OPTIMIZING CELL LYSIS FOR ENHANCED RELEASE OF A RECOMBINANT PNEUMOCOCCAL PROTEIN: A COMPARATIVE STUDY OF SURFACTANTS

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ABSTRACT

Streptococcus pneumoniae is a Gram-positive encapsulated bacterium, known for causing both invasive and non-invasive pneumococcal infections such as pneumonia and meningitis. This study aimed to optimize the cell disruption method for PdT-producing *Escherichia coli* biomass using lysis buffers containing Triton X-100, Tergitol 15-S-9, and Virodex TXR2 (0.1% each). High-pressure homogenization was employed and lysis progression was monitored via optical density, and subsequent analyses included Bradford assay for total protein measurement and SDS-PAGE with densitometry for PdT-specific quantification. Tergitol demonstrated superior relative amount of PdT compared to Triton, highlighting its potential to replace Triton, which has been considered of high concern to the environment, for bioprocessing applications. This finding not only suggests similar protein yield and purity but also underscores the potential environmental benefits of using alternative surfactants in biotechnological processes.

Keywords: Cell lysis, Recombinant protein, Pneumococcus, Protein release, Detergent.

1 INTRODUCTION

Streptococcus pneumoniae is a Gram-positive encapsulated bacterium of the genus *Streptococcus* and family *Streptococcaceae*. Under the microscope, it appears as diplococci or short chains and, as part of its metabolism, is aerotolerant, and alpha-hemolytic[1]. It causes invasive pneumococcal infections such as pneumonia, bacteremia/sepsis and meningitis, as well as non-invasive infections, including community-acquired pneumonia, sinusitis and acute otitis media. Currently, *S. pneumoniae* is a leading cause of fatal infections, notably bacterial pneumonia and meningitis [2].

A polysaccharide capsule envelops the entire surface of pneumococci during exponential growth, concealing internal structures and leading to serotype-specific reactions with anti-capsular antibodies, thereby classifying pneumococci into serotypes. Up to now, over 100 distinct serotypes have been identified[3]. Although pneumococcal vaccines prevent diseases caused by *S. pneumoniae*, they are composed of capsular polysaccharides and only offer protection against serotypes included in their formulation, typically limited to up to 20 serotypes in conjugate vaccines. In order to overcome this constraint, various approaches for new vaccines have been proposed, focusing on conserved protein antigens across serotypes for serotype-independent protection, such as the genetically detoxified pneumolysin (PdT) [4,5].

In the case of recombinant proteins produced in *Escherichia coli*, several challenges arise in downstream processing, including their predominantly intracellular production, demanding cell lysis to release the target-molecule [6]. During cell lysis, a surfactant is used for chemical disruption, with Triton X-100 being one of the most utilized detergents. However, Triton X-100 has been listed as a substance of high concern by the European Chemicals Agency due to its endocrine-disrupting properties, resulting in restrictions on its use with a deadline for cessation in January 2019, requiring now authorization for continued use [7]. Moreover, this surfactant carries a H410 hazard statement, indicating high risk to aquatic life [8]. This has led to a significant impact in the biopharmaceutical industry, which uses Triton in different processes [9], and prompted companies to seek alternatives to minimize environmental and public health impacts [7], replacing Triton X-100 by formulations like Virodex TXR2 and Tergitol 15-S-9 for cell lysis. In this sense, this work aims to identify a substitute surfactant for Triton X-100 in the cell lysis of PdT purification process.

2 MATERIAL & METHODS

Cell disruption was performed by resuspending 10 g of *E. coli* biomass in 300 mL of lysis buffer (composition detailed in Table 1). The PdT-producing *E. coli* was obtained and cultured according to the methods described by Fusco et al. (2024). The surfactant was added directly to the lysis buffer, resulting in three different buffers prepared with three detergents: Triton X-100 (Sigma-Aldrich, USA), Tergitol 15-S-9 (Dow Chemical Company, USA) and Virodex TXR2 (Croda, UK), all of them at 0.1%(m/v) concentration. Following buffer preparation, the biomass was kept on ice and then loaded into a high-pressure homogenizer (PandaPLUS 2000, GEA Group, Germany), set at 70 bar (first pressure valve) and 700 bar (second pressure valve). During the homogenization, samples of 1 mL were collected from the reservoir after each cycle, totalizing 5 cycles for each detergent.

Lysis progression was evaluated by measuring the optical density (OD) of lysate samples in a spectrophotometer at 600 nm. The samples were centrifuged for 30 min at 13,000 rpm and the supernatants were quantified for total protein concentration according to the Bradford protein assay method, utilizing a Pierce™ Bradford Plus Protein Assay Kit (Thermo Fisher, USA).

Specific PdT concentration was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry to measure the percentage of PdT band in relation to the sum of all other bands in the lane.

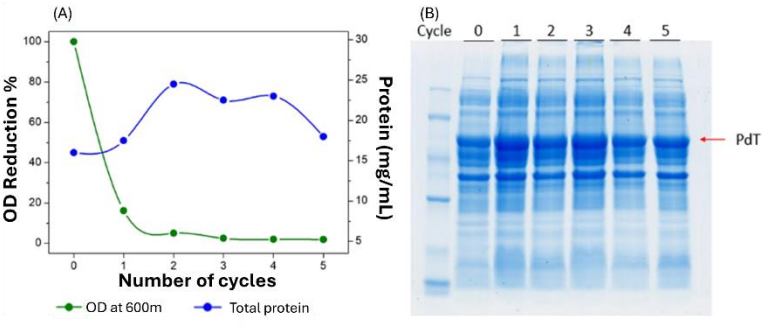
Table 1 Lysis buffer composition

Compound	Concentration
Protease inhibitor cocktail (Roche)	One tablet
Bis-tris buffer pH 7.0	10 mM
EDTA	1 mM
Surfactant	0.10%(m/v)

3 RESULTS & DISCUSSION

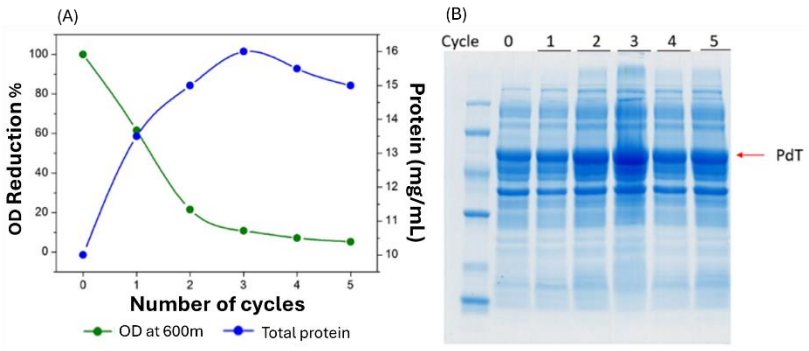
Cell lysis performed with Triton X-100 served as a standard for comparing the other cell lysates, as it is the target for replacement. For this surfactant, we determined 2 cycles as the optimal for maximum cell disruption, as indicated by the decrease in OD (Figure 1A). Additionally, it was crucial to evaluate the number of cycles resulting in the highest release of PdT protein into the supernatant, which in this case was also 2 cycles. Figure 1B displays the SDS-PAGE of all supernatant samples, with 0 indicating the sample before starting the homogeneizer and the following numbers indicating the next cycles in sequence. Release of PdT was observed in all cycles, as indicted by the presence of the band indicated by the red arrow.

Figure 1 (A) plot of OD reduction (%) and total protein concentration in the supernatant vs.number of cycles (B) SDS-PAGE Triton X-100



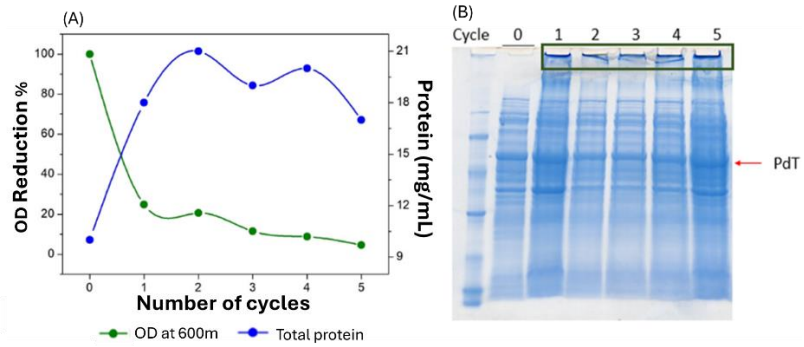
Experiments performed with Tergitol 15-S-9 showed results closest to that with Triton. It required 3 cycles to reach a plateau in total protein concentrarion released into the supernatant, and the results indicated that the release of total and specific protein was similar to the values obtained with Triton X-100 (Figure 2).

Figure 2 (A) plot of OD reduction (%) and total protein concentration in the supernatant vs. number of cycles (B) SDS-Page Tergitol 15-S-9



Experiments performed with Virodex TXR2 proved to be less effective compared to the other surfactants. It required 2 cycles to reach a plateau in total protein concentration (Figure 3A). However, the greatest cause of inefficiency of this surfactant is related to the release of PdT: whereas for the other lysates the maximum PdT relative amount in the supernatant was between 25% and 28%, Virodex TXR2 achieved a maximum of only 14% of PdT relative amount (Table 2). It is noteworthy that even before mechanical lysis began (cycle 0), there was less PdT in the Virodex supernatant compared to the others (Table 2). This could be due to aggregation caused by interactions between PdT and Virodex TXR2. This is supported by accumulations on the top of SDS-PAGE wells, as shown in Figure 3B (highlighted by a green box). These bands indicate the presence of large molecules (possibly aggregates) that were not able to migrate through the gel, which was not observed in the other experiments. Detergent-protein interactions that facilitate aggregation have been described in the literature [10]. Additionally, PdT has shown aggregation with other types of detergents (for other purposes) in experiments conducted in this laboratory.

Figure 3 (A) plot of OD reduction (%) and total protein concentration in the supernatant vs. number of cycles (B) SDS-Page Virodex TXR2



Evaluating the presence of the target-protein is critical to assess the effectiveness of the detergent specifically for PdT. All surfactants started to disrupt the cells at the cycle 0 and, although 3 passages were required to reach the plateau of lysed cells with Tergitol, it achieved the highest PdT relative amount of 28% in the first passage (Table 2). Despite the high relative amount reached with Tergitol, the PdT concentration was still greater in all passages with Triton, peaking at 1.18 mg/mL in the second cycle, showing the most favorable results for this protein. This demonstrates that while Tergitol can potentially replace Triton, it does not achieve the same level of efficiency. Virodex had the lowest percentages and PdT concentrations, thus it can not be used to replace Triton for this protein.

Table 2 PdT exact and relative concentration at each cycle for each detergent

Cycle	SDS-PAGE band relative amount (%)			PdT (mg/mL)		
	Virodex	Triton	Tergitol	Virodex	Triton	Tergitol
0	11%	24%	25%	0.23	0.79	0.52
1	12%	25%	28%	0.42	0.88	0.78
2	14%	24%	26%	0.59	1.18	0.80
3	8%	23%	25%	0.30	1.02	0.81
4	3%	27%	25%	0.12	1.24	0.79
5	9%	26%	26%	0.31	0.94	0.77

4 CONCLUSION

In conclusion, our results showed that lysis buffer composition should be specifically tailored for each biomolecule of interest, since different surfactants can interact with the target-molecule, as it was observed for Virodex TXR2 and PdT. Moreover, we have identified Tergitol 15-S-9 as the best replacement for Triton X-100 in the cell lysis targeting PdT, resulting in similar process times and even superior efficiency in releasing PdT with higher relative amount than Triton.

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ACKNOWLEDGEMENTS

The present work was developed with financial support from CNPq through grants 105844/2024-1 and 310973/2022-8.