



Γεωπονικό Πανεπιστήμιο Αθηνών
Agricultural University of Athens

The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

Konstantinos Papadimitriou, Eugenia Manolopoulou, Marina Georgalaki, Rania Anastasiou,
Anastasios Aktypis, Georgia Zoumpopoulou, and Effie Tsakalidou

Agricultural University of Athens, Department of Food Science and Technology,
Laboratory of Dairy Research, Athens, Greece

XXXII Annual Meeting
of the European Culture
Collections' Organization

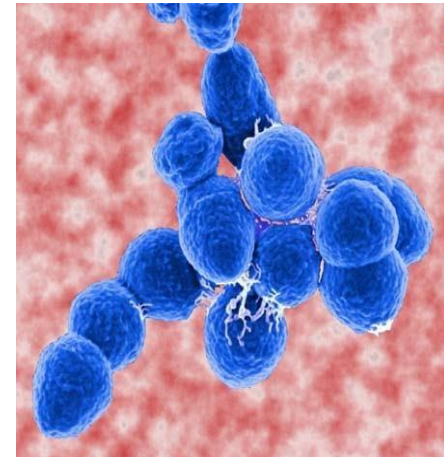
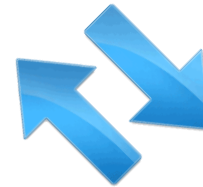


The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

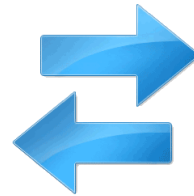
The ACA-DC Collection



Fermented foods



Lactic acid bacteria



The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future



The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- A **krater** (in Greek: κρατήρ, kratēr, from the verb κεράννυμι, keránnymi, "to mix") is a large vase used to mix wine and water in Ancient Greece
- In ancient Greece, the **symposium** (Greek συμπόσιον symposion, from συμπίνειν sympinein, "to drink together") was a drinking party



The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- Advanced agriculture and animal breeding



The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

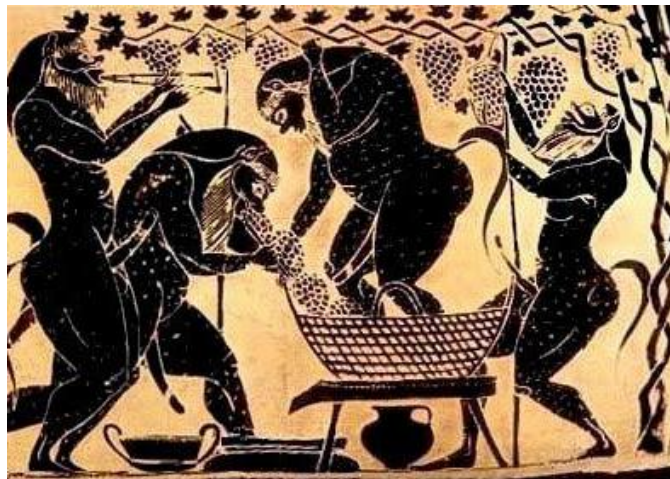
- The **agora** (Ancient Greek: Ἀγορά, Agorá) was a central spot in ancient Greek city-states. The literal meaning of the word is "gathering place" or "assembly"
- The agora had a twin function as a political and commercial space including **advanced food trading** practices



The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- **Fermented foods** were already established

Wine

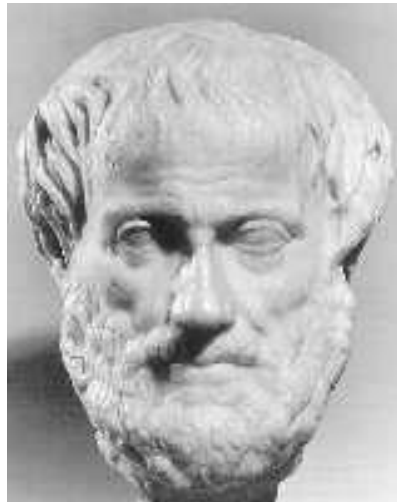
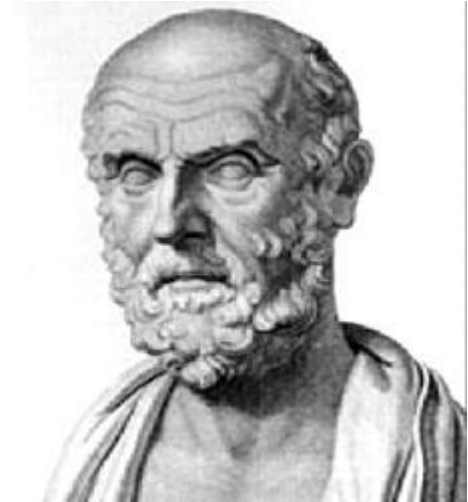


The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- **Fermented foods** were already established

Vinegar

Around 400 BC, Hippocrates, the father of modern medicine, prescribed vinegar mixed with honey for a variety of illnesses, including coughs and colds



Around 350 BC the pickling process was also known to the Ancient Greeks. Aristotle is reported to have praised pickled cucumbers

The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- **Fermented foods** were already established

Olives



The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- **Fermented foods** were already established

Bread



The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- **Fermented foods** were already established

Dairy

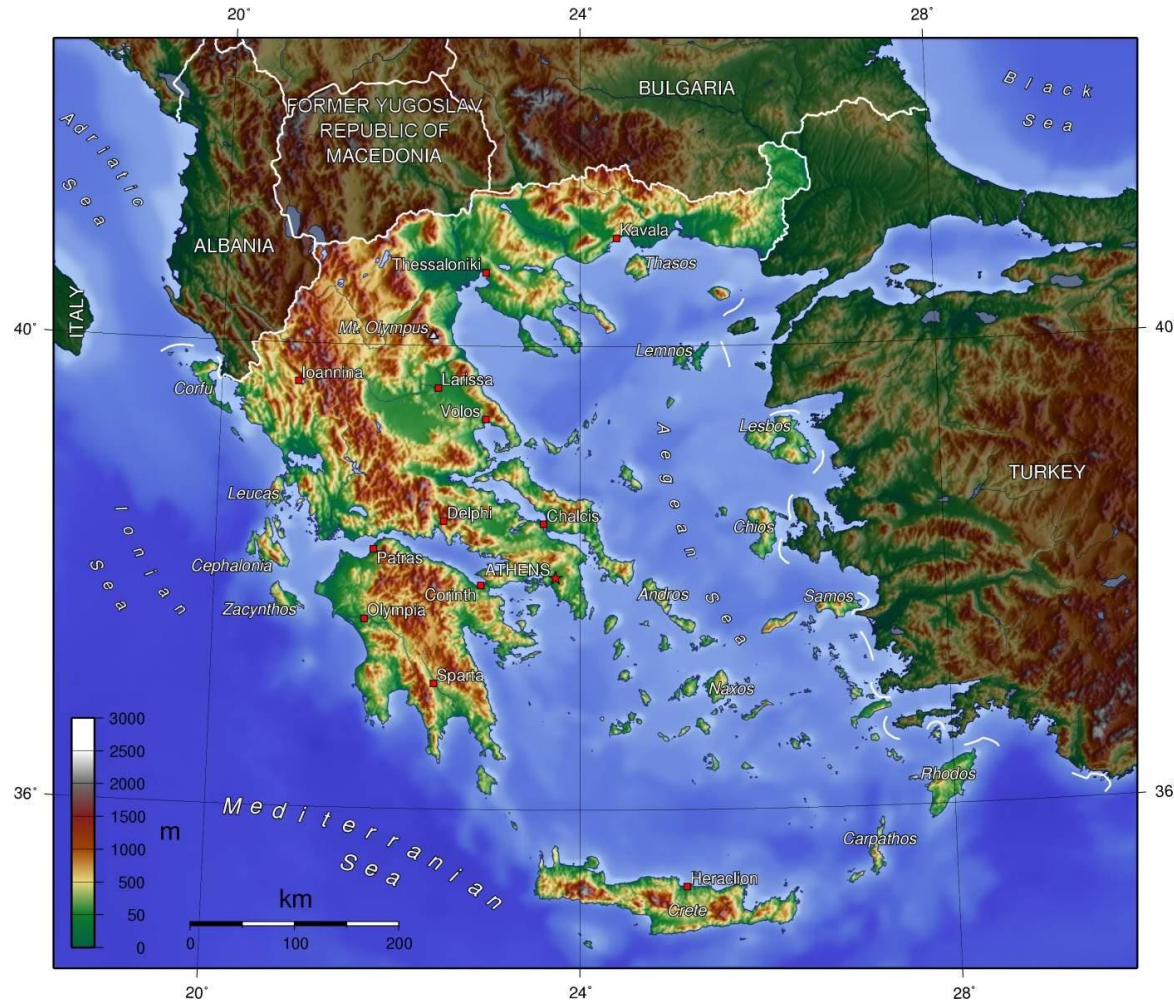


“...milk left in buckets or jars coagulated, and took the form of the woven basket where it was placed when separated from the liquid, ready to be eaten the following day...”.

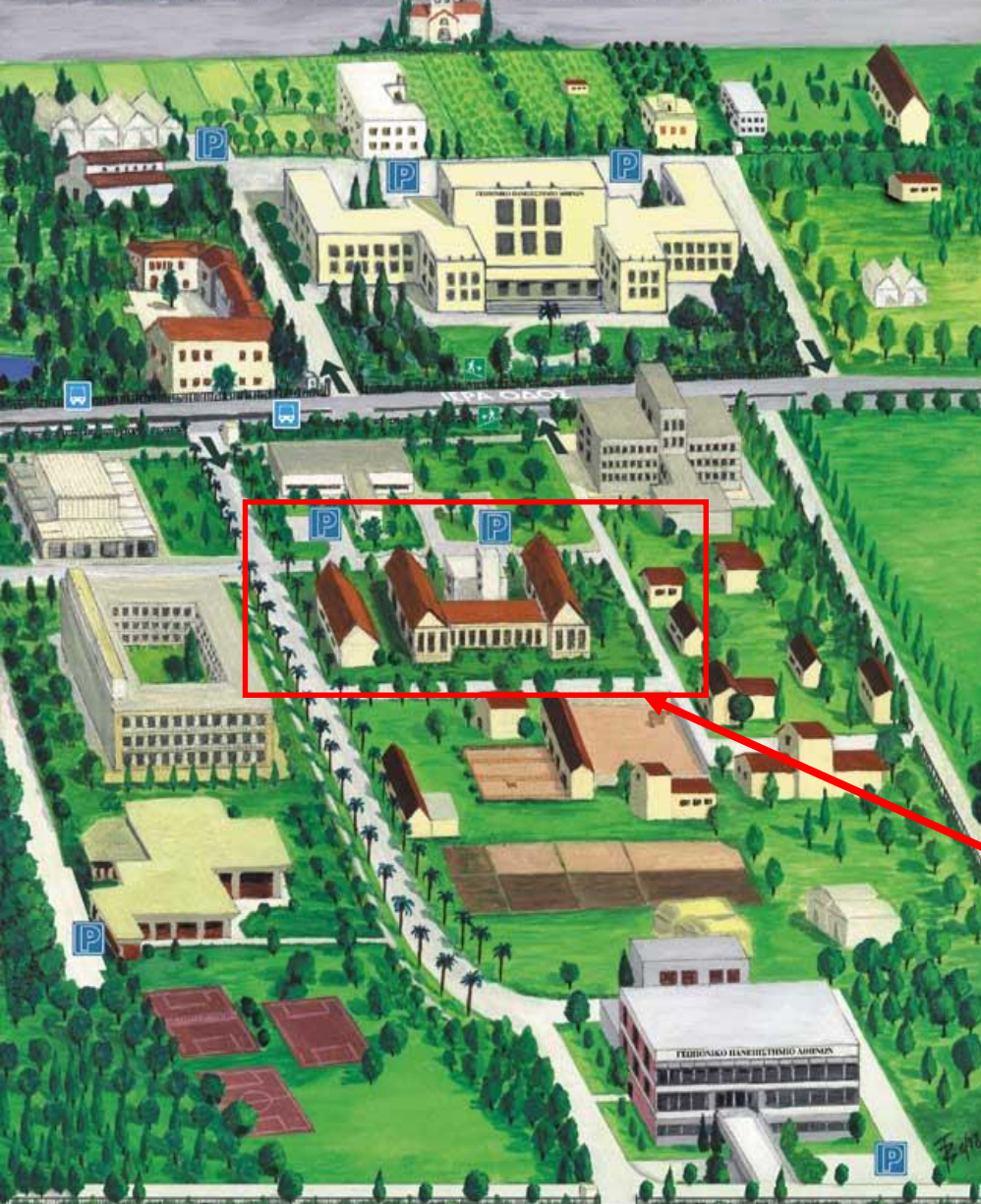
Homer in the *Odyssey*, 8th century BC

The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- The fate of **fermented foods** was most probably influenced by the Greek terrain
- Eco- Sosio- pockets for product development



ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ



Agricultural University of Athens



Crop Sciences

Animal Sciences

Agricultural Biotechnology

Food Science and Technology

Natural Resources Management &

Agricultural Engineering

Rural Economics & Development

General Sciences

**Laboratory
of Dairy Research**

The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- The ACA-DC Collection of Microorganisms was established in 1987

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
- Novel species described
Streptococcus macedonicus (Kasseri cheese, 1998)
Lactobacillus zymae (Sourdough, 2005)
- Unusual species
Lactobacillus acidipiscis
Lactobacillus rennini
isolated from Kopanisti and Mana Kopanisti (2006)

The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future



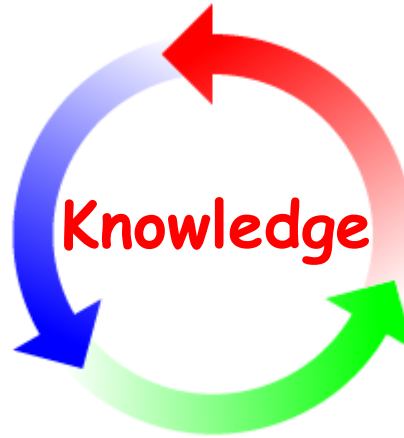
Fermented dairies



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 (antimicrobial peptides)
- probiotics

The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

- Knowledge acquired is disseminated back to the producers and the society
- Associated opportunities to solve major technological hurdles in
 - food flavour and texture
 - food safety
 - probiotics
- It is the nature of our collection that raises interest
 - traditional products
 - “wild isolates” with important and frequently unexpected traits
- Industrial Partners
 - Fonterra (New Zealand)
 - Chr. Hansen (Denmark)
 - Applied Maths (Belgium)

The ACA-DC Collection - Snapshots of the Past and a Glimpse into the Future

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

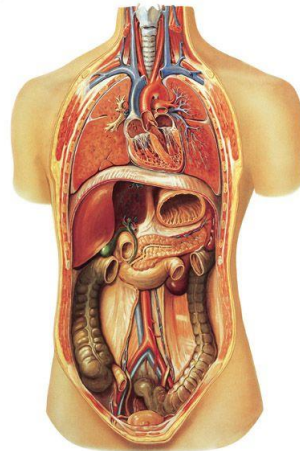
- **Lactic acid bacteria (LAB) are routinely used in food fermentations and as probiotics**
- **Besides LAB that are of a benign nature there are several LAB that are notorious pathogens (e.g. Enterococci and Streptococci)**
- **Streptococci are an extreme example since today only *S. thermophilus* is considered to be a non-pathogenic and food-grade species when compared to GAS, GBS and *S. pneumoniae* (Bolotin et al. Nature Biotechnol. 2004)**
- **However, *S. thermophilus* is not the only streptococcal species found in fermented food especially of dairy origin...**

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

- **Streptococci in milk**

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

S. equinus
(formerly *S. bovis*)



colon cancer
infective
endocarditis
septicemia
meningitis

???

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

• Streptococci in milk

• *Streptococcus macedonicus* which was originally isolated and characterized from traditional Greek Kasserri 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

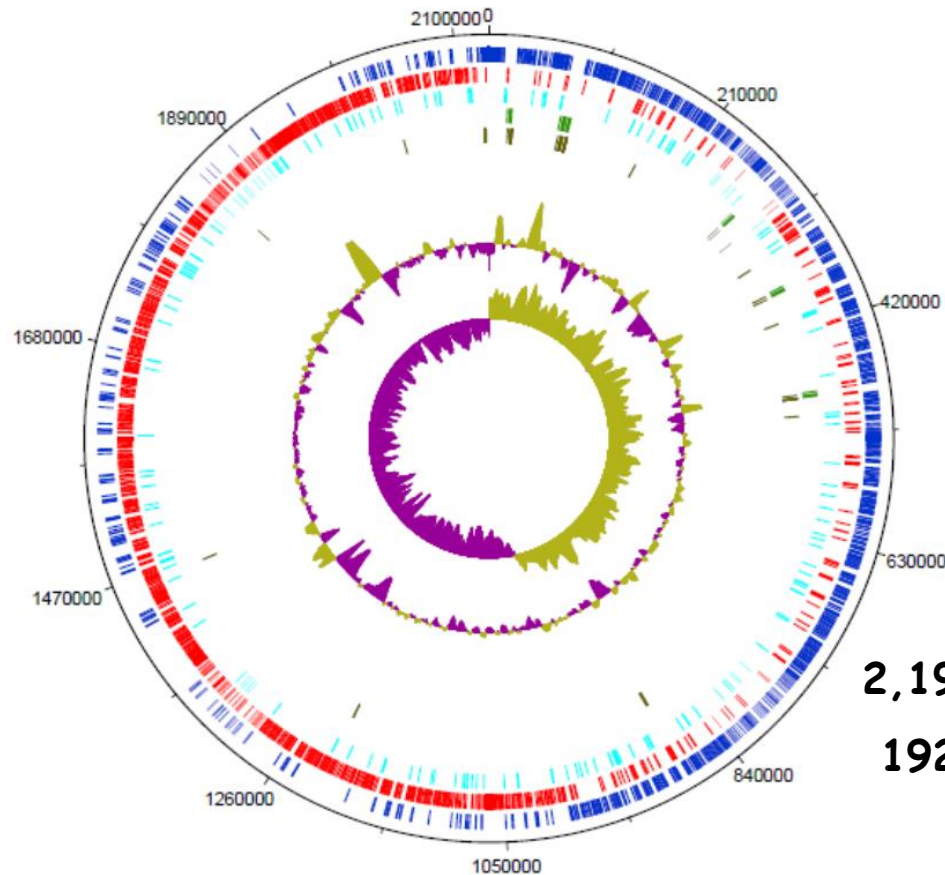
The aim of this work was to sequence the complete genome of the dairy isolate *S. macedonicus* ACA-DC 198 in order to assess *in silico* the pathogenic potential of the species and its potential adaptation to the milk environment

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

•Annotating the genome of *S. macedonicus* ACA-DC 198

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

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

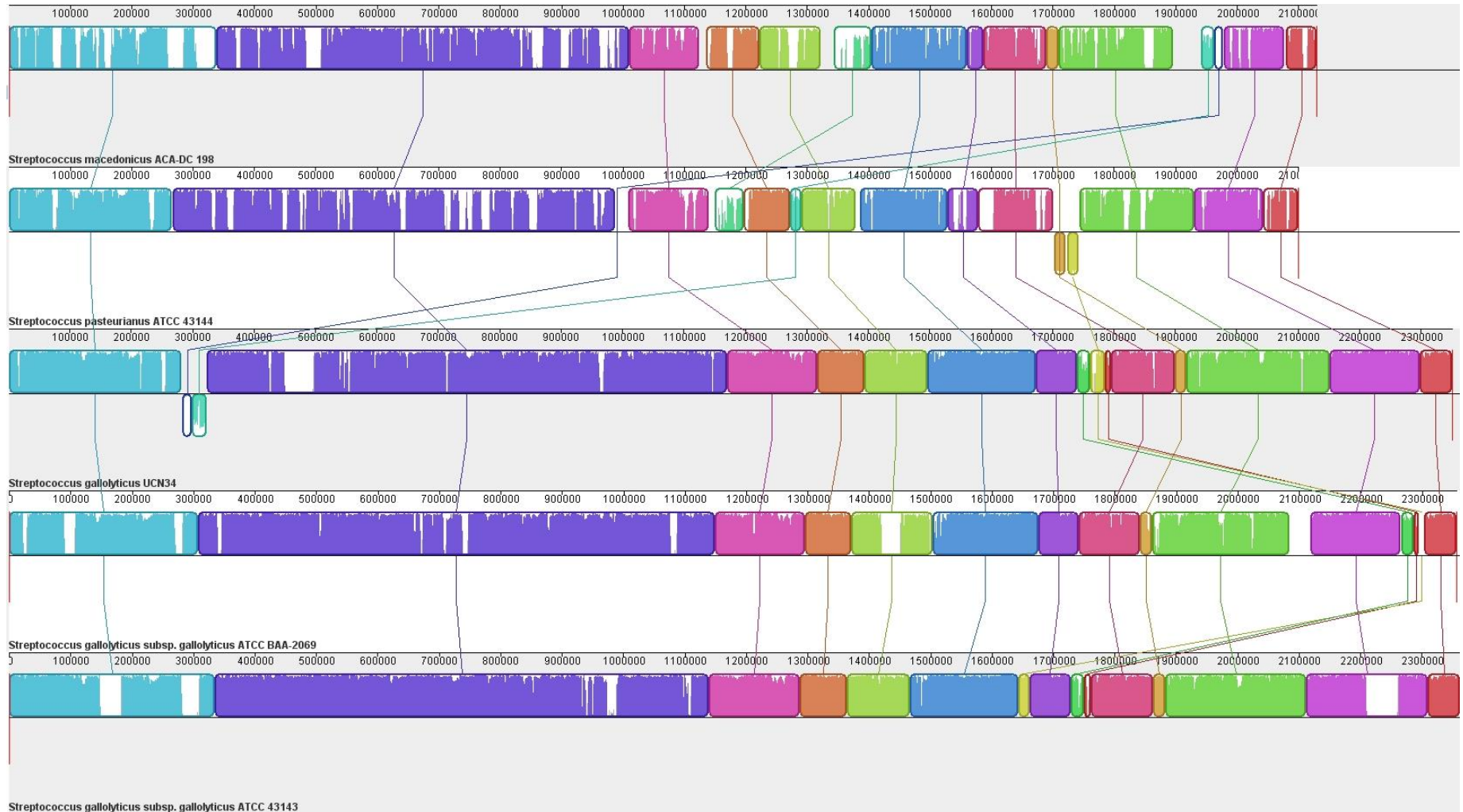
•Comparative genomics of *S. macedonicus* ACA-DC 198

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)

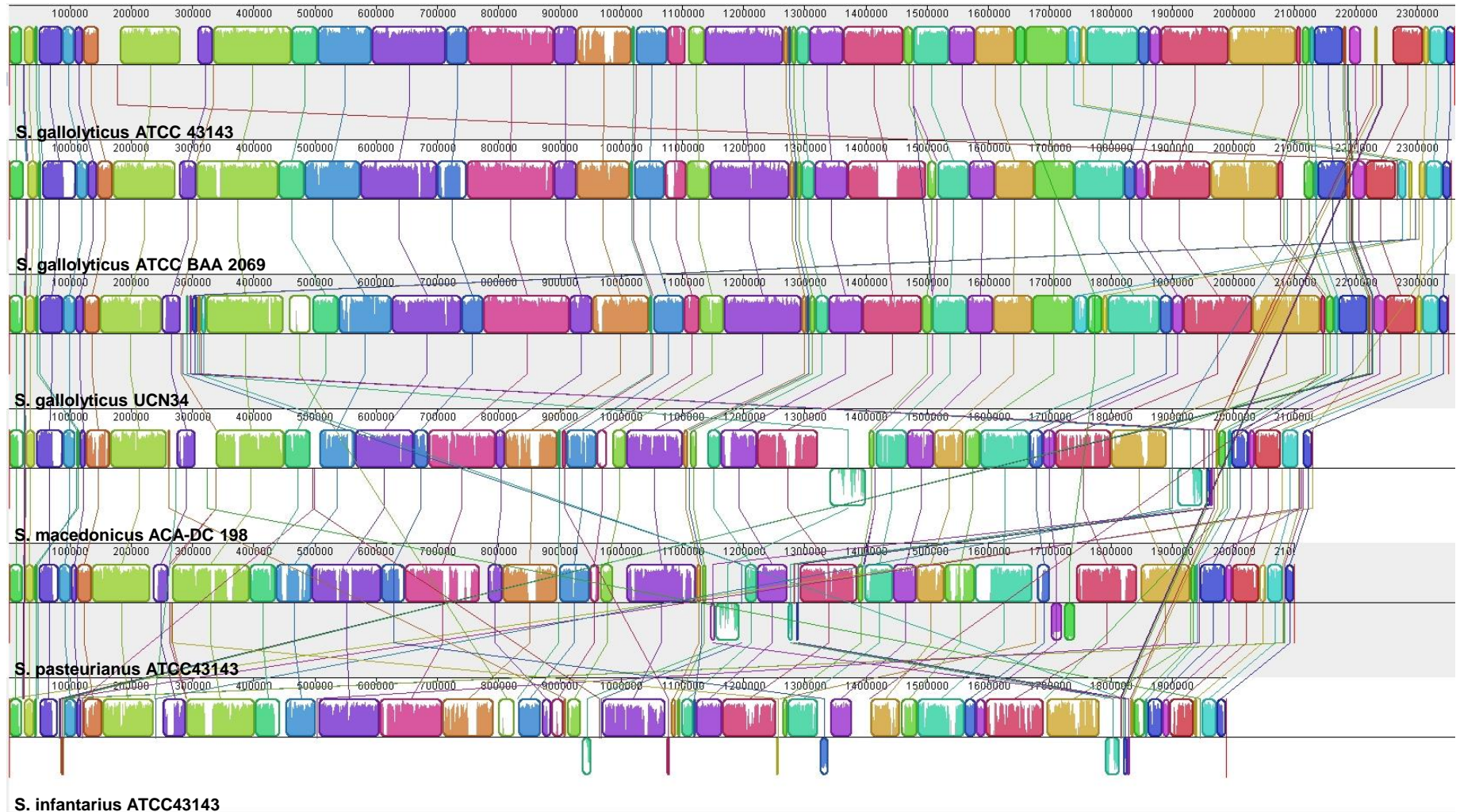
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

Comparative genomics of *S. macedonicus* ACA-DC 198



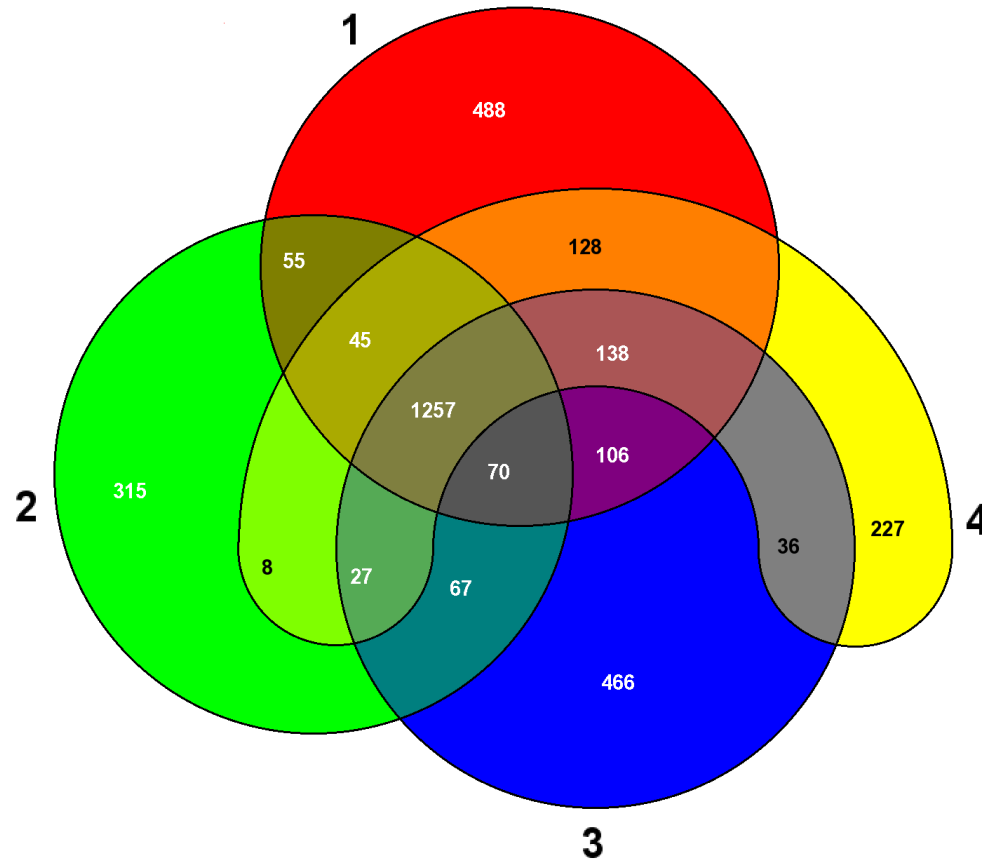
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

Comparative genomics of *S. macedonicus* ACA-DC 198



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

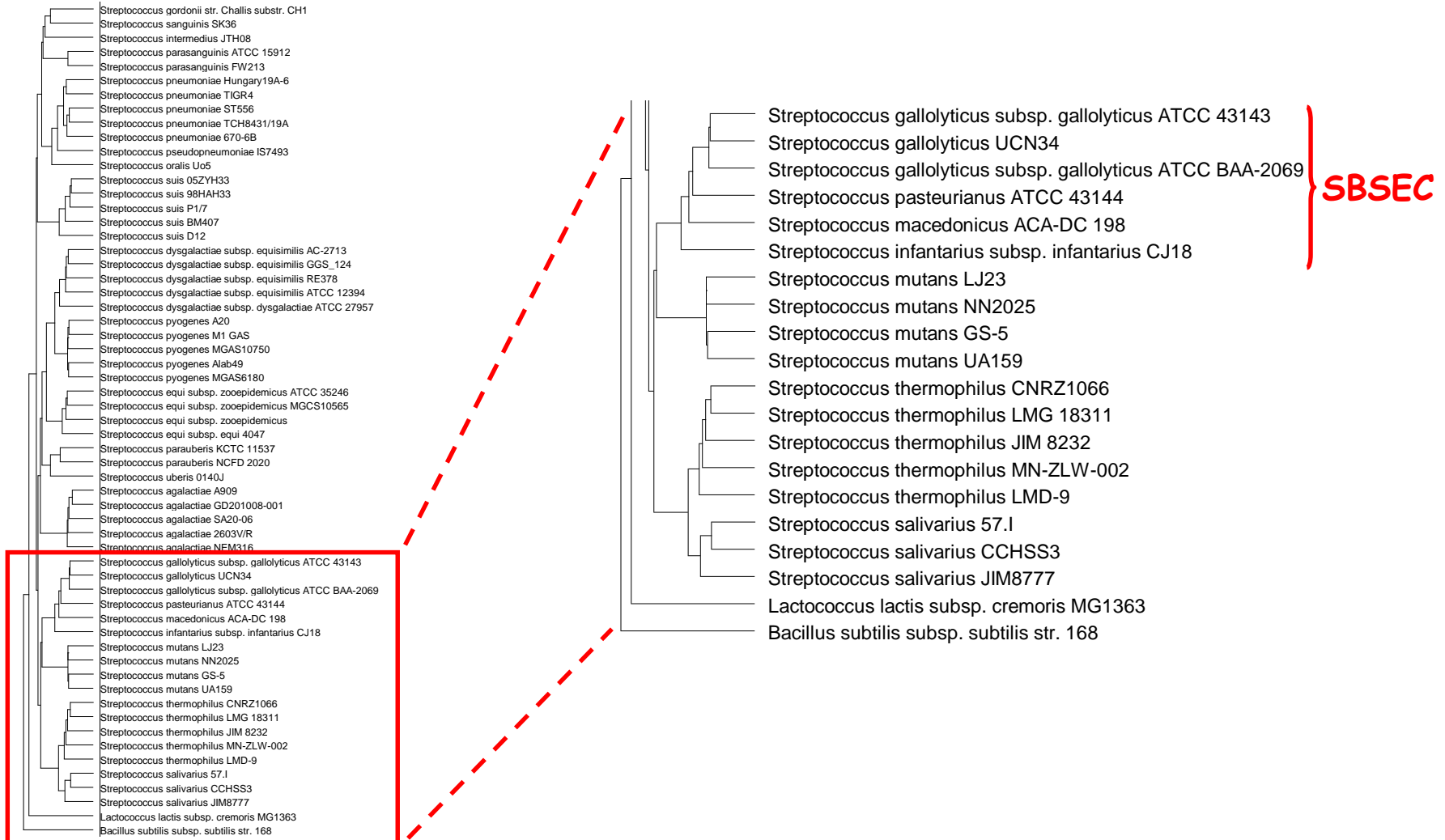


Core genome: **57.3%**

- 1: *Streptococcus_gallolyticus_subsp_gallolyticus_ATCC_43143_DNA*
- 2: *Streptococcus_infantarius_subsp_infantarius_CJ18*
- 3: *Streptococcus_macedonicus_ACA-DC_198_main_chromosome*
- 4: *Streptococcus_pasteurianus_ATCC_43144_NC_015600*

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



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)

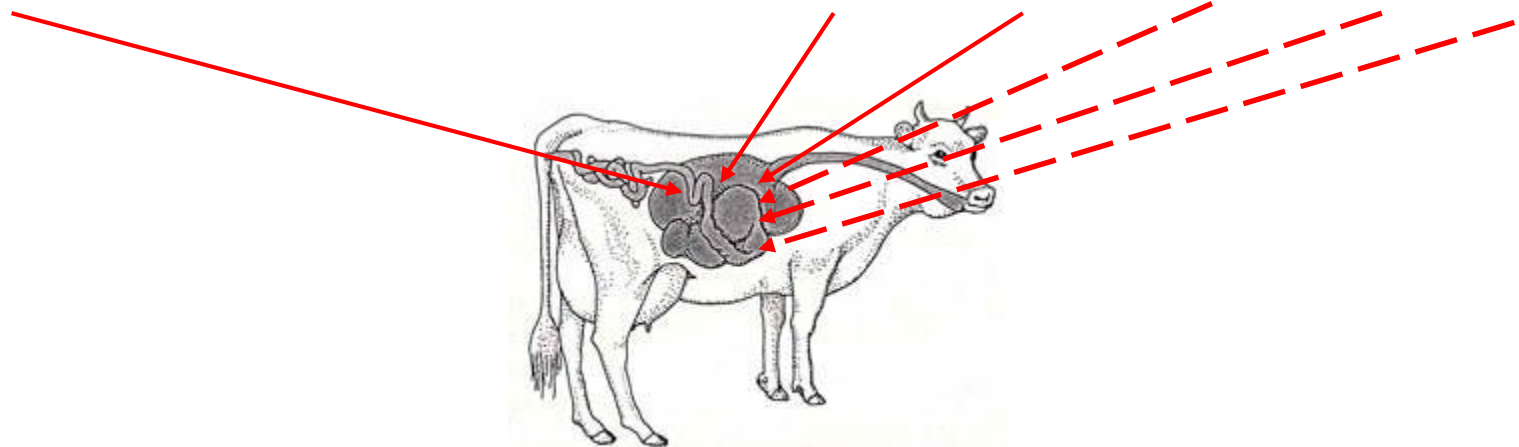
* not reported

- 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

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

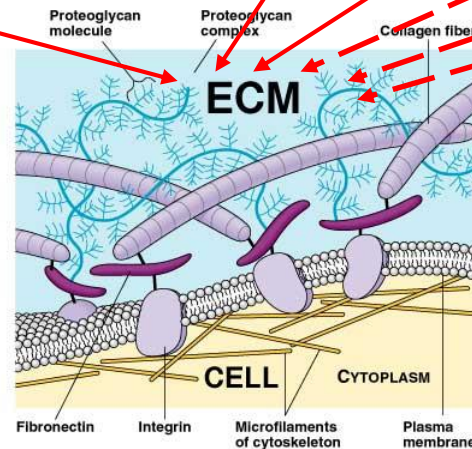
•Pathogenicity genes presence/absence

1. Virulence factors have been rarely investigated in *S. bovis* despite its known pathogenicity
2. *In silico* analysis of *S. gallolyticus* revealed that it contains three pilus gene clusters (*pil1*, *pil2*, *pil3*), which may mediate its binding to the extracellular matrix (ECM)
3. Each pilus operon consists of two adhesins belonging to the MSCRAMM (microbial surface recognizing adhesive matrix molecules) family and a sortase C necessary for the polymerization of the adhesin filaments into a pilus
4. Danne et al. (J Infect Dis. 2011) recently demonstrated that *pil1* is most probably the major factor for adhesion of *S. gallolyticus* to ECM

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

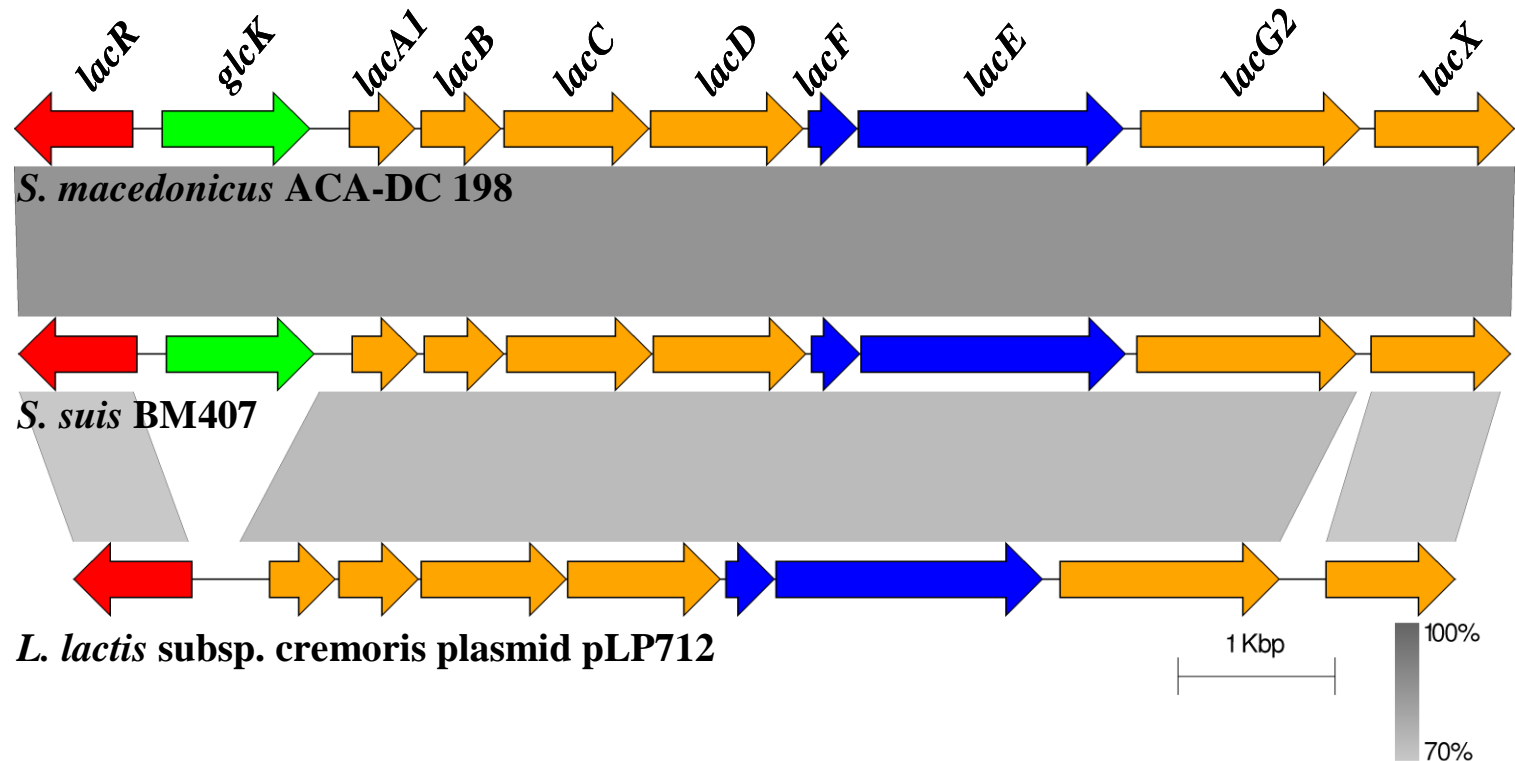
Virulence genes presence/absence

<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)	✓	✓	✓	✓	✓



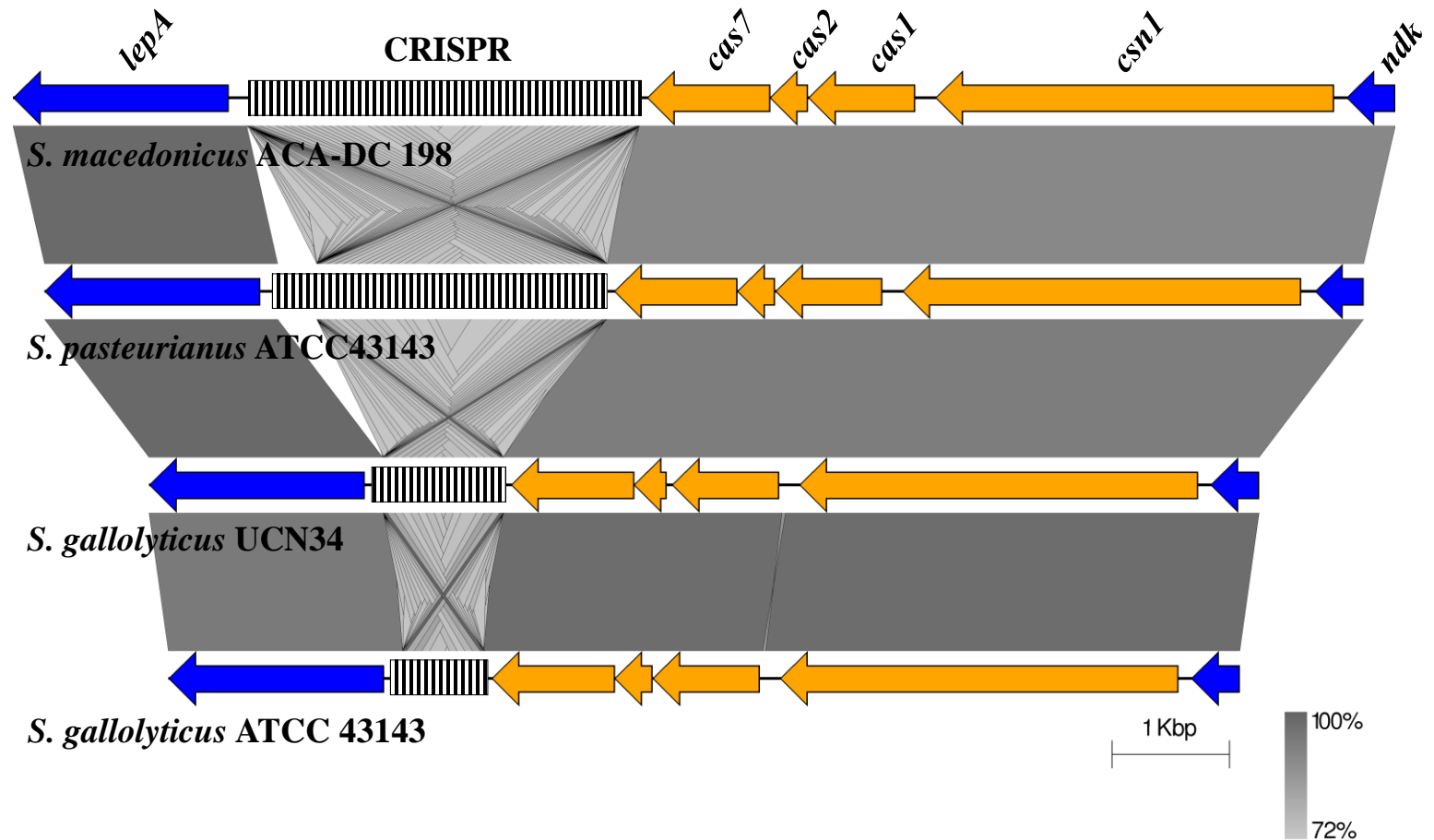
Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

- 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

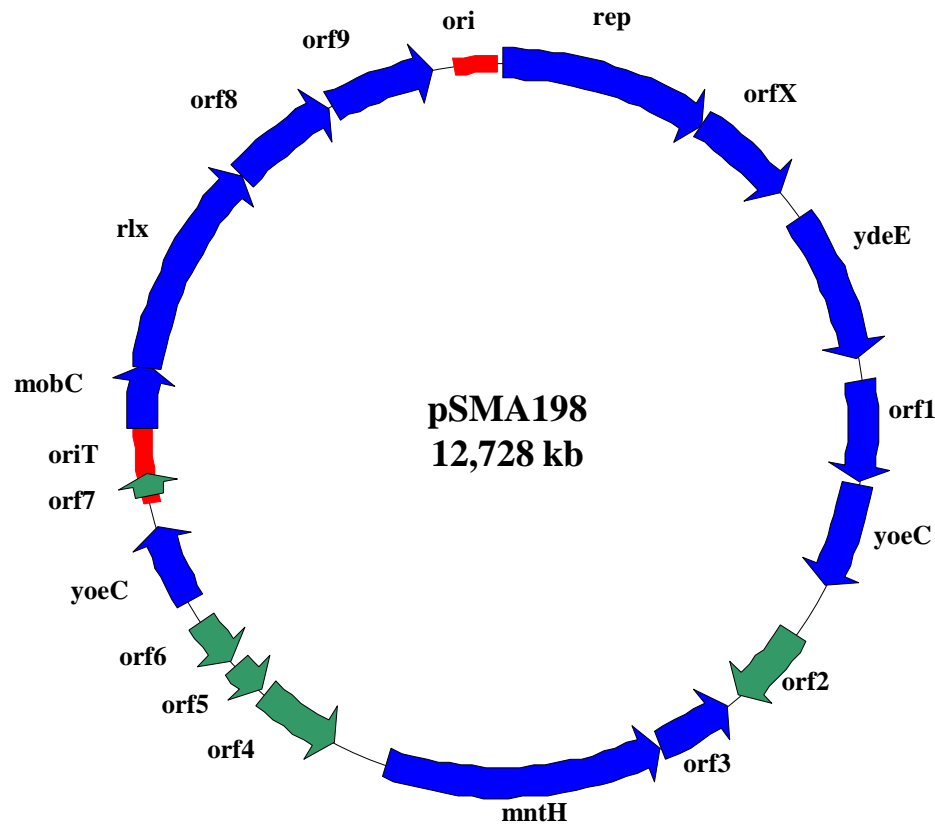


Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

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

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

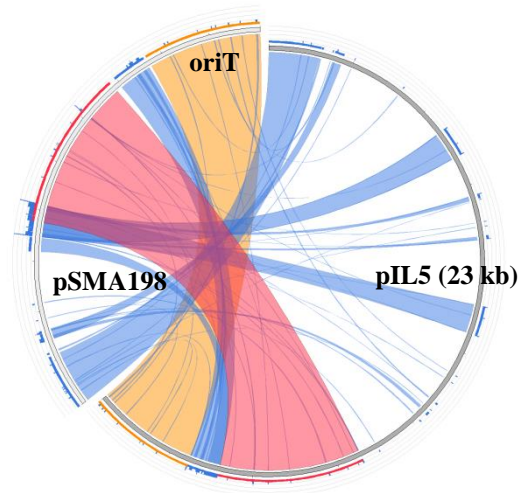
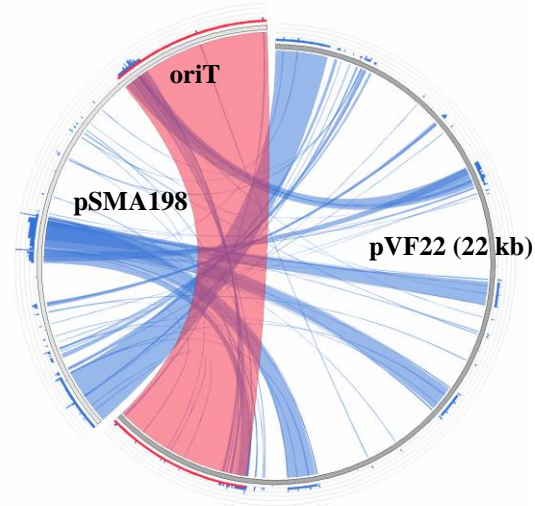
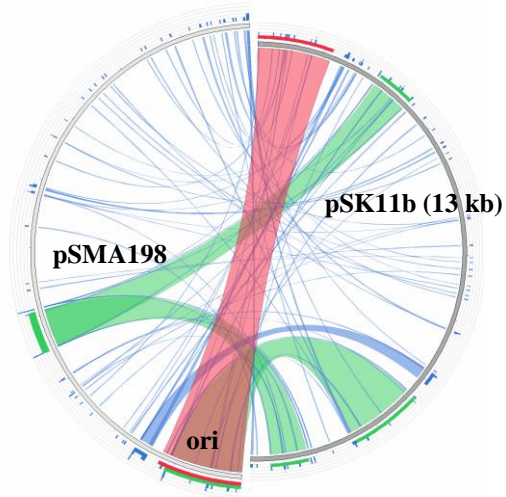
1. pSMA198 belongs to the narrow host range pCI305/pWV02 family of lactococcal plasmids and it is the first such plasmid to be reported in *Streptococcus*

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

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

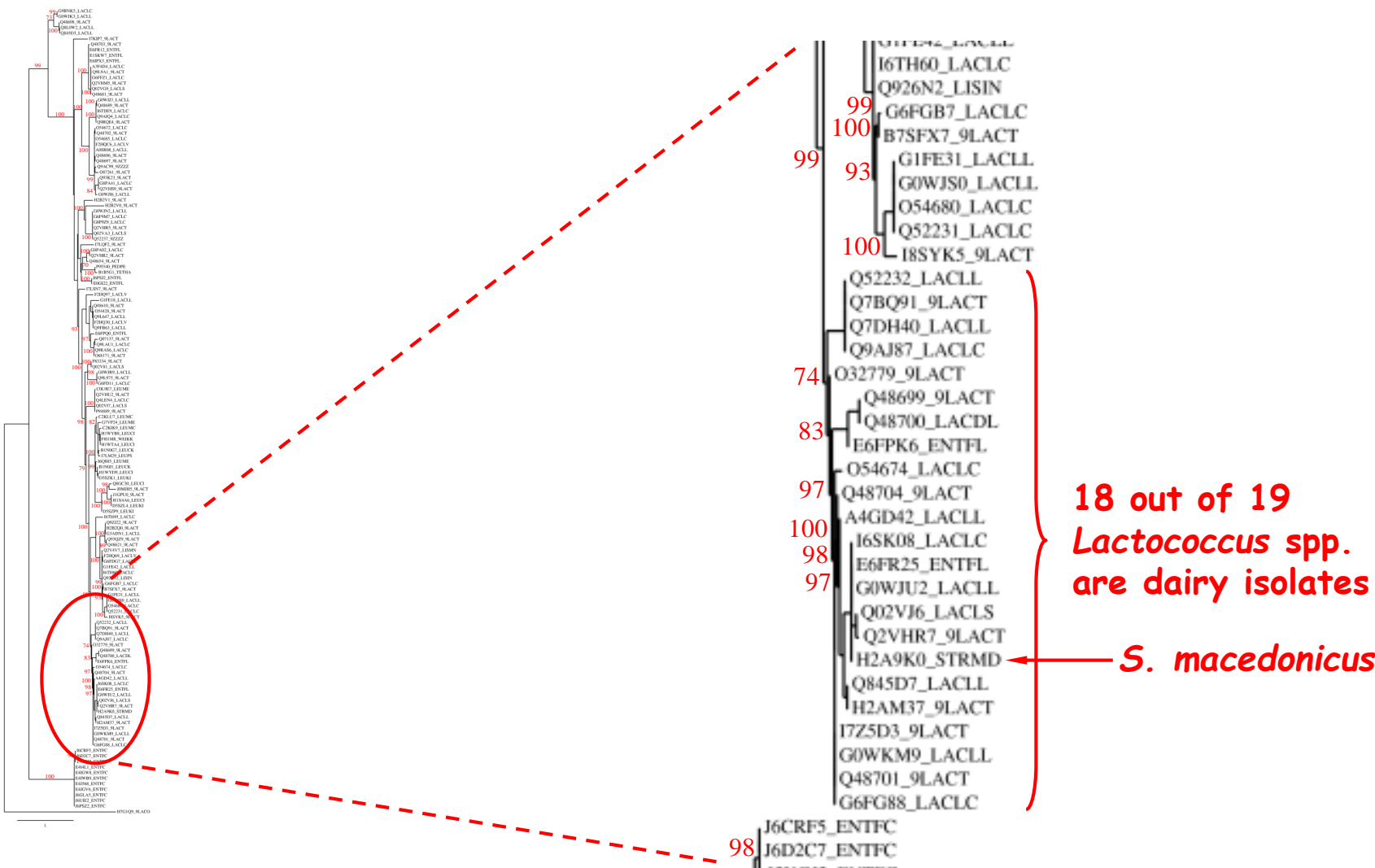
- Comparative analysis of pSMA198 with its related plasmids



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



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

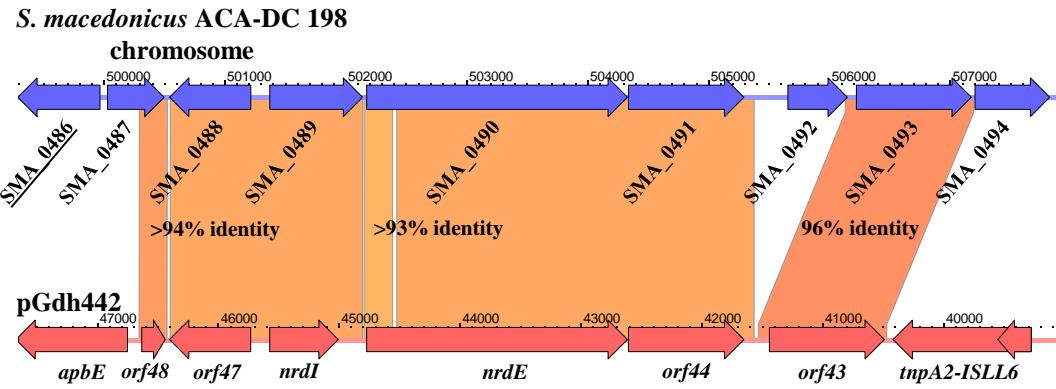
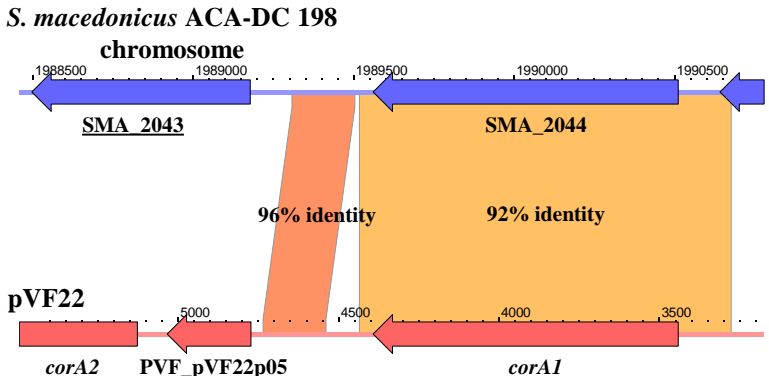
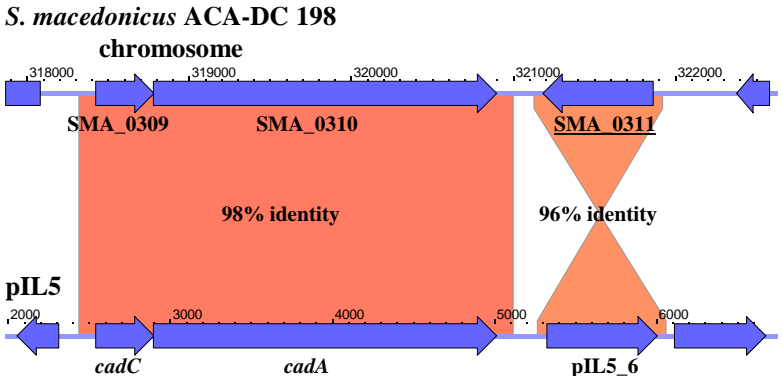
Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

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

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

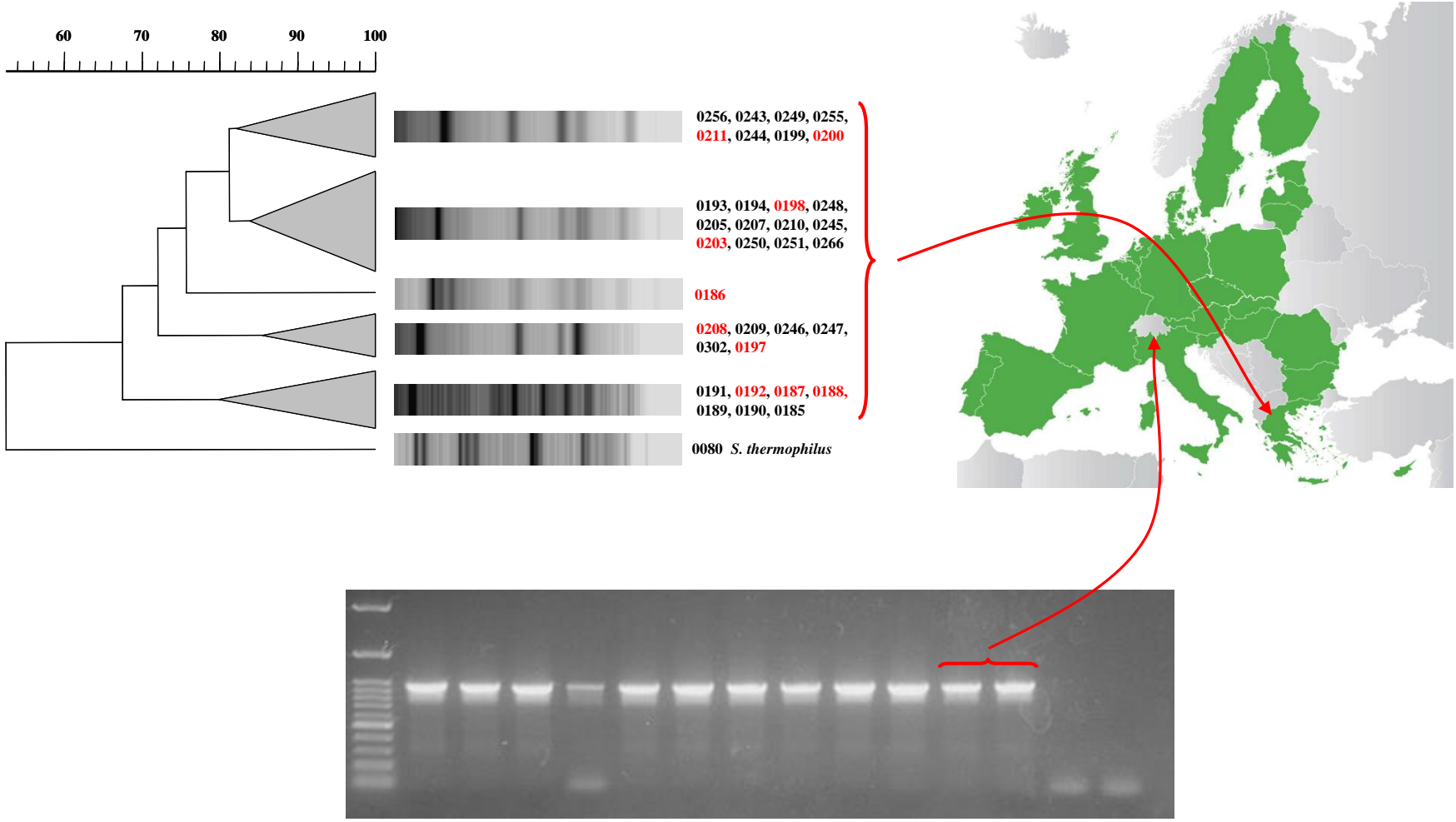
Analysis of the lactococcal plasmid pSMA198 points towards the habituation of *Streptococcus macedonicus* ACA-DC 198 to the dairy environment

- S. macedonicus* chromosome 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*



Conclusions

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*
- There are concrete genetic traits supporting that the species is habituated in the dairy environment

Validation with functional analysis is necessary

The future of the ACA-DC collection

1. To apply novel approaches in the characterization of the microbial ecosystems
2. To identify the “gene pool” of our collection
3. To keep expanding in a rational manner

Acknowledgments

The present work was cofinanced by the European Social Fund and the National resources EPEAEK and YPEPTH through the Thales project



Thank you for your attention!!

