

Milk adaptation and pathogenic potential among members of the *Streptococcus bovis*/*Streptococcus equinus* complex

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Abstract

The *Streptococcus bovis*/*Streptococcus equinus* complex (SBSEC) consists of species commonly found in the gastrointestinal tract (GIT) of herbivores. Certain members of the complex like *Streptococcus macedonicus* and *Streptococcus infantarius* are frequently isolated from traditional fermented foods, mostly of dairy origin. However, some species of this complex, like *Streptococcus gallolyticus* and *Streptococcus pasteurianus* are known pathogens of humans involved in a range of diseases including endocarditis, meningitis, bacteremia, colon cancer etc. Analysis of the genome of *S. macedonicus* strain ACA-DC 198 isolated from traditional Greek Kasser cheese revealed important traits of adaptation to the dairy environment. The strain contains a significant percentage of potential pseudogenes indicating that it may have evolved through genome decay processes. *Streptococcus macedonicus* has an extra gene cluster for lactose and galactose metabolism unique among the SBSEC members and a typical proteolytic system required for casein hydrolysis. Furthermore, we found evidence in the genome of *S. macedonicus* suggesting HGT (horizontal gene transfer) events with potential donors *Lactococcus lactis* and *Streptococcus thermophilus*. Perhaps the most pronounced among these HGT events is the presence in *S. macedonicus* of pSMA198 which belongs to the lactococcal pCI305/pWV02 family of plasmids. Our analysis suggests that pSMA198 may have been acquired by *S. macedonicus* from *L. lactis*. Analogous traits have been reported for *S. infantarius* suggesting that at least these two strains present adaptations to the dairy environment among SBSEC. Finally, we were able to identify a number of potential virulence factors (VFs) within the SBSEC members whose presence varied among species of the complex. *Streptococcus macedonicus* and *S. infantarius* miss some of the VFs present in *S. gallolyticus* suggesting a diminished pathogenic potential for the two species.

Results and Discussion

Figure 1 - The circular map of the genome of *Streptococcus macedonicus* ACA-DC 198. Genomic features appearing from the periphery to the centre of the map: 1. Forward CDSs (blue); 2. Reverse CDSs (red); 3. Putative pseudogenes (cyan); 4. rRNA genes (orange); 5. tRNA genes (green); 6. %GC plot; 7. GC skew.

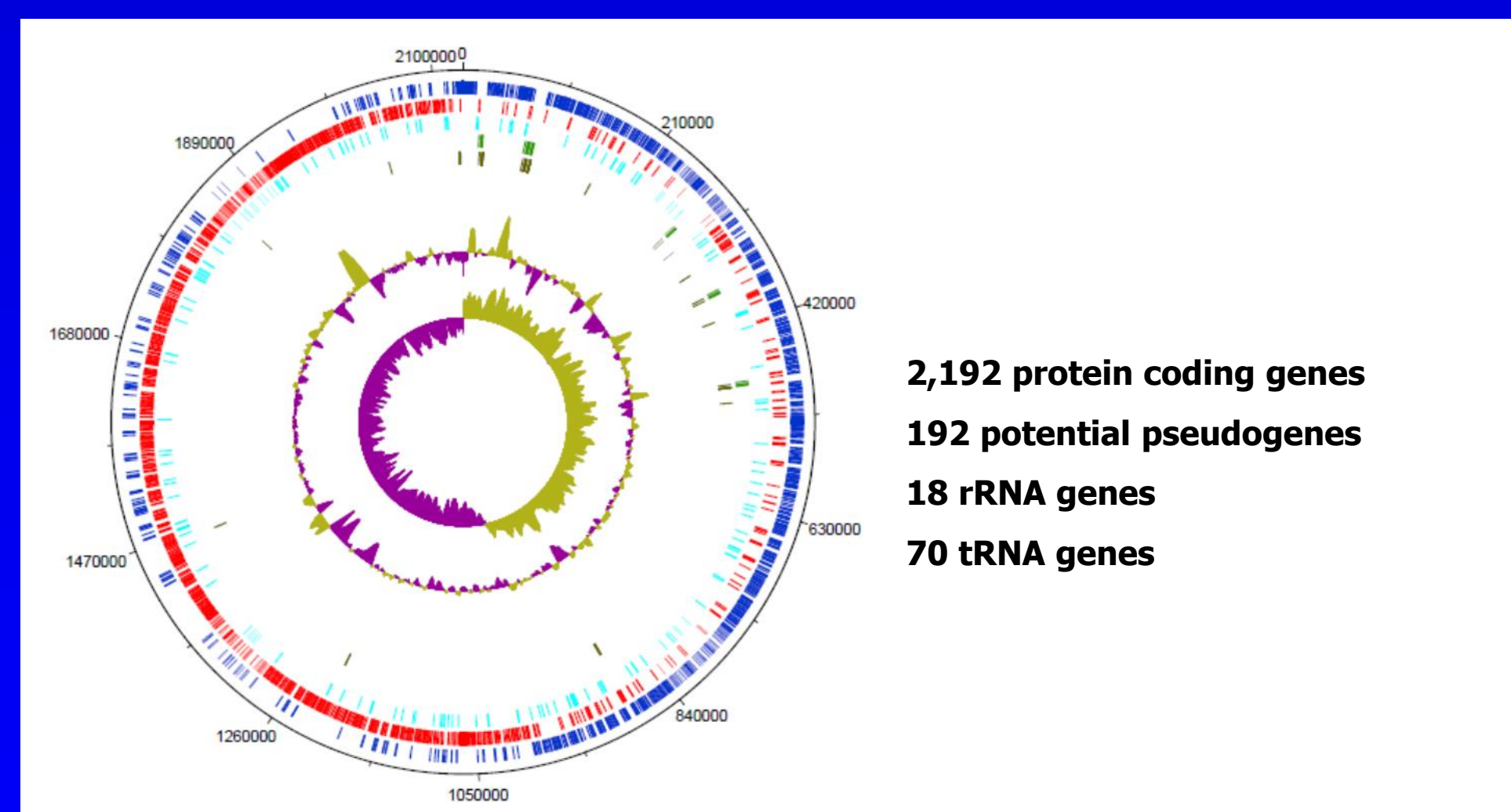


Figure 2 - Chromosome alignments of the *Streptococcus bovis*/*Streptococcus equinus* complex members as calculated by progressiveMauve. Chromosome alignments among *Streptococcus gallolyticus*, *Streptococcus macedonicus* and *Streptococcus pasteurianus* (A) and all the aforementioned streptococci and *Streptococcus infantarius* (B). Local collinear blocks (LCBs) of conserved sequences among the strains are represented by rectangles of the same colour. Connecting lines can be used to visualize synteny or rearrangement. LCBs positioned above or under the chromosome (black line) correspond to the forward and reverse orientation, respectively. The level of conservation is equivalent to the level of vertical colour filling within the LCBs (e.g. white regions are strain-specific). Sequences not placed within an LCB are unique for the particular strain.

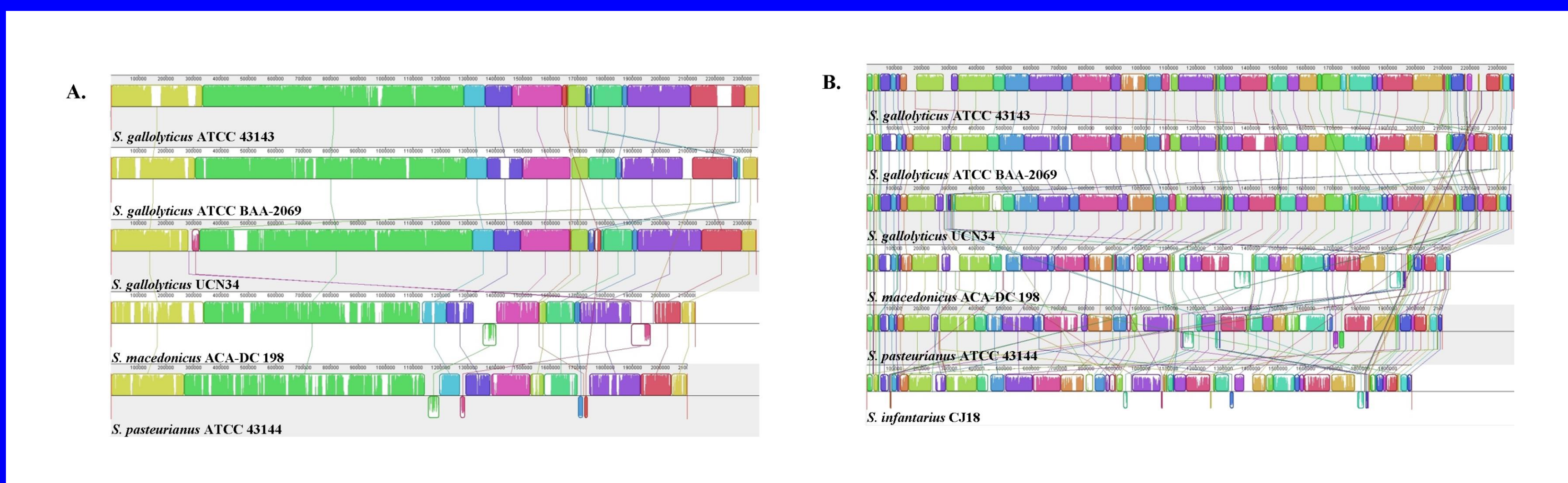


Figure 3 - Core genome analysis of members of the *Streptococcus bovis*/*Streptococcus equinus* complex. Whole CDS Venn diagrams of *Streptococcus gallolyticus*, *Streptococcus macedonicus* and *Streptococcus pasteurianus* (A) or *Streptococcus gallolyticus*, *Streptococcus infantarius*, *Streptococcus macedonicus* and *Streptococcus pasteurianus* (B). In (B) *Streptococcus gallolyticus* ATCC 43143 was selected as a representative of the *Streptococcus gallolyticus* species, since it has the longest genome size among the three sequenced strains.

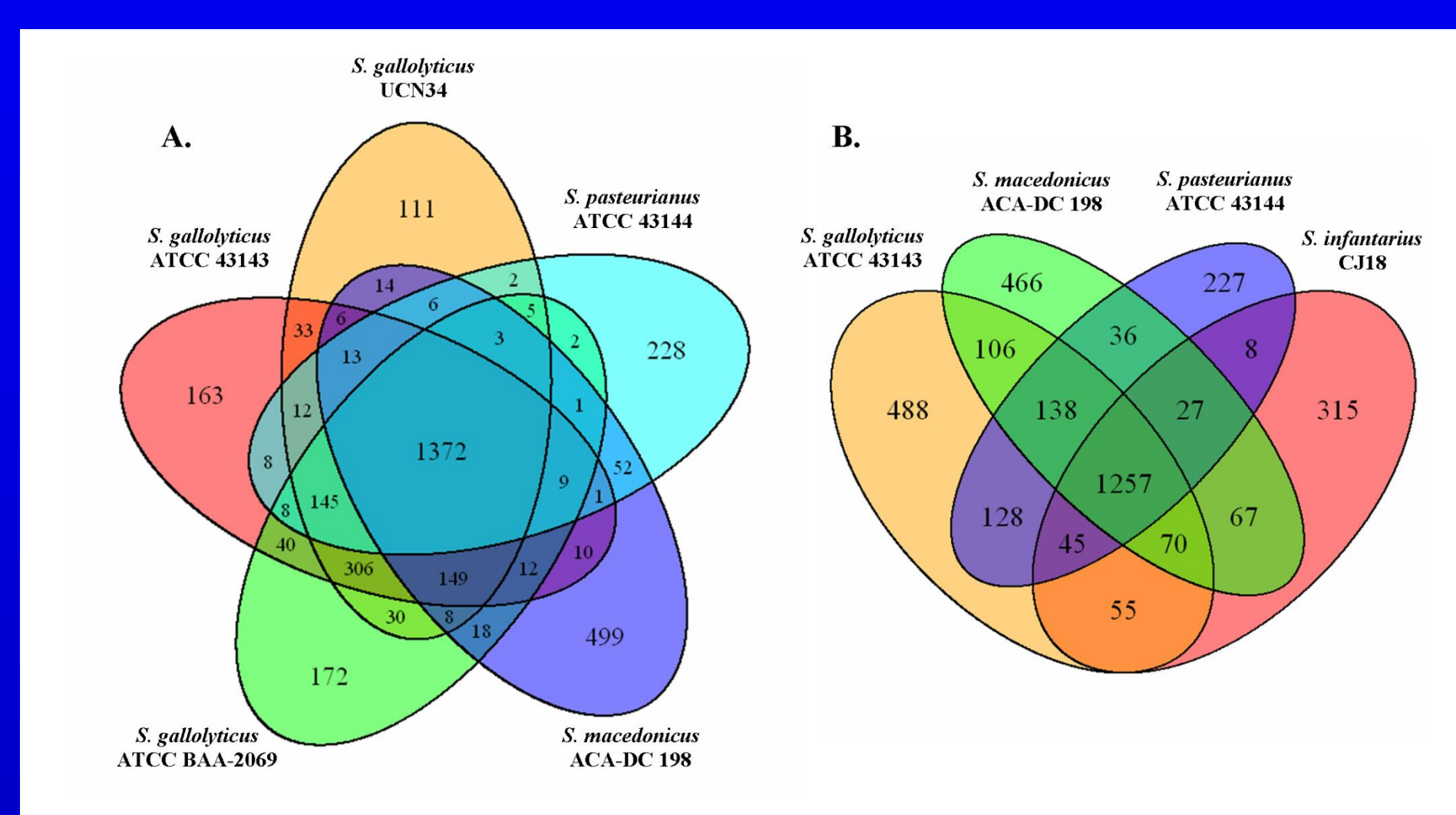


Figure 4 - The extra gene cluster for lactose and galactose metabolism found solely in *Streptococcus macedonicus* among the SBSEC members.

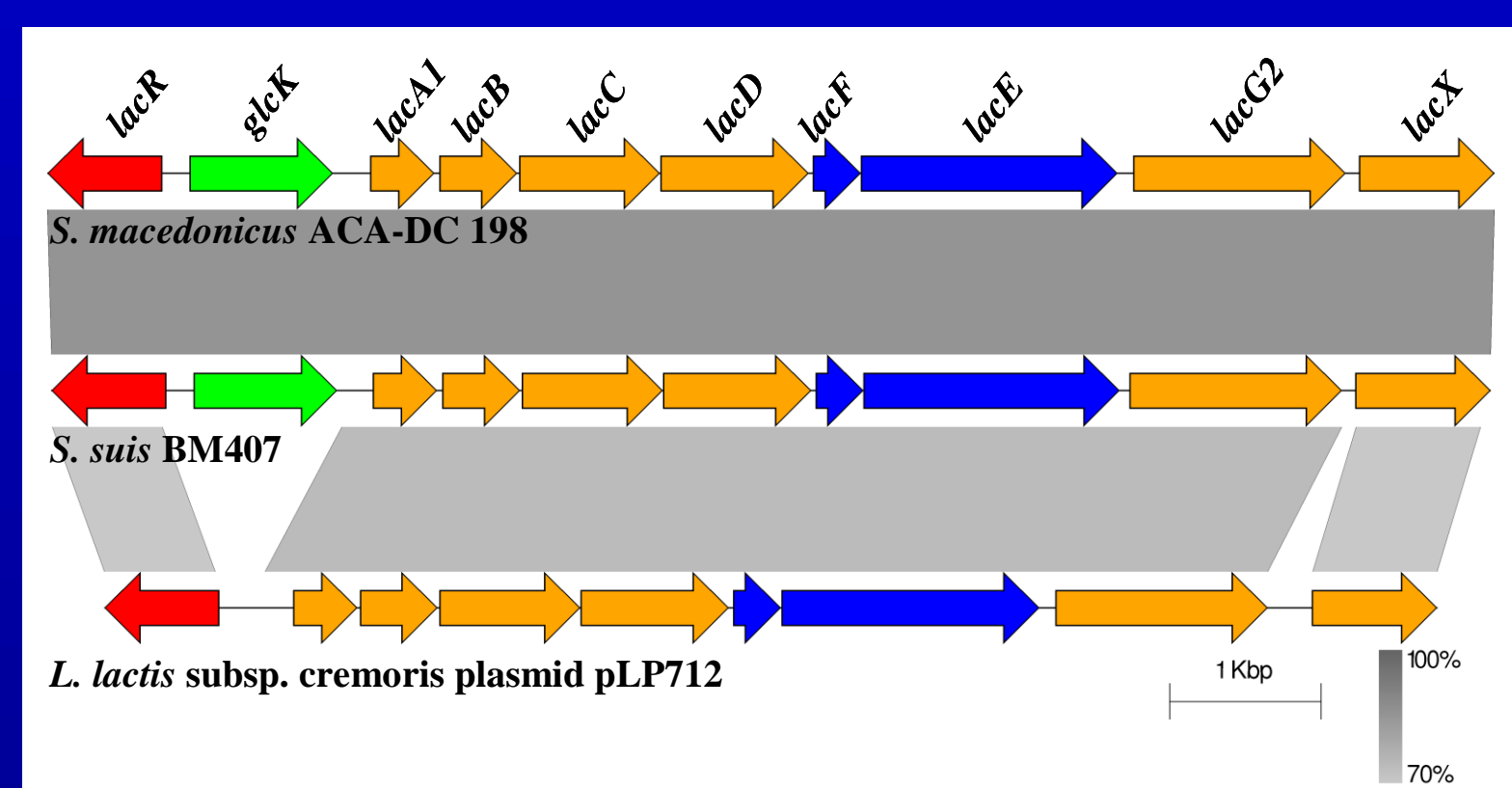


Table 1 - Genes within the *Streptococcus bovis*/*Streptococcus equinus* complex potentially involved in adaptation to the rumen.

S. gallolyticus UCN 34 locus_tag	gene	function	S. gallolyticus ATCC BAA 2069	S. gallolyticus ATCC 43143	S. macedonicus ACA-DC 198	S. pasteurianus ATCC 43144	S. infantarius C718
gallo_0112	fruA	fructan hydrolase	✓	✓	✓	✓	✓
gallo_0330	-	beta-1,4-endoglucanase (cellulase)	✓	✓	✓	✓	✓
gallo_0757	-	alpha-amylase	✓	✓	✓	✓	✓
gallo_0162	-	mannase	✓	✓	✓	✓	✓
gallo_0189	-	endo-beta-1,4-galactanase	✓	✓	pseudo	✓	pseudo
gallo_1577	-	pectate lyase	✓	✓	✓	✓	✓
gallo_1578	-	pectate lyase	✓	✓	✓	✓	✓
gallo_1632	amyE	alpha-amylase	✓	✓	✓	✓	✓
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	✓	✓

Figure 5 - Map of plasmid pSMA198 isolated from *Streptococcus macedonicus* and its annotation.

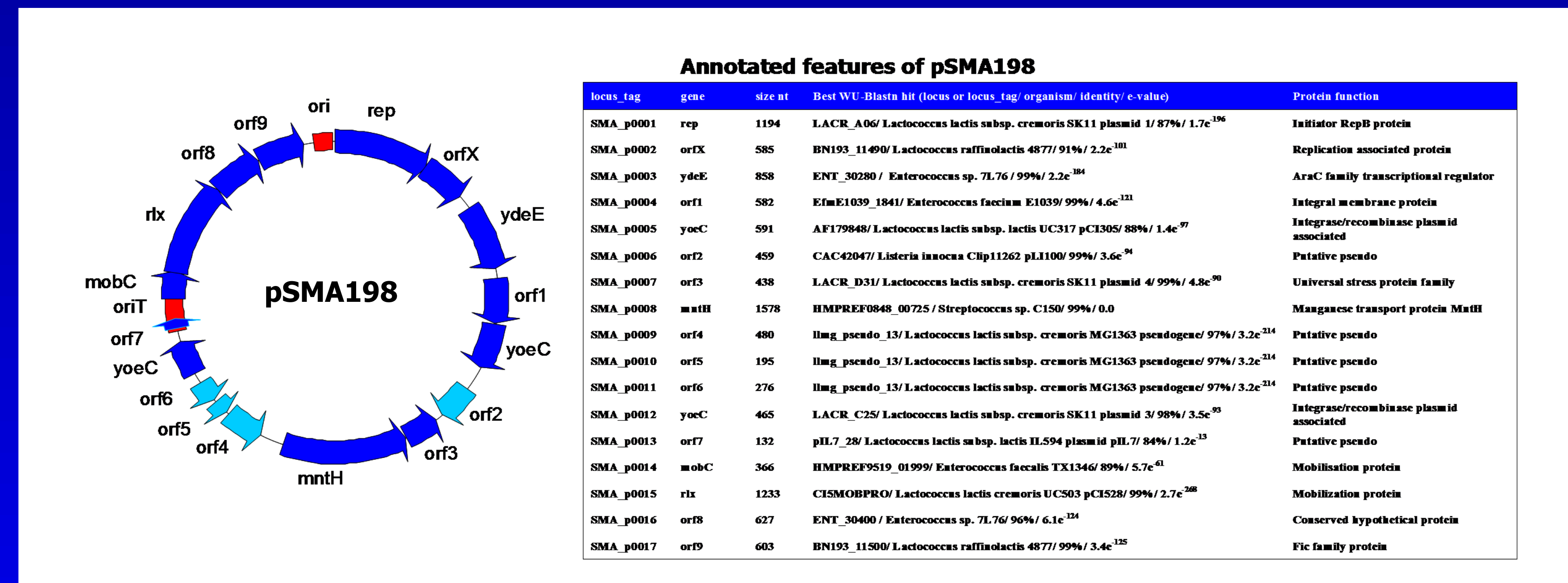


Figure 6 - Sequence alignment in a circular fashion of pSMA198 against the lactococcal pSK11b (A), pVF22 (B) and pIL5 (C) of dairy origin. Local alignments produced by BLAST are presented using ribbons whose color corresponds to four quartiles of the alignment's bitscore (red: top 25%, orange: second 25%, green: third 25% and blue: worst 25%). In order to aid orientation, the position of the ori or oriT of pSMA198 has been added in the figures. (D) Maximum likelihood tree of the pSMA198 Rep generated using the Phylogeny.fr pipeline.

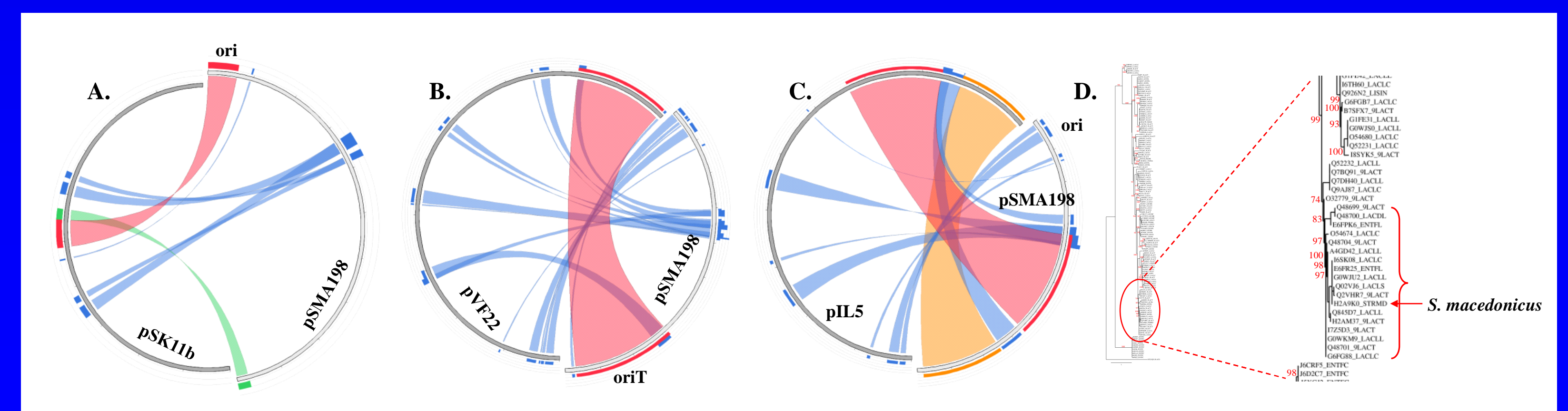


Figure 7 - Sequence alignment of chromosomal regions of *Streptococcus macedonicus* ACA-DC 198 against pIL5 (A), pVF22 (B) and pGdh442 (C) performed using Kodon. The flanking transposase gene showing high identity to orf2 (SMA_p0006) is underlined. Colored areas between the sequences correspond to different levels of identity that is depicted within the areas.

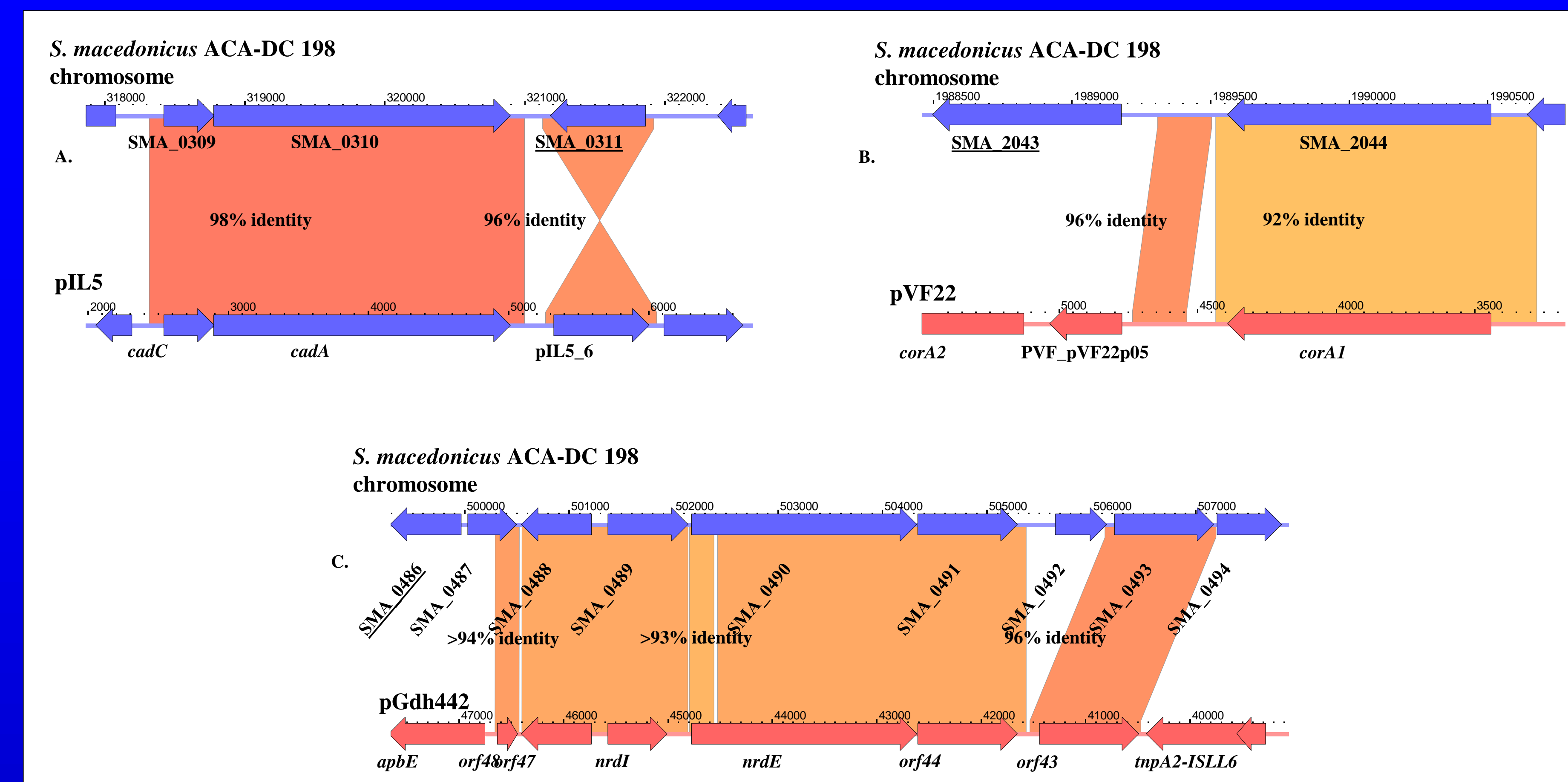


Table 2 - Genes in the *Streptococcus bovis*/*Streptococcus equinus* complex identified as putative virulence factors

S. gallolyticus UCN 34 locus_tag	gene	function	S. gallolyticus ATCC BAA 2069	S. gallolyticus ATCC 43143	S. macedonicus ACA-DC 198	S. pasteurianus ATCC 43144	S. infantarius C718
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)	✓	✓	✓	✓	✓

Bibliography

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• Papadimitriou K., T. Plakas, R. Anastasiou, S. Ferreira, P. Supply, P. Renault, N. C. Papandreou, B. Pot, and E. Tsakalidou (Under review) Analysis of the lactococcal plasmid pSMA198 found in *Streptococcus macedonicus* ACA-DC 198 points towards the habituation of the strain to the dairy environment.

Acknowledgments

This work was financially supported by the THALES Program (Funded by the Greek General Secretariat for Research and Technology).