

TITLE: GENETIC AND PHYSIOLOGICAL ABILITIES OF TENSIOACTIVE ANTIMICROBIAL METABOLITES PRODUCTION BY *Staphylococcus warneri* TRPF4, ISOLATED FROM A BRAZILIAN OCEANIC ISLAND

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ABSTRACT:

Staphylococcus warneri is a Gram-positive and coagulase-negative species that holds a biotechnological potential as starter culture for fermented foods and for antimicrobial compounds production, like 10-ketostearic and a large range of bacteriocins, like warnericin, warnerin, nukacin and delta-lysin. In this work, the antimicrobial potential of tensioactive metabolites produced by a *Staphylococcus warneri* TRPF4, a strain isolated from the coast of Trindade Island – Brazil – was investigated. For the tensioactive metabolites production, the microorganism was cultivated in TSB at 30 °C for 48 h under 200 rpm. Its ability to produce tensioactive molecules was detected by oil displacement test and by superficial tension measurement. For extracting the tensioactive metabolites, cells were removed by centrifugation (15000 × g, 15 min, 4 °C) and the supernatant was acidified to pH 2,0 with 6,0 N HCl. The precipitate was recovered by centrifugation (15000 × g, 20 min, 4 °C) and the pH was adjusted to 7,0 with 6,0 N NaOH. The raw extract resulting was lyophilized. The antimicrobial activity of the raw extract (0,1 to 1,0 mg.mL⁻¹) was tested against 15 pathogenic bacterial strains for animals in Mueller-Hinton Broth at 37 °C for 24 h, according Clinical and Laboratory Standards Institute (CLSI). To verify the presence of genes encoding the tensioactive bacteriocins warnericin and delta-lysin, the *S. warneri* TRPF4 genome was sequenced on an Illumina MiSeq. The sequenced reads were trimmed for length (minimum, 50 bp) and quality (minimum score, Q20) and *de novo* assembled to contigs using CLC Genomics Workbench version 6.5.1 (CLC bio). The presence of bacteriocins coding genes in the assembled to contigs was accessed by the software BAGEL3. The *S. warneri* TRPF4 tensioactive metabolites were able to reduce the superficial tension of the cultivate to 33,3363 ± 0,2939 mN/m and displacing oil (2,5 ± 0,0 cm halo). The raw extract (0,1 mg.mL⁻¹) presented inhibitory effect of 98,20 ± 1,18 % (expressed as percent inhibition over control) against *Legionella pneumophila* ATCC 33155. The sequence analysis on BAGEL3 demonstrated the presence of gene clusters encoding warnericin RC, an anti-*Legionella* bacteriocin, and delta-lysin, both recognized as tensioactive bacteriocins. The results indicate that *S. warneri* TRPF4 produces the tensioactive bacteriocins warnericin and delta-lysin and can be used for the control of the causative agent of Legionnaires' disease.

Keywords: *Staphylococcus warneri*, antimicrobial, tensioactive bacteriocins.

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