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DEVELOPMENT OF A PRODUCTION PROCESS FOR A RECOMBINANT PROTEIN PNEUMOCOCCAL VACCINE

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1. INTRODUCTION

Pneumonia represents important infection in children under fiver years old. Even with efficient vaccines, this disease in 2015 was globally responsible for 15% of all deaths in children and *Streptococcus pneumoniae* is the most common infectious agent (WHO, 2015).

Bio-Manguinhos/Oswaldo Cruz Foundation supplies in Brazil the 10-valent pneumococcal vaccine that contains 10 of the 92 pathogen´s serotypes. This vaccine is obtained through fermentations and purification of the capsular polysaccharides of each serotype, followed by chemical couplings to specific carrier proteins.

Based on protein expression in prokaryotic system and purification, this work presents a proposal for a industrial process of only one recombinant protein antigen, the PsaA (pneumococcal surface adhesin A, 37 kDa), potentially capable to act against most of all prevalent serotypes of *S. pneumoniae*.

2. AIMS OF THE WORK

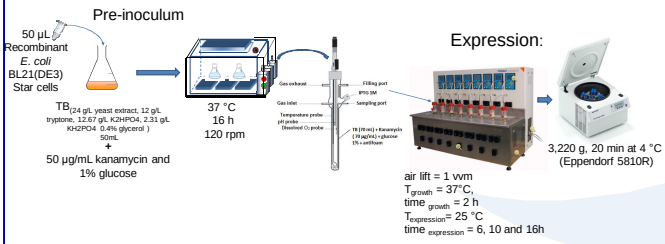
The aim of this work was to propose a process to obtain this recombinant antigen, describing fermentation, purification and formulation steps which could be scalable to be converted in an industrial process.

3. EXPERIMENTAL STEPS

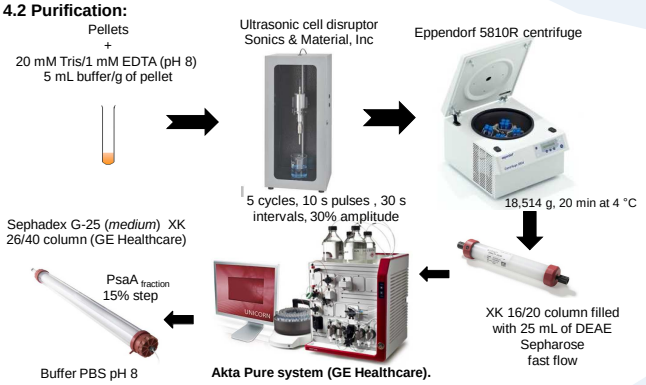
Protein expression → Purification → Formulation

4. MATERIALS AND METHODS

4.1 Protein expression:

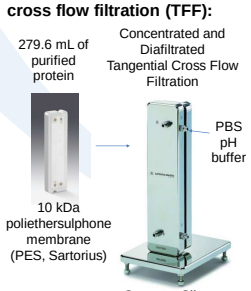


4.2 Purification:

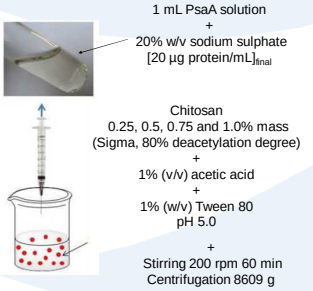


Stepwise gradient: 4%, 15% and 100%
Buffer A : 20 mM Tris/1 mM EDTA (pH 8)
Buffer B : 20 mM Tris/1 mM EDTA (pH 8) 1M NaCl.

4.3 Dessalting using tangential cross flow filtration (TFF):

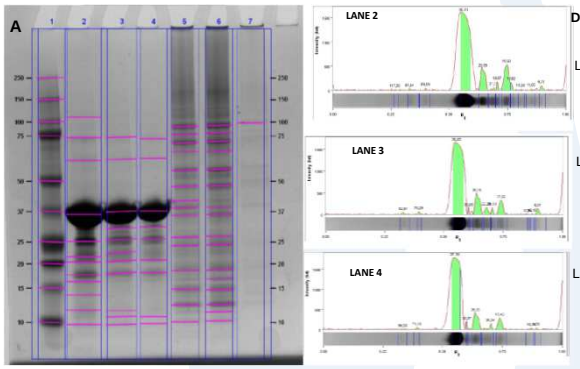


4.4 Formulation:



5. RESULTS AND DISCUSSION

Protein expression

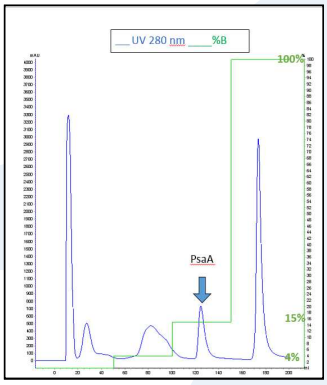


PreCasting NuvexNuPAGE 4-12% denaturanting gel of samples from DEAE Sepharose purification. Lane 1: molecular weigh standard. Lanes 2, 3,4: extract from 16, 6 and 10 hours cultivation respectively at 15% elution gradient. Lanes 5, 6, 7: extracts from 16, 6 and 10 hours expression respectively at 100% elution. Same mass protein was applied on the gel.

Purification

PsaA recovery in different time expression conditions when eluted at 15% of NaCl in gradient DEAE Sepharose fast flow elution

Expression (h)	Mass Injected (mg)	Mass eluted (mg)	Recovery %	Homogenity %
16	120.9	45.23	37.4	68.1
10	124.3	44.79	36.04	77.6
6	98.7	20.21	29.6	73.7

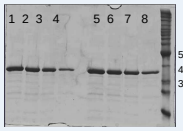


Stepwise gradient elution of PsaA 10-h expression in DEAE Sepharose FF. Vol inj. 5 mL, flow rate 5 mL/min (VC 25mL)

Dessalting technique	G-25	TFF
mL initial	15	279.6
mg/mL initial	5.25	1.98
mg initial	78.75	553.61
mL final	35	205.91
mg/mL final	2.08	2.19
mg final	72.8	450.94
Recovery (%)	**92.4**	**81.4**

Overall recovery for each 100 mg total protein		
mg initial	mg	mg
DEAE FF (average)	34.3	34.3
Dessalting G25	31.7	----
Desalting TFF*	-----	27.9

* Initial result – technique not optimized



SDS page (12.5%) analysis after desalting. Samples with 20,15,10 and 5 µg total protein

Lanes 1 - 4 ;G25
Lanes 5 8 10 kDa membrane

Formulation

$$\text{Encapsulation Efficiency (EE)}\% = \frac{([\text{protein}]_{\text{initial}} - [\text{protein}]_{\text{supernatant}}) * 100}{[\text{protein}]_{\text{initial}}}$$

Chitosan (% w/v)	EE (%)
1.0	50.28
0.75	34.86
0.50	15.58
0.25	11.48

6. CONCLUSIONS

These data demonstrate that it´s possible to develop a recombinant antigen production process considering to reduce the expression time in 60% from original process, proposed in LARENTIS *et al* (2011). Furthermore *E. coli* fermentation presents several advantages compared to native form production in *S. pneumoniae*. Purification steps could be performed with techniques with large-scale application achieving yield around 30% with high purity levels. Formulation is a proposal for new approach for bacterial vaccines administration that could be more acceptable for children and elderly, the most affected group of this infection.

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