Jade Passey, Madridge J Food Technol. 2017
http://dx.doi.org/10.18689/2577-4182.a1.003

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International Probiotics and Antimicrobial Proteins Conference

November 6-8, 2017 Barcelona, Spain

Lactobacilli Probiotics as a Potential Control Strategy for Avian Intestinal Spirochaetosis

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Brachyspira species are the causative agents of avian intestinal spirochaetosis (AIS). AIS is a gastrointestinal disease whereby *Brachyspira* colonise the caeca and colo-rectum of poultry, primarily layer hens, resulting in diarrhoeal disease and consequently a 6-10% reduction in egg production, poor egg quality and faecally stained eggs. It is estimated that AIS costs the UK poultry industry £18 million per annum. Prevalence of *Brachyspira* in the UK has increased significantly in recent years, with up to 90% of free range hens and 74% of barn hens testing positive for *Brachyspira* species. Furthermore emerging antimicrobial resistance is a concern. Therefore, a better understanding of the pathobiology and novel measures to mitigate this economically important disease are urgently required.

Eight avian *Brachyspira* isolates from the four clinically relevant species, *B. pilosicoli, B. intermedia, B. alvinipulli* and *B. innocens* were selected and subjected to genetic and metabolic analysis using the Biology system and next-generation whole genome sequencing, in order to further understand the genus.

Additionally, to investigate the mechanisms by which *Lactobacillus* may mitigate AIS, sixteen *Lactobacillus* isolates were isolated from chicken faeces and characterised using 16S rRNA sequencing, biochemistry and next-generation sequencing. *Lactobacillus* isolates were screened for *in vitro* antimicrobial activity against *Brachyspira* species. These studies indicated that the cell free supernatant (CFS) from all *Lactobacillus* strains significantly inhibited *Brachyspira* growth in a pH-dependent manner ($p \leq 0.01$). Furthermore, the CFS from three *Lactobacillus* strains consistently inhibited the growth of all test *Brachyspira* strains in a pH-independent manner ($p \leq 0.01$). This suggests that the inhibition of *Brachyspira* is not solely attributed to acidity. Subsequently, nuclear magnetic resonance spectroscopy (NMR) was used to identify metabolites secreted into the CFS which may have the potential to inhibit *Brachyspira.* In addition to lactic and acetic acid, other metabolites such as acetoin were identified as having potential antimicrobial properties.

Furthermore, these three *Lactobacillus* isolates were able to physically interact with *Brachyspira* in order to significantly impair growth and motility ($p \leq 0.05$).

Collectively, these data suggest that physical interactions between *Lactobacillus* and *Brachyspira* are important factors in mitigation of disease and that secreted compounds from *Lactobacillus* have inhibitory effects against *Brachyspira*. Further study to explore these mechanisms is required.