Food related streptococci beyond *Streptococcus thermophilus*: friends or foes?

A comparative genomics approach

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Laying the background: Fermented foods and Streptococci

Commensal species including:

- GAS
- GBS
- *Streptococcus pneumoniae*

*Streptococcus thermophilus*
Laying the background: Fermented foods and Streptococci

Identification of streptococci from Greek Kasseri cheese and description of *Streptococcus macedonicus* sp. nov.

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Streptococci that can be found growing in milk belong to the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC).
Sequencing the genome of *S. macedonicus* ACA-DC 198

The aim of this work was to sequence the complete genome of the dairy isolate *S. macedonicus* ACA-DC 198 in order to assess *in silico* its adaptation to the milk environment and its pathogenic potential.
Assessing the adaptation to milk and the pathogenic potential of the dairy *Streptococcus macedonicus* ACA-DC 198 through comparative genomics

A. Complete genome sequencing and annotation of *S. macedonicus*

B. Comparative genomics of *S. macedonicus* against related streptococci

C. Assessing the adaptation of *S. macedonicus* to the milk environment

D. Assessing the pathogenic potential *S. macedonicus*
Complete genome sequencing and annotation of *S. macedonicus*

- Annotation was performed with the BaSys and the RAST pipelines and Kodon software.
- Final corrections and quality assessment was performed using GenePRIMP.

2,192 protein coding genes
192 potential pseudogenes
18 rRNA genes
70 tRNA genes

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Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis/S. equinus* complex

In the SBSEC there are currently five additional complete genome sequences available:

- *S. gallolyticus* UCN34 (human blood)
- *S. gallolyticus* ATCC BAA 2069 (human blood)
- *S. gallolyticus* ATCC 43143 (human blood)
- *S. pasteurianus* ATCC 43144 (human blood)
- *S. infantarius* CJ18 (suusac fermented camel milk)
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis/S. equinus* complex
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Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis/S. equinus* complex
### Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis/S. equinus* complex

- **Some additional characteristics of the genomes under investigation**

<table>
<thead>
<tr>
<th>Species</th>
<th>Genome size (Mb)</th>
<th>No. of protein coding genes</th>
<th>No. of potential pseudogenes/ (% percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. gallolyticus</em> ATCC BAA 2069</td>
<td>2.35</td>
<td>2329</td>
<td>nr*/(nr)</td>
</tr>
<tr>
<td><em>S. gallolyticus</em> ATCC 43143</td>
<td>2.36</td>
<td>2287</td>
<td>41/(1.8)</td>
</tr>
<tr>
<td><em>S. gallolyticus</em> UCN34</td>
<td>2.35</td>
<td>2251</td>
<td>28/(1.2)</td>
</tr>
<tr>
<td><em>S. macedonicus</em> ACA-DC 198</td>
<td>2.13</td>
<td>2192</td>
<td>192/(8.7)</td>
</tr>
<tr>
<td><em>S. pasteurianus</em> ATCC 43144</td>
<td>2.10</td>
<td>1869</td>
<td>157/(7.7)</td>
</tr>
<tr>
<td><em>S. infantarius</em> CJ18</td>
<td>1.98</td>
<td>1964</td>
<td>nr/(4.6)</td>
</tr>
</tbody>
</table>

* not reported

1. *S. macedonicus*, *S. pasteurianus* and *S. infantarius* genomes are being shaped by selective pressures that favor extensive gene loss events and genome decay processes when compared to the *S. gallolyticus* genome

2. This property (i.e. genome decay) has been linked to the adaptation of bacteria to rich in nutrients environments as in the case of *S. thermophilus* adaptation to the milk environment
Assessing the adaptation of *S. macedonicus* to the milk environment

A. Complete genome sequencing and annotation of *S. macedonicus*

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D. Assessing the pathogenic potential *S. macedonicus*
**Assessing the adaptation of *S. macedonicus* to the milk environment**

- *S. macedonicus* has deviated from the rumen environment
Assessing the adaptation of *S. macedonicus* to the milk environment

- *S. macedonicus* has an extra gene cluster responsible for lactose and galactose catabolism

![Gene cluster diagram]

- S. macedonicus ACA-DC 198
- S. suis BM407
- L. lactis subsp. cremoris plasmid pLP712
Assessing the adaptation of *S. macedonicus* to the milk environment

- *S. macedonicus* is particularly equipped with defenses against phages

<table>
<thead>
<tr>
<th>CRISPR label</th>
<th>Position</th>
<th>Nbr Spacers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRISPR_1</td>
<td>1507890, 1508913</td>
<td>15</td>
</tr>
<tr>
<td>CRISPR_2</td>
<td>1515490, 1516317</td>
<td>12</td>
</tr>
<tr>
<td>CRISPR_3</td>
<td>1484496, 1486444, 1477224, 1477919</td>
<td>29, 10</td>
</tr>
<tr>
<td>CRISPR_4</td>
<td>1515726, 1516570</td>
<td>12</td>
</tr>
<tr>
<td>CRISPR_5</td>
<td>1395041, 1397515</td>
<td>37</td>
</tr>
<tr>
<td>CRISPR_6</td>
<td>1412482, 1415817</td>
<td>50</td>
</tr>
<tr>
<td>CRISPR_7</td>
<td>1273106, 1273801</td>
<td>10</td>
</tr>
</tbody>
</table>

- Spacers 3, 5, 17, 46 in *S. macedonicus* CRISPR provide immunity against phages of the dairy *S. thermophilus* and *L. lactis*
Assessing the adaptation of *S. macedonicus* to the milk environment

- The *S. macedonicus* plasmid pSMA198 belongs to the narrow host range pCI305 family of lactococcal plasmids.
Assessing the adaptation of *S. macedonicus* to the milk environment

- The *S. macedonicus* plasmid pSMA198 belongs to the narrow host range pCI305 family of lactococcal plasmids

18 out of 19 *Lactococcus* spp. are dairy isolates

*S. macedonicus*
Assessing the adaptation to milk and the pathogenic potential of the dairy Streptococcus macedonicus ACA-DC 198 through comparative genomics

A. Complete genome sequencing and annotation of S. macedonicus

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D. Assessing the pathogenic potential S. macedonicus
### Assessing the pathogenic potential *S. macedonicus*

- *S. macedonicus* shows a diminished potential to bind to the extracellular matrix of the host

<table>
<thead>
<tr>
<th>S. gallolyticus UCN 34</th>
<th>gene</th>
<th>function</th>
<th>S. gallolyticus ATCC BAA 2069</th>
<th>S. gallolyticus ATCC 43143</th>
<th>S. macedonicus ACA-DC 198</th>
<th>S. pasteurianus ATCC 43144</th>
<th>S. infantarius CJ18</th>
</tr>
</thead>
<tbody>
<tr>
<td>gallo_2179</td>
<td>accessory pilin (pil1)</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gallo_2178</td>
<td>major pilin (pil1)</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gallo_2177</td>
<td>sortase C (pil1)</td>
<td>✓</td>
<td>✓</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gallo_1570</td>
<td>accessory pilin (pil2)</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gallo_1569</td>
<td>major pilin (pil2)</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
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<tr>
<td>gallo_1568</td>
<td>sortase C (pil2)</td>
<td>✓</td>
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<tr>
<td>gallo_2040</td>
<td>accessory pilin (pil3)</td>
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<tr>
<td>gallo_2039</td>
<td>major pilin (pil3)</td>
<td>✓</td>
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<tr>
<td>gallo_2038</td>
<td>sortase C (pil3)</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Diagram:**
- *ECM* (Extracellular Matrix)
- *CELL*
- *Proteoglycan molecule*
- *Proteoglycan complex*
- *Collagen fibril*

**Legend:**
- Fibronectin
- Integrin
- Microfilaments of cytoskeleton
- Plasma membrane
Assessing the pathogenic potential *S. macedonicus*

- *S. macedonicus* has retained the hemolysin encoding genes

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th><em>S. gallolyticus UCN34</em></th>
<th><em>S. gallolyticus ATCC 43143</em></th>
<th><em>S. gallolyticus ATCC BAA-2069</em></th>
<th><em>S. pasteurianus ATCC 43144</em></th>
<th><em>S. macedonicus ACA-DC 198</em></th>
<th><em>S. infantarius CJ18</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>hemolysin TLY</td>
<td>GALLO_0630</td>
<td>SGGB_0605</td>
<td>SGGBAA2069_c05730</td>
<td>SGPB_0499</td>
<td>SMA_0591</td>
<td>Sinf_0511</td>
</tr>
<tr>
<td>hemolysin III</td>
<td>GALLO_1262</td>
<td>SGGB_1256</td>
<td>SGGBAA2069_c12530</td>
<td>SGPB_1172</td>
<td>SMA_1191</td>
<td>Sinf_1093</td>
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<tr>
<td>hemolysin A</td>
<td>GALLO_1799</td>
<td>SGGB_1786</td>
<td>SGGBAA2069_c17570</td>
<td>SGPB_1603</td>
<td>SMA_1706</td>
<td>Sinf_1530</td>
</tr>
</tbody>
</table>
Conclusions

1. *Streptococcus macedonicus* is evolving under genome decay processes suggesting adaptation to a rich in nutrients environment

2. Our analysis supports that the species shows traits of adaptation to the dairy environment

3. Even though *S. macedonicus* shows a diminished pathogenic potential compared to *S. gallolyticus*, several pathogenicity traits are still conserved
This work was performed in collaboration with:

- Prof. Stavros Hamodrakas
- Dr. Bruno Pot
- Dr. Philippe Supply
Acknowledgments

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Thank you for your attention!!!