

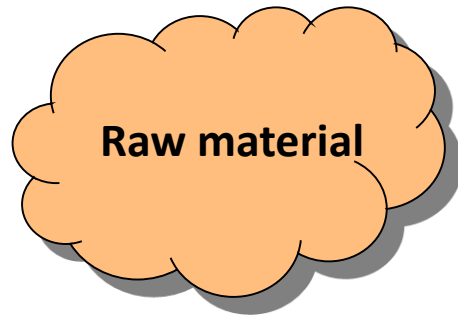
# Assessing the adaptation to milk and the pathogenic potential of the dairy *Streptococcus macedonicus* ACA-DC 198 through comparative genomics

**Konstantinos Papadimitriou<sup>1</sup>, Rania Anastasiou<sup>1</sup>, Eleni Mavrogonatou<sup>2</sup>, Jochen Blom<sup>3</sup>, Nikos C. Papandreou<sup>4</sup>, Stavros J. Hamodrakas<sup>4</sup>, Stéphanie Ferreira<sup>5</sup>, Philip Supply<sup>5, 6, 7, 8, 9</sup>, Bruno Pot<sup>6, 7, 8, 9</sup>, Effie Tsakalidou<sup>1</sup>**

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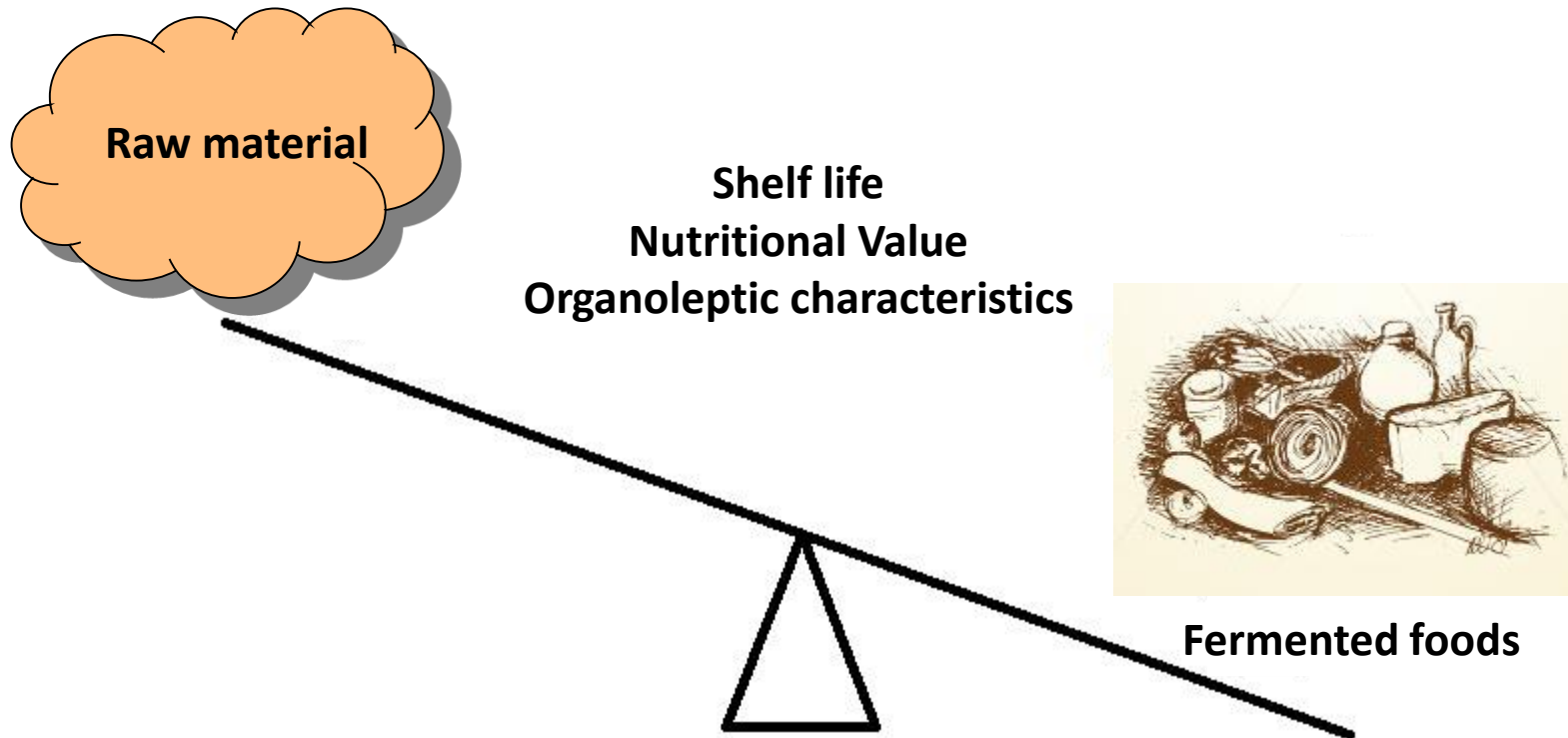
# Laying the background: Fermented foods and Microorganisms



**lactic acid bacteria**



# Laying the background: Fermented foods and Microorganisms



**Fermented foods**



# Laying the background: Fermented foods and Microorganisms



Traditionally fermented dairies



Fermenting ecosystem



Experimental dairy production

**Selection of novel  
starter cultures**

## 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



## Laying the background: Fermented foods and Microorganisms

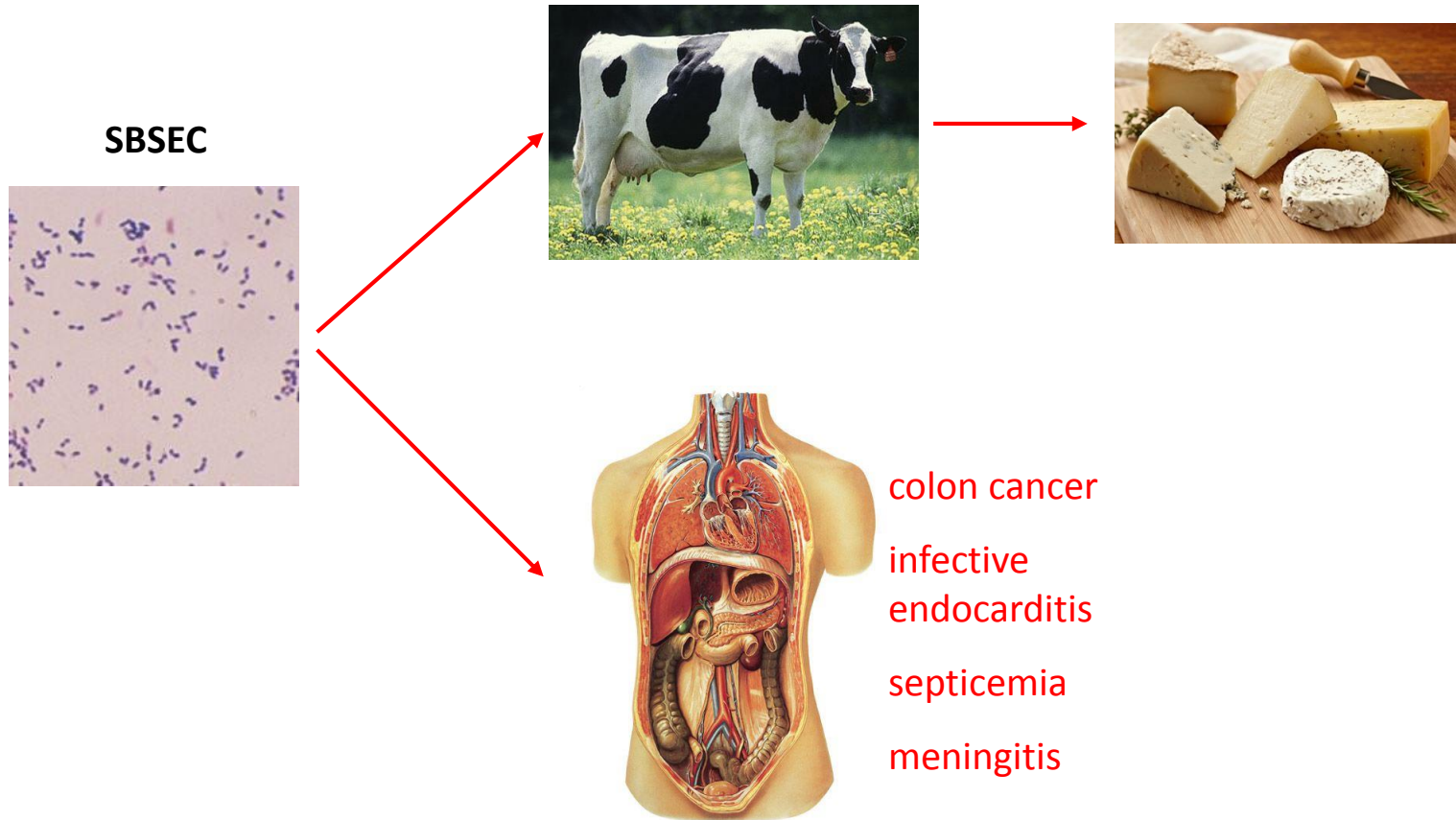
- **Lactic Acid Bacteria: Gram-positive, low-GC, rods or cocci, non-sporulating, catalase-negative, aerotolerant but not aerophilic and they produce lactic acid as the major metabolic end-product of carbohydrate fermentation**
  - **Carnobacterium**
  - **Enterococcus**
  - **Lactococcus**
  - **Lactobacillus**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

## Laying the background: Fermented foods and Microorganisms

- The *Streptococcus* genus (*sensu stricto*) mainly consists of commensals of humans and animals including both non-pathogenic and pathogenic bacteria
- *Streptococcus thermophilus* is the only species in the genus used as a starter culture in food fermentations since all known lactic streptococci belong now to the *Lactococcus* genus
- *Streptococcus macedonicus* which was originally isolated and characterized from traditional Greek Kasserri cheese (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).

# Laying the background: Fermented foods and Microorganisms

- 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**





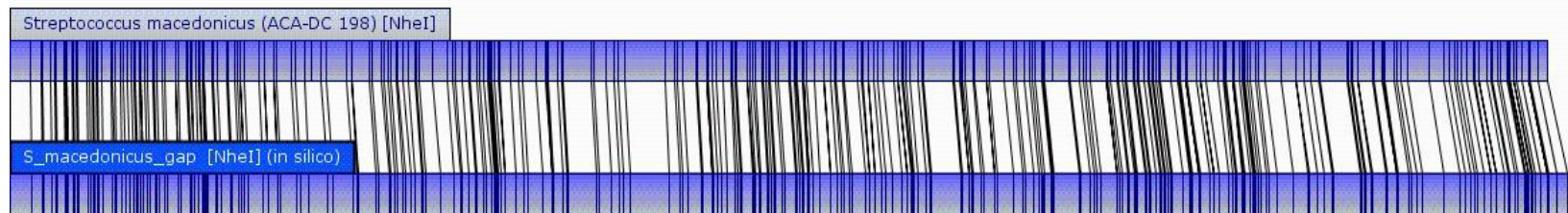
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- D. Assessing the pathogenic potential *S. macedonicus*



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

- 1<sup>st</sup> step: shotgun pyrosequencing with 454 GS-FLX titanium (>100 contigs)
- 2<sup>nd</sup> step: 3kb paired-end pyrosequencing with 454 GS-FLX titanium (7 scaffolds)
- 3<sup>rd</sup> step: gap-closure and polishing with Illumina sequencing using the HiSeq 2000
- 4<sup>th</sup> step: validation of the overall assembly (>200X coverage) with an *NheI* optical map

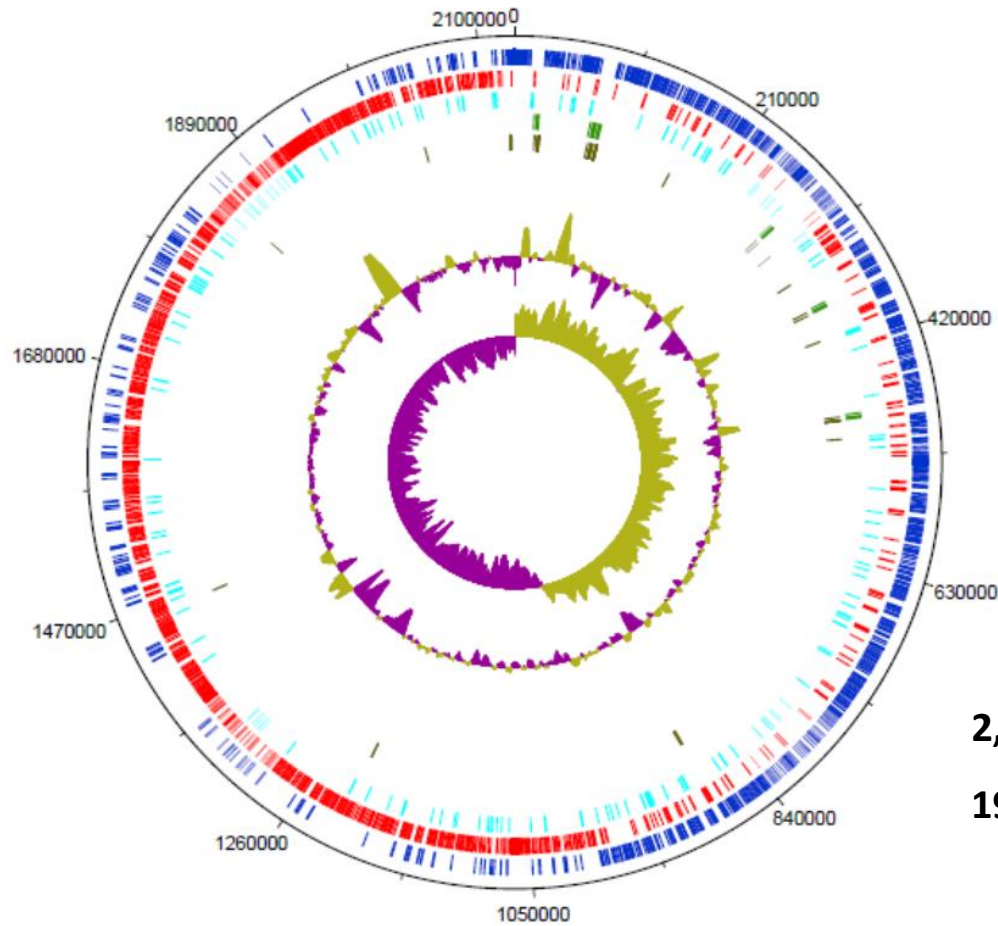


**Chromosome: 2,130,034 bp**

**Plasmid: 12,728 bp (pSMA198)**

# 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**



8th Conference of the Hellenic Society for Computational Biology and Bioinformatics (HSCBB13)

Department of computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece, 22-11-2013

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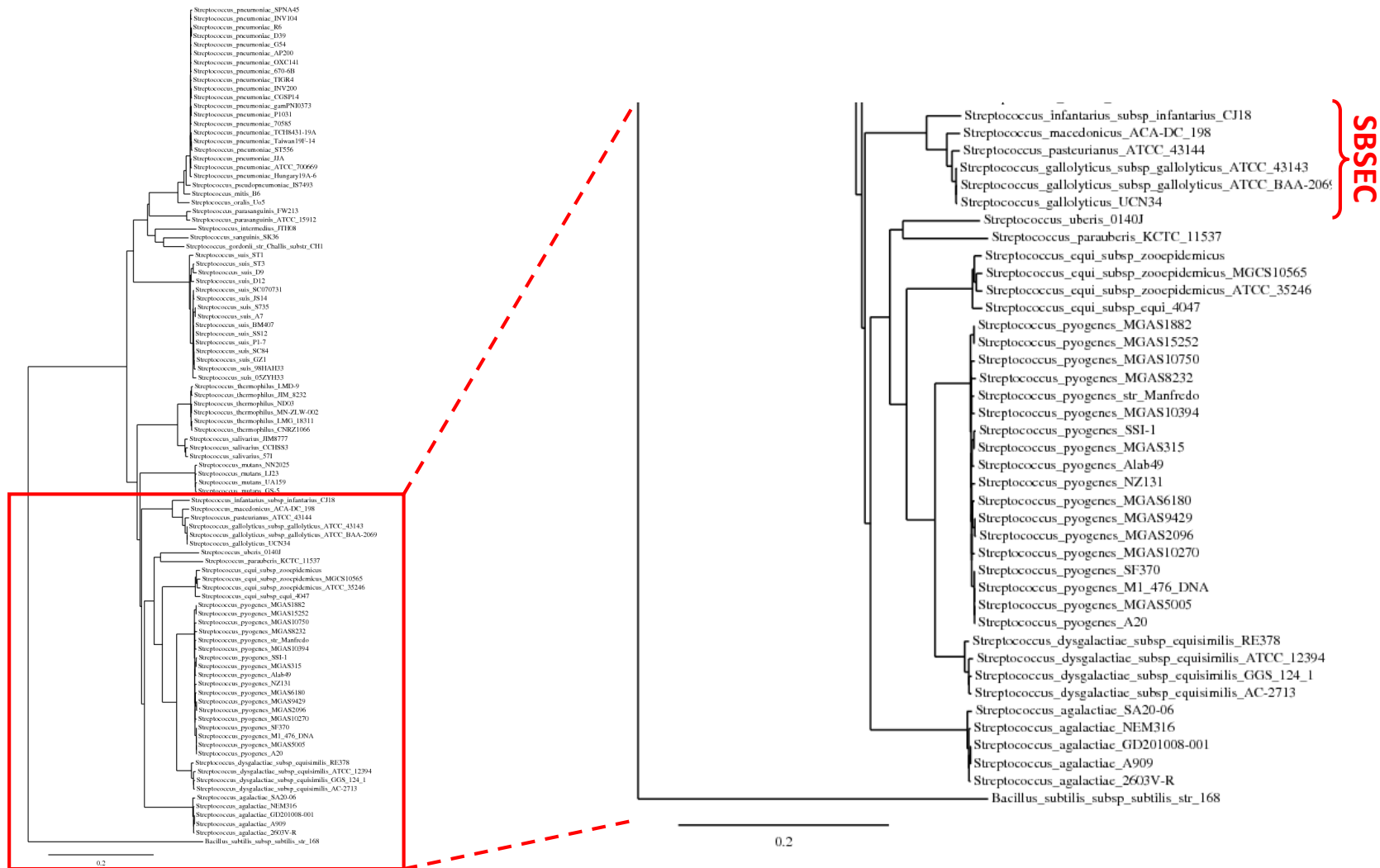
# Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

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

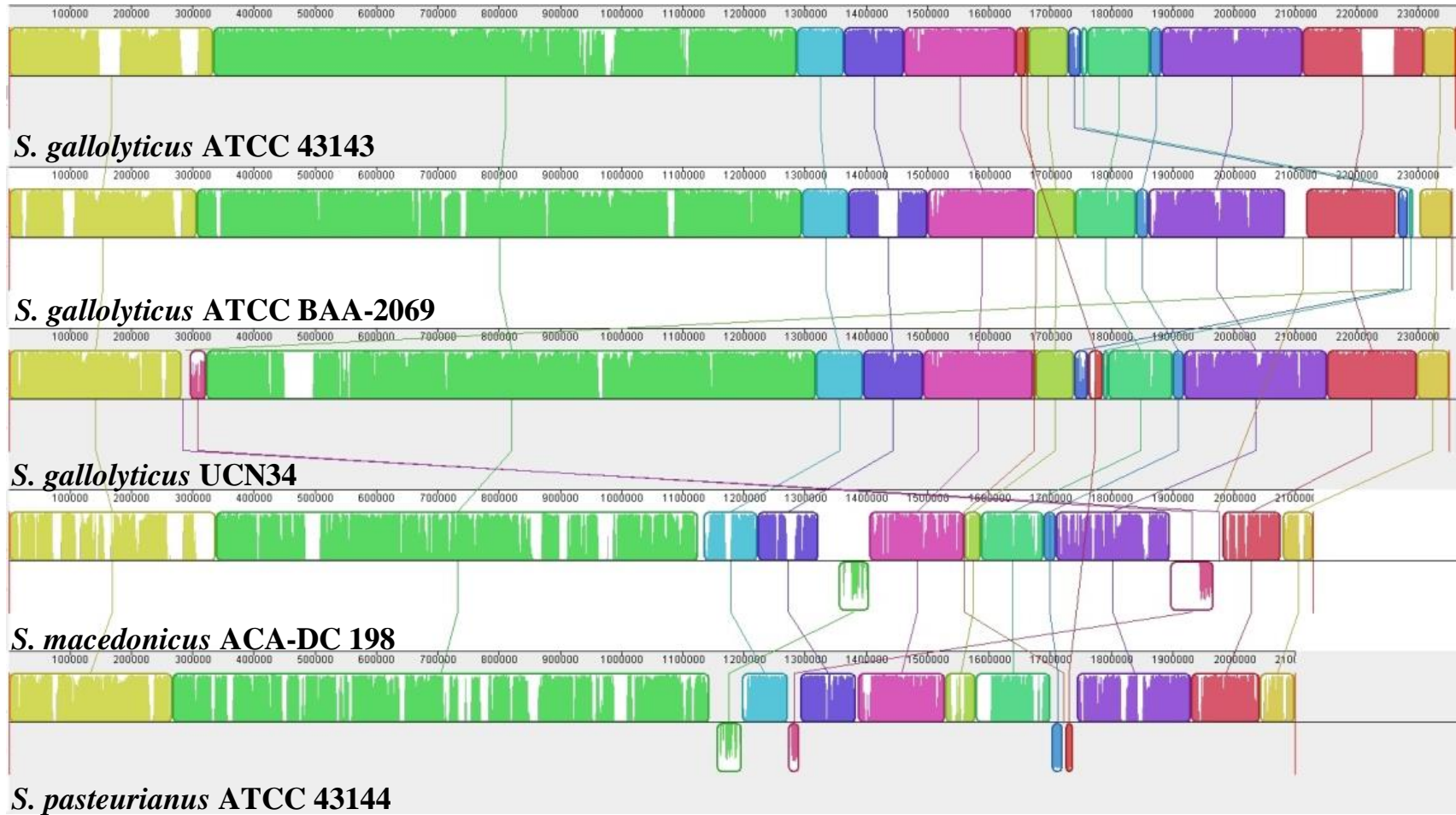
- *S. gallolyticus* UCN34 (human blood)
- *S. gallolyticus* ATCC BAA 2069 (human blood)
- *S. gallolyticus* ATCC 43143 (human blood)
- *S. pasteurianus* ATCC 43144 (human blood)
- *S. infantarius* CJ18 (suusac fermented camel milk)



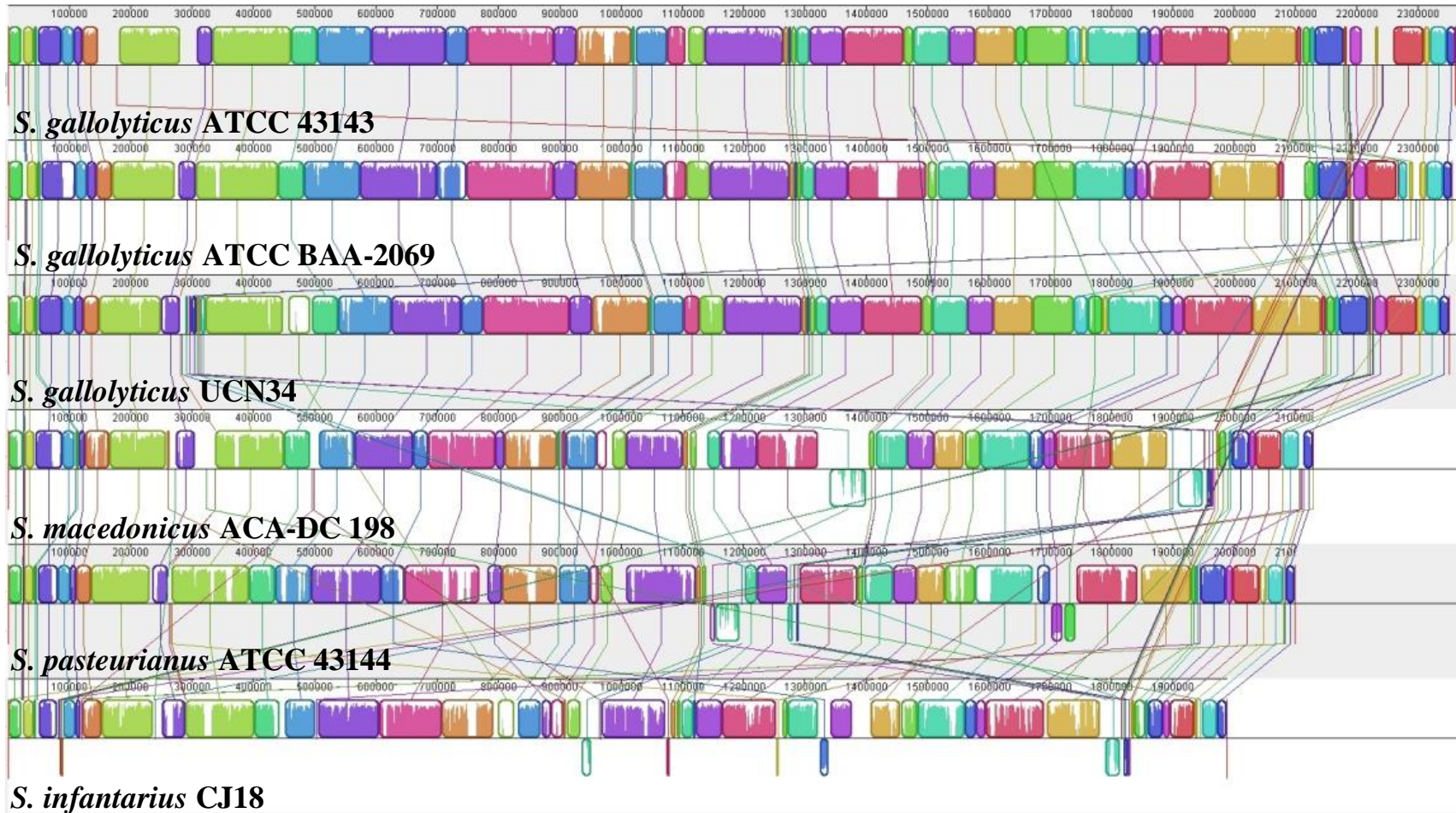
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# Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex



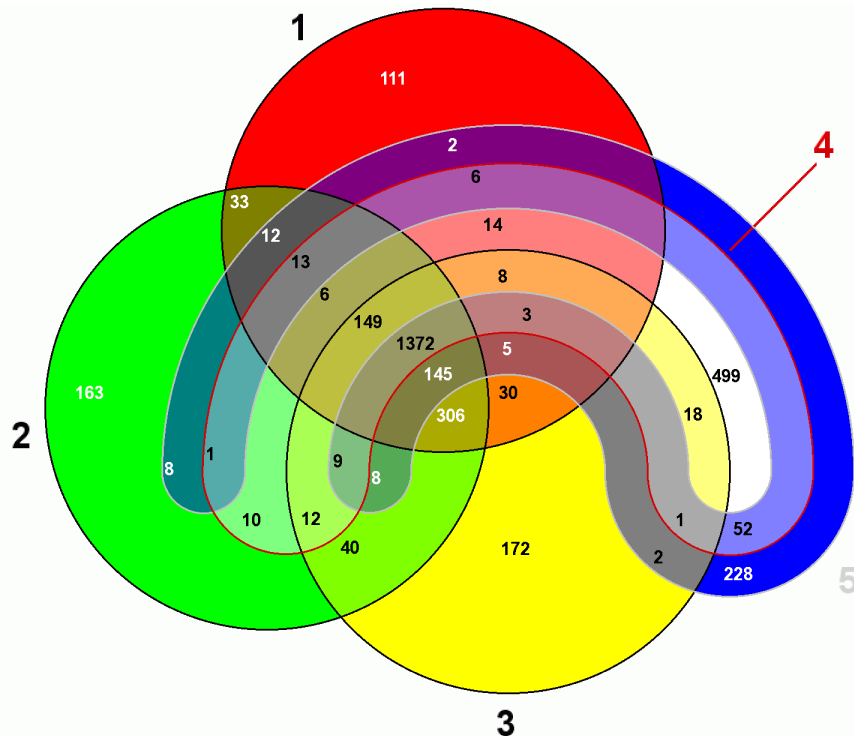
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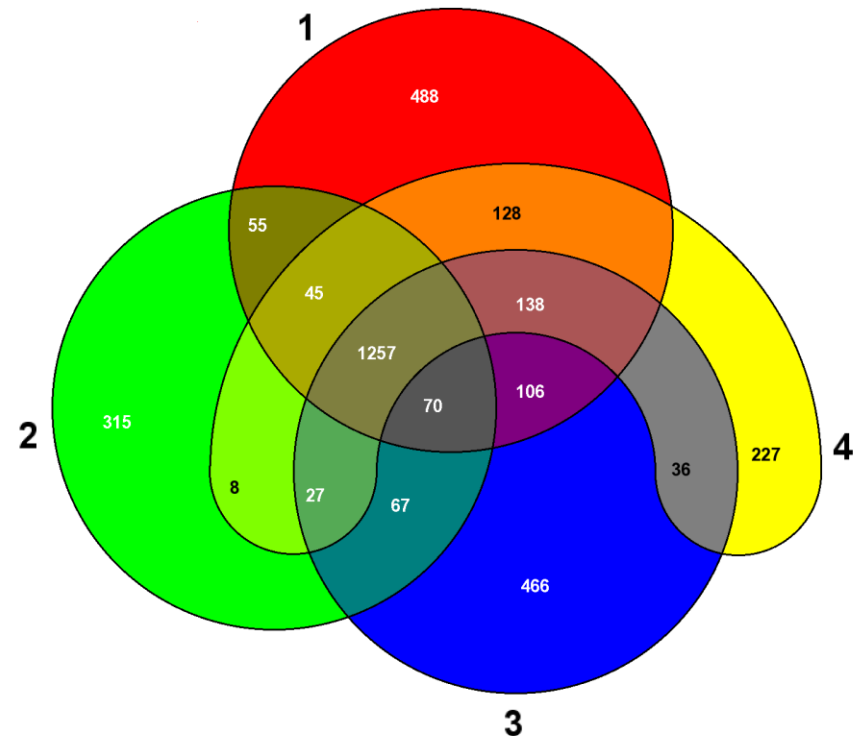
# Comparative genomics of *S. macedonicus* ACA-DC 198 against related species within the *S. bovis*/*S. equinus* complex

Core genome: 62.6%



- 1: *Streptococcus\_gallolyticus*\_UCN34\_NC\_013798
- 2: *Streptococcus\_gallolyticus*\_subsp\_gallolyticus\_ATCC\_43143\_DNA
- 3: *Streptococcus\_gallolyticus*\_subsp\_gallolyticus\_ATCC\_BAA-2069\_NC\_015215
- 4: *Streptococcus\_macedonicus*\_ACA-DC\_198\_main\_chromosome
- 5: *Streptococcus\_pasteurianus*\_ATCC\_43144\_NC\_015600

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

- 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



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

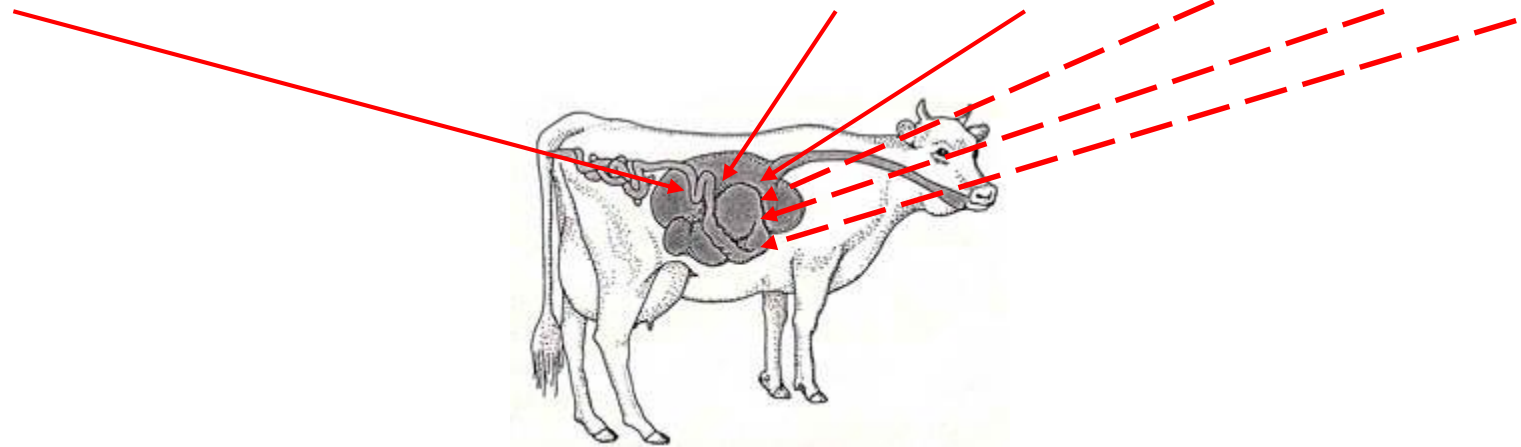
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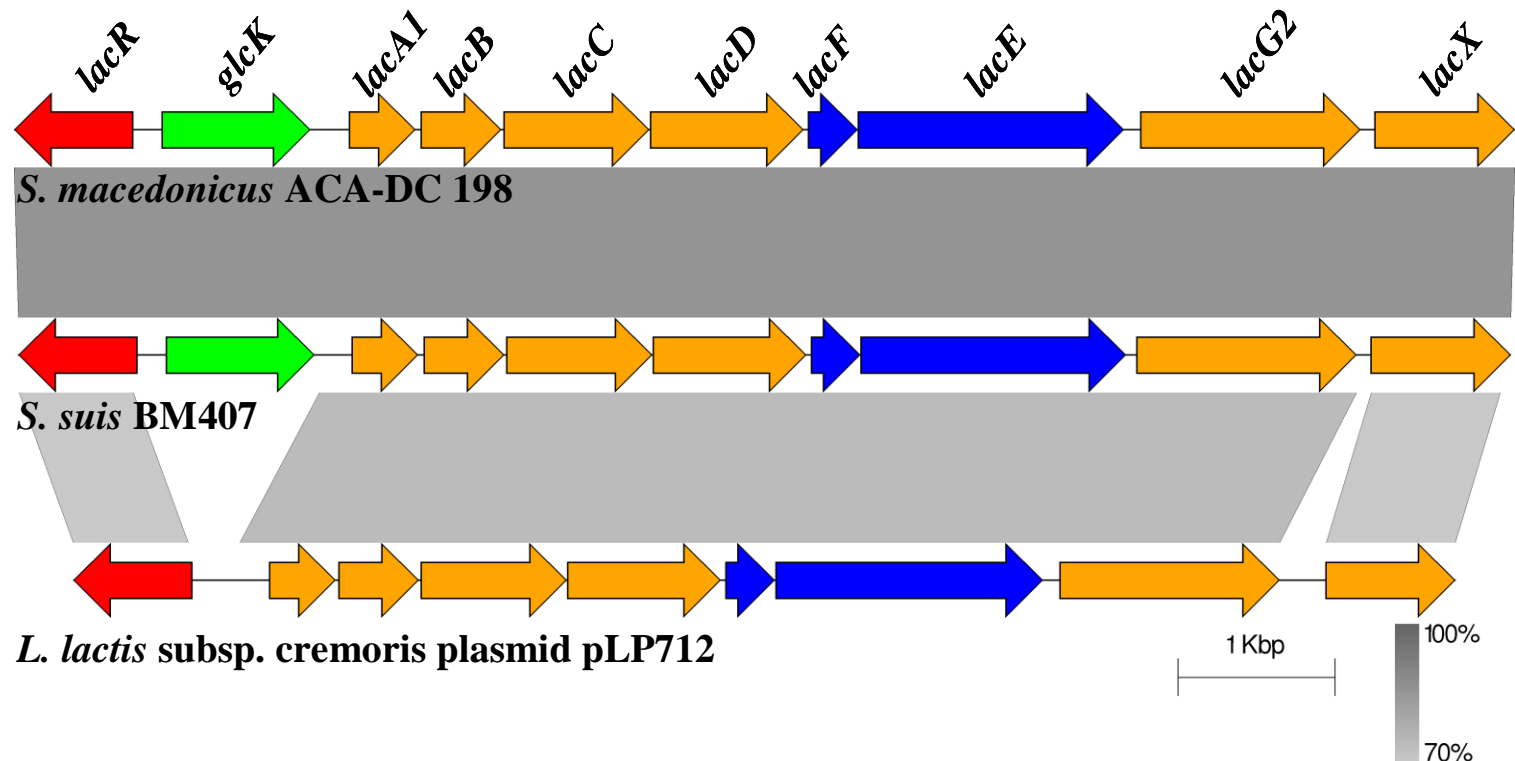
- *S. macedonicus* has deviated from the rumen environment

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

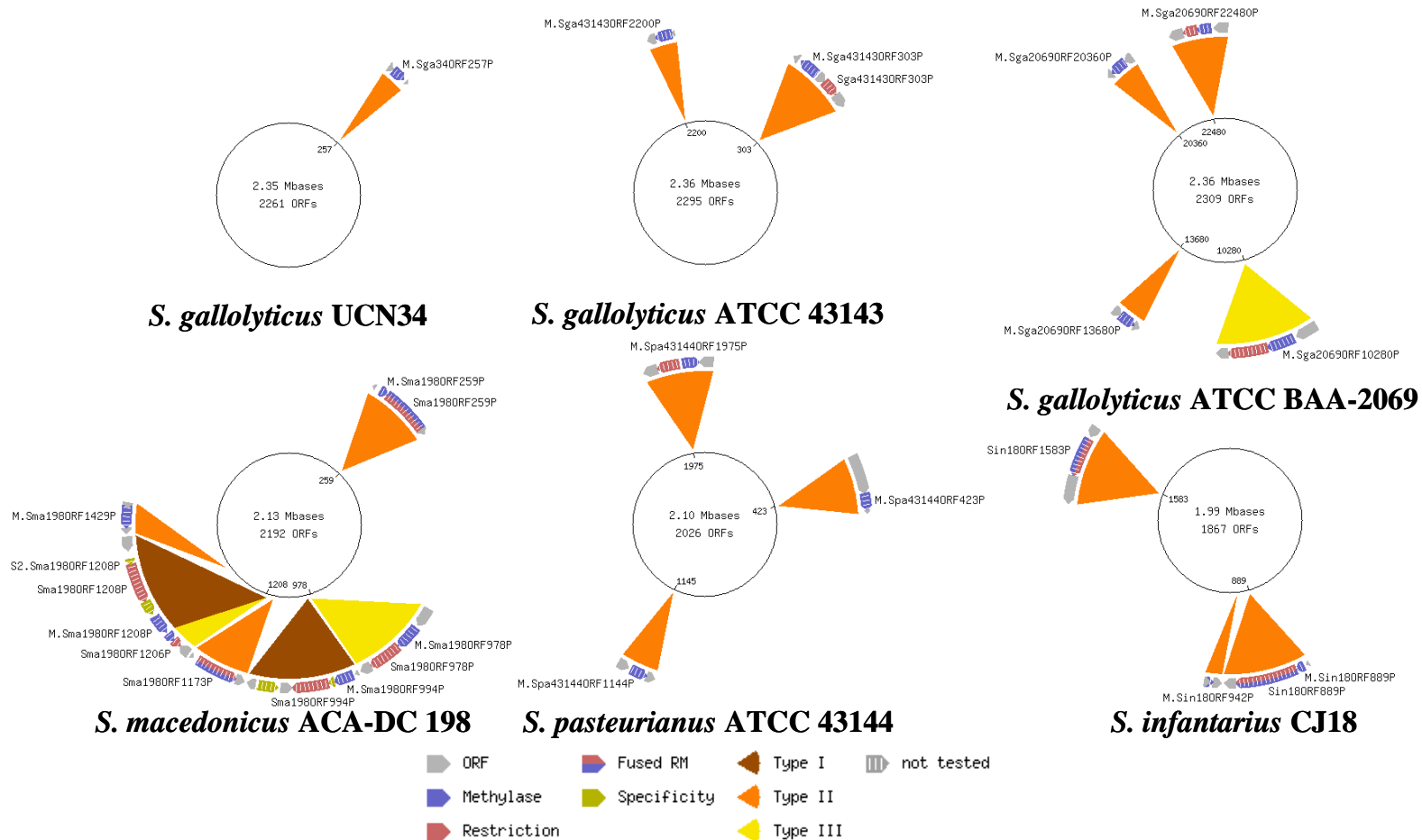
	<i>Streptococcus gallolyticus</i> UCN34		<i>Streptococcus gallolyticus</i> subsp. <i>gallolyticus</i> ATCC 43143		<i>Streptococcus gallolyticus</i> subsp. <i>gallolyticus</i> ATCC BAA-2069		<i>Streptococcus pasteurianus</i> ATCC 43144		<i>Streptococcus macedonicus</i> ACA-DC 198		<i>Streptococcus infantarius</i> subsp. <i>infantarius</i> CJ18	
CRISPR label	Position <sup>a</sup>	Nbr Spacers <sup>b</sup>	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*



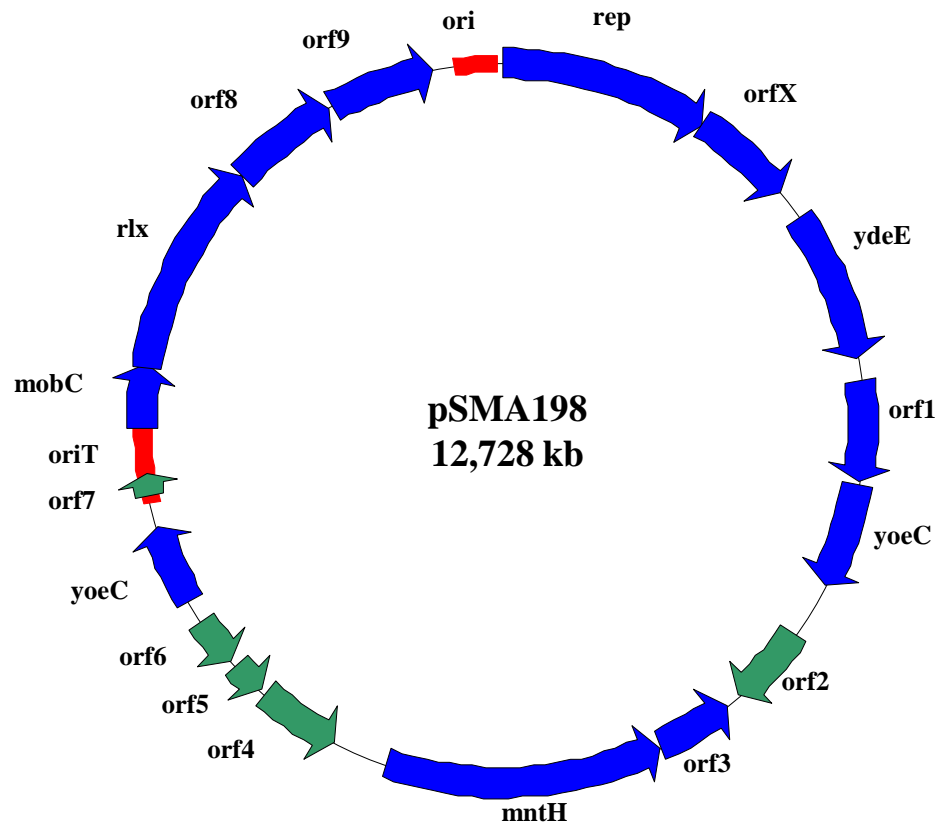
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# Assessing the adaptation of *S. macedonicus* to the milk environment

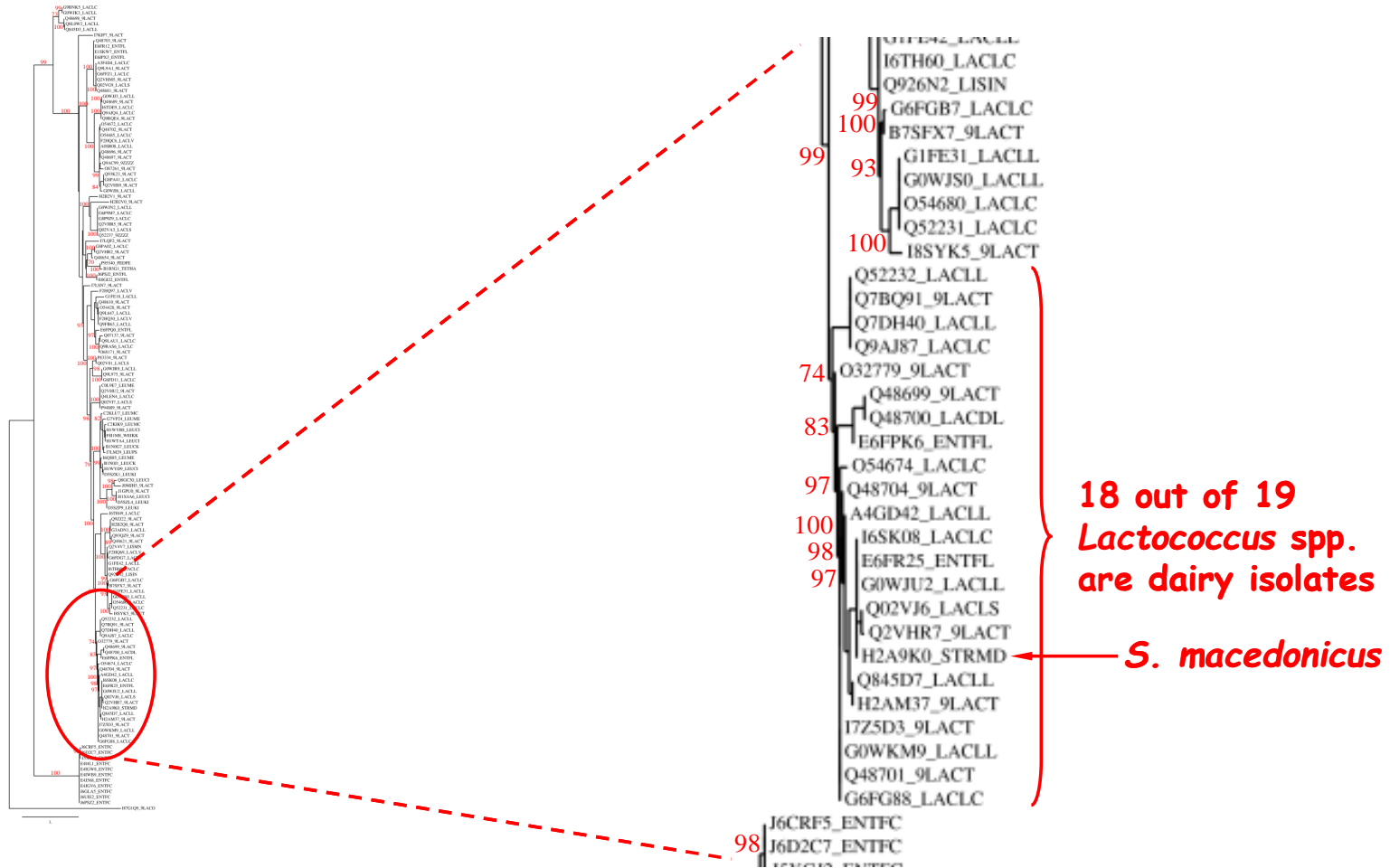
- The *S. macedonicus* plasmid pSMA198 belongs to the narrow host range pCI305 family of lactococcal plasmids





# 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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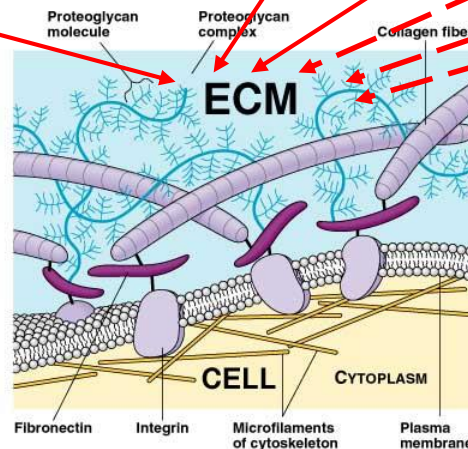
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# Assessing the pathogenic potential *S. macedonicus*

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

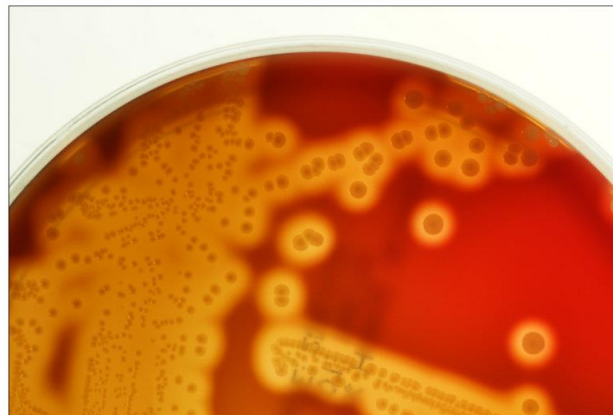
<i>S. gallolyticus</i> UCN 34			<i>S. gallolyticus</i>	<i>S. gallolyticus</i>	<i>S. macedonicus</i>	<i>S. pasteurianus</i>	<i>S. infantarius</i>
locus_tag	gene	function	ATCC BAA 2069	ATCC 43143	ACA-DC 198	ATCC 43144	CJ18
gallo_2179	-	accessory pilin (pil1)	✓	✓	-	-	-
gallo_2178	-	major pilin (pil1)	✓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

**Functional analysis needed!!!**

**This work was performed in collaboration with:**

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

## 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!!!**

