Production and Chemical Modification of Pneumococcal Capsular Polysaccharide Serotype 6B in a Preparative Scale.

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Effective vaccines against *S. pneumoniae* are constituted by capsular polysaccharides (PS) from prevalent serotypes free or conjugated to a carrier protein (tetanus toxoid or inactivated diphtheria toxin). In order to reduce the number of conjugated PS we are planning to use a pneumococcal surface exposed protein, PspA, as carrier to PS from the three most prevalent serotypes in Brazil - 14, 6B and 1. Preparative scale PS production and modification is an important step in this project. *S. pneumoniae* 6B was fermented in bioreactor (5L-BioFlo 2000) through three different methods - batch, fed-batch and continuous system with cell-recycle. The total PS productivity in the supernatant varied from 930 to 1300 gr. PS from the supernatant was purified by the method previously developed in our laboratory. The final product characteristics were: purification yield > 80%, MW > 500 kDa, protein content <2%, nucleotide content <2%, all in accordance to WHO. The PS size was reduced to 20 KDa by acid hydrolysis with HCl. Then, it was oxidized by sodium metaperiodate to create an aldehyde radical. The radical introduced was capable to be linked with the group amine that is present in the adipic acid dihydrazide molecule through Schiff's base formation. After this, the PS was derivatized and its terminal amine can be coupled with the carboxyl group of PspA to the formation of the conjugate.

Keys Words: Conjugate Vaccine, *Streptococcus pneumoniae*, PS6B, PspA.

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