	0.496194	5.40E-05	0.248889	0.71939603
Soy_I1L8Z3_I1L8Z3_SOYBN	0.12887835	3.31E-08	0.180339	0.662547848	0.203909	0.551944287
Soy_I1L934_I1L934_SOYBN	0.10767362	1.24E-05	0.901981	0.12526941	0.700723	0.353278843
Soy_I1L947_I1L947_SOYBN	0.86038862	0.09949	0.610758	0.590201909	0.229707	0.762555987
Soy_I1LDR2_I1LDR2_SOYBN	0.96941516	5.56E-08	0.03594	0.048955483	0.159478	0.608361683
Soy_I1LZA5_I1LZA5_SOYBN	0.09390334	3.36E-06	0.926094	0.000747926	0.805267	0.722322747
Soy_I1M167_I1M167_SOYBN	9.35E-14	5.46E-07	0.710015	1.70E-07	0.240773	0.121786548
Soy_I1M2K9_I1M2K9_SOYBN	0.16042612	0.004774	0.951936	0.180417555	0.140753	0.721175677
Soy_I1M315_I1M315_SOYBN	0.05858768	7.52E-06	0.156093	0.088920972	0.70395	0.253551137
Soy_I1M3R5_I1M3R5_SOYBN	0.03471943	0.116422	0.910562	0.597112611	0.816695	0.036250325
Soy_I1M596_I1M596_SOYBN	0.23753565	2.86E-11	0.202327	0.015763678	0.825441	0.281679255
Soy_I1M8Q5_I1M8Q5_SOYBN	0.50051232	0.152487	0.112161	0.223882149	0.919652	0.396941194
Soy_I1MB71_I1MB71_SOYBN	0.04346574	0.059095	0.029667	0.342120215	0.000949	0.810308707
Soy_I1MC39_I1MC39_SOYBN	0.84667987	6.75E-08	0.030679	0.040854046	0.179477	0.653779565
Soy_I1MDT4_I1MDT4_SOYBN	0.12304921	3.22E-05	0.067819	0.041013871	0.022512	0.747280006
Soy_I1MG42_I1MG42_SOYBN	2.85E-09	0.000184	0.380493	0.000636263	0.171502	0.561431561
Soy_I1MGV5_I1MGV5_SOYBN	0.01871077	0.050609	0.202339	0.157798731	0.635347	0.888103685
Soy_I1MJN2_I1MJN2_SOYBN	0.04698789	0.00319	0.721543	0.088757828	0.901766	0.03632611
Soy_I1ML66_I1ML66_SOYBN	4.71E-07	7.71E-12	0.172093	7.71E-12	0.172093	0.189697132
Soy_I1MN39_I1MN39_SOYBN	0.819786	1.46E-07	0.983174	0.964476736	0.575468	0.986866176
Soy_I1MZ84_I1MZ84_SOYBN	0.60674032	1.42E-06	0.034511	0.617348687	0.924554	0.038414498
Soy_I1N0F1_I1N0F1_SOYBN	0.65916427	9.80E-11	0.250979	0.809707242	0.823433	0.248721233
Soy_I1N1Y6_I1N1Y6_SOYBN	0.38996971	1.39E-09	0.128416	0.918164752	0.511093	0.428446688
Soy_I1N898_I1N898_SOYBN	0.00279278	8.03E-09	0.959144	0.030576108	0.122234	0.033242032
Soy_I1NFS4_I1NFS4_SOYBN	0.02506527	3.82E-07	0.687368	0.002177043	0.889598	0.6353954
Soy_K7K577_K7K577_SOYBN	0.67768035	0.185447	0.803567	0.213387265	0.773628	0.831718928
Soy_K7K753_K7K753_SOYBN	0.65402144	0.000685	0.137618	0.641693615	0.522622	0.598635804
Soy_K7KPN3_K7KPN3_SOYBN	0.28272402	2.12E-10	0.682023	0.437142093	0.934122	0.994934088
Soy_K7M7G3_K7M7G3_SOYBN	0.0003041	0.00029	0.414593	0.000289618	0.414593	0.905428773
Soy_K7MDZ1_K7MDZ1_SOYBN	0.86750704	6.21E-06	0.814734	0.998750279	0.596191	0.625714844
Soy_K7MJX0_K7MJX0_SOYBN	0.00193607	4.11E-05	0.952024	0.068755079	0.924083	0.113877919
Soy_K7MNB1_K7MNB1_SOYBN	0.18724984	0.129002	0.18725	0.106042656	0.030694	0.106042656
Soy_K7MPA9_K7MPA9_SOYBN	3.72E-05	3.82E-05	0.628341	8.93E-05	0.477146	0.95098816

Soy_K7MVD3_K7MVD3_SOYBN	0.05230421	9.50E-08	0.178407	0.681063919	0.11011	0.70746213
Soy_M1FPG4_M1FPG4_SOYBN	0.11554397	0.105317	0.573078	0.057199354	0.95278	0.67450137
Soy_P02580_ACT3_SOYBN	0.60281598	2.61E-08	0.363125	0.301994045	0.042108	0.501412426
Soy_P02581_ACT1_SOYBN	0.11773277	3.21E-06	0.18285	0.076967061	0.006634	0.480287176
Soy_P02858_GLYG4_SOYBN	0.48725403	0.000298	0.863905	0.021163885	0.556429	0.339352089
Soy_P04347_GLYG5_SOYBN	0.48280185	0.172339	0.397002	0.16185779	0.186182	0.211973147
Soy_P04776_GLYG1_SOYBN	0.88139922	1.16E-07	0.566548	0.19510089	0.915783	0.039988142
Soy_P08170_LOX1_SOYBN	0.06176556	1.63E-13	0.159034	0.008900831	0.149219	0.528945693
Soy_P09186_LOX3_SOYBN	0.63813769	0.50108	0.764402	0.012864561	0.599495	0.430301955
Soy_P0DO15_GLCA2_SOYBN	0.15519201	0.024067	0.937715	0.049337871	0.990746	0.143956638
Soy_P11827_GLCAP_SOYBN	0.09930934	0.127912	0.675687	0.055965831	0.834387	0.297214516
Soy_P11828_GLYG3_SOYBN	0.42021142	4.36E-07	0.976634	0.017259436	0.981036	0.105943012
Soy_P19594_2SS_SOYBN	0.05299786	6.53E-09	0.014679	0.003783954	0.686625	0.001529675
Soy_P25272_KTI1_SOYBN	0.83180356	1.58E-06	0.68555	0.707074934	0.371543	0.22180086
Soy_P25273_KTI2_SOYBN	0.22120694	0.00023	0.873951	0.673568239	0.637276	0.414617049
Soy_P29530_OLEO1_SOYBN	0.03099616	0.691471	0.193269	0.006487073	0.686587	0.705340523
Soy_P29531_OLEO2_SOYBN	0.0626425	0.165247	0.171442	0.00725269	0.614084	0.582069997
Soy_P34811_EFGC1_SOYBN	0.08081105	0.115481	0.75714	0.720097971	0.222459	0.015746154
Soy_P46280_EFTU2_SOYBN	5.78E-13	1.90E-08	0.213991	8.57E-07	0.70198	0.128775772
Soy_P54774_CDC48_SOYBN	0.09555568	3.41E-09	0.141693	0.587500914	0.216915	0.465744264
Soy_P62302_RS13_SOYBN	0.45963389	8.47E-08	0.016758	0.917193786	0.405143	0.177781051
Soy_Q01915_ATPAM_SOYBN	0.04449461	0.000365	0.80323	0.626507099	0.603446	0.290389215
Soy_Q04672_SBP_SOYBN	0.60581545	6.01E-07	0.482607	0.06462551	0.423653	0.297766129
Soy_Q2PMS8_ATPA_SOYBN	0.08128191	0.022624	0.175827	0.033948066	0.650099	0.397959315
Soy_Q2PMV0_ATPB_SOYBN	4.12E-06	2.65E-09	0.753185	0.000102446	0.614409	0.760738642
Soy_Q39898_Q39898_SOYBN	0.35771506	1.80E-13	0.957224	0.003602735	0.186027	0.289858349
Soy_Q8W171_CYP1_SOYBN	0.16551991	7.03E-05	0.366026	0.502271836	0.552422	0.595287154
Soy_Q96453_1433D_SOYBN	0.05867072	9.54E-06	0.118438	0.116520312	0.758594	0.225316532
Soy_Q96558_UGDH1_SOYBN	0.30739747	2.19E-07	0.777717	0.43241296	0.017384	0.326076776

Protein	Mouse_Type	Location	Sex	Type_Location	Type_Sex	Location_Sex
Cow_A0A140T8C6_A0A140T8C6_BOVIN	0.918975282	0.82160002	0.09440689	0.105193554	0.909943	0.715375681
Cow_A0A3Q1LL67_A0A3Q1LL67_BOVIN	0.567129122	0.03654	0.31579531	0.887481504	0.90164	0.030939809
Cow_A0A3Q1LNQ6_A0A3Q1LNQ6_BOVIN	0.717866926	0.00672832	0.86368549	0.735022551	0.86793	0.315559759
Cow_A0A3Q1LPH5_A0A3Q1LPH5_BOVIN	0.334859358	5.70E-09	0.21022515	0.511460334	0.783255	0.585803859
Cow_A0A3Q1LSB7_A0A3Q1LSB7_BOVIN	0.081076869	2.60E-06	0.14582243	0.070753294	0.92021	0.24986674
Cow_A0A3Q1LU30_A0A3Q1LU30_BOVIN	0.002693791	0.00657051	0.66398604	0.576048538	0.004825	0.438237521
Cow_A0A3Q1LV05_A0A3Q1LV05_BOVIN	0.101166993	3.76E-11	0.02453953	0.442448364	0.804193	0.000110925
Cow_A0A3Q1LVC7_A0A3Q1LVC7_BOVIN	0.003717718	6.93E-05	0.62500214	0.645550528	0.304249	0.311981637
Cow_A0A3Q1LW91_A0A3Q1LW91_BOVIN	0.803372079	1.32E-09	0.58843765	0.965656244	0.261495	0.87542699
Cow_A0A3Q1LWG5_A0A3Q1LWG5_BOVIN	0.708267777	5.57E-06	0.41771242	0.862392474	0.432606	0.317025017
Cow_A0A3Q1LYE8_A0A3Q1LYE8_BOVIN	0.590858396	0.42126239	0.81531516	0.222789316	0.38053	0.549511522
Cow_A0A3Q1LZB8_A0A3Q1LZB8_BOVIN	0.010439674	3.06E-06	0.83802406	0.037150298	0.741613	0.954750746
Cow_A0A3Q1M032_A0A3Q1M032_BOVIN	0.216013682	0.16343667	0.18287707	0.642427472	0.720269	0.40239985
Cow_A0A3Q1M0W3_A0A3Q1M0W3_BOVIN	0.931975694	3.08E-17	0.24049807	0.008456443	0.981145	0.391867762
Cow_A0A3Q1M2Q2_A0A3Q1M2Q2_BOVIN	0.620033857	0.00224564	0.8911662	0.750832042	0.807613	0.059271485
Cow_A0A3Q1M3L6_A0A3Q1M3L6_BOVIN	0.423043977	0.18745772	0.19333339	0.910484479	0.971241	0.619894754
Cow_A0A3Q1M5R4_A0A3Q1M5R4_BOVIN	0.23456799	0.0067958	0.09389501	0.625722453	0.133748	0.140300894
Cow_A0A3Q1M701_A0A3Q1M701_BOVIN	0.592974172	0.4243566	0.8112127	0.221179459	0.375026	0.548738728
Cow_A0A3Q1M8Q4_A0A3Q1M8Q4_BOVIN	0.667214101	0.00014427	0.59896574	0.038915976	0.668103	0.975725927
Cow_A0A3Q1M9I9_A0A3Q1M9I9_BOVIN	0.877953276	7.33E-08	0.28356881	0.558710498	0.052232	0.470278529
Cow_A0A3Q1MBL4_A0A3Q1MBL4_BOVIN	0.40675978	1.26E-06	0.15209459	0.030027751	0.561231	0.195721039
Cow_A0A3Q1MFL7_A0A3Q1MFL7_BOVIN	0.097484786	0.00136286	0.26823147	0.855899846	0.594912	0.318411079
Cow_A0A3Q1MG98_A0A3Q1MG98_BOVIN	0.000452068	1.20E-06	0.11453451	0.000103341	0.807801	0.004842305
Cow_A0A3Q1MGZ4_A0A3Q1MGZ4_BOVIN	0.244515576	6.00E-12	0.70544972	0.081352902	0.701194	0.46484769
Cow_A0A3Q1MH46_A0A3Q1MH46_BOVIN	0.007700601	2.64E-05	0.63100086	0.636943996	0.206943	0.005240214
Cow_A0A3Q1MHC4_A0A3Q1MHC4_BOVIN	0.525379593	3.91E-08	0.78011998	0.281135201	0.796056	0.019075108
Cow_A0A3Q1MHE5_A0A3Q1MHE5_BOVIN	0.456790949	9.59E-08	0.62295113	0.866008717	0.962271	0.001718048
Cow_A0A3Q1MJ66_A0A3Q1MJ66_BOVIN	0.013418296	0.11677509	0.44611474	0.092290077	0.283122	0.166685289
Cow_A0A3Q1MJE5_A0A3Q1MJE5_BOVIN	0.59946053	5.63E-05	0.07538466	0.820449423	0.200614	0.353451234
Cow_A0A3Q1MM86_A0A3Q1MM86_BOVIN	0.899340917	4.31E-08	0.59536505	0.57991295	0.986005	0.790149727
Cow_A0A3Q1MP70_A0A3Q1MP70_BOVIN	0.365602379	0.00016668	0.86295843	0.457701504	0.149494	0.20974323

Cow_A0A3Q1MPF1_A0A3Q1MPF1_BOVIN	0.048079923	1.91E-05	0.5841457	0.445333837	0.263969	0.540082922
Cow_A0A3Q1MQU2_A0A3Q1MQU2_BOVIN	0.014414149	3.18E-08	0.64256273	0.0451253	0.031524	0.014491141
Cow_A0A3Q1MR17_A0A3Q1MR17_BOVIN	0.088498767	0.0071704	0.31806368	0.84580748	0.170295	0.706364491
Cow_A0A3Q1MR76_A0A3Q1MR76_BOVIN	0.000621858	0.16750808	0.08933573	0.193890715	0.00353	0.007926129
Cow_A0A3Q1MRU0_A0A3Q1MRU0_BOVIN	0.097562842	9.39E-08	0.85954808	0.466311208	0.27935	0.222472228
Cow_A0A3Q1MU93_A0A3Q1MU93_BOVIN	0.414424228	0.14605366	0.6714102	0.753097247	0.737111	0.840325051
Cow_A0A3Q1MUN0_A0A3Q1MUN0_BOVIN	0.033809422	0.00033005	0.40066958	0.002740797	0.495694	0.292688657
Cow_A0A3Q1MUU4_A0A3Q1MUU4_BOVIN	0.924754555	6.57E-08	0.61134408	0.523671583	0.411627	0.324587158
Cow_A0A3Q1MWG7_A0A3Q1MWG7_BOVIN	0.539855861	3.32E-06	0.82409654	0.003820231	0.616227	0.012881415
Cow_A0A3Q1MXQ0_A0A3Q1MXQ0_BOVIN	0.140740309	9.09E-09	0.84958591	0.44946432	0.082033	0.534900798
Cow_A0A3Q1NB06_A0A3Q1NB06_BOVIN	0.429275283	5.50E-06	0.44475563	0.948674428	0.306012	0.952588916
Cow_A0A3Q1NJ70_A0A3Q1NJ70_BOVIN	0.227253231	2.57E-07	0.55051178	0.803613593	0.762762	0.368081205
Cow_A0A3Q1NKS6_A0A3Q1NKS6_BOVIN	0.134861104	4.41E-09	0.34507061	0.613787978	0.774237	0.16717989
Cow_A0A3S5ZPB0_A0A3S5ZPB0_BOVIN	0.166681426	1.17E-05	0.80839929	0.980739964	0.184848	0.848189942
Cow_A0A3S5ZPM3_A0A3S5ZPM3_BOVIN	0.030845557	1.12E-06	0.61680568	0.565347959	0.126887	0.565518922
Cow_A0A452DHW7_A0A452DHW7_BOVIN	0.473396108	0.0008598	0.08258915	0.718350517	0.082673	0.670936337
Cow_A0A452DID1_A0A452DID1_BOVIN	0.434399727	0.02615118	0.31279394	0.014814591	0.66541	0.055162306
Cow_A0A452DID6_A0A452DID6_BOVIN	0.002371672	1.70E-10	0.79397139	0.013953181	0.635576	0.334286729
Cow_A0A452DIK7_A0A452DIK7_BOVIN	0.039872914	3.19E-05	0.65254451	0.643073804	0.025428	0.335895615
Cow_A0A452DIU9_A0A452DIU9_BOVIN	0.502800487	2.67E-08	0.76202543	0.420689822	0.920346	0.085599049
Cow_A0A452DIX8_A0A452DIX8_BOVIN	0.60619149	1.52E-05	0.10449666	0.112793461	0.858429	0.308228343
Cow_A0A452DJA8_A0A452DJA8_BOVIN	0.462155354	5.54E-05	0.66916256	0.144509828	0.59812	0.113267334
Cow_A0A452DJG2_A0A452DJG2_BOVIN	0.438703648	7.85E-06	0.46382073	0.686344111	0.310875	0.076190845
Cow_A1L595_K1C17_BOVIN	0.278579432	0.00051763	0.26505358	0.542470828	0.987415	0.106991125
Cow_A2VDL6_AT1A2_BOVIN	0.024420195	2.59E-07	0.13561621	0.57156101	0.023631	0.132324135
Cow_A3KMY1_A3KMY1_BOVIN	0.000813254	4.89E-19	0.17255801	4.04E-07	0.795993	0.830381023
Cow_A3KN04_A3KN04_BOVIN	0.163217661	7.38E-06	0.37455911	0.417953173	0.467921	0.210914712
Cow_A3KN27_K2C74_BOVIN	0.001545242	0.024236	0.95955837	0.321510868	0.15521	0.495587354
Cow_A4FV08_GNPI1_BOVIN	0.003389634	8.28E-10	0.00692652	0.011092579	0.985247	0.00028224
Cow_A4FV54_RAB8A_BOVIN	0.044366607	1.11E-05	0.72162894	0.769974036	0.320887	0.007480592
Cow_A4IFP2_A4IFP2_BOVIN	0.026311106	0.00027479	0.64534105	0.03790462	0.452364	0.039833656
Cow_A5D7A0_EFHD2_BOVIN	0.585948703	2.61E-06	0.14684925	0.982345186	0.580955	0.571069867
Cow_A5D7D5_A5D7D5_BOVIN	0.886265283	0.5837141	0.58240274	0.31492992	0.336564	0.201153673
Cow_A5D984_A5D984_BOVIN	0.712252231	9.04E-08	0.19303212	0.685290156	0.04097	0.194679708

Cow_A5PJZ1_SCMC1_BOVIN	0.403559159	0.00962287	0.64687332	0.933430635	0.386057	0.393693879
Cow_A6QLU1_GPDM_BOVIN	0.319571522	7.57E-10	0.38405432	0.536479232	0.528082	0.20068618
Cow_A6QNX5_K2C78_BOVIN	0.924124155	0.10596931	0.14584348	0.64778544	0.675938	0.194009348
Cow_A6QP90_A6QP90_BOVIN	0.152074463	1.77E-07	0.74450534	0.324914997	0.970816	0.140858229
Cow_A6QPB7_A6QPB7_BOVIN	0.018371863	7.59E-11	0.8342996	0.04793199	0.301433	0.367126422
Cow_A6QQ54_A6QQ54_BOVIN	0.479587941	1.44E-06	0.9396828	0.36261963	0.65756	0.107215659
Cow_A7MBG1_A7MBG1_BOVIN	0.419049328	2.18E-09	0.33024821	0.019235406	0.632425	0.339954628
Cow_A7MBI5_A7MBI5_BOVIN	0.400232092	6.22E-06	0.10488053	0.081101811	0.38345	0.097149958
Cow_A7YWK3_K2C73_BOVIN	0.119292019	0.01287343	0.84729237	0.841304199	0.009784	0.079582758
Cow_E1B975_E1B975_BOVIN	0.221400543	0.00148398	0.92383553	0.842141816	0.244942	0.076758872
Cow_E1B991_E1B991_BOVIN	0.09241757	0.44932538	0.65787132	0.157695226	0.946449	0.458762847
Cow_E1BBY7_E1BBY7_BOVIN	0.18901825	1.77E-09	0.75900074	0.002130658	0.658757	0.057133107
Cow_E1BDG9_E1BDG9_BOVIN	0.959213126	0.03279728	0.25175215	0.12299949	0.594424	0.809025906
Cow_E1BEI6_E1BEI6_BOVIN	0.643953789	0.00511049	0.13534269	0.514688924	0.231037	0.00604298
Cow_E1BFG1_E1BFG1_BOVIN	0.268104913	6.48E-12	0.43343265	0.156600988	0.657441	0.214760248
Cow_E1BGH5_E1BGH5_BOVIN	0.22708831	3.53E-05	0.15141337	0.000278948	0.215607	0.576276887
Cow_E1BHA5_E1BHA5_BOVIN	0.736626573	7.85E-07	0.06320734	0.250793733	0.064515	0.741276227
Cow_E1BJA2_E1BJA2_BOVIN	0.67196435	2.75E-11	0.0065907	0.98016738	0.154007	0.000258376
Cow_E1BK89_E1BK89_BOVIN	3.23E-05	0.02724782	0.88311657	0.122144112	0.801319	0.412761047
Cow_E1BKD8_E1BKD8_BOVIN	0.819922836	2.50E-07	0.78908885	0.093507597	0.945433	0.383140824
Cow_E1BKZ1_E1BKZ1_BOVIN	0.055768069	1.09E-06	0.60534247	0.004106277	0.061655	0.123744726
Cow_E1BL29_E1BL29_BOVIN	0.413920401	0.04325073	0.36358906	0.216912682	0.316954	0.144507248
Cow_E1BMG4_E1BMG4_BOVIN	0.035350601	0.03240413	0.0571447	0.004365183	0.084112	0.14370744
Cow_E1BNA9_E1BNA9_BOVIN	0.563523717	0.01984449	0.87967109	0.594441061	0.044772	0.972285896
Cow_E1BP05_E1BP05_BOVIN	0.052421845	0.15592291	0.84744904	0.381556949	0.289897	0.640876608
Cow_E1BPL8_E1BPL8_BOVIN	0.843715752	5.90E-05	0.4761607	0.028946471	0.321566	0.277693462
Cow_F1MBF6_F1MBF6_BOVIN	0.111310999	2.35E-07	0.39753218	0.405411444	0.288495	0.148152435
Cow_F1MC11_F1MC11_BOVIN	0.667084433	0.00189069	0.21218759	0.248996129	0.631642	0.367832986
Cow_F1MD24_F1MD24_BOVIN	0.238223458	3.16E-07	0.47779879	0.632517258	0.472341	0.914280589
Cow_F1MDA1_F1MDA1_BOVIN	0.634709086	3.97E-09	0.17148308	0.908482769	0.416938	0.131009021
Cow_F1MDX5_F1MDX5_BOVIN	0.887749426	7.25E-06	0.75810994	0.689387291	0.24652	0.590339001
Cow_F1MFW9_F1MFW9_BOVIN	0.075837694	0.00069406	0.19356172	0.843563755	0.779852	0.328184762
Cow_F1MI98_F1MI98_BOVIN	0.611008774	1.21E-12	0.85959805	0.072112019	0.759094	0.149069538
Cow_F1MIM3_F1MIM3_BOVIN	0.431326359	1.15E-10	0.38354346	0.754764587	0.985832	0.274712019
Cow_F1MJM0_ZN326_BOVIN	0.59770437	0.14337594	0.23351629	0.460022069	0.623972	0.387984346
Cow_F1MJU1_F1MJU1_BOVIN	0.114783679	0.00531702	0.72922148	0.344478131	0.374508	0.130285462
Cow_F1MKB7_F1MKB7_BOVIN	0.15670628	3.85E-09	0.91474268	0.016200056	0.401565	0.004447432
Cow_F1MKE7_F1MKE7_BOVIN	0.059753865	1.91E-06	0.79692205	0.133729846	0.614273	0.138040936
Cow_F1MKV7_F1MKV7_BOVIN	0.260201333	0.00014569	0.17984827	0.404833681	0.764275	0.005982813
Cow_F1ML89_F1ML89_BOVIN	0.611978778	3.80E-09	0.84626732	0.823041038	0.944402	0.499711346

Cow_F1MLQ1_F1MLQ1_BOVIN	0.851518522	5.22E-05	0.4531972	0.503714312	0.515609	0.221679599
Cow_F1MLR9_F1MLR9_BOVIN	0.895388625	2.47E-07	0.4175453	0.03439802	0.067186	0.059591333
Cow_F1MLW8_F1MLW8_BOVIN	0.434733708	0.21319847	0.95386069	0.080840142	0.481621	0.431381374
Cow_F1MLX9_F1MLX9_BOVIN	0.809792837	3.86E-09	0.62857699	0.128567359	0.989043	0.690454019
Cow_F1MM79_F1MM79_BOVIN	0.336179695	1.09E-05	0.27530699	0.053686607	0.975206	0.039384458
Cow_F1MP06_F1MP06_BOVIN	0.299164428	6.22E-10	0.61850217	0.145503109	0.928206	0.808164615
Cow_F1MP21_F1MP21_BOVIN	0.046938244	2.93E-07	0.83963969	0.459003735	0.837119	0.566279551
Cow_F1MQ37_F1MQ37_BOVIN	0.838189557	5.96E-08	0.78279395	0.219633912	0.577349	0.298328206
Cow_F1MQJ0_F1MQJ0_BOVIN	0.159671238	0.00370455	0.87904157	0.657439005	0.823799	0.084281098
Cow_F1MSF5_F1MSF5_BOVIN	0.008369118	0.000443	0.31859834	0.004180304	0.623393	0.570660327
Cow_F1MTR1_F1MTR1_BOVIN	0.569929132	8.63E-07	0.97259699	0.806500773	0.621518	0.392942554
Cow_F1MTV9_F1MTV9_BOVIN	0.772297642	0.11718234	0.11876785	0.173661302	0.019733	0.495525594
Cow_F1MUP9_F1MUP9_BOVIN	0.666636565	4.80E-09	0.37476338	0.001297519	0.819272	0.006918345
Cow_F1MUU9_F1MUU9_BOVIN	0.099436705	0.45827876	0.58783953	0.53372753	0.528129	0.762213312
Cow_F1MWD3_F1MWD3_BOVIN	0.796286458	8.83E-07	0.39678474	0.949028626	0.516724	0.542367824
Cow_F1MWU9_F1MWU9_BOVIN	0.425263635	0.00105939	0.46344791	0.858205499	0.05354	0.063845346
Cow_F1MXX6_F1MXX6_BOVIN	0.208523404	0.04833083	0.07994463	0.418634084	0.318199	0.05684385
Cow_F1N1R7_F1N1R7_BOVIN	0.025084782	0.03065221	0.99103579	0.030652213	0.930075	0.994939452
Cow_F1N206_F1N206_BOVIN	0.648879575	4.05E-07	0.61440711	0.995057974	0.975028	0.066421183
Cow_F1N2L9_F1N2L9_BOVIN	0.420807927	2.63E-07	0.4687442	0.765593951	0.301756	0.00532901
Cow_F1N362_F1N362_BOVIN	0.015716613	0.08651901	0.90012237	0.150461336	0.287781	0.484020878
Cow_F1N3J3_F1N3J3_BOVIN	0.414435002	9.41E-06	0.32427067	0.82443557	0.200765	0.0173184
Cow_F1N566_F1N566_BOVIN	0.753041116	6.98E-17	0.47921862	0.000423356	0.607951	0.681772034
Cow_F1N6H4_F1N6H4_BOVIN	0.0677141	0.17846029	0.46985497	0.148421508	0.55349	0.797043157
Cow_F1N726_F1N726_BOVIN	0.753930202	0.10669566	0.69021822	0.001834464	0.297153	0.000496874
Cow_F6RYN2_F6RYN2_BOVIN	0.128660885	1.36E-06	0.04420642	0.221824747	0.138418	0.077451415
Cow_F6S1Q0_F6S1Q0_BOVIN	0.079011383	0.00501263	0.17562232	0.887375002	0.95687	0.725648362
Cow_G3MWX6_G3MWX6_BOVIN	0.810935173	1.80E-07	0.96819662	0.093157782	0.228797	0.07577007
Cow_G3MX98_G3MX98_BOVIN	0.887587658	0.00033635	0.04415902	0.000524888	0.099425	0.298107144
Cow_G3MYN4_G3MYN4_BOVIN	0.272584566	2.36E-08	0.50567568	0.136178434	0.690678	0.267882649
Cow_G3MZ19_G3MZ19_BOVIN	0.046836132	0.00188486	0.74441655	0.021456006	0.778963	0.033770386
Cow_G3MZR4_G3MZR4_BOVIN	0.352884106	0.0008069	0.4329894	0.037197682	0.600531	0.037258427
Cow_G3N0V2_G3N0V2_BOVIN	0.129563902	0.15823518	0.4754448	0.102824434	0.438683	0.411403604
Cow_G3X6I0_G3X6I0_BOVIN	0.119654965	8.55E-05	0.20206502	0.025768116	0.449941	0.349285931
Cow_G5E5T5_G5E5T5_BOVIN	0.303251825	0.40714055	0.1797314	0.658756302	0.565243	0.352836461
Cow_G5E6E8_G5E6E8_BOVIN	0.066211995	0.70127821	0.76371791	0.605403162	0.495303	0.882102111
Cow_G5E6I9_G5E6I9_BOVIN	0.515273897	0.02244303	0.92372476	0.167599942	0.169193	0.230901738
Cow_M0QVZ8_M0QVZ8_BOVIN	0.181966543	0.64447451	0.0931541	0.959154968	0.22868	0.560580034

Supplementary Table 2. CheckM summary statistics of MAGs from the four metagenomic sequencing samples.

Sample	**Completeness**	**Contamination**	**Strain Heterogeneity**
Cage 1	497	0.9	8.1
Cage 2	53.4	1.6	9.8
Cage 3	49.0	0.6	9.9
Cage 4	46.3	1.26	8.0

Supplementary Table 3. Quast summary statistics of MAGs for each sample.

Quast Statistic	Cage 1 Average	Cage 2 Average	Cage 3 Average	Cage 4 Average
# contigs (>= 0 bp)	98.9	117.5	101.6	114.3
# contigs (>= 5000 bp)	53	70.1	56.1	58
# contigs (>= 50000 bp)	6.2	5.5	5.6	5.1
Total length (>= 0 bp)	1509554.3	1637777.3	1480529.8	1391308.3
Total length (>= 5000 bp)	1348767.9	1469136.3	1319729.5	1193563.8
Total length (>= 10000 bp)	1180725	1232527.5	1140166.2	981182.8
Total length (>= 50000 bp)	708603.8	610108.1	612349.3	532295.4
# contigs	98.9	117.5	101.6	114.3
Largest contig	151881	151696.9	141379	137730.9
Total length	1509554.3	1637777.3	1480529.8	1391308.3
GC (%)	52.5	53.3	53.2	54.4
N50	83526.1	78166.8	79037.1	73770.7

Supplementary Table 4. MAG taxonomic information generated from BAT and CheckM.

ID	BAT: phylum	BAT: class	BAT: order	BAT: family	BAT: genus	BAT: species	CheckM Classification
bin.1.Cage1	Firmicutes: 0.73	Clostridia: 0.52	Clostridiales: 0.52	not classified	not classified	not classified	root (UID1)
bin.10.Cage1	Firmicutes: 0.86	Clostridia: 0.78	Clostridiales: 0.78	Lachnospiraceae: 0.51	NA	Lachnospiraceae bacterium 3-2: 0.37	f__Lachnospiraceae (UID1256)
bin.100.Cage1	Firmicutes: 0.86	Clostridia: 0.76	Clostridiales: 0.76	not classified	not classified	not classified	f__Lachnospiraceae (UID1256)
bin.101.Cage1	Firmicutes: 0.93	Clostridia: 0.86	Clostridiales: 0.85	Oscillospiraceae: 0.72	Oscillibacter: 0.69	Oscillibacter sp. 1-3: 0.65	k__Bacteria (UID203)
bin.102.Cage1	Firmicutes: 0.99	Clostridia: 0.95	Clostridiales: 0.95	Clostridiaceae: 0.90	Clostridium: 0.90	Clostridium sp. ASF356: 0.90	k__Bacteria (UID203)
bin.103.Cage1	Proteobacteria: 1.00	Betaproteobacteria: 0.61	Burkholderiales: 0.61	not classified	not classified	not classified	k__Bacteria (UID203)
bin.104.Cage1	Firmicutes: 0.90	Clostridia: 0.82	Clostridiales: 0.82	Clostridiaceae: 0.76	Clostridium: 0.76	not classified	o__Clostridiales (UID1212)
bin.105.Cage1	Firmicutes: 0.90	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.106.Cage1	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.107.Cage1	Actinobacteria: 0.99	Actinobacteria: 0.99	Bifidobacteriales: 0.92	Bifidobacteriaceae: 0.92	Bifidobacterium: 0.92	Bifidobacterium pseudolongum: 0.77	f__Bifidobacteriaceae (UID1460)
bin.108.Cage1	Firmicutes: 0.64	Clostridia: 0.46	Clostridiales: 0.46	not classified	not classified	not classified	root (UID1)
bin.109.Cage1	Bacteroidetes: 0.99	Bacteroidia: 0.99	Bacteroidales: 0.99	Muribaculaceae: 0.93	not classified	not classified	k__Bacteria (UID203)
bin.11.Cage1	not classified	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.110.Cage1	Firmicutes: 0.80	Clostridia: 0.72	Clostridiales: 0.72	not classified	not classified	not classified	root (UID1)
bin.111.Cage1	Bacteroidetes: 0.96	Bacteroidia: 0.95	Bacteroidales: 0.95	Rikenellaceae: 0.85	Alistipes: 0.85	not classified	p__Bacteroidetes (UID2605)
bin.112.Cage1	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.113.Cage1	Bacteroidetes: 0.86	Bacteroidia: 0.86	Bacteroidales: 0.86	Muribaculaceae: 0.61	not classified	not classified	root (UID1)
bin.114.Cage1	Bacteroidetes: 0.79	Bacteroidia: 0.79	Bacteroidales: 0.79	not classified	not classified	not classified	root (UID1)
bin.115.Cage1	Bacteroidetes: 0.72	Bacteroidia: 0.72	Bacteroidales: 0.72	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.116.Cage1	Firmicutes: 0.51	Clostridia: 0.36	Clostridiales: 0.35	not classified	not classified	not classified	root (UID1)

bin.117.Cage1	Firmicutes: 0.83	NA	NA	NA	NA	Firmicutes bacterium CAG:582: 0.56	k__Bacteria (UID2329)
bin.118.Cage1	Bacteroidetes: 0.66	Bacteroidia: 0.65	Bacteroidales: 0.65	not classified	not classified	not classified	k__Bacteria (UID203)
bin.119.Cage1	Bacteroidetes: 0.74	Bacteroidia: 0.74	Bacteroidales: 0.74	Muribaculaceae: 0.39	not classified	not classified	root (UID1)
bin.12.Cage1	Bacteroidetes: 0.76	Bacteroidia: 0.75	Bacteroidales: 0.75	Muribaculaceae: 0.36	not classified	not classified	k__Bacteria (UID203)
bin.120.Cage1	Firmicutes: 0.87	Clostridia: 0.78	Clostridiales: 0.77	Oscillospiraceae: 0.61	Oscillibacter: 0.35	not classified	o__Clostridiales (UID1212)
bin.121.Cage1	Firmicutes: 0.79	Clostridia: 0.31	not classified	not classified	not classified	not classified	c__Clostridia (UID1118)
bin.122.Cage1	Firmicutes: 0.99	Clostridia: 0.99	Clostridiales: 0.98	Ruminococcaceae: 0.98	Anaerotruncus: 0.97	not classified	o__Clostridiales (UID1212)
bin.123.Cage1	Firmicutes: 0.61	Clostridia: 0.47	Clostridiales: 0.47	not classified	not classified	not classified	root (UID1)
bin.13.Cage1	Firmicutes: 0.81	Clostridia: 0.47	Clostridiales: 0.47	not classified	not classified	not classified	f__Lachnospiraceae (UID1256)
bin.14.Cage1	Firmicutes: 0.91	Clostridia: 0.86	Clostridiales: 0.85	Oscillospiraceae: 0.71	Oscillibacter: 0.63	Oscillibacter sp. 1-3: 0.56	o__Clostridiales (UID1212)
bin.15.Cage1	Firmicutes: 0.73	Clostridia: 0.53	Clostridiales: 0.51	not classified	not classified	not classified	k__Bacteria (UID203)
bin.16.Cage1	Firmicutes: 0.79	Clostridia: 0.70	Clostridiales: 0.67	Ruminococcaceae: 0.41	not classified	not classified	k__Bacteria (UID203)
bin.17.Cage1	Firmicutes: 0.84	Clostridia: 0.79	Clostridiales: 0.77	not classified	not classified	not classified	k__Bacteria (UID203)
bin.18.Cage1	not classified	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.19.Cage1	Bacteroidetes: 1.00	Bacteroidia: 1.00	Bacteroidales: 1.00	Muribaculaceae: 0.99	not classified	not classified	k__Bacteria (UID203)
bin.2.Cage1	Firmicutes: 0.51	Clostridia: 0.38	Clostridiales: 0.38	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.20.Cage1	Firmicutes: 0.31	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.21.Cage1	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.95	Bacteroides: 0.95	not classified	root (UID1)
bin.22.Cage1	Firmicutes: 0.91	Clostridia: 0.86	Clostridiales: 0.85	Ruminococcaceae: 0.70	not classified	not classified	o__Clostridiales (UID1212)
bin.23.Cage1	Tenericutes: 0.69	Mollicutes: 0.69	Mycoplasmatales: 0.68	Mycoplasmataceae: 0.68	Mycoplasma: 0.68	Mycoplasma sp. CAG:776: 0.67	k__Bacteria (UID2329)
bin.24.Cage1	Firmicutes: 0.73	Clostridia: 0.34	Clostridiales: 0.32	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.25.Cage1	Bacteroidetes: 0.56	Bacteroidia: 0.54	Bacteroidales: 0.53	not classified	not classified	not classified	k__Bacteria (UID203)

bin.26.Cage1	Firmicutes: 0.86	Clostridia: 0.78	Clostridiales: 0.77	Oscillospiraceae: 0.60	Oscillibacter: 0.33	not classified	o__Clostridiales (UID1212)
bin.27.Cage1	Firmicutes: 0.98	Clostridia: 0.95	Clostridiales: 0.95	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.28.Cage1	Firmicutes: 1.00	Erysipelotrichia: 0.98	Erysipelotrichales: 0.98	Erysipelotrichaceae: 0.98	Faecalibaculum: 0.97	Faecalibaculum rodentium*: 0.97	k__Bacteria (UID2372)
bin.29.Cage1	Firmicutes: 0.80	Clostridia: 0.68	Clostridiales: 0.65	Ruminococcaceae: 0.32	not classified	not classified	o__Clostridiales (UID1212)
bin.3.Cage1	Firmicutes: 0.86	Clostridia: 0.75	Clostridiales: 0.75	Oscillospiraceae: 0.54	Oscillibacter: 0.36	not classified	k__Bacteria (UID203)
bin.30.Cage1	Firmicutes: 0.66	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.31.Cage1	Firmicutes: 0.77	Clostridia: 0.37	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.32.Cage1	Firmicutes: 0.70	Clostridia: 0.57	Clostridiales: 0.54	not classified	not classified	not classified	k__Bacteria (UID203)
bin.33.Cage1	not classified	not classified	not classified	not classified	not classified	not classified	root (UID1)
bin.34.Cage1	Firmicutes: 1.00	Clostridia: 1.00	Clostridiales: 1.00	Oscillospiraceae: 0.99	Oscillibacter: 0.99	Oscillibacter sp. 1-3: 0.98	k__Bacteria (UID203)
bin.35.Cage1	Firmicutes: 0.81	Clostridia: 0.35	not classified	not classified	not classified	not classified	root (UID1)
bin.36.Cage1	Firmicutes: 0.96	Clostridia: 0.93	Clostridiales: 0.93	Oscillospiraceae: 0.88	Oscillibacter: 0.79	Oscillibacter sp. 1-3: 0.74	k__Bacteria (UID203)
bin.37.Cage1	Firmicutes: 0.68	Clostridia: 0.42	Clostridiales: 0.35	not classified	not classified	not classified	c__Clostridia (UID1085)
bin.38.Cage1	NA	NA	NA	NA	NA	bacterium 1XD8-92: 0.86	f__Lachnospiraceae (UID1255)
bin.39.Cage1	Firmicutes: 0.46	Clostridia: 0.38	Clostridiales: 0.38	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.4.Cage1	Bacteroidetes: 1.00	Bacteroidia: 1.00	Bacteroidales: 1.00	Muribaculaceae: 0.96	not classified	not classified	o__Bacteroidales (UID2617)
bin.40.Cage1	Firmicutes: 0.90	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.41.Cage1	Firmicutes: 0.89	Clostridia: 0.86	Clostridiales: 0.86	Lachnospiraceae: 0.78	NA	Lachnospiraceae bacterium A4: 0.51	root (UID1)
bin.42.Cage1	Firmicutes: 0.49	Clostridia: 0.42	Clostridiales: 0.42	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.43.Cage1	Firmicutes: 0.31	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.44.Cage1	Firmicutes: 0.36	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.45.Cage1	Firmicutes: 0.80	Clostridia: 0.75	Clostridiales: 0.75	not classified	not classified	not classified	o__Clostridiales (UID1212)

bin.46.Cage1	not classified	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.47.Cage1	Firmicutes: 0.75	Clostridia: 0.59	Clostridiales: 0.58	not classified	not classified	not classified	k__Bacteria (UID203)
bin.48.Cage1	Firmicutes: 0.80	Clostridia: 0.73	Clostridiales: 0.71	Ruminococcaceae: 0.48	not classified	not classified	o__Clostridiales (UID1212)
bin.49.Cage1	Proteobacteria: 0.98	Betaproteobacteria: 0.34	Burkholderiales: 0.34	not classified	not classified	not classified	p__Proteobacteria (UID3887)
bin.5.Cage1	Firmicutes: 0.92	Clostridia: 0.48	Clostridiales: 0.48	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.50.Cage1	Bacteroidetes: 0.64	Bacteroidia: 0.63	Bacteroidales: 0.63	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.51.Cage1	Firmicutes: 0.93	Clostridia: 0.86	Clostridiales: 0.85	Clostridiaceae: 0.80	Clostridium: 0.80	not classified	o__Clostridiales (UID1212)
bin.52.Cage1	Firmicutes: 0.77	Clostridia: 0.63	Clostridiales: 0.61	Ruminococcaceae: 0.31	not classified	not classified	o__Clostridiales (UID1212)
bin.53.Cage1	Firmicutes: 0.90	Clostridia: 0.83	Clostridiales: 0.83	Oscillospiraceae: 0.68	Oscillibacter: 0.34	not classified	k__Bacteria (UID203)
bin.54.Cage1	Firmicutes: 0.71	Clostridia: 0.37	Clostridiales: 0.35	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.55.Cage1	Firmicutes: 0.87	Clostridia: 0.79	Clostridiales: 0.78	Oscillospiraceae: 0.62	Oscillibacter: 0.33	not classified	o__Clostridiales (UID1212)
bin.56.Cage1	Bacteroidetes: 0.95	Bacteroidia: 0.95	Bacteroidales: 0.95	Bacteroidaceae: 0.86	Bacteroides: 0.86	not classified	k__Bacteria (UID203)
bin.57.Cage1	Firmicutes: 0.74	Clostridia: 0.34	Clostridiales: 0.32	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.58.Cage1	NA	NA	NA	NA	NA	bacterium 1xD42-87: 0.68	f__Lachnospiraceae (UID1286)
bin.59.Cage1	Firmicutes: 0.59	Clostridia: 0.46	Clostridiales: 0.46	not classified	not classified	not classified	root (UID1)
bin.6.Cage1	Bacteroidetes: 0.82	Bacteroidia: 0.82	Bacteroidales: 0.80	Muribaculaceae: 0.40	not classified	not classified	k__Bacteria (UID203)
bin.60.Cage1	Firmicutes: 0.76	Clostridia: 0.38	Clostridiales: 0.35	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.61.Cage1	Firmicutes: 0.87	Clostridia: 0.82	Clostridiales: 0.82	Eubacteriaceae: 0.55	Eubacterium: 0.55	Eubacterium plexicaudatum: 0.54	k__Bacteria (UID203)
bin.62.Cage1	Bacteroidetes: 0.80	Bacteroidia: 0.80	Bacteroidales: 0.79	Muribaculaceae: 0.40	not classified	not classified	o__Bacteroidales (UID2617)
bin.63.Cage1	Bacteroidetes: 0.44	Bacteroidia: 0.43	Bacteroidales: 0.43	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.64.Cage1	Firmicutes: 0.89	Clostridia: 0.49	Clostridiales: 0.49	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.65.Cage1	Bacteroidetes: 0.60	Bacteroidia: 0.59	Bacteroidales: 0.58	not classified	not classified	not classified	o__Bacteroidales (UID2617)

bin.66.Cage1	Proteobacteria: 1.00	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.67.Cage1	Bacteroidetes: 0.78	Bacteroidia: 0.78	Bacteroidales: 0.78	Muribaculaceae: 0.46	NA	Muribaculaceae bacterium Isolate-013 (NCI): 0.33	root (UID1)
bin.68.Cage1	Firmicutes: 0.75	Clostridia: 0.54	Clostridiales: 0.53	not classified	not classified	not classified	k__Bacteria (UID203)
bin.69.Cage1	Firmicutes: 0.79	Clostridia: 0.37	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.7.Cage1	Firmicutes: 0.71	Clostridia: 0.59	Clostridiales: 0.58	not classified	not classified	not classified	o__Clostridiales (UID1226)
bin.70.Cage1	Firmicutes: 0.79	Clostridia: 0.71	Clostridiales: 0.69	Ruminococcaceae: 0.47	Angelakisella: 0.31	Angelakisella massiliensis*: 0.31	o__Clostridiales (UID1212)
bin.71.Cage1	Firmicutes: 0.79	Clostridia: 0.70	Clostridiales: 0.69	Ruminococcaceae: 0.32	not classified	not classified	k__Bacteria (UID203)
bin.72.Cage1	Actinobacteria: 1.00	Actinobacteria: 1.00	Bifidobacteriales: 0.92	Bifidobacteriaceae: 0.92	Bifidobacterium: 0.92	Bifidobacterium pseudolongum: 0.80	k__Bacteria (UID203)
bin.73.Cage1	Firmicutes: 0.88	Clostridia: 0.80	Clostridiales: 0.79	Oscillospiraceae: 0.62	Oscillibacter: 0.36	not classified	k__Bacteria (UID203)
bin.74.Cage1	Firmicutes: 0.75	Clostridia: 0.36	Clostridiales: 0.33	not classified	not classified	not classified	k__Bacteria (UID203)
bin.75.Cage1	Firmicutes: 0.39	not classified	not classified	not classified	not classified	not classified	root (UID1)
bin.76.Cage1	Firmicutes: 0.36	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.77.Cage1	Firmicutes: 0.41	Clostridia: 0.35	Clostridiales: 0.35	not classified	not classified	not classified	k__Bacteria (UID203)
bin.78.Cage1	Bacteroidetes: 0.99	Bacteroidia: 0.99	Bacteroidales: 0.99	Muribaculaceae: 0.78	not classified	not classified	root (UID1)
bin.79.Cage1	Firmicutes: 0.90	Clostridia: 0.87	Clostridiales: 0.87	not classified	not classified	not classified	root (UID1)
bin.8.Cage1	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.92	Bacteroides: 0.92	not classified	root (UID1)
bin.80.Cage1	Firmicutes: 0.70	not classified	not classified	not classified	not classified	not classified	c__Clostridia (UID1118)
bin.81.Cage1	Verrucomicrobia: 0.99	Verrucomicrobiae: 0.99	Verrucomicrobiales: 0.99	Akkermansiaceae: 0.99	Akkermansia*: 0.99	not classified	k__Bacteria (UID2982)
bin.82.Cage1	Firmicutes: 0.80	Clostridia: 0.74	Clostridiales: 0.74	Lachnospiraceae: 0.41	Roseburia: 0.36	not classified	k__Bacteria (UID203)
bin.83.Cage1	Firmicutes: 0.96	Clostridia: 0.93	Clostridiales: 0.93	Oscillospiraceae: 0.88	Oscillibacter: 0.84	Oscillibacter sp. 1-3: 0.80	o__Clostridiales (UID1212)
bin.84.Cage1	Firmicutes: 0.93	NA	NA	NA	NA	Firmicutes bacterium: 0.64	o__Thermoanaerobacterales (UID1420)
bin.85.Cage1	Firmicutes: 0.49	Clostridia: 0.41	Clostridiales: 0.41	not classified	not classified	not classified	k__Bacteria (UID203)

bin.86.Cage1	Firmicutes: 0.98	Bacilli: 0.98	Lactobacillales: 0.98	Streptococcaceae: 0.98	Lactococcus: 0.98	Lactococcus lactis: 0.91	k__Bacteria (UID203)
bin.87.Cage1	Firmicutes: 0.93	Clostridia: 0.88	Clostridiales: 0.88	Eubacteriaceae: 0.78	Eubacterium: 0.78	not classified	o__Clostridiales (UID1212)
bin.88.Cage1	Firmicutes: 0.38	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1286)
bin.89.Cage1	not classified	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.9.Cage1	Firmicutes: 0.91	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.90.Cage1	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.94	Bacteroides: 0.94	not classified	k__Bacteria (UID203)
bin.91.Cage1	Bacteroidetes: 0.97	Bacteroidia: 0.97	Bacteroidales: 0.97	Bacteroidaceae: 0.97	Bacteroides: 0.97	not classified	k__Bacteria (UID203)
bin.92.Cage1	Firmicutes: 0.96	Bacilli: 0.95	Lactobacillales: 0.95	Streptococcaceae: 0.95	Lactococcus: 0.95	Lactococcus lactis: 0.80	o__Lactobacillales (UID543)
bin.93.Cage1	Firmicutes: 0.78	Clostridia: 0.64	Clostridiales: 0.61	Ruminococcaceae: 0.30	not classified	not classified	o__Clostridiales (UID1212)
bin.94.Cage1	Firmicutes: 0.76	Clostridia: 0.37	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.95.Cage1	Firmicutes: 1.00	Erysipelotrichia: 0.97	Erysipelotrichales: 0.97	Erysipelotrichaceae: 0.97	Dubosiella: 0.95	Dubosiella newyorkensis*: 0.95	k__Bacteria (UID2372)
bin.96.Cage1	Firmicutes: 0.85	Clostridia: 0.73	Clostridiales: 0.71	Ruminococcaceae: 0.43	Pseudoflavonifractor: 0.39	Pseudoflavonifractor sp. Marseille-P3106: 0.38	k__Bacteria (UID203)
bin.97.Cage1	Firmicutes: 0.74	Clostridia: 0.36	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.98.Cage1	Firmicutes: 0.77	Clostridia: 0.72	Clostridiales: 0.72	Lachnospiraceae: 0.42	Roseburia: 0.30	not classified	f__Lachnospiraceae (UID1286)
bin.99.Cage1	Firmicutes: 0.34	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1286)
bin.1.Cage2	Firmicutes: 0.30	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.10.Cage2	Firmicutes: 0.99	Clostridia: 0.99	Clostridiales: 0.99	Ruminococcaceae: 0.99	Anaerotruncus: 0.99	not classified	o__Clostridiales (UID1212)
bin.100.Cage2	Firmicutes: 0.87	Clostridia: 0.78	Clostridiales: 0.74	Ruminococcaceae: 0.41	Pseudoflavonifractor: 0.36	Pseudoflavonifractor sp. Marseille-P3106: 0.35	o__Clostridiales (UID1212)
bin.101.Cage2	Firmicutes: 0.98	Clostridia: 0.96	Clostridiales: 0.96	Oscillospiraceae: 0.91	Oscillibacter: 0.91	Oscillibacter sp. 1-3: 0.91	k__Bacteria (UID203)
bin.102.Cage2	Proteobacteria: 1.00	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.103.Cage2	Firmicutes: 0.86	Clostridia: 0.32	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.104.Cage2	Firmicutes: 0.91	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)

bin.105.Cage2	Firmicutes: 0.85	Clostridia: 0.74	Clostridiales: 0.72	Ruminococcaceae: 0.45	Pseudoflavonifractor: 0.40	Pseudoflavonifractor sp. Marseille-P3106: 0.40	o__Clostridiales (UID1212)
bin.106.Cage2	Firmicutes: 0.74	Clostridia: 0.35	Clostridiales: 0.33	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.107.Cage2	Firmicutes: 0.78	Clostridia: 0.66	Clostridiales: 0.63	Ruminococcaceae: 0.30	not classified	not classified	o__Clostridiales (UID1212)
bin.108.Cage2	Firmicutes: 0.80	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.109.Cage2	Bacteroidetes: 0.98	Bacteroidia: 0.98	Bacteroidales: 0.98	Muribaculaceae: 0.83	not classified	not classified	o__Bacteroidales (UID2617)
bin.11.Cage2	Firmicutes: 0.35	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.110.Cage2	Firmicutes: 0.76	Clostridia: 0.39	Clostridiales: 0.37	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.111.Cage2	Firmicutes: 0.79	Clostridia: 0.75	Clostridiales: 0.75	not classified	not classified	not classified	o__Clostridiales (UID1226)
bin.112.Cage2	Firmicutes: 0.69	Clostridia: 0.57	Clostridiales: 0.51	not classified	not classified	not classified	k__Bacteria (UID203)
bin.113.Cage2	Firmicutes: 0.98	Erysipelotrichia: 0.90	Erysipelotrichales: 0.90	Erysipelotrichaceae: 0.90	Turicibacter: 0.90	not classified	c__Bacilli (UID285)
bin.114.Cage2	Firmicutes: 0.64	Clostridia: 0.54	Clostridiales: 0.54	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.115.Cage2	Firmicutes: 0.90	Clostridia: 0.82	Clostridiales: 0.81	Lachnospiraceae: 0.57	NA	Lachnospiraceae bacterium 3-2: 0.47	f__Lachnospiraceae (UID1256)
bin.116.Cage2	Firmicutes: 0.54	Clostridia: 0.34	Clostridiales: 0.33	not classified	not classified	not classified	k__Bacteria (UID203)
bin.117.Cage2	Firmicutes: 0.75	Clostridia: 0.33	Clostridiales: 0.30	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.118.Cage2	Firmicutes: 0.64	Clostridia: 0.51	Clostridiales: 0.50	not classified	not classified	not classified	root (UID1)
bin.12.Cage2	Tenericutes: 0.38	Mollicutes: 0.37	Anaeroplasmatales: 0.35	Anaeroplasmataceae: 0.35	Anaeroplasma: 0.35	Anaeroplasma bactoclasticum: 0.35	k__Bacteria (UID2328)
bin.13.Cage2	Firmicutes: 0.64	Clostridia: 0.58	Clostridiales: 0.58	not classified	not classified	not classified	root (UID1)
bin.14.Cage2	Firmicutes: 0.44	Clostridia: 0.36	Clostridiales: 0.36	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.15.Cage2	Proteobacteria: 0.98	Betaproteobacteria: 0.60	Burkholderiales: 0.60	not classified	not classified	not classified	k__Bacteria (UID203)
bin.16.Cage2	Firmicutes: 0.80	Clostridia: 0.72	Clostridiales: 0.70	Ruminococcaceae: 0.46	not classified	not classified	k__Bacteria (UID203)
bin.17.Cage2	Firmicutes: 0.78	Clostridia: 0.39	Clostridiales: 0.36	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.18.Cage2	Firmicutes: 0.34	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)

bin.19.Cage2	Firmicutes: 0.46	Clostridia: 0.39	Clostridiales: 0.38	not classified	not classified	not classified	f__Lachnospiraceae (UID1286)
bin.2.Cage2	Firmicutes: 0.32	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.20.Cage2	Firmicutes: 0.72	Clostridia: 0.35	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.21.Cage2	Firmicutes: 0.93	Clostridia: 0.89	Clostridiales: 0.89	Clostridiaceae: 0.84	Clostridium: 0.83	not classified	o__Clostridiales (UID1212)
bin.22.Cage2	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.23.Cage2	Firmicutes: 0.78	Clostridia: 0.65	Clostridiales: 0.63	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.24.Cage2	Firmicutes: 0.91	Clostridia: 0.83	Clostridiales: 0.78	Ruminococcaceae: 0.52	Pseudoflavonifractor: 0.48	Pseudoflavonifractor sp. Marseille-P3106: 0.47	k__Bacteria (UID203)
bin.25.Cage2	Firmicutes: 0.76	Clostridia: 0.40	Clostridiales: 0.37	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.26.Cage2	Firmicutes: 0.50	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.27.Cage2	Firmicutes: 0.75	Clostridia: 0.62	Clostridiales: 0.60	Ruminococcaceae: 0.30	not classified	not classified	o__Clostridiales (UID1212)
bin.28.Cage2	Firmicutes: 0.74	Clostridia: 0.60	Clostridiales: 0.58	not classified	not classified	not classified	k__Bacteria (UID203)
bin.29.Cage2	Bacteroidetes: 0.88	Bacteroidia: 0.88	Bacteroidales: 0.88	Muribaculaceae: 0.55	not classified	not classified	k__Bacteria (UID203)
bin.3.Cage2	Firmicutes: 0.78	Clostridia: 0.37	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.30.Cage2	Firmicutes: 0.82	Clostridia: 0.34	not classified	not classified	not classified	not classified	c__Clostridia (UID1118)
bin.31.Cage2	Firmicutes: 0.79	Clostridia: 0.71	Clostridiales: 0.71	Lachnospiraceae: 0.44	Roseburia: 0.37	not classified	k__Bacteria (UID203)
bin.32.Cage2	Firmicutes: 0.39	Clostridia: 0.34	Clostridiales: 0.34	not classified	not classified	not classified	k__Bacteria (UID203)
bin.33.Cage2	Bacteroidetes: 0.93	Bacteroidia: 0.92	Bacteroidales: 0.92	Muribaculaceae: 0.79	not classified	not classified	o__Bacteroidales (UID2617)
bin.34.Cage2	Firmicutes: 0.79	Clostridia: 0.70	Clostridiales: 0.68	Ruminococcaceae: 0.46	Angelakisella: 0.31	Angelakisella massiliensis*: 0.31	o__Clostridiales (UID1212)
bin.35.Cage2	Firmicutes: 0.89	Clostridia: 0.57	Clostridiales: 0.56	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.36.Cage2	Firmicutes: 0.99	Bacilli: 0.98	Lactobacillales: 0.54	Lactobacillaceae: 0.54	Lactobacillus: 0.54	not classified	g__Lactobacillus (UID380)
bin.37.Cage2	Firmicutes: 0.83	Clostridia: 0.70	Clostridiales: 0.67	NA	NA	Clostridiales bacterium: 0.38	k__Bacteria (UID203)
bin.38.Cage2	Firmicutes: 0.35	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)

bin.39.Cage2	Firmicutes: 0.35	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.4.Cage2	Firmicutes: 0.94	Clostridia: 0.83	Clostridiales: 0.83	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.40.Cage2	Firmicutes: 0.80	Clostridia: 0.66	Clostridiales: 0.64	Ruminococcaceae: 0.31	not classified	not classified	o__Clostridiales (UID1212)
bin.41.Cage2	Firmicutes: 0.73	Clostridia: 0.59	Clostridiales: 0.59	not classified	not classified	not classified	root (UID1)
bin.42.Cage2	Firmicutes: 0.90	Clostridia: 0.85	Clostridiales: 0.85	Ruminococcaceae: 0.70	not classified	not classified	o__Clostridiales (UID1212)
bin.43.Cage2	Bacteroidetes: 0.97	Bacteroidia: 0.97	Bacteroidales: 0.97	Bacteroidaceae: 0.97	Bacteroides: 0.97	not classified	k__Bacteria (UID203)
bin.44.Cage2	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.45.Cage2	Firmicutes: 0.91	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.46.Cage2	Firmicutes: 0.73	Clostridia: 0.53	Clostridiales: 0.53	not classified	not classified	not classified	root (UID1)
bin.47.Cage2	Bacteroidetes: 0.63	Bacteroidia: 0.63	Bacteroidales: 0.63	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.48.Cage2	Firmicutes: 0.99	Clostridia: 0.99	Clostridiales: 0.99	Peptostreptococcaceae: 0.96	Romboutsia: 0.92	Romboutsia ilealis: 0.42	k__Bacteria (UID203)
bin.49.Cage2	Firmicutes: 0.96	Clostridia: 0.93	Clostridiales: 0.93	Oscillospiraceae: 0.89	Oscillibacter: 0.84	Oscillibacter sp. 1-3: 0.81	o__Clostridiales (UID1212)
bin.5.Cage2	Firmicutes: 0.79	Clostridia: 0.64	Clostridiales: 0.61	Ruminococcaceae: 0.30	not classified	not classified	o__Clostridiales (UID1212)
bin.50.Cage2	Firmicutes: 0.77	Clostridia: 0.39	Clostridiales: 0.39	not classified	not classified	not classified	root (UID1)
bin.51.Cage2	Firmicutes: 0.83	Clostridia: 0.69	Clostridiales: 0.65	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.52.Cage2	Bacteroidetes: 0.98	Bacteroidia: 0.98	Bacteroidales: 0.98	Bacteroidaceae: 0.98	Bacteroides: 0.98	not classified	root (UID1)
bin.53.Cage2	Firmicutes: 0.69	Clostridia: 0.41	Clostridiales: 0.34	not classified	not classified	not classified	c__Clostridia (UID1085)
bin.54.Cage2	NA	NA	NA	NA	NA	bacterium 1XD8-76: 0.64	f__Lachnospiraceae (UID1255)
bin.55.Cage2	Firmicutes: 0.99	Clostridia: 0.98	Clostridiales: 0.98	Oscillospiraceae: 0.97	Oscillibacter: 0.96	Oscillibacter sp. 1-3: 0.96	o__Clostridiales (UID1212)
bin.56.Cage2	Bacteroidetes: 1.00	Bacteroidia: 1.00	Bacteroidales: 1.00	Muribaculaceae: 0.97	not classified	not classified	o__Bacteroidales (UID2617)
bin.57.Cage2	Firmicutes: 0.74	Clostridia: 0.36	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.58.Cage2	Actinobacteria: 0.52	Coriobacteriia: 0.52	Eggerthellales: 0.51	Eggerthellaceae: 0.51	Enterorhabdus: 0.44	not classified	p__Actinobacteria (UID2112)

bin.59.Cage2	NA	NA	NA	NA	NA	bacterium D16-50: 0.94	f__Lachnospiraceae (UID1255)
bin.6.Cage2	Firmicutes: 0.44	Clostridia: 0.37	Clostridiales: 0.37	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.60.Cage2	Firmicutes: 0.87	Clostridia: 0.78	Clostridiales: 0.77	Oscillospiraceae: 0.57	Oscillibacter: 0.33	not classified	o__Clostridiales (UID1212)
bin.61.Cage2	Firmicutes: 0.91	Clostridia: 0.51	Clostridiales: 0.50	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.62.Cage2	Firmicutes: 0.81	Clostridia: 0.71	Clostridiales: 0.71	not classified	not classified	not classified	root (UID1)
bin.63.Cage2	Firmicutes: 0.82	Clostridia: 0.46	Clostridiales: 0.46	not classified	not classified	not classified	f__Lachnospiraceae (UID1256)
bin.64.Cage2	Firmicutes: 0.47	Clostridia: 0.41	Clostridiales: 0.41	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.65.Cage2	Firmicutes: 0.74	Clostridia: 0.56	Clostridiales: 0.55	not classified	not classified	not classified	root (UID1)
bin.66.Cage2	Firmicutes: 0.90	Clostridia: 0.81	Clostridiales: 0.80	Oscillospiraceae: 0.62	not classified	not classified	root (UID1)
bin.67.Cage2	Firmicutes: 0.88	Clostridia: 0.80	Clostridiales: 0.79	Oscillospiraceae: 0.60	Oscillibacter: 0.34	not classified	o__Clostridiales (UID1212)
bin.68.Cage2	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.69.Cage2	Firmicutes: 0.59	Clostridia: 0.51	Clostridiales: 0.51	Lachnospiraceae: 0.31	not classified	not classified	root (UID1)
bin.7.Cage2	Firmicutes: 0.75	Clostridia: 0.56	Clostridiales: 0.40	not classified	not classified	not classified	k__Bacteria (UID203)
bin.70.Cage2	Bacteroidetes: 0.99	Bacteroidia: 0.99	Bacteroidales: 0.99	Muribaculaceae: 0.92	not classified	not classified	k__Bacteria (UID203)
bin.71.Cage2	Firmicutes: 0.32	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.72.Cage2	Firmicutes: 0.92	Bacilli: 0.90	Lactobacillales: 0.90	Streptococcaceae: 0.89	Lactococcus: 0.89	Lactococcus lactis: 0.78	o__Lactobacillales (UID543)
bin.73.Cage2	Firmicutes: 0.87	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.74.Cage2	Proteobacteria: 0.99	Betaproteobacteria: 0.37	Burkholderiales: 0.37	not classified	not classified	not classified	k__Bacteria (UID203)
bin.75.Cage2	Firmicutes: 0.78	Clostridia: 0.71	Clostridiales: 0.70	not classified	not classified	not classified	root (UID1)
bin.76.Cage2	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.92	Bacteroides: 0.92	not classified	root (UID1)
bin.77.Cage2	NA	NA	NA	NA	NA	bacterium 1xD42-67: 0.82	o__Clostridiales (UID1212)
bin.78.Cage2	Firmicutes: 0.76	Clostridia: 0.38	Clostridiales: 0.35	not classified	not classified	not classified	k__Bacteria (UID203)

bin.79.Cage2	Firmicutes: 0.95	Clostridia: 0.90	Clostridiales: 0.90	Oscillospiraceae: 0.83	Oscillibacter: 0.80	Oscillibacter sp. 1-3: 0.76	k__Bacteria (UID203)
bin.8.Cage2	Firmicutes: 0.69	Clostridia: 0.53	Clostridiales: 0.52	not classified	not classified	not classified	root (UID1)
bin.80.Cage2	Firmicutes: 0.43	Clostridia: 0.34	Clostridiales: 0.34	not classified	not classified	not classified	root (UID1)
bin.81.Cage2	Bacteroidetes: 0.95	Bacteroidia: 0.95	Bacteroidales: 0.95	Bacteroidaceae: 0.87	Bacteroides: 0.87	not classified	k__Bacteria (UID203)
bin.82.Cage2	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.94	Bacteroides: 0.94	not classified	k__Bacteria (UID203)
bin.83.Cage2	Firmicutes: 0.48	Clostridia: 0.37	Clostridiales: 0.37	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.84.Cage2	Firmicutes: 0.88	Clostridia: 0.83	Clostridiales: 0.82	Oscillospiraceae: 0.70	Oscillibacter: 0.63	Oscillibacter sp. 1-3: 0.57	o__Clostridiales (UID1212)
bin.85.Cage2	Firmicutes: 0.71	Clostridia: 0.59	Clostridiales: 0.59	Lachnospiraceae: 0.32	not classified	not classified	root (UID1)
bin.86.Cage2	Firmicutes: 0.79	Clostridia: 0.70	Clostridiales: 0.68	Ruminococcaceae: 0.45	Angelakisella: 0.30	Angelakisella massiliensis*: 0.30	o__Clostridiales (UID1212)
bin.87.Cage2	Firmicutes: 0.88	Clostridia: 0.79	Clostridiales: 0.79	Oscillospiraceae: 0.64	Oscillibacter: 0.35	not classified	o__Clostridiales (UID1212)
bin.88.Cage2	Firmicutes: 0.46	Clostridia: 0.40	Clostridiales: 0.40	not classified	not classified	not classified	f__Lachnospiraceae (UID1286)
bin.89.Cage2	Proteobacteria: 0.99	not classified	not classified	not classified	not classified	not classified	p__Proteobacteria (UID3887)
bin.9.Cage2	Firmicutes: 0.71	Clostridia: 0.36	Clostridiales: 0.31	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.90.Cage2	Firmicutes: 0.79	Clostridia: 0.58	Clostridiales: 0.58	not classified	not classified	not classified	k__Bacteria (UID203)
bin.91.Cage2	Firmicutes: 0.75	Clostridia: 0.37	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.92.Cage2	Firmicutes: 0.90	Clostridia: 0.81	Clostridiales: 0.78	Ruminococcaceae: 0.50	Pseudoflavonifractor: 0.45	Pseudoflavonifractor sp. Marseille-P3106: 0.45	k__Bacteria (UID203)
bin.93.Cage2	Firmicutes: 0.79	Clostridia: 0.70	Clostridiales: 0.67	Ruminococcaceae: 0.49	Angelakisella: 0.36	Angelakisella massiliensis*: 0.36	k__Bacteria (UID203)
bin.94.Cage2	Firmicutes: 0.81	Clostridia: 0.76	Clostridiales: 0.76	Lachnospiraceae: 0.46	Roseburia: 0.38	not classified	f__Lachnospiraceae (UID1286)
bin.95.Cage2	Bacteroidetes: 0.92	Bacteroidia: 0.92	Bacteroidales: 0.91	Muribaculaceae: 0.65	not classified	not classified	k__Bacteria (UID203)
bin.96.Cage2	Firmicutes: 0.80	Clostridia: 0.73	Clostridiales: 0.73	Lachnospiraceae: 0.42	Roseburia: 0.36	not classified	f__Lachnospiraceae (UID1286)
bin.97.Cage2	Firmicutes: 0.72	Clostridia: 0.58	Clostridiales: 0.35	not classified	not classified	not classified	k__Bacteria (UID203)
bin.98.Cage2	Firmicutes: 0.80	Clostridia: 0.39	Clostridiales: 0.37	not classified	not classified	not classified	root (UID1)

bin.99.Cage2	Verrucomicrobia: 0.99	Verrucomicrobiae: 0.99	Verrucomicrobiales: 0.99	Akkermansiaceae: 0.99	Akkermansia*: 0.99	not classified	k__Bacteria (UID2982)
bin.1.Cage3	Firmicutes: 0.74	Clostridia: 0.37	Clostridiales: 0.35	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.10.Cage3	Firmicutes: 0.44	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.100.Cage3	Firmicutes: 0.68	Clostridia: 0.42	Clostridiales: 0.35	not classified	not classified	not classified	c__Clostridia (UID1085)
bin.101.Cage3	Firmicutes: 0.92	Clostridia: 0.87	Clostridiales: 0.87	Ruminococcaceae: 0.77	not classified	not classified	o__Clostridiales (UID1212)
bin.102.Cage3	Firmicutes: 0.88	Clostridia: 0.77	Clostridiales: 0.76	Oscillospiraceae: 0.49	Oscillibacter: 0.31	not classified	k__Bacteria (UID203)
bin.103.Cage3	Firmicutes: 0.80	Clostridia: 0.74	Clostridiales: 0.73	Ruminococcaceae: 0.49	Angelakisella: 0.34	Angelakisella massiliensis*: 0.34	o__Clostridiales (UID1212)
bin.104.Cage3	Firmicutes: 0.87	Clostridia: 0.78	Clostridiales: 0.77	Oscillospiraceae: 0.61	Oscillibacter: 0.34	not classified	o__Clostridiales (UID1212)
bin.105.Cage3	Firmicutes: 0.78	Clostridia: 0.70	Clostridiales: 0.69	Oscillospiraceae: 0.47	not classified	not classified	root (UID1)
bin.106.Cage3	Firmicutes: 0.31	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.107.Cage3	Firmicutes: 0.94	Clostridia: 0.88	Clostridiales: 0.85	Ruminococcaceae: 0.55	Pseudoflavonifractor: 0.48	Pseudoflavonifractor sp. Marseille-P3106: 0.48	k__Bacteria (UID203)
bin.108.Cage3	Firmicutes: 0.90	Clostridia: 0.83	Clostridiales: 0.82	NA	NA	uncultured Clostridiales bacterium*: 0.31	root (UID1)
bin.109.Cage3	Bacteroidetes: 0.64	Bacteroidia: 0.63	Bacteroidales: 0.63	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.11.Cage3	Firmicutes: 0.81	Clostridia: 0.38	Clostridiales: 0.34	not classified	not classified	not classified	root (UID1)
bin.110.Cage3	Firmicutes: 0.74	Clostridia: 0.35	Clostridiales: 0.33	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.111.Cage3	Firmicutes: 0.72	Clostridia: 0.36	Clostridiales: 0.35	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.112.Cage3	Bacteroidetes: 1.00	Bacteroidia: 1.00	Bacteroidales: 1.00	Muribaculaceae: 0.96	not classified	not classified	o__Bacteroidales (UID2617)
bin.113.Cage3	Firmicutes: 0.76	Clostridia: 0.65	Clostridiales: 0.63	not classified	not classified	not classified	root (UID1)
bin.114.Cage3	Firmicutes: 0.62	Clostridia: 0.46	Clostridiales: 0.44	not classified	not classified	not classified	root (UID1)
bin.115.Cage3	Firmicutes: 0.92	Clostridia: 0.53	Clostridiales: 0.53	not classified	not classified	not classified	k__Bacteria (UID203)
bin.116.Cage3	Bacteroidetes: 0.82	Bacteroidia: 0.80	Bacteroidales: 0.80	Muribaculaceae: 0.45	not classified	not classified	root (UID1)
bin.117.Cage3	Firmicutes: 0.66	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)

bin.118.Cage3	Firmicutes: 0.99	Clostridia: 0.98	Clostridiales: 0.98	Ruminococcaceae: 0.97	Anaerotruncus: 0.97	not classified	o__Clostridiales (UID1212)
bin.119.Cage3	Firmicutes: 0.91	Clostridia: 0.84	Clostridiales: 0.84	Clostridiaceae: 0.78	Clostridium: 0.78	not classified	o__Clostridiales (UID1212)
bin.12.Cage3	Firmicutes: 0.32	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.120.Cage3	Firmicutes: 0.69	Clostridia: 0.46	Clostridiales: 0.45	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.121.Cage3	Bacteroidetes: 0.63	Bacteroidia: 0.62	Bacteroidales: 0.61	not classified	not classified	not classified	k__Bacteria (UID203)
bin.122.Cage3	Firmicutes: 0.46	Clostridia: 0.39	Clostridiales: 0.38	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.13.Cage3	Firmicutes: 0.76	Clostridia: 0.41	Clostridiales: 0.38	not classified	not classified	not classified	k__Bacteria (UID203)
bin.14.Cage3	Firmicutes: 0.80	Clostridia: 0.72	Clostridiales: 0.71	Ruminococcaceae: 0.48	not classified	not classified	o__Clostridiales (UID1212)
bin.15.Cage3	Firmicutes: 0.57	Clostridia: 0.53	Clostridiales: 0.53	not classified	not classified	not classified	root (UID1)
bin.16.Cage3	Firmicutes: 0.96	Clostridia: 0.93	Clostridiales: 0.93	Oscillospiraceae: 0.88	Oscillibacter: 0.83	Oscillibacter sp. 1-3: 0.80	o__Clostridiales (UID1212)
bin.17.Cage3	Firmicutes: 1.00	Erysipelotrichia: 0.99	Erysipelotrichales: 0.99	Erysipelotrichaceae: 0.99	Faecalibaculum: 0.99	Faecalibaculum rodentium*: 0.99	k__Bacteria (UID203)
bin.18.Cage3	Firmicutes: 0.78	Clostridia: 0.72	Clostridiales: 0.72	Lachnospiraceae: 0.42	Roseburia: 0.34	not classified	f__Lachnospiraceae (UID1286)
bin.19.Cage3	Proteobacteria: 0.98	Betaproteobacteria: 0.38	Burkholderiales: 0.38	not classified	not classified	not classified	k__Bacteria (UID203)
bin.2.Cage3	Firmicutes: 0.31	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.20.Cage3	Firmicutes: 0.87	Clostridia: 0.80	Clostridiales: 0.79	Oscillospiraceae: 0.61	Oscillibacter: 0.33	not classified	o__Clostridiales (UID1212)
bin.21.Cage3	Firmicutes: 0.96	Clostridia: 0.90	Clostridiales: 0.90	Clostridiaceae: 0.84	Clostridium: 0.84	Clostridium sp. ASF356: 0.83	o__Clostridiales (UID1212)
bin.22.Cage3	Firmicutes: 0.79	Clostridia: 0.57	Clostridiales: 0.56	not classified	not classified	not classified	k__Bacteria (UID203)
bin.23.Cage3	Firmicutes: 0.93	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.24.Cage3	Bacteroidetes: 0.76	Bacteroidia: 0.75	Bacteroidales: 0.75	Muribaculaceae: 0.36	not classified	not classified	k__Bacteria (UID203)
bin.25.Cage3	Bacteroidetes: 0.56	Bacteroidia: 0.55	Bacteroidales: 0.55	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.26.Cage3	Firmicutes: 0.83	Clostridia: 0.62	Clostridiales: 0.60	not classified	not classified	not classified	root (UID1)
bin.27.Cage3	Firmicutes: 0.34	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)

bin.28.Cage3	Firmicutes: 0.94	Clostridia: 0.90	Clostridiales: 0.90	Lachnospiraceae: 0.67	NA	Lachnospiraceae bacterium 3-2: 0.56	f__Lachnospiraceae (UID1256)
bin.29.Cage3	Firmicutes: 0.88	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.3.Cage3	Firmicutes: 0.88	Clostridia: 0.83	Clostridiales: 0.80	Ruminococcaceae: 0.45	Pseudoflavonifractor: 0.41	Pseudoflavonifractor sp. Marseille-P3106: 0.40	k__Bacteria (UID203)
bin.30.Cage3	Firmicutes: 0.89	Clostridia: 0.84	Clostridiales: 0.83	Ruminococcaceae: 0.66	not classified	not classified	o__Clostridiales (UID1212)
bin.31.Cage3	Firmicutes: 0.82	NA	NA	NA	NA	Firmicutes bacterium CAG:582: 0.58	k__Bacteria (UID2329)
bin.32.Cage3	Proteobacteria: 1.00	not classified	not classified	not classified	not classified	not classified	p__Proteobacteria (UID3887)
bin.33.Cage3	Firmicutes: 0.71	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.34.Cage3	Firmicutes: 0.80	Clostridia: 0.68	Clostridiales: 0.65	Ruminococcaceae: 0.32	not classified	not classified	o__Clostridiales (UID1212)
bin.35.Cage3	Firmicutes: 0.74	Clostridia: 0.35	Clostridiales: 0.33	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.36.Cage3	Firmicutes: 0.92	Clostridia: 0.48	Clostridiales: 0.47	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.37.Cage3	Tenericutes: 0.69	Mollicutes: 0.69	Mycoplasmatales: 0.69	Mycoplasmataceae: 0.69	Mycoplasma: 0.69	Mycoplasma sp. CAG:776: 0.68	k__Bacteria (UID2329)
bin.38.Cage3	Firmicutes: 1.00	Erysipelotrichia: 0.97	Erysipelotrichales: 0.97	Erysipelotrichaceae: 0.97	Dubosiella: 0.95	Dubosiella newyorkensis*: 0.95	k__Bacteria (UID2372)
bin.39.Cage3	Firmicutes: 0.37	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1286)
bin.4.Cage3	Firmicutes: 0.85	Clostridia: 0.76	Clostridiales: 0.76	not classified	not classified	not classified	f__Lachnospiraceae (UID1256)
bin.40.Cage3	Firmicutes: 0.97	Bacilli: 0.96	Lactobacillales: 0.96	Streptococcaceae: 0.96	Lactococcus: 0.96	Lactococcus lactis: 0.85	o__Lactobacillales (UID543)
bin.41.Cage3	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.94	Bacteroides: 0.94	not classified	k__Bacteria (UID203)
bin.42.Cage3	Firmicutes: 0.80	Clostridia: 0.31	not classified	not classified	not classified	not classified	c__Clostridia (UID1118)
bin.43.Cage3	Firmicutes: 0.37	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.44.Cage3	Bacteroidetes: 0.69	Bacteroidia: 0.68	Bacteroidales: 0.68	Muribaculaceae: 0.31	not classified	not classified	o__Bacteroidales (UID2617)
bin.45.Cage3	Bacteroidetes: 0.72	Bacteroidia: 0.71	Bacteroidales: 0.71	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.46.Cage3	Firmicutes: 0.68	Clostridia: 0.54	Clostridiales: 0.51	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.47.Cage3	Firmicutes: 0.51	Clostridia: 0.38	Clostridiales: 0.38	not classified	not classified	not classified	o__Clostridiales (UID1212)

bin.48.Cage3	Firmicutes: 0.74	Clostridia: 0.61	Clostridiales: 0.61	not classified	not classified	not classified	root (UID1)
bin.49.Cage3	Firmicutes: 0.99	Clostridia: 0.99	Clostridiales: 0.99	Oscillospiraceae: 0.98	Oscillibacter: 0.97	Oscillibacter sp. 1-3: 0.97	k__Bacteria (UID203)
bin.5.Cage3	Firmicutes: 0.69	not classified	not classified	not classified	not classified	not classified	c__Clostridia (UID1118)
bin.50.Cage3	Firmicutes: 0.92	Clostridia: 0.82	Clostridiales: 0.77	Ruminococcaceae: 0.49	Pseudoflavonifractor: 0.46	Pseudoflavonifractor sp. Marseille-P3106: 0.45	k__Bacteria (UID203)
bin.51.Cage3	Firmicutes: 0.88	Clostridia: 0.85	Clostridiales: 0.85	Lachnospiraceae: 0.80	NA	Lachnospiraceae bacterium A4: 0.48	f__Lachnospiraceae (UID1255)
bin.52.Cage3	Firmicutes: 0.97	Clostridia: 0.94	Clostridiales: 0.94	Oscillospiraceae: 0.89	Oscillibacter: 0.88	Oscillibacter sp. 1-3: 0.88	o__Clostridiales (UID1212)
bin.53.Cage3	Firmicutes: 0.50	Clostridia: 0.43	Clostridiales: 0.42	not classified	not classified	not classified	k__Bacteria (UID203)
bin.54.Cage3	Firmicutes: 0.93	Clostridia: 0.87	Clostridiales: 0.87	Oscillospiraceae: 0.74	Oscillibacter: 0.71	Oscillibacter sp. 1-3: 0.67	k__Bacteria (UID203)
bin.55.Cage3	Firmicutes: 0.85	NA	NA	NA	NA	Firmicutes bacterium CAG:475: 0.49	o__Clostridiales (UID1120)
bin.56.Cage3	Firmicutes: 0.66	Clostridia: 0.41	Clostridiales: 0.41	not classified	not classified	not classified	root (UID1)
bin.57.Cage3	Actinobacteria: 0.99	Actinobacteria: 0.99	Bifidobacteriales: 0.92	Bifidobacteriaceae: 0.92	Bifidobacterium: 0.92	Bifidobacterium pseudolongum: 0.77	f__Bifidobacteriaceae (UID1460)
bin.58.Cage3	NA	NA	NA	NA	NA	bacterium 1xD42-87: 0.63	f__Lachnospiraceae (UID1255)
bin.59.Cage3	Firmicutes: 0.78	Clostridia: 0.73	Clostridiales: 0.73	Eubacteriaceae: 0.45	Eubacterium: 0.45	Eubacterium plexicaudatum: 0.45	f__Lachnospiraceae (UID1255)
bin.6.Cage3	Firmicutes: 0.99	Erysipelotrichia: 0.97	Erysipelotrichales: 0.97	Erysipelotrichaceae: 0.97	Faecalibaculum: 0.96	Faecalibaculum rodentium*: 0.96	k__Bacteria (UID2372)
bin.60.Cage3	Bacteroidetes: 0.80	Bacteroidia: 0.80	Bacteroidales: 0.79	Muribaculaceae: 0.40	not classified	not classified	o__Bacteroidales (UID2617)
bin.61.Cage3	NA	NA	NA	NA	NA	bacterium D16-50: 0.94	k__Bacteria (UID203)
bin.62.Cage3	Bacteroidetes: 0.79	Bacteroidia: 0.77	Bacteroidales: 0.76	Muribaculaceae: 0.32	not classified	not classified	root (UID1)
bin.63.Cage3	Bacteroidetes: 0.86	Bacteroidia: 0.86	Bacteroidales: 0.86	Muribaculaceae: 0.61	not classified	not classified	root (UID1)
bin.64.Cage3	Firmicutes: 0.84	Clostridia: 0.71	Clostridiales: 0.69	Ruminococcaceae: 0.42	Pseudoflavonifractor: 0.37	Pseudoflavonifractor sp. Marseille-P3106: 0.36	o__Clostridiales (UID1212)
bin.65.Cage3	Firmicutes: 0.86	Clostridia: 0.78	Clostridiales: 0.77	Oscillospiraceae: 0.60	Oscillibacter: 0.33	not classified	o__Clostridiales (UID1212)
bin.66.Cage3	Firmicutes: 0.48	Clostridia: 0.40	Clostridiales: 0.40	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.67.Cage3	Firmicutes: 0.32	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)

bin.68.Cage3	Firmicutes: 0.77	Clostridia: 0.64	Clostridiales: 0.62	Ruminococcaceae: 0.34	not classified	not classified	o__Clostridiales (UID1212)
bin.69.Cage3	Firmicutes: 0.80	Clostridia: 0.47	Clostridiales: 0.47	not classified	not classified	not classified	f__Lachnospiraceae (UID1256)
bin.7.Cage3	Firmicutes: 0.31	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.70.Cage3	Firmicutes: 0.79	Clostridia: 0.73	Clostridiales: 0.73	Lachnospiraceae: 0.43	Roseburia: 0.36	not classified	f__Lachnospiraceae (UID1286)
bin.71.Cage3	Firmicutes: 0.77	Clostridia: 0.62	Clostridiales: 0.60	Ruminococcaceae: 0.30	not classified	not classified	o__Clostridiales (UID1212)
bin.72.Cage3	Bacteroidetes: 0.98	Bacteroidia: 0.98	Bacteroidales: 0.98	Muribaculaceae: 0.92	not classified	not classified	k__Bacteria (UID203)
bin.73.Cage3	Proteobacteria: 1.00	Betaproteobacteria: 0.62	Burkholderiales: 0.62	not classified	not classified	not classified	k__Bacteria (UID203)
bin.74.Cage3	Bacteroidetes: 0.99	Bacteroidia: 0.99	Bacteroidales: 0.99	Muribaculaceae: 0.79	not classified	not classified	root (UID1)
bin.75.Cage3	Firmicutes: 0.75	Clostridia: 0.39	Clostridiales: 0.36	not classified	not classified	not classified	k__Bacteria (UID203)
bin.76.Cage3	Verrucomicrobia: 0.99	Verrucomicrobiae: 0.99	Verrucomicrobiales: 0.99	Akkermansiaceae: 0.99	Akkermansia*: 0.99	not classified	k__Bacteria (UID2982)
bin.77.Cage3	Firmicutes: 0.90	Clostridia: 0.87	Clostridiales: 0.87	Lachnospiraceae: 0.78	NA	Lachnospiraceae bacterium A4: 0.49	f__Lachnospiraceae (UID1286)
bin.78.Cage3	Firmicutes: 0.90	Clostridia: 0.85	Clostridiales: 0.84	Oscillospiraceae: 0.67	Oscillibacter: 0.61	Oscillibacter sp. 1-3: 0.51	k__Bacteria (UID203)
bin.79.Cage3	Firmicutes: 0.88	Clostridia: 0.81	Clostridiales: 0.80	Oscillospiraceae: 0.66	Oscillibacter: 0.36	not classified	k__Bacteria (UID203)
bin.8.Cage3	Firmicutes: 0.94	Clostridia: 0.90	Clostridiales: 0.90	Oscillospiraceae: 0.82	Oscillibacter: 0.73	Oscillibacter sp. 1-3: 0.67	o__Clostridiales (UID1212)
bin.80.Cage3	Firmicutes: 0.75	Clostridia: 0.65	Clostridiales: 0.64	Ruminococcaceae: 0.39	not classified	not classified	o__Clostridiales (UID1212)
bin.81.Cage3	Firmicutes: 0.92	Clostridia: 0.79	Clostridiales: 0.75	Ruminococcaceae: 0.45	Pseudoflavonifractor: 0.37	Pseudoflavonifractor sp. Marseille-P3106: 0.37	k__Bacteria (UID203)
bin.82.Cage3	Bacteroidetes: 0.97	Bacteroidia: 0.97	Bacteroidales: 0.97	Bacteroidaceae: 0.97	Bacteroides: 0.97	not classified	k__Bacteria (UID203)
bin.83.Cage3	Firmicutes: 0.83	Clostridia: 0.77	Clostridiales: 0.77	not classified	not classified	not classified	root (UID1)
bin.84.Cage3	Firmicutes: 0.41	Clostridia: 0.35	Clostridiales: 0.35	not classified	not classified	not classified	k__Bacteria (UID203)
bin.85.Cage3	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.86.Cage3	Actinobacteria: 0.43	Coriobacteriia: 0.43	not classified	not classified	not classified	not classified	p__Actinobacteria (UID2112)
bin.87.Cage3	Firmicutes: 0.81	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)

bin.88.Cage3	Bacteroidetes: 0.61	Bacteroidia: 0.60	Bacteroidales: 0.59	not classified	not classified	not classified	k__Bacteria (UID203)
bin.89.Cage3	Firmicutes: 0.98	Clostridia: 0.95	Clostridiales: 0.95	not classified	not classified	not classified	k__Bacteria (UID203)
bin.9.Cage3	Bacteroidetes: 0.96	Bacteroidia: 0.95	Bacteroidales: 0.95	Rikenellaceae: 0.85	Alistipes: 0.85	not classified	p__Bacteroidetes (UID2605)
bin.90.Cage3	Firmicutes: 0.82	Clostridia: 0.69	Clostridiales: 0.69	not classified	not classified	not classified	root (UID1)
bin.91.Cage3	Firmicutes: 0.66	Clostridia: 0.58	Clostridiales: 0.58	Lachnospiraceae: 0.34	not classified	not classified	root (UID1)
bin.92.Cage3	Bacteroidetes: 0.59	Bacteroidia: 0.58	Bacteroidales: 0.58	not classified	not classified	not classified	k__Bacteria (UID203)
bin.93.Cage3	Firmicutes: 0.90	Clostridia: 0.82	Clostridiales: 0.81	Oscillospiraceae: 0.69	Oscillibacter: 0.36	not classified	k__Bacteria (UID203)
bin.94.Cage3	Firmicutes: 0.75	Clostridia: 0.38	Clostridiales: 0.35	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.95.Cage3	NA	NA	NA	NA	NA	bacterium 1XD8-92: 0.89	f__Lachnospiraceae (UID1255)
bin.96.Cage3	Firmicutes: 0.80	Clostridia: 0.38	Clostridiales: 0.34	not classified	not classified	not classified	k__Bacteria (UID203)
bin.97.Cage3	Firmicutes: 0.70	Clostridia: 0.52	Clostridiales: 0.52	not classified	not classified	not classified	root (UID1)
bin.98.Cage3	Bacteroidetes: 0.95	Bacteroidia: 0.95	Bacteroidales: 0.95	Bacteroidaceae: 0.88	Bacteroides: 0.88	not classified	k__Bacteria (UID203)
bin.99.Cage3	Bacteroidetes: 0.78	Bacteroidia: 0.78	Bacteroidales: 0.78	Muribaculaceae: 0.46	NA	Muribaculaceae bacterium Isolate-013 (NCI): 0.33	root (UID1)
bin.1.Cage4	Firmicutes: 0.80	Clostridia: 0.72	Clostridiales: 0.69	Ruminococcaceae: 0.47	Angelakisella: 0.33	Angelakisella massiliensis*: 0.33	o__Clostridiales (UID1212)
bin.10.Cage4	Bacteroidetes: 0.59	Bacteroidia: 0.58	Bacteroidales: 0.58	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.100.Cage4	Firmicutes: 0.91	Clostridia: 0.86	Clostridiales: 0.85	Ruminococcaceae: 0.71	not classified	not classified	o__Clostridiales (UID1212)
bin.101.Cage4	Firmicutes: 0.92	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.102.Cage4	Bacteroidetes: 0.99	Bacteroidia: 0.99	Bacteroidales: 0.99	Muribaculaceae: 0.94	not classified	not classified	k__Bacteria (UID203)
bin.103.Cage4	NA	NA	NA	NA	NA	bacterium 1XD8-76: 0.68	f__Lachnospiraceae (UID1255)
bin.104.Cage4	Firmicutes: 0.81	Clostridia: 0.34	not classified	not classified	not classified	not classified	c__Clostridia (UID1118)
bin.105.Cage4	Firmicutes: 0.72	Clostridia: 0.37	Clostridiales: 0.36	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.106.Cage4	Firmicutes: 0.48	Clostridia: 0.40	Clostridiales: 0.39	not classified	not classified	not classified	k__Bacteria (UID203)

bin.107.Cage4	Firmicutes: 0.91	Clostridia: 0.50	Clostridiales: 0.50	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.108.Cage4	Bacteroidetes: 0.98	Bacteroidia: 0.98	Bacteroidales: 0.98	Bacteroidaceae: 0.98	Bacteroides: 0.98	not classified	root (UID1)
bin.109.Cage4	Firmicutes: 0.38	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.11.Cage4	Bacteroidetes: 0.95	Bacteroidia: 0.95	Bacteroidales: 0.95	Bacteroidaceae: 0.90	Bacteroides: 0.90	not classified	k__Bacteria (UID203)
bin.110.Cage4	Firmicutes: 0.83	Clostridia: 0.73	Clostridiales: 0.70	NA	NA	Clostridiales bacterium: 0.35	o__Clostridiales (UID1120)
bin.111.Cage4	Firmicutes: 0.78	Clostridia: 0.53	Clostridiales: 0.52	not classified	not classified	not classified	k__Bacteria (UID203)
bin.112.Cage4	Firmicutes: 0.33	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.113.Cage4	Firmicutes: 0.96	Clostridia: 0.92	Clostridiales: 0.92	Oscillospiraceae: 0.86	Oscillibacter: 0.83	Oscillibacter sp. 1-3: 0.80	o__Clostridiales (UID1212)
bin.114.Cage4	Firmicutes: 0.56	Clostridia: 0.49	Clostridiales: 0.48	not classified	not classified	not classified	g__Bacteroides (UID2691)
bin.115.Cage4	Firmicutes: 0.83	Clostridia: 0.34	not classified	not classified	not classified	not classified	c__Clostridia (UID1118)
bin.116.Cage4	Firmicutes: 0.46	Clostridia: 0.40	Clostridiales: 0.40	not classified	not classified	not classified	f__Lachnospiraceae (UID1286)
bin.117.Cage4	Firmicutes: 0.99	Clostridia: 0.99	Clostridiales: 0.99	Oscillospiraceae: 0.94	Oscillibacter: 0.93	Oscillibacter sp. 1-3: 0.93	k__Bacteria (UID203)
bin.118.Cage4	Firmicutes: 0.85	Clostridia: 0.77	Clostridiales: 0.76	not classified	not classified	not classified	root (UID1)
bin.119.Cage4	Proteobacteria: 0.98	Betaproteobacteria: 0.40	Burkholderiales: 0.40	not classified	not classified	not classified	p__Proteobacteria (UID3887)
bin.12.Cage4	Firmicutes: 0.81	Clostridia: 0.76	Clostridiales: 0.76	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.120.Cage4	Firmicutes: 0.33	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.121.Cage4	Firmicutes: 0.46	Clostridia: 0.30	Clostridiales: 0.30	not classified	not classified	not classified	root (UID1)
bin.122.Cage4	Firmicutes: 0.78	Clostridia: 0.66	Clostridiales: 0.63	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.123.Cage4	Firmicutes: 0.46	Clostridia: 0.37	Clostridiales: 0.37	not classified	not classified	not classified	k__Bacteria (UID203)
bin.13.Cage4	Proteobacteria: 1.00	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.14.Cage4	Bacteroidetes: 0.64	Bacteroidia: 0.64	Bacteroidales: 0.64	not classified	not classified	not classified	o__Bacteroidales (UID2617)
bin.15.Cage4	Firmicutes: 0.93	NA	NA	NA	NA	Firmicutes bacterium: 0.59	c__Clostridia (UID1118)

bin.16.Cage4	Firmicutes: 0.98	Clostridia: 0.98	Clostridiales: 0.98	Peptostreptococcaceae: 0.94	Romboutsia: 0.90	Romboutsia ilealis: 0.41	o__Clostridiales (UID1120)
bin.17.Cage4	Firmicutes: 0.73	Clostridia: 0.61	Clostridiales: 0.61	Lachnospiraceae: 0.39	not classified	not classified	root (UID1)
bin.18.Cage4	Firmicutes: 0.82	Clostridia: 0.69	Clostridiales: 0.65	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.19.Cage4	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.2.Cage4	Firmicutes: 0.79	Clostridia: 0.70	Clostridiales: 0.68	Ruminococcaceae: 0.44	not classified	not classified	k__Bacteria (UID203)
bin.20.Cage4	Firmicutes: 0.40	Clostridia: 0.31	Clostridiales: 0.31	not classified	not classified	not classified	k__Bacteria (UID203)
bin.21.Cage4	Firmicutes: 0.93	Clostridia: 0.89	Clostridiales: 0.88	Oscillospiraceae: 0.78	Oscillibacter: 0.70	Oscillibacter sp. 1-3: 0.63	o__Clostridiales (UID1212)
bin.22.Cage4	Firmicutes: 0.87	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.23.Cage4	Firmicutes: 0.34	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.24.Cage4	Firmicutes: 0.78	Clostridia: 0.37	Clostridiales: 0.35	not classified	not classified	not classified	k__Bacteria (UID203)
bin.25.Cage4	Firmicutes: 0.88	Clostridia: 0.72	Clostridiales: 0.72	not classified	not classified	not classified	root (UID1)
bin.26.Cage4	Firmicutes: 0.54	Clostridia: 0.44	Clostridiales: 0.44	not classified	not classified	not classified	k__Bacteria (UID203)
bin.27.Cage4	Firmicutes: 0.72	Clostridia: 0.33	Clostridiales: 0.32	not classified	not classified	not classified	k__Bacteria (UID203)
bin.28.Cage4	Firmicutes: 0.87	Clostridia: 0.78	Clostridiales: 0.77	Oscillospiraceae: 0.57	Oscillibacter: 0.32	not classified	o__Clostridiales (UID1212)
bin.29.Cage4	Firmicutes: 0.36	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.3.Cage4	Firmicutes: 0.89	Clostridia: 0.57	Clostridiales: 0.57	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.30.Cage4	Firmicutes: 0.95	Bacilli: 0.94	Lactobacillales: 0.94	Streptococcaceae: 0.93	Lactococcus: 0.93	Lactococcus lactis: 0.78	o__Lactobacillales (UID543)
bin.31.Cage4	Firmicutes: 0.38	Clostridia: 0.31	Clostridiales: 0.30	not classified	not classified	not classified	k__Bacteria (UID203)
bin.32.Cage4	Firmicutes: 0.95	Clostridia: 0.88	Clostridiales: 0.88	Oscillospiraceae: 0.84	Oscillibacter: 0.80	Oscillibacter sp. 1-3: 0.74	k__Bacteria (UID203)
bin.33.Cage4	Firmicutes: 0.76	Clostridia: 0.69	Clostridiales: 0.69	not classified	not classified	not classified	o__Clostridiales (UID1226)
bin.34.Cage4	Firmicutes: 0.74	Clostridia: 0.38	Clostridiales: 0.36	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.35.Cage4	Firmicutes: 0.63	Clostridia: 0.44	Clostridiales: 0.44	not classified	not classified	not classified	k__Bacteria (UID203)

bin.36.Cage4	Bacteroidetes: 0.92	Bacteroidia: 0.92	Bacteroidales: 0.91	Muribaculaceae: 0.65	not classified	not classified	k__Bacteria (UID203)
bin.37.Cage4	Firmicutes: 0.95	Clostridia: 0.83	Clostridiales: 0.83	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.38.Cage4	Firmicutes: 0.42	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.39.Cage4	Firmicutes: 0.91	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.4.Cage4	Firmicutes: 1.00	Clostridia: 1.00	Clostridiales: 1.00	Ruminococcaceae: 1.00	Anaerotruncus: 0.99	not classified	k__Bacteria (UID203)
bin.40.Cage4	Bacteroidetes: 0.98	Bacteroidia: 0.98	Bacteroidales: 0.98	Muribaculaceae: 0.75	not classified	not classified	k__Bacteria (UID203)
bin.41.Cage4	Firmicutes: 0.79	Clostridia: 0.65	Clostridiales: 0.63	not classified	not classified	not classified	k__Bacteria (UID203)
bin.42.Cage4	Firmicutes: 0.46	Clostridia: 0.38	Clostridiales: 0.38	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.43.Cage4	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.94	Bacteroides: 0.94	not classified	k__Bacteria (UID203)
bin.44.Cage4	Firmicutes: 0.87	Clostridia: 0.78	Clostridiales: 0.78	Oscillospiraceae: 0.60	Oscillibacter: 0.34	not classified	o__Clostridiales (UID1212)
bin.45.Cage4	Firmicutes: 0.89	Clostridia: 0.81	Clostridiales: 0.80	Oscillospiraceae: 0.66	Oscillibacter: 0.35	not classified	k__Bacteria (UID203)
bin.46.Cage4	Firmicutes: 0.64	Clostridia: 0.46	Clostridiales: 0.46	not classified	not classified	not classified	root (UID1)
bin.47.Cage4	Bacteroidetes: 1.00	Bacteroidia: 1.00	Bacteroidales: 1.00	Muribaculaceae: 0.96	not classified	not classified	o__Bacteroidales (UID2617)
bin.48.Cage4	Firmicutes: 0.84	Clostridia: 0.54	Clostridiales: 0.53	Ruminococcaceae: 0.31	not classified	not classified	k__Bacteria (UID203)
bin.49.Cage4	Firmicutes: 0.96	Clostridia: 0.94	Clostridiales: 0.94	Oscillospiraceae: 0.89	Oscillibacter: 0.85	Oscillibacter sp. 1-3: 0.81	o__Clostridiales (UID1212)
bin.5.Cage4	Firmicutes: 0.31	not classified	not classified	not classified	not classified	not classified	root (UID1)
bin.50.Cage4	not classified	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.51.Cage4	Firmicutes: 0.72	Clostridia: 0.34	Clostridiales: 0.32	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.52.Cage4	Firmicutes: 0.98	Bacilli: 0.98	Lactobacillales: 0.98	Streptococcaceae: 0.98	Lactococcus: 0.98	Lactococcus lactis: 0.91	k__Bacteria (UID203)
bin.53.Cage4	Bacteroidetes: 0.96	Bacteroidia: 0.96	Bacteroidales: 0.96	Bacteroidaceae: 0.92	Bacteroides: 0.92	not classified	root (UID1)
bin.54.Cage4	Firmicutes: 0.74	Clostridia: 0.35	Clostridiales: 0.33	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.55.Cage4	Firmicutes: 0.99	Clostridia: 0.98	Clostridiales: 0.98	Oscillospiraceae: 0.97	Oscillibacter: 0.96	Oscillibacter sp. 1-3: 0.95	o__Clostridiales (UID1212)

bin.56.Cage4	Firmicutes: 0.92	Clostridia: 0.86	Clostridiales: 0.86	Oscillospiraceae: 0.68	Oscillibacter: 0.66	Oscillibacter sp. 1-3: 0.62	k__Bacteria (UID203)
bin.57.Cage4	Verrucomicrobia: 0.99	Verrucomicrobiae: 0.99	Verrucomicrobiales: 0.99	Akkermansiaceae: 0.99	Akkermansia*: 0.99	not classified	k__Bacteria (UID2982)
bin.58.Cage4	Firmicutes: 0.75	Clostridia: 0.61	Clostridiales: 0.59	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.59.Cage4	Firmicutes: 0.76	Clostridia: 0.36	Clostridiales: 0.34	not classified	not classified	not classified	k__Bacteria (UID203)
bin.6.Cage4	Bacteroidetes: 0.92	Bacteroidia: 0.92	Bacteroidales: 0.92	Bacteroidaceae: 0.50	Bacteroides: 0.50	not classified	root (UID1)
bin.60.Cage4	Firmicutes: 0.79	Clostridia: 0.65	Clostridiales: 0.62	Ruminococcaceae: 0.31	not classified	not classified	o__Clostridiales (UID1212)
bin.61.Cage4	Firmicutes: 0.81	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.62.Cage4	Firmicutes: 0.77	Clostridia: 0.65	Clostridiales: 0.62	Ruminococcaceae: 0.30	not classified	not classified	o__Clostridiales (UID1212)
bin.63.Cage4	Firmicutes: 0.99	Bacilli: 0.99	Lactobacillales: 0.57	Lactobacillaceae: 0.57	Lactobacillus: 0.57	not classified	g__Lactobacillus (UID380)
bin.64.Cage4	Firmicutes: 0.74	Clostridia: 0.64	Clostridiales: 0.62	Ruminococcaceae: 0.38	not classified	not classified	o__Clostridiales (UID1212)
bin.65.Cage4	Firmicutes: 0.85	Clostridia: 0.43	Clostridiales: 0.43	not classified	not classified	not classified	f__Lachnospiraceae (UID1256)
bin.66.Cage4	Bacteroidetes: 0.97	Bacteroidia: 0.97	Bacteroidales: 0.97	Bacteroidaceae: 0.97	Bacteroides: 0.97	not classified	k__Bacteria (UID203)
bin.67.Cage4	Firmicutes: 0.85	Clostridia: 0.72	Clostridiales: 0.71	NA	Intestinimonas: 0.32	Intestinimonas timonensis: 0.32	root (UID1)
bin.68.Cage4	Firmicutes: 0.74	Clostridia: 0.38	Clostridiales: 0.37	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.69.Cage4	Firmicutes: 0.86	Clostridia: 0.75	Clostridiales: 0.72	Ruminococcaceae: 0.45	Pseudoflavonifractor: 0.40	Pseudoflavonifractor sp. Marseille-P3106: 0.40	o__Clostridiales (UID1212)
bin.7.Cage4	Firmicutes: 0.77	Clostridia: 0.64	Clostridiales: 0.37	not classified	not classified	not classified	k__Bacteria (UID203)
bin.70.Cage4	Firmicutes: 0.86	Clostridia: 0.71	Clostridiales: 0.71	not classified	not classified	not classified	root (UID1)
bin.71.Cage4	Firmicutes: 0.79	Clostridia: 0.58	Clostridiales: 0.58	not classified	not classified	not classified	k__Bacteria (UID203)
bin.72.Cage4	Firmicutes: 0.75	Clostridia: 0.37	Clostridiales: 0.34	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.73.Cage4	NA	NA	NA	NA	NA	bacterium D16-50: 0.94	f__Lachnospiraceae (UID1255)
bin.74.Cage4	Firmicutes: 0.89	Clostridia: 0.83	Clostridiales: 0.82	Oscillospiraceae: 0.69	Oscillibacter: 0.40	not classified	k__Bacteria (UID203)
bin.75.Cage4	Firmicutes: 0.68	Clostridia: 0.58	Clostridiales: 0.51	not classified	not classified	not classified	k__Bacteria (UID203)

bin.76.Cage4	Firmicutes: 0.78	Clostridia: 0.33	Clostridiales: 0.30	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.77.Cage4	not classified	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.78.Cage4	Proteobacteria: 1.00	Betaproteobacteria: 0.62	Burkholderiales: 0.62	not classified	not classified	not classified	k__Bacteria (UID203)
bin.79.Cage4	Firmicutes: 0.62	Clostridia: 0.54	Clostridiales: 0.53	Lachnospiraceae: 0.30	not classified	not classified	root (UID1)
bin.8.Cage4	NA	NA	NA	NA	NA	bacterium 1xD42-67: 0.89	k__Bacteria (UID203)
bin.80.Cage4	Firmicutes: 0.33	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.81.Cage4	Firmicutes: 0.36	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.82.Cage4	Firmicutes: 0.79	Clostridia: 0.70	Clostridiales: 0.68	Ruminococcaceae: 0.45	Angelakisella: 0.31	Angelakisella massiliensis*: 0.31	o__Clostridiales (UID1212)
bin.83.Cage4	Firmicutes: 0.80	Clostridia: 0.67	Clostridiales: 0.65	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.84.Cage4	Firmicutes: 0.87	Bacilli: 0.86	Bacillales: 0.86	Staphylococcaceae: 0.85	Staphylococcus: 0.85	Staphylococcus lentus: 0.77	k__Bacteria (UID203)
bin.85.Cage4	Firmicutes: 0.75	Clostridia: 0.38	Clostridiales: 0.35	not classified	not classified	not classified	o__Clostridiales (UID1212)
bin.86.Cage4	Firmicutes: 0.85	Clostridia: 0.37	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.87.Cage4	Bacteroidetes: 0.88	Bacteroidia: 0.88	Bacteroidales: 0.88	Muribaculaceae: 0.55	not classified	not classified	k__Bacteria (UID203)
bin.88.Cage4	Firmicutes: 0.66	Clostridia: 0.38	not classified	not classified	not classified	not classified	c__Clostridia (UID1085)
bin.89.Cage4	Actinobacteria: 0.55	Coriobacteriia: 0.55	Eggerthellales: 0.54	Eggerthellaceae: 0.54	Enterorhabdus: 0.46	Enterorhabdus caecimuris: 0.31	p__Actinobacteria (UID2112)
bin.9.Cage4	Bacteroidetes: 0.93	Bacteroidia: 0.92	Bacteroidales: 0.92	Muribaculaceae: 0.79	not classified	not classified	o__Bacteroidales (UID2617)
bin.90.Cage4	Firmicutes: 0.79	Clostridia: 0.72	Clostridiales: 0.72	Lachnospiraceae: 0.43	Roseburia: 0.35	not classified	f__Lachnospiraceae (UID1255)
bin.91.Cage4	Firmicutes: 0.67	Clostridia: 0.30	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.92.Cage4	Firmicutes: 0.80	Clostridia: 0.79	Clostridiales: 0.79	not classified	not classified	not classified	k__Bacteria (UID203)
bin.93.Cage4	not classified	not classified	not classified	not classified	not classified	not classified	k__Bacteria (UID203)
bin.94.Cage4	Firmicutes: 0.87	Clostridia: 0.76	Clostridiales: 0.76	Lachnospiraceae: 0.50	NA	Lachnospiraceae bacterium 3-2: 0.42	root (UID1)
bin.95.Cage4	NA	NA	NA	NA	NA	bacterium 1XD8-92: 0.98	root (UID1)

bin.96.Cage4	Firmicutes: 0.85	Clostridia: 0.80	Clostridiales: 0.80	not classified	not classified	not classified	k__Bacteria (UID203)
bin.97.Cage4	Firmicutes: 0.98	Clostridia: 0.94	Clostridiales: 0.94	Oscillospiraceae: 0.92	Oscillibacter: 0.92	Oscillibacter sp. 1-3: 0.92	root (UID1)
bin.98.Cage4	not classified	not classified	not classified	not classified	not classified	not classified	f__Lachnospiraceae (UID1255)
bin.99.Cage4	Firmicutes: 0.32	not classified	not classified	not classified	not classified	not classified	o__Clostridiales (UID1212)

Supplementary Table 5. Composition of the 20% soy or casein protein diets.

	Soy Based Diet	**Casein Based Diet**
Teklad Diet Number	**TD.190249**	
	g/kg	**g/kg**
Protein	230 (isolated soy protein)	230 (lactic casein)
Sucrose	436.1	434.7
Corn Starch	200	200
Corn Oil	52.3	52.3
Cellulose (Fiber)	37.86	37.86
Vitamin Mix, Teklad (40060)	10	10
Mineral Mix, Ca-P Deficient (79055)	13.37	13.37
Calcium Phosphate, dibasic	15.26	16.66
Calcium Carbonate	5.1	5.1
Ethoxyquin, antioxidant	0.01	0.01
Protein (% by weight)	20	20
Protein (% of kcal)	21.2	21.2
Carbohydrate (% by weight)	62.3	61.9
Carbohydrate (% by kcal	65.8	65.7
Fat (% by weight)	5.5	5.5
Fat (% by kcal)	13	13
kcal/g diet	3.8	3.8

Appendix 2 – Additional Scientific Contributions During My M.S. Training

Microbe-dependent heterosis in maize

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Abstract:

Hybrids account for nearly all commercially planted varieties of maize and many other crop plants, because crosses between inbred lines of these species produce F_1 offspring that greatly outperform their parents. The mechanisms underlying this phenomenon, called heterosis or hybrid vigor, are not well understood despite over a century of intensive research[1]. The leading hypotheses—which focus on quantitative genetic mechanisms (dominance, overdominance, and epistasis) and molecular mechanisms (gene dosage and transcriptional regulation)—have been able to explain some but not all of the observed patterns of heterosis. However, possible ecological drivers of heterosis have largely been ignored. Here we show that heterosis of root biomass and germination in maize is strongly dependent on the belowground microbial environment. We found that, in some cases, inbred lines perform as well by these criteria as their F_1 offspring under sterile conditions, but that heterosis can be restored by inoculation with a simple community of seven bacterial strains. We observed the same pattern for seedlings inoculated with autoclaved vs. live soil slurries in a growth chamber, and for plants grown in fumigated vs. untreated soil in the field. Together, our results demonstrate a novel, ecological mechanism for heterosis whereby soil microbes generally impair the germination and early growth of inbred but not hybrid maize.

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