Inactivation of farR causes high rhodomyrtone resistance and increased pathogenicity in staphylococcus aureus

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Abstract
Rhodomyrtone (Rom) is an acylphloroglucinol antibiotic originally isolated from leaves of Rhodomyrtus tomentosa. Rom targets the bacterial membrane and is active against a wide range of Gram-positive bacteria but the exact mode of action remains obscure. Here we isolated and characterized a spontaneous Rom-resistant mutant from the model strain Staphylococcus aureus HG001 (RomR) to learn more about the resistance mechanism. We showed that Rom-resistance is based on a single point mutation in the coding region of farR [regulator of fatty acid (FA) resistance] that causes an amino acid change from Cys to Arg at position 116 in FarR, that affects FarR activity. Comparative transcriptome analysis revealed that mutated farR affects transcription of many genes in distinct pathways. FarR represses for example the expression of its own gene (farR), its flanking gene farE (effector of FA resistance), and other global regulators such as agr and sarA. All these genes were consequently upregulated in the RomR clone. Particularly the upregulation of agr and sarA leads to increased expression of virulence genes rendering the RomR clone [...]
Fig. S1. Phenotypic expression of hemolysin, lipase and protein A in HG001 and the RomR clone. (A) Hemolytic activity on sheep blood agar; strains were grown for 24 h. The halo around the colonies shows essentially alpha hemolytic activity due to lysed erythrocytes. (B) Lipase zymogram of culture supernatants, the strains were grown for 8 and 16 h in TSB. (C) Western blot of Protein A, the strains were grown for 8, 16 and 24 h in TSB.
Fig. S2. **FarE transmembrane domain prediction.** According to the prediction of transmembrane helices software (TMHMM Server v.2.0), FarE is supposed to be a membrane protein with 12 transmembrane domains.
Fig. S3. Expression patterns of farR and farE of *S. aureus* HG001 under various experimental conditions. The transcriptome of *S. aureus* was analyzed by strand-specific tiling arrays (Mäder et al., 2016). Graphs of condition-dependent gene expression levels were obtained from the Expression Data Browser at [http://genome.jouy.inra.fr/cgi-bin/aeb/index.py](http://genome.jouy.inra.fr/cgi-bin/aeb/index.py). The genespecific pages of the Expression Data Browser, which can be retrieved using the "Locus Tag" field, show additional information including the respective chromosomal region with transcription profiles, mapped transcription units and newly identified RNA features.
Fig. S4. GC analysis of the fatty acids isolated by total lipid extraction from the supernatant of different *S. aureus* strains after 16 h culture in TSB. This is the representative of two independent experiments. 0.2 mg/ml C12 FA was used as internal standard.