Biotechnology Techniques **13:** 391–393, 1999.

Purification of polysaccharides from a biofilm matrix by selective precipitation of proteins

R. Oliveira*, F. Marques & J. Azeredo
Centro de Engenharia Biológica – IBQF, Universidade do Minho, 4700 Braga, Portugal
**Author for correspondence (Fax: +53 678986)*

Received 26 April 1999; Accepted 11 May 1999

Key words: differential precipitation, polysaccharides, proteins

Abstract

The objective was to obtain the polysaccharide fraction present in the extracted polymeric matrix of a biofilm by differential precipitation of the protein content. This was tried by pH adjustment and by addition of trichloroacetic acid (TCA). The best yield of protein precipitation was with 20% (w/v) TCA, with more than 97% of the protein removed and without affecting the amount of polysaccharides in solution.

Introduction

The increasing commercial importance of bacterial polysaccharides is directing efforts to the development of rapid, efficient and easily scaleable techniques for their purification. In the present situation, the objective of obtaining a purified fraction of polysaccharides arose from the need to quantify the total amount of polysaccharides present in the polymeric matrix of bacterial biofilms. Usually bacterial polysaccharides are quantified by the phenol/sulfuric acid method (Dubois *et al.* 1956), using glucose as standard. The values so obtained can only be used in comparative terms, because the response of the method depends on the standard used and glucose overestimates the actual amount of polysaccharides. To have the absolute value it is necessary to use the polysaccharide as standard, obviously after purification.

Other authors have reported methods for the rapid isolation of polysaccharides from contaminating proteins but in different circumstances. Doco *et al.* (1991) used coupled anion exchange and gel permeation high-performance liquid chromatography for the separation and quantification (with pure polysaccharide as standard) of polysaccharides produced by acid lactic bacteria in fermented skim milk. Beri and Rollings (1995) described a technique relying on the adjustment of the solution pH to remove by precipitation the protein fraction of alkali extracts of *Mucor rouxii*.

This paper describes the precipitation of proteins by trichloroacetic acid (TCA) and compares the results with the precipitation by pH adjustment.

Materials and methods

Bacterial biofilm

The biofilm used was formed by a strain of *Pseudomonas fluorescens* on PMMA plates of 2 cm × 2 cm suspended inside a fermenter of 2 l containing a medium of glucose 5 g l^{-1}, peptone 2.5 g l^{-1} and yeast extract 1.25 g l^{-1}.

Matrix extraction

The polymeric matrix was extracted immersing the plates with biofilm in a beaker containing 5 ml of EDTA 1.5%, during 3 h at 5 °C. The extract was dialysed against deionized water using tubing with a cut-off of 14 kDa.

Precipitation by pH adjustment

The initial pH of the extracts was 7.17 and the adjustment of other pH values was performed by the addition

of 1 M NaOH or 1 M HCl. The pH was adjusted to a new value and after 2 h the precipitate was recovered by vacuum filtration through a membrane of 0.45 μm. The filtrate was assayed for total protein content and polysaccharides. The procedure was repeated for another pH value.

Precipitation with TCA

To optimise the efficiency of precipitation by TCA, different concentrations were assayed: 10, 15, 20 and 25% (w/v). The precipitate was recovered by filtration and the filtrate was assayed for total protein content and polysaccharides.

Quantitative methods

The polysaccharides were assayed by the phenol-sulfuric acid method (Dubois *et al.* 1956) using glucose as standard. The total protein content was determined by the Lowry modified method, using the SIGMA P5656 kit and BSA (bovine serum albumin) as standard.

Results and discussion

The total protein content of the extracted solutions used in the precipitation assays was 1.12 mg l^{-1} (BSA as standard).

Two types of procedures were followed for precipitation by pH adjustment. In one case the pH was raised by the addition of NaOH, followed by a pH decrease via the addition of HCl. In the other one this order was inverted. The results obtained are presented in Figure 1.

The first procedure enabled to precipitate in average 79% of the total protein content. In the second case the protein elimination was slightly lower, about 76%. In both situations the polysaccharide content of the extracted solution remained almost unchanged. The small variations observed are within the expected experimental deviations.

The results obtained with the different concentrations of TCA are expressed in Figure 2, in terms of the amounts of proteins and polysaccharides remaining in solution.

As it can be observed, the total polysaccharide content of the solution is not significantly affected by any of the TCA concentrations assayed. As protein precipitation is concerned, all the TCA concentrations

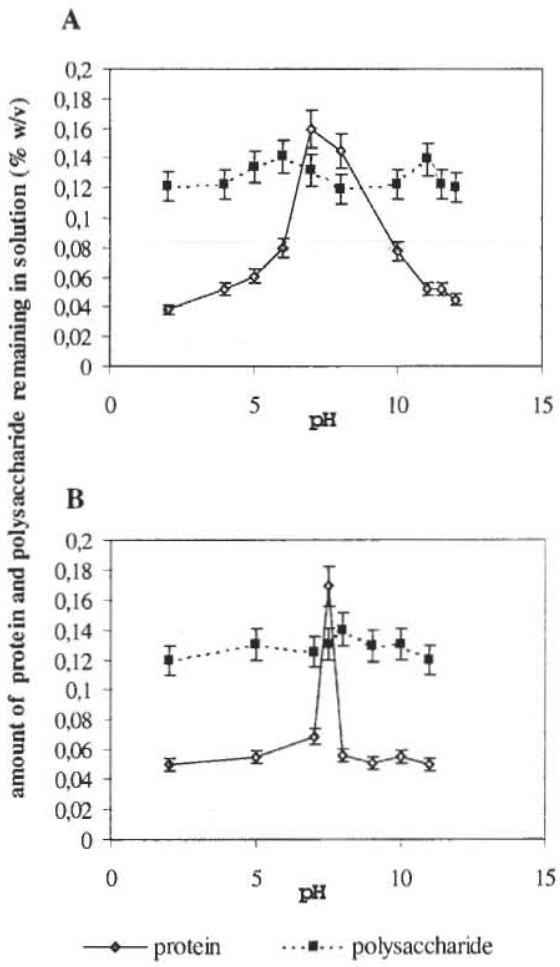

Fig. 1. Amount of proteins and polysaccharides remaining in solution after precipitation by pH adjustment. (A) Addition of NaOH followed by HCl. (B) Addition of HCl followed by NaOH.

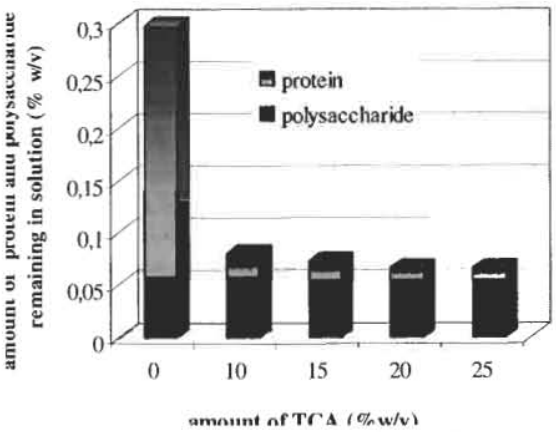

Fig. 2. Amount of proteins and polysaccharides remaining in solution after precipitation wiht different concentrations of TCA.

assayed led to precipitation yields above 90%. However, 20% (w/v) TCA proved to be the most efficient concentration, removing more than 97% of the total protein content. This means that it is not expectable to obtain higher yields by increasing the TCA concentration, which is corroborated by the results obtained with 25% TCA.

It is worth noting that TCA also precipitates DNA. This was verified by assaying the precipitation of different standard solutions of DNA, because in old biofilms DNA is likely to be present in the polymeric matrix.

Conclusion

TCA proved to be a very efficient and selective precipitating agent. In a concentration of 20% w/v it promotes the precipitation of almost all the protein content without affecting the amount of polysaccharides present in the solution.

References

Beri RG, Rollings JE (1995) A technique for the rapid removal of proteins from cell extracts containing polysaccharides. *Biotechnol. Tech.* **9**: 783–786.

Doco T, Carcano D, Ramos P, Loones A, Fournet B (1991) Rapid isolation and estimation of polysaccharide from fermented skim milk with *Streptococcus salivarius* subsp. *thermophilus* by coupled anion exchange and gel-permeation high-performance liquid chromatography. *J. Dairy Res.* **5**: 147–150.

Dubois, M, Gilles, KA, Hamilton, JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350-356.