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## ANTIBACTERIAL ACTIVITY OF MARINE BACTERIA METABOLITES AGAINST S. AUREUS ISOLATED FROM BOVINE MASTITIS

## <u>Thamires Martins</u><sup>1</sup>, Claudia Schinke<sup>1</sup>, Itamar Soares de Melo<sup>2</sup>, Felix Guillermo Reyes Reyes<sup>1</sup>

<sup>1</sup>University of Campinas – UNICAMP, Campinas -SP, Brazil; <sup>2</sup>Embrapa Environment, Jaguariúna – SP, Brazil; martins\_thamires@hotmail.com

Abstract: Bovine mastitis is the most common disease among dairy cattle and responsible for large economic losses worldwide. Staphylococcus aureus is one of the main agents causing mastitis. The failure of current conventional treatments for this disease is stimulating the search for new antimicrobial agents against this pathogen [1]. Marine bacteria are considered to be a potent source of new compounds for drug development. Therefore, we evaluated the antibacterial activity of metabolites produced by several marine actinobacteria isolated from marine sponges collected from São Pedro and São Paulo islands, Brazil, against S. aureus isolated from bovine mastitis. One isolate, which had previously shown inhibitory activity in disk diffusion assays, was inoculated in glucose-yeast extract broth added of marine salts and incubated at room temperature (20-23°C) for 38 days in static culture. The broth was centrifuged for 15 min at 8500 rpm and the supernatant was discarded. The cell pellet was extracted with methanol ( $3 \times 5$  mL), filtered and the solvent was evaporated to dryness. A 96-well microtiter plate was prepared with the methanol crude extract diluted in Brain Heart Infusion (BHI) broth at concentrations 8,62 mg mL<sup>-1</sup>, 17,25 mg mL<sup>-1</sup> and 34,50 mg mL<sup>-1</sup>, and ampicillin at 55  $\mu$ g mL<sup>-1</sup> was the positive control. S. aureus overnight culture was added to the microtiter plate at a final bacterial concentration of  $2,5 \times 10^5$  CFU mL<sup>-1</sup>. Wells containing only BHI and the pathogen served as negative control. The microplate was incubated for 24 hours at 35-37°C and growth inhibition was determined by measuring the optical density at 425nm. The experiment was performed in triplicate. S. aureus growth was inhibited by the three different concentrations of the methanol crude extract. No growth occurred with ampicilin. Antibacterial activity of methanolic extracts of marine bacteria has been reported before [2]. As the lowest concentration (8,62 mg mL<sup>-1</sup>) was enough to inhibit the pathogen, our results suggest that metabolites produced by this marine actinobacteria show potential use in the treatment of mastitis caused by S. aureus.

## **References:**

[1] Oliver, S.P. and Murinda, S. E. 2012. Antimicrobial resistance of mastitis pathogens. Vet. Clin. Food Anim. 28: 165–185.

[2] Isnansetyo, A., and Kamei, Y. 2003. MC21-A, a bactericidal antibiotic produced by a new marine bacterium, Pseudoalteromonas phenolica sp. nov. O-BC30T, against methicillin-resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy, 47(2), 480-488.