1

Common Definitions

1.1 Microbiome

There are multiple functional definitions of the term "microbiome." According to the Human Microbiome Consortium, the microbiome is considered as the community of all microbes recovered from a particular habitat or ecosystem [1]. These microscopic communities, including bacteria, fungi, and viruses, can be found in all living things, including plants, and are found in every different imaginable habitat, from life-forms to soils and bodies of water [2, 3]. Microbiomes can be found on outer surfaces, particularly as biofilms, and within several body systems of animals including the respiratory tract, reproductive organs, integumentary, oral cavity, urinary tract, neurological pathways via the brain-gut axis, and the gastrointestinal (GI) tract. Over 30 trillion microbes may reside within the GI system alone [4, 5]. This list is not exhaustive, as this area of knowledge is relatively novel, and innovations allow us to discover microbiomes in organs and systems once thought to be sterile. The total cumulative microbiomes in a human host may weigh as much as 1–3% body mass [4].

While some common trends are being observed in current research, microbiomes are unique for each individual with their diversity and density affected by several intrinsic (genetics, age, sex) and extrinsic (environment, physiological state, antibiotic therapy, health and nutrition) factors [6]. These incredibly diverse communities shape the health of the host and influence its physiology, through multiple complex

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Companion website: www.wiley.com/go/saar/1e

pathways, including influencing remote organ and immune responses. A main focus of research is on the microbiomes of the GI tract, and how perturbations of these complex communities are associated with multiple health conditions in humans: depression, autism spectrum disorder, oral health, chronic obstructive pulmonary disease (COPD), asthma, pneumonia, dermatological, obesity, cardiovascular, diabetes, rheumatoid arthritis, hepatic associated disorders, cancer, inflammatory bowel disease (IBD), and infection due to bacterial translocation [4–7]. Microbiome communities affect the status of, and rely on, each other for daily functions, communicating through the release of metabolites – products of microbial fermentation [8]. One recognized influence is the cumulative genetic material, the metagenome, of all the microbes in one animal's singular microbiome. A metagenome may contain over 200 times the number of genes in a host's genome; therefore, the level of influence these genes have over host gene expression is one explanation for the microbiota influence on the host's physiological systems [8].

The development of new innovative research tools allows us to see, understand, and evaluate previously unidentifiable concepts regarding the body's microbiomes. Some obstacles that remain with identifying and determining the effects of microbiomes are reproducing their environment, including food sources, to enhance growth and preventing the death of the microbes when sampling. Research is also limited at this time, with many research projects utilizing small study groups, which are not always representative of the wider population, or reproducible in future projects. This is a common limitation for quantitative research [9].

1.2 Microbiota

The microbiota may be defined as the individual bacteria, fungi, viruses, and protozoa that can be found in a microbiome community. Microbes pre-date the Earth's eukaryotic biodiversity and are numerous, diverse, and ubiquitous. They have adapted to live in extreme environments such as the high pressure, as in the deep ocean, extreme heat, or chemical exposure. Different types of bacteria survive in both aerobic and/or anaerobic environments. Environmental differences are one reason that it has been difficult to identify microbiota in discovered and undiscovered communities [8]. Those that live in an anaerobic environment may

have a shorter survival rate when removed, for example in biopsy samples, and then brought into an aerobic environment. It is estimated that only 20–30% of organisms are culturable, which leaves a large group of microbiota that are unidentified through routine culture [10]. The main phyla composing the gut microbiome vary from species to species, but *Fusobacteria, Bacteroidetes,* and *Firmicutes,* as well as, to a lesser extent, *Proteobacteria* and *Actinobacteria*, are typically prevalent in dogs and cats [3, 11].

Communication occurs between the microbes within their microbiome and with host body systems, which in turn can change or influence the physiology of the host. The host relies on the microbiota to complete functions that may not be encoded in their genes to complete [5]. The roles of microbiota are complex and may change as resource availabilities change [8]. Currently, we understand that microbiota plays roles in the production of vitamins, mineral absorption, structural integrity of barriers, metabolism of nondigestible products and provision of energy sources (short-chain fatty acids – SCFAs), interactions with or involved in the production of chemical and neurotransmitter metabolites affecting other organs of the body (bidirectional axis), host genomic expression, inflammatory processes, intestinal permeability, immune function, and food intake and energy expenditure [4, 6, 8, 11–16].

1.3 Pathogens

Pathogens are defined as a biological agent that causes disease or illness to its host. Although in the minority, these microbes are generally known to cause illness, at least in certain circumstances. Pathogens can be divided into five groups: viruses, bacteria, fungi, protozoa, and helminths [17]. Characteristics of pathogens are the mode of transmission, mechanism of replication, pathogenesis (how it causes diseases), and ability to elicit a response. Depending on the pathogen, replication may occur in the intracellular and/or extracellular compartments, while host defense mechanisms work to destroy the pathogen and stop its growth. Common canine and feline pathogens are summarized by group in Table 1.1.

Pathobionts are commensal microbes that can be present at low levels in healthy microbiomes without causing harm to the host but can be pathogenic under certain circumstances [10]. While a general previous

Table 1.1 Common canine and feline pathogens.

Common causes of disease in dogs and cats			
Viruses	DNA viruses	Adenoviruses	Canine adenoviruses
		Herpesviruses	Canine herpesvirus
			Feline herpesvirus
		Parvoviruses	Canine parvovirus
			Feline panleukopenia virus
	RNA viruses	Orthomyxoviruses	Canine influenza
		Paramyxoviruses	Canine distemper virus
			Canine parainfluenza virus
		Coronaviruses	Canine respiratory coronavirus
			Feline enteric coronavirus
		Picornaviruses	Feline calicivirus
		Rhabdoviruses	Rabies
		Retroviruses	Feline leukemia virus
			Feline immunodeficiency virus
Bacteria	Gram +ve cocci	Staphylococci	*Staphylococcus* spp.
		Streptococci	*Streptococcus* spp.
	Gram −ve cocci		*Bartonella henselae*
	Gram +ve bacilli		*Corynebacteria*
			Bacillus anthracis
			Listeria monocytogenes
	Gram −ve bacilli		*Bordetella bronchiseptica*
			Yersinia pestis
	Anaerobes	Clostridia	*Clostridia* spp.
	Spirochetes		*Borrelia burgdorferi*
			Leptospira interrogans
	Rickettsials		*Ehrlichia canis*
			Anaplasma spp.

Table 1.1 (Continued)

Common causes of disease in dogs and cats		
	Chlamydias	*Chlamydophila felis*
	Mycoplasmas	*Mycoplasma haemocanis*
		Mycoplasma felis
Fungi		*Candida albicans*
		Cryptococcus neoformans
		Aspergillus
		Histoplasma capsulatum
		Coccidioides immitis
Protozoa		*Giardia* spp.
		Leishmania
		Babesia spp.
		Hepatozoon canis
		Cystoisospra
		Cryptosporidium
		Toxoplasma gondii
Helminths (worms)	Nematodes (roundworms)	*Dirofilaria immitis*
		Toxocara spp.
		Toxascaris leonina
		Uncinaria stenocephala
		Trichuris vulpis
		Ancylostoma spp.
		Capillaria spp.
	Cestodes (tapeworms)	*Taenia* spp.
		Echinococcus spp.
		Sarcocystis

Source: Adapted from Alexander et al. [18], Inpankaew et al. [19], Day et al. [20], Riley et al. [21], Millán and Rodriíguez [22], Biek et al. [23] and Villeneuve et al. [24].

concept was a simple overgrowth of a pathogenic bacteria was the cause of dysbiosis, new information shows that a barrier dysfunction plays a larger role in pathogenic bacteria being allowed to either colonize or translocate (cross the surface of an epithelial barrier) causing illness in the host [10, 17]. In some circumstances, it may be a combination of genetics along with the presence of specific microbiota or metabolites that lead to disease or illness in the host. The immune response cannot eliminate most pathogens, and most pathogens are not universally lethal as this would affect the long-term survival of that pathogen [17]. However, some pathogens may cause an attack on the immune response that can affect other microbiomes in the body and may be detrimental for the host [25, 26].

1.4 Symbiosis

Symbiosis describes a relationship or interaction between two organisms of different types, and the specific classification of symbiosis depends on whether either or both organisms benefit from the relationship [27]. These different species inhabit the same spaces and share or compete for the same resources. They interact in a variety of ways, known collectively as symbiosis. There are five main symbiotic relationships: mutualism, commensalism, predation, parasitism, and competition. Table 1.2 shows examples of each.

1) Mutualism – All species benefit from positive effects focusing on protection from pathogens and/or the provision of nutrients [28]. An example of mutualism is the dependence of symbionts (microbiotas) on resources (cellulose) that are not utilized by the host. Another example is *Bifidobacterium longum* (subspecies *infantis* – *B. infantis*), which is found in human breast milk. This breast milk contains 30% of calories coming from oligosaccharides that cannot be digested by the infant and are instead digested by *B. infantis* in the GI tract. *Bifidobacterium* and *Lactobacillus* are generally regarded as beneficial microbes because of their ability to exclude harmful bacteria by producing various antimicrobial agents [26].
2) Predation – One species benefits from consuming another species. When looking for means to precisely modulate microbiomes, predation

Table 1.2 Examples of symbiotic relationships.

Strongly positive	Obligate mutualism	Lichen
	Strong mutualism	Most vertically transmitted gut mutualists and their host
	Moderate mutualism	Clownfish and anemones
	Marginal mutualism	Ants and aphids
Neutral	Commensalism	Clown fish and anemones
	Benign parasitism	*Dipylidium caninum* and canids
	Conspicuous parasitism	Many GI nematodes and their host
	Severe parasitism	Parvovirus and their host
Strongly negative	Lethal parasitism	*Dirofilaria immitis* and their host

Source: Adapted from Swain Ewald and Ewald [28].

is being researched as a promising approach [29]. Bacteriophages (phages) are viruses that prey on bacteria [30]. Phages can enter a bacterium and rapidly multiply producing hundreds of new viruses. Benefits of using predation as an approach in modulating microbiomes are the ability to (i) deliberately perturb specific bacteria, (ii) develop a deeper understanding of interbacterial and bacterial-mammalian host interactions, and (iii) be able to plan and create reproducible approaches to remodel microbiota for therapeutic purposes [29].

3) Parasitism – One species benefits from living with, on, or in a host species at the expense of the host. Some negative effects of parasitism are vitamin deficiency, immunopathy, tissue damage, and mortality [28]. While parasitism is generally thought of to have a negative effect, there are some instances where utilization of select organisms may induce a more positive effect on the host, particularly in chronic infections. In mice, chronic intestinal helminth infection has been documented to increase susceptibility to co-infection, and lower the efficacy of vaccination, while also downregulating allergic immune responses to harmless antigens creating protection against allergic diseases [29, 31].
4) Competition – Different species benefit from limited resources in the same ecosystem at the expense of each other. Competition exclusion is

a common trait of some probiotics. These nonpathogenic bacterial cultures are used to reduce colonization or decrease populations of pathogenic bacteria [32]. Competitive exclusion for intestinal bacteria is the bacteria to bacteria competition for available nutrients and mucosal adhesion sites [33]. They can also change the environment to make it less suitable for their competitors. Bacteria may also displace pathogens by taking up space in the biofilm or mucosa, inhibit the adhesion of pathogens, and decrease the competitor's ability to attach to receptor sites. The level of effectiveness of a bacterium using this relationship depends on the strain, species, and genus to receive reproducible results. Another form of competition exclusion is the production of debilitating metabolites by beneficial bacteria. Bacteriocins are one example of antimicrobial metabolites, which affect the pathogens but not the bacteriocins themselves. There are three main classes of bacteriocins based on their structure and function [34]:

i) Class I – small peptides possessing lanthionine residues
ii) Class II – which is heat-stable and does not contain lanthionine residues
iii) Class III referred to as bacteriolysins – which are large, heat-labile murein hydrolases

5) Commensalism – One species benefits with no net positive or negative effect to the other [35]. Many discussions about the microbiota in microbiomes regard the relationship between the host and the microbiota to be commensal. This term when used in a simplistic definition does not consider the complexity of these relationships. One paper describes commensalism as the dividing line between parasitic and mutualistic associations, with the overall effects of this relationship as being positive or negative, difficult to accurately assess [28]. Additionally, because these effects are not able to be precisely measured, it is difficult to always place microbiota appropriately on this continuum of mutualistic to commensal to parasitic. This group of microorganisms is called indeterminate symbionts. There are situations where some bacteria can move from being mutualistic to parasitic in different circumstances. These ambisymbionts can be identified as either mutualistic or parasitic, whereas indeterminate symbionts have an uncertain net effect.

Positive "commensal relationships" are providing the host with essential nutrients, metabolism of indigestible compounds, protection against colonization of

Box 1.1 Positive Effects of Host–Symbiote Relationship

- Providing the host with essential nutrients
- Metabolism of indigestible compounds
- Protection against colonization of opportunistic pathogens
- Contribution to the development of the intestinal architecture
- Stimulation of the immune system

opportunistic pathogens, contribution to the development of the intestinal architecture, and stimulation of the immune system [36] (Box 1.1).

1.5 Dysbiosis

If there is a microbial shift, brought on by genetics of the host, infectious illnesses, diets, or the prolonged use of antibiotics or other bacteria-destroying medications, these perturbations to the structure of the community is referred to as dysbiosis [10, 37]. When this occurs, normal interactions stop, resulting in the host body being more susceptible to disease [10]. Perturbation of normal host microbiota has been associated with several pathologies in dogs and cats, including chronic enteropathies, idiopathic IBD, acute hemorrhagic diarrhea syndrome, small bowel strictures or adhesions, neoplasia, chronic intussusception, hypothyroidism, diabetic autonomic neuropathy, scleroderma, abnormal migrating motor complexes, atrophic gastritis, and exocrine pancreatic insufficiency [3]. Table 1.3 lists some dysbiotic conditions recognized in humans.

Three types of dysbiosis can occur concurrently [10]:

1) Loss of beneficial microbial organisms – There are several mechanisms where commensal bacteria can positively influence host biology to prevent disease. Beneficial microbiota influences the host immune response with the tolerance of resident commensals being governed in part by T regulatory (Treg) cells, which are a specialized subset of T lymphocytes. When beneficial microbiota ferments dietary products, they can produce SCFAs, which have been shown to regulate the Treg pool to protect the body from inflammation and disease states such as colitis. Beneficial bacteria may also directly reduce

Table 1.3 Dysbiosis-associated conditions in humans.

Periodontal disease
Neurological diseases – depression/anxiety
Respiratory diseases
Dermatological diseases
Obesity
Diabetes
Arthritis and joint diseases
Inflammatory bowel diseases
Allergic diseases
Autism

Source: Adapted from DeGruttola et al. [38] and Sudhakara et al. [39].

inflammation by targeting cytokines and play a role in regulating invariant Natural Killer T cells (NKT) cells, which influence lipid antigens along with innate and adaptive inflammation.

2) Expansion of pathobionts – Pathobionts may create harmful effects when given the opportunity to expand. Multiple studies have demonstrated how pathobionts can increase their numbers by taking advantage of an inflamed environment. For example, when animals are treated with antibiotics, then have colitis induced using dioctyl sodium sulfosuccinate, a multi-drug-resistant stain of *Escherichia coli,* increases in numbers and can penetrate the intestinal mucosal barrier and translocate causing sepsis.
3) Loss of diversity – With multiple varieties of microbiota eliciting health benefits to the host, having a more diverse and complex pool of organisms has been shown to provide maximum benefits. Some studies have observed a difference in disease processes later in life associated with lower microbial diversity at a crucial development stage.

1.6 Probiotics

The Food and Agriculture Organization of the United Nations and the World Health Organization defined a probiotic as "live microorganisms, which when administered in adequate amounts, confer a health benefit

upon the host" [40, 41]. Probiotics in the form of cultured and fermented food sources (examples: yogurt, sauerkraut) have been anecdotally known to have health benefits, with research now looking at specific microbiota strains, species, and subspecies of bacteria, correlating them with direct benefits via commensal interactions with the GI tract resident microbiota [42]. Probiotics may be utilized to manage GI dysbiosis and have been shown in some cases to strengthen the immune response. Some probiotics can stimulate the production of anti-inflammatory substances and may participate in communication with other organs via bidirectional communication [42] (Figure 1.1).

There are characteristics of probiotics that allow them to be beneficial:

1) The microbiota in the probiotic must be alive and viable at the time of consumption [40, 43].
2) It must be able to survive varying environments through GI transit including gastric acid and be resistant to digestion by intestinal enzymes [40, 43].
3) The probiotic being used should be considered safe [40, 43].
4) Probiotics must either enhance the commensal bacteria or suppress the growth or colonization of pathogenic bacteria. There are three main actions through competition and exclusion that probiotics protect the host from pathogenic bacteria:
   i) Bacteria to bacteria competition for available nutrients [32]
   ii) Competition for space and acting as a physical barrier prevent pathogens from attaching to the gut surface [33, 43] – the action

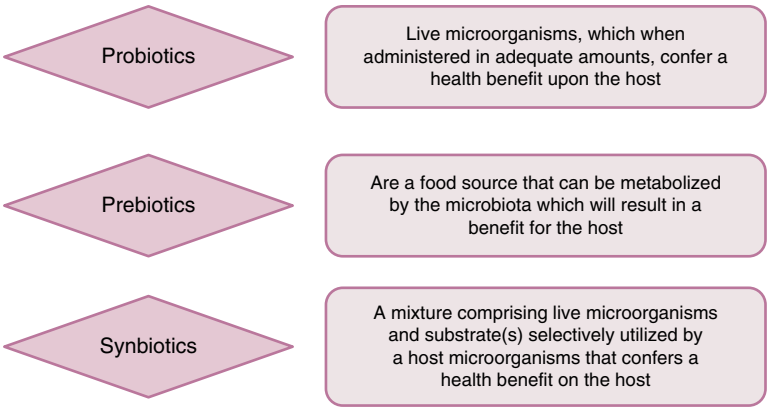

Figure 1.1 Definitions of probiotics, prebiotics, and synbiotics.

of the probiotic bacteria may be to adhere to the intestinal epithelia or receptors of the GI tract preventing the colonization of pathogenic bacteria

iii) Secretion of antimicrobial metabolites or mediators such as bacteriocins [34, 43]

5) The result of giving a probiotic should enhance the overall health of the pet. One example of positive action is the fermentation of undigested nutrients resulting in the production of SCFAs [44, 45]. SCFAs nourish enterocytes, increasing the health of the intestinal wall. SCFAs when produced in larger volumes can lower intestinal pH inhibiting the growth of pathogenic bacteria that prefer a more alkaline environment.

The main uses of probiotics are to:

1) Promote a positive change in a GI microbiome – utilizing the actions listed above the probiotic microbiota may elicit a change back to the host's normal diversity and density of microbiota [45].
2) Stimulate or enhance an immune response, without being proinflammatory – the modulation of the host GI immune system both locally and systemically via their interaction with the resident microbiota, GI epithelia, and gut immune cells [43, 46].
3) Increase or stimulate the production of neurochemicals and interaction with the gut-brain axis [47]. Probiotics that act as psychobiotics are utilized to affect many neurochemical disorders such as anxiety in pets.
4) Modulation of the immune system, help in stress management, protection from infection, improve growth and development, control allergies, and manage obesity [44].

There are times when the use of probiotics is possibly contraindicated. In an instance where a pet has a compromised GI barrier (barrier dysfunction), it is not recommended to take oral dosages of probiotics (live bacteria) as there is a rare, yet severe risk of bacterial translocation and sepsis, particularly in unregulated products [48].

In North America, as long as the probiotic label does not indicate any health claims, probiotics are not considered to be pharmaceuticals, and the classes of nutraceuticals or supplements are not recognized for animals and therefore do not have the same regulations placed upon them as pharmaceuticals, with regards to viability studies, dosing, and expiry

of products. This lack of regulation could result in inconsistencies in the product contents with concerns that:

1) The product is not what is indicated on the label [49].
2) The bacteria may not be viable or in an carrier promoting survival to the target location [44].
3) The product may not provide benefits and may be pathogenic [37, 44, 49].
4) The bacteria are not listed at the subspecies level and may not result in the same or any benefits for the host [37].
5) The product may not contain sufficient bacteria to elicit a change [50].
6) The product may not be tested to identify the specific genus and dosage, nor have been tested for safety, stability, and consistent positive results [37, 50].
7) The strain used may release harmful metabolites [43].
8) The strain may be prone to transmit antibiotic resistance [44, 51].
9) Bacterial translocation may result in infection [44, 48].

1.7 Prebiotics

As opposed to probiotics, which consist of viable organisms, prebiotics are nutrients predominantly undigestible by the host animal that serve as a food source to be metabolized by the microbiota, resulting in benefit to the host [40, 45, 52]. These food sources consist of nutrients that can be fermented by the microbiota, which increases the survivability of beneficial microbes. Each species of microbiota has preferential food sources. Sources of indigestible nutrients are mainly from carbohydrates, with psyllium husk and/or yeast cell walls being a common ingredient used in commercial prebiotics at this time. As microbiota ferment cellulose, they may create the metabolites butyrate and acetate, SCFAs that are energy sources for colonocytes, and provide multiple other beneficial effects [44, 45].

To be classified as a prebiotic, the substrate must meet certain criteria [53]:

1) Resistant to digestion – does not break down in the acidic pH of the stomach, cannot be hydrolyzed by digestive enzymes, and is not absorbed in the upper GI tract.
2) Fermentable – it is fermentable by intestinal microbiota.

3) Produces a positive result – there is selective growth and/or activity of the intestinal microbiota that conveys a health benefit to the host.

There are different types of prebiotics: oligosaccharides like fructo-oligosaccharide (FOS), oligofructose, mannose-oligosaccharide (MOS), galacto-oligosaccharide (GOS), pectic oligosaccharide (POS), inulin, and glucan and noncarbohydrate compounds such as flavanols. Fructans, pectins, inulin, and flavanols are predominantly derived from vegetation like fruits and vegetables, GOS can be derived from lactose or synthetic lactulose, and resistant starches and glucan may be derived from plants and yeast cells [53].

1.8 Synbiotics

Compounds comprising of a mixture of both live microorganisms (probiotics) and a substrate selectively utilized by beneficial microorganisms that confer a health benefit to the host (prebiotic) are termed synbiotics [54]. The International Scientific Association for Probiotics and Prebiotics (ISAPP) has created another class of synbiotics called synergistic synbiotics, defined as "a synbiotic for which the substrate is designed to be selectively utilized by the co-administered microorganisms" [54]. These terms should be reserved for products where the prebiotic will selectively favor the specific probiotic organisms.

Reasons to feed a specific prebiotic with the probiotic are to [53]:

1) Selectively support the growth of the preferred microbiota once in the desired location (GI tract)
2) Aid in the metabolism of one or more health-promoting bacteria

1.9 Biological Markers (Biomarkers) and Their Measurement

Biomarkers are cellular, biochemical, or molecular indicators detectable in biological media that suggest changes to normal or pathological processes, as well as responses to a therapeutic intervention [55]. When assessing a healthy patient or a patient experiencing a disease state, biomarkers as a tool can aid in the prediction, cause, diagnosis, progression,

regression, or outcome of treatment. Biomarkers can be divided into Exposure, where the level of risk is predicted, and Disease, where screening, diagnosis, and progression are monitored (Chart slide 13). The methods of detection and measurement of biomarkers can also be divided into targeted or untargeted, depending on the aim of the study [56]. When relationships between specific biomarkers and outcomes are known, they are typically measured by targeted processes, meaning quantitative measurements of specific metabolites are made. Alternatively, untargeted approaches allow for a more discovery-based approach, where a potentially vast number of individual or classes of biomarkers may be measured.

1.9.1 Genes, the Genome, and Genomics

The genome is the complete set of deoxyribonucleic acid (DNA) bearing all the genetic information of an organism [57]. The genes encoded in the DNA provide instructions to the cell on how to make each specific protein, which is then utilized to carry out functions in the body.

Proteins are synthesized through two processes:

1) Transcription – the synthesis of DNA into functional forms of ribonucleic acid (RNA), including mRNA, tRNA, rRNA, and noncoding RNA, which are used in the translation process.
2) Translation – the synthesis of mRNA into an amino acid sequence. The final polypeptide chain can then further undergo post-translation modifications along with subsequent enzymatic and nonenzymatic alterations to increase the number of protein species.

Genomes are complex and can be influenced by environmental factors. The microbiome and their respective genome have been shown to play a major role in human health and disease.

Genome-wide association studies (GWAS) is a scientific collaboration to create a large database to look for similar variants across the human genome, intending to determine links between genotypic and phenotypic variabilities [58]. Microbiome genome-wide association studies (mGWAS) explain the interaction of host genetic variation with the microbiome.

Genomics refers to the study of an organism's genome, the entire complement of genes, including interactions between genes with both each

other and the organism's environment [59, 60]. This may include analysis of all nucleotide sequences in the genome, and the structure, function, evolution, and mapping of the genome, in order to understand the entire genetic information of the organism. Next-generation sequencing technology enables the investigation of the complex interaction between host genetics and microbial communities [58].

In comparison to genomics that study the genome of a singular organism, metagenomics is the study of a collection of genomes from a community of organisms [61]. This platform may be used to examine all DNA sequences across multiple organisms, particularly useful when culture or separation of microbes is not feasible or required.

Lastly, epigenetics refers to the study of how internal and external factors can influence the expression of genes [62–64]. Epigenetic changes are reversible and do not alter the sequence of DNA, or genotype, but instead affect the way the encoding of the DNA is interpreted – the phenotype.

Three ways epigenetics can affect gene expression are:

1) DNA methylation – a chemical group is added to a specific place on the DNA where it "blocks" the proteins "read" the gene [63, 64]. The chemical group can then be removed by a process called demethylation. To "silence" or turn genes "off," methylation is used while demethylation turns genes "on."
2) Histone (chromatin) modification – histones are proteins that allow or stop DNA from being "read" depending on how tightly the DNA is wrapped around it [64]. Chromatin is the complex of histones and DNA combined. Chemical groups are added or removed from histones to alter the wrap. A wrapped gene is considered "off," while an unwrapped gene is considered "on" or able to be "read."
3) Noncoding RNA – while coding RNA is used to make proteins, noncoding RNA helps control gene expression by attaching to and breaking down coding RNA so it cannot be used to make proteins [63]. Noncoding RNA can also modify histones with the use of other proteins to influence whether the gene is "on" or "off."

Epigenetics can change as part of growth and development, with some changes being reversible. These changes can affect an organism's health by affecting the immune system, developing neoplasia due to mutations, and affecting fetal epigenetics based on the maternal environment and behavior during pregnancy [64]. Known associated illnesses include

cognitive dysfunction, respiratory, cardiovascular, reproductive, autoimmune, neurobehavioral illnesses along with a wide variety of behaviors and cancers. Epigenetic modifications can be induced by several drivers, including exposure to toxins, nutrition during pregnancy and early development, and behavioral influences such as maternal-neonatal care, mental health, and the aging process.

1.9.2 Metabolites, the Metabolome, and Metabolomics

Microbial metabolism and fermentation results in production of small molecular substrates, intermediates, and products, collectively termed metabolites [65]. The vast diversity of metabolites are produced by microbiota from the metabolism or fermentation of macronutrients and micronutrients, which then can interact with multiple body systems. The effect of specific microbiota-derived metabolites depends on multiple factors including the strain of microbiota, the food source provided, the volume of metabolites produced, and the health status of the host (Figure 1.2).

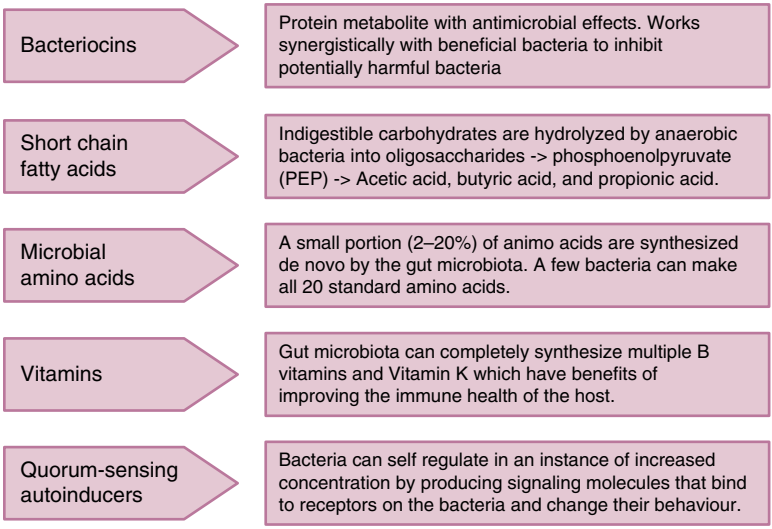

Figure 1.2 The five metabolites derived from the gut microbiota and their main function.

There are currently 5 gut microbiota-derived metabolites identified:

1) Bacteriocins – A protein metabolite that has antimicrobial effects and works synergistically with beneficial bacteria to inhibit potentially harmful bacteria. The synergy of bacteriocins and antibiotics may help address the problem of antimicrobial resistance as a future approach in the treatment of infectious diseases [34].
2) Short-chain fatty acids – Indigestible carbohydrates such as some fiber sources are hydrolyzed by anaerobic bacteria into oligosaccharides that are then converted into phosphoenolpyruvate (PEP) and finally into acetic acid, butyric acid, and propionic acid depending on the bacteria of origin [66]. These metabolites in particular have been highly studied for their effect on the host [45].
3) Microbial amino acids – While most amino acids in the intestines originate from the consumption of nitrogenous ingredients (animal and plant sources along with from the host muscle tissue), a small portion is synthesized *de novo* by the gut microbiota. A few bacteria can make all 20 of the standard amino acids, contributing to the host's amino acid homeostasis [66]. Microbial synthesized lysine, in particular, has been shown to contribute 2–20% of the total circulating volume in a study in humans, pigs, and rats.
4) Vitamins – Some vitamins can be completely synthesized by the gut microbiota including multiple B vitamins and vitamin K, which have benefits at improving immune health for the host – though sites of production and absorption must be considered [67].
5) Quorum-sensing autoinducers – Over time there is a gradual and accumulative increase in bacteria concentrations in a certain area. The bacteria will self-regulate by producing and releasing signaling molecules to detect the bacterial concentration. The signaling molecules will bind to receptors on the bacteria and ultimately change the behavior of the bacteria [66].

Metabolites have a variety of functions:

1) Key factors in a variety of host to microbiota and cell to cell communication [65]
2) Metabolite signaling through a series of innate immune receptors affecting host immunity [65, 68], along with regulation of the adaptive immune cell development (T lymphocytes) [66]

3) Drive changes in the composition and function of the microbiota through signaling (quorum sensing) [66, 68]
4) Production of SCFAs, which provide multiple benefits to the host [45, 68]
5) Participate in various physiological processes, including energy metabolism [65]
6) Represent potential biomarkers for early diagnosis of multiple disorders [65]
7) Directly kill pathogens by:
   i) disrupting bacterial cell structures.
   ii) interfering with bacterial DNA, RNA, and protein metabolism.
   iii) resource competition between commensal bacteria and pathogens.
   iv) affect cell adhesion and biofilm formation.
   v) regulation of the immune system by activating innate immunity [66]
8) Production of vitamins and metabolism of some minerals [67]

The phrase "metabolome" refers to the biochemical environment derived from the symbiosis of nutrient-rich milieu provided by the host and the products and metabolites produced by the microbiota [69]. It provides a functional interpretation of cellular activity and physiological status.

The metabolome includes simple amino acids and related amines, lipids, sugars, nucleotides, vitamins, and other intermediary metabolites. The molecules in a metabolome will change depending on the organism being studied and what chemical reactions are occurring in the cell. Metabolomics analyses include sensitive chromatographic methods coupled with mass spectrometry – gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) – as well as nuclear magnetic resonance to identify and quantify compounds in the metabolome [59].

The study of the metabolome is termed metabolomics. This platform includes analytical profiling, quantification, and comparison of metabolites present in biological samples [56, 65].

Metabolomics offers a window into metabolic mechanisms through the use of analytical chemistry and multivariate data analysis [59]. Examples of metabolomics in disease research include certain types of

neoplasia with dogs and the use of dogs as a translation model for humans, with the data possibly being bidirectionally beneficial [70, 71].

Due to the complexity of the metabolome, there are two main types of analytic platforms used in metabolomics [56, 59, 71–73]:

1) Mass spectrometry – achieves a high sensitivity in the analysis. Mass spectrometry may require extensive sample preparation, which can result in the loss of certain compounds. This technique can be limited with respect to the range of metabolite detection per sample and require different preparations of multiple samples to detect maximum number of metabolites. Types of mass spectrometry include:
   i) Gas chromatography-mass spectrometry (GC-MS) – oldest tool for qualitative metabolic profiling while providing high chromatographic resolution
   ii) Liquid chromatography-mass spectrometry (LC-MS) – high sensitivity and provides information regarding metabolite structure.
   iii) Capillary electrophoresis-mass spectrometry (CE-MS) – high efficiency allowing separation of chemically diverse metabolites in smaller sample volumes with little to no pretreatment required.
2) Nuclear magnetic resonance (NMR) – quantitative, highly reproducible nondestructive technique. Requires comparatively large sample volumes, but little to no pretreatment of samples, and a range of metabolites can be analyzed in a single sample. Specificity is limited as metabolite resonances may overlap.

These technological advances allow for large quantities of data to be obtained at multiple levels – organelle, cell, tissue/fluid, organ, and entire organism, which provide information on biomolecular functions. Databases for metabolomes and metabolic analyses are available through online platforms:

1) The Human Metabolome Database – https://hmdb.ca/ [74]
2) The Livestock Metabolome Database – https://lmdb.ca/ [75]
3) The Bovine Metabolome Database – https://bovinedb.ca/ [76]
4) Metaboanalyst – https://www.metaboanalyst.ca [77]

1.9.2.1 Metabonomics

A specific branch of metabolomics, termed metabonomics, is dedicated to the study of how the metabolic profile of biological systems change in

response to alterations, such as environmental exposures, pathophysiologic events, and nutrition [56, 71, 78]. Thus, this profile is determined by both host genetics and exogenous factors. The term is not always used; however, often the term metabolomics is used to include what could be more strictly defined as metabonomics.

1.9.3 The Proteome and Proteomics

The proteome is the complete set of proteins expressed by an organism [79]. It represents the expression of an organism's genome and actively changes in response to various factors, including the organism's developmental stage, and other internal and external conditions.

"a large-scale study of protein properties produced by the cell. This includes the expression level, post-transcriptional modification, and protein interaction, in order to obtain a global view of disease processes or cellular processes at the protein level." Proteomics aims to catalog the entire protein products of the human genome. Other specific "omics" studies exist, such as lipidomics, which is the study of biological lipids [56].

There are three main strategies of proteomics showing to have an impact:

1) Protein–protein linkage maps
2) Genomic DNA sequences of peptide sequences from mass spectrometry
3) Quantitative protein expression 42.2

Glossary

Biological marker (biomarker) – cellular, biochemical, or molecular alterations that are measurable in biological media such as tissues, cells, or fluids; include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention

Commensalism – a form of symbiosis where one species lives with, on, or in a host and provides no benefit or detriment to the host

Competition – different species using similarly limited resources in the same ecosystem

Dysbiosis – a change to the composition of resident commensal communities relative to the community found in healthy individuals

Genome – the complete set of DNA (genetic information) in an organism; contains all the information needed to build and maintain that organism throughout its life

Genomics – the study of an organism's genome (genes), including interactions of those genes with each other and the organism's environment

Epigenetics – the study of how behaviors and environment can cause changes that affect the way genes work

Metabolites – small molecular substrates, intermediates, and products of metabolism

Metabolome – the collection of small compound metabolites in an organism

Metabolomics – an analytical profiling technique for measuring and comparing large numbers of metabolites present in biological samples

Metabonomics – the quantitative study of how the metabolic profile of biologic systems change in response to alterations caused by pathophysiologic stimuli, toxic exposures, or dietary changes

Metagenomic – the study of a collection of genetic material (genomes) from a mixed community of organisms

Microbiome – the microbiome is the genetic material of all the microbes – bacteria, fungi, protozoa, and viruses – that live in a particular ecosystem. These microscopic communities can be found in all biological systems, including inside humans and other animals, along with residing in plants, soils, and oceans

Microbiota – the individual bacteria, fungi, virus, and protozoa that make up the microbiome community

Mutualism – both (or all) species benefit in a mutualistic symbiotic relationship

Parasitism – a symbiotic relationship in which one species lives with, on, or in a host species at the expense of the host

Pathogens – a biological agent that causes disease or illness to its host

Prebiotics – food sources that can be utilized by the microbiota and in turn result in a benefit for the host; typically indigestible to the host but rapidly fermented by the host's microbiota

Predation – a symbiotic relationship in which one species hunts, kills, and consumes (an)other species

Probiotics – viable microbes intended to be beneficial to the commensal microbiota
Proteome – the complement of proteins produced by a cell
Proteomics – large-scale study of proteins produced by a cell; includes the expression level, post-transcriptional modification, and protein interaction in order to obtain a global view of disease processes or cellular processes at the protein level
Short-chain fatty acids (SCFAs) – metabolites of microbial fermentation – predominantly acetic acid, butyric acid, and propionic acid
Symbiosis – any relationship or interaction between two dissimilar organisms, with the specific kind of symbiosis depending on whether either or both organisms benefit from the relationship
Symbiotic – a product consisting of a probiotic and prebiotic in combination

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