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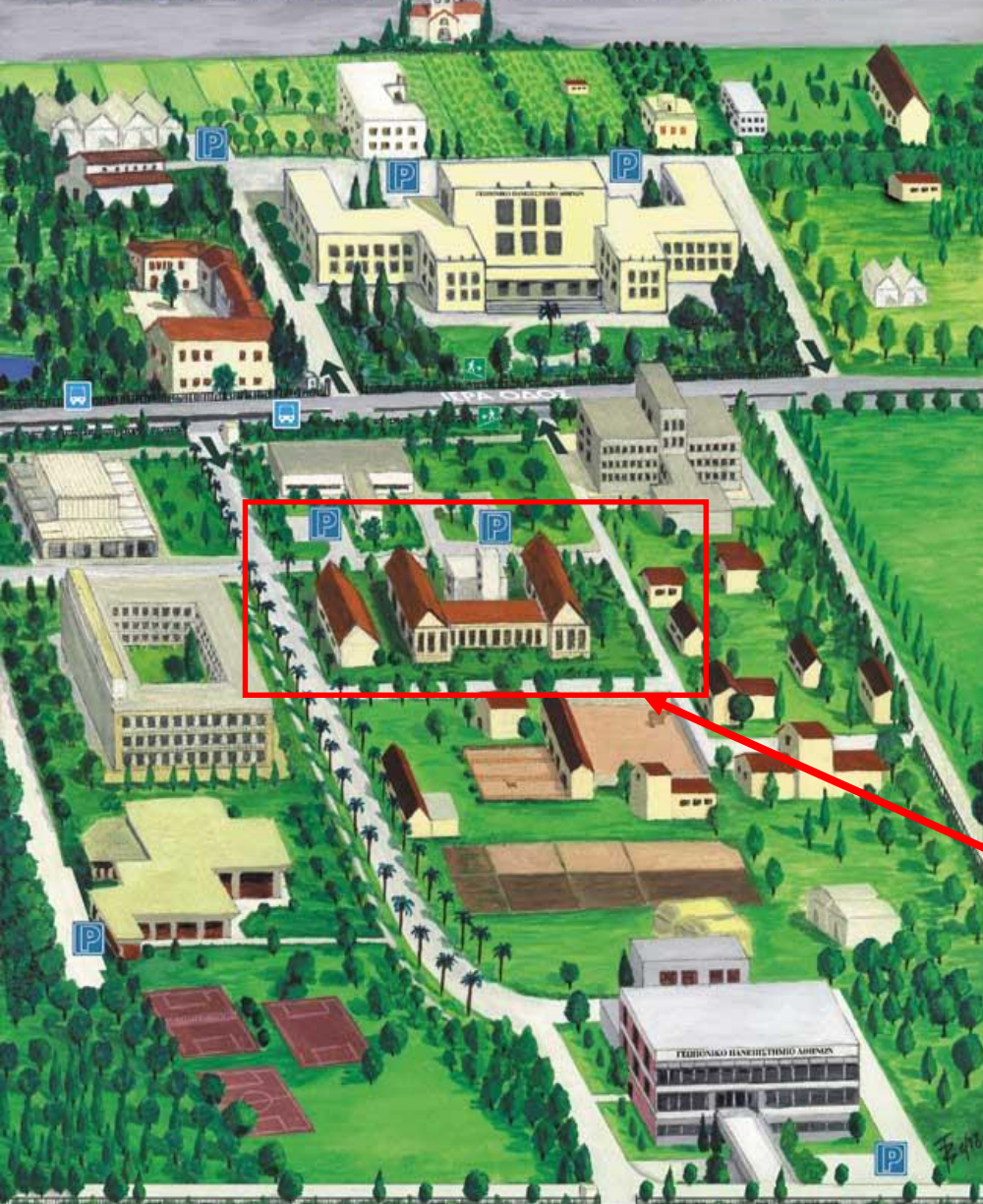
Assessing the safety of dairy streptococci beyond *Streptococcus thermophilus* based on recent genomic evidence for the *Streptococcus bovis*/*Streptococcus equinus* complex

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ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ



Agricultural University of Athens



**Department of Food Science and
Human Nutrition**

**Laboratory
of Dairy Research**

Laying the background: Fermented foods and Microorganisms

- **Traditional Fermented products**
 - **“Spontaneous” fermentation**
 - **“Back-Slopping”**: Fermented product to fresh raw material
- **New Technology (Today’s industrial products)**
 - **Use of Starter Cultures** : well-defined, single or multi-species microbial populations added directly to the raw material to carry the necessary fermentation that would result in the **“best” possible product**

BUT WHERE DO WE FIND STARTER CULTURES???

Laying the background: Fermented foods and Microorganisms



Fermented dairies



Fermenting ecosystem



Experimental dairy production



Basic Research

- Taxonomy
- metabolism
- physiology
- genetics and genomics
- bioinformatics

Technological properties

- flavour bio-generation
- bio-texturant molecule development
- bio-preservative molecule production (bacteriocins)
- probiotics

Laying the background: Fermented foods and Microorganisms

- **The ACA-DC Collection of Microorganisms was established in 1987 by Prof. George Kalantzopoulos**

The Mission

...to systematically explore and preserve the microbial diversity of Greek traditional foods...

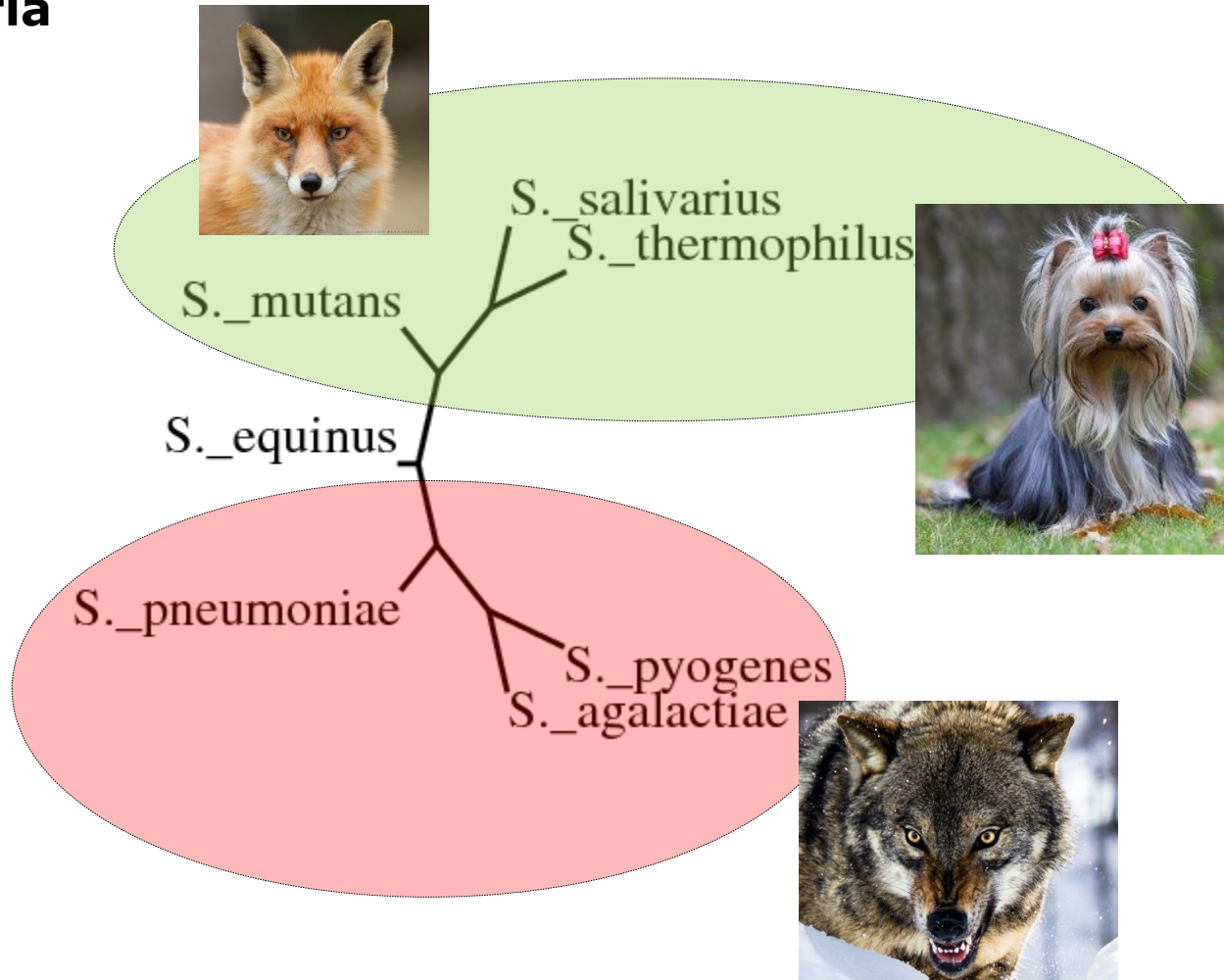
- **More than 1000 holdings,
mainly lactic acid bacteria (LAB)
mainly from dairy products**

Laying the background: Fermented foods and Microorganisms

- **Lactic Acid Bacteria: Gram-positive, low-GC, rods or cocci, non-sporulating, catalase-negative, aerotolerant but not aerophilic and they produce lactic acid as the major metabolic end-product of carbohydrate fermentation**
 - **Carnobacterium**
 - **Enterococcus**
 - **Lactococcus**
 - **Lactobacillus**
 - **Lactosphaera**
 - **Leuconostoc**
 - **Oenococcus**
 - **Pediococcus**
 - **Streptococcus** ←
 - **Teragenococcus**
 - **Vagococcus**
 - **Weissella**

Laying the background: Pathogenicity within the *Streptococcus* genus and *Streptococcus thermophilus*

- The *Streptococcus* genus is a food-related genus within lactic acid bacteria



Laying the background: Pathogenicity within the *Streptococcus* genus and *Streptococcus thermophilus*

- Domestication of *S. thermophilus*



Ancestor A



Ancestor B



S. thermophilus

Virulence



**65-23x 10⁶ B.C.
Paleocene**



**10,000 B.C.
Neolithic**

Laying the background: Pathogenicity within the *Streptococcus* genus and *Streptococcus thermophilus*

- Evolutionary traits of adaptation in milk of *Streptococcus thermophilus*
 - i. 10% pseudogenes (degenerative evolution process)
 - ii. diminished number of biosynthetic capabilities
 - ii. loss of genes related to pathogenicity
 - iv. acquisition of genes related to technological traits

Bolotin et al. Nat Biotechnol 2004

Hols et al. FEMS Microbiol Rev 2005

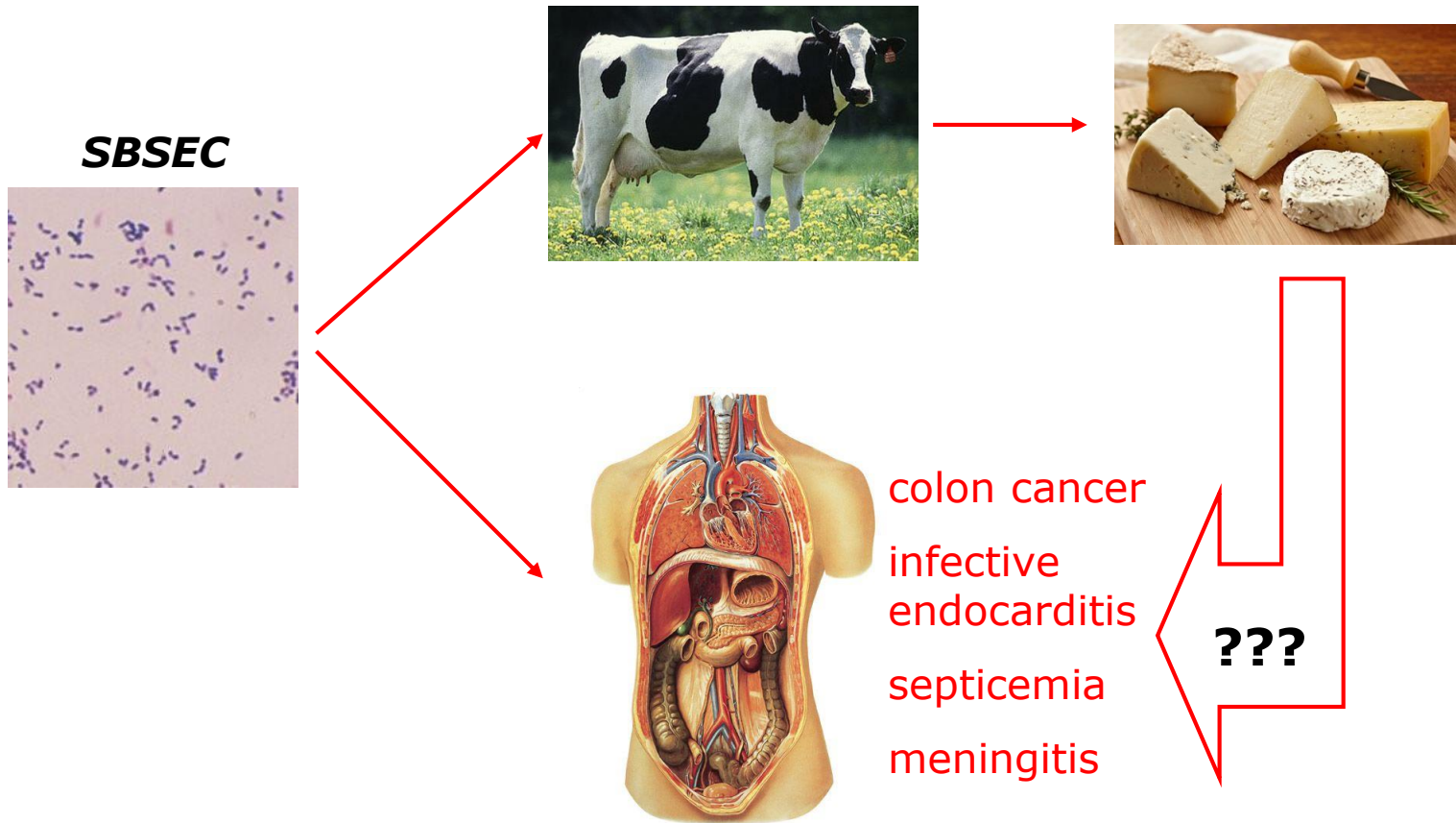
Papadimitriou et al. COFS 2015

- However, *S. thermophilus* is not the only streptococcal species found in fermented food especially of dairy origin...

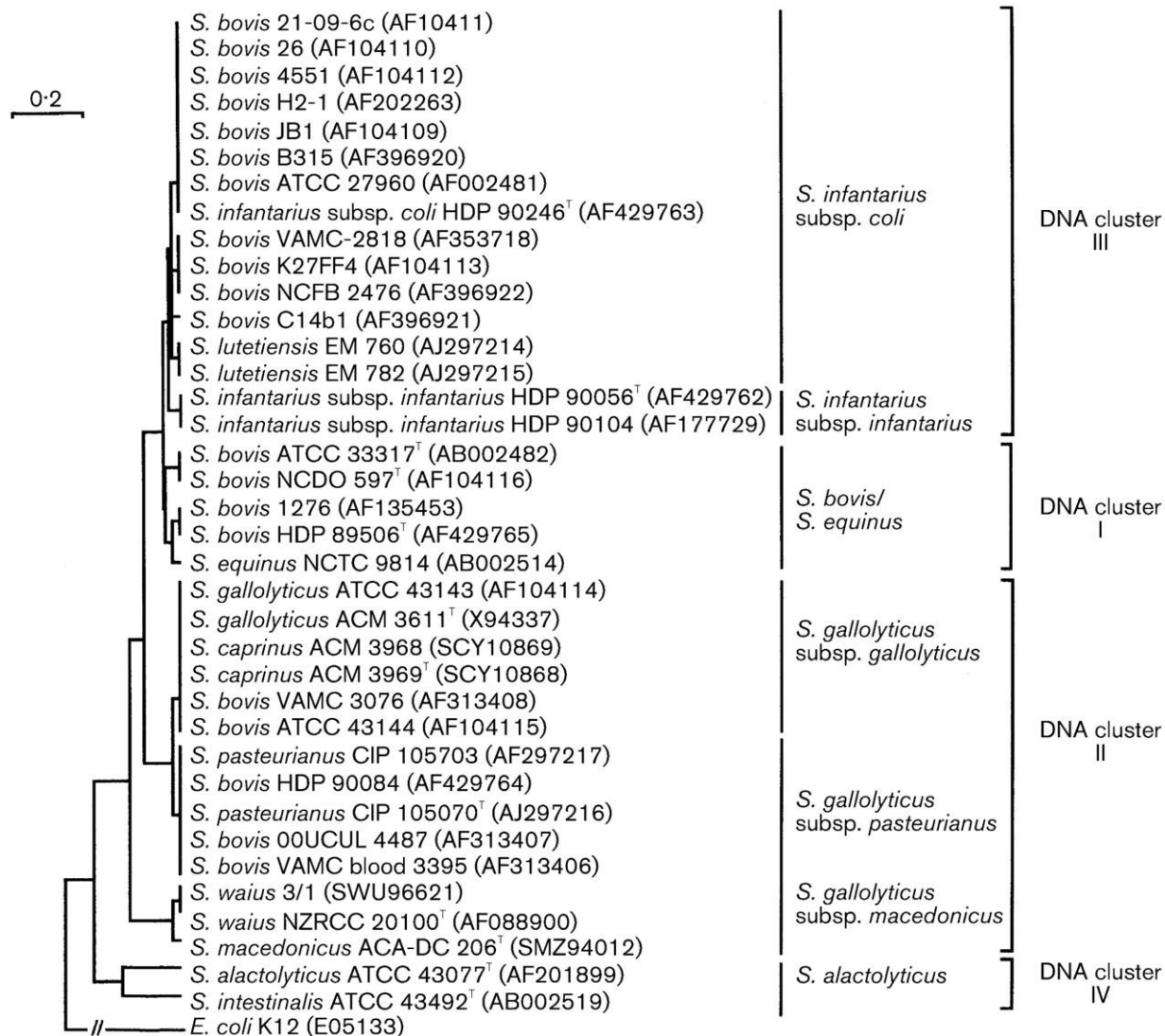


Sequencing the genome of *S. macedonicus* ACA-DC 198

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



Laying the background: The *S. bovis*/*S. equinus* (SBSEC) complex



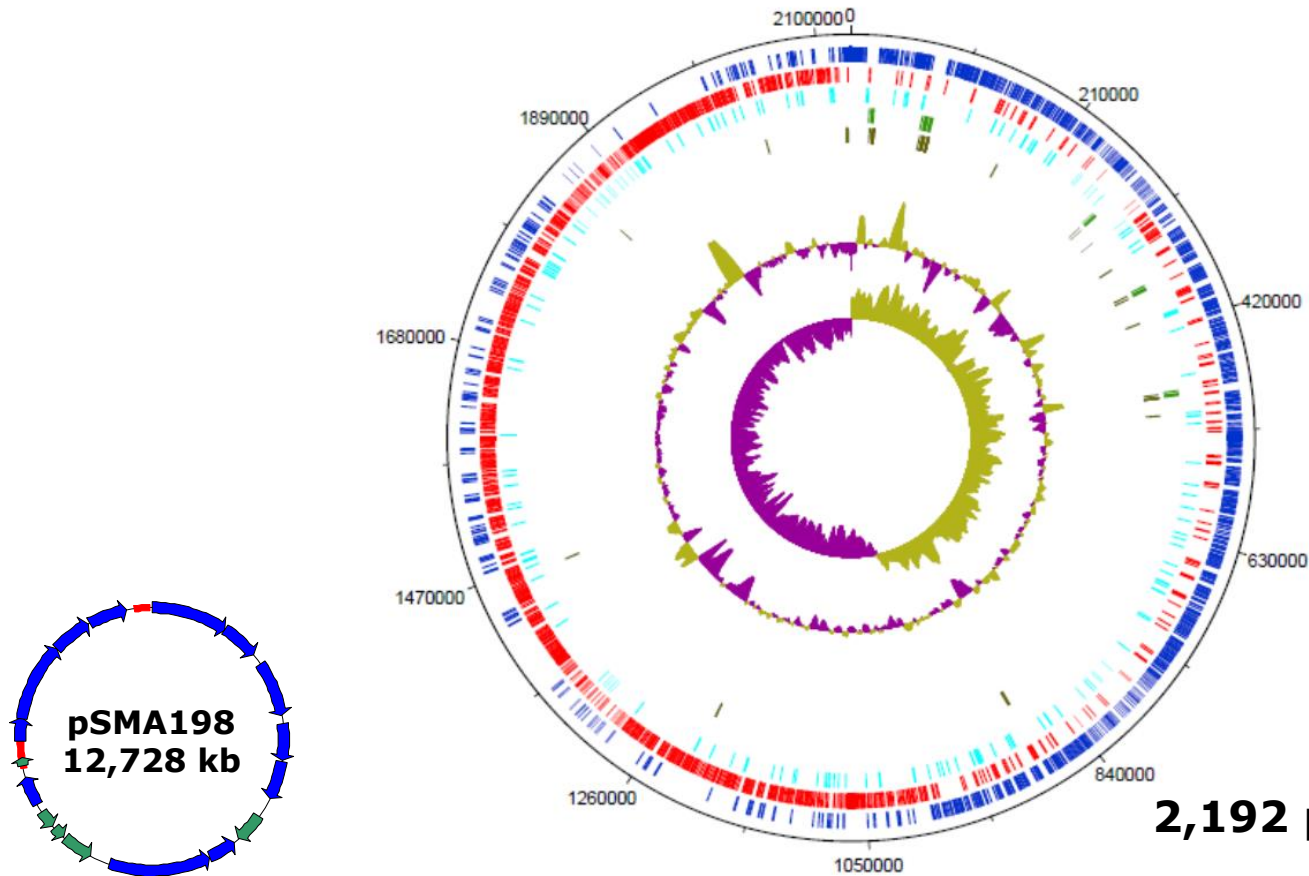
Sequencing the genome of *S. macedonicus* ACA-DC 198

- ***Streptococcus macedonicus*** which was originally isolated and characterized from traditional Greek Kasserli cheese belongs to the SBSEC (Tsakalidou et al. Int J Syst Bacteriol. 1998) and seems to have milk and dairy products as its primary ecological niche (De Vuyst and Tsakalidou Int Dairy J. 2008).
- The close phylogenetic relationship of *S. macedonicus* with pathogenic species of the SBSEC like *S. gallolyticus* and *S. pasteurianus* according to current taxonomy raises concern about the safety of the species

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* the pathogenic potential of the species and its potential adaptation to the milk environment

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

- **Genome map of *S. macedonicus***



2,192 protein coding genes

192 potential pseudogenes

18 rRNA genes

70 tRNA genes

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

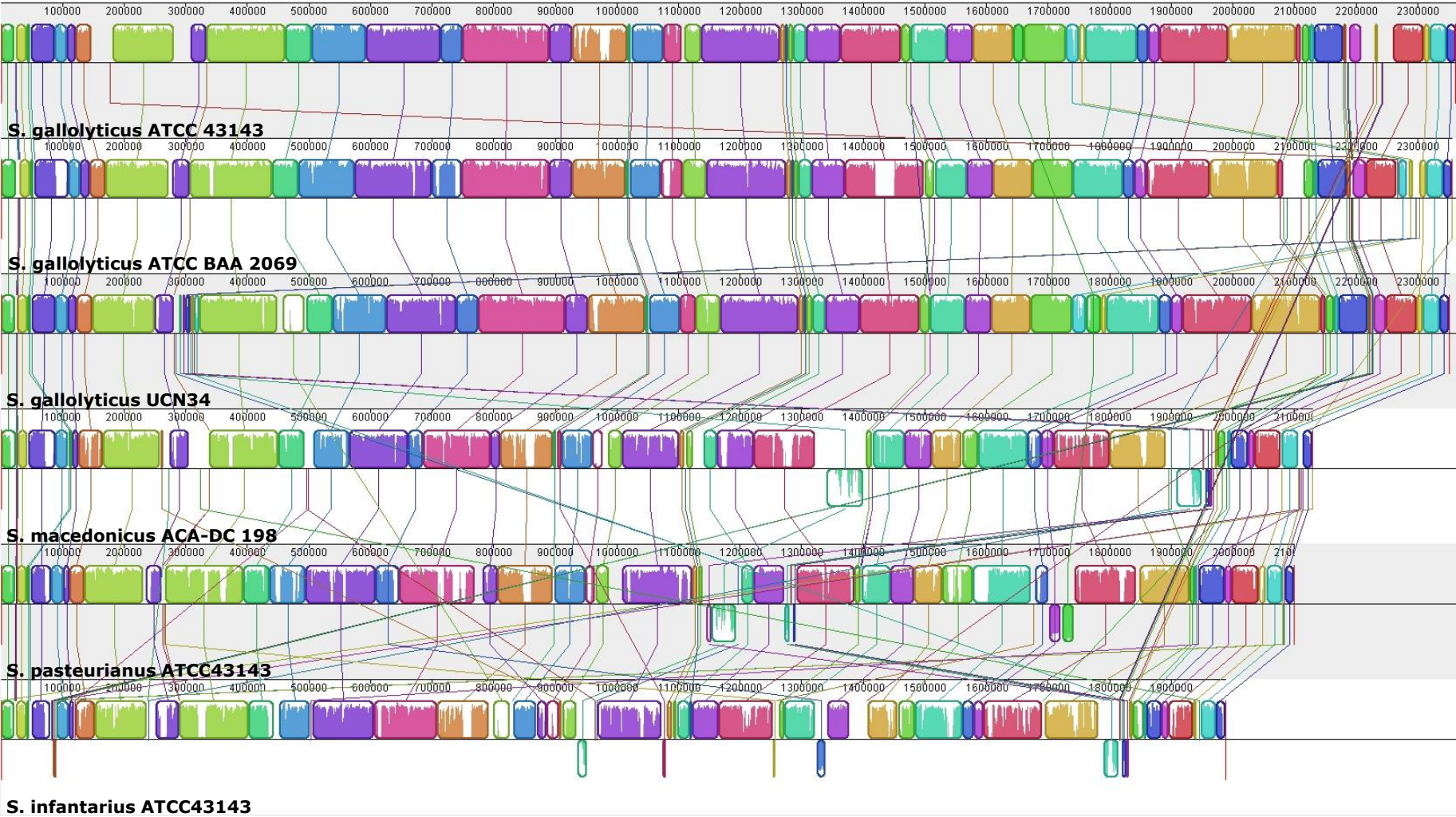
In the SBSEC there are currently five additional complete genome sequences available:

- 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)**

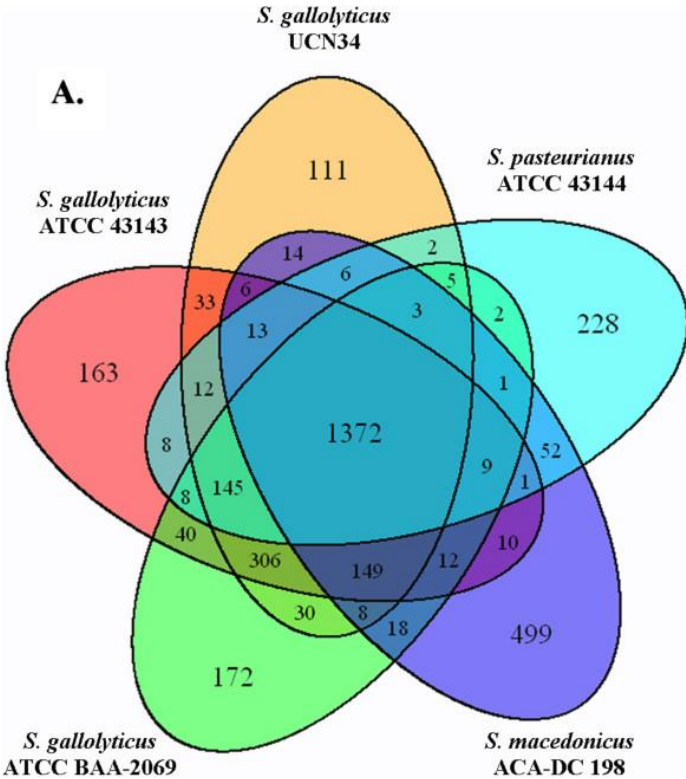
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex



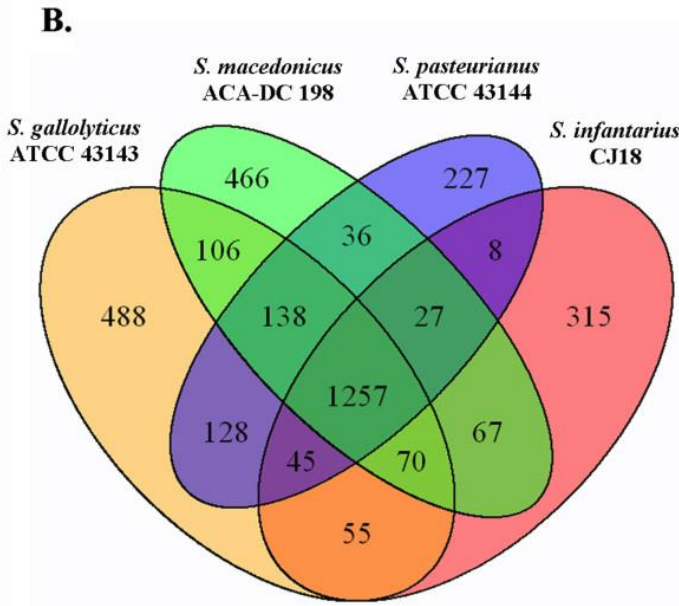
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex



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



Core genome:
62.6%



Core genome:
57.3%

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

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

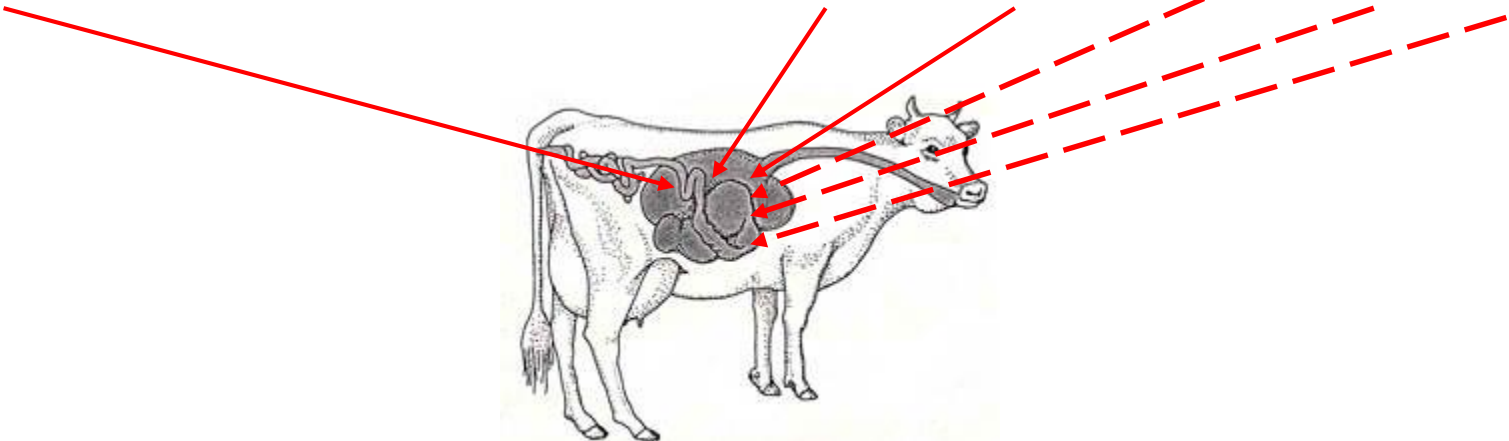
1. *S. macedonicus*, *S. pasteurianus* and *S. infantarius* genomes are being shaped by selective pressures that favor extensive gene loss events and genome decay processes when compared to the *S. gallolyticus* genome

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

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

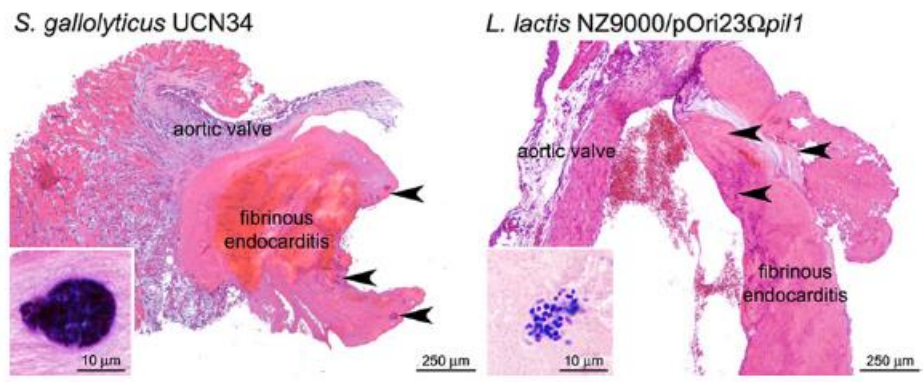
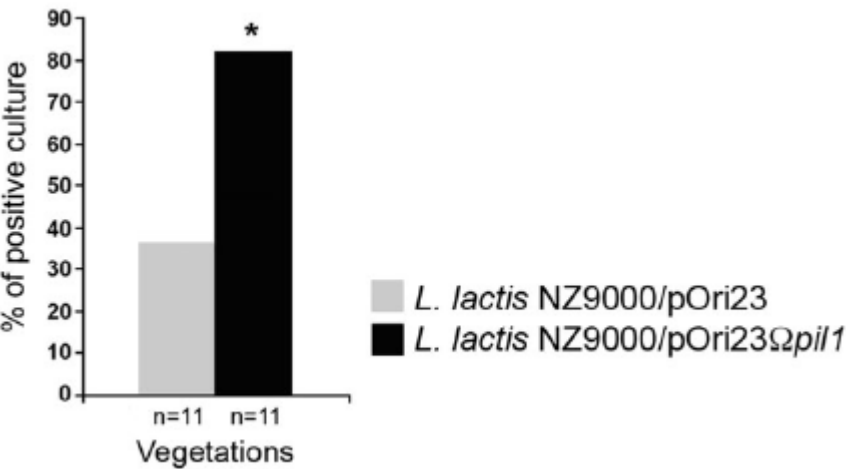
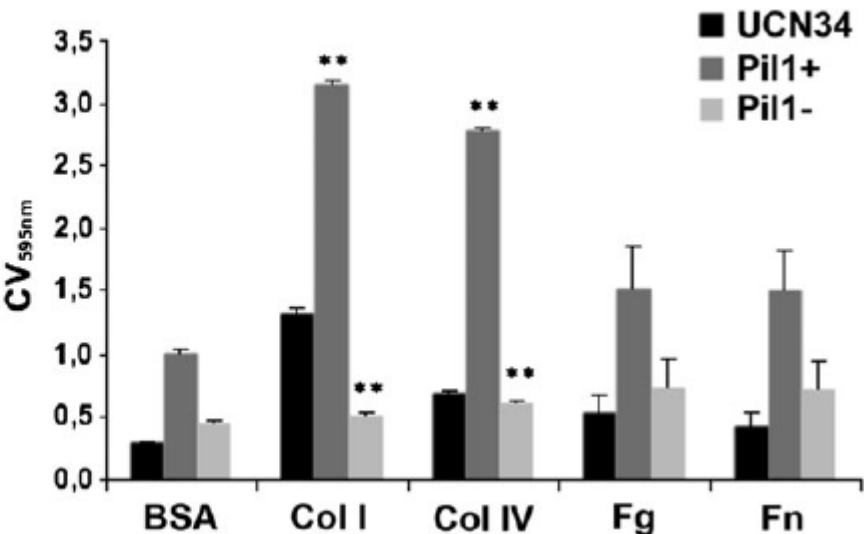
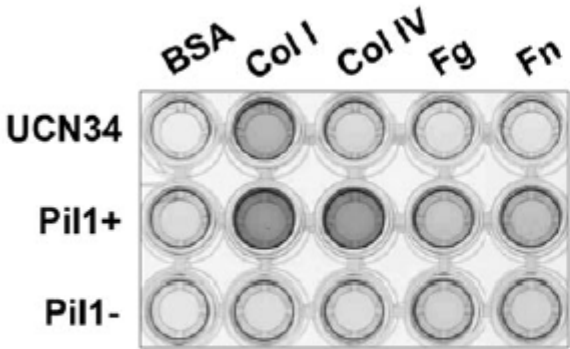
• **Niche-specific genes presence/absence**

<i>S. gallolyticus</i> UCN 34 locus_tag	gene	function	<i>S. gallolyticus</i> ATCC BAA 2069	<i>S. gallolyticus</i> ATCC 43143	<i>S. macedonicus</i> ACA-DC 198	<i>S. pasteurianus</i> ATCC 43144	<i>S. infantarius</i> 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	✓	✓	pseudo	✓	-
gallo_0818	bsh	bile salt hydrolase	✓	✓	pseudo	✓	✓



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

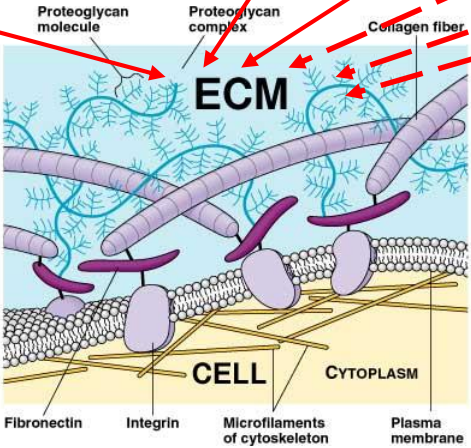
Pathogenicity genes presence/absence



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

• Virulence genes presence/absence

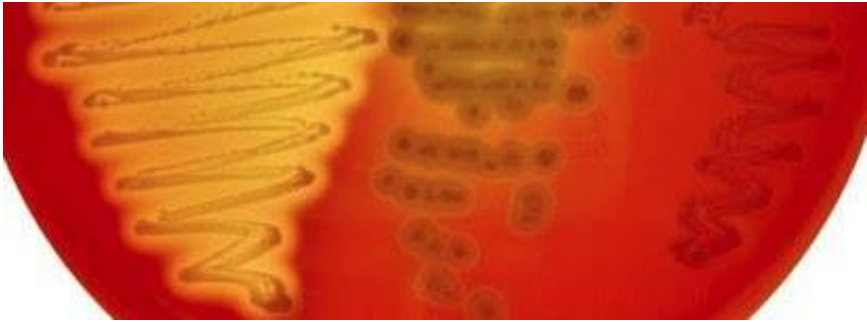
<i>S. gallolyticus</i> UCN 34 locus_tag	gene	function	<i>S. gallolyticus</i> ATCC BAA 2069	<i>S. gallolyticus</i> ATCC 43143	<i>S. macedonicus</i> ACA-DC 198	<i>S. pasteurianus</i> ATCC 43144	<i>S. infantarius</i> CJ18
gallo_2179	-	accessory pilin (pil1)	✓	✓	-	-	-
gallo_2178	-	major pilin (pil1)	✓	✓	-	-	-
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)	✓	✓	✓	✓	✓



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

- Virulence genes presence/absence**

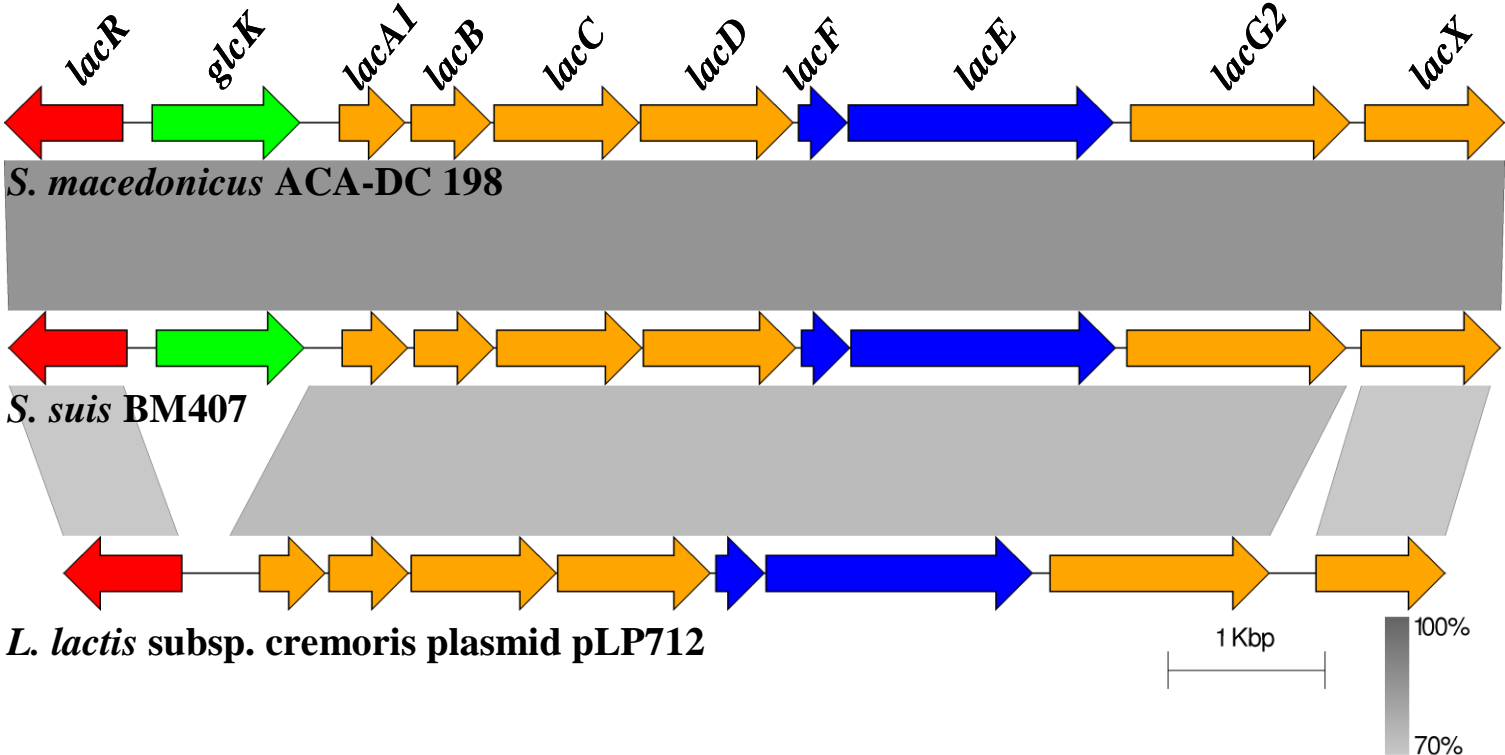
<i>S. gallolyticus</i> UCN 34 locus_tag	gene	function	<i>S. gallolyticus</i> ATCC BAA 2069	<i>S. gallolyticus</i> ATCC 43143	<i>S. macedonicus</i> ACA-DC 198	<i>S. pasteurianus</i> ATCC 43144	<i>S. infantarius</i> CJ18
gallo_0630	-	hemolysin TLY	✓	✓	✓	✓	✓
gallo_1262	-	hemolysin III	✓	✓	✓	✓	✓
gallo_1799	-	hemolysin A	✓	✓	✓	✓	✓



Hinse et al. BMC Genomics 2011
 Papadimitriou et al. BMC Genomics 2014

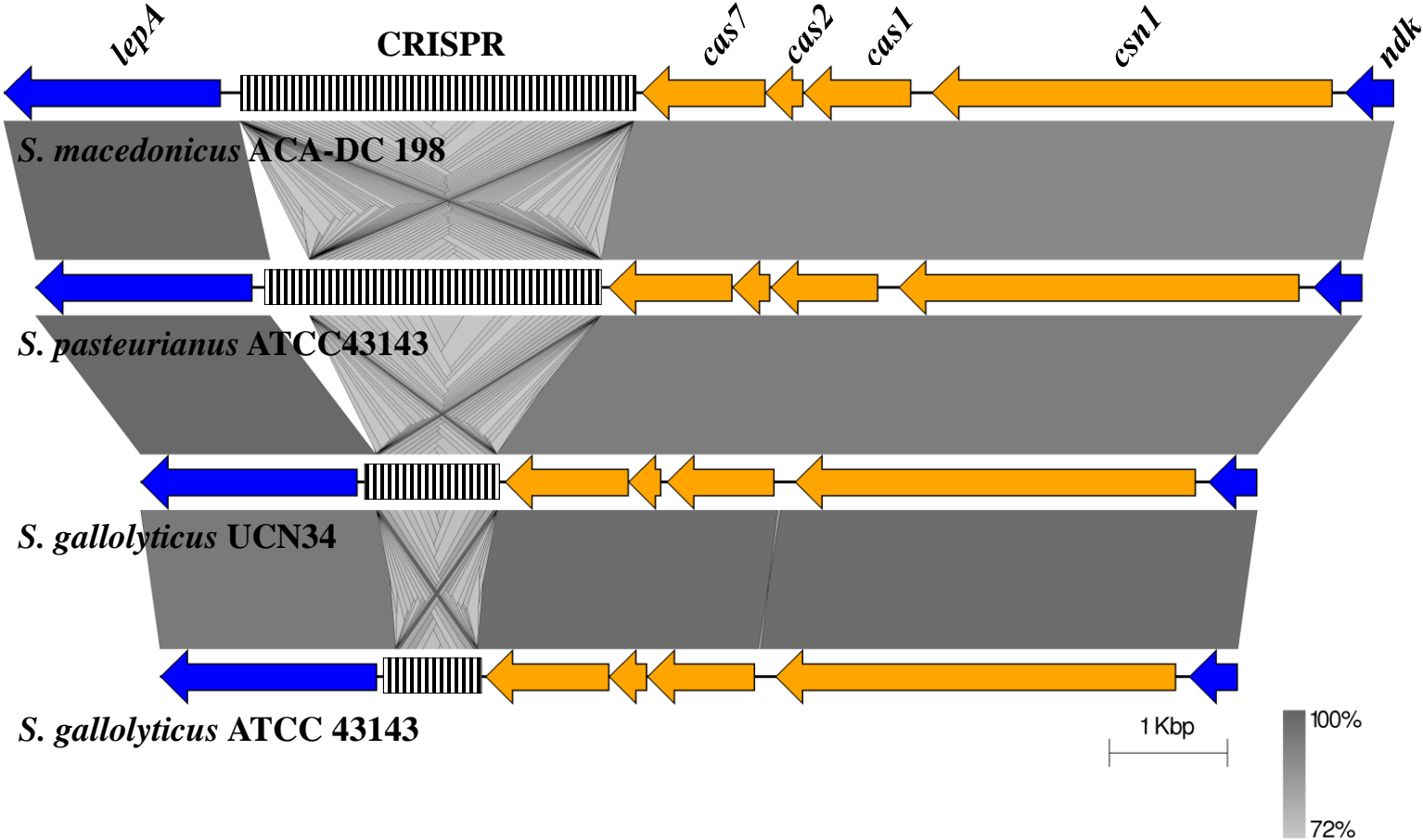
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

- Traits supporting adaptation to the milk environment



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

- Traits supporting adaptation to the milk environment

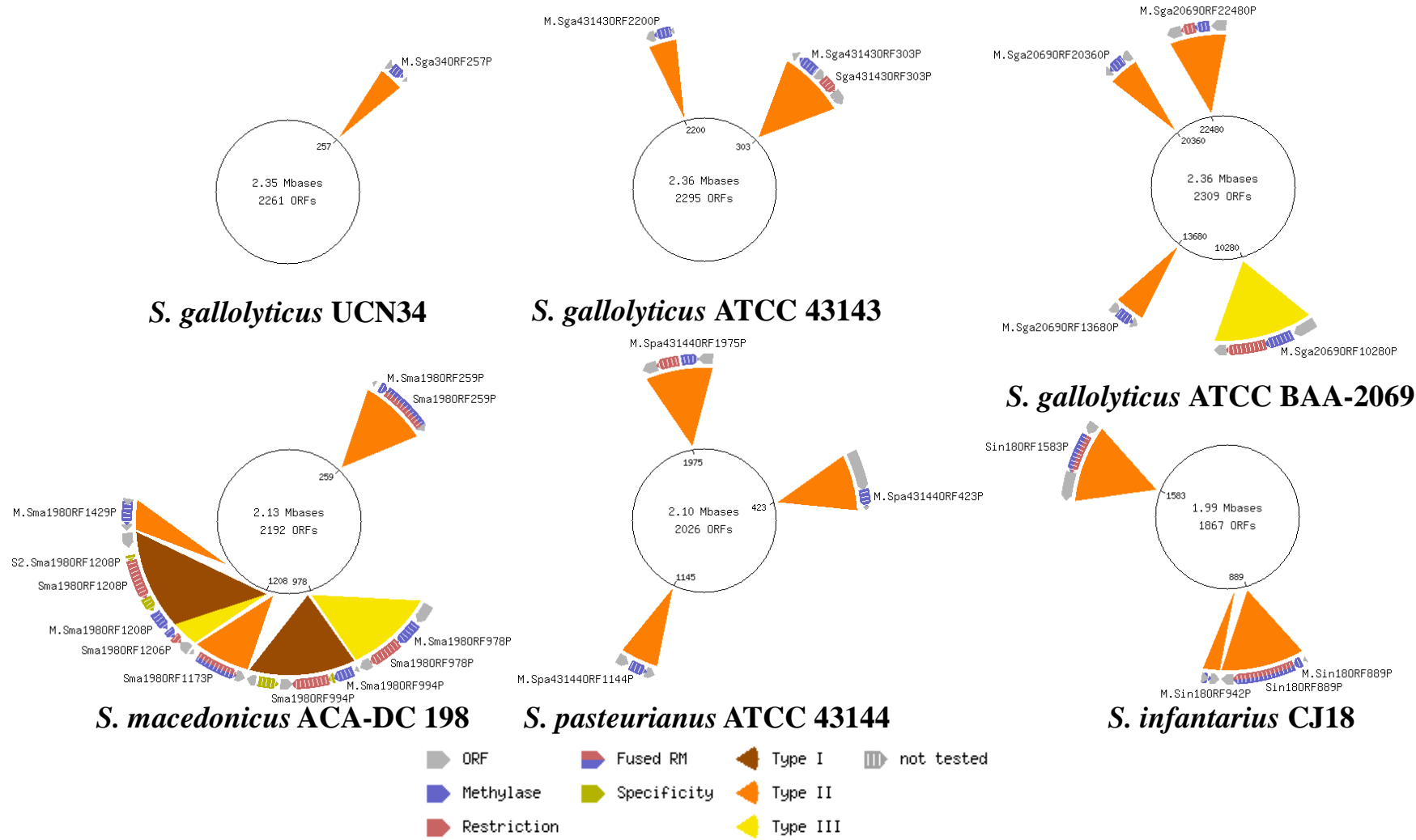


Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

- **Traits supporting adaptation to the milk environment**
- **In *S. macedonicus* spacers 3, 5, 17, 46 provide immunity against phages of the dairy *S. thermophilus* and *L. lactis***

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

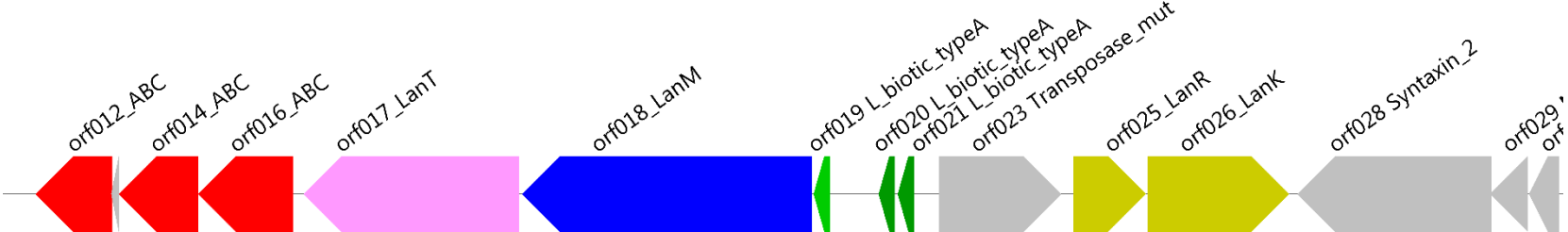
Traits supporting adaptation to the milk environment



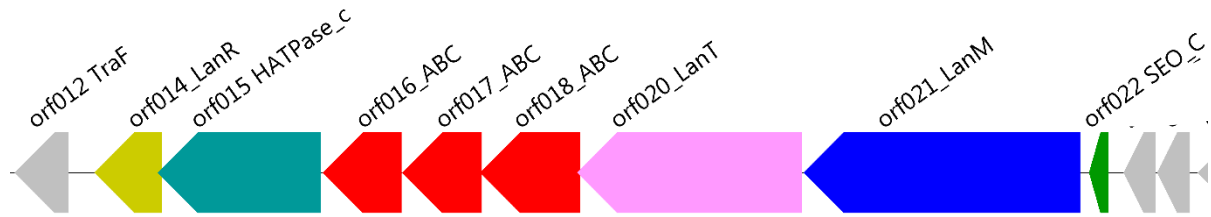
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

- **Traits supporting adaptation to the milk environment**

- **Macedocin (Papadelli et al. FEMS Microbiol lett. 2007)**



- **Macedovicin (Georgalaki et al. Food Microbiol. 2013)**



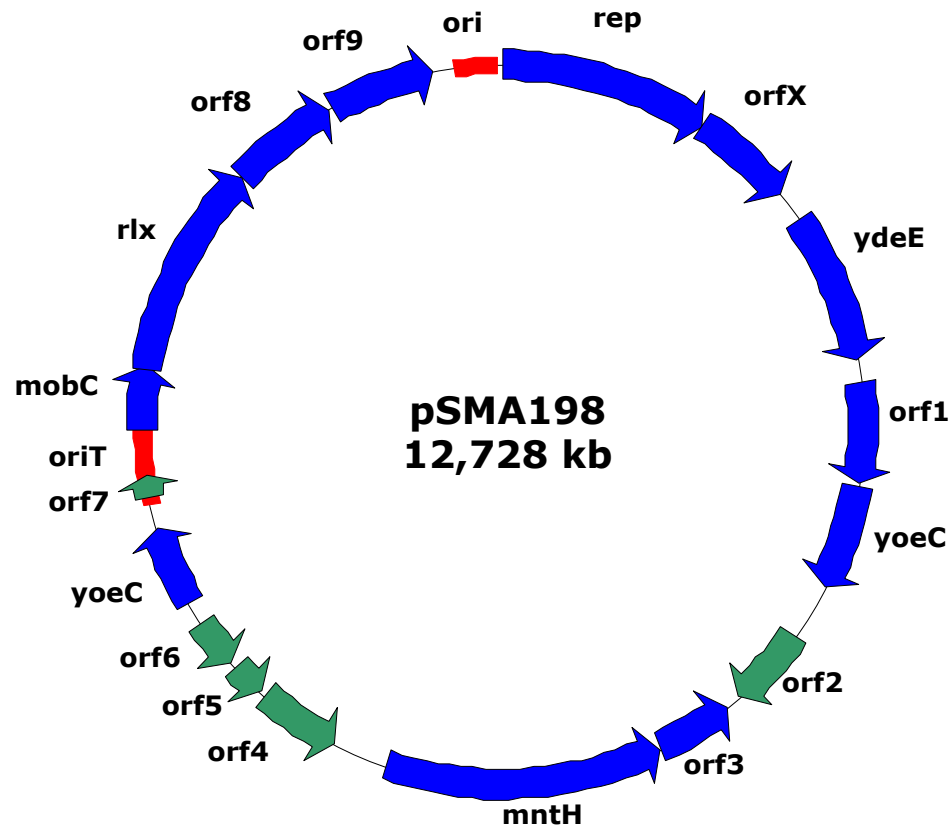
- **OpuC (L-carnitine and choline) ABC transporter**

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

- **Traits supporting adaptation to the milk environment**
 - **Proteolytic system (no CEP, PepE/PepG, PepI/PepR/PepL)**
 - **Vitamin auxotrophy (e.g. biotin, pantothenate and riboflavin)**

Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

- The presence of plasmid pSMA198 in *Streptococcus macedonicus* provided us with unexpected clues about the habituation of the species to the milk environment!!!



Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

1. pSMA198 belongs to the narrow host range pCI305/pWV02 family of lactococcal plasmids and it is the first such plasmid to be reported in *Streptococcus*

Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

Multiple sequence alignment of the pSMA198 Rep with RepB proteins of the pCI305 family

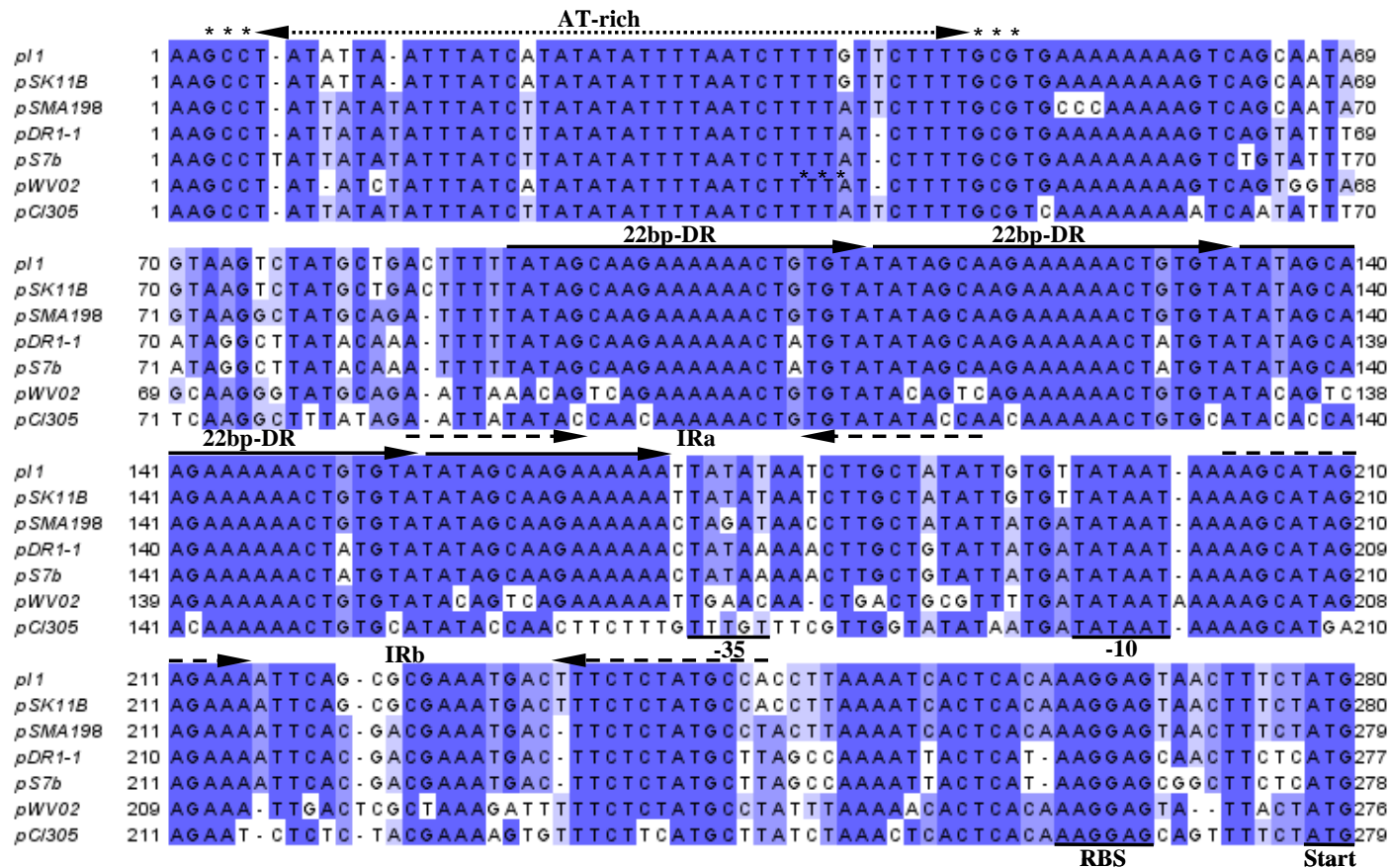
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pWV02          1 MPSTAEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSK EELFTFFKVDNDKHSRFKQAVAKMQEQAFFE 100
LLCRE1631_02531 1 MSIIPEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pIL7          1 MPIISEKQNNQKQVLTNLNELEKRRVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pGL3          1 MSSITEKAKNQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pS7a         1 MSSITEKEENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pDBORO       1 MLIITEKQENKQVLTNLNELEKRRVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pK214        1 MSIIPEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKAE LFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
Ent_faecalis_TX1346 1 MSSIIPEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pCI305       1 MSSISKNQEPNQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSK EELFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pSK11B      1 MSSIIPEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pLACR1      1 MSSIIPEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pSMA198     1 MSSIIPEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
pVS40       1 MSIIPEKQENQKQVLTNLNLSKRKVVVEHNSLITSIAKMDKTP LKMFELAVSCINTEEPPKDNVYLSKRDLFAFKVSDNDKHSRFKQAVEKMQKQAAF 100
-----
pWV02          101 IKEEAGKGFKFRRLP IPTVEWTDYDDKVMIRFNQDIMPYLIELNENFSKYALSEIMELNSKYSII LYLKWSMNYNQYEHYSNKGGRRAEQVESYRNP 200
LLCRE1631_02531 101 IKEEAGKGFKFKSIVP IPYVEWTDYNDVYKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSVKGGRRAEQVESYRNP 200
pIL7          101 IKEEAGKGFKFKSIVP IPYVEWTDYNDVYKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSVKGGRRAEQVESYRNP 200
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pS7a         101 IKEEAGKGFKFKSIVP IPYVEWTDYHDEVKIEFHREIMPYLINLKKNFQHALSEISELNSKYSII LYLWLSMNYNQYEHYSVKGGRADQVESYRNP 200
pDBORO       101 IKEEAGKGFKFKSIVP IPYVEWTDYHDEVKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSVKGGRRAEQVESYRNP 200
pK214        101 IKEEAGKGFKFKSIVP IPYVEWTDYNDVYKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSNKGGRRAEQVESYRNP 200
Ent_faecalis_TX1346 101 IKEEAGKGFKFKSIVP IPYVEWTDYHDEVKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSTKGGRRAEQVESYRNP 200
pCI305       101 IKEEAGKGFKFRSIVP IPYVEWTDYHDDVYKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSVKGGRREEQVESYRNP 200
pSK11B      101 IKEEAGKGFKFKSIVP IPYVEWTDYHDEVKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSTKGGRRAEQVESYRNP 200
pLACR1      101 IKEEAGKGFKFKSIVP IPYVEWTDYHDEVKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSTKGGRRAEQVESYRNP 200
pSMA198     101 IKEEAGKGFKFKSIVP IPYVEWTDYHDEVKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSVKGGRRAEQVESYRNP 200
pVS40       101 IKEEAGKGFKFKSIVP IPYVEWTDYNDVYKIEFHREIMPYLINLKKNFQHALSDIAELNSKYSII LYLWLSMNYNQYEHYSVKGGRRAEQVESYRNP 200
-----
pWV02          201 SVKELRITDVTVNEYRHFPHFNWILKKP LEEINAHTSFNVSYEKIKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
LLCRE1631_02531 201 SIKELRIMTDTVNSYKQFTRFNNDV LKTP LDEINAHTSFNVTYDKIKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pIL7          201 SIKELRIMTDTIKEYSRFQS FENRV LKNAVVEITQHTSFNVSYEKVKKGRSIDSIVFHI TKKRADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pGL3          201 SVKELRIMTDTVNEYRFTNFTFKKILDIPLKEINDNTTFKYSYEKVKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pS7a         201 SIKELRIMTDTVNEYRFTNFTFKKILDIPLKEINDNTTFKYSYEKVKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pDBORO       201 TVKELRVMTDTVNEYKRMFQTEWIL EKLPLAEINAHTSFNVSYEKVKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pK214        201 KYKEMRLMTDTVNEYKYNWDRYIL LKNS LKEINAHTSFNVTYDKIKKGRSIDSIVFHI TKKRADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
Ent_faecalis_TX1346 201 SIKELRIMTDTVNEYRFSLETWILKKP LEEINAHTSFNVTYDKIKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pCI305       201 SMRELREMTDTVDEYPRFDRLEHRV LKEPIEINHTSFNVTYDKIKKGRSIDSIVFHI TKKRADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pSK11B      201 SIKELRIMTDTVNEYRFSLETWILKKP LEEINDHTSFNVTYDKIKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pLACR1      201 SIKELRIMTDTVNEYRFSLETWILKKP LEEINDHTSFNVTYDKIKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pSMA198     201 SIKELRIMTDTVNEYRFSLETWILKKP LEEINAHTSFNVTYDKIKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
pVS40       201 SIKELRIMTDTVNSYKQFTRFNNDV LKTP LDEINAHTSFNVTYDKIKKGRSIDSIVFHI EKKRMADDSYKLEDQYVIEGKKAKEETEKLDTYTESMOSP 300
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pWV02          301 TKLLLENMLLSPYEMTDALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 383
LLCRE1631_02531 301 TKLLMEHFLLSYLDLMDKKILAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 383
pIL7          301 TRLLIENFLLSPLEMTDTALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 383
pGL3          301 TRLLIENFLLSPLEMTDTALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 383
pS7a         301 TRLLIENFLLSPLEMTDTALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 383
pDBORO       301 TKLLIENFLLSPLEMTDTALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 383
pK214        301 TKLLLENFLLSPYEMTDPAIMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 384
Ent_faecalis_TX1346 301 TOI LLENMLLSPYEMTDALMAGLQAHVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 384
pCI305       301 TRLLIENFLLSPLEMTDTALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 386
pSK11B      301 TOI LLENMLLSPYEMTDALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 386
pLACR1      301 TOI LLENMLLSPYEMTDALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 386
pSMA198     301 TKLLLDNMLLSYEMTDALMAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 397
pVS40       301 TKLLMEHFLLSYLDLMDKKILAGLQKNVYPL YDELKELRGLNGVKDHLSYVSSKQEAYSKRNVAKYLKKAIEQYLP TVKRQDL 399

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Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

Multiple sequence alignment of the pSMA198 ori with the respective sequences of plasmids of the pCI305 family

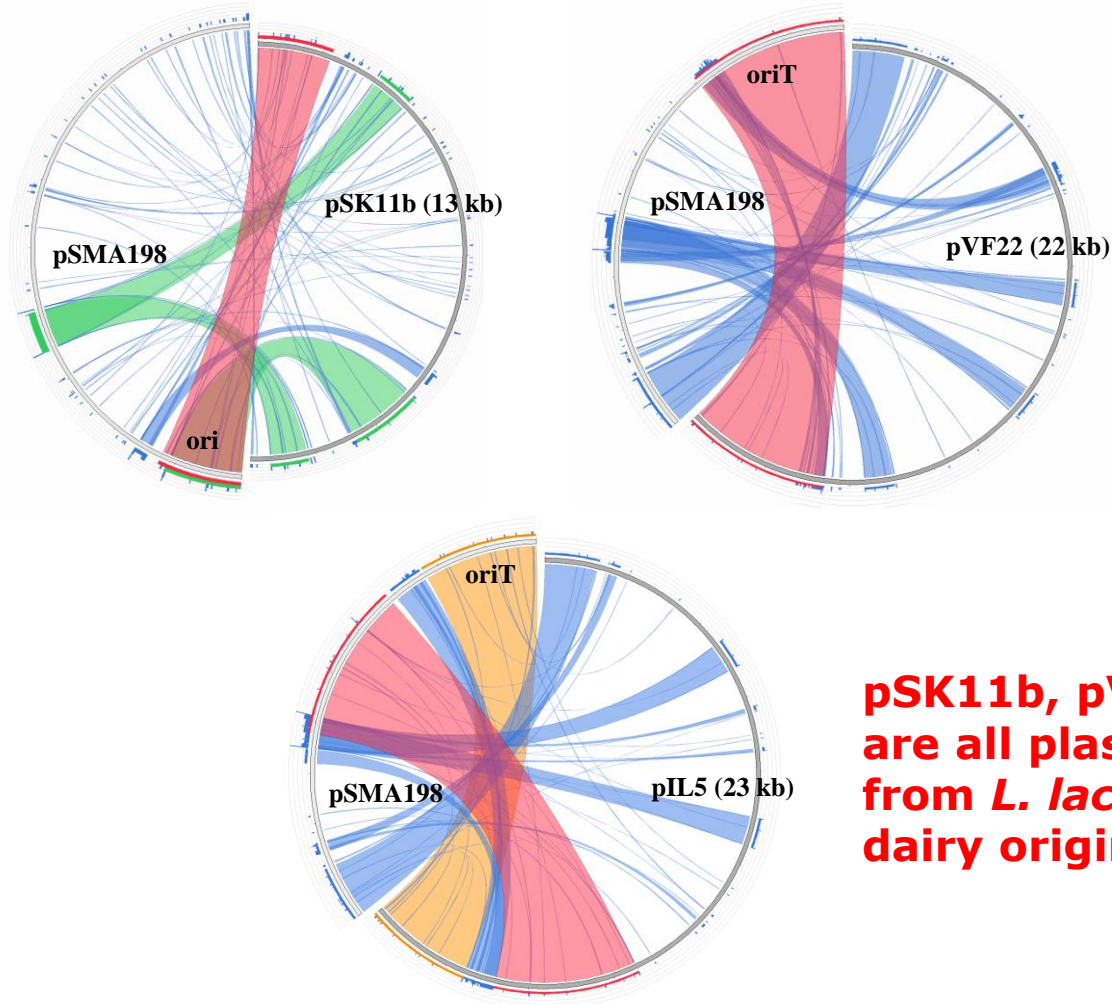


Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

- 1. pSMA198 belongs to the narrow host range pCI305 family of lactococcal plasmids and it is the first such plasmid to be reported in *Streptococcus***
- 2. *S. macedonicus* acquired pSMA198 from *L. lactis* and this acquisition took place most probably in the dairy environment**

Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

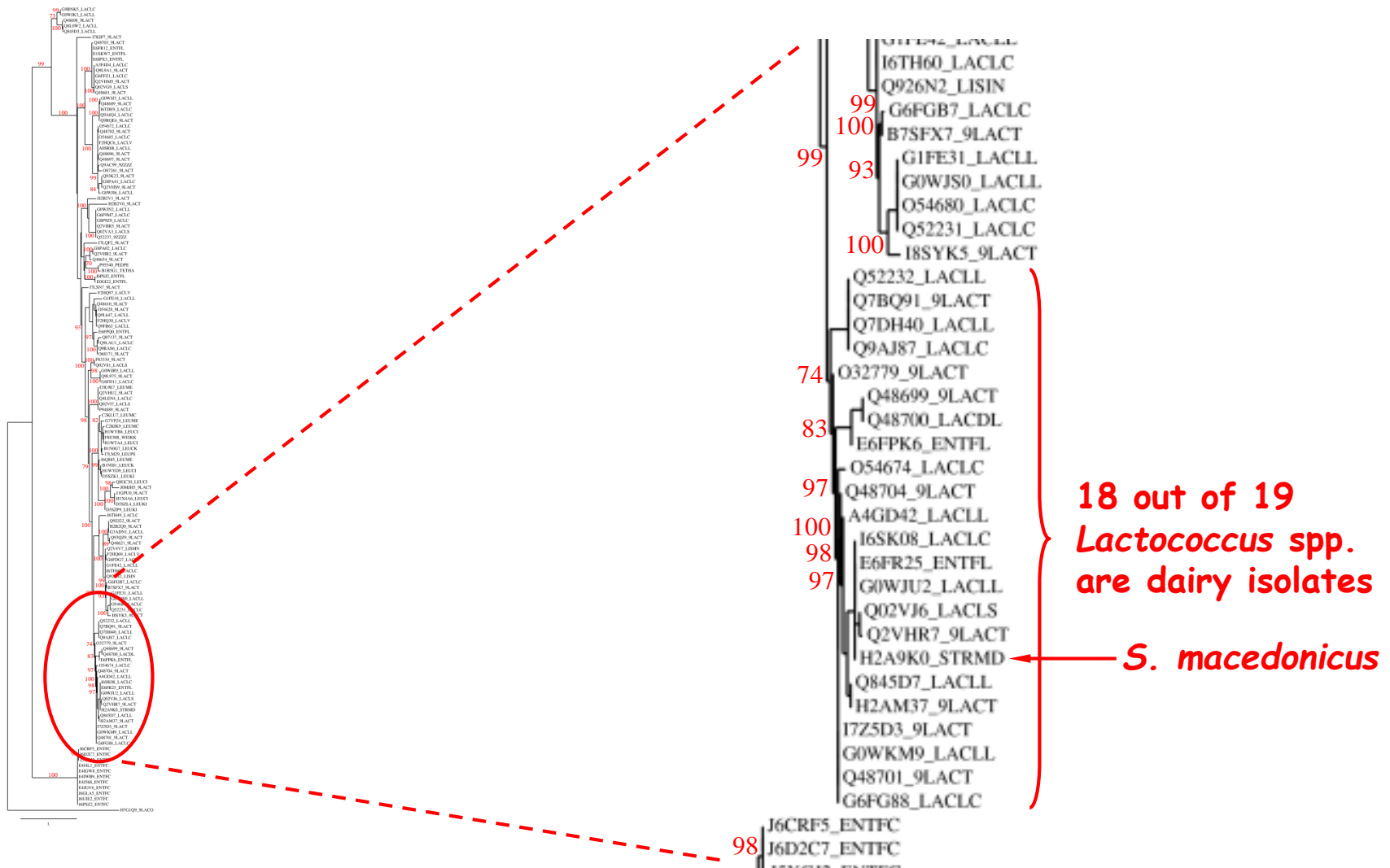
- **Comparative analysis of pSMA198 with its related plasmids**



pSK11b, pVF22 and pIL5 are all plasmids isolated from *L. lactis* strains of dairy origin

Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

- Phylogenetic analysis of the pSMA198 Rep with its related proteins

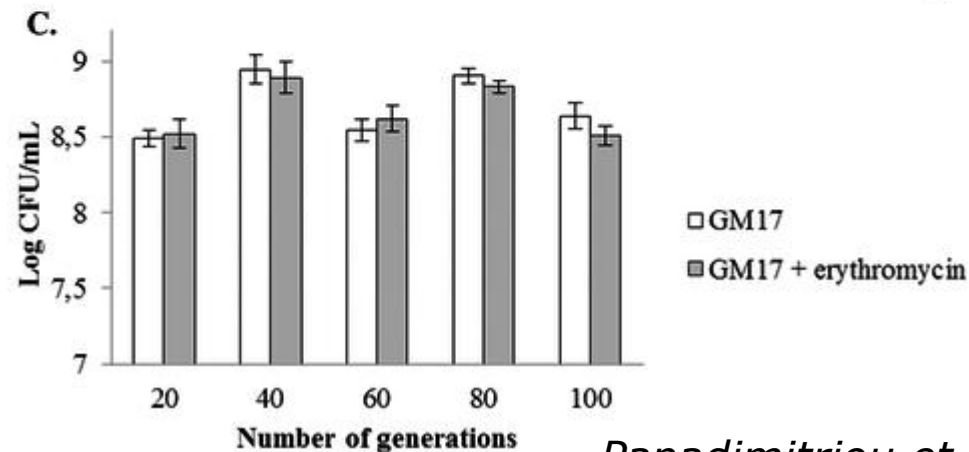
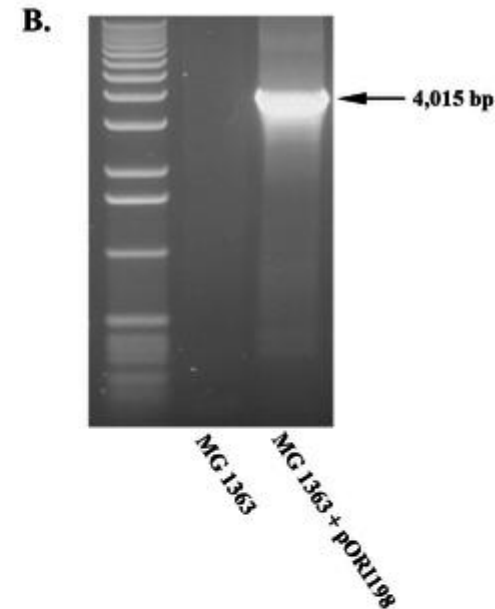
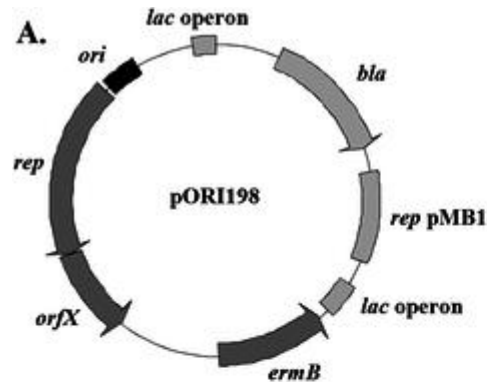


18 out of 19
Lactococcus spp.
are dairy isolates

S. macedonicus

Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

• Cloning and stability of the pSMA198 *ori* in *L. lactis* MG1363



Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

- 1. pSMA198 belongs to the narrow host range pCI305 family of lactococcal plasmids and it is the first such plasmid to be reported in *Streptococcus***
- 2. *S. macedonicus* acquired pSMA198 from *L. lactis* and this acquisition took place most probably in the dairy environment**
- 3. The acquisition of pSMA198 is most probably not a recent event**

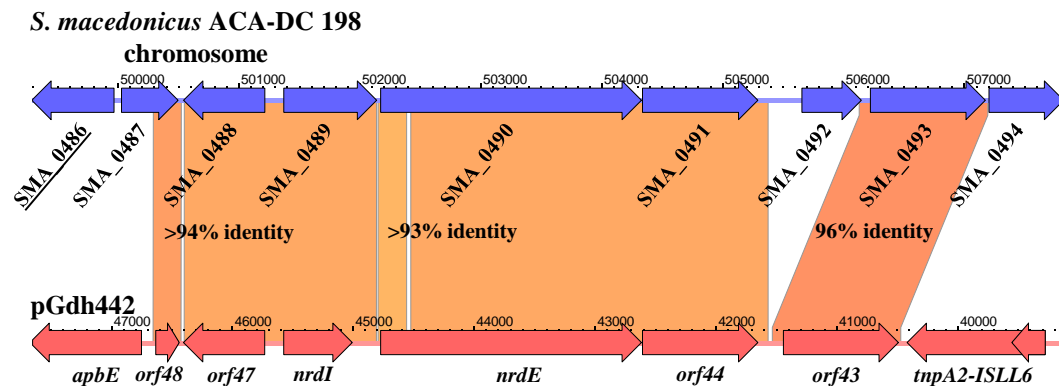
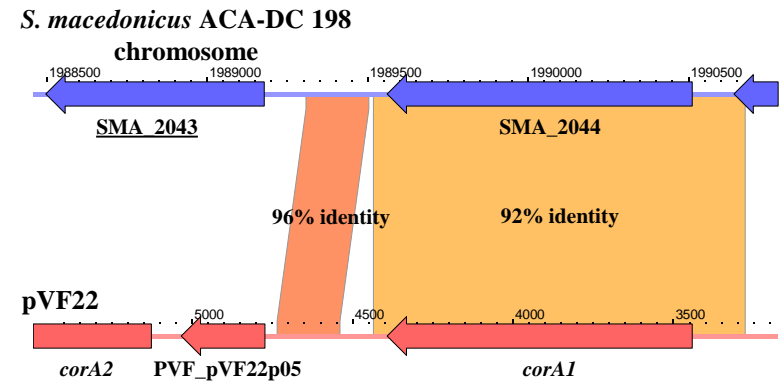
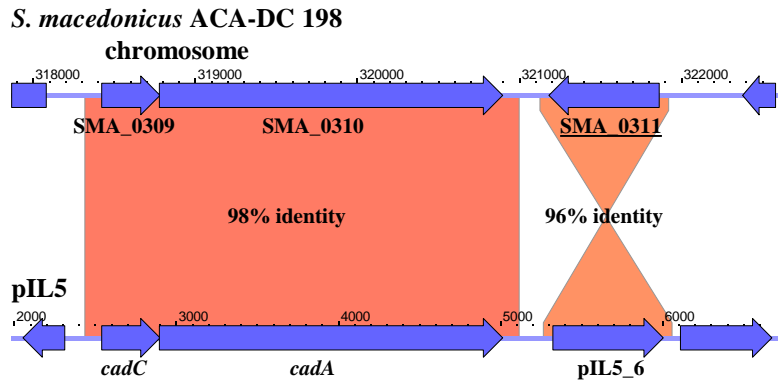
Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

• Increased percentage of pseudogenes (5 out of 17) found in pSMA198

locus_tag	gene	size nt	Best WU-Blastn hit (locus or locus_tag/ organism/ identity/ e-value)	Protein function
SMA_p0001	<i>rep</i>	1194	LACR_A06/ <i>Lactococcus lactis</i> subsp. <i>cremoris</i> SK11 plasmid 1/ 87%/ 1.8e ⁻¹⁹⁶	Initiator RepB protein
SMA_p0002	<i>orfX</i>	585	BN193_11490/ <i>Lactococcus raffinolactis</i> 4877/ 91%/ 2.3e ⁻¹⁰¹	Replication associated protein
SMA_p0003	<i>ydeE</i>	858	ENT_30280 / <i>Enterococcus</i> sp. 7L76 / 99%/ 2.3e ⁻¹⁸⁴	AraC family transcriptional regulator
SMA_p0004	<i>orf1</i>	582	EfmE1039_1841/ <i>Enterococcus faecium</i> E1039/ 99%/ 4.7e ⁻¹²¹	Integral membrane protein
SMA_p0005	<i>yoeC</i>	591	AF179848/ <i>Lactococcus lactis</i> subsp. <i>lactis</i> UC317 pCI305/ 88%/ 1.4e ⁻⁹⁷	Integrase/recombinase plasmid associated
SMA_p0006	<i>orf2</i>	459	CAC42047/ <i>Listeria innocua</i> Clip11262 pLI100/ 99%/ 3.7e ⁻⁹⁴	Putative pseudo ←
SMA_p0007	<i>orf3</i>	438	LACR_D31/ <i>Lactococcus lactis</i> subsp. <i>cremoris</i> SK11 plasmid 4/ 99%/ 4.9e ⁻⁹⁰	Universal stress protein family
SMA_p0008	<i>mntH</i>	1578	HMPREF0848_00725 / <i>Streptococcus</i> sp. C150/ 99%/ 0.0	Manganese transport protein MntH
SMA_p0009	<i>orf4</i>	480	llmg_pseudo_13/ <i>Lactococcus lactis</i> subsp. <i>cremoris</i> MG1363 pseudogene/ 97%/ 3.3e ⁻²¹⁴	Putative pseudo ←
SMA_p0010	<i>orf5</i>	195	llmg_pseudo_13/ <i>Lactococcus lactis</i> subsp. <i>cremoris</i> MG1363 pseudogene/ 97%/ 3.3e ⁻²¹⁴	Putative pseudo ←
SMA_p0011	<i>orf6</i>	276	llmg_pseudo_13/ <i>Lactococcus lactis</i> subsp. <i>cremoris</i> MG1363 pseudogene/ 97%/ 3.3e ⁻²¹⁴	Putative pseudo ←
SMA_p0012	<i>yoeC</i>	465	GMD1E_00300 / <i>Enterococcus</i> sp. GMD1E / 98%/ 2.2e ⁻⁹³	Integrase/recombinase plasmid associated
SMA_p0013	<i>orf7</i>	132	pIL7_28/ <i>Lactococcus lactis</i> subsp. <i>lactis</i> IL594 plasmid pIL7/ 84%/ 1.3e ⁻¹³	Putative pseudo ←
SMA_p0014	<i>mobC</i>	366	HMPREF9519_01999/ <i>Enterococcus faecalis</i> TX1346/ 89%/ 5.9e ⁻⁶¹	Mobilization protein
SMA_p0015	<i>rlx</i>	1233	CI5MOBPRO/ <i>Lactococcus lactis</i> subsp. <i>cremoris</i> UC503 pCI528/ 99%/ 3.4e ⁻²⁶⁸	Mobilization protein
SMA_p0016	<i>orf8</i>	627	ENT_30400 / <i>Enterococcus</i> sp. 7L76/ 96%/ 6.3e ⁻¹²⁴	Conserved hypothetical protein
SMA_p0017	<i>orf9</i>	603	BN193_11500/ <i>Lactococcus raffinolactis</i> 4877/ 99%/ 3.5e ⁻¹²⁵	Fic family protein

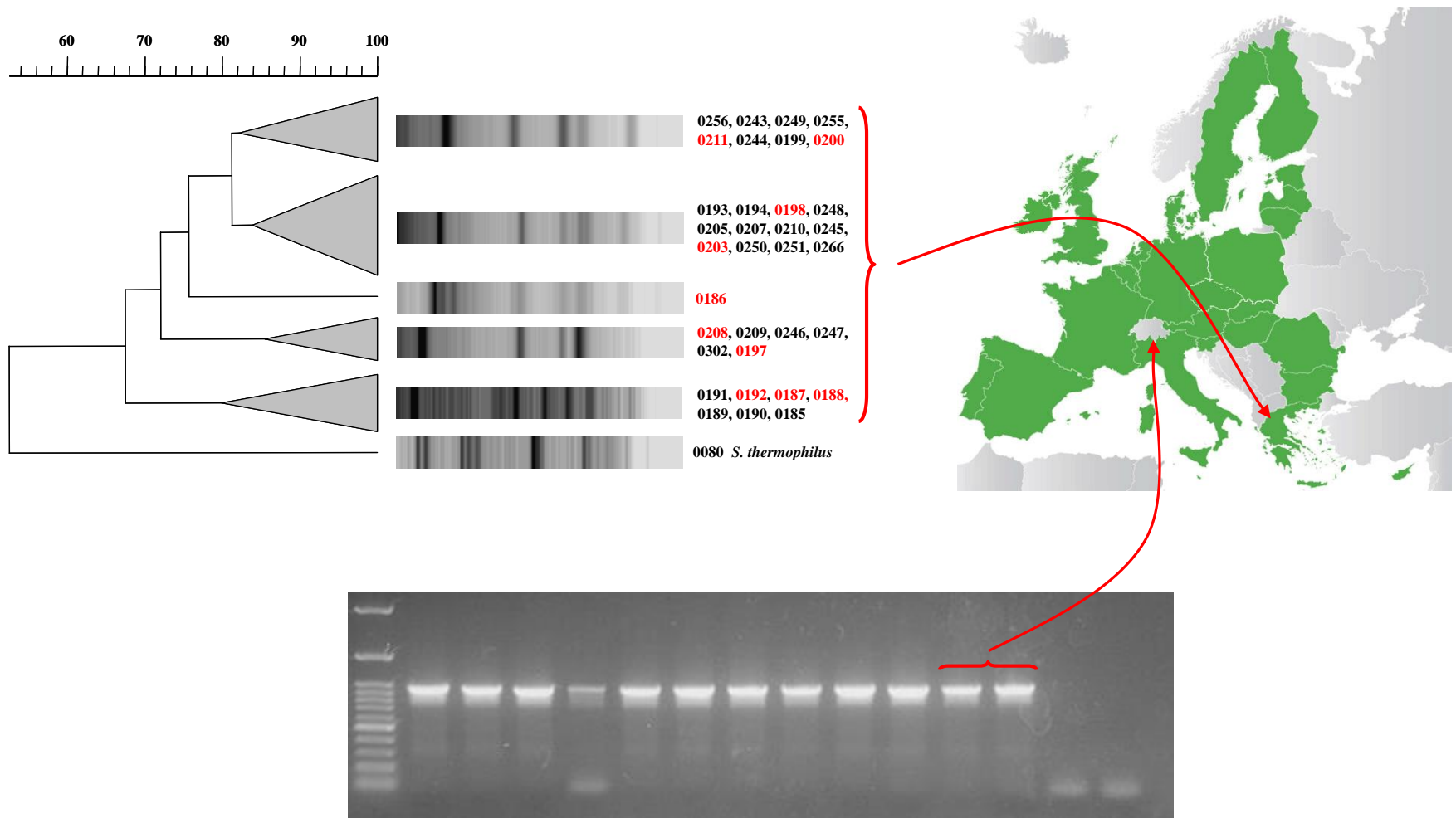
Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

- S. macedonicus* chromosomal loci potentially originating from pSMA198**

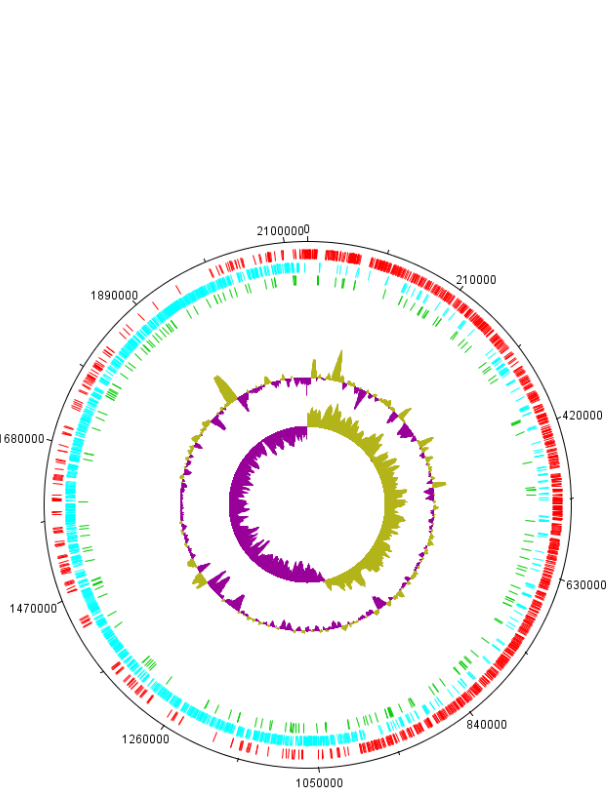


Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

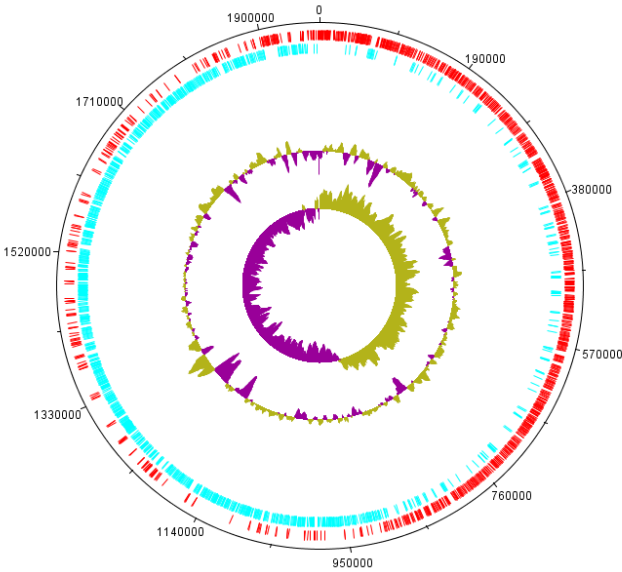
- The distribution of pSMA198 in different strains of *S. macedonicus*



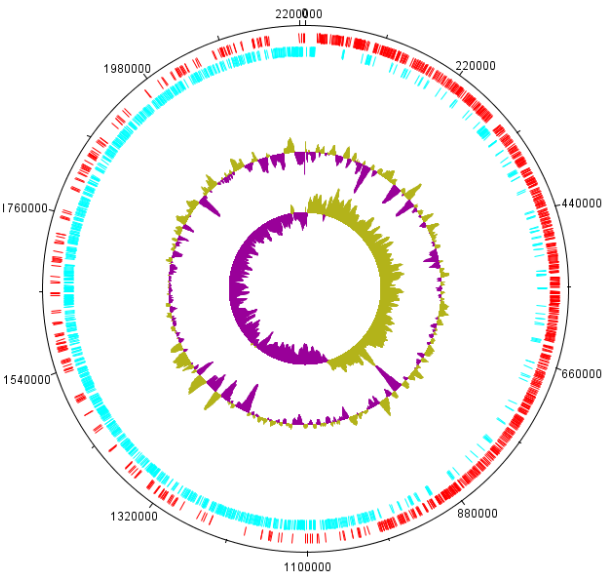
Comparative genomics of three *Streptococcus macedonicus* strains



ACA-DC 198



679



33MO

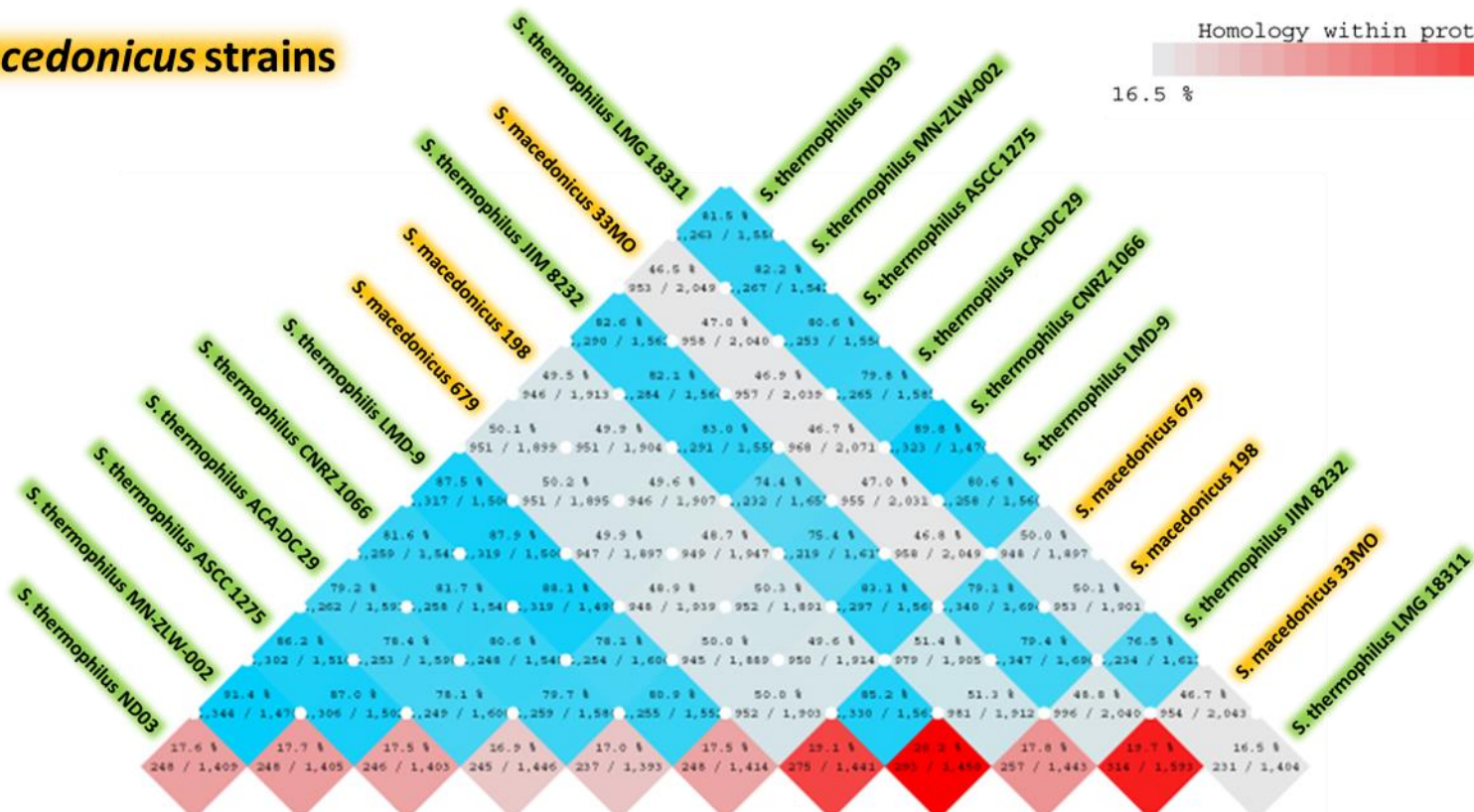
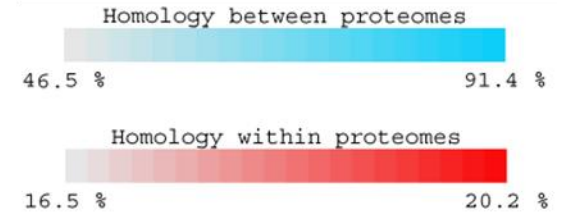
Comparative genomics of three *Streptococcus macedonicus* strains



Comparative genomics between strains of *S. thermophilus* and *S. macedonicus*

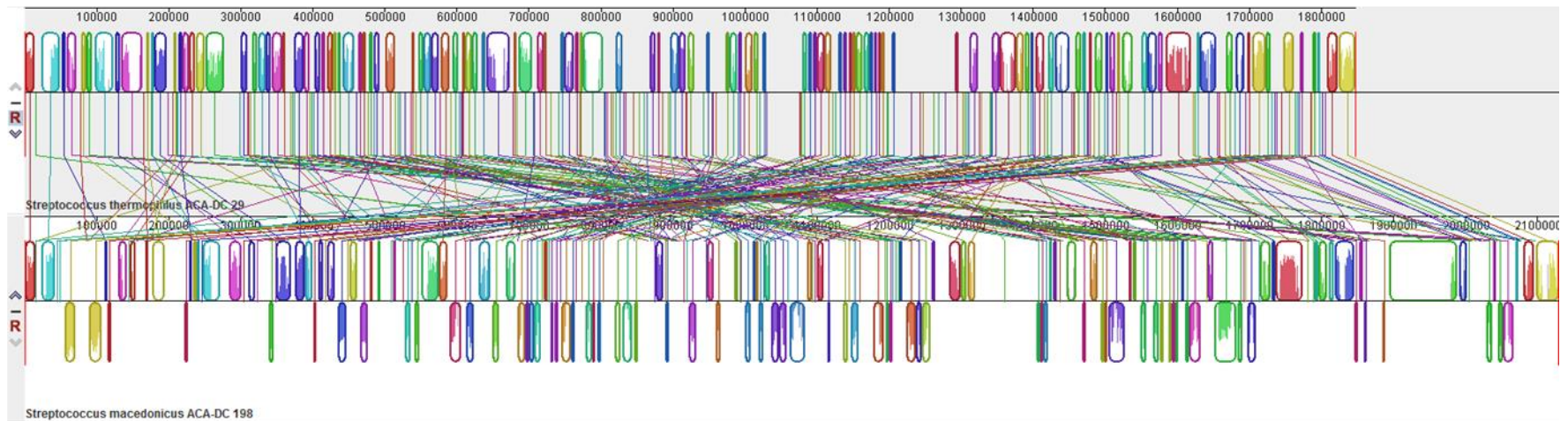
***S. thermophilus* strains**

***S. macedonicus* strains**



Comparative genomics between strains of *S. thermophilus* and *S. macedonicus*

S. thermophilus ACA-DC 29



S. macedonicus ACA-DC 198

Conclusions

- 1. There are streptococci beyond *S. thermophilus* that can be found in milk and dairy products**
- 2. *In silico* analysis of *S. macedonicus* ACA-DC 198 suggests that:**
 - It shows a diminished capacity to live and survive in the GI tract of herbivores**
 - It has a diminished pathogenic potential compared to *S. gallolyticus***
- 3. There are concrete genetic traits supporting that the species is habituated in the dairy environment**
- 4. *S. thermophilus* and *S. macedonicus* are diverge species but they both present traits of adaptation to the milk environment**

Functional analysis needed!!!

This work was performed in collaboration with:

- **Dr. Bruno Pot (Institute Pasteur in Lille, Lille, FR)**
- **Dr. Pierre Renault (Micalis Institute, INRA-CRJ, Jouy-en-Josas, FR)**
- **Prof. Stavros Hamodrakas (Department of Biology, UOA, GR)**
- **Dr. Philippe Supply (Institute Pasteur in Lille, Lille, FR)**

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Thank you for your attention!!!

