Lactic Acid Bacteria-based probiotic

The selection of individual enteric bacteria capable of inhibiting Salmonella growth in vitro and the ability of selected oxygen tolerant bacteria, in combination, to protect normal flora from Salmonella infection following challenge. Concurrently, they also worked toward the isolation, selection, further evaluation and combination of LAB to control additional foodborne pathogens. Extensive laboratory and field research conducted with this defined LAB culture has demonstrated accelerated development of normal microflora in chicken and turkeys, providing increased resistance to Salmonella age infections (Fennell et al., 2006). J. P. Higgins et al., 2007; J. P. Higgins et al., 2006; J. P. Higgins et al., 2010; S. H. Higgins et al., 2008; Vacanti et al., 2006). Published experimental and commercial studies have shown that these selected probiotic bacteria are able to reduce idiopathic diarrhea in commercial turkey breeding houses (H. J. Higgins et al., 2005). Large scale commercial trials indicated that appropriate administration of this probiotic mixture to turkeys and chickens increased production and reduced costs of production (Torniello-Rodriguez et al., 2008; Torniello-Rodriguez et al., 2007; June et al., 2007; June et al., 2005; Vacanti et al., 2006c). These data have clearly demonstrated that selection of therapeutically efficacious probiotic cultures with minimal performance benefits in poultry is possible, and that defined cultures sometimes can provide an alternative to conventional antimicrobial therapy. Two products developed in our laboratory include Fornisin B17® (B17) and Sporulin® [http://www.fornisin.com] for more information.

B17 to BMD for Control of Necrotic Enteritis in a Laboratory Challenge Model

**Introduction**

In the United States, a number of performance-enhancing antimicrobials are still permitted for use in broiler and turkey production. Increasing regulatory pressures and consumer preferences have driven a marked increase in poultry meat production when labeled as “Raised Without Antibiotics,” a label that includes interferences for coccidiosis control. While attempts to control human foodborne pathogens, poultry producers are simultaneously challenged to improve production in the face of increasing food costs while using fewer antibiotics due to increased resistance of antimicrobial usage. Sustainable alternatives to antibiotic growth promoters for animal production include probiotics or direct feed modifiers (DFM) consisting of live or dead organisms and spore(s). Probiotics and DFM used in animal feed are becoming accepted as potential alternatives to antibiotics for use as growth promoters, and in some cases, for control of specific enteric pathogens. The most common type of probiotics that have been independently effective involve some, but not all, probiotics and bacteria (LAB). These bacteria are found normally in the gastrointestinal tracts (GIT) of vertebrates and invertebrates, and the use of some LAB cultures are able to restore the normal microflora within the gut (Bending & Applied, 1990). Lactic acid bacteria include the genera Lactobacillus, Bifidobacterium, and others that have long been associated with health benefits and which have been used for fermentation of certain foods. While speculation of members of these groups is difficult and inconsistent, these organisms are considered amendably safe and are not associated with disease in healthy animals or humans (Teller et al., 2008). A second classification of probiotic cultures are those microorganisms that are not normally found in the GIT (such as allelochemical flow). For example, spore-forming bacteria, normally members of the genus Bacillus, are not normally found in high numbers in the GIT, but clearly can enhance performance and disease resistance under some conditions.

**Lactic Acid Bacteria-based probiotic**

In the presence of the vaccines shown by the development of the LAB probiotics for use in commercial poultry as described here, there is still an urgent need for commercial probiotics that are shelf-stable, cost-effective and feed-stable (tolerance to heat polymerization process) to meet compliance and widespread adoption. Among the large number of probiotic products to use today some are bacterial spore formers, mostly of the genus Bacillus. Used primarily in their spore form, some though not all have been shown to prevent selected gastrointestinal disorders and the diversity of species used and their applications are expanding. There is scientific evidence suggesting that some but not all isolates of ingested B. subtilis spores can, in fact, germinate in the small intestines (Casal & Cutting, 2002; Casal & Cutting, 2002; Univ. & Cutting, 2003; Univ. & Cutting, 2001). Together, these studies not only show that spores are not transient passengers in the gut, but that they have an intimate interaction with the host cells or microorganisms that can enhance their potential probiotic effect. Several commercial spore-forming Bacillus cultures have been shown to reduce food borne pathogens (Ajumma et al., 2010). Holohan (2010) and associates identified with achieving various concentrations of spores in food have greatly limited commercial acceptance in the animal industry (Hog et al., 2005).

Importantly, well-designed commercial trials have indicated that Bacillus cultures used as effective as a well-established LAB-based probiotic for Salmonella reduction in poultry (Rodriguez R.E. et al., 2010; Shrivastava et al., 2011), and was equal to treatments for prevention of experimental necrotic enteritis, and was able to markedly reduce necrotic enteritis lesions in large scale food trials (unpublished data). Other isolates or combinations of isolates with increased potency and efficacy may be identified with continued research. Some of these environmental Bacillus isolates have been evaluated in vivo for antimicrobial activity against selected bacterial pathogens, heat stability, and the ability to grow to high numbers. Unpublished experimental evaluations have confirmed improved body weight gain, as well as Salmonella, in vitro Lactobacilli probiotics reduction in commercial turkey and broiler operations when compared with medicated (intestine) or control medicated respectively. Indeed, preliminary data suggests that these isolates could be an effective alternative to antibiotic growth promoters for commercial poultry.

Immunological efficacy of amplification and operation is absolutely essential to gain widespread industry acceptance of a cost-effective probiotic for more modern foodborne intervention, as well as cost effectiveness.Reasonably, both vaccination and growth rates have been demonstrated, which may lead to new efficiencies for commercial amplification and manufacture of a cost-effective product at very high spore counts (Rodriguez R.E. et al., 2010). In order to select even more effective isolates, current research is focused on the mechanistic action of new Bacillus cultures. Preliminary studies indicate a potential mechanism in these new Bacillus cultures that may partially involve rapid activation of innate host immune mechanisms (system or response) in chickens and turkeys (unpublished data). This data provides an exciting possibility for identification of vastly superior and more potent probiotics in the near future.

**Microarray Analysis**

We have recently investigated the effects of B17 with or without Salomona challenge on transcriptional profiling of cecal gene expression in chickens (Higgins, 2013). This study, in addition to broiler chickens were challenged with Salmonella enterica serovar Enteritidis (SE) and treated 18 h later with the poultry-derived, LAB culture (B17, discussed above). Cecal were collected 24h after infection and RNA isolation for macroarray analysis of gene expression. As both 12 and 24 h, SE was significantly reduced in chicks treated with the probiotics as compared with the birds challenged with only SE (P < 0.05). Microarray analysis revealed gene expression differences among all treatment groups. At 12 h, 170 genes were exposed at significantly different levels (P < 0.05) with the most differential expression of 1-2 fold. At 24 h, the number of differentially regulated genes with a minimum 1-2 fold change was 201.

**Discussion**

Work in our laboratories during the last 12 years has resulted in development and commercialization of two products that are globally available and used in several countries for reduction of enteric bacterial infections, including those associated with food-borne pathogens. Furthermore, these products have been shown to reduce Salmonella infections in both field and laboratory evaluations. Emphasis, effects are seen on diarrhea-related lesions and mortality in birds 2-3 weeks post-treatment. These observations revealed that at both 12 and 14 d, host genes associated with the nuclear factor kappa B (NF-κB) signaling pathways. Salmonella infection analysis, probiotic-induced differential regulation of the genes growth Arrest-specific 2 (GAS2) and cytokine-rich, angiogenic inducer (A3 (CPI96)) may result in increased apoptosis in the cecum of chickens. Because Salmonella is an intracellular pathogen, we suggest that increased apoptosis may be a mechanism by which the probiotic cultures reduce Salmonella infection. These may be the first evidence indicating that highly efficacious probiotics/DPM treatments may suppress both host gene expression and associated pathways and may provide clues for selection of even more effective strategies for replacing antibiotics.