Effect of sulphate supplementation on polysaccharide production and composition in two *Porphyridium cruentum* strains

Sousa A. M.; Silva C.; de J-Raposo M. F.; Morais R. M.
e-mail: rmmorais@esb.ucp.pt

CATÓLICA
UNIVERSIDADE CATÓLICA PORTUGUESA | PORTO
Escola Superior de Biotecnologia

Porphyridium polysaccharide

Introduction

The marine microalga *Porphyridium cruentum* is of high commercial value because of its products. The main compounds produced are sulphated exopolysaccharides (EPS), polyunsaturated fatty acids (PUFA) and phycoerythrin, a red proteinaceous pigment (1). The easy growth conditions make the use of this alga very appealing.
Many studies have been conducted to determine the chemical and physical properties of EPS due to its antiviral activity as described by Huleihel M. *et al*[2]. This activity seems to be directly correlated with the polysaccharide degree of sulphation (3).
The aim of this study was to improve polysaccharide production by *P. cruentum*. Different sulphate ions concentrations were tested. Further studies are going to be carried out to determine the effect of the sulphate supplementation in the polysaccharide structure.

Material and Methods

Two *Porphyridium cruentum* strains, one from Department of Biotechnology Engineering of Ben-Gurion University - Negev, Israel (ISR) and the other from Department of Microbiology - Pharmacy Faculty of University of Santiago, Spain (ESP), were grown in batch cultures in sterile conditions. The cells were cultured in 1L flasks, with constant temperature (25ºC), continuously illuminated (30mE·s^{-1}·m^{-2}) and aerated with sterile air. The growth was monitored by dry weight. The supplementation of sulphate ions was conducted by addition of magnesium sulphate and magnesium chloride was used as magnesium control.

The EPS extraction was accomplished by precipitation with two volumes of ethanol. After centrifuging the culture at 10000rpm, 4-10ºC for 20-30min, the sample was placed in a water bath at 80ºC and after 1 hour it was filtrated. The EPS was lyophilized before and after a dialysis procedure. The carbohydrate and uronic acid composition were determined colometrically with a Shimadzu UV-1601 by the phenol-sulphuric acid (4) and MHDP (5) methods using galactose and glucuronic acid as standards, respectively. The sulphate content was determined turbidimetrically as barium sulphate (6) using the same spectrometer mention above.

Results and Discussion

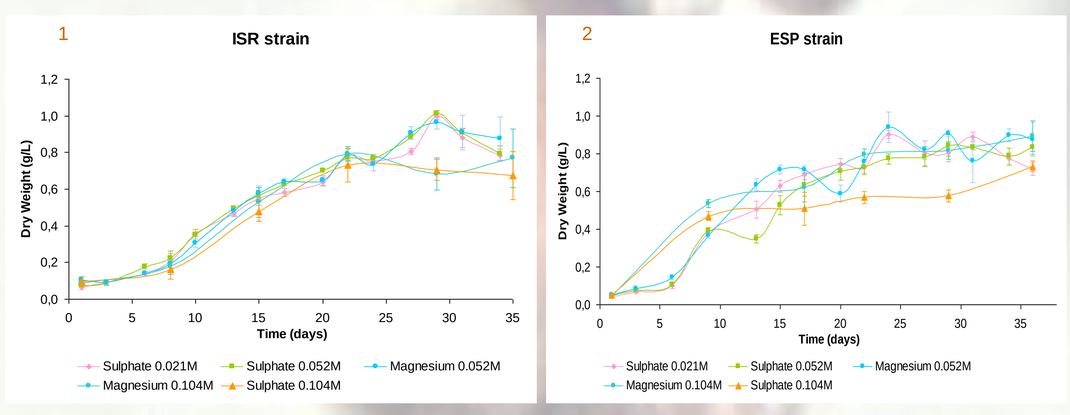

Figure 1 – Growth curves of two strains of *Porphyridium cruentum*, ISR and ESP, in different culture media determined by the dry weight. Each value is the average of three replicates. Two magnesium sulphate and magnesium chloride concentrations were tested 0,052M and 0,104M and compared with a initial concentration of magnesium sulphate 0,021M. 1 – ISR strain; 2 – ESP strain.

Table 1 – Yield of EPS extraction of IRS strain of *Porphyridium cruentum* specie in different culture media determined by EPS dry weight (g) per culture volume (L). Two magnesium sulphate and magnesium chloride concentrations were tested 0,052M and 0,104M and compared with a initial concentration of magnesium sulphate 0,021M.

Culture Media	Concentration (M)	Extraction yield: $W_{EPS}/V_{culture}$ (g/L)
$MgSO_4$	0,021 (control)	0,53
	0,052	0,43
	0,104	0,57
$MgCl_2$	0,052	0,53
	0,104	0,52

Studies of supplementation of the medium with sulphate ion (Figures 1 and 2) showed that the 2,5 fold magnesium sulphate supplementation (0,052M) does not influence significantly the biomass production in the two strains studied here. The five fold magnesium sulphate supplementation (0,104M) presents even a lower value of biomass than all the others concentrations tested.

With respect to the EPS production by *P. cruentum* ISR strain in all media (Table 1) it was lower than in 0,021M magnesium sulphate concentration with one exception: the five fold supplemented medium in which the amount of produced EPS was slightly higher (approximately 1,1 fold). Polysaccharides extraction of ESP strain are being carried out.

Preliminary studies to investigate the structure of the obtained polysaccharides of ISR strain revealed that the supplementations produce slightly different carbohydrate and uronic acid contents and mostly it is associated with an increasing of the sulphate content.

Further studies need to be conducted but the addition of sulphate to the growth medium seems to be a simple and promising approach to improve polysaccharide applications, such as antiviral activity. This can simplify the industrial process because the microalgae seem to incorporate the sulphate and thus any additional chemical reactions after EPS extraction would not be necessary.

Acknowledgments

The authors acknowledge the financial support of project: Desenvolvimento de Aplicações para Polissacarídeos de *Porphyridium* com Actividade Antiviral (nº ADI/2006/M2.3/0032) and Professors S. Arad and J. Fabregas for providing the *P. cruentum* strains.

Bibliography

(1) Spolaore P., Joannis-Cassan C., Duran E. and Isambert A.; 2006; Commercial Application of Microalgae; *Journal of Bioscience and Bioengineering*: 101, 87-96.
(2) Huleihel M., Ishanu V., Tal J. and Arad M. S.; 2002; Activity of *Porphyridium sp.* Polysaccharide against herpes simplex viruses in vitro and in vivo; *Journal of Biochemical and Biophysical methods*: 50, 189-200.
(3) Geresh S., Mamontov A. and Weinstein J.; 2002; Sulfation of the extracellular polysaccharides of red microalgae: preparation, characterization and properties; *Journal of Biochemical and Biophysical Methods*: 50, 179-187.
(4) Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A. and Smith F.; 1956; Colorimetric method for determinations of sugars and related substances; *Analytical Chemistry*: 28, 350-356.
(5) Blumenkrantz N. e Asboe-Hansen G.; 1973; New method for quantitative determination of uronics acids; *Analitical Biochemistry*: 54, 484-489.
(6) Dodgson K. S. and Price R. G.; 1962; A note on the determination of the ester sulphate content of the polysaccharides; *Biochemistry Journal*: 84, 106-110.