POSTER PRESENTATIONS

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ANTI-SALMONELLA LACTIC ACID BACTERIA FROM PORCINE INTESTINAL SOURCES

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Summary: The aim of this study was to isolate lactic acid bacteria (LAB) with anti-Salmonella activity from the porcine gastrointestinal tract (GIT) and to characterise these for potentially probiotic properties using in vitro assays. Porcine caecal and faecal samples were screened for the presence of anti-Salmonella LAB; the ten most promising isolates belonged to the genera Lactobacillus and Pediococcus. The LAB exhibited large variation in their ability to survive in simulated gastric juice at pH 1.85. While Lactobacillus acidophilus species survived at up to 80% for 30 min, Lb. pentosus species declined to less than 0.001%. All isolates tolerated porcine bile at a concentration of 0.3%, with some capable of growth in the presence of up to 5% bile. The ability of the LAB isolates to prevent Salmonella invasion of intestinal epithelial cells varied, with reductions of 55% (Lb. acidophilus spp.) to 82% (Lb. salivarius spp.) observed. The data demonstrates that some porcine intestinal LAB isolates may offer potential as probiotics for the reduction of Salmonella carriage in pigs.

Keywords: probiotic, pigs, Salmonella, Lactobacillus, pathogen

Introduction: Probiotics offer potential to reduce intestinal Salmonella carriage in pigs. Potentially probiotic bacterial cultures must possess certain properties if they are to function effectively in the intestine. Of prime importance amongst these is the ability to survive passage through the GIT; consequently, acid and bile tolerance are important criteria, as well as an ability to exert the probiotic effect at the target site. In this study, LAB isolates were characterized in relation to potentially probiotic traits using a range of in vitro procedures.

Materials and Methods: LAB were initially isolated from porcine faecal and caecal samples by growth in Brain Heart Infusion (BHI) broth and both liquid and solid de Man Rogosa Sharpe (MRS) medium. Isolates with anti-Salmonella activity were identified by spot plating onto MRS agar and, following overnight incubation at 37°C, overlaying with a lawn of Salmonella Typhimurium in BHI agar; these isolates were then stocked. The ten isolates with greatest anti-Salmonella activity were identified and further characterized. Tolerance to gastric juice was examined by suspending overnight cultures in synthetic gastric juice adjusted to pH 1.85 and incubating at 37°C. After 30 min, viability was measured by plating on MRS agar. The synthetic gastric juice solution consisted of 3.5 g/l D-glucose, 2.05 g/l NaCl, 0.6 g/l KH₂PO₄, 0.11 g/l CaCl₂, 0.37 g/l KCl, 0.05 g/l porcine bile, 0.1g/l lysozyme and 13.3 mg/l pepsin. Tolerance to bile was assayed by streaking cultures onto MRS plates containing porcine bile at concentrations of 0 – 5% (w/v) and examining the plates for growth after 72 h. The ability of the isolates to prevent invasion of HT-29 human intestinal epithelial cells by Salmonella was investigated. Volumes (1 ml) of overnight isolate resuspended in Dulbecco’s Modified Eagle Medium (DMEM) were added to wells of washed confluent HT-29 cells in six-well Corning tissue culture plates and incubated at 37°C for 1 h. One ml volumes of similarly-treated Salmonella Typhimurium DT104 cells, adjusted to give a multiplicity of infection ratio of 100:1, were then added and incubation continued for another hour. Bacterial cells were then removed from wells and DMEM containing 100 mg/ml gentamicin was added and plates were incubated for a further hour. The monolayers were then washed several times with phosphate buffered saline (PBS) and lysed; the resultant lysate was serially diluted and
Salmonella counts were obtained by plating on tryptic soya agar (TSA) plates. Control wells were treated similarly except that DMEM was added instead of isolate suspension.

**Results:** Initial examination of approximately 6000 colonies resulted in 173 isolates giving zones of inhibition with a radius of greater than 5 mm. The ten isolates exhibiting the greatest anti-Salmonella activity in these assays were identified by 16S rRNA sequencing (Table 1). Survival of these isolates in synthetic gastric juice (pH 1.85) varied from 0.004% to 79.29% after 30 min exposure (Table 1). 

*Lb. acidophilus* 4 survived better than any other isolate; indeed its survival (79.29%) was approximately 200 times greater than that of *Lb. salivarius* 18, the next best surviving isolate. *Lb. salivarius* strains performed well, with isolates DPC6005, 7.3 and 18 surviving relatively well. All isolates tested grew in conditions of 0.3% bile, while the maximum level tolerated varied from this concentration up to 5% (Table 1). *Lb. acidophilus* 4 and *Lb. pentosus* DPC6004 both grew in the presence of 5% bile. Several isolates, particularly *Lb. acidophilus* 4, were associated with a cloudy zone of precipitation in the medium which may be indicative of bile salt hydrolase production (Dashkevicz and Feighner, 1989). All isolates tested adhered to HT-29 intestinal cells (data not shown). When examined for their ability to prevent *Salmonella* invasion of HT-29 cells where the control represented 100% *Salmonella* invasion, levels of invasion in the presence of isolates varied from 18.57% to 46.02% (Table 1). The greatest reduction in invasion was associated with the *Lb. salivarius* strains DPC6005, 7.3 and M7.2. Isolates *Lb. acidophilus* 4 and *Lb. pentosus* DPC6004 were the least effective in inhibiting *in vitro* *Salmonella* invasion (Table 1).

<table>
<thead>
<tr>
<th>Isolate</th>
<th><em>Salmonella</em> inhibition</th>
<th>Gastric juice survival</th>
<th>Bile Tolerance</th>
<th>Invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lb. murinus</em> DPC6002</td>
<td>6.3</td>
<td>0.0029&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3</td>
<td>21.96</td>
</tr>
<tr>
<td><em>Lb. murinus</em> DPC6003</td>
<td>6</td>
<td>0.0021&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3</td>
<td>21.76</td>
</tr>
<tr>
<td><em>Lb. pentosus</em> DPC6004</td>
<td>6.7</td>
<td>0.0024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0</td>
<td>44.17</td>
</tr>
<tr>
<td><em>Lb. salivarius</em> DPC6005</td>
<td>9</td>
<td>0.0391&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5</td>
<td>18.57</td>
</tr>
<tr>
<td><em>P. pentosaceaeus</em> DPC6006</td>
<td>7.3</td>
<td>0.0026&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0</td>
<td>26.87</td>
</tr>
<tr>
<td><em>Lb. acidophilus</em> 4</td>
<td>6</td>
<td>79.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.0</td>
<td>46.02</td>
</tr>
<tr>
<td><em>Lb. agilis</em> 13</td>
<td>9</td>
<td>0.0004&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.3</td>
<td>36.84</td>
</tr>
<tr>
<td><em>Lb. salivarius</em> 18</td>
<td>6.8</td>
<td>0.3732&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.3</td>
<td>30.47</td>
</tr>
<tr>
<td><em>Lb. salivarius</em> 7.3</td>
<td>7</td>
<td>0.023&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3</td>
<td>20.89</td>
</tr>
<tr>
<td><em>Lb. salivarius</em> M7.2</td>
<td>8.3</td>
<td>0.0028&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.3</td>
<td>21.33</td>
</tr>
<tr>
<td>Control</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>1</sup>Radius (mm) of zones of inhibition produced by isolates in plate assays with *Salmonella* Typhimurium.

<sup>2</sup>Results are expressed as percentage survival after 30 minutes in synthetic juice, pH 1.85. Different superscripts represent values statistically different from each other (P < 0.05).

<sup>3</sup>Values represent the maximum concentration of bile (% w/v) at which growth was observed on MR<sub>S</sub> plates.

<sup>4</sup>Inhibition by porcine isolates of *Salmonella* invasion of HT-29 intestinal epithelial cells. Results are expressed as percentage invasion in the presence of cultures (control = 100% *Salmonella* invasion).

**Discussion:** In this study, ten porcine bacterial intestinal isolates were selected on the basis of anti-*Salmonella* activity and investigated for their probiotic potential. The first major hurdle bacteria must overcome in the GIT is the low pH of the gastric contents. In simulated gastric transit studies using synthetic gastric juice at pH 1.85, *Lb. acidophilus* 4 exhibited the strongest survival after 30 min; this is in agreement with previous reports linking *Lb. acidophilus* with good survival under acidic conditions (Marteau et al., 1997). This isolate also survived well in the presence of bile, a major impediment to bacterial survival in the small intestine; however, it was also apparently a producer of bile salt hydrolase, which has been linked to colorectal cancer (Nagengast et al., 1995). Levels of *Salmonella* invasion of intestinal epithelial cells in the presence of isolates spanned a relatively narrow range,
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Effects of commercial feed additives on Porcine intestinal microflora

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Summary: The objective of this study was to assess the effects of commercially available feed additives on the gut microflora of finishing pigs. Pigs received either a barley-based control diet, or an experimental diet supplemented with mannanoligosaccharide (BioMOS™), fumaric acid, or a commercially available acid/salt mixture (Bact-A-Cid™) for four weeks prior to slaughter. Dietary supplementation with fumaric acid (20 g/kg) resulted in the greatest effects on gut microflora composition. Following 28 days of treatment, faecal coliforms and lactobacilli numbers were reduced in the fumaric acid-fed animals (P<0.05). In addition, there was a ten-fold reduction in lactobacilli in the caecum and colon due to fumaric acid treatment (P<0.05). The data indicate that supplementation with fumaric acid caused a desirable change in coliform numbers. However, given that Lactobacillus are considered beneficial microorganisms in the mammalian intestine, the reduction in lactobacilli counts as a result of fumaric acid supplementation warrants further investigation.

Keywords: pigs, gastrointestinal tract, bacteria, acid, mannanoligosaccharide

Introduction: A possible means of controlling carcass contamination with salmonellae and reducing consequent public health concerns, is to reduce intestinal carriage of the organism in finishing pigs by incorporation of additives into the feed. Reduced numbers of pathogenic bacteria in weanling pigs supplemented with organic acids and salts (Canibe et al., 2001; Tsiloyiannis et al., 2001) has been attributed to reduced pH and dissociation of acid molecules in the cell cytoplasm. Dietary supplementation with mannanoligosaccharide (MOS) or D-mannose has been shown to effectively reduce colonisation by Salmonella Typhimurium in broiler chicks (Spring et al., 2000), and Escherichia coli K88 in piglets (White et al., 2002) by reduced adherence of pathogenic bacteria to epithelial cells.

Conclusions: The results of the present study support the potential use of some porcine intestinal LAB as probiotics for the reduction of Salmonella carriage in the pig GIT.

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References: