Probiotics and Prebiotics in Animal Health and Food Safety

Diana Di Gioia
Bruno Biavati
Editors

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Diana Di Gioia and Bruno Biavati

1 Probiotics and Prebiotics: An Overview on Recent Trends

Georgia Zoumpopoulou, Maria Kazou, Voula Alexandraki,
Angeliki Angelopoulou, Konstantinos Papadimitriou,
Bruno Pot, and Effie Tsakalidou

1.1 Introduction

Nowadays, the pro- and prebiotic concept is very well known regarding human applications related to preserving or restoring health. However, applications in feed are far less documented. While prebiotics should be considered a more recent concept, the history of probiotics is long and interesting. Thousands of years ago, man discovered already the benefits of "fermented" foods, e.g., in extending shelf life of fresh food products (Ozen and Dinleyici 2015; Gogineni et al. 2013). This observation was extended to the fermentation of feed as well. Ancient evidence from Egyptian drawings and old Carthusian silos shows that more than 1000 years ago, farmers already knew that silage was an excellent way to preserve summer crops for their animals during winter times (Mannetje 2010). It took, however, until the early twentieth century before the Nobel Prize winner Ellie Metchnikoff for the first time defined and studied the role of the fermenting bacteria in health (Metchnikoff 1908). While at that time the "probiotic" concept (live microorganisms that can promote health) was born, it took until 2001 for the concept to be acceptably defined. This

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Georgia Zoumpopoulou, Maria Kazou, Voula Alexandraki, and Angeliki Angelopoulou contributed equally to this work.

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was achieved by an expert panel composed by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), who defined probiotics as "Live microorganisms that when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). Followed by FAO/WHO (2002) and recently revised by Hill et al. (2014), this definition today is widely accepted by the scientific community and most governmental institutions.

In the area of animal nutrition, however, in the USA in 1989, also the term "direct-fed microbials" (DFM) was introduced by the Food and Drug Administration (FDA). DFM were, similarly as probiotics, defined as "a source of live (viable), naturally occurring microorganisms." Clearly, the health aspect was not maintained in that definition. Consequently, while manufacturers were required to use this term on their ingredient lists, FDA did not allow them to make therapeutic claims. FDA together with the Association of American Feed Control Officials (AAFCO) published a list of microbial species that could be used in DFM products (AAFCO 1999). The interest in DFM was already raised in the 1950s when a positive growth response was noted in animals fed with antibiotics, suggesting that the suppression of pathogens had a positive impact on the animal health and improved its growth response. It was not until much later that it was shown that a healthy intestinal microbiota consists of a certain balance between potentially beneficial commensal and potentially pathogenic bacteria and that supplementation with "beneficial" bacteria might evoke a similar positive growth response, without the need to use antibiotics that left traces in the meat and caused resistance problems (Landers et al. 2012; Tzivara et al. 2013). In general, nowadays, two categories of DFM applications can be distinguished: (1) feed inoculants (e.g., for silage or high-moisture grain), intended to ferment the feed substrate and modify the digestibility and safety of the feed component, and (2) beneficial, viable microorganisms added to feed to improve the health parameters of the consuming animal.

The prebiotic concept is much younger and was first introduced by Gibson and Roberfroid (1995) as "Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health." As for probiotics, the original definition has been modified frequently, but so far no consensus has been reached. Bindels et al. (2015) proposed the definition "A non-digestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host." In the latter definition, the "selectivity" is no longer a criterion, and the link with the metabolic degradation of the compound and the results on the ecology of the gut microbiota or the observed physiological effects are more deeply stressed.

Nowadays, in both the pro- and prebiotic definitions, the importance for health (whether man or animal) occupies a central position. Traditional applications of probiotics have been mostly performed through fermented dairy products and were mainly directed to usual digestive processes. However, more recently other foods and food supplements have gained much more attention, increasing the worldwide probiotic market, which exceeded 30 billion dollar in 2015 and expected to account for 52 billion by 2020 (Market Research Report 2016). Moreover, the explosion of the metagenomics approach to study the microbiota niches in man and animal, and their exploration in terms of health and disease, has opened a completely new range of applications, situated in the pharmaceutical field on top of the nutritional field. The applications of pro- and prebiotics in pet animals and their exploitation in farm animals, in aquaculture, or even in plants are equally expanding. The global probiotics in the animal feed market are expected to attain 4.71 billion dollars by 2021 (Market Report 2016). While the potential of these applications is high at the prophylactic as well as therapeutic level, there are currently a number of technological, microbiological, and regulatory bottlenecks, which slow down the developments in the field and which will be explored further in this chapter.

The technological challenges for probiotics are mainly related to the requirement that the microorganisms should be viable at the end of the product's shelf life. While spray-dried vegetative cells know a faster decline in numbers over time compared to freeze-dried, the latter is considerably more expensive, which, for agricultural applications, is a considerable bottleneck. The use of spore-forming bacteria has, therefore, been proposed successfully. Spores are better in resisting environmental conditions, such as heat, moisture, and pH changes. Upon digestion by the animals, they will germinate into active vegetative cells. Because of their resistance properties, they are frequently added to pelleted diets, where they survive the thermal treatments that are often necessary in feed compacting and pelleting.

The strain selection. Probiotic effects are known to be strain specific (FAO/WHO 2001); therefore, it is important that strains intended for a particular application in a particular animal are carefully selected. Selection criteria will differ depending on the animal species (e.g., farm versus pet animals) and desired application (e.g., growth promotion versus anti-infection).

The cost issue. The above research requires a considerable amount of effort, the cost of which has to be borne by the farmer. As financial margins in animal husbandry are shrinking, the cost of probiotics does become an issue. A number of older studies have shown, however, that the supplementation with a mixture of lactobacilli could improve egg production and feed efficiency by 3.03 and 7.41%, respectively, in Leghorn hens, while a large-scale study comprising 101,615 commercial hens showed an egg production increase from 69.5 to 72.2% with a feed reduction from 1.75 to 1.69 kg. For further examples in turkey, pigs, and ruminants, see Ezema (2013). The use of probiotics in pet animals, mainly for health or animal well-being reasons, however, has a different economic reasoning and might be more difficult to calculate in this simple way. In estimating the cost of probiotics in animals, it might also be important to consider arguments related to the reduction of antibiotic usage. In the European Union (EU), in 2006, a ban on the use of antibiotics as growth promoters was introduced (European Parliament and Council 2003). This ban did reduce antibiotic resistance development but also increased the general infection rate in husbandry installations (Bywater et al. 2005; Casewell et al. 2003).

The short life span of, e.g., broiler chicken, reaching slaughter weight after 5–7 weeks, leaves little time for probiotics to contribute to the development of a mature immune system, a process, which takes up to 6 weeks. Therefore, rather than being fed with probiotics, hen and broiler chicks are most often vaccinated against infectious pathogens, such as *Salmonella* or a variety of viruses (Breytenbach 1999). Vaccines are mostly administered via drinking water or spraying. However, given the need for fast growing (weight gains of over 50 g per day), selected probiotics could be used for weight gain purpose.

The regulatory aspect for probiotics is complex. In human applications, different countries allow different levels of health claims. While in the EU currently no health claims are approved for human applications with probiotics, except for yogurt in relation to lactose intolerance, the use of probiotics in animals is well regulated. Manufacturers of probiotics will need to provide evidence of the identity, safety, and efficacy of the product, which will be assessed by a committee of experts (European Parliament and Council 2003). When approved, probiotic products can be labelled and marketed as “gut flora stabilizers” under the category “zootechnical additives,” which is one of the five categories of feed additives defined by this Regulation (EC) No 1831/2003: (1) technological additives, (2) sensory additives, (3) nutritional additives, (4) zootechnical additives, and (5) coccidiostats and histomonostats (European Parliament and Council 2003). In the USA, the Center for Veterinary Medicine (CVM) within the FDA is responsible for the recognition of safety, effectiveness, labelling, and distribution of livestock feeds, pet foods, veterinary drugs, and devices. As mentioned before, the FDA uses the term DFM for probiotics used in animal feed, “products that are purported to contain live (viable) micro-organisms (bacteria and/or yeast)” (FDA 2015). For more detailed information on global regulations, see FAO (2016).

In order to convince the legislator to recognize the benefits of probiotics at a much broader scale and promote their acceptability by the farmer and the consumer, the clarification of the mechanisms underlying the beneficial properties is extremely important. These mechanisms can be more generic in nature, e.g., the production of organic acids, the reduction of toxic amines, or more strain specific, e.g., the production of antimicrobials, such as bacteriocins or hydrogen peroxide (explaining the competitive exclusion of pathogens, most often observed for probiotics), or the production of specific enzymes like amylases, lipases, proteases, and glycosidases, which can assist digestion. Other mechanisms, such as the stimulation of immune responses are strain specific, may be limited to a specific period during the development of the animal, and efficacy is mostly depending on the animal species. Some of these mechanisms are further discussed in this chapter.

1.2 Polygastric Animals

Ruminants, the most widely distributed group of mammals on Earth, currently add up to about 150 domestic and wild species, while economic interest lies mainly in the breeding of cattle, sheep, goats, and water buffaloes. Ruminants are able to assimilate nutrients from low-quality plant-based feeds, through their digestive tract, which is uniquely designed and includes, in contrast to other mammals, a four-compartmentalized stomach consisting of the rumen, the reticulum, the omasum, and the abomasum. From the physiological point of view, each chamber performs different processes. The microbial fermentation of the fibers and solid feeds takes place in the rumen, while the liquids are transferred to the reticulum, which serves also to the entrapment of large feed particles, regurgitated subsequently for optimal digestion. In the omasum the liquids are filtered and various nutrients are being absorbed, and, finally, in the abomasum the enzymatic digestion of the feed takes place (Hofmann 1989). While the ruminant gastrointestinal tract (GIT) consists of different niches, the vast microbial diversity is observed in the rumen, where the microbial fermentation of the feed is carried out. The rumen microbiome is composed predominately of bacterial species but also of methanogenic archaea, flagellated and ciliated protozoa, fungi, and bacteriophages (Chaucheyras-Durand and Ossa 2014) with populations at a level of 10^{10} (bacteria), 10^{8} (protozoa), 10^{7} (archaea), and 10^{3} (fungal spores) colony-forming units per mL of rumen fluid (Deusch et al. 2015).

The optimized ruminal fermentation is essential in supporting health and productivity in the ruminants, since several physiological parameters of farm animals are highly correlated with the abundance of various bacterial members of the rumen microbiome (Jami et al. 2014). Toward this, the systematic use of antibiotics was gradually adopted as a common practice in animal husbandry, targeting, inter alia, the beneficial manipulation of ruminal metabolism. Nevertheless, their rampant use as growth promoters in animal feed during the last decades gradually raised concerns, not only for the antibiotic residues in animal products and the emergence of drug-resistant microorganisms but also for the well-being of the animals themselves. In recent years, probiotics and DFM are widely used in the livestock production, especially in the EU, where the use of antibiotics in this field has been completely prohibited (Landers et al. 2012; Papatsiros et al. 2013). However, numerous countries, e.g., China, the USA, Australia, etc., still employ antibiotics in livestock production, and an unprecedented increase in usage rate during the next decade is foreseen, mainly in developing countries (Van Boeckel et al. 2015).

The application of probiotics and DFM in ruminant productivity and health includes treatment of digestive disorders and reduction of gut pathogens (Wisener et al. 2015), stabilization of the ruminal pH (Chiquette et al. 2008), enhanced animal performance, increased feed conversion efficiency and fiber digestibility (Zhang et al. 2015b), improved milk yield and composition (Ayad et al. 2013; Maragkoudakis et al. 2010), stimulation of the immune system (Spaniol et al. 2015), treatment of mastitis (Espeche et al. 2012), and methane mitigation (Alazzeh et al. 2012). The potential of some probiotics to bind mutagens either present in feeds or formed due to stress or GIT infections has been recently also reported (Apas et al. 2014). The vast majority of the applications concern cows and the pre-ruminant life of calves, whereas the number of respective studies for lambs, sheep, and goats has increased over the last years. The probiotic preparations are delivered to ruminants mainly orally, directly, or in the feed. However, the oral administration may compromise the probiotic efficacy due to the adverse conditions prevailing in the GIT. For ensuring the stability and viability of probiotics, the microencapsulation technology has come into use, providing protection and controlled deliverance of the probiotic preparation in the GIT (Qi et al. 2011).

An overview of the respective literature reveals the broad applicability of the well-studied lactic acid bacteria (LAB) as probiotics and DFM in ruminants. The use of *Lactobacillus*, *Enterococcus*, *Streptococcus*, and *Bifidobacterium* species has been reported. Besides LAB, several other microorganisms have been studied for their probiotic potential in ruminants. These include lactic acid utilizers, such as *Propionibacterium* and strains of *Megasphaera elsdenii*, as well as other bacteria, such as *Escherichia coli*, *Bacillus*, and fibrolytic *Prevotella* species (Dhama et al. 2008; Puniya et al. 2015; Rafat and Hussain 2013; Seo et al. 2010). In two recent studies, the use of cellulolytic *Ruminococcus* species in buffaloes and reindeers resulted in the beneficial modulation of their rumen microbiome (Kumar and Sirohi 2013; Praesteng et al. 2013). While most bacterial probiotics are highly efficacious in pre-ruminant calves, probiotic yeasts and fungi, such as *Saccharomyces cerevisiae* and *Aspergillus oryzae*, respectively, have shown greater benefits for adult ruminants (Nagaraja 2012). It has been demonstrated that their use positively influences certain bacterial populations and the fermentation patterns in the rumen (Pinloche et al. 2013). Furthermore, non-live products from fermentations of probiotic microorganisms have been efficiently employed in ruminants (Bernard 2015). In a recent study, it has been demonstrated that there was no evident benefit from the supplementation of live LAB when compared to the administration of non-live probiotic extracts (Jenkins and Jenkins 2014). Among the various studies performed, even kefir has been examined as a probiotic supplement in ruminants, but its administration did not affect significantly the physiological parameters of the animals (Atasoglu et al. 2010).

The interest for identifying candidate probiotics for ruminants is gradually focusing on the autochthonous microorganisms from the various niches of the target animal and toward their ensuing use in the digestive tract of the animal, which served as the initial isolation source (Fraga et al. 2014; Nader-Macias et al. 2008). For example, comparison of the probiotic characteristics among isolates from dairy products and animal rumen revealed that the latter were more tolerant to bile salts and exhibited higher inhibition against pathogens (Jose et al. 2015). These findings show that the adaptation of the microorganisms to a specific ecosystem could play a significant role in the selection of probiotic candidates and that the probiotic efficacy of selected isolates might depend to some extent on the original host. Furthermore, the use of rumen inhabitants as probiotics will result in enhancing the existing beneficial gut microbiota, which seems to be a milder method of gut microbiome manipulation than introducing ecosystem-irrelevant microbes (Kumar and Sirohi 2013). Therefore, the niches of the ruminant GIT constitute a rich and diverse reservoir for mining potentially novel probiotics (Tellez et al. 2015). The boost in the development of high-throughput sequencing techniques revealed an abundance of non-culturable bacteria in the rumen ecosystem in comparison to data obtained by conventional microbiology (Kim et al. 2011b). The recent accumulation of metagenomics studies on the rumen microbiome can provide a vast body of information concerning not only the composition and the function of the respective microbiota but also its interaction with the host and its feed (Morgavi et al. 2013).

The concept of using bacteriophages for manipulating certain microbial populations in ruminants has been also studied (Callaway et al. 2008; Sheng et al. 2006). Although phages present high host specificity, their efficient application requires the identification of the bacterial target in the rumen. To prevent bacterial resistance, the use of phage cocktails is recommended. In a recent study, a cocktail of designed bacteriophages was successfully employed in rats as a biocontrol means against the gut pathogen *E. coli*, suggesting further testing for possible use in ruminants (Abdulamir et al. 2014). An effective treatment demands the monitoring of the developing resistance mechanisms, the use of newly isolated phages from the rumen environment, and even the development of new phages in the laboratories. Furthermore, the use of isolated lysins instead of whole bacteriophages could be a promising alternative. However, there are only few data available about the rumen virome. Recent studies on the rumen bacteriophages and their interactions with the rumen bacteria constitute an initial attempt to study the rumen virome in depth, helping to obtain new insights probably exploitable in the manipulation of the rumen microbiome (Berg Miller et al. 2012; Ross et al. 2013). The detailed characterization of the rumen virome would be of great significance, since the endemic ruminal phages could prove to be either a useful tool (Hallewell et al. 2014) or a drawback for the probiotic interventions in the animals (Kropinski et al. 2012). Additionally, further research is needed regarding the potential risk associated with the use of phages in lactating ruminants and the possible contamination of milk and dairy products. If the adverse effect on dairy manufacturing is demonstrated, their application could be limited to meat-producing animals.

The application of probiotics and DFM could also play a decisive role in the mitigation of rumen methanogenesis, since the reduction of the enteric methane emissions could be attained through the enhancement of rumen fermentation efficiency and the augmentation of animals' productivity (Karakurt et al. 2012). The environmental impact of the ruminant-derived methane is of considerable importance for the sustainability of livestock, since it is accountable for 25% of the global methane emissions produced by anthropogenic activities (Buddle et al. 2011). The use of probiotic acetogenic bacteria and yeasts, mainly *S. cerevisiae*, for decreasing rumen's methane emissions has been studied with promising results (Jeyanathan et al. 2014). Another interesting aspect is the use of probiotics for controlling specifically the protozoal population in the rumen, since it has been reported that methanogens found both attached and inside ciliate protozoal cells are responsible for 9–37% of the enteric methane production (Jeyanathan et al. 2014; Martin et al. 2010). The proportional correlation among rumen protozoa and methane emission has been confirmed using a meta-analysis approach (Guyader et al. 2014). Recently, the availability of genome projects on rumen methanogens can provide information about the dominant microorganisms implicated in methane production, e.g., methanogenic archaea (Leahy et al. 2013), leading to a more targeted selection of probiotics and DFM.

The use of recombinant microorganisms with probiotic properties in ruminants has been also documented. The most successful study concerns the genetically modified bacterium *Butyrivibrio fibrisolvens*, in which a dehalogenase for fluoroacetate encoding gene from a *Moraxella* soil species was introduced (Gregg et al. 1994). The modified organism was able to degrade the toxic fluoroacetate present in forage plants. The results were encouraging since the microorganism survived in the rumen of sheep and cattle without the loss of the respective gene (Gregg et al. 1998; Padmanabha et al. 2004). The same species was also used for the creation of a recombinant xylanolytic strain. A plasmid containing a xylanase gene from *Neocallimastix patriciarum* was successfully inserted into *Butyrivibrio fibrisolvens* (Xue et al. 1997), and although the modified microorganism had enhanced capacity for xylan degradation, it failed to persist in the rumen (Krause et al. 2001). The recent information obtained from various sequencing projects and databases reveals the abundance of specialized microorganisms in the rumen. Thus, it would be difficult for genetically engineered superbugs to fully colonize the ruminal microbial ecosystem and exert on the host the benefits for which they have been designed (Krause et al. 2013).

Similar to probiotics, prebiotics, which are nondigestible oligosaccharides, are also effective in altering the composition and activity of the microbiome in the GIT, since they constitute suitable substrates for the enhancement of certain beneficial ruminal microorganisms. However, the ability of ruminants to catabolize most of the common prebiotic compounds creates a limitation in the use of prebiotics as growth promoters in ruminant production. In addition, several nondigestible oligosaccharides found naturally in plant cell wall are included in feeds normally used in ruminant rations (Gaggia et al. 2010), making the implementation of prebiotics in ruminants possibly unnecessary. The administration of prebiotics seems to be beneficial on very young ruminants, since these substrates may contribute to the formation of a desirable intestinal community, which may further improve the performance of older animals (Uyeno et al. 2015). The advance of rumen-protective technologies providing shielding from ruminal digestion, such as encapsulation, may become useful tools for the eventual use of selected prebiotics in ruminant feed, as it has been reported for probiotics (Mustapha et al. 2013).

Despite the wide applicability of probiotics and to a lesser extent of prebiotics in ruminant production and the promising results obtained from various studies, reproducibility issues are raised, since experimental data acquired are often inconsistent (Uyeno et al. 2015). A wide variety of factors, such as the growth environment, the animal species and breed, the age and physiological state of the animal, the diet, the nature of the probiotic preparation used (e.g., type of microorganism, live culture, or lyophilized cells), and even its dose, seem to affect the outcomes of probiotics' utilization in livestock. Obviously, comprehensive research is needed for the reliable and viable use of probiotics and prebiotics in ruminant production.

1.3 Monogastric Animals

Monogastrics are classified as animals having one simple or single-chambered stomach with the main agricultural species being pigs, poultry, and horses. The gut microbiota of pigs mainly consists of bacteria, while a small percentage of archaea mostly *Methanomicrobia* and *Thermococci* have been also identified (Isaacson and

Kim 2012; Lamendella et al. 2011). In the poultry GIT, 13 phyla of bacteria were discovered with *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* being the more representative ones with up to 900 and 500 species in chicken and turkey gut, respectively. Of all the species found, only 117 out of 900 and 69 out of 500 are established genera of bacteria with the most predominant genera in both chicken and turkey being *Clostridium*, *Ruminococcus*, *Lactobacillus*, and *Bacteroides*. Besides bacteria, the poultry GIT is also inhabited by methanogenic archaea, fungi, and viruses (Pan and Yu 2014; Yeoman et al. 2012). Furthermore, the horse GIT is inhabited by bacteria as well, but archaea, fungi, and protozoa are also present (Daly et al. 2001).

The composition and activity of intestinal microbiota have a crucial impact on the animal health, growth, and performance as a whole. After the ban of antibiotics as animal growth promoters in the European Union, Korea, and Japan, probiotics gained ground as they present a variety of beneficial effects including, among others, promotion of gut health and homeostasis (Hou et al. 2015). Costs, however, have been a major bottleneck for their routine use.

Over the years, probiotics have been used in a number of different ways in livestock, but in the 1960s, it was demonstrated for the first time that *Lactobacillus* strains were able to improve the growth performance of pigs (Ahasan et al. 2015). The most frequently used probiotics in monogastric animals are yeasts (*Saccharomyces boulardii* and *S. cerevisiae*) and bacteria (*Lactobacillus* spp., *Enterococcus* spp., *Pediococcus* spp., *Bacillus* spp.) targeting the cecum and the colon. The most common benefits of probiotics in monogastric animals are the increase of body weight, the reduction of the risk of diarrhea, the improvement of feed efficiency, and diet digestibility (Ahasan et al. 2015). Furthermore, probiotics have been assigned to play a significant role in providing supportive care to piglets during their initial part of life, while probiotics like *Enterococcus faecium* and *Bacillus subtilis* can reduce the concentration of ammonia in the excreta of poultry (Dhama et al. 2008). There are many microorganisms to be considered as potential probiotics, but only a limited number of microorganisms seem to satisfy the necessary criteria.

In order to identify and detect the GIT microbiota from the animal gut and feces, several techniques have been developed based on biochemical, microbiological, immunological, and molecular biological features. Among them, the expansion of high-throughput sequencing techniques exposed the plethora of non-culturable bacteria enabling the comprehensive characterization of the intestinal microbiota of poultry and other monogastric animals (Danzeisen et al. 2011; Kim et al. 2011a). A full understanding of the intestinal microbiota and the genomic functions of its members, i.e., microbiome, will lead to the development of targeted probiotic strains and novel or improved strategies for effective microbiota modulation (Chambers and Gong 2011; Choi et al. 2015; Pan and Yu 2014; Umu et al. 2015). Next-generation sequencing studies on broilers and pig's gut microbiota shed light on the age-related bacterial diversity revealing the importance of gut modulation to improve animal health (Kim et al. 2011a; Mohd Shaufi et al. 2015). Compared to the other monogastric animals, there is only a limited number of studies characterizing the equine gut microbiota using culture-independent methods (Daly and

Shirazi-Beechey 2003; Hastie et al. 2008; Shepherd et al. 2012; Yamano et al. 2008). However, as these techniques have been recently developed, the results are not always reliable (Sachsenröder et al. 2014).

Although the native gut microbiota is commonly used as a pool for probiotic candidates, the use of genetically modified strains as probiotics in monogastric animals is of ongoing interest (Sieo et al. 2005). A species commonly used for genetic engineering in poultry is *Lactobacillus reuteri*. A lot of research has been conducted using strains of this species expressing heterologous genes in a poultry diet with encouraging results on the growth performance and welfare of animals (Li et al. 2014; Liu et al. 2005, 2007; Yu et al. 2008). Since genetic engineering approaches have positive results in poultry, research is currently focusing on genetically modified strains capable of expressing more than one heterologous genes (Wang et al. 2014). Apart from poultry, genetically engineered probiotics are also used in pigs either therapeutically, e.g., in pancreatic insufficiency, or as feed additives enhancing livestock production (Drouault et al. 2002; Yin et al. 2010).

The idea of using bacteriophages to manage or eliminate zoonotic bacteria in poultry husbandry has been established as a cost-effective approach with significant advantages compared to antibiotics. The chicken gut microbial imbalance frequently caused by broad-spectrum antibiotics is avoided using host-specific bacteriophages. These bacteriophages are naturally self-limiting as they replicate only in the target bacteria and only as long as the bacteria are present (Atterbury et al. 2007). Recently, due to the advantages of bacteriophage biology, a lot of successful research has been made in broiler chickens indicating the ability of host-specific bacteriophages alone or in combination with probiotics to reduce colonization of *Salmonella* and *Campylobacter* (Atterbury et al. 2007; Bardina et al. 2012; Loc Carrillo et al. 2005; Marietto-Gonçalves et al. 2014). It is important that both *Salmonella* and *Campylobacter* phages can be isolated from poultry feces and farm environment resulting in gut microbial stability (Atterbury et al. 2007). Additionally, the use of lytic bacteriophages to prevent or treat colibacillosis in broilers has also been studied (El-Gohary et al. 2014; Lau et al. 2010; Oliveira et al. 2010). It is worth noting that although the successful use of phage therapy in swine dates back to 1920s, it recently regained the attention of the research community (Zhang et al. 2015a). A limited number of studies on pigs indicate that the use of bacteriophages could be a successful strategy against various species of *Salmonella* (Albino et al. 2014; Callaway et al. 2011). In general, a cocktail of phages that use different receptors on the host cell is more effective in reducing pathogens compared to pure phages and also delays the formation of phage resistance (Goodridge 2010). Bacteriophages are used not only therapeutically but also as growth promoters in pigs and poultry (Gebru et al. 2010; Kim et al. 2014a; Wang et al. 2013; Zhao et al. 2012). Yan et al. (2012) suggested that a bacteriophage diet can be used as an antibiotic alternative on growth performance of pigs, and in some cases bacteriophages appeared more effective than probiotics on the performance of growing pigs, as indicated by Kim et al. (2014b). In addition, the further understanding of the biology underlying phage therapy, safe practice, quality control, and accumulation of knowledge and experience remains future challenges (Chambers and Gong 2011).

It should be noted, however, that the oral use of probiotics or bacteriophages can be effective only if they manage to survive during the passage through the digestive system. Therefore, a successful delivery system is of utmost importance. A number of studies have been performed on poultry or swine simulated GIT conditions showing that a microencapsulation technique can protect the bacteriophages or probiotics against gastric environment (Ma et al. 2008; Musikasang et al. 2009; Ross et al. 2008). In a similar study, a microencapsulated phage cocktail administered to swine feed remained effective after the passage through the GIT and successfully reduced *Salmonella* colonization (Saez et al. 2011). The same results were observed in poultry with a cocktail of liposome-encapsulated bacteriophages (Colom et al. 2015).

Although the concept of functional foods has been introduced a long time ago, scientific evidence for the use of prebiotics in animal feed exists from the late 1990s for poultry and pigs (Hajati and Rezaei 2010). The majority of research in prebiotics has been performed in poultry, as this is the most studied monogastric animal. Prebiotics were found to increase the stool volume of chicken by regulating intestinal microbiota through selective stimulation of beneficial bacteria and inhibiting undesirable bacteria, such as *Salmonella* (Park et al. 2013; Totton et al. 2012). The most common prebiotics used in monogastric animals are inulin, fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS), and galacto-oligosaccharides (GOS), with GOS being the less investigated in the poultry industry compared to FOS (Park et al. 2013). It is difficult, however, to draw conclusions for the prebiotic effects in animals from the published studies due to the wide variety of these studies regarding subjects, age, diet, outcome parameters, substances tested, dose, and duration of the experiments (Allaart et al. 2013; Samanta et al. 2013). Such inconsistent results have been mainly recorded after the use of MOS to reduce the intestinal numbers of *Clostridium perfringens* in poultry and after the use of inulin as prebiotic to improve growth performance of layers and broilers, indicating that the effects are both dose- and diet-dependent (Allaart et al. 2013; Biggs et al. 2007; Ortiz et al. 2009; Yusrizal and Chen 2003). The application of prebiotics in animal feed is a relatively recent effort, and although the results are promising, many issues must be solved, such as the establishment of the efficacy of prebiotics in routine diets of livestock. The advanced techniques like next-generation sequencing could be very useful to substantiate any prebiotic effect on animal microbiota, while at the same time future research of prebiotics in livestock should be focused on immunological aspects, changes at the gut epithelium, and at livestock product quality (Samanta et al. 2013).

1.4 Aquaculture

Aquaculture is the farming of aquatic organisms, and it involves the cultivation of freshwater and saltwater populations under controlled conditions. Compared to commercial fishing, this activity allows a selective increase in the production of species used for human consumption, industry, or sport fishing. Due to overfishing of wild populations, aquaculture has become an economic activity of great importance around the world (FAO 2012). The possibility to use feed supplements to improve animal health, welfare, and productivity also addressed the manipulation of the GIT microbial ecosystem in fish (Chaucheyras-Durand and Durand 2010). To our knowledge, the first empirical application of probiotics in aquaculture (Kozasa 1986) is relatively recent and built on their benefits exerted on humans and poultry. Additionally, the first study on prebiotics in aquaculture was reported in 1995 (Hanley et al. 1995). However, a growing number of scientific papers currently deal with probiotics and prebiotics in aquaculture, passing from their empirical use to a science-based approach.

When looking for probiotics intended for an aquatic usage, it is important to consider certain influencing factors that are fundamentally different from probiotics used in, e.g., mammals. Aquatic animals have a much closer relationship with their external environment (Kesarcodi-Watson et al. 2008). This intensive interaction between the environment and the farmed aquatic animals inspired some to change the definition of probiotics when applied to aquaculture. One of these "alternative" definitions proposed is "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment" (Verschuere et al. 2000). Apart from the FAO/WHO requirement of probiotics to be a live culture, this and other definitions are a (too) lengthy way of describing a probiotic, e.g., "a microorganism or components thereof that is/are beneficial to the health of the host" (Irianto and Austin 2002). The most recent definition identifies aquaculture-probiotics as "live or dead, or even a component of the microorganism that acts under different modes of action in conferring beneficial effects to the host or to its environment" (Lazado and Caipang 2014), again in contradiction with the generally accepted definition of probiotics (FAO/WHO 2002). In fact, nowadays, there is no definition of aquaculture-probiotics that is accepted by the majority of the aquaculture community. Hence, it is important, if really required, to develop a definition of aquaculture-probiotics that is eliminating ambiguity on the term and makes clear the difference with the FAO/WHO definition for "classical" probiotics, used in human and other animals.

The development of probiotics applicable to commercial use in aquaculture is a multistep and multidisciplinary process requiring both empirical and fundamental research, full-scale field trials, and an economic assessment of its use. Defined procedural strategies have been proposed on the selection and evaluation of probiotic candidates for farmed aquatic animals (Lazado et al. 2015).

A good pool of candidate probiotics is of major importance in the selection process, and for aquaculture it is vital to examine isolates that are both autochthonous and allochthonous to the aquatic environment (Gatesoupe 2008). Whereas humans and terrestrial farm animals tend to have an intestinal microbiota dominated by Gram-positive obligate or facultative anaerobes, that of aquatic animals consists mainly of Gram-negative aerobes as well as obligate and facultative anaerobic bacteria (Vine et al. 2006). Bacteria, such as *Vibrio*, *Pseudomonas*, and *Acinetobacter* constitute the predominant indigenous microbiota of a variety of marine fish species and crustaceans

(Pandiyan et al. 2013), while, in contrast to saltwater species, the indigenous microbiota of freshwater animals is dominated by members of the genera *Aeromonas* and *Plesiomonas*, representatives of the family *Enterobacteriaceae*, and obligate anaerobic bacteria of the genera *Bacteroides*, *Fusobacterium*, and *Eubacterium* (Sakata 1990). LAB are generally subdominant in aquatic organisms and represented essentially by the genus *Carnobacterium* (Balcázar et al. 2006). Interestingly, despite the indigenous Gram-negative species, probiotics used in aquaculture belong mainly to the Gram-positive genera *Bacillus*, *Enterococcus*, *Lactobacillus*, and *Carnobacterium* as well as to yeast species when used as biological control or immunostimulatory agents. In contrast, probiotics used as antimicrobials in aquaculture belong essentially to the aforementioned Gram-negative genera (De et al. 2014). Nevertheless, recently, the Gram-positive bacteria belonging to *Streptomyces* genus demonstrated promising results as probiotics in aquaculture regarding not only the production of antagonistic and antimicrobial compounds against pathogens but also the improved growth of the aquatic organisms (Tan et al. 2016).

In the past, the information available on the intestinal microbiota of aquatic species was based on the use of conventional culture-dependent methods. Nowadays, molecular-based approaches are used successfully for the analysis of bacterial communities (Martínez Cruz et al. 2012): (1) 16S rDNA clone libraries (Han et al. 2010; Iehata et al. 2015); (2) fingerprinting methods, such as denaturing gradient gel electrophoresis (DGGE) (McIntosh et al. 2008; Sun et al. 2012) and temporal temperature gradient electrophoresis (TTGE) (Navarrete et al. 2010); and (3) fluorescent in situ hybridization (FISH) (Payne et al. 2007). Also, in a limited number of recent studies, next-generation sequencing (NGS) 16S-amplicon metagenomics-based approaches have been used and reveal a far greater level of diversity in the gut microbiota of animals than previous studies that lacked an NGS approach (Zhou et al. 2014).

The use of gnotobiotic systems (animals cultured in axenic conditions or with a known reconstituted microbiota) can be an excellent tool to extend the understanding of mechanisms involved in host-microbe interactions of cultured animals (Dimitroglou et al. 2011). This approach in parallel to the use of mutant strains, e.g., non-motile *Pseudomonas* mutants (Rawls et al. 2007) or yeast mutants (Soltanian et al. 2007), led to the clarification of genes involved in specific probiotic mechanisms in fishes and crustaceans in the past, respectively. Further mechanistic understanding might also result from the use of tissue- or cell- specific mutants expressing green fluorescent protein (GFP) or GFP variants as a powerful method for in situ monitoring of the presence and behavior of microbes that are intentionally introduced into the host organisms (Mulero et al. 2007). According to Tinh et al. (2008), GFP translational fusions of genes of interest in probiotics might provide additional data on gene functioning when introduced into translucent larvae.

The ability of probiotics to affect the ontogenetic development of animals by interfering with their gonad differentiation and maturation or progression to puberty and aging gains interest for future studies (Avella et al. 2010). Indeed, microarray analysis was used in the past to evaluate alterations on the expression of genes involved in immune response, protein folding, cytoskeletal/structural proteins, and other vital cellular processes such as lipid metabolism, cell proliferation, and apoptosis in aquatic organisms (Rodriguez-Lanetty et al. 2006; Skugor et al. 2008).

The genomic information that is generated from the sequences of known probiotic bacteria provides clear understanding on the inherent probiotic properties (Ventura et al. 2012). In aquaculture, the concept of probiogenomics is not yet widely recognized or applied, although recently the relevance of this perspective in aquaculture has been raised (Lazado and Caipang 2014).

Features correlated to certain modes of probiotic action in the aquatic environment are under investigation (Kesarcodi-Watson et al. 2008). Enhancement of colonization resistance and/or direct inhibitory activity against pathogens are considered important factors when probiotics are used for the prevention of bacterial diseases (Balcázar et al. 2006). Potential probiotics can also be correlated to the growth promotion of cultivated fishes by producing a variety of extracellular enzymes (i.e., proteases, lipases, carbohydrases, phosphatases, esterases, and peptidases) that facilitate the efficient absorption of nutrients (Bairagi et al. 2002; Giri et al. 2013). For instance, the use of plant protein sources in the diets (Gatlin et al. 2007) led to the investigation of the metabolic capabilities of probiotics, such as the degradation of anti-nutritional factors, a feature interrelated with the improvement of the nutritional value of the feed of aquatic animals in the past (Refstie et al. 2005). Immunomodulation by probiotics has also gained great attention, and the assessment of phagocytic, respiratory burst, lysozyme, serum peroxidase, and complement activities and modulation of cytokine production have been referred to as potential strategies for finding novel probiotic strains for aquaculture (Akhter et al. 2015; Magnadottir 2010; Nayak 2010). Furthermore, the theory that probiotic bacteria, through biofilm formation, enhance the survival rate and growth performance in aquatic organisms (Pandey et al. 2014) has been also established (Boutin et al. 2013).

Taking into consideration that probiotics for aquaculture are marketed in two forms, dry and liquid (Sahu et al. 2008), an appropriate route of delivery of the probiotic to the host should be proposed. So far, literature refers to several ways in which probiotics can be provided to the host or added to its aquatic environment, such as addition via live food, bathing, and addition to culture water and to artificial diet (Balcázar et al. 2006). Bioencapsulation of probiotics has also been demonstrated to be a more effective way to introduce probiotics in the animal gut; in the case of some allochthonous bacteria, this may be the only efficient route (Pintado et al. 2014).

The current literature is heavily focused on the bacterial microbiota, and considerably less information is available on indigenous yeast, bacteriophages, archaea, microalgae, and protozoans in aquaculture. Although it is debatable whether or not bacteriophages constitute bona fide probiotics, their influence on indigenous and/or probiotic bacteria must be taken into account for future studies, especially after the “kill the winner” hypothesis about their important role in shaping the mammalian gut microbiota (Mills et al. 2013). Moreover, bacteriophage therapy has been suggested in the past as an alternative for the prevention and treatment of microbial diseases in aquaculture (Nakai and Park 2002). Even if many recent studies indicate their promising application (Oliveira et al. 2012), caution must be taken for their use in the future (Madhusudana Rao and Lalitha 2015).

Despite the potential benefits of prebiotics to health and performance as noted in various terrestrial species, less information is available about the effect of prebiotics in aquatic organisms. The most common prebiotics used in aquatic species are inulin, FOS, short-chain FOS (scFOS), mannan-oligosaccharides (MOS), trans-galacto-oligosaccharides (TOS), GOS, xylo-oligosaccharides (XOS), arabinoxylo-oligosaccharides (AXOS), iso-malto-oligosaccharides (IMO), and various commercial products containing multiple prebiotic combinations. Prebiotic applications in aquaculture improve animal growth performance and survival, feed conversion and digestibility, GIT enzyme activities and GIT morphology, as well as the suppression of potentially pathogenic bacteria due to the presence of beneficial gut bacteria (Ganguly et al. 2013; Ringø et al. 2014). The role of prebiotics as immunostimulants in aquaculture is also well studied with promising results (Akhter et al. 2015; Song et al. 2014).

One major issue that needs to be addressed is whether the prebiotic supplementation effect can vary in regard to age- and size-related responses, appropriate doses, and timing of administration. The life stage of the animal was highlighted in a study where inulin was used (Hoseinifar et al. 2010). Furthermore, the surrounding environment, i.e., water temperature and salinity and oxygen availability, might have greater influences than the diet on animal health or potentially confound interpretations of the prebiotic findings (Daniels and Hoseinifar 2014). Further research is needed in order to differentiate the health-promoting effects from potentially deleterious responses toward prebiotics (Ringø et al. 2010) as observed for oligosaccharides from soybeans, causing diarrhea in Atlantic salmon (Refstie et al. 2005).

Another recent concept with regard to the manipulation of gut microbiota of animals in aquaculture are synbiotics. The use of synbiotics is an important approach in order to explore in what way prebiotic administration may seed and maintain probiotic strains as the dominant species in the fish GIT (Rurangwa et al. 2009). Despite recent progress in the field of synbiotic administration in aquaculture, there is limited information available on different aspects of synbiotics on fish species (Llewellyn et al. 2014). To our knowledge, few studies so far have investigated the effect of synbiotics only in fish species since the first one in 2009 (Cerezuela et al. 2011). In those studies, probiotics belonging to the genera *Enterococcus*, *Bacillus*, and *Pediococcus*, as well as FOS and MOS prebiotics, were used. The studied fish species were rainbow trout (Mehrabi et al. 2012; Rodriguez-Estrada et al. 2009), Japanese flounder (Ye et al. 2011), yellow croaker (Ai et al. 2011), cobia (Geng et al. 2011), sea bream (Cerezuela et al. 2013), Nile tilapia (Aly et al. 2008), and Atlantic salmon (Abid et al. 2013), indicating better growth, feed efficiency ratio, improved immune responses, and disease resistance of aquatic animals after synbiotic supplementation.

1.5 Pets

Companion animals have high numbers of microorganisms in the GIT, which in fact exceed in quantity those living in the human gut. Nonetheless, both cats and dogs have distinct bacterial species that also vary among different dog and cat breeds, gut niches, and geographical areas. Microbial diversity and concentration increase along the length of the GIT. The prevalent bacterial phyla in the colon and feces of both dogs and cats are represented by *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Fusobacteria* as well as *Eubacterium* in cats. The microbial differences between dogs and cats are manifested in the microbial groups and on the species levels (Grzeskowiak et al. 2015). Molecular fingerprinting has revealed that every individual pet has a unique and stable microbial ecosystem (Suchodolski 2011). A recent metagenomics approach estimated that, besides bacteria, the feline GIT microbiota comprises 0.02% fungi, 0.09% archaea, and 0.09% viruses with 99% of them being bacteriophages. The most commonly observed archaeal phyla belonged to *Crenarchaeota* and *Euryarchaeota*, with the most abundant families being *Desulfurococcaceae* (54.8% of sequences), *Methanobacteriaceae* (40.6%), *Methanosarcinaceae* (5%), and *Halobacteriaceae* (2.7%) (Tun et al. 2012). According to Handl et al. (2011), among fungi, *Aspergillus* and *Saccharomyces* are the most abundant genera in the feline GIT microbiota. As for other animals, any disturbances within the gut microbiota of the pets may lead to the development of a multitude of diseases and disorders, such as diarrhea, allergies, obesity, and stress symptoms (Lee and Hase 2014).

Possible benefits of the probiotic use in pets include modulation of the immune system, assistance in stress maintenance, protection from infections caused by enteropathogens, increased growth and development, control of allergic disorders, and recently obesity (Grzeskowiak et al. 2015).

So far, the common mode of administration of probiotics to pets is oral by adding them to the pets' feed (Arslan et al. 2012; Biagi et al. 2013; Bybee et al. 2011; Hutchins et al. 2013; Strompfova et al. 2012). Regarding the genera, which are used as probiotics in companion animals, these include mainly *Bacillus* spp. (Biourge et al. 1998; Gonzalez-Ortiz et al. 2013), *Lactobacillus* spp. (Gómez-Gallego et al. 2016; Kumar et al. 2017; Marsella et al. 2012; Ohshima-Terada et al. 2015; Strompfova et al. 2012) *Bifidobacterium* spp. (Biagi et al. 2013), and *Enterococcus faecium* (Bybee et al. 2011; Gonzalez-Ortiz et al. 2013), and only recently scientists started using as probiotics *Weissella confusa* (Manninen et al. 2006) and *Streptococcus thermophilus* (Arslan et al. 2012).

In order to enhance survival of probiotics during passage through the GIT of pets, encapsulation of bacteria has been used so that a larger number of viable bacteria can reach the intestine. Starch, alginate, carrageenan, and chitosan are included among the hydrocolloids used to encapsulate or to obtain films and coatings (González-Forte et al. 2014; Ma et al. 2015).

The use of prebiotics in companion animal nutrition was reviewed comprehensively by Swanson et al. (2002). Studies evaluating prebiotics have utilized several outcome variables to assess efficacy in canine and feline diets, including (1) food intake, (2) fecal output, (3) stool consistency, (4) macronutrient digestibility (ideal and total tract apparent digestibility), (5) fermentative end products, (6) immune indices, and (7) intestinal microbial populations (Fahey and Vester 2009). From the limited number of reports in the field, it appears that prebiotic supplementation has several beneficial effects on the GIT of dogs and cats, such as positive shifts in microbial populations, decreases in fecal protein catabolites, and changes in immune status. However, more research is required to determine optimal doses, life stages most likely to benefit, and disease states likely to be avoided or treated with prebiotic supplementation. In the future, experiments must also investigate prebiotic supplementation on animals at different life stages and disease states.

Among prebiotics, FOS are the most studied in dogs and cats. They have been used to alleviate small intestinal bacterial overgrowth (Willard et al. 1994), to promote reduction of clostridia, to increase bifidobacteria and lactobacilli populations (Sparkes et al. 1998; Swanson et al. 2002; Twomey et al. 2003), and to reduce the concentrations of protein catabolites produced in the colon (Swanson et al. 2002). MOS may beneficially change the enteric microbiota, since its addition to the diet can reduce *Clostridium perfringens* counts in dog feces (Strickling et al. 2000). In vitro studies suggest that MOS are moderately fermentable by canine and feline microbiota (Vickers et al. 2001) being thus a source of energy to lactate-producing bacteria. This explains the reduced fecal pH and fecal ammonia excretion verified in dogs, improving indices of colonic health (Zentek et al. 2002). Furthermore, Swanson and Fahey (2006) reported the immunomodulatory effect of MOS in dogs, in particular on the concentrations of IgA, IgG, and plasma lymphocytes. The use of MOS and FOS in diseased or immunocompromised animals (Apanavicius et al. 2007; Gouveia et al. 2006) has revealed a protective effect for immunocompromised dogs.

Molecular techniques have been also employed to evaluate the effect of prebiotics on the GIT microbial consortia in cats and dogs (Middelbos et al. 2007; Vanhoutte et al. 2005). Vanhoutte et al. (2005) observed potential alterations in fecal microbiota of seven adult healthy dogs related to the administration of oligofructose and inulin. Middelbos et al. (2007) appraised spray-dried yeast cell wall (YCW) supplementation of diets to adult dogs where it was noted that YCW altered digest flow through the intestinal tract, decreased quadratically total white blood cell and eosinophil counts, and was responsible for the reduction of fecal microbial populations. However, further work is necessary to confirm the above results and also elucidate the effect of prebiotics in other diseases, such as inflammatory bowel disease, small intestinal bacterial overgrowth, etc.

Regarding the combination of probiotics and prebiotics, Swanson et al. (2002) were the first to study the effect of synbiotics, namely, administration of FOS and/or *Lactobacillus acidophilus*, on the gut microbial populations, end products, and nutrient digestibility in healthy adult dogs. It was shown that FOS enhanced indices of gut health by positively reshaping gut microbial ecology and fecal protein catabolites, whereas *Lactobacillus acidophilus* was more effective when fed in combination with FOS rather than fed alone. Later on, Ogue-Bon et al. (2010) showed that GOS supplementation in dogs can sustain the growth of *Bifidobacterium bifidum*, when used as a synbiotic combination, while Biagi et al. (2013) reported that the combination of GOS with a strain of *Bifidobacterium pseudocatenulatum* had some positive effects on the intestinal microbiota in cats.

1.6 Bees

As a pollinator, the honeybee, *Apis mellifera*, is a key species for agricultural production and contributes to the human food supply (Aizen et al. 2008; Klein et al. 2007). Recent losses of *A. mellifera* and bumble bees (genus *Bombus*), and the potential association of these declines with various infectious agents, call for a better understanding of the bees' microbiota (Evans and Schwarz 2011; Genersch 2010). Honeybees pool resources, divide labor, and communicate in highly structured social colonies. Sterile female worker bees dominate within colonies, in which they initially clean cells, rear brood, and store food; then they leave the hive and search for pollen and nectar (Seeley 1985). Independent studies of bacterial community profiles based on 16S rRNA sequences demonstrate that workers of *A. mellifera* and some *Bombus* species consistently harbor an offbeat gut microbiota not shared with solitary bees (Cox-Foster et al. 2007; Koch and Schmid-Hempel 2011; Martinson et al. 2011). This microbiota consists of eight distinct species or phylotypes, i.e., closely related strains with ≥97% sequence identity in 16S rRNA sequences, hereafter referred to as species. These include three Gram-positive species, namely, two closely related *Firmicutes* within *Lactobacillus* and one within *Bifidobacterium*, and five Gram-negative species, namely, one β-proteobacterium with the Candidatus name "*Snodgrassella alvi*," two closely related γ-proteobacteria, one with the Candidatus name "*Gilliamella apicola*," and two α-proteobacteria (Martinson et al. 2012).

The application of probiotics in bees is achieved through feeding, with *Lactobacillus* and *Bifidobacterium* being the major genera used until now. Machova et al. (1997) were the first who added probiotics, without specifying the microorganisms used though, into sugar syrup in order to feed honeybees (*Apis mellifera*) and noticed that this ameliorated bee survival. The next attempt was not until 7 years later, and it was demonstrated that probiotics including *Bifidobacterium infantis*, *B. longum*, *B. breve*, *Lactobacillus rhamnosus*, *L. acidophilus*, *L. reuteri*, *L. casei*, and *L. plantarum* enhance immune responses in bees by stimulating the production of antimicrobial peptides against *Paenibacillus* and *Ascosphaera apis* infections (Evans and Lopez 2004). Kaznowski et al. (2005) used *Lactobacillus* spp., *Pediococcus acidilactici*, *B. bifidum*, and *E. faecium* as supplements to pollen substitute in feeding honeybees. It was shown that in order to accomplish increase in dry mass and crude fat level, it was sufficient to supply probiotics only in the beginning of the feeding period, directly after bee emergence. These results have been confirmed by Kazimierczak-Baryczko and Szymaś (2006), who used the same species and showed that the addition of probiotics in pollen substitute prolonged bee life span and stimulated the growth of the faucial gland and fat body. Moreover, administration of *Lactobacillus* spp., *Bifidobacterium* spp., *Saccharomyces boulardii*, and *Streptococcus thermophilus* through sugar syrup resulted in better colony development, a longer life span, and enhanced development of wax production (Patruica et al. 2011a, b, 2012, 2013; Patruica and Mot 2012). It seems, however, that in order to be efficient, probiotics have to be tailored for bees (Johnson et al. 2014).

In recent years, molecular methods offer great potential for the phylogenetic identification of probiotic microorganisms in bees (Mattila et al. 2012; Olofsson and Vasquez 2008; Tajabadi et al. 2013). Olofsson and Vasquez (2008) detected and identified novel LAB, mainly lactobacilli, as well as bifidobacteria in the honey stomach of honeybees by employing 16S rRNA sequencing. Using the same method, Tajabadi et al. (2013) detected *Lactobacillus* spp. in *Apis dorsata* honeycomb, which could be explored as a source of new bacteria with probiotic potential in honeybees. Moreover, deeper comprehension of the complex host-microbial interactions might also result from the use of tissue- or cell- specific mutants expressing GFP or GFP variants. In this direction, Hyrsl et al. (2015) have successfully used a mutant of *Photorabdum luminscens* that expressed GFP in order to track the nematobacterial infection in bees.

Conclusions

The overview of the existing literature regarding the studies performed so far with probiotics and prebiotics in monogastric and polygastric animals, aquaculture, pets, and bees highlights (a) the variety of microorganisms, comprising both bacteria, which are mainly lactic acid bacteria and yeasts, and to a lesser degree the array of oligosaccharides, mainly inulin, FOS, MOS, and GOS, employed; (b) the administration method, e.g., feed, water, and aquatic environment (for fish); (c) the origin of strains, although mainly allochthonous; (d) the target body function, e.g., balance of GIT microbiota, reduction of diarrhea risk, improvement of feed efficiency, and diet digestibility to increase body weight, growth and development, immune system, assistance in stress maintenance, protection from infections, control of allergic disorders, obesity, etc.; and (e) the assays used for the elucidation of these beneficial actions.

Despite the stimulating data accumulated so far, further studies are needed using more standardized protocols. These protocols should consider, among others, the age and size of the animal, the rearing conditions, the dose and composition of the active compound used, the route and matrix of delivery, etc. This will allow a more reliable comparison of results, thus facilitating the consistency and conclusions that can be drawn about the beneficial impact of both probiotics and prebiotics. Moreover, the application of advanced techniques, e.g., metagenomics, to shed full light to the indigenous microbiota of the animal under consideration, comprising a complex mixture of indigenous bacteria, yeasts, bacteriophages, archaea, microalgae, and protozoans, will drive the use of autochthonous and/or animal tailored probiotic strains. Indeed, a full understanding of the intestinal microbiota and the genomic functions of its members will lead to the development of targeted probiotic strains and novel or improved strategies for effective microbiota modulation. It should be also stressed that the detailed characterization of the animal virome would be of great significance, since the endemic phages could prove to be either a useful tool or a drawback for the probiotic interventions in animals. Additionally, the use of well-targeted recombinant probiotics is expected to receive further attention in the near future.

Furthermore, it should be stressed that the elucidation and interpretation of the mechanisms underlying the beneficial properties are extremely important. Learning from the human applications of probiotics and prebiotics, these mechanisms can be either of generic nature or strain specific, while some mechanisms may be limited to a specific life stage during the development of the animal, and their efficacy critically depends on the animal species. For instance, the use of gnotobiotic systems can be an excellent tool to extend the understanding of mechanisms involved in host-microbe interactions in animals and to study cause and consequence of specific interventions. Thus, this field needs further investigation, which will help to understand the interactions between probiotics and prebiotics as well as the interactions of the host with either probiotics and prebiotics separately or in combination.

Unravelling and overcoming problems existing at the science level are needed to facilitate the applications and subsequently smoothen the regulatory actions. The legislator needs to clearly recognize the benefits of probiotics and prebiotics at a much broader scale in order to promote their acceptability by the farmer and the consumer.

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