Title: STUDIES ON THE BIOCHEMISTRY OF *STAPHYLOCOCCUS* SP. BIOFILM FORMATION

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Abstract

The *Staphylococcus* genus is known for its medically important species, like *S. aureus*. Those microorganisms may play a role as opportunistic pathogens in the urinary, respiratory and digestive tract, mostly in hospitalized individuals. It is noteworthy the bacterial ability to produce biofilms, an structure that impairs antimicrobials and the immune system to reach the pathogen. The extracellular adhesion protein (Eap) is an important protein in the initial step of the biofilm formation, since it binds to plasma proteins and extracellular matrix of the host tissue. Eap redocks the bacterial surface by binding to a neutral phosphatase (NPase) located on cell wall, promoting the adhesion. Phosphatases can dephosphorylate a huge variety of substrates, but it is uncertain if the catalytic property of NPase is involved in the biofilm formation. To better understand the biofilm formation and provide new insights for fighting staphylococcal infection, the relationship between NPase activity and the biofilm formation ability is studied here. We analyzed 33 strains previously isolated from meat food obtained in Rio de Janeiro city by our laboratory. The ability to form a single-species biofilm was tested semi-quantitatively by crystal violet method using 96-well microplate. The NPase activity was colorimetric determined based on the hydrolysis of the artificial substrate *p*-nitrophenylphosphate (*p*-NPP) to the yellowish *p*-nitrophenolate (*p*-NP), for 1h. For each enzymatic assay, it was used roughly 6×10^8 CFU/ml, as determined by comparison to McFarland 2.0 standard. Twenty two strains were strong biofilm producers, while 6 moderately produced biofilms and with 5 strains it was detected a weak biofilm after 24h of incubation in BHI broth under a 100 rpm agitation. Three strains were already tested for NPase activity in order to validate the method: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 12600 e *Staphylococcus epidermidis* ATCC 12228. These strains were moderate biofilm producers under the conditions indicated above and showed a NPase activity of 12.27 ± 0.67, 5.80 ± 1.71 and 20.88 ± 6.61 nmol *p*-NP/h (mean $\pm$ SE, n = 2), respectively. NPase activity assays are currently in progress with all the strains isolated from food and will be compared to these three activities. Based on this standard strains levels of NPase activity and their ability to form biofilms, the NPase role on biofilm formation is closer to an understanding.

Key-words: *Staphylococcus*, biofilm, extracellular phosphatase, adhesion

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