Deciphering the RNA targetome of SprF1 antitoxin to elucidate its role in *Staphylococcus aureus* antibiotic persistence

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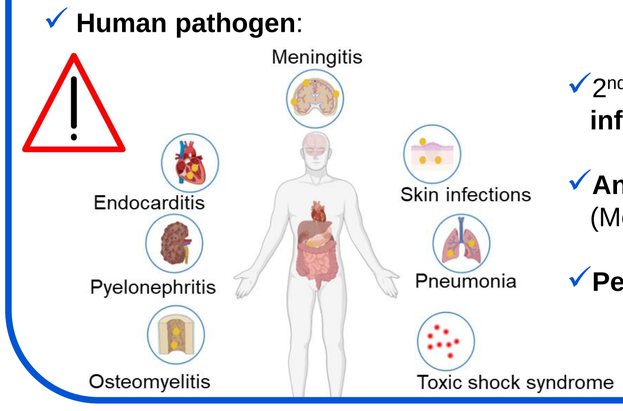
Bacterial RNAs & Medicine

Staphylococcus aureus

- ✓ Gram-positive cocci
- ✓ Human commensal (30% of the population)
- ✓ **Human pathogen**:

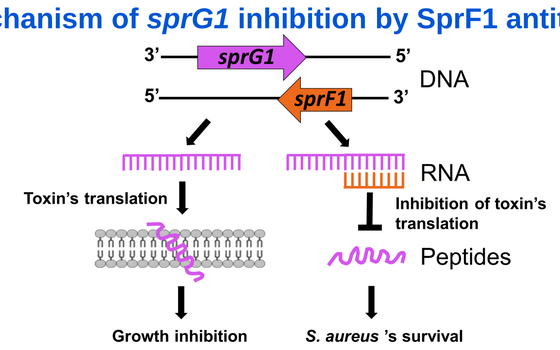


- ✓ 2nd cause of **nosocomial infections** in France
- ✓ **Antibiotics resistance** (Methicillin, Vancomycin)
- ✓ **Persistent bacteria**

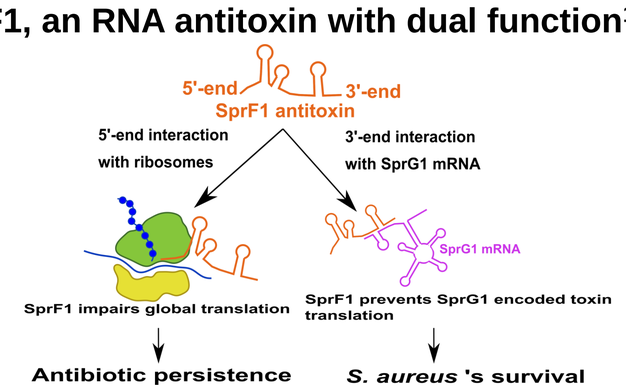
SprG1/SprF1 toxin-antitoxin system (TA)

- ✓ Type I TA system of *S. aureus*
- ✓ SprF1 ➔ RNA antitoxin
- ✓ SprG1 ➔ Peptide toxin

Mechanism of *sprG1* inhibition by SprF1 antitoxin



SprF1, an RNA antitoxin with dual function[1]

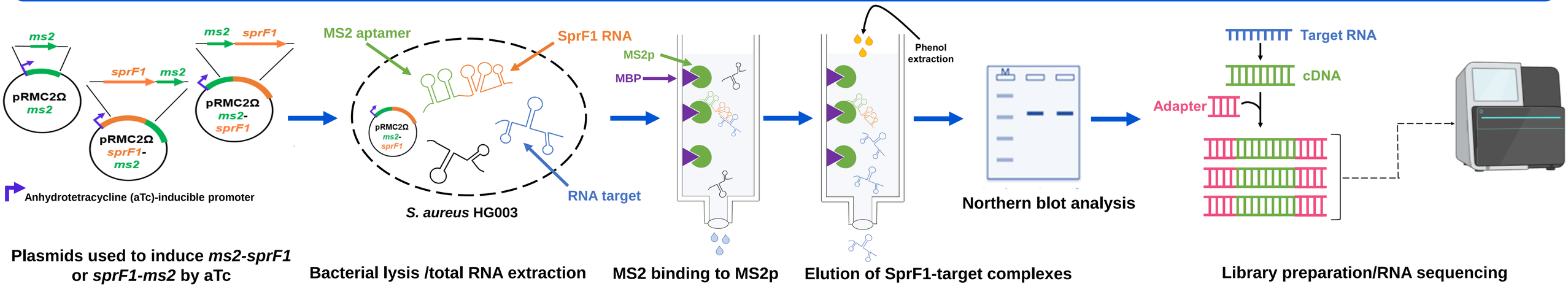


What are persistent bacteria?

➔ Subpopulation of transiently antibiotic-tolerant slow-growing bacteria that can resume growth after a lethal stress

AIM ➔ Identify SprF1 novel molecular targets using MAPS approach to better understand its role in antibiotic persistence

MS2-affinity purification coupled with RNA sequencing (MAPS)[2]



Plasmids used to induce *ms2-sprF1* or *sprF1-ms2* by aTc | Bacterial lysis /total RNA extraction | MS2 binding to MS2p | Elution of SprF1-target complexes | Library preparation/RNA sequencing

Results

1 Is MS2-SprF1 or SprF1-MS2 induction toxic for bacteria?

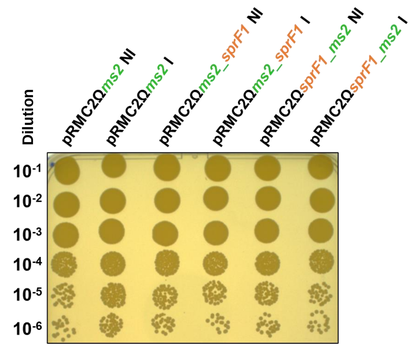


Figure 1: **Viability test on BHI agar plate**. After 2 h of growth, *S. aureus* containing pRMC2Ω*ms2,* pRMC2Ω*ms2-sprF1* or pRMC2Ω*sprF1-ms2* plasmids were induced (I: 0.5 µM aTc) or not (NI: ethanol) for 5 min at 37°C. Serial dilutions were spotted on BHI agar plate.

➔ **Induction of MS2 RNA is not toxic for bacteria.**

2 Are MS2 RNA constructs induced by aTc?

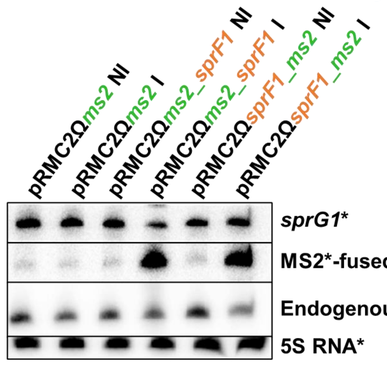


Figure 2: **Expression of MS2-SprF1 and SprF1-MS2 upon aTc induction.** Total RNAs were extracted after 2 h of growth and 5 min of induction with aTc (I) or ethanol (NI). RNAs expression was analyzed by northern blot .

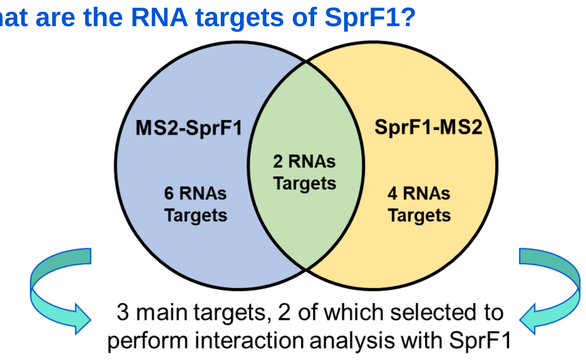
➔ **5 min of aTc induction allows significant MS2-SprF1 and SprF1-MS2 induction.**

3 Does MS2 aptamer bind MBP-MS2p protein?

Figure 3: Northern blot analysis of RNAs recovered during MAPS. Radiolabeled *sprG1* and MS2 RNA probes were used to detect *sprG1*, MS2-SprF1 and SprF1-MS2 RNAs recovered during the different MAPS steps: flow-through (non specific), washes (wash 6) and elution (target fraction).

➔ **MS2-SprF1 and SprF1-MS2 bind MBP-MS2p protein and are enriched in target fraction.**

4 What are the RNA targets of SprF1?



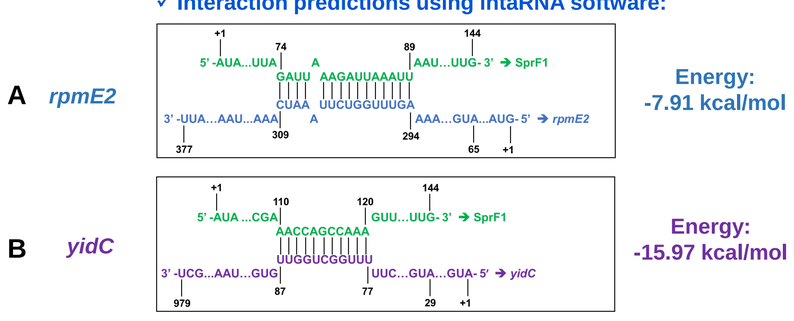
Gene ID	Gene name	Product	RNA fold change		P-value	
SAOUHSC_02176	*sprG1*	SprG1 toxin	12.13	24.87	4,45E-06	0,0003477
SAOUHSC_02361	*rpmE2*	50S ribosomal protein L31	17.09		6.96E-24	
SAOUHSC_02327	*yidC*	Protein insertase?	8.68	6.72	5.66E-06	3.24E-05

Figure 4: **RNA sequencing results of SprF1 targets.** Libraries of RNAs extracted from *S. aureus* HG003 containing pRMC2Ω*ms2,* pRMC2Ω*ms2-sprF1* or pRMC2Ω*sprF1-ms2* plasmids obtained following MAPS were sequenced on an Illumina MiSeq instrument. Enrichment of RNA targets was calculated using the HTSeq/DESeq2 pipeline for pRMC2Ω*ms2-sprF1 (blue circle)* or pRMC2Ω*sprF1-ms2 (yellow circle)* baits. Common targets between the two analyses are in green.

➔ ***sprG1* is one of the common target of MS2-SprF1 and SprF1-MS2 analysis. Recovery of *sprG1* validates the MAPS. *rpmE2* and *yidC* are 2 novel potential targets of SprF1.**

5 Can SprF1 bind *rpmE2* and *yidC* mRNAs?

✓ **Interaction predictions using intaRNA software:**



✓ **Electrophoretic mobility shift assay:**

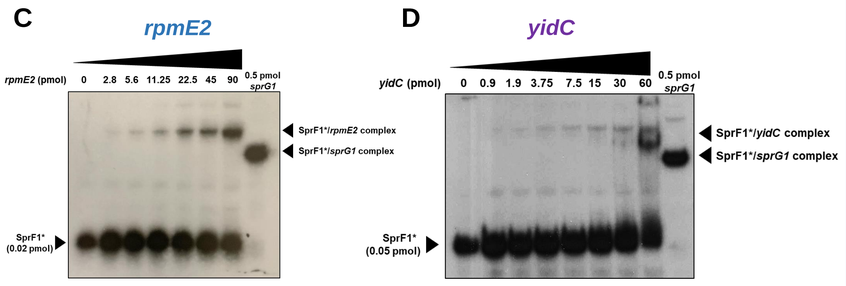
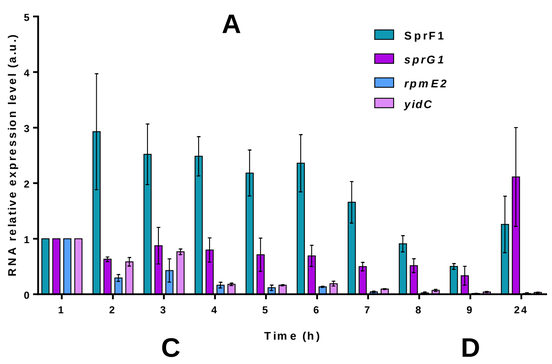


Figure 5: **Interaction analysis between SprF1 and *rpmE2* or *yidC* mRNAs.** Prediction of SprF1 and *rpmE2* mRNA interaction (**A**) or SprF1 and *yidC* mRNA interaction (**B**) using intaRNA. Electrophoretic mobility shift assay of radiolabeled SprF1 with unlabeled *rpmE2* (**C**) or unlabeled *yidC* (**D**). Unlabeled *sprG1* is used as a binding control.

➔ **SprF1 binds *rpmE2* and *yidC* with low affinity. Need for a chaperone?**

6 What are the kinetics of RNA expression during growth?



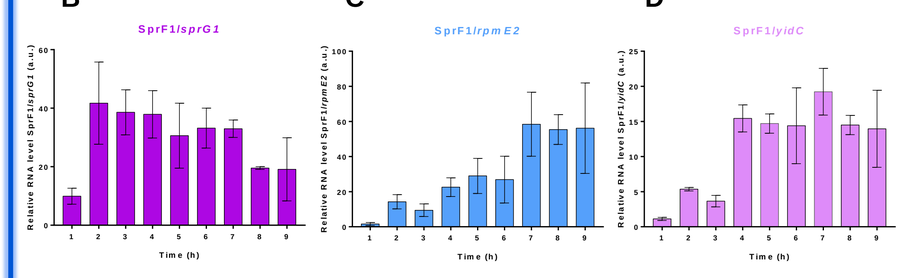


Figure 6: **SprF1, *sprG1*, *rpmE2* and *yidC* RNA relative expression**. SprF1, *sprG1*, *rpmE2* and *yidC* expression profile during *S. aureus* HG003 growth normalized with *gyrB* RNA (**A**). Relative RNA level of SprF1 compared to *sprG1* (**B**), *rpmE2* (**C**) and *yidC* (**D**).

➔ **SprF1 and *sprG1* expressions are stable during growth. Expressions of *rpmE2* and *yidC* are maximal at the beginning of growth and then decrease. The level of SprF1 is always higher than the level of its targets suggesting that SprF1 may interact with several targets during growth.**

7 Does SprF1 modulate *rpmE2* or *yidC* RNA expression?

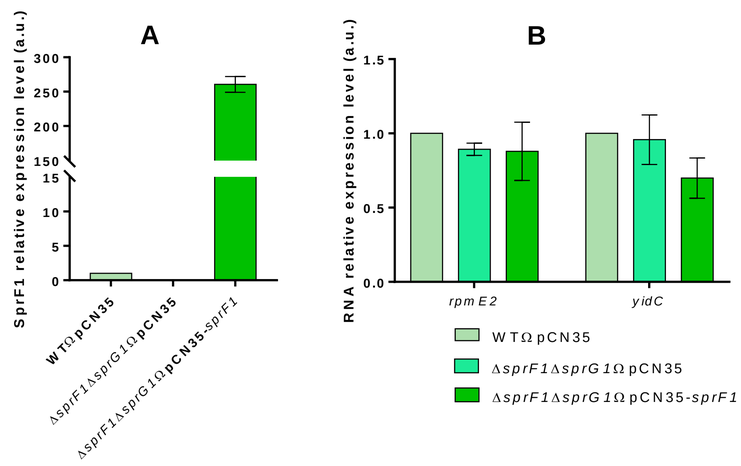


Figure 7: **Effect of SprF1 deletion or overexpression on *rpmE2* and *yidC* expression**. Relative expression of SprF1 (**A**), *rpmE2* and *yidC* (**B**) RNAs after 2 h of growth in HG003 WTΩpCN35, Δ*sprF1*Δ*sprG1*ΩpCN35 and Δ*sprF1*Δ*sprG1*ΩpCN35-*sprF1* overexpressing SprF1 strains. Data were normalized with *gyrB* RNA.

➔ **SprF1 does not alter the expression level of *rpmE2* and *yidC*. SprF1 does not appear to act at the transcriptional or post-transcriptional level on these targets.**

Conclusions

This work shows that dual function RNA antitoxin SprF1 has the potential to bind other targets than *sprG1* and ribosomes. Using MAPS, we identified 11 novel mRNA targets candidates, two of which showing high enrichment and putative interaction sites. Preliminary electrophoretic mobility shift assay analyses revealed that SprF1 binds *rpmE2* and *yidC in vitro.* Further analysis will be needed to validate the predicting interactions sites between SprF1/*rpme2* and SprF1/*yidC*.

Future directions

- ➢ Does SprF1 interaction with *rpmE2* and *yidC* mRNAs influence the translation of these proteins?
- ➢ Does *yidC* encode a functional protein insertase in *S. aureus*?
- ➢ Investigate biological function and relation with persistence and cell wall damage

1. Pinel-Marie ML & *al*.. RNA antitoxin SprF1 binds ribosomes to attenuate translation and promote persister cell formation in *Staphylococcus aureus* (2021). Nat Microbiol. 2. Lalaouna D & *al*. Identification of unknown RNA partners using MAPS (2017). Methods.