



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

International Journal of Systematic Bacteriology (1998), 48, 519–527

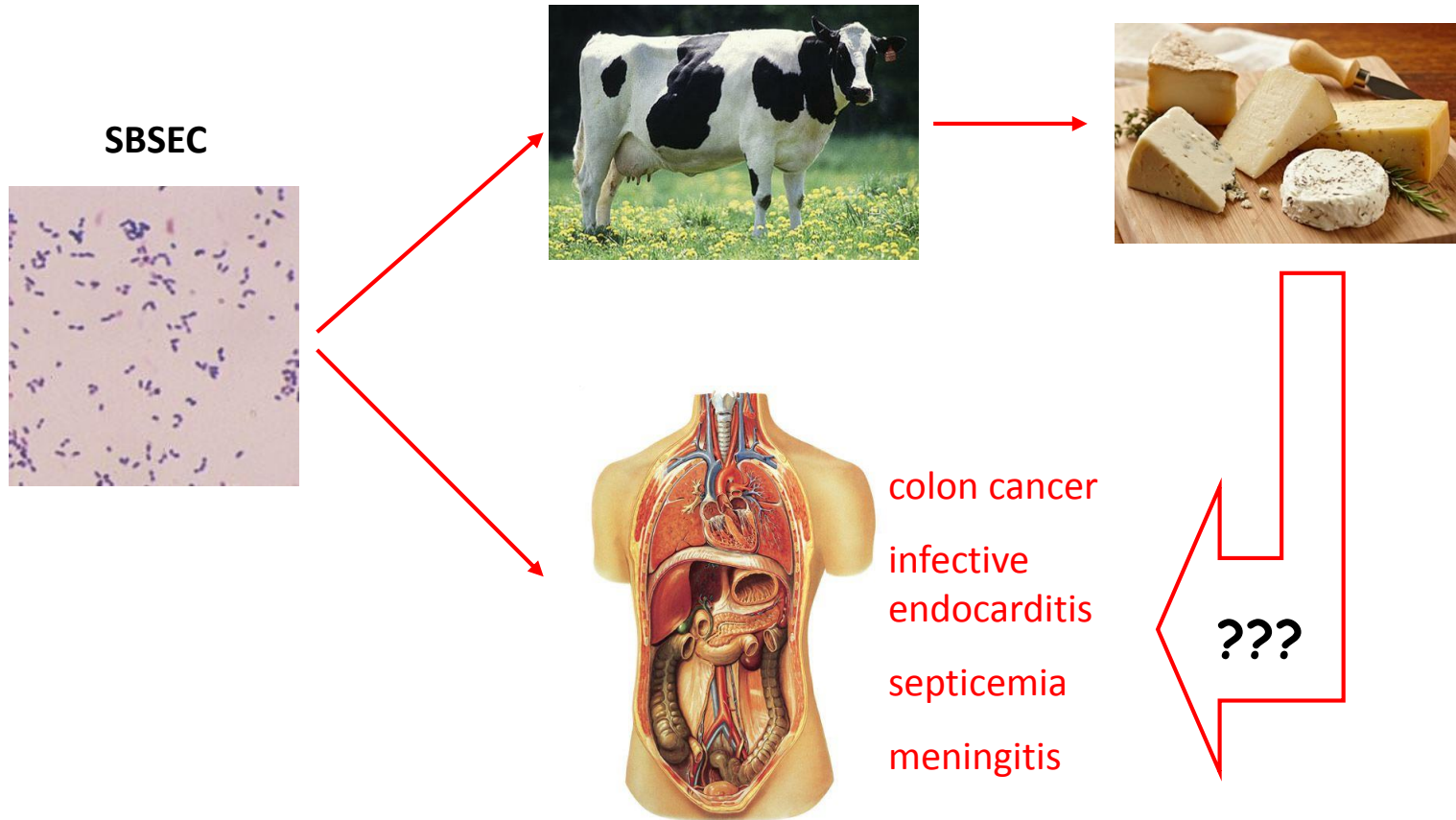
Printed in Great Britain

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

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L. A. Devriese,⁴ G. Kalantzopoulos,¹ K. H. Schleifer³ and K. Kersters²

Laying the background: Fermented foods and Streptococci

- Streptococci that can be found growing in milk belong to the *Streptococcus bovis*/*Streptococcus equinus* complex (SBSEC)



Herrera et al. Anaerobe 2009

Sequencing the genome of *S. macedonicus* ACA-DC 198

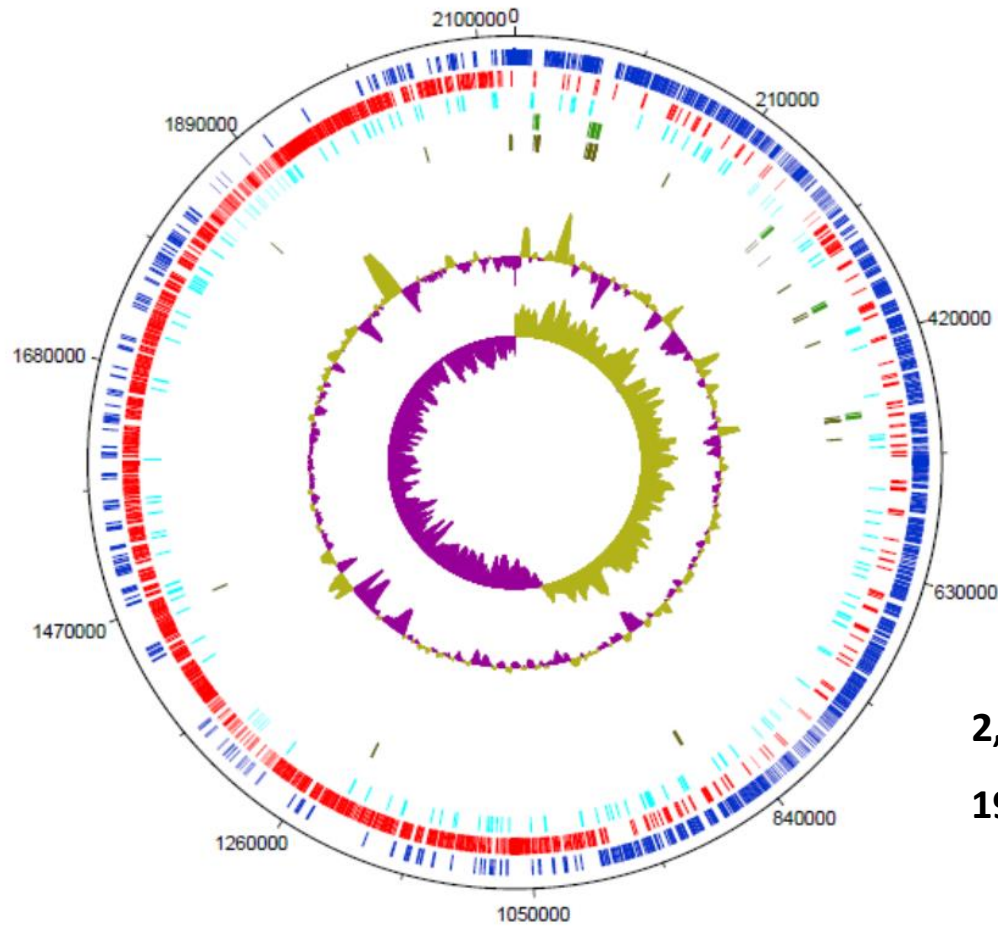
The aim of this work was to sequence the complete genome of the dairy isolate *S. macedonicus* ACA-DC 198 in order to assess *in silico* its adaptation to the milk environment and its pathogenic potential

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

Papadimitriou et al. J Bacteriol. 2012

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

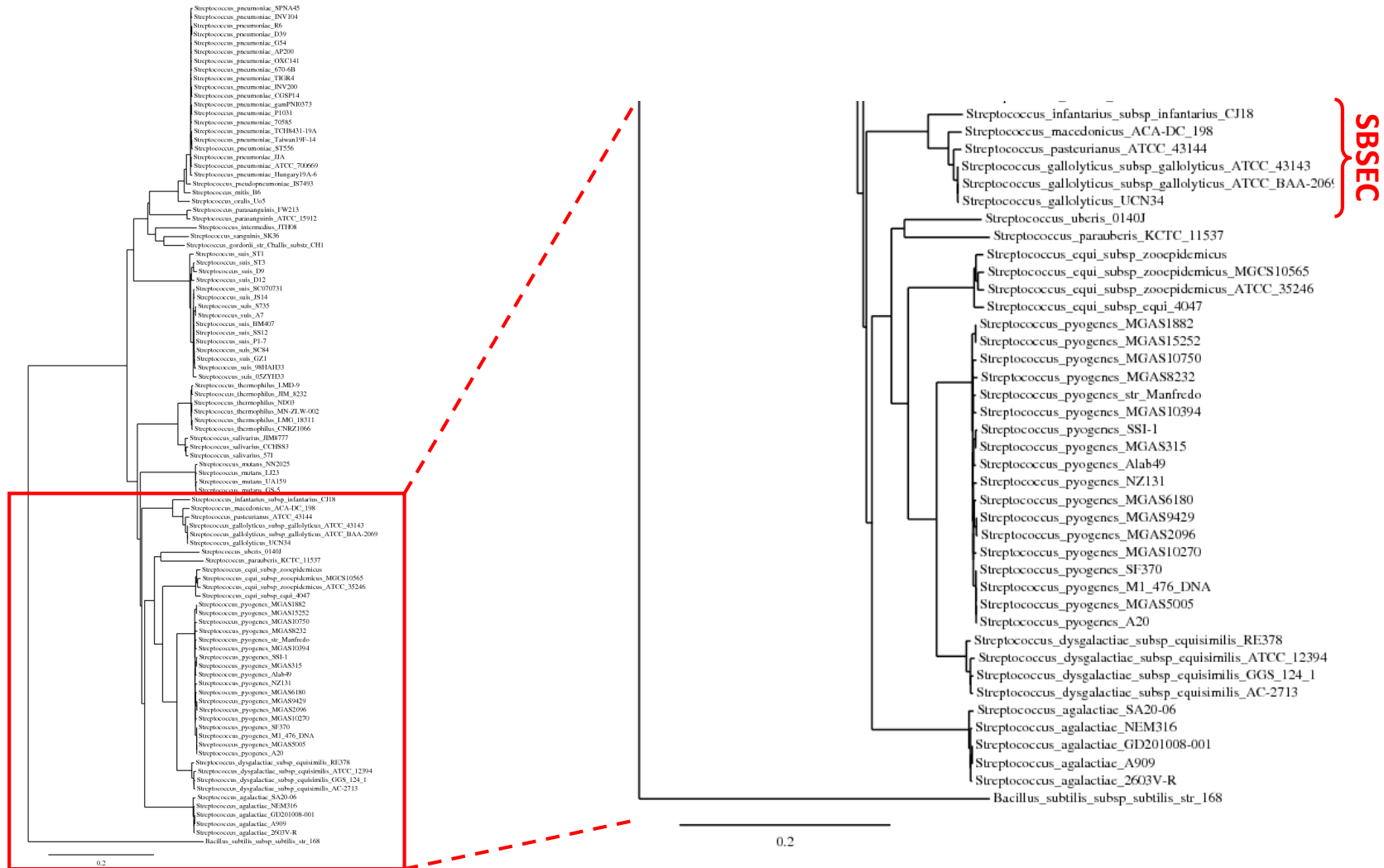
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Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

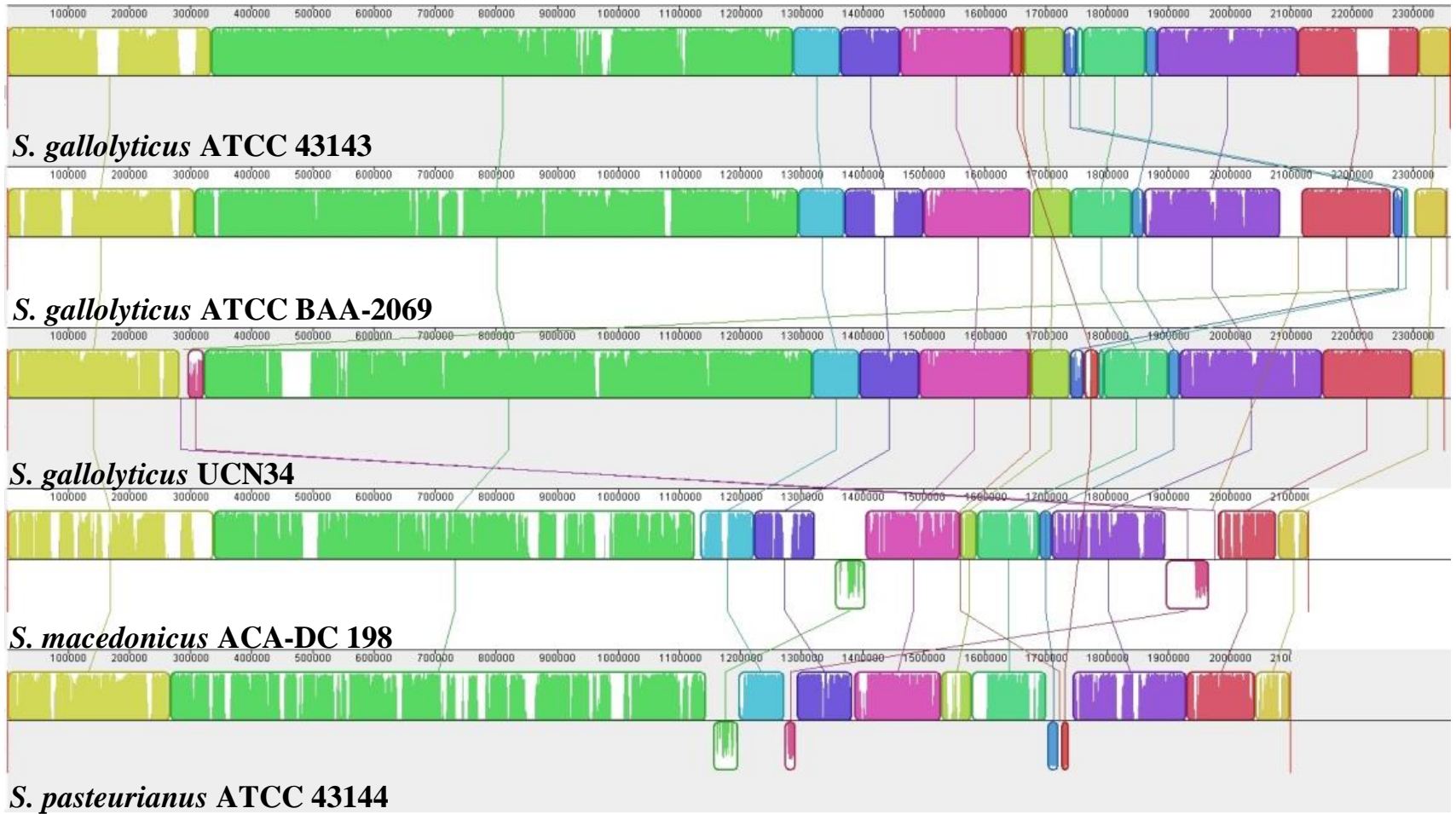
In the SBSEC there are currently five additional complete genome sequences available:

- *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)

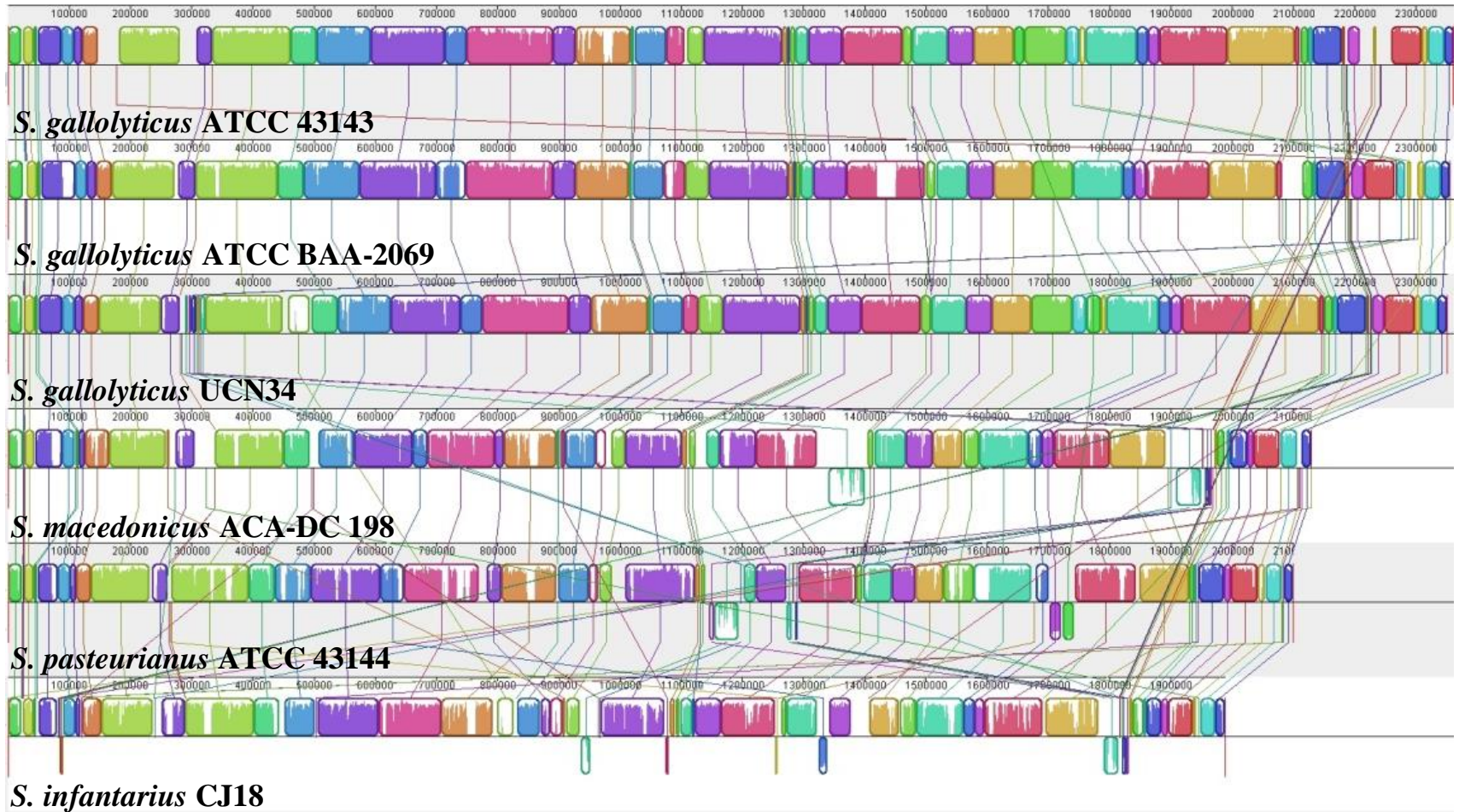
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex



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

• Some additional characteristics of the genomes under investigation

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

* not reported

- 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**
- 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**

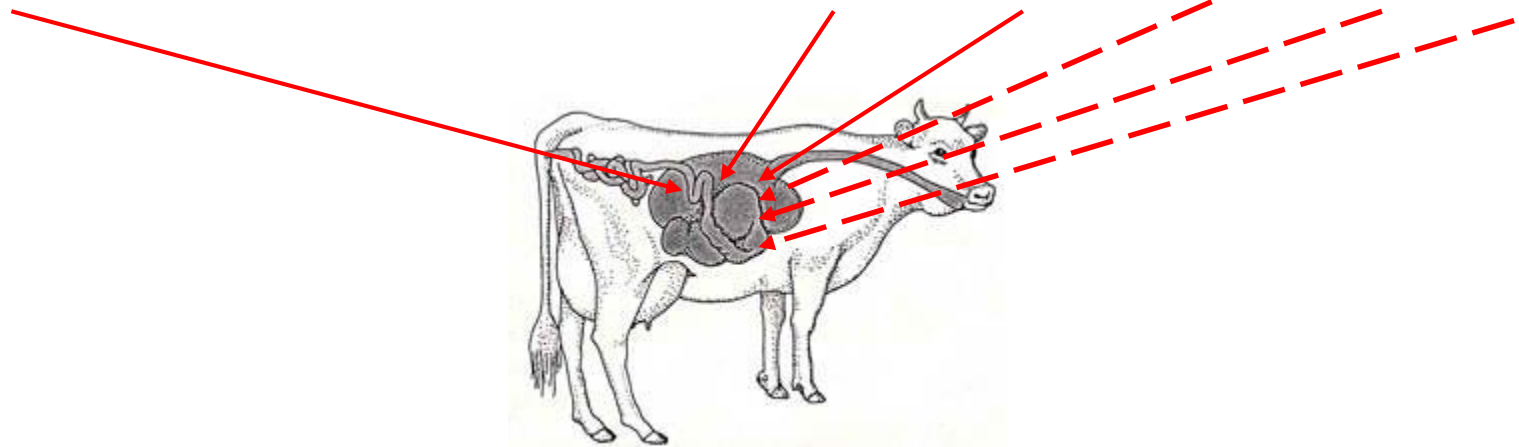
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Assessing the adaptation of *S. macedonicus* to the milk environment

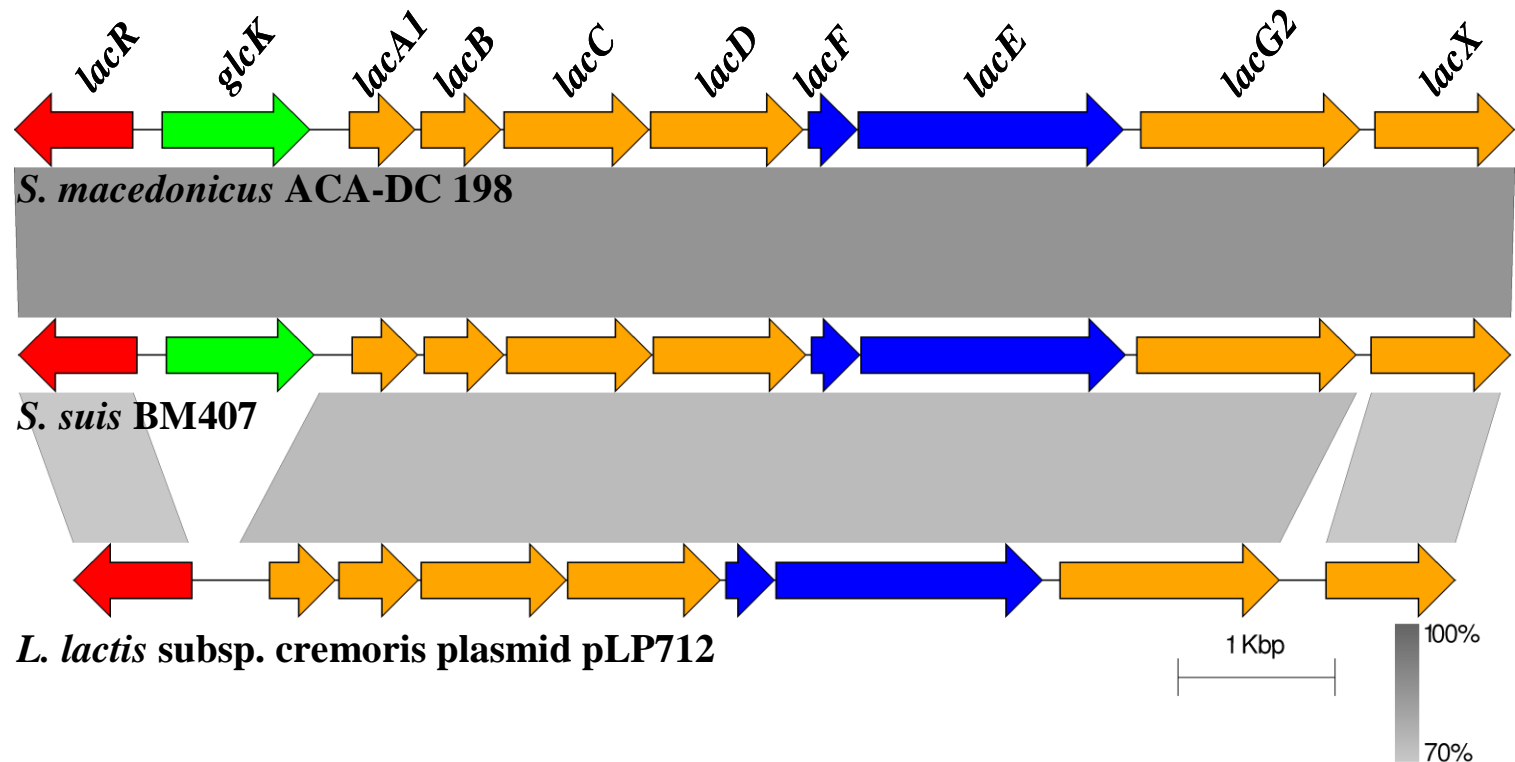
- S. macedonicus* has deviated from the rumen environment

| S. gallolyticus UCN 34 | | | S. gallolyticus | S. gallolyticus | S. macedonicus | S. pasteurianus | S. infantarius |
|------------------------|-----------------|------------------------------------|-----------------|-----------------|----------------|-----------------|----------------|
| locus_tag | gene | function | ATCC BAA 2069 | ATCC 43143 | ACA-DC 198 | ATCC 43144 | CJ18 |
| gallo_0112 | fruA | fructan hydrolase | ✓ | ✓ | - | - | - |
| gallo_0330 | - | beta-1,4-endoglucanase (cellulase) | ✓ | ✓ | - | - | - |
| gallo_0757 | - | α-amylase | ✓ | ✓ | - | - | - |
| gallo_0162 | - | mannase | ✓ | ✓ | - | - | pseudo |
| gallo_0189 | - | endo-beta-1,4-galactanase | ✓ | ✓ | pseudo | ✓ | pseudo |
| gallo_1577 | - | pectate lyase | ✓ | ✓ | ✓ | pseudo | ✓ |
| gallo_1578 | - | pectate lyase | ✓ | ✓ | ✓ | pseudo | ✓ |
| gallo_1632 | amyE | α-amylase | ✓ | ✓ | ✓ | pseudo | ✓ |
| gallo_0933 | tanA | tanins degradation | ✓ | ✓ | - | - | - |
| gallo_1609 | similar to tanA | tanins degradation | ✓ | ✓ | - | - | - |
| gallo_2106 | padC | gallic acid decarboxylation | ✓ | ✓ | ✓ | ✓ | - |
| gallo_0906 | | gallic acid decarboxylation | ✓ | ✓ | | ✓ | - |
| gallo_0818 | bsh | bile salt hydrolase | ✓ | ✓ | pseudo | ✓ | ✓ |



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

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



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

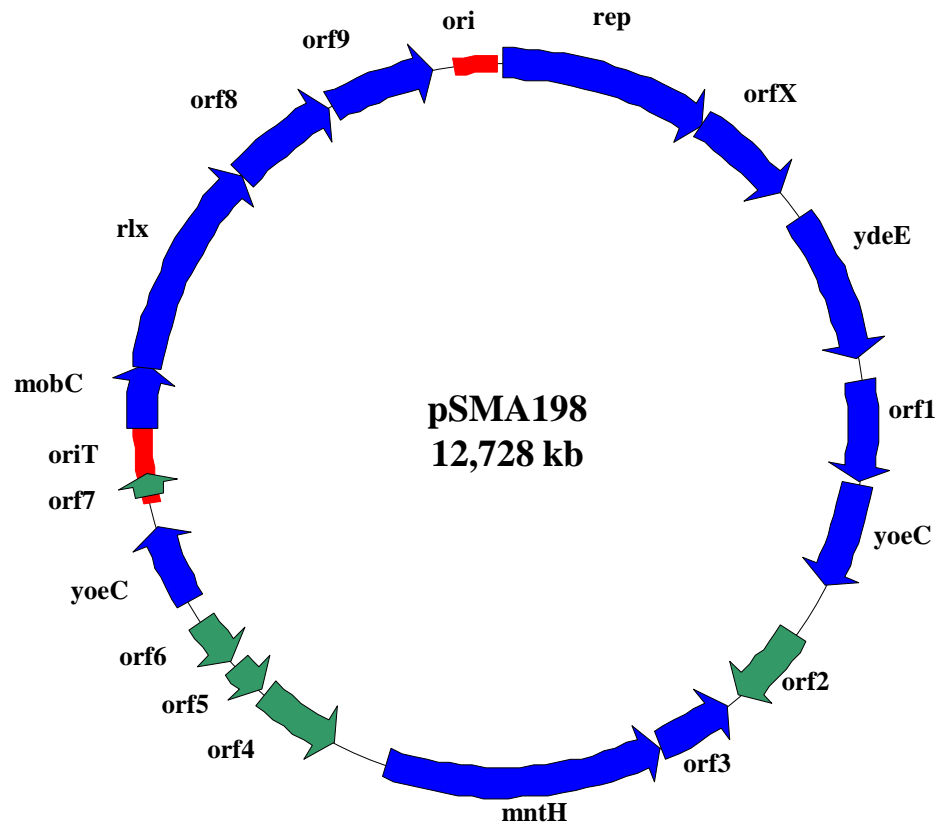
- S. macedonicus* is particularly equipped with defenses against phages

| | <i>Streptococcus gallolyticus</i> UCN34 | | <i>Streptococcus gallolyticus</i> subsp. <i>gallolyticus</i> ATCC 43143 | | <i>Streptococcus gallolyticus</i> subsp. <i>gallolyticus</i> ATCC BAA-2069 | | <i>Streptococcus pasteurianus</i> ATCC 43144 | | <i>Streptococcus macedonicus</i> ACA-DC 198 | | <i>Streptococcus infantarius</i> subsp. <i>infantarius</i> CJ18 | |
|--------------|---|--------------------------|---|-------------|--|-------------|--|-------------|---|-------------|---|-------------|
| CRISPR label | Position ^a | Nbr Spacers ^b | Position | Nbr Spacers | Position | Nbr Spacers | Position | Nbr Spacers | Position | Nbr Spacers | Position | Nbr Spacers |
| CRISPR_1 | 1507890, 1508913 | 15 | | | | | | | 1412482, 1415817 | 50 | | |
| CRISPR_2 | 1515490, 1516317 | 12 | 1484496, 1486444 | 29 | 1517213, 1518237 | 15 | | | | | | |
| CRISPR_3 | | | 1477224, 1477919 | 10 | | | | | | | | |
| CRISPR_4 | | | | | 1515726, 1516570 | 12 | | | | | | |
| CRISPR_5 | | | | | | | | | | | 1273106, 1273801 | 10 |
| CRISPR_6 | | | | | | | 1395041, 1397515 | 37 | | | | |

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

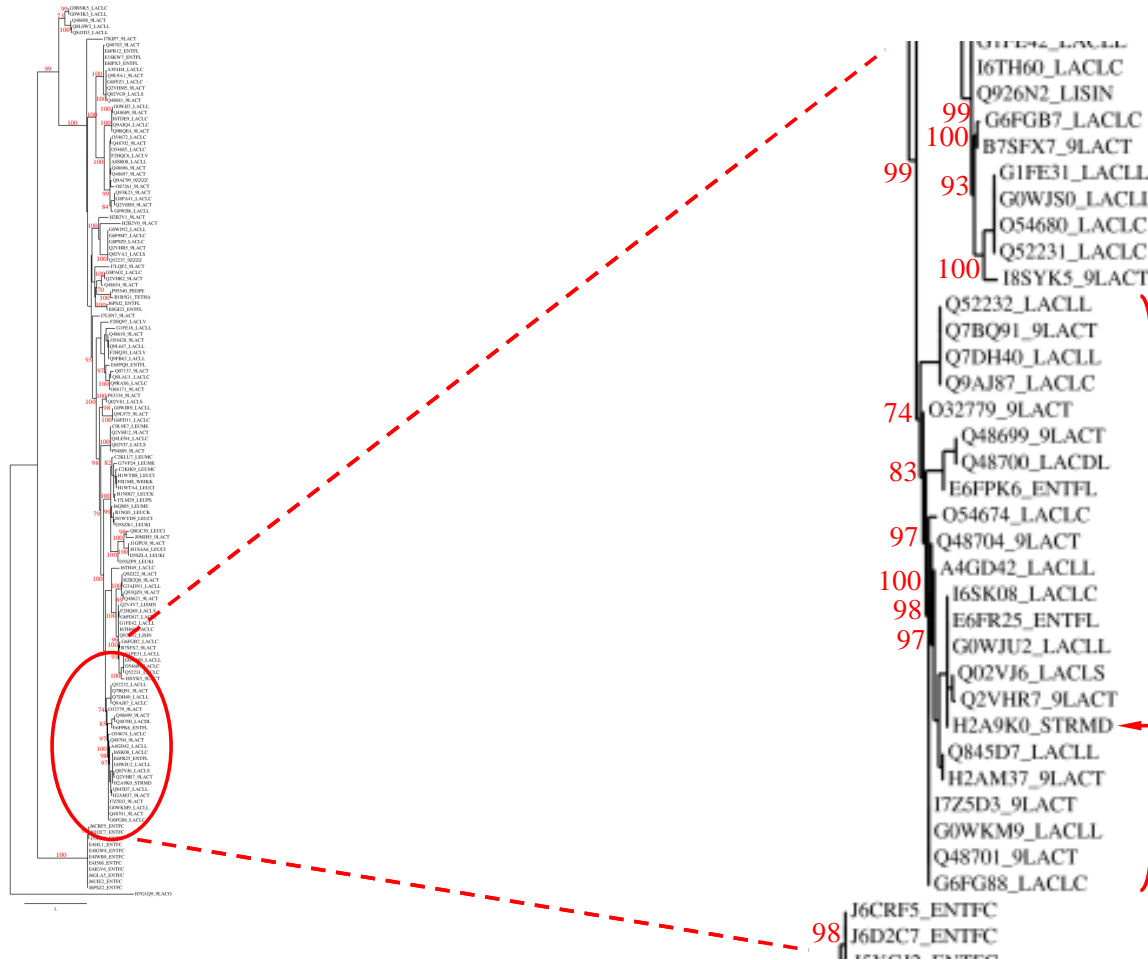
Assessing the adaptation of *S. macedonicus* to the milk environment

- 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

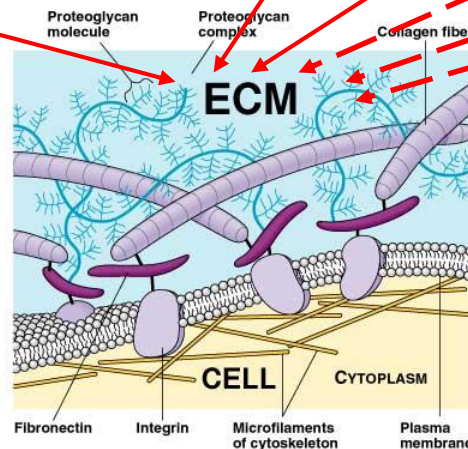
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Assessing the pathogenic potential *S. macedonicus*

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

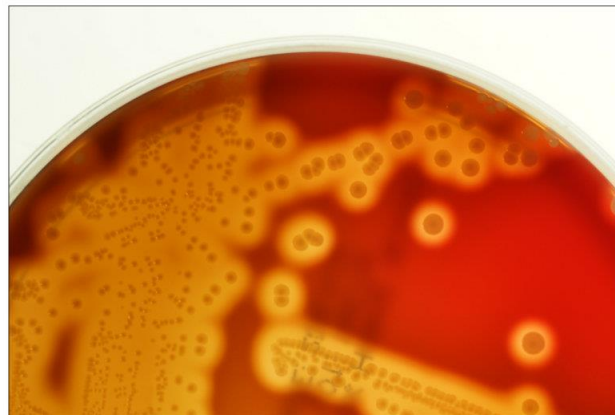
| <i>S. gallolyticus</i> UCN 34 | | | <i>S. gallolyticus</i> | <i>S. gallolyticus</i> | <i>S. macedonicus</i> | <i>S. pasteurianus</i> | <i>S. infantarius</i> |
|-------------------------------|------|------------------------|------------------------|------------------------|-----------------------|------------------------|-----------------------|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



Assessing the pathogenic potential *S. macedonicus*

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

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



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**

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Thank you for your attention!!!

