Probiotic microorganisms: 100 years of innovation and efficacy. Modes of action.

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Summary

Benefits from probiotic microorganisms have been recognized for over 100 years with use in poultry for 50 years. Fuller (1989) redefined probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Benefits derived from this improved intestinal microbial balance could be reflected in performance or prevention of pathogen colonization. Probiotic microorganisms use in poultry production has been widely accepted and new opportunities arose from the 2006 EU ban on antimicrobial growth promoters. The majority of microbial products for compound feeds are made up from a relatively small number of microorganisms that are normally present in the GI tract.. They include non-sporulated bacteria, sporulated bacteria, fungi or yeasts; and presented from single to multi strain products. A review on the proposed modes of action is presented including recent approaches to quorum sensing interference.

Keywords

probiotic microorganisms, direct fed microbials, poultry, modes of action

Introduction and history

Elie Metchnikoff established in 1908 the basis of thinking for the development of what we now call probiotic microorganisms or direct fed microbials. Rusch (2002) presented a complete overview and history of the term. The origin of the term is credited to Werner Kollath who proposed in 1953 the term "Probiotika" to designate "active substances that are essential for a healthy development of life". More in the line of what we now define as probiotic microorganisms, Kolb (1955) proposed the probiotic therapy by administering symbiont cultures to prevent the deleterious effects of antibiotics. It was later used in an entirely different context by Lilley and Stillwell (1965), and Sperti (1971) to describe substances secreted by one microorganism which stimulated the growth of another (several species of protozoa, during their logarithmic phases of growth, produce substances that prolong the logarithmic phase in other species –more in line with the present definition of quorum sensing-; the term probiotic was also used in contrast with antibiotic). Parker (1974) made a definition closer to the present approach, and widely accepted: "organisms and substances which contribute to intestinal microflora balance". Several years later, Fuller (1989) redefined probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance", excluding dead organisms and other substances from the definition.

However, the term direct-fed microbials (DFM) was preferred in the US, and in 1989 the Food and Drug Administration (FDA) required manufacturers to use it rather than probiotic (Miles and Bootwalla, 1991). The FDA defined direct-fed microbials as a "source of live

(viable) naturally occurring microorganisms", and therefore included bacteria, fungi and yeast.

Competitive exclusion refers to the reduction of intestinal colonization by enteric pathogens such as *Salmonella* and *Campylobacter* by the microflora already present in the gastrointestinal tract, not necessarily improving performance. According to Garlich (1999), Greenberg used the term Competitive Exclusion for the first time in 1969 to describe the control over Salmonella by using other bacteria in Calliphoridae fly larvae. Nurmi and Rantala (1973) applied this concept in poultry and they were the first authors to apply the idea to protect chickens against Salmonella infection by inoculation with microflora from adult birds.

In the 1980's the most used probiotic microorganisms for animal feeding belonged to three bacterial and one yeast genera: *Lactobacillus* spp. (several species); *Streptococcus faecium*, *S. faecalis* and *S. salivarius*; *Bacillus cereus* var. toyoi and *B. subtilis*; *Saccharomyces boulardii,* and *S. cerevisiae*. At least 20 different biological preparations were on the market in the European Union (EU) countries at that time, being *Streptococcus faecium*, *Lactobacillus acidophilus* and *Bacillus cereus* var. toyoi the most widely distributed (at least in 8 countries each), with the latter the first probiotic microorganism authorized as a feed additive in the EU (in April 1994).

The legislation in the EU on probiotic microorganisms feed additives, including safety assessments and the Qualified Presumption of Safety (QPS) concept of microorganisms in food and feed, were comprehensibly compiled by Anadón *et al.* (2006). The same year 2006 marked the end of the use of antimicrobials as growth promoters (AGP) in the EU, and a new opportunity for further widening the use of probiotic microorganisms. The long term effects

of this ban might be inferred from Dibner and Richards (2005), who presented a review on voluntary and legislative bans of AGP, and the experience of animal producers following the 1998 ban on antimicrobials in Denmark. They concluded that replacement for AGP involves the use of multiple products in the diet and management changes to maintain animal productivity.

The Qualified Presumption of Safety (QPS) concept of microorganisms in food and feed requires a special mention. The Scientific Committee on Animal Nutrition (SCAN) expressed its position on safety assessment and regulatory aspects of microorganisms in feed and food applications in 2003 (SCAN, 2003). This system, similar in concept and purpose to the Generally Recognized As Safe (GRAS) definition used by the FDA in the USA, but adapted to the different regulatory practices in Europe, was firstly presented as a working paper for public consultation in 2003, and later debated in an European Food Safety Agency (EFSA) Scientific Colloquium on December 2004 in Brussels, Belgium. The report of this colloquium was published in 2005 (EFSA, 2005) and reflected that QPS might provide a mechanism to recognize and give weight to prior knowledge when assessing the safety of microorganisms in food and feed production.

Probiotic microorganisms for poultry

According to Jernigan *et al.* (1985) there are two different types of bacteria which can establish in the digestive tract. The first exists in close association with the gut epithelium and the second occurs free in the gut lumen. Therefore, the adhesion capacity of the microorganism would not be indispensable, and probiotic microorganisms for poultry can be designed either to establish beneficial organisms absent from the gastrointestinal tract or to provide other beneficial bacteria. Probiotic microorganisms might be directed to act in the

crop and the anterior regions of the gastrointestinal tract or mainly at the caeca. However, it is likely that either case, to some extent, they will be effective throughout the gut. Lactobacillus preparations are among the first group: they colonize the crop and small intestine, and exert their antibacterial effects against potential pathogens. *Lactobacillus* spp. may produce large amounts of lactate from carbohydrates and can withstand a high degree of acidity which is usually fatal to other bacteria. Lev and Briggs reported as earlier as 1956 that after feeding a lactobacillus culture to chicks a balanced lactic acid microflora was established in the GI tract within 24 hours.

The majority of microbial products for compound feeds are made up from a relatively small number of microorganisms: *Lactobacillus* spp. (mainly *L. acidophilus*); *Streptococcus faecium*; *Bacillus* spp.; and yeasts, especially *Saccharomyces* species. *Lactobacillus* species and *S. faecium* are normally present in the GI tract, while *Bacillus* species and yeasts are only sporadically present in the gut microflora.

Direct fed probiotic microorganism species like the lactic acid bacteria are relatively fragile. They have to be technologically protected as they do not easily tolerate the heat and pressure of feed processing (without protection, lactobacilli only resist up to 52ºC, yeasts up to 63ºC, and streptococci up to 71ºC.). However, the spores of certain *Bacillus* species are more resistant and they easily survive the pelleting process during feed manufacture. Moreover, these *Bacillus* species seem to have growth promoting effects beyond the "balancing" or "stabilizing" effects of the lactic acid bacteria, especially in pigs (Sögaard and Suhr-Jessen, 1990).

According to SCAN (2000), microbial products "able to affect or stabilize the gut flora of target animals do so only when the natural flora is in some way disturbed". Adding a

probiotic microorganism becomes a preventive measure against any detrimental effect on performance originated through the intestinal flora, and it "is reasonable to expect a microbial product not to affect animal performance where there is no significant disturbance to the flora".

Reviews on results obtained by the use of probiotic microorganisms in poultry have been published recently, and readers are referred to them for an extensive overview (Simon and Jadamus, 2002; Edens, 2003; Patterson and Burkholder, 2003; Schneitz, 2005; Revolledo *et al.*, 2006; Flint and Garner, 2009). The present review focuses on the proposed modes of action.

Mode of action of probiotic microorganisms

One of the main interests in animal production is the relationship between nutrition and gut health, especially in the small intestine. Digestion, absorption and intestinal barrier (the first line of defense against pathogens) in monogastric animals should be optimized to spend the minimal amount of nutrients for immune or anti-inflammatory responses while achieving the maximal production performance. The quality of the barrier function of the intestinal epithelium (the mucus layer, the glycocalyx and the enterocytes) warrants an optimal first line of defense. This quality is determined by host's genetics and the intestinal environment with its microflora. A normal gastrointestinal tract requires a balance of its bacterial population. This balance within the gastrointestinal tract is challenged when animals are subjected to stressful conditions such as hot weather and humidity, feed changes or imbalances, mycotoxin contamination, transportation, molting, etc. Pathogenic bacteria become harmful either through mucosal invasion or toxins production or both. Feeding probiotic microorganisms continuously to animals has been found to maintain the beneficial intestinal microflora. For

instance, several studies have demonstrated the *in vivo* efficiency of *B.* toyoi in modulating the intestinal microflora, sustaining beneficial bacteria such as lactobacilli and decreasing the presence of potential pathogens like *E. coli* and *S.* Enteritidis (Jadamus *et al.*, 2000, 2002; Simon *et al.*, 2002; Taras *et al.*, 2005; Vilà *et al.*, 2009). These findings were confirmed *in vitro* and the mechanisms of action elucidated (Calvo *et al.*, 2007): *B.* toyoi exhibited a wide range of enzymatic activities that possess a destructive activity upon gram-negative bacteria (esterases, hydrolases, phosphatases…) and possibly interfere with protein synthesis (leucine arylamidase, valine arylamidase).

The gastrointestinal tract of broilers is sterile at hatching, and immediately bacteria from the environment or the diet colonize it. After this first colonization, new bacterial species have more difficulties to colonize. A wide range of dietary factors affect the composition of the microflora. This leads to new micro-ecological conditions that allow a better colonization of some species due to improved adhesion or growth rate. Ingested bacterial species could colonize the gastrointestinal tract. This is the case when probiotic microorganisms are administered to the animals. Using probiotic microorganisms shortens the period needed to stabilize the microflora. This microflora regulation may serve three purposes: improve feed conversion and weight gain; improve the intestinal health and immune competence of the animals and suppress food-borne pathogens such as Salmonella and Campylobacter species (which is interesting for the production of "safe" meat and meat products).

In "natural" conditions, the microflora colonizing the gastrointestinal tract few days after birth consists of 400 to 500 different bacterial strains for a total count of 10^{14} bacteria. The microflora consists of transient bacteria which temporarily reside in the tract, and indigenous bacteria that colonize the intestinal tract permanently. Colonization by a bacterial species is

defined as "a bacterial population in the gastrointestinal tract which is stable in size and occurrence over time, without the need for periodic reintroduction of bacteria by repeated oral doses or other means" (Snel *et al.*, 2002). Therefore, colonizing bacteria multiply in a particular niche, at a rate equal or superior to their rate of washout or elimination. Certain species of the microflora can influence the expression of glycoconjugates of epithelial cells that may serve as receptors for adhesion of other bacteria, positively or negatively influencing in this way colonization by other species.

Snel *et al.* (2002) gave a detailed list of the mechanisms by which the microflora can contribute to intestinal health of animals and man: growth promotion; improvement of the mucosal architecture; degradation of unfermentable substrates into digestible components; improvement of intestinal and general health; breakdown of cytotoxic substances; production of vitamins; suppression of pathogens; competition for nutrients; competition for adhesion sites at the mucosal epithelium; stimulation of intestinal motility; stimulation of the immune system; production of volatile fatty acids; production of antimicrobial substances.

Microbial management practices aim to stimulate beneficial bacteria and/or suppress detrimental bacteria. This is done by suppression of certain species by including antibiotics (no longer allowed in the EU), and alternatively short chain fatty acids. Another option is to promote beneficial species in the microflora by feeding the animal suitable substrates such as oligosaccharides or other prebiotic fibres; or directly by adding beneficial bacteria to the diet of the animals.

Fuller (1977) established that lactobacillus in the crop was important in maintaining the microbial balance and also exerted its influence on the small intestine, being the inhibitory effect against *E. coli* not due to pH alone, as organic acids (such as lactic acid and acetic acid)

affecting membrane structure and oxidative metabolism; other antibacterial factors have been found to be produced *in vitro* by lactobacilli such as hydrogen peroxide, antibiotic and bacteriocin-like substances. Bacillus species also produce a large number of antimicrobials, including bacteriocin and bacteriocin-like substances. Once swallowed, food is temporarily stored in the crop where a predominantly lactic acid fermentation takes place. The pH is fairly low, and a simple microflora is present compared with that of the caeca: predominant microorganisms are lactobacilli, that produce lactic and acetic acids decreasing the pH of crop contents to 4-5 in a healthy chicken. The pH of the proventriculus and gizzard is much lower (pH 1-2) and microbial survival depends on acid tolerance.

The relatively high flow rate of the fluid content of duodenum implies little multiplication of the microorganisms. The caeca contain a thick viscous fluid, and allow the highest viable bacterial counts (counts of 10^{11} g^{-1} of contents) and most complex microflora. Most of the microorganisms present are obligate anaerobes: gram-positive, anaerobic cocci, comprise up to one third of the total; other major components include gram-negative, non-sporing rods such as the *Bacteriodaceae* (one fifth of the total); *Clostridium* spp. and bifidobacteria only represent one tenth of the total; while lower numbers of facultative anaerobes including *E. coli*, Salmonella, and Klebsiella are frequently present. Diet has more influence on the flora of the first part of the gastrointestinal tract, while little changes occur in the caeca. In the absence of stress or major selective pressures, the adult intestinal flora is relatively stable and difficult to change simply by oral administration of microorganisms. It would be easier to establish a beneficial organism soon after hatching before other organisms are able to colonize (Barrow, 1992).

Therefore, the intestinal microecology is very complex as the gastrointestinal tract is not uniform and consists of small ecological niches within which most bacteria grow in colonies enclosed in the glycocalyx, forming biofilms on the surfaces of both tissue and digesta. Bacteria are attached to their nutritive substrates by chemotaxis or form colonies in locations with high concentrations of nutrients. Tissues exposed to extreme concentrations of acids may be colonized by single species of bacteria (lactobacilli) or yeast, as special adhesion mechanisms or acid resistance are required. However, most non secretory epithelia are colonized by a rich mixture of bacteria, the majority of bacteria being associated with the viscous layer of the mucosa. They must be able to digest enzymatically the mucinous glycoproteins and to use the degradation products such as carbon, energy and nitrogen *in situ*. Also they have to overcome peristalsis in the small intestine, as well as the turnover of epithelial cells, colonizing new surfaces. The digestion of the mucus layer represents a metabolic expense for the host, as it has to replace it by continuously secreting more mucus.

As explained above, animals in "natural" conditions have in their gastrointestinal tract, and few days after birth, a population of microorganisms that protects them against disease. However, commercial production tends to limit the contact with the mother and provide unnatural environmental conditions. Modern animal husbandry, intensive or semi-intensive, brings numerous stresses. Chicks have no access to the mother and when they hatch they might be challenged by potential pathogens in the hatchery. Then they go into the brooding stage where challenge from microflora may vary from almost *nil*, due to extremely hygienic conditions, or an excessive one if the environment is dirty.

The result is that the gut microflora is deficient in some of the normal components that could provide resistance to disease. Even the microflora of more adult birds can be affected by diet,

coccidiostats or antimicrobial products, and stress. Stress affects the animals from the very beginning: travel with temperature stress or dehydration; overcrowding that leads to excessive bacterial challenge; vaccination; deficient supplies of feed or water; chilling or poor ventilation in the house; bad litter; sudden environmental fluctuations and airborne challenges that bring clinical and sub-clinical infections, etc. The use of probiotic microorganisms tries to repair these deficiencies restoring the full protective capacity of the microflora.

Probiotic microorganisms may be administered to the animal in several forms, either directly or through feed or water; continuous or multiple dosing is essential to obtain the full effect. In order to be effective, probiotic microorganisms have to be stable for long periods under normal storage and feed production conditions, and must be able to survive in the intestine of the target species to produce its beneficial effect.

In relation to the nutritional, metabolic and immunological point of view, according to Vanbelle *et al.* (1990) an ideal probiotic microorganism desirably must fulfill, the following requirements: be resistant against digestive enzymes, lysozyme, the low pH in the stomach for a few hours, also to bile salts; produce a sufficient decrease in the pH of the gut to avoid the development of pathogens and reduce the production of toxins; produce antibiotics and be resistant to in feed antimicrobials (coccidiostats); attach to the brush border cells or colonization of mucous and glycocalix, although this characteristic is not strictly necessary; obviously be present in a viable state resistant to product/feed processing and storage; and confer immune stimulation to the host. From a practical standpoint, Delbecque (1991) pointed out that the adhesion capacity of the microorganism is not indispensable, as adhesive strains disappear one week after finishing their administration; also, bioregulation by the probiotic microorganism requires an adaptation period of at least 2 weeks.

Secretion of bacteriocins

Lactobacilli and *B.* cereus have been reported to produce various types of antibiotics (Fox, 1988; Oscáriz *et al.*, 1999; Risøen *et al.*, 2004). *Lactobacillus acidophilus* produces acidophilin, lactocidin, and acidolin, and *L. plantarum* produces lactolin. Nisin and diplococcin are among the anti-metabolites produced by streptococci. *Bacillus cereus* produces bacteriocin-like substances that inhibit closely related *Bacillus* spp. and species such as *Staphylococcus aureus* and *Micrococcus luteus*; and presents high activity in the pH range of 2.0-9.0 (Risøen *et al.*, 2004). Additionally, some of the lactobacilli produce sufficient hydrogen peroxide to inhibit various microorganisms. Acidophilin, acidolin, lactobacillin, and lactocidin have demonstrated an *in vitro* inhibitory activity against Bacillus, Klebsiella, Pseudomonas, Proteus, Salmonella, Shigella, Staphylococcus, and Vibrio species and enteropathogenic *E. coli*.

In any case probiotic microorganisms are not an alternative to antibiotic treatment for acute diseases and should not be considered a "wonder medicine" against any specific disorder. Probiotic microorganisms do aid feed conversion and can be used prophylactically against enteritis. In the truest sense they are not growth promotants, but rather "growth permitants", allowing the host to best express its genetic potential.

Immunomodulation

The normal microflora of an animal has a significant impact on the body's immune system. The numbers of intraepithelial lymphocytes, plasma cells, and Peyer's patches are lower in germ-free animals than in conventional animals.

Dunham *et al.* (1993) reported that birds treated with *L. reuteri* had longer ileal villi and deeper crypts than control birds, which is a response associated with enhanced T-cell

function, and increased production of anti-Salmonella IgM antibodies. Nahashon *et al.* (1994b) found that Lactobacillus supplementation of layers diets increased cellularity of Peyer's patches in the ileum indicating a stimulation of the mucosal immune system that responded to antigenic stimuli by secreting immunoglobulin (IgA).

Khajarern and Ratanasethakul (1998) stated that when used continuously, probiotic microorganisms also served to reinforce the non specific immune system of animals, decreasing the need of anti-infectious treatments. In trials with broiler breeders, they showed that a supplementation of *B.* toyoi in feed under practical farming conditions improved not only some zootechnical variables, but also the humoral immune response. They detected higher titers and average mean values with the Newcastle Disease Haemagglutination Inhibition test (ND HI) and with Infectious Bursal Disease Virus (IBD) for the probiotic-fed birds during the four-month study. *B.* toyoi have been also demonstrated to improve humoral response in mice and piglets (Coppola *et al.*, 2005; Scharek *et al.*, 2007b) and improve systemic and intestinal immunity in piglets (Scharek *et al.*, 2007a; Schierack *et al.*, 2007).

Zulkifli *et al.* (2000) assessed the effect of antimicrobial growth promoters and probiotic microorganisms in two strains of broiler chickens (Shaver and Hubbard) also on antibody production against Newcastle disease vaccine. They supplemented feed by either 50 mg/kg oxytetracycline or 10^6 CFU/g of a lactobacillus culture and submitted birds to heat stress (36±1ºC for 3 hours daily from day 21 to 42). Before heat exposure, antibody production was not influenced by chickens' strain or feeding treatment. In contrast, after heat exposure, a significant interaction was observed: Hubbard chicks fed probiotic microorganism exhibited a greater antibody response than those given the control diet; while feeding treatments had no effect on antibody response of Shaver chickens.

Kabir *et al.* (2004) found a significantly (P<0.01) higher antibody production against SRBC, and higher spleen and bursa weights, in experimental birds provided with a multi-strain probiotic through drinking water (a product containing nine strains of several bacterial, fungal and yeast species: *L. plantarum*, *L. bulgaricus*, *L. acidophilus*, *L. rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopessi*) as compared to control birds (6.0 $\log_2$ *vs.* 2.8 $\log_2$ respectively).

Khaksefidi and Ghoorchi (2006) evaluated the influence of dietary supplementation of probiotic *Bacillus subtilis* on performance and immunocompetence in broiler chicks. At 7, 14, 21 and 28 days of age, twenty birds per dietary group were injected intravenously (brachial vein) with 0.1ml of 0.5% sheep red blood cell (SRBC). The probiotic microorganism had positive effect on production and persistency of antibody in response to SRBC antigen. Also, antibody production against Newcastle disease virus in probiotic microorganism supplemented group was significantly higher at 10 days of post immunization compared to control. The results suggested that the use of probiotic containing *Bacillus subtilis* had positive effect on performance and immune system of broiler.

Higgins *et al.* (2007) hypothesized that the innate immune system of chickens, specifically macrophages, played a role in reduction of *Salmonella* Enteritidis colonization with probiotic treatment (lactobacillus-based probiotic culture FM-B11). Chicks were challenged or not with *S.* Enteritidis at day of hatch and treated or not with the probiotic culture 1 hour later in a factorial design. Probiotic microorganism treatment on the day of hatch reduced (P<0.05) cecal *S.* Enteritidis recovery as compared with the control treatment, but the modest differences detected in two out of four experiments, and the fact that those differences were not repeatedly detectable, suggested them that the macrophage-related changes were not

322 solely responsible for the reductions of *S.* Enteritidis following probiotic microorganism
323 treatment.

324 Interference with quorum sensing signaling agents

325 Bacteria communicate to each other using chemical signaling molecules (called autoinducers).
326 This phenomenon is known as quorum sensing, which allows bacteria to measure the
327 population density, nutrient concentration and other ecological characteristics, and to
328 coordinately control the gene expression of the entire community in response to changes in
329 cell number or niche conditions (Schauder and Bassler, 2001).

330 Highly specific as well as universal quorum sensing languages exist which enable bacteria to
331 communicate within and between species. Also, prokaryotic and eukaryotic mechanisms that
332 interfere with bacterial quorum sensing have evolved. Specifically, the secretion of enzymes
333 that degrade the autoinducers, or the production of autoinducer antagonists, are recourses used
334 by competitor bacteria and susceptible hosts to render quorum sensing bacteria mute and deaf,
335 respectively. Analogous synthetic strategies are being explored for the development of novel
336 antimicrobial therapies (Schauder and Bassler, 2001) and might also be used by probiotic
337 microorganisms.

338 Most gram negative bacteria use N-acylhomoserine lactone (AHL) signals to monitor their
339 own population density. A *luxR*-like protein is responsible for recognition of the AHL
340 autoinducer; this protein of the *luxR* type have a domain for binding AHL and a second
341 domain for binding DNA, and subsequently activates the transcription of downstream target
342 genes. The proteins of the *luxI* type catalyze the final step in AHL synthesis, each *luxI*
343 homolog makes a specific AHL, which differ primarily in the acyl chain length and the nature

of the substituents at the C-3 position. In addition to the *luxI* family of AHL synthases, other synthases types have been described (Michael *et al.*, 2001).

In general, each bacterial cell in a population produces AHL, and as the population density increases, the concentration of AHL also increases. Above a threshold concentration, the LuxR homolog binds AHL and activates transcription of target genes (one of the target genes is often the *luxI* homolog, which results in a positive feedback) (Michael *et al.*, 2001).

Many bacterial behaviors have been shown to be regulated by AHLs, including plasmid conjugal transfer, protein secretion, synthesis of exoenzymes, cytotoxins, antibiotics, and capsular exopolysaccharide, biofilm formation, and motility (Michael *et al.*, 2001).

Escherichia coli and *Salmonella enterica* serovar Typhimurium encode a single *luxR* homolog named *sdiA*. Virulence functions in γ-proteobacterial pathogens are controlled by a transcription factor encoded by *uvrY* orthologs. However, *Escherichia* spp., *Salmonella* spp., and *Klebsiella* spp., are the only genera that present this gene downstream of *sdiA*. It is also surprising that although these three genera possess a copy of *sdiA*, they are not known to synthesize the AHLs that are typically detected by *luxR* homologs. In fact, there are no AHL synthase genes (*luxI* or *luxLM* homologs) in any of the available genome sequences for these organisms. Therefore, Escherichia, Salmonella, and Klebsiella appear to be unusual with regard to quorum sensing in that they encode a putative AHL receptor, SdiA, but not an AHL synthase (Michael *et al.*, 2001).

Production of autoinducers is not limited to pathogenic bacteria. Many commensal and potentially probiotic bacteria such as lactobacillus, bifidobacterium, or *B. cereus* strains, i.e., possess a *luxS* homologue and can produce autoinducers (Auger *et al.*, 2006; Lebeer *et al.*, 2007). The toxicity of *E. coli* O157:H7 is developed once the bacteria have attached to host

intestinal epithelial cells, and genes involved in attachment are directly activated by quorum sensing. The (human) probiotic *Lactobacillus acidophilus* La-5 secretes a molecule that acts inhibiting the quorum sensing signals or directly interacts with bacterial transcriptionals regulators, controlling the transcription of *E. coli* O157 genes involved in colonization and avoiding bacteria toxicity (Medellin-Peña *et al.*, 2007). Degradation of AHL by *B. cereus* has also been described by Medina-Martinez *et al.* (2007). Cerdà-Cuéllar *et al.* (2009) also demonstrated the ability of *B.* toyoi to degrade AHL, partly explaining the action mechanisms of this probiotic microorganism.

From the previous papers it can be concluded that quorum sensing regulates the virulence expression in some microorganisms and probiotics may interfere with this signaling system avoiding the onset of virulence.

Implications

The demonstration of the importance for animal's health of the normal microflora was vital for the development of probiotic microorganisms in poultry production. In practice it is difficult to keep flocks clean of pathogens, even if chicks come free of infection from the hatchery. In the case of Salmonella, it may be that some birds are undetected carriers and start shedding when stressed, or that Salmonella remained in the house or came through the feed, trucks, visitors, air, wildlife or personnel. Problems may arise if a challenge with these microorganisms happens before a normal microflora has been established. Additionally, if birds require treatment for any disease, the chemotherapeutic or antibiotic used may cause a disruption in the intestinal microflora and Salmonella or Campylobacter may be allowed to infect or emerge.

Feeding probiotic microorganisms on a continuous basis and from the very beginning helps to develop and stabilize very soon (after hatching or disruption) a "competent" microflora that should successfully avoid the proliferation of pathogens, especially when these stresses might come.

The microflora of the gastrointestinal tract of the chickens is crucial to avoid colonization by potential pathogens; interference with quorum sensing signals may play an important role on that. The principal locations of risk are the crop, the first site for colonization following ingestion, and the caeca, the main colonization site for most pathogens including Salmonella and Campylobacter. Although Salmonella is firstly thought as the food poisoning cause in the public mind, Campylobacter species actually cause more outbreaks in man than Salmonella.

Meat and meat products may pose a risk if contaminated with pathogenic microorganisms such as Salmonella and Campylobacter. To improve food safety, the industry is requested to decrease the level of contamination to zero or at least to acceptable levels. Several intervention strategies are been applied starting at the breeding and farm level through the final product. Part of these intervention strategies are the use of probiotic microorganisms and competitive exclusion microflora, used for prophylactic and curative purposes.

Gut microfloral enzymes are also beneficial to the nutrition of the host because they increase the digestion of nutrients, especially in the lower intestine, and suppress ammonia production and urease activity, which in turn can improve animal health and enhance growth because ammonia produced by ureolysis in the intestinal mucosa may significantly damage the surface of the cells.

Other effects of probiotic microorganisms include enterotoxin neutralization or synthesis inhibition by interfering with quorum sensing signals and stimulation of the immune system.

Enterotoxins produced by pathogenic bacteria may be neutralized by substances produced by a probiotic microorganism or signaling molecules for transcription might be degraded before reaching its target. Lactobacilli could be important in the development of immune competence in animals, especially when protection must be acquired against antigens that will probably cause gut inflammatory reactions.

Conclusions

Evidence suggests that probiotic microorganisms affect the gut flora of target animals and consequently not only improve performance, but also immune and health status of the animal. The response obtained might also be reflected in greater consistency in performance rather than any overall improvement as stated by the SCAN (2000); this might happen when animals are raised in good conditions. Therefore, "using a probiotic microorganism can also be seen as providing an insurance policy against any detrimental effect on performance mediated through the intestinal flora" (SCAN, 2000).

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