OPTIMIZATION OF DIGESTION OF LIVING CELLS OF STREPTOCOCCUS PNEUMONIAE FOR SEARCHING PROTEIN VACCINE CANDIDATES

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Streptococcus pneumoniae (pneumococcus) is a Gram-positive bacterial inhabitant of the human respiratory tract, which is pathogenic in certain conditions, thus causing high morbidity and mortality. Pneumococcal infections have increased in frequency and severity over the past decade because of the emergence of multidrug-resistant strains. Moreover, the available vaccines are developed against the polysaccharide capsule, but their degree of effectiveness is not high, as they do not cover most of the 92 serotypes described to date. This makes protein vaccine development of great interest since surface proteins antigenically conserved in clinically relevant serotypes would be more effective immunogens, and thus might lead to more effective vaccines and at lower cost. Proteomics has become a fast and reliable tool for vaccine candidate selection. However, in pneumococcus, the high rate of lysis occurrence hampers the proteomic analysis of surface protein fractions. In this work, we have optimized the conditions for pneumococcal lysis control, and applied a well-described method for protein vaccine candidate selection in Gram-positive organisms, consisting of digestion of living cells followed by LC/MS/MS analysis. This strategy allows a quick selection of the most abundant and exposed candidates, which are the most promising to raise an immune response. To limit cytoplasmic contamination coming from cell lysis, we have cultured the R6 reference strain in different conditions. Culturing in a chemically-defined medium (CDM) with ethanolamine was the condition in which the greatest number of surface proteins with minor cytoplasmic contamination was reached. Then, we analyzed a set of clinical isolates representing those most prevalent and invasive in Spain in the last years. Those identified surface proteins common to most or all the isolates would be the best candidates for testing their protection capacity in animal models.