Identification of bile salt hydrolase inhibitors, the promising alternative to antibiotic growth promoters

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Antibiotic growth promoters (AGPs)

- AGPs: a group of antibiotics used as feed additives (low dose) in food animal industry for more than five decades.
- AGPs can improve daily weight gain and feed efficiency of various food animals.
Food safety and public health concerns for AGP usage

• Emergence and prevalence of drug-resistant foodborne pathogens are correlated with the use of AGPs.

• AGP ban
  – 1998 in Denmark
  – 2006 European Union

• To date, there is a worldwide trend of limiting AGP use in food animals.

• Ending the use of AGPs creates challenges for the animal feed industries.
Mode of action of AGPs

• The precise mechanisms are still not clear.
• It is widely accepted that AGP usage affects gut microbiota and results in an optimal and balanced microbiota for enhanced growth performance.
• Examination of the effect of AGP on intestinal microbiota is important for developing novel alternatives to AGPs.
AGP usage and chicken intestinal microbiota

- **Experiment**
  - Diet:
    - Non-medicated (control)
    - Medicated
      - Day 1-32 → salinomycin & bacitracin
      - Day 33-42 → virginiamycin

- **Body weights** determined on days 7, 14, 21, 32, 42.
  - At day 14, 32 and 42, one bird from each pen whose body weight was nearest the mean for the pen was removed for intestinal sample collection.
Effect of AGP on gut microbiota
Culture-independent approaches

- Phylochip
- 16S rDNA libraries analysis

Luminal fecal samples collected at day 42 from ileum of 8 birds (4 control, 4 medicated) were used for rDNA library construction (768 clones).

<table>
<thead>
<tr>
<th>Bacterial identity</th>
<th>Number of Clones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Lactobacillales</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>45</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>3</td>
</tr>
<tr>
<td>Erysipelotrichales</td>
<td></td>
</tr>
<tr>
<td>Turicibacter</td>
<td>199</td>
</tr>
<tr>
<td>Clostridiales</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcaceae</td>
<td>120</td>
</tr>
<tr>
<td>Total</td>
<td>369</td>
</tr>
</tbody>
</table>
AGPs reduced the population of *Lactobacillus* in the chicken intestine

- *L. salivarius*: the dominant lactic acid bacterium present in the intestine
- *Lactobacillus* species are the major commensals that produce bile salt hydrolase (BSH) in the intestine.

Bile Salt Hydrolase (BSH)

- **Bile salts**: synthesized in the liver and conjugated with either glycine or taurine prior to secretion
- The conjugated bile salts are needed to maintain efficient lipid digestion and absorption
- Function of BSH

![Bile Salt Structure](image)

The growth-promoting effect of AGPs was highly correlated with the decreased BSH activity in the intestine


• Knarreborg et al., 2004: *J Nutr* **134**:1487-1492.

• Guban et al., 2006: *Poult Sci* **85**:2186-2194.
Hypothesis

Inhibition of BSH activity using specific inhibitors is a promising approach to promote feed efficiency and weight gain in food animals.
**Lactobacillus salivarius** NRRL B-30514

- A chicken isolate that produces bacteriocin
- Display potent BSH activity to hydrolyze conjugated bile salts

*Stern et al.* 2006. Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. *Antimicrob Agents Chemother* **50**:3111-3116.
Whole Genome Sequencing of *L. salivarius* NRRL B-30514 (454 FLX Titanium)

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
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<tbody>
<tr>
<td>Total number of reads</td>
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<tr>
<td>Total number of bases</td>
<td>96,071,065</td>
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<tr>
<td>Average read length</td>
<td>357</td>
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<td>No. of total bases in all contigs</td>
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<tr>
<td>No. of total bases in large contigs</td>
<td>1,892,975</td>
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<tr>
<td>No. of all contigs</td>
<td>108</td>
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<tr>
<td>No. of large contigs</td>
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<tr>
<td>Size (bp) of large contigs</td>
<td>502 to 170,991</td>
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<td>Average coverage depth fold</td>
<td>50</td>
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Automatic annotation by the RAST server (http://rast.nmpdr.org/)

# of ORFs: 1878
Identification of two BSH genes

- **BSH1**: contig 107
- **BSH2**: contig 7
Expression and Purification of rBSH

Lane 1: Cell lysate w/o IPTG induction.

Lane 2: Cell lysate w IPTG induction

Lane 3: Purified His-tagged rBSH
**Activity and kinetics of the rBSH for different bile salts**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>BSH activity µmol/min/mg</th>
<th>Relative activity (%)</th>
<th>$K_m$ (mM)</th>
<th>$k_{cat}$ (min$^{-1}$)</th>
<th>$k_{cat}/K_m$ (min$^{-1}$mM$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCA</td>
<td>7.7±0.48</td>
<td>41.9</td>
<td>1.71</td>
<td>532</td>
<td>311</td>
</tr>
<tr>
<td>GDCA</td>
<td>4.0±0.54</td>
<td>22.3</td>
<td>1.15</td>
<td>382</td>
<td>332</td>
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<tr>
<td>GCDCDA</td>
<td>17.7±1.18</td>
<td>100</td>
<td>2.48</td>
<td>938</td>
<td>378</td>
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<tr>
<td>TCA</td>
<td>5.6±0.33</td>
<td>31.4</td>
<td>3.21</td>
<td>585</td>
<td>182</td>
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<tr>
<td>TDCA</td>
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<td>252</td>
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<tr>
<td>TCDCDA</td>
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<td>2.53</td>
<td>510</td>
<td>201</td>
</tr>
</tbody>
</table>

The BSH displayed efficient hydrolysis activity for both glycoconjugated and tauroconjugated bile salts.
Effect of pH and Tm on BSH Activity

BSH maximum activity occurred at pH 5.5

The BSH has the highest activity at around 41°C
Inhibitory effect of various feed additives on the BSH activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition</th>
<th>Compound</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuCl$_2$</td>
<td>98.1</td>
<td>CuSO$_4$</td>
<td>91.7</td>
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<tr>
<td>ZnCl$_2$</td>
<td>68.3</td>
<td>ZnSO$_4$</td>
<td>89.5</td>
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<tr>
<td>MnCl$_2$</td>
<td>68.1</td>
<td>MnSO$_4$</td>
<td>83.1</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>73.0</td>
<td>FeSO$_4$</td>
<td>96.1</td>
</tr>
<tr>
<td>KCl</td>
<td>25.9</td>
<td>NaSeO$_3$</td>
<td>93.1</td>
</tr>
<tr>
<td>NaCl</td>
<td>27.7</td>
<td>NaSO$_4$</td>
<td>27.7</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>25.7</td>
<td>MgSO$_4$</td>
<td>31.3</td>
</tr>
<tr>
<td>NaIO$_3$</td>
<td>88.8</td>
<td>KIO$_3$</td>
<td>92.9</td>
</tr>
<tr>
<td>CoCl$_2$</td>
<td>95.9</td>
<td>NaHCO$_3$</td>
<td>20.6</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>22.4</td>
<td>Vitamin C</td>
<td>21.8</td>
</tr>
</tbody>
</table>
Copper/zinc have been used at high concentrations to aid in feed efficiency and growth promotion.

**Poultry**

**Swine**
Discovery of potent, safe, and cost-effective BSH inhibitors

• Screen more compounds including emerging feed additives (e.g. dietary plant bioactives)
• High-throughput screening (HTS)
  – We have developed a rapid, convenient, and effective HTS system.
Conclusions

• Identified and characterized a BSH with broad substrate specificity from a chicken *L. salivarius* strain.

• Established a solid platform for us to discover novel BSH inhibitors, the promising feed additives to replace AGPs for enhancing the productivity and sustainability of food animals.
Acknowledgment

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Questions?