



## Review Article

Using probiotics to improve swine gut health and nutrient utilization<sup>☆</sup>Shengfa F. Liao<sup>a, \*</sup>, Martin Nyachoti<sup>b</sup><sup>a</sup> Department of Animal and Dairy Sciences, Mississippi State University, MS 39762, USA<sup>b</sup> Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

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## ABSTRACT

To maintain a healthy gut is definitely key for a pig to digest and absorb dietary nutrients efficiently. A balanced microbiota (i.e., a healthy micro-ecosystem) is an indispensable constituent of a healthy gut. Probiotics, the live microorganisms which, when administered in adequate amounts, confer good health benefits onto the host, are a category of feed additives that can be used to replenish the gut microbial population while recuperating the host immune system. Besides their antitoxin and diarrhea reduction effects, dietary supplementation of probiotics can improve gut health, nutrient digestibilities and, therefore, benefit nutrient utilization and growth performance of pigs. Current knowledge in the literature pertinent to the beneficial effects of utilizing various probiotics for swine production has been comprehensively reviewed, and the safety and the risk issues related to probiotic usage have also been discussed in this paper. Considering that the foremost cost in a swine operation is feed cost, feed efficiency holds a very special, if not the paramount, significance in commercial swine production. Globally, the swine industry along with other animal industries is moving towards restricting and eventually a total ban on the usage of antibiotic growth promoters. Therefore, selection of an ideal alternative to the in-feed antibiotics to compensate for the lost benefits due to the ban on the antibiotic usage is urgently needed to support the industry for profitable and sustainable swine production. As is understood, a decision on this selection is not easy to make. Thus, this review paper aims to provide some much needed up-to-date knowledge and comprehensive references for swine nutritionists and producers to refer to before making prudent decisions and for scientists and researchers to develop better commercial products.

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## 1. Introduction

A major task of raising pigs for producing pork is to feed the pigs. The cost on feed represents more than two-thirds of the total operation cost in pig production. Therefore, enhancing feed

efficiency (i.e., the efficiency of converting feed mass into pig body mass) is very critical for the profitability of producing pigs (Patience, 2012). To enhance the feed efficiency, that is to improve the metabolic utilization of dietary nutrients by a pig, relies heavily on a healthy gut or gastro-intestinal tract (GIT), because only a healthy gut can result in a better feed digestion and a better nutrient absorption via its epithelial membranes (Ewing, 2008; Willing et al., 2012).

Beyond its physiological function as the alimentary canal for nutrient digestion and absorption, pig's GIT is also one of the largest organs that helps animal's immune function, because by nature the gut is animal's first line of defense against the microbial pressure from its environment, especially the invasive pathogens from the GIT lumen (Veizaj-Delia and Pirushi, 2012). Activation of the GIT immune system incurs the direct cost of producing a diverse set of specialized immune cells (comprising more than 70% of the body's immune cells) and signaling molecules, as well as the losses in the efficiency of GIT digestive function (Willing et al., 2012). Therefore,

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only a healthy gut can lead to a healthy pig, allowing a pig to thrive throughout its lifespan well without sickness or falling back. And only a healthy pig can utilize dietary nutrients efficiently for tissue accretion, and lead to a better production performance and, thus, a higher return on investment for swine producers. In this regard, ensuring a healthy gut is an “all-the-time deal” in swine production practices (Taylor-Pickard and Spring, 2008; Hubbard Feeds, 2014).

## 2. Microbiota and a healthy gut

Like for all mammals including humans, a healthy gut of a pig is inhabited with hundreds of species of microorganisms, which together form a microbial community often referred to as microflora or, more appropriately, microbiota (Jonsson and Conway, 1992; Leser et al., 2002; Sears, 2005; Fohse et al., 2016). Microorganisms begin to colonize the sterile gut of a newborn pig right after birth, a process called microbial succession. A fully developed microbiota in a gut is established within weeks after birth (Tortuero et al., 1995; Bauer et al., 2006; Kim and Isaacson, 2015). An established gut microbiota is a complex micro-ecosystem composed of approximately 1,014 microorganisms (most of them are bacteria), which co-exist with the pig as the host (Kim and Isaacson, 2015). When this co-existence (also known as symbiosis) is balanced, the gut of the pig will be normal and healthy, and functions well (Willing et al., 2012). Animals raised in the absence of bacteria show profound retardation in the development of adult gut morphology, digestive physiology, and normal immune function (Kenny et al., 2011).

Management of intestinal micro-ecosystem is one of the common strategies applied to prevent diarrhea, improve health status, and enhance growth performance of pigs in modern intensive production systems (Williams et al., 2001; Bauer et al., 2006; Zimmermann et al., 2016). Under natural environments, harmful microorganisms can enter and colonize the pig GIT (called dysbiosis) and produce waste products which are toxic and can lead to gas bloating, diarrhea, constipation, ulcers, or more serious events like poisoning (Cho et al., 2011). In this situation, the pig cannot utilize dietary nutrients efficiently and cannot grow well (Willing et al., 2012). A more detailed discussion regarding the role of gut microbiota in swine health and disease can be found in a recent review article authored by Fohse et al. (2016).

The processes of nutrient digestion in pig GIT, in the simplest way, include enzymatic hydrolysis and microbial fermentation of feedstuffs. Although pigs rely heavily on the process of nutrient hydrolysis by endogenous digestive enzymes, the microbial fermentation (especially, in the hind gut) contributes a great deal (Williams et al., 2001). The gut microbiota provides a critical support to the host in areas including vitamin and co-factor production, usage of otherwise indigestible feed ingredients, detoxification of feed components, coating the gut with a benign microbiota to physically exclude pathogens, production of natural antibiotics and antifungals, maintenance of gut barrier function, and promotion of anti-inflammatory response (Kenny et al., 2011). Therefore, the composition of gut microbiota significantly impacts on gut health, dietary nutrient utilization, and whole body health of the pig.

## 3. Strategies for promoting gut health and regulation on antibiotic usage

Although the modern intensive systems have advanced swine production efficiency, they also create suitable conditions for propagation and transmission of harmful bacteria or pathogens, which cause pathogenic stress to the pig (Lee et al., 2016). The early weaning practice (at 14 to 21 days of age) widely adopted in the

industry reduces the chance of young piglets to be infected by the pathogens from lactating sows, but this practice also deprives piglets of more opportunities to acquire a protective gut microbiota from the mother, leaving the GIT unprotected against the colonization by pathogenic microorganisms (Guerra and Castro, 2009). Although it is not impossible that 3 weeks are long enough for microbes to be established in a piglet's gut, a modern management interest is to better solutions to achieve a well-balanced gut microbiota that is a healthy gut micro-ecosystem optimal for animal to digest feed, absorb nutrients, and grow tissues (Taras et al., 2007).

As is known, the gut microbiota can be manipulated by dietary means using feed additives such as organic and inorganic acids, enzymes, antibiotics, prebiotics, probiotics, mold inhibitors, botanical products (de Lange et al., 2010; Le Bon et al., 2010; Heo et al., 2013; Sezen, 2013). The use of antibiotics has been an integral part of modern swine operation worldwide ever since early 1950s (Dibner and Richards, 2005). Veterinary uses of antibiotics in pig production include not only the therapeutic and prophylactic uses, but also the administration at subtherapeutic levels to stabilize the gut microbiota and enhance pig growth performance (Adjiri-Awere and van Lunen, 2005; Guerra and Castro, 2009). In reality, the use of antibiotics in swine production is the most studied of all livestock species because the subtherapeutic use of antibiotics can greatly improve pig growth rate, reduce morbidity and mortality, and improve production and reproduction performance (Cromwell, 2002; Thacker, 2013). Because the subtherapeutic use of antibiotics can promote animal growth performance and, therefore, many antibiotics that are used in this regard are referred as antibiotic growth promoters (AGP; Dibner and Richards, 2005; Guerra and Castro, 2009).

Nevertheless, research on the AGP modes of action showed that they may affect not only the potentially harmful but also the benign gut microorganisms (Adjiri-Awere and van Lunen, 2005; Dibner and Richards, 2005; Guerra and Castro, 2009). Therefore, there are 2 major concerns regarding the use of AGP for farm animals. One is the chemical residues from such antibiotics which may be found in animal products as foreign substances that should not have any place in the food chain. The other is that the antibiotics used for animals were the same as those used in human medicine (Casewell et al., 2003; Dibner and Richards, 2005). The use of AGP was then incriminated as contributing to selection pressure, resistance reservoirs, and transmission routes (Gersema and Helling, 1986; Wegener, 2003). Following the ban on AGP use in Sweden in 1986, and the ban on avoparcin and virginiamycin in Denmark in 1995 and 1998, respectively, the European Union (EU) banned the use of avoparcin in 1997 and the four remaining AGP (bacitracin, spiramycin, tylosin, and virginiamycin) in 1999, on the basis of the “Precautionary Principle” (Casewell et al., 2003; Dibner and Richards, 2005). The EU total ban on the use AGP in animal feed entered into effect on January 1, 2006 (European Commission, 2005).

North America, following the actions of the EU, has started moving towards restricting or a total ban on the use of AGP because of the general public concern and potential international trade barriers to the meat products from livestock industries. In the United States (US), recommendations to reduce or eliminate the use of AGP were made in 2 reports by the Institute of Medicine in 1980 and 1989, respectively, in one report by the Council for Agricultural Science and Technology in 1981, and in another by the Committee on Drug Use in Food Animals in 1998 (Dibner and Richards, 2005). The World Health Organization (WHO) published a report in 1997 on the medical impact of the use of antimicrobials in food animals, suggesting a link between the 2 on an epidemiological basis (Dibner and Richards, 2005). WHO suggested again in

the year of 2000 that the use of AGP that are in the classes also used in human medicine be terminated or rapidly phased out, by legislation if necessary, unless and until risk assessments are carried out (Dibner and Richards, 2005).

In the year of 2003, the US Food and Drug Administration (FDA) released Guidance 152, which made recommendations on how to best develop new animal drugs with regard to the potential impacts on human health (FDA, 2003). In 2010, the FDA released Guidance 209, which suggested limiting livestock use of antibiotics that are medically important to humans (FDA, 2012). On December 11, 2013, FDA released Guidance 213, which initiated a 3-year transition process to complete its food-animal antibiotic strategy (FDA, 2013). This guidance eliminates over-the-counter status of these medications and increases veterinary oversight for on-farm therapeutic use by requiring a veterinary feed directive (VFD) for feed applications and a prescription for water treatments. On January 1, 2017 this new regulation has taken effect in the US (NPB, 2015).

As is known, digestive disorders are common problems at times of stress (e.g., at weaning) and the highest death loss of post-weaned pigs is from diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC). The other zoonotic pathogens, such as *Salmonella*, can also cause swine herd health problems. Because of these common pathogenic bacteria, the ban on AGP usage can significantly decrease pig health status, feed efficiency, and growth performance, especially during the post-weaning stage, and this ban at the same time gave rise to broad interests in and popularity of another family of feed additives – an alternative to antibiotics (Seal et al., 2013; Thacker, 2013), the probiotics (Reid and Friendship, 2002; Guerra and Castro, 2009).

#### 4. Probiotics: What are they?

##### 4.1. Definition and brief history

The word “probiotic” was derived from the Greek meaning “for life” or “in favor of life”. Although it has had several different meanings or definitions over the years (Sperti, 1971; Fuller, 1989, 1992; Azizpour et al., 2009), probiotics are now defined by a joint FAO/WHO working group as “live microorganisms which, when administered in adequate amounts, confer a good health benefit on the host” by improving its intestinal microbial balance (Kenny et al., 2011; Bajagai et al., 2016). Theoretically, the word, probiotic, is only a generic term, and the commercial products may contain bacterial cultures, yeast cells, or both that stimulate the microorganisms capable of modifying the GIT environment to improve the health status and feed efficiency of the host (Yirga, 2015). Another scientific term, direct-fed microbials (DFM), are often used interchangeably with the term of probiotics, but in fact these 2 terms are not truly synonymous. Many probiotic products also contain enzymes and/or crude extracts in addition to live microorganisms. The Office of Regulatory Affairs of the US FDA and the Association of American Feed Control Officials (AAFCO) have defined DFM as feed “products that are purported to contain live (viable) microorganisms (bacteria and/or yeast)” (Bajagai et al., 2016) and the microorganisms should be those that are naturally occurring (McAllister et al., 2011). For regulatory purposes, DFM are considered either as fermentation products or yeast products (Bajagai et al., 2016). In this review, those probiotic microorganisms and commercial probiotic products that can be used for animals, especially for pigs, are discussed.

Historically, humans started to consume live microorganisms with food as early as civilization began, likely with fermented milk being the first food containing live bacteria (Fuller, 1992; Yirga, 2015). However, the beneficial effects of consuming fermented

milk on human health were only scientifically recognized at the beginning of the 20th century (Muralidhara et al., 1977; Yirga, 2015). In 1907, a Russian zoologist, Professor Metchnikoff, was firstly attributed, in his book titled “The Prolongation of Life: Optimistic Studies”, the noted longevity of certain Bulgarian peasants to their high consumption of milk products fermented with lactic acid bacteria (LAB; Metchnikoff, 1908). At about the same time, Dr. Henry Tissier, a French pediatrician, observed that children with diarrhea had lower numbers of bacteria with a peculiar “Y” shape in their stools than healthy children. He then suggested that the ‘bifid’ bacteria from healthy children could be given to diarrhea patients to restore their gut microflora (Azizpour et al., 2009).

Various studies following the year of 1908 demonstrated that the intestinal microbiota had several physiological functions including metabolic, trophic and protective functions (Yirga, 2015). The first clinical trials focused on the effect of probiotics on constipation were conducted in 1930s (Azizpour et al., 2009). In the 1960s the term, probiotic, was introduced for live microorganisms that, as food supplements or feed additives, beneficially affect the intestinal microbial balance of humans and animals (Fuller, 1992; Jorgensen and Hansen, 2006). In the 1970s, probiotics were started to be incorporated into animal feed to increase animal growth performance, health status, and resistance to diseases (Yirga, 2015). In the 1980s, the concept of probiotics was becoming a proven solution to improve animal gut health (Veizaj-Delia and Pirushi, 2012) and production performance (Busch et al., 2004; Yirga, 2015), but the positive effects were observed not in all pig experiments (Zimmermann et al., 2016). As a matter of fact, swine producers do expect a feed additive to have reliable and consistent effects and probiotics are no exception (Jorgensen and Hansen, 2006).

Pork is the most consumed meat in the world. Considering the legislations that prohibit the use of antibiotics as AGP and the high consumer's demand for safe pork, the inclusion of alternative feed additives in lieu of antibiotics in swine diets is definitely required to support profitable and sustainable swine production (Yirga, 2015; Zimmermann et al., 2016). Different from antibiotics which destroy the harmful bacteria as well as some desirable species, probiotics are designed to encourage certain benign strains or species of bacteria in the gut at the expense of less desirable ones. In this regard, the use of probiotics as nutritional modifiers is preferable. However, more defined research for developing better probiotic products as alternative AGP for the global swine industry and a broader education on the use of these products are still needed.

##### 4.2. Probiotic microorganisms and commercial products

There are a wide array of microorganisms that have been studied as probiotics, which leads to numerous commercial products that are being promoted and marketed as food supplements for humans or feed additives for farm animals (Ahasan et al., 2015). Commercial strains of probiotic species are usually isolated from the intestinal microflora of the intended users (e.g., human, pig or chicken) and selected on the basis of criteria such as resistance to stomach acids and bile salts, ability to colonize the intestine or antagonize potentially pathogenic microorganisms (Fuller, 1992; Azizpour et al., 2009; Cho et al., 2011). The most commonly used bacteria are *Bacillus*, *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Pediococcus*, and *Streptococcus* (Yirga, 2015). The commonly called LAB comprise various genera of bacteria, including *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Lactosphaera*, *Leuconostoc*, *Melisso-coccus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, and *Enterococcus*, and this LAB group are of gram-positive, acid-tolerant, generally non-sporulating, non-respiring rod (bacillus) or spherical (coccus) shaped (Yang et al., 2015a). They are associated by their common

metabolic and physiological characteristics, one of which is to produce lactic acid as their major metabolic end-product of carbohydrate fermentation (Yang et al., 2015a).

Although the advantages of using more than one species of bacteria in a single commercial product has not been established clearly (Zhao et al., 2013), most commercial products contain more than one species or more than one strain of a species, and others even contain viable yeast or other fungi. Numerous types of commercial products and their manufacturers are listed in a FAO technical paper authored by Bajagai et al. (2016). Striking differences do exist among different commercial products due to the origins, properties, and modes of action of different microorganisms. Table 1 presents the microorganisms that are commonly used in animal feed (Yirga, 2015; Bajagai et al., 2016). For the convenience of communication in research, development, and application practices, those commonly used microorganisms can be classified into different groups according to different criteria (Bajagai et al., 2016):

- 1) Single- vs. multi-species/strain probiotics: The composition of probiotic products ranges from a single-species/strain to multi-species/strain microorganisms. Examples of single-species probiotics include Bro-bio-fair (*Saccharomyces servisia*) and Anta Pro EF (*Enterococcus faecium*), while multi-species probiotics include PrimaLac (contains *Lactobacillus* spp., *E. faecium*, and *Bifidobacterium thermophilum*); Microguard (contains various species of *Lactobacillus*, *Bacillus*, *Streptococcus*, *Bifidobacterium*, and *Saccharomyces*); and PoultryStar ME (contains *E. faecium*, *Lactobacillus reuteri*, *L. salivarius*, and *Pediococcus acidilactici*).
- 2) Bacterial vs. non-bacterial probiotics: With the exception of certain yeast and fungal probiotics, most of the microorganisms

used are bacteria. Examples of bacterial probiotics are several species of *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and *Enterococcus*. Non-bacterial (yeast or fungal) probiotics include *Aspergillus oryzae*, *Candida pintolopesii*, *Saccharomyces boulardii*, and *S. cerevisiae*.

- 3) Spore forming vs. non-spore forming probiotics: Although non-spore forming *Lactobacillus* and *Bifidobacterium* strains predominated the market initially, spore forming bacteria, such as *Bacillus subtilis* and *B. amyloliquefaciens*, are now being increasingly used. Sporulation is an excellent way of bacteria to protect themselves against damaging factors from environment, such as heat, desiccation and UV radiation (Setlow, 2006). From this standpoint, several advantages about using spore forming probiotics can be speculated. For instance, the issues of shelf life and storage conditions are less critical when considering that spores can remain viable for hundreds of years. Another main advantage is that they can be easily incorporated into animal feed tolerating handling and pelleting processes with minimal reduction in viability (Lorenzoni, 2010). Similarly, passage through the stomach should not be a problem for spores.
- 4) Allochthonous vs. autochthonous probiotics: The microorganisms used as probiotics, which are not normally present in the GIT of animals, are referred to as allochthonous (e.g., yeasts), while the microorganisms normally present as indigenous inhabitants of the GIT are referred to as autochthonous probiotics (e.g., *Lactobacillus* and *Bifidobacterium*).

For research and development in the field of animal nutrition, the major steps in the manufacture, the commercial labelling requirements, and the global regulatory guidelines for the

**Table 1**  
List of the microorganisms commonly used as probiotics in animal feed.<sup>1</sup>

Genus	Species	References
<i>Bacillus</i>	<i>B. amyloliquefaciens</i> ; <i>B. cereus</i> ; <i>B. coagulans</i> ; <i>B. licheniformis</i> ; <i>B. megaterium</i> ; <i>B. mesentericus</i> ; <i>B. polymyxa</i> ; <i>B. subtilis</i> ; <i>B. toyonensis</i>	Yirga, 2015; Bajagai et al., 2016
<i>Brevibacillus</i>	<i>B. laterosporus</i>	Bajagai et al., 2016
<i>Bifidobacterium</i>	<i>B. adolescentis</i> ; <i>B. animalis</i> ; <i>B. bifidum</i> ; <i>B. bifidus</i> ; <i>B. infantis</i> ; <i>B. lactis</i> ; <i>B. longum</i> ; <i>B. pseudolongum</i> ; <i>B. thermophilum</i>	Azizpour et al., 2009; Yirga, 2015; Bajagai et al., 2016
<i>Candida</i>	<i>C. pintolopesii</i> ; <i>C. utilis</i>	Pan et al., 2011; Ibrahim et al., 2012; Bajagai et al., 2016
<i>Clostridium</i>	<i>C. butyricum</i>	Meng et al., 2010; Bajagai et al., 2016
<i>Escherichia</i>	<i>E. coli</i>	Bhandari et al., 2010; Bajagai et al., 2016
<i>Enterococcus</i>	<i>E. faecium</i> ; <i>E. faecalis</i>	Yirga, 2015; Bajagai et al., 2016
<i>Lactobacillus</i>	<i>L. acidophilus</i> ; <i>L. amylovorus</i> ; <i>L. brevis</i> ; <i>L. bulgaricus</i> ; <i>L. casei</i> ; <i>L. cellobiosus</i> ; <i>L. curvatus</i> ; <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> ; <i>L. farciminis</i> ; <i>L. farciminis</i> ; <i>L. fermentum</i> ; <i>L. gallinarum</i> ; <i>L. jensenii</i> ; <i>L. lactis</i> ; <i>L. paracasei</i> ; <i>L. plantarum</i> ; <i>L. reuteri</i> ; <i>L. rhamnosus</i> ; <i>L. salivarius</i> ; <i>L. sobrius</i> ; <i>L. thermophilus</i>	Azizpour et al., 2009; Lv et al., 2015; Yirga, 2015; Bajagai et al., 2016
<i>Lactococcus</i>	<i>L. lactis</i>	Yirga, 2015; Bajagai et al., 2016
<i>Leuconostoc</i>	<i>L. citreum</i> ; <i>L. lactis</i> ; <i>L. mesenteroides</i>	Yirga, 2015
<i>Megasphaera</i>	<i>M. elsdenii</i>	Bajagai et al., 2016
<i>Pediococcus</i>	<i>P. acidilactici</i> ; <i>P. parvulus</i> ; <i>P. pentosaceus</i> subsp. <i>pentosaceus</i>	Daudelin et al., 2011; Yirga, 2015; Bajagai et al., 2016
<i>Prevotella</i>	<i>P. bryantii</i>	Bajagai et al., 2016
<i>Propionibacterium</i>	<i>P. acidipropionici</i> ; <i>P. freudenreichii</i> ; <i>P. jensenii</i> ; <i>P. shermanii</i>	Bajagai et al., 2016
<i>Saccharomyces</i>	<i>S. boulardii</i> <sup>2</sup> ; <i>S. cerevisiae</i> ; <i>S. pastorianus</i> ( <i>S. carlsbergensis</i> ); <i>S. servisia</i>	Le Bon et al., 2010; Lv et al., 2015; Yirga, 2015; Bajagai et al., 2016
<i>Streptococcus</i>	<i>S. bovis</i> ; <i>S. cremoris</i> ; <i>S. diacetyllactis</i> ; <i>S. faecalis</i> ; <i>S. faecium</i> ; <i>S. gallolyticus</i> ; <i>S. infantarius</i> ; <i>S. intermedius</i> ; <i>S. salivarius</i> subsp. <i>thermophilus</i>	Pollmann et al., 1980; Azizpour et al., 2009; Yirga, 2015; Bajagai et al., 2016
<i>Aspergillus</i>	<i>A. oryzae</i> ; <i>A. niger</i>	Yirga, 2015; Bajagai et al., 2016

<sup>1</sup> The commonly called lactic acid bacteria (LAB) comprise *Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp., *Lactosphaera* spp., *Leuconostoc* spp., *Melissoctococcus* spp., *Oenococcus* spp., *Pediococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. (Yang et al., 2015a).

<sup>2</sup> Some microbiologists consider *S. boulardii* as a subspecies or variant of *S. cerevisiae*.

production, processing, storage, transport, and distribution of commercial probiotic products have been summarized in a FAO document paper (Bajagai et al., 2016). Jonsson and Conway (1992) described some practical usage of various forms of probiotic preparations for the swine feeding practices.

#### 4.3. Effects of probiotics on swine production

The use of probiotics for human health and farm animal production has been widely reported in the literature. Even though most of the earlier studies suffered from lack of rigorous experimental design, microbial strain characterization, sufficient treatment duration, and/or host microbiota description, many recent studies have shown that humans and animals fed probiotics have altered intestinal microbiota, increased intestinal immunity, improved resistance to disease, reduced shedding of pathogens and disease symptoms, and improved health status (Bhandari et al., 2010; Kenny et al., 2011; Upadhaya et al., 2015; Yirga, 2015). Probiotics sometimes are given to animals that have been therapeutically treated with antibiotics or other drugs, to re-colonize a gut that may have been depopulated by the therapeutic treatment (Hughes and Heritage, 2002; Pamer, 2016).

For improving production efficiency, the modern swine industry adapted some advanced, but unnatural, husbandry practices that could induce certain stress to the pig, causing changes in the composition of intestinal microbiota and thus compromising pig's resistance to pathogens (Fuller, 1992). Although it is still not consistent in the literature and difficult to make generalizations in terms of the effects of using probiotics on pig production due to the variation in the microbial strains used, the doses applied, the treatment duration, as well as the husbandry practices, most of the reports have shown that administration of probiotic strains, either separately or in combination, significantly improved the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) of pigs.

As early as in 1970s, some studies showed that the *Lactobacillus* supplements improved ADG and FCR in swine, while others observed no significant response (Pollmann et al., 1980; Fuller, 1989). Pollmann et al. (1980) reported that *Lactobacillus*

*acidophilus* supplement improved the ADG and FCR in starter pigs, but not in growing-finishing pigs. They suggested that the lack of effect in the older pigs might have been due to the use of a different diet; the grower-finisher diet was less complex than the diet used for the starter pigs (Fuller, 1989). Huang et al. (2004) demonstrated that dietary lactobacilli supplementation improved ADFI of the weaning pigs during the first 2 weeks, increased ADG and ADFI during the second week, and had no effect during the third week post-treatment. Similarly, Le Bon et al. (2010) reported that their probiotic regimen had positive effects on FCR of weaned pigs. Although the villus length, crypt depth, mucus-producing cell counts, and the thickness of the mucus layer of the small intestine remained unaffected after 4 weeks of probiotic treatment, the *E. coli* counts in the gut were reduced dramatically and transiently when compared to the non-treated pigs. More studies of probiotic effects on the production performance of pigs, including suckling, weaning, growing, and finishing pigs, have been reviewed in details by Cho et al. (2011) and Bajagai et al. (2016). Some representative results summarized from recent literature are shown in Table 2.

Studies on the effects of probiotics on the reproductive performance of swine are relatively limited. However, some studies, as summarized by Ahasan et al. (2015), showed that some probiotic species (in the genera of *Bacillus*, *Lactobacillus*, and *Streptococcus*, for example) improved the colostrum quality, milk quality and quantity, litter size and vitality, and piglet body weight. Alexopoulos et al. (2004) reported that the pregnant sows fed BioPlus 2B (containing *Bacillus licheniformis* and *B. subtilis*) from 2 weeks before expected farrowing date and during lactation period improved the performance of the litters, with reduced piglet diarrhea, reduced pre-weaning mortality, and increased weaning weights. The decreased weight loss in sows during lactation and the production of milk with higher fat and protein contents were the suggested reasons for the improved health and performance of the piglets (Alexopoulos et al., 2004). Another reason might be the improved microbial environment surrounding the sows and the piglets.

It should be pointed out that the use of different doses and different strains of microbial species, the differences in swine husbandry practices (nutrition, feed types, housing, etc.), and the

**Table 2**  
Effects of probiotics on growth performance of pigs.

Microorganisms	ADG	FCR	ADFI	Age group	Reference
<i>B. subtilis</i>	S (+)	S (-)	NS	Growing–finishing pigs	Meng et al., 2010
<i>C. butyricum</i>					
<i>L. acidophilus</i>	S (+)	NS	S (-)	Weaned piglets	lv et al., 2015
<i>S. cerevisiae</i>					
<i>L. acidophilus</i>	S (+)	NS	NS	Growing pigs	Bajagai et al., 2016
<i>S. cerevisiae</i>					
<i>B. subtilis</i>					
<i>L. plantarum</i> ATCC 4336	S (+)	NS	NS	Weaned piglets	Bajagai et al., 2016
<i>L. fermentum</i> DSM 20016					
<i>E. faecium</i> ATCC 19434					
<i>E. faecium</i> EK13	NS	–	–	Newborn piglets	Bajagai et al., 2016
<i>Bi. longum</i> AH1206	NS	NS	–	Neonatal piglets	Bajagai et al., 2016
<i>B. licheniformis</i>	S (+)	S (-)	–	Weaned piglets	Bajagai et al., 2016
<i>B. subtilis</i>	S (+)	S (-)	NS	Growing pigs	Bajagai et al., 2016
<i>B. licheniformis</i>					
<i>B. subtilis</i>	S (+)	S (-)	NS	Grower finisher pigs	Bajagai et al., 2016
<i>B. licheniformis</i>					
<i>B. subtilis</i> MA139	NS	S (-)	NS	Weaned piglets	Bajagai et al., 2016
<i>B. toyonensis</i>	S (+)	S (-)	S (+)	Weaning piglets	Bajagai et al., 2016
<i>B. licheniformis</i>	NS	S (-)	NS	Growing–finishing pigs	Davis et al., 2008
<i>B. subtilis</i>					
<i>S. cerevisiae</i> subsp. <i>boulardii</i> CNCM I-1079	–	S (-)	–	Weaned piglets	Le Bon et al., 2010

ADG = average daily gain; FCR = feed conversion ratio; ADFI = average daily feed intake; S (+) = significantly increased; S (-) = significantly decreased; NS = non-significant; – = not studied.

different breeds and ages of pigs tested may all help to explain some contrasting results concerning the same microbial species of probiotics in the literature (Bajagai et al., 2016). To assess the disputed probiotic effects on ADG and FCR of pigs, a meta-analysis of 67 and 60 experiments (published during the years of 1980 to 2015) has been conducted, respectively (Zimmermann et al., 2016). Dietary supplementation of probiotics significantly increased ADG by 29.9 g/day (summarized from 32 studies with 67 experiments and 4,122 pigs) and significantly improved FCR by saving 0.096 kg feed required for each kilogram of body weight gain (summarized from 29 studies with 60 experiments and 4,011 pigs). The results of subgroup analyses are shown in Table 3. The meta-analysis results also showed that the application of probiotics to pigs during their first stage of growth and in the finishing period resulted in greater ADG and better FCR (Zimmermann et al., 2016) suggesting that these additives are more beneficial at certain stages of growth.

**Table 3**  
Effects of probiotics on the average daily gain (ADG) and feed conversion ratio (FCR) of different groups of pigs.<sup>1</sup>

Item	Difference in means <sup>2</sup>	95% confidence intervals <sup>2</sup>	No. of experiments <sup>3</sup>
ADG, g/day			
Weaning to 18 kg	35.6	17.1 to 54.2	43
Growing, 18 to 50 kg	24.1	–2.21 to 50.4	8
Finishing, >50 kg	19.7	13.7 to 25.6	12
Lactation period	11.1	–2.1 to 24.3	4
FCR, kg/kg (feed/body weight)			
Weaning to 18 kg	–0.10	–0.14 to –0.07	38
Growing, 18 to 50 kg	–0.12	–0.22 to –0.01	8
Finishing, >50 kg	–0.10	–0.13 to –0.06	12
Lactation period	0.00	–0.04 to 0.04	2

<sup>1</sup> Data were compiled from Zimmermann et al. (2016).

<sup>2</sup> The effect measure used to present the results was the difference in means between the probiotic treatment and controls with 95% confidence intervals using a random effects model.

<sup>3</sup> The number of experiments from which the values were calculated.

## 5. Modes of action of probiotics in pigs

In the early days, the beneficial effect of probiotics on human health was linked to the modification of the colon bacterial

community, as Metchnikoff (1908) postulated that many human ills were due to the overgrowth of undesirable colonic bacteria. In pigs, the probiotic effect may mainly target the colon and cecum where an abundant and diverse microbial population is harbored (Chaucheyras-Durand and Durand, 2010). Unlike antibiotics, probiotics are believed to improve animal overall health by increasing the population of desirable microbes in the gut. Modern work, however, has indicated that the mechanism by which the beneficial effects of probiotics have must be more comprehensive and have not been fully elucidated (Wohlgenuth et al., 2010). Although different probiotics may influence the gut environment somewhat differently, the functional mechanisms in general can be summarized into 5 aspects: 1) modulation of gut microbiota, 2) modulation of host immune responses, 3) diarrhea reduction and antitoxin effects, 4) modulation of nutrient digestibility, and 5) some other actions (Pollmann, 1986; Ng et al., 2009; Yirga, 2015), and these diverse functional mechanisms or modes of action (Table 4) are credited to different types of probiotics (Oelschlaeger, 2010; Cho et al., 2011). In the following sections these modes of action are discussed in more details.

### 5.1. Modulation of gut microbiota

Probiotics are believed to improve the overall health of animals by preventing gut microbiota imbalance and improving gut health via modifying the gut microbial population (Veizaj-Delia and Pirushi, 2012; Lescheid, 2014), because introducing beneficial microorganisms can repair the deficiencies of benign microorganisms in the gut, restore or improve pig's resistance to diseases. This beneficial effect in turn can lead pigs with better capacity of nutrient digestion and absorption, and better nutrient utilization and production performance (Kenny et al., 2011; Yirga, 2015).

Bajagai et al. (2016) summarized several studies on weanling pigs and reported that probiotics increased the counts of LAB and decreased *Clostridium*, *E. coli*, and *Enterobacterium* spp. in swine gut. Yang et al. (2009) reported that selenium-enriched probiotics (*Candida utilis*, *L. acidophilus*, *Lactobacillus rhamnosus*, and *Streptococcus thermophilus*) can strongly antagonize pathogenic *E. coli* either *in vitro* or *in vivo* (in mice). The *E. coli* levels in the weaned pigs were reduced transiently but dramatically after 4 weeks supplementation with *Saccharomyces cerevisiae* ssp. *boulardii* and

**Table 4**  
Five modes of summarized action of various probiotics on animal or human gut health and function.<sup>1</sup>

Item	Description
Modulation of gut microbiota	
Competitive exclusion	Competing for adhesion sites on the gastro-intestinal wall
Direct antimicrobial inhibition	Competing for organic substrates or nutrients in the gut Producing substances that have bactericidal or bacteriostatic properties Decreasing luminal pH via probiotic fermentative activity Inhibiting the growth of Gram-negative bacteria by the hydrogen peroxide produced Affecting the metabolism and toxin production of the pathogenic microorganisms
Modulation of host immune responses	Improving gut innate immunity through restitution of intestinal barrier integrity and function Improving gut innate immunity through increasing gut mucus production or chloride secretion Stimulating or suppressing animal acquired immune responses Influencing animal immune system by products-like metabolites, cell wall components, and DNA
Diarrhea reduction and antitoxin effects	Inhibiting toxin expression in pathogenic bacteria Neutralizing the enterotoxins produced by pathogenic bacteria
Modulation of nutrient digestibilities	By the high fermentative activity of probiotics Increasing digestive enzyme production and activities Affecting the absorption and secretion activities of swine gut Producing some vitamins
Other modes of action	Antioxidative activity and alleviation of stress Altering bacterial and host gene expression

<sup>1</sup> Data were summarized from Pollmann (1986), Ng et al. (2009), Oelschlaeger (2010), Cho et al. (2011), and Yirga (2015).

*P. acidilactici* compared to the pigs fed non-supplemented diet (Le Bon et al., 2010). Pospíšková et al. (2013) reported that the counts of *E. coli* and *C. perfringens* in the feces of weaned sows fed a monoculture of *E. faecium* were significantly reduced. As described below there are 2 main mechanisms involved in the modulation of gut microbiota or gut micro-ecosystem, namely competitive exclusion and direct antimicrobial inhibition.

### 5.1.1. Competitive exclusion

Competitive exclusion is defined as the action of normal microbiota that protects the gut against the establishment of harmful microorganisms and decreases the risk of intestinal infections and disorders in pigs. Hillman and colleagues reported that the growth of *E. coli* was successfully inhibited by different strains of lactobacilli (cited in Yirga, 2015). In piglets, attachment of *E. coli* to the small intestinal epithelia was reported to be inhibited by dietary supplementation with *E. faecium* and a colicin-producing *E. coli* based probiotic (Bhandari et al., 2010). Daudelin et al. (2011) also reported that the administration of *P. acidilactici* or *S. cerevisiae boulardii* limited the attachment of *E. coli* (harbouring the F4/K88 fimbriae) to the ileal mucosa, a key step in the pathogenesis by this pathogen.

The concept of competitive exclusion indicates that the cultures of selected benign microorganisms compete with harmful microorganisms in the gut for adhesion sites and organic substrates. The adhesion of probiotic microorganisms to the GIT wall could prevent its colonization by pathogenic microorganisms (Pollmann, 1986; Cho et al., 2011). It is known that detrimental bacteria need to have attached to the GIT wall to exert their harmful effects on the host (Yirga, 2015; Bajagai et al., 2016). Therefore, an expected effect of probiotics is an increase in normal microbiota colonization with inhibition of the adhesion of harmful bacteria to the intestinal epithelia (Pollmann et al., 1980; Herfías et al., 1999), thereby blocking receptor sites against the pathogen attachment (Yang et al., 2015a). By doing so, the probiotic bacteria exclude pathogens and thus prevent them from causing infection. Certain species within the microbiota may influence the expression of glycol conjugates (a class of fucosylated glycoproteins) on intestinal epithelia that serve as receptors for the adhesion of bacteria (Umesaki et al., 1997; Yirga, 2015).

The concept of competitive exclusion also indicates that probiotics compete with pathogenic bacteria for nutrients and nutrient absorption sites (Yang et al., 2015a). The competition for energy and nutrients (mainly the carbon source) between probiotic and other bacteria could result in a growth suppression of pathogenic ones. The gut is such a rich source of nutrients that it may seem unlikely that microorganisms would find insufficient nutrients for growth, but it should be noted that an environment has only to be deficient in one essential nutrient to inhibit microbial growth. In addition, the ability to rapidly utilize the energy source may reduce the log phase of bacterial growth and make it impossible for the bacteria to resist the flushing effect exerted by gut peristalsis (Cho et al., 2011; Yirga, 2015).

### 5.1.2. Direct antimicrobial inhibition

Certain probiotic organisms, once established in the gut, may produce substances that have bactericidal or bacteriostatic properties, which can suppress the colonization of host intestine by undesirable microorganisms including both gram-positive and gram-negative bacteria. This microbial antagonism action can counteract the disruption of the host gut microbial equilibrium by harmful microbes and lead to a good eubiotic status (Pollmann et al., 1980; Cho et al., 2011; Bajagai et al., 2016).

Many probiotic bacteria, especially LAB, ferment carbohydrates such as lactose, to produce short chain fatty acids such as lactic and acetic acids, thereby dropping the luminal pH to a level that

harmful bacteria cannot tolerate (Pollmann et al., 1980; Bajagai et al., 2016). Some species also produce hydrogen peroxide, which inhibits the growth of gram-negative bacteria (Yirga, 2015; Bajagai et al., 2016). A decrease in gut pH by these substances may partially offset the low secretion of hydrochloric acid in the stomach of weanling piglets (Kenny et al., 2011; Yirga, 2015).

Besides organic acids, a variety of other substances including antioxidants, antimicrobial peptides (defensins), reuterin, bacteriocins, and microcin can also be produced by probiotic bacteria. These substances may not only reduce the number of viable pathogenic organisms but may also affect bacterial metabolism and toxin production (Ng et al., 2009; Murali et al., 2010; Hou et al., 2015). Bacteriocins produced by LAB have been reported to be able to permeate the outer membrane of gram-negative bacteria, and subsequently inactivating them in conjunction with other enhancing anti-microbial environmental factors, such as low temperatures, organic acids and detergents (Alakomi et al., 2000). Microcin produced by probiotic *E. coli* can limit the growth of competitors in an inflamed intestine, including commensal *E. coli*, adherent-invasive *E. coli*, and the related pathogen *Salmonella enterica* (Setia et al., 2009; Bhandari et al., 2010; Krause et al., 2010; Sassone-Corsi et al., 2016).

## 5.2. Modulation of host immune responses

The mechanism of replenishing of gut microbial population through probiotics has gone beyond the benefits of maintaining a balanced micro-ecosystem to recuperating host immune systems responsible for both innate immune responses and acquired immune responses (Daudelin et al., 2011).

### 5.2.1. Enhancing gut innate immunity

The gastro-intestinal lumen contains benign nutrients and microorganisms, but also harmful substances such as harmful microorganisms, toxic materials, and some foreign antigens (Takahashi et al., 1998; Willing et al., 2012). Epithelial cells in the GIT mucosa create a selectively permeable barrier between the lumen environment and the internal body tissues. This barrier is the first line of host defense against harmful microbes in the GIT; however, stress or disease conditions can disrupt this barrier (Willing et al., 2012; Bajagai et al., 2016; Lee et al., 2016).

Restitution of the GIT mucosa barrier function by probiotics has been observed in both *in vitro* and *in vivo* models (García-Lafuente et al., 2001; Madsen et al., 2001). Certain probiotics can influence the intestinal mucosal cell–cell interactions and cellular “stability” by enhancing the function of intestinal barrier through modulation of the phosphorylation of cytoskeletal and tight junction proteins (Ng et al., 2009; Willing et al., 2012). Although the details of this mode of action are still not very clear, the action was thought to be related to the alterations in the secretion of mucus or chlorides, or the changes in the expression of tight junction proteins by epithelial cells (Ng et al., 2009; Yang et al., 2015b).

It was reported that an epithelial cell line (i.e., IPEC-J2) isolated from neonatal piglet mid-jejunum is a valuable *in vitro* model for studying the interactions between microorganisms and the host (Liu et al., 2010; Brosnahan and Brown, 2012). Using this model, Liu et al. (2010) found that the *L. acidophilus* or *L. rhamnosus* GG treatment of the cells did not reduce the replication of porcine rotavirus, but the *L. rhamnosus* GG alone treatment post-rotavirus infection reduced the mucin secretion response induced by the virus. The *L. acidophilus* treatment prior to the virus infection increased the interleukin 6 (IL-6) response to the infection, whereas the *L. rhamnosus* GG treatment post-rotavirus infection down-regulated the IL-6 response (Liu et al., 2010). Wu et al. (2016) investigated the protective effects and related mechanisms of

*Lactobacillus plantarum* on epithelial barrier damages induced by ETEC K88 in the differentiated IPEC-J2 cells, and demonstrated that *L. plantarum* effectively diminished the *E. coli* induced upregulation of *IL-8* and *TNF- $\alpha$*  gene expression, and thereby protected the cells against epithelial barrier damage through sustaining the gene expression and the subsequent contents of critical tight junction proteins including claudin-1, occluding, and zonula occludens.

Although it is not very clear how a host differentially recognizes the pathogenic, the commensal, and the probiotic bacteria that respectively result in immune activation, immune tolerance, and immune activation or deactivation (Vinderola et al., 2005; Hardy et al., 2013), recent studies revealed that some bacterial macromolecules, called microbe-associated molecular patterns (MAMPs), are key ligands or factors in the beneficial microorganism-host crosstalk. These MAMPs can interact with pattern recognition receptors (PRRs) of the host GIT mucosa (Lebeer et al., 2010). Both the host intestinal epithelial and dendritic cells are crucial players in the innate (as well as the acquired) immunity, and can respond to and interact with gut microorganisms by means of their PRRs that detect MAMPs, such as bacterial DNA, long surface appendages, lipoteichoic acids, and polysaccharides (Rachmilewitz et al., 2004; Lebeer et al., 2010). The best studied host PRRs are Toll-like receptors (TLRs) which are transmembrane proteins present at the cell surface, or on the membrane of endocytic vesicles or other intracellular organelles (Lebeer et al., 2010; Gu et al., 2016). Various studies in the biomedical fields with murine models have suggested that the signaling interactions between the innate PRRs of gastrointestinal cells and the MAMPs of probiotics contribute largely to the stabilization of host mucosal immunity (Rachmilewitz et al., 2004; Vinderola et al., 2005; Maldonado et al., 2015).

### 5.2.2. Stimulation or suppression of acquired immunity

Human and animal immune responses should be stimulated in some cases (for example, in infection and immune-deficiency situations) but be suppressed in others (for example, in allergy and autoimmune disease situations) depending on the clinical conditions (Borchers et al., 2009). Research has showed that the normal gut microbiota can function as immunomodulators to support animal's defense systems against invading pathogens by stimulating gastrointestinal immune response, and this immunomodulation effect may aid the immune system development via stimulating antibody production and increasing phagocytic activity (Yirga, 2015). Fuller (1992) explained 2 ways in which the immune system is stimulated: 1) They can either migrate through the gut wall as viable cells or multiply to a limited extent, and 2) the antigens released by the dead organisms are absorbed and directly stimulate the host immune system. It is the product of this change that further induces the immune response (Yirga, 2015).

Through various cytokine cascade reactions, the immune responses of pigs to the probiotic administration are reflected in both local intestinal immunity and whole body systemic immunity (Herías et al., 1999; Gan et al., 2014; Schierack et al., 2007). Early in 1995, Tortuero et al. reported that the mixture of *Streptococcus faecium* and *Lactobacillus casei* (but not *L. bulgaricus*) increased the IL-2 concentration in the ileal tissue of pigs, indicating an increased intestinal local immunity. Lactobacilli can colonize and adhere to the GIT epithelia forming a protective membrane against pathogenic microorganisms while at the same time modulating immunity via stimulating epithelial lymphocytes (Yu et al., 2008). Oral administration of *Bifidobacterium longum* and other LAB have been found to increase the total amount of intestinal mucosal IgA (Takahashi et al., 1998; Vitini et al., 2000). Likewise, rats colonized with both *L. plantarum* and *E. coli* had higher levels of total serum IgA and marginally higher levels of IgM and IgA antibody against *E. coli* than those colonized with *E. coli* alone (Herías et al., 1999).

Probiotics can provide defense to the cells by inducing anti-inflammatory cytokines and reducing pro-inflammatory cytokines from the enterocytes and the intestinal immune cells which were recruited to the inflammation sites by the probiotics (O'Hara et al., 2006; Cho et al., 2011). Some strains of *Lactobacillus* are able to recruit various cytokines, and thus they are capable of acting as immunomodulators by enhancing macrophage activity, increasing the local antibody levels, inducing interferon production, and activating killer cells (Cho et al., 2011). It was reported that feeding *Lactobacillus fermentum* to piglets induced an increase in the pro-inflammatory cytokines and the percentage of CD41 lymphocyte subset in the blood (Cho et al., 2011). *L. casei* has been reported to have immunoadjuvant activity (Perdigón et al., 1995).

Recent studies revealed that the increase in intestinal IgA is mainly caused by the proliferation of IgA-producing B cells in the GIT lumen without increase in the population of CD4<sup>+</sup> T-cells (Vitini et al., 2000; Vinderola et al., 2005). The probiotic bacteria enhanced the secretion of IL-6 by intestinal epithelial cells which resulted in differentiation of B-cells responsible for producing IgA and IgM (Vinderola et al., 2005; Goodrich and McGee, 1999). As is known, IgA plays a crucial role in the clearance of foreign pathogens via combination with mucins in the GIT (Takahashi et al., 1998).

Schierack et al. (2007), however, demonstrated that the population of intraepithelial CD8<sup>+</sup> T cells was enhanced in the piglets fed *Bacillus cereus* var. *toyoi*, and the numbers of  $\gamma\delta$  T cells tended to be higher in the intestinal epithelium at the time of weaning (day 28), and *Lamina propria* lymphocytes were also influenced by the probiotic treatment. Application of *B. cereus* var. *toyoi* also resulted in more CD25<sup>+</sup> lymphocytes and  $\gamma\delta$  T cells in the post-weaning piglets. Dietary *E. faecium* (NCIMB 10415) supplementation was reported to have enhanced the course of infection in weaning pigs challenged with *Salmonella* serovar Typhimurium (DT104) and increased the production of specific antibodies against *Salmonella* (Szabó et al., 2009).

*Influencing the immune system by probiotic chemical components:* Probiotics can also influence the host immune system by products-like metabolites, cell wall components, and DNA. Obviously, immune modulatory effects might be even achieved with dead probiotic bacteria or just probiotic-derived components like peptidoglycan fragments (Oelschlaeger, 2010). When the acquired immune system is engaged following exposure to viable probiotic bacteria or to bacterium-derived components, any hostile bacteria are also noticed, following the increased surveillance by leukocytes, and thus potential pathogens are eliminated (Hughes and Heritage, 2002).

Currently, there appears to be some relationship between the ability of a microbial strain to translocate and the ability to be immunogenic (Fuller, 1992). However, it is difficult to completely conclude that probiotics contribute to the host immune system significantly as they are not intended to eradicate the invasive pathogens in the GIT. The main reason behind this caveat is that probiotics differ from antibiotics in that they are not intended to eradicate invasive pathogens in the GIT. Therefore, such observed improvements or positive effects are always somewhat compromised due to animal's immune system status and various applied situations (Cho et al., 2011).

### 5.3. Diarrhea reduction and antitoxin effects

Diarrhea is the most critical problem of piglets during the first weeks post-weaning and, therefore, the reduction of diarrhea incidence by probiotics has been frequently studied (Hill et al., 1970; Simon, 2005; Campbell et al., 2013; Trckova et al., 2014). Approximately 80% of the reported studies detected reductions of diarrhea incidence in piglets receiving probiotics, and this effect was independent of microorganism types, such as *B. cereus*,

*E. faecium*, *L. lactis*, or *P. acidilactici* (Muralidhara et al., 1977; Simon, 2005). Taras et al. (2006) investigated the effects of long-term application of *E. faecium* (NCIMB 10415) on performance and health characteristics of sows and offspring. They reported that probiotic supplementation reduced the rate of post-weaning diarrhea (21% vs. 38%) and the overall pre-weaning mortality (16.2% vs. 22.3%). In agreement with this are 3 separate studies in piglets (2 with *E. faecium* NCIMB 10415, one with *B. cereus* var. *toyoi*), where a reduced diarrhea incidence was recorded (Simon, 2005). Giang et al. (2012) found that addition of *B. subtilis* H4 or together with *S. boulardii* Sb to the LAB strains (*E. faecium* 6H2, *L. acidophilus* C3, *Pediococcus pentosaceus* D7, and *L. fermentum* NC1) resulted in a lowered incidence of diarrhea.

Similarly, Bhandari et al. (2010) reported that a colicin-producing *E. coli* probiotic prevented the adhesion of ETEC K88 to gut mucosa, which prevented the ETEC-challenged weaned pigs from diarrhea. Furthermore, dietary supplementation of micro-encapsulated *Enterococcus faecalis* (CG1.0007) probiotic significantly reduced the incidences of diarrhea in the weaned piglets orally challenged with ETEC (Chen et al., 2016). In addition, Lv et al. (2015) reported that the diarrhea incidence of weaned piglets raised under high ambient temperature was significantly reduced because of dietary supplementation of probiotics or selenium-enriched probiotics (*L. acidophilus* and *S. cerevisiae*).

It is known that the enterotoxins produced by pathogenic bacteria could cause intestinal fluid loss and diarrhea. The effectiveness of certain probiotics in reducing diarrhea incidence is most likely because of their ability to protect the animal against bacterial toxins, perhaps the most important virulence factors to the GIT. Some enterotoxins produced by pathogenic bacteria can be neutralized by some substances produced by probiotics (Yirga, 2015). The probiotic yeast *S. boulardii* also showed effective protection against *Clostridium difficile* toxin A in the murine ileal loop model as well as in cell culture assays (Chen et al., 2006a,b).

A protection can also result from inhibition of toxin expression in pathogens (Oelschlaeger, 2010). Mice treated with *Bifidobacterium breve* (Yakult strain) survived whereas 90% of the mice in the control group died after challenge with enterohaemorrhagic *E. coli* serotype O157:H7. Some *in vitro* studies implied that the high concentration of acetic acid produced by the Yakult strain is responsible for the inhibition of Shiga toxin expression (Asahara et al., 2004). Carey et al. (2008) using an *in vitro* study confirmed that 15 different probiotic lactobacilli strains inhibited Shiga toxin 2A expression via production of organic acids at sub-bactericidal concentrations for *E. coli* O157:H7.

Additionally, some probiotics can prevent amine synthesis by harmful bacteria (Pollmann et al., 1980). Coliform bacteria decarboxylate amino acids to produce amines, which are toxic, can irritate the gut, and are concurrent with the diarrhea incidence. If desirable bacteria such as probiotics prevent the coliforms from proliferating, then amine production will be prevented (Hill et al., 1970; Pollmann, 1986; Yirga, 2015).

Promotion of favorable GIT microbiota in swine can be attributed to the reduction of gut pathogenic infections and diarrhea incidence. The diarrhea reduction as described above at least implies less veterinary interventions for the animal, which may be cost-saving for swine producers and therefore a further aspect of production efficiency (Simon, 2005).

#### 5.4. Modulation of nutrient digestibilities

Various studies demonstrated that probiotics can improve the digestibilities of dry matter, organic matter, energy, crude protein, crude fiber, and phosphorus. Huang et al. (2004) found that the apparent digestibilities of crude protein and phosphorus were

increased in weaned pigs fed a corn and soybean meal based diet supplemented with 0.1% of the complex lactobacilli preparation, and their analyzed bacterial content was  $2.4 \times 10^5$  colony forming units (cfu) per gram of diet. Yu et al. (2008) demonstrated that *L. fermentum* (a better candidate in the study) at a dietary concentration of  $5.8 \times 10^7$  cfu/g maximized the crude protein digestibility of weaned pigs among the dietary concentrations from  $3.2 \times 10^6$  to  $2.9 \times 10^8$  cfu/g. Similarly, Meng et al. (2010) reported that growing-finishing pigs fed probiotics (a mixture of spray-dried spore-forming *B. subtilis* and *Clostridium butyricum* endospores) showed improved crude protein and energy digestibilities compared to those with non-probiotic treated pigs. Zhao and Kim (2015) found that the direct-fed 0.1% *L. reuteri* and *L. plantarum* complex ( $1 \times 10^6$  cfu/g) improved apparent total tract digestibilities of nitrogen and energy at the end of the 4-week treatment.

Giang et al. (2010) demonstrated that diets supplemented with three different LAB complexes increased the apparent ileal digestibilities of organic matter, crude protein, and crude fiber, and the apparent total tract digestibilities of crude protein and crude fiber in the first 2 weeks post-weaning. The 3 bacterial complexes were Complex 1 comprised of *E. faecium* 6H2 at  $3 \times 10^8$  cfu/g, *L. acidophilus* C3 at  $4 \times 10^6$  cfu/g, and *P. pentosaceus* D7 at  $3 \times 10^6$  cfu/g; Complex 2 comprised of *E. faecium* 6H2 at  $3 \times 10^8$  cfu/g, *L. acidophilus* C3 at  $4 \times 10^6$  cfu/g, and *L. plantarum* 1K8 at  $2 \times 10^6$  cfu/g; and Complex 3 comprised of *L. acidophilus* C3 at  $4 \times 10^6$  cfu/g, *L. plantarum* 1K8 at  $2 \times 10^6$  cfu/g, and *L. plantarum* 3K2 at  $7 \times 10^6$  cfu/g. In a later study, Giang et al. (2012) found that addition of *B. subtilis* H4 or together with *S. boulardii* Sb to the LAB strains (*E. faecium* 6H2, *L. acidophilus* C3, *P. pentosaceus* D7, and *L. fermentum* NC1) resulted in improved organic matter and crude protein digestibilities, which suggested that a multi-bacterial mixture including bacteria other than LAB may have benefits over a mixture of LAB only, and that the inclusion of yeast may have additional benefits, in terms of probiotic effects in weaned piglets.

The improvement in the digestibilities of dietary nutrients in pigs by probiotics may be, in a large part, due to the increased production and activities of digestive enzymes in the gut by probiotics since probiotics possess a high fermentative activity and can enhance gut digestion (Cho et al., 2011; Upadhaya et al., 2015). Lactobacilli, for example, are known to produce lactic acid and proteolytic enzymes that can enhance nutrient digestion in the GIT (Yu et al., 2008). Collington et al. (1990) reported that the activities of sucrase, lactase and tripeptidase (not dipeptidase though) in the small intestine of pre-weaned piglets were increased in response to Probios, a commercial probiotic product containing *L. plantarum*, *L. acidophilus*, *L. casei*, and *S. faecium*. In a study carried out by Kim et al. (2007) in pigs to screen LAB that produce active enzymes including amylase, lipase, phytase, and protease, *Lactobacillus* sp. PSC101 was selected as a strong candidate because of its production of these enzymes and resistance to both acid and bile. Spore forming bacteria, such as *Bacillus amyloliquefaciens*, can produce extracellular enzymes including  $\alpha$ -amylase, cellulase, proteases, and metalloproteases (Lee et al., 2008; Bajagai et al., 2016). These increased enzyme activities in the GIT of pigs fed probiotics could be attributed to either the production of enzymes by the probiotics themselves or the induced change in the gut micro-ecosystem and hence the enzyme production (Bajagai et al., 2016).

Probiotics may also affect the absorption and secretion activities of swine gut. A slightly higher L-glutamine transport and increased ion secretion were observed in *B. cereus* or *E. faecium* treated pigs at 28 days of age (Kenny et al., 2011). Cai et al. (2015) reported that the pigs (15 to 42 days post-weaning) fed a DFM product had longer duodenum, jejunum, and ileal ( $P = 0.17$ ) villi compared with the control pigs. The DFM product was formulated to provide  $1.5 \times 10^5$  cfu spores per gram of feed based on one strain of

*B. subtilis* and 2 strains of *B. amyloliquefaciens* in equal proportions. Increased length of intestinal villi means increased nutrient absorption surface by the small intestine.

Other postulated effects of probiotics in terms of swine nutrition also include a beneficial interaction with bile salts and a greater vitamin production (Hughes and Heritage, 2002; Oelschlaeger, 2010; Yirga, 2015).

### 5.5. Other modes of action

Antioxidative activity and alleviation of stress: pigs in the modern industrial farming system are frequently exposed to oxidative stress that can result in decreased immune function and chronic diseases due to oxidative damage. It has been shown that some LAB (such as *Bi. longum* and *L. fermentum*) can produce antioxidants, scavenge free radicals (*in vitro*), and could thus be used to alleviate host oxidative stress (Hou et al., 2015; Yang et al., 2015a).

Wang et al. (2009) reported that supplementation of *L. fermentum* improved the antioxidant status of growing–finishing pigs (50 to 90 kg) as evidenced by increased serum levels of antioxidant enzymes which were superoxide dismutase and glutathione peroxidase, and decreased serum and muscle levels of malondialdehyde. In weanling pigs, Wang et al. (2013) showed that diquat injection decreased pig performance and increased the plasma levels of cortisol, adrenaline, carbonyl, and malondialdehyde. *L. fermentum* I5007 supplementation improved the antioxidative defense system, alleviated the damage caused by diquat, and enhanced the pig performance.

Altering bacterial and host gene expression: bacteria communicate cell to cell through the secretion of chemical signals, called autoinducers that are produced in response to the changes in cell-population density. This process of bacterial communication, called quorum sensing, can affect the behavior of both bacterial and host cells (Hughes and Sperandio, 2008; Bajagai et al., 2016). Through quorum sensing probiotics may affect pathogenic bacteria and influence their pathogenicity. Extracellular secretion of a chemical signal, autoinducer-2, by human enterohaemorrhagic *E. coli* O157:H7 was substantially inhibited by fermentation products from *L. acidophilus* La-5, resulting in suppression of the virulence gene (LEE – locus of enterocyte effacement) expression *in vitro*. This suppression disrupts the quorum sensing and eventually prevents the colonization of host GIT by *E. coli* O157:H7 (Medellin-Peña et al., 2007; Bajagai et al., 2016).

As a final point, although the probiotic modes of action have been summarized in five aspects as discussed above, not all of the actions have been satisfactorily explained through thorough scientific research. To explain the beneficial effects of probiotics, some mechanisms that have been proposed based on developing metabolic activity comprise both direct and especially indirect effects, and it is very likely that the positive results reported in different *in vivo* studies are due to a combination of some, if not all, of these actions (Yirga, 2015).

## 6. Safety and risk issues related to probiotic usage

### 6.1. Risk assessment for using probiotics

Most publications regarding probiotics in the literature dealt with probiotics efficacy and safety rather than their risks in practical usage. However, as with any other new feed additives, swine producers and the general public do have some concerns over the usage of probiotics, as well as some speculation over the negative effects of probiotics, if any, on pig performance (Pollmann, 1986). According to Doron and Snyderman (2015) and Bajagai et al. (2016), any microorganisms considered for use as probiotics in swine diets should be assessed against the following risks:

- 1) Gastrointestinal or systemic infection of the pigs fed probiotics.
- 2) Gastrointestinal or systemic infection of the handlers of pigs and/or the pig feed.
- 3) Sensitization of skin, eye, and/or mucus membranes of the probiotic handlers.
- 4) Human food “contamination” and the infection (gastrointestinal or systemic) of the humans consuming pork products produced from the pigs fed probiotics.
- 5) Release of infectious microorganisms or noxious substances to the environment from the pig production system.
- 6) Hyper-stimulation of the immune systems of the pig.
- 7) Transfer of antibiotic resistance from probiotics to other pathogenic microorganisms.
- 8) Detrimental metabolic or toxic effects on the host due to the production of toxins by probiotic microorganisms.

The list of risks above is by no means complete and in any priority order. Nonetheless, the most serious risks posed by probiotics in animal feed can be: first, the transfer of antibiotic resistance due to the presence of transmissible antibiotic resistance genes/determinants in some probiotic bacteria; and secondly, the infections from probiotic microorganisms and the presence of enterotoxins and emetic toxins in probiotic bacteria (Bajagai et al., 2016).

For product development consideration, the microorganisms need to be identified to the strain level (Fig. 1; Fuller, 1992; Borchers et al., 2009). A particular strain should have not been associated with any infection in humans or pigs. Likewise, a putative probiotic microorganisms should not harbor any transferable antibiotic resistance genes. Any microorganisms that either produce toxins or cause hyper-stimulation of the immune system of a host are generally not suitable for use (Bajagai et al., 2016).

### 6.2. Safety issues related to probiotic usage

Although generally considered safe, there is little evidence showing that probiotics are absolutely safe, and it has been generally agreed that “zero risk does not exist” (Marteau, 2001). Information about the safety of probiotics was mostly based on

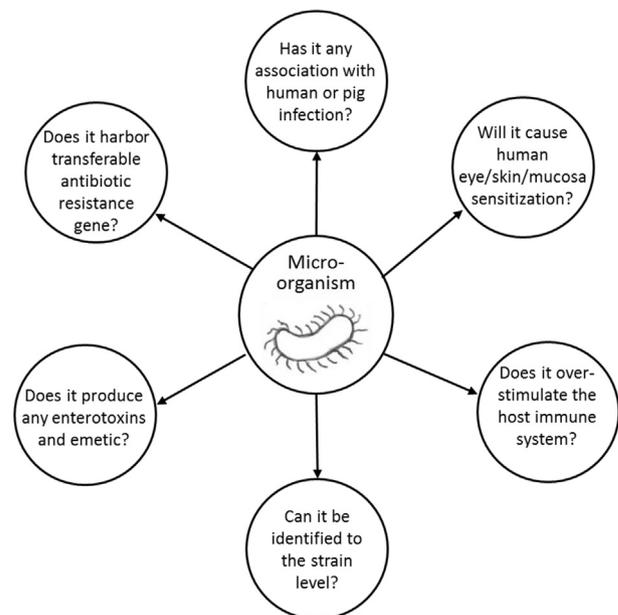


Fig. 1. Major questions to be addressed when assessing the safety of, and the risks associated with, the microorganisms being considered for use as probiotics in animal feed (adapted from Bajagai et al., 2016).

*Lactobacillus* and *Bifidobacterium* bacteria (Shanahan, 2012). Considering an array of microorganisms that could be used as probiotics, uncertainty always exist about the safety of the microorganisms to be used. According to Shanahan (2012) and FAO (2012), probiotic developers should pay special attention to the following 4 issues which, in general, are the limitations of many current claims made on probiotic safety:

- 1) No probiotic can be regarded as 100% safe or with zero risk, as is the case with drugs.
- 2) The adverse effects and the severity of the effects of a probiotic product could be context specific and depend on the physiological state and susceptibility (immunity) of the host. Therefore, a probiotic strain deemed to be safe in one condition may not be safe in another. For example, an immunologically compromised host could be at greater risk than a normal, healthy host.
- 3) Safety assessment and information on one particular probiotic strain cannot be generalized to other similar probiotics (even within a species), as each product requires risk and safety assessment on a case-by-case basis.
- 4) Public awareness about the risks of probiotic usage is limited, and there is a need for proper risk-benefit analyses and communication of the analysis results to probiotic users or the general public.

## 7. Concluding remarks

The sub-therapeutic use of antibiotics as AGP to improve growth and efficiency of farm animal production has been restricted or banned in more than 30 countries, but the application of these AGP in feed to prevent diarrhea and improve production performance of pigs is still a common practice in other parts of the world. Thus, the substitution of AGP with acceptable alternatives, such as probiotics, to address the issue of antibiotic resistance is very critical for public health and the global swine production.

The intention of this paper was to review the current knowledge in the literature regarding the effects of utilizing various probiotics for swine production. From the literature it can be seen that, depending on the products used and the animal husbandry practices applied, feeding probiotics to pigs can improve pig gut health, nutrient digestibilities, and growth performance. Using probiotics is generally regarded as safe to pigs, humans, and the environment, and it does not run high risks of introducing foreign chemicals or hazardous substances into food products of animal origin. Therefore, there exists a significant potential of using probiotics to replace the AGP currently still in use in many parts of the world.

A great deal of work on the efficacy of probiotics in human health has been broadly conducted (Zuccotti et al., 2008; Veizaj-Delia and Pirushi, 2012; Lescheid, 2014), and certain aspects of the work can be surely applied to the pig, particularly the mechanistic studies looking at the interaction between probiotics and the host mucosal surfaces or the pathogenic bacteria (Kenny et al., 2011). However, this “human model” for pig production does not always give a complete insight into the efficacy of probiotics in terms of production and reproduction parameters commonly used in the swine industry (Kenny et al., 2011). Therefore, more research into optimizing varied commercial products and the corresponding feeding regimen or strategies is strongly suggested. Thanks to the knowledge advancement in the areas of GIT microbiota and the modes of action of probiotics, upgraded or new probiotic products including designer probiotics or next-generation probiotics (Oelschlaeger, 2010; Pamer, 2016) are expected to be available in the near future. However, to be effectively used to support a profitable and sustainable global swine production, it is critical to take into consideration both the efficacy and the safety of probiotic usage.

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