The study of the pheromone exporter PptAB reveals crosstalk between different quorum sensing systems in *Streptococcus thermophilus*

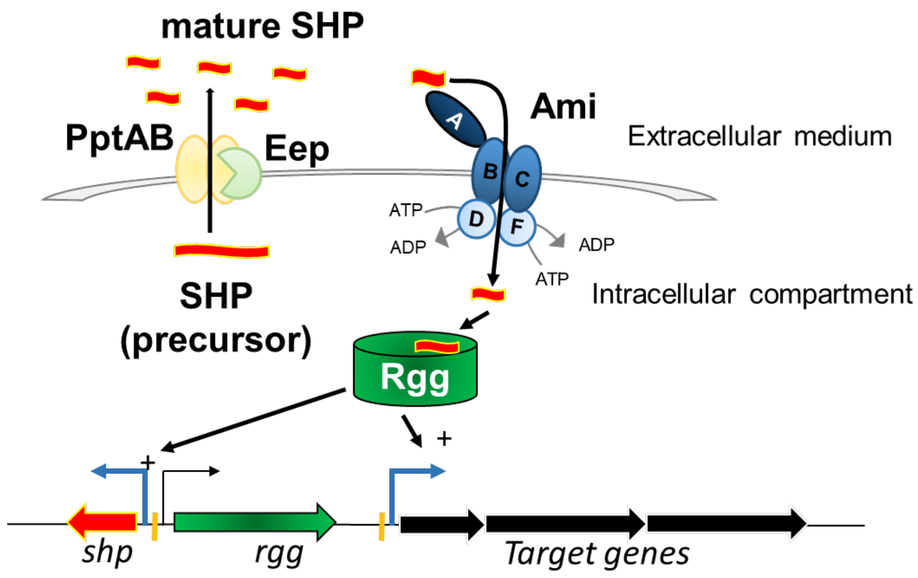
Abarna Lingeswaran[1], Alexia Royer[1], Coralie Metton[1], Céline Henry[2], Véronique Monnet[1,2*], Vincent Juillard[1], Rozenn Gardan[1]

[1] ComBac, Micalis Institute, INRAE, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[2] PAPPSO, Micalis Institute, INRAE, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France

* veronique.monnet@inrae.fr

Introduction

In Gram+ bacteria, oligopeptides serve as the signaling molecules of quorum sensing (QS) mechanisms. They are secreted, matured and detected. In the case of SHP/Rgg mechanisms, peptides (SHP) need to be imported back to interact with their cognate regulators (Rgg) that modulate the expression of target genes involved in crucial functions such as virulence or competence.



The SHP/Rgg QS mechanism in *S. thermophilus*

We have described such a SHP/Rgg mechanism in *S. thermophilus*. The SHPs are matured by the membrane protease Eep, exported by the ABC transporter PptAB and re-imported by the oligopeptide transporter Ami. Interestingly, this mechanism is widespread in streptococci and *S. thermophilus* has accumulated several copies of SHP/Rgg systems in its genome. The objective of this work was to characterize all targets of the 5 different systems present in *S. thermophilus* strain LMD-9.

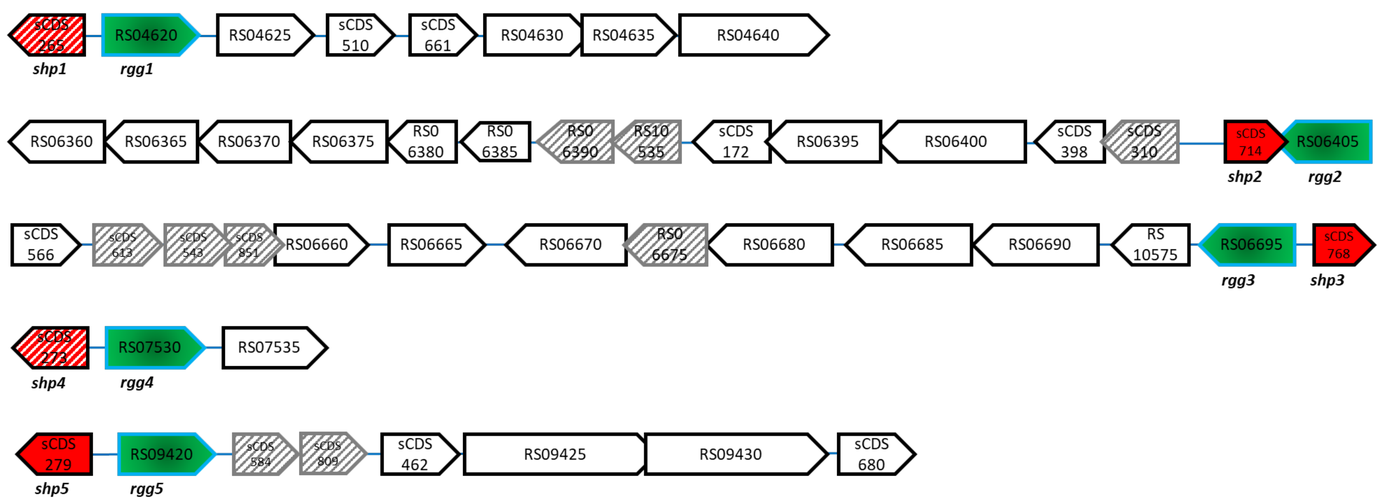
Results

1. Genes regulated by a SHP/Rgg mechanism in *S. thermophilus* strain LMD-9 are mainly located downstream of the *rgg* genes

We compared the transcriptome of strain LMD-9 vs its isogenic Δ*pptAB* mutant* using a deep sequencing approach (RNAseq):

• the expression of genes located downstream of the 5 *rgg* genes was downregulated in the Δ*pptAB* mutant (in white on the figure; genes hatched in grey were unaffected),

• 2 *shp* out of 5 were strongly controlled (*shp*2 and *shp*3) and one was slightly controlled (*shp*5) by PptAB and thus by QS (in red). The 2 remaining *shp* transcripts were not detected by this approach (in red, hatched in white),



Organizational schematic of the genes controlled by PptAB

→ Two *shp* genes (*shp1* and *shp4*) do not seem to be expressed although the genes located dowstream their cognate *rgg* genes are controlled by QS

* Both strains were inactivated for *comR* encoding the regulator of the competence as (i) the activity of ComR is positively controlled by QS via PptAB, (ii) the growth rate of a ΔcomR or ΔpptAB mutant is impaired compared to a WT strain. Thus, both strains (ΔcomR and ΔcomR ΔpptAB) had a strictly identitical growth rate.

2. Three SHPs are detected by mass spectrometry in strain LMD-9

We only detected three SHPs out of the five (SHP2, SHP3 and SHP5; in grey in the table) by LC-MS/MS from culture supernatants in accordance with the results of the RNAseq approach.

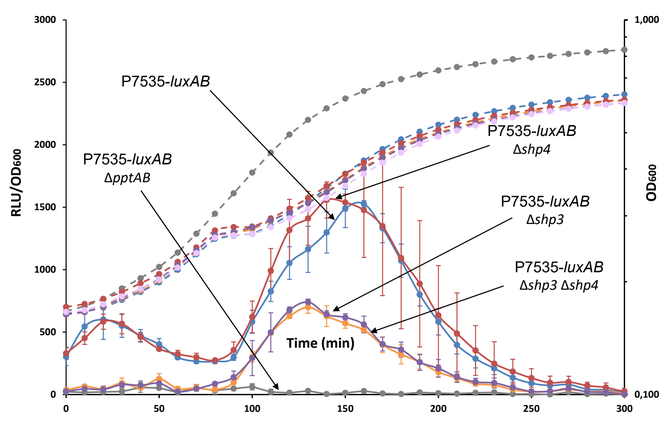
SHP	AA Sequence of the native SHPs*	$[M+H]^+_{theorical}$	$[M+H]^+_{experimental}$
SHP1	MNKESFLAILLLIF**ESIIVIAVG**	900.54010	
SHP2	MKKVIAIFLFIQTVVVI**DIIIFPPFG**	1018.56084	1018.56165
SHP3	MKKQILLTLLLVVF**EGIIVIVVG**	898.56084	898.56189
SHP4	MKKQKLLLLVVLVC**EGIIVILVG**	912.57649	
SHP5	MNKKALFSLLFVIL**EGIIVIGVG**	856.51389	856.51599

* The AA sequence of the mature forms of the SHPs is underlined.

Working hypothesis:
as the SHP of two loci (1 and 4) were apparently not synthesized although QS target genes were expressed (RNAseq results), we hypothesized that other SHPs were involved in the regulation of the both loci. We chose the target of locus SHP4/Rgg4 (gene 7535) to study this phenomenon.

3. SHP3 is involved in the regulation of the locus *shp4/rgg4 target (7535)*

We studied the regulation of gene7535 using a transcriptional fusion with the reporter LuxAB (P7535-luxAB). We hypothesized that the SHP3/Rgg3 system could be involved in this regulation because the sequence of the promoters of gene RS10775 (the target of the SHP3/Rgg3 system) and 7535 are identical.



Growth and luciferase activity levels of different strains grown in CDM

The P7535-*luxAB* transcriptional fusion was introduced in different genetic background.

• Inactivation of SHP4 did not affect p7535 activity (red curve),

• Inactivation of SHP3 only partially affected p7535 activity (orange curve).

→ The expression of the gene 7535 does not seem to be controlled by SPH4 but is controlled by SHP3 and at least another SHP.

Conclusion

• We have demonstrated cross-talk between the SHP/Rgg systems of *S. thermophilus* strain LMD-9, i.e. that the targets of a locus can be controlled by a distal SHP.

Perspectives

• We will seek which SHPs regulates the 5 Rgg and will assess the affinity of the different pairs with the objective to have a global view of this regulation QS network in *S. thermophilus*.

• We will study the function of the targets of these systems

• We hope that these results will help to better understand these SHP/Rgg mechanisms in streptococci with the objective of finely and precisely manipulating them without collateral effects.

References

Lingeswaran, A. et al. Export of Rgg quorum sensing peptides is mediated by the PptAB ABC transporter in Streptococcus thermophilus strain LMD-9. Genes 11, doi:10.3390/genes11091096 (2020).