Appendix file

A multifaceted small RNA modulates gene expression upon glucose limitation in *Staphylococcus aureus*.

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APPENDIX MATERIALS AND METHODS

Plasmids and Strains Constructions

To delete *rsa*I in HG001, we amplified 967 nucleotides and 1000 nucleotides respectively upstream and downstream to the deleted region by using the following specific primers: Teg24-5'F-MluI and Teg24-5'R-NcoI (for the 5'-region PCR fragment), Teg24-3'F-NcoI and Teg24-3'R-BglII (for the 3'-region PCR fragment) (Table S2). The 5′- and 3'-region fragments were digested with *Nco*I, ligated and re-amplified using primers Teg24-5'F-*Mlu*I and Teg24-3'R-*Bgl*II. The generated 1.96 kb fragment was digested using *Bgl*II and *Mlu*I restriction enzymes and cloned into the plasmid pMAD CM (Memmi et al., 2008). The resulting plasmid was transformed into *E. coli* strain DH5α for subsequent electroporation into *S. aureus* strain RN4220. Clones were selected on Mueller-Hinton broth (MHB) supplemented with 10 µg/mL chloramphenicol and the plasmid was transduced in *S. aureus* strain HG001. Plasmid integration into the chromosome was selected after growth at a non-permissive temperature (42°C) while maintaining antibiotic selective pressure. A second recombination event was screened at the permissive temperature (30°C). Recombinant genomes were checked by PCR and sequenced using appropriate primers (Table S2). The mutant allele for deletion of *rsa*I gene in *S. aureus* 132 strain was generated by two-step overlapping PCR using the appropriate oligonucleotides (Table S2). The resulting PCR product was purified from agarose gel using NucleoSpin® Gel and PCR Clean-up Macherey-Nagel kit and cloned into a LIC-modified pMAD vector using the Ligation-independent cloning kit (Novagen) (Arnaud et al., 2004). The pMAD-Δ*rsaI* plasmid was introduced into *S. aureus* 132 strain by electroporation (Lee, 1995). Then, gene deletion was performed by a two-step procedure replacing the wild type chromosomic region by the corresponding mutant allele contained into the pMAD plasmid (Valle et al. 2003). The resulting modified strain was verified by PCR and fully sequenced using Sanger sequencing (Table S2).

Plasmids pES::*rsa*I and pES::*rsa*I mut5 were constructed as followed. The promoter of *bla*Z gene (P*bla*Z) was excised using *Sph*I and *BamH*I from the pCN57 and cloned into pCN47 generating the pES plasmid (Charpentier et al., 2004). The wild type *rsa*I gene was amplified by PCR using the *rsa*I_*BamH*I and *rsa*I_*EcoR*I oligonucleotides. The RsaI sRNA carrying the mutation at the predicted IcaR binding site was generated by two-step overlapping PCR using appropriate oligonucleotides (Table S2). The resulting RsaI and RsaI_mut5 PCR products were purified from agarose gel using NucleoSpin® Gel and PCR Clean-up Macherey-Nagel kit, ligated into the pJET 1.2 vector (Thermo Scientific) and cloned in *E. coli* XL1-Blue (Stratagene). The pJET-*rsa*I and pJET *rsa*I_mut5 plasmids were purified from overnight cultures with the NucleoSpin® Plasmid Macherey-Nagel kit. The DNA fragments were excised with *BamH*I and *EcoR*I FastDigest restriction enzymes (Thermo

Scientific), purified from agarose gel, and ligated using the Rapid Ligation Kit (Thermo Scientific) into a pES plasmid digested with the same enzymes. The generated pES-*rsa*I and pES_*rsa*I_mut5 plasmids were sequenced for verification and then introduced into *S. aureus* 132 derivative strains by electroporation (Lee, 1995).

MAPS experiments and RNA-seq analysis

To analyse the effect of specific mutations in RsaI on the target recognition, MAPS approach was performed on a set of experiments including 1 MS2 control, 1 wild-type MS2-RsaI, and one experiment for each RsaI mutant (MS2-RsaI mut2 carrying deletion of C22 to G29, and MS2-RsaI mut4 carrying deletion of nucleotides 75 to 113). To get a more relevant analysis of the data, we have also included the previous experiments performed on the MS2-RsaI. Therefore, in total seven MAPS experiments (2 MS2 controls, 3 MS2-RsaI, 1 MS2-RsaI mut2 and 1 MS2-RsaI mut4) have been analysed together using Deseq2. For this analysis, we have considered four categorical variables or factors (MS2 contr, MS2-RsaI-, MS2-RsaI mut2 and MS2-RsaI mut4). Because the MS2 purifications were done in two separate days (1 MS2 control and two MS2-RsaI in one experiment; 1 MS2 control, 1 MS2-RsaI, 1 MS2-RsaI mut2 and 1 MS2-RsaI mut4 in the other sets of experiments), some aleatory variables were expected to occur. The data pairing has been performed by assigning a blocking factor (batch effect) in order to be able to directly compare the obtained fold changes. Hence, the shrinkage estimation for dispersions and fold changes has been calculated considering the seven samples together. Under these conditions, the amount of shrinkage was significantly enhanced when compared to the analysis presented in Tables 1 and S3. It is inversely proportional to the residual degrees of freedom = number of samples - number of parameters to estimate. In this case, we added two more parameters, the groups MS2-RsaI mut2 and MS2-RsaI mut4, and four more samples. Because the shrinkage is increased, we applied a lower cut-off for the consideration of the significantly enriched RNAs, which is 3 for this analysis.

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