TITLE: Proteomic analysis of *Staphylococcus saprophyticus* during macrophage infection

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ABSTRACT

The Gram-positive bacterium *Staphylococcus saprophyticus*, one of the coagulase-negative staphylococci, is the second most common causative agent of urinary tract infection, affecting mainly sexually active women. Furthermore, *S. saprophyticus* can cause acute diseases as pyelonephritis, sepsis, nephrolithiasis, endocarditis, urethritis, epididymitis and prostatitis. Because of their ability to perform phagocytosis, the first line of defence of the immune system, macrophages play a critical role in the development of infection. Besides phagocytosis, macrophages play a significant role in nonspecific defence (innate immunity) and also help initiate specific defence mechanisms (adaptive immunity) by recruiting other immune cells. This work aims to identify the *S. saprophyticus* proteins that are expressed or repressed during macrophage infection. For this purpose, the analysis were performed using the *S. saprophyticus* strain ATCC 15305 and macrophages from cell line J744. The standardization of the phagocytosis assays was performed in different times: 30, 60, 90 and 120 minutes, using 10^6 macrophages per cell of *S. saprophyticus*. The time chosen for the experiments was 120 minutes, because at that time it was possible to recover a larger amount of *S. saprophyticus* cells. The peptides obtained after the macrophage infections were treated by trypsin, reduced, alkylated and identified by nano-chromatography using a nanoACQUITY UPLC™ system (Waters) coupled to a SYNAPT Q-TOF mass spectrometer (Waters). The homology analysis was performed using the software ProteinLynx 2.3 (Waters). The identified proteins are being analysed through LocateP and NCBI databases. Three knockout mutants of the isaA, ssaA and fur genes of *S. saprophyticus*, obtained through protoplast transformation, are in the process of development. These mutant strains will also be used in phagocytosis assays. The results of this research help to elucidate the strategies and machineries used by *S. saprophyticus* during the invasion/adhesion of macrophages, leading us to understand how the pathogenic bacteria *S. saprophyticus* behaves during the interaction with macrophages and how it can dribble the host's immune system.

Keywords: *Staphylococcus saprophyticus*, proteomics, macrophage infection, phagocytosis, knockout mutants.

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