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Evaluation of in-situ fatty acid extraction protocols for the analysis of Staphylococcal cell membrane associated fatty acids by gas chromatography

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Abstract:

The composition and integrity of the bacterial cytoplasmic membrane is critical to the survival of staphylococci in dynamic environments and it is important to investigate how the cell membrane responds to changes in the environmental conditions. The staphylococcal membrane differs from eukaryotic and many other bacterial cell membranes by having a high abundance of branch fatty acids and relatively few unsaturated fatty acids. The range of available methods for extraction and efficient analyses of staphylococcal fatty acids was initially appraised to identify the best potential procedures for appraisal. *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213) was grown under optimal conditions to generate a cell biomass to compare the efficiencies of three approaches to extract and prepare methyl esters of the membrane fatty acids: (1) acidic direct transesterification of lipids, (2) modified basic direct transesterification of membrane lipids with adjusted reaction times and temperatures, and (3) base catalysed hydrolysis followed by acid catalysed esterification in two separate chemical reactions (MIDI process). All methods were able to extract fatty acids from the cell mass effectively where these lipids represented approximately 5% of the cellular dry mass. The acidic transesterification method had the least number of steps, the lowest coefficient of variation at 6.7% and good resistance to tolerating water. Basic transesterification was the least accurate method showing the highest coefficient of variation