BIOSYNTHESIS OF HIGH CONCENTRATIONS OF AN EXOPOLYSACCHARIDE DURING THE CULTIVATION OF THE MICROALGA *BOTRYOCOCUS BRAUNII*

H. L. Fernandes*, M. M. Tomé
Departamento de Energias Renováveis - Laboratório Nacional de Engenharia e Tecnologia Industrial
1699 Lisboa Codex, Portugal

Laboratório de Engenharia Bioquímica - Instituto Superior Técnico
1096 Lisboa Codex, Portugal

SUMMARY

A non-axenic strain of the microalga *Botryococcus braunii* Kützing, isolated from a small lake in Portugal, when cultured at 25°C in mineral medium and under continuous illumination, showed a poor production of hydrocarbons (5% of the dry biomass) but excreted remarkably high quantities of an exopolysaccharide (4.4-4.5g/l) into the medium. The production of soluble polysaccharide with galactose, fucose and uronic acid residues, follows growth. The role of the mucoid contaminating bacteria in polysaccharide production in the mixed culture was unproven.

INTRODUCTION

Photosynthetic microorganisms (microalgae and cyanobacteria) are increasingly considered as potential sources of useful products which range from biomass to fine chemicals and fuels (Aaronson; *et al.*, 1980; Klausner, 1986; Borowitzka and Borowitzka, 1988). So far, the main commercial products obtained from microalgae are proteins from *Spirulina*, *Chlorella* and *Scenedesmus* and β-carotene and glycerol from *Dunaliella* (Richmond, 1986; Tapie and Bernard, 1988). A successful test was undertaken with the mass culture of the exopolysaccharide producer soil microalga *Chlamydomonas mexicana* used as a soil conditioner. An increase in water retention ranging from 2-5% above untreated soils and the reduction of erosion was reported (Kroen and Rayburn, 1984; Metting and Rayburn, 1983). Long chain hydrocarbons, produced in high yields by the colonial green microalga *Botryococcus braunii* (Maxwell *et al.*, 1968), have received considerable attention (Casadevall *et al.*, 1985; Wolf, 1983; Wake and Hillen, 1981) and different varieties with very close morphologies but producing different types of hydrocarbons have been reported (Metzger *et al.*, 1987).

Increasing attention has been received by the exopolysaccharides produced in large quantities by a wide range of microorganisms due to their commercial application as industrial gums and to their participation in pathogenic and symbiotic processes in plants and animals and the general interactions between microorganisms and their environment. The exopolysaccharide from *Porphyridium cruentum* was considered commercially attractive and an economic analysis based on data of polysaccharide productivity and processing, collected over a five month period, was prepared for a plant size of 2 000 metric tons/year (Anderson and Eakin, 1986). Strains of *Botryococcus braunii* were also reported as being exopolysaccharide producers, although with low yields (Allard *et al.*, 1987; Casadevall *et al.*, 1985). The biosynthesis of extracellular polysaccharides by microorganisms, mainly of the economically important gums produced by bacteria (Sandford and Baird, 1983), is at present a subject of very active research.

During a screening for microalga strains capable of producing new and interesting exopolysaccharides, a fresh-water strain of *Botryococcus braunii* Kützing isolated from a small lake in Portugal was selected for further study. Like other microalgae isolated from natural environments, this strain was associated with bacteria. Two of its most frequent contaminating bacteria strains were...
mucoid and also produced exocellular polysaccharides. There is evidence that bacteria exert considerable influence, either antagonistic or beneficial, on algal growth and metabolites production. The relationships occurring in algal-bacterial systems depend on the species involved and on culture conditions (Bell, 1983; DeLucca and McCracken, 1977; Chirac et al., 1985; Weimann, 1970). Bacteria may obtain nutritional benefit from the organic carbon compounds photosynthetically derived and released by algae and can also stimulate algal growth by the release of CO₂, vitamins, assimilable nitrogen derivatives, inorganic nutrients or by positively influencing the pH. Bacteria may compete with algal growth for limiting nutrients and by the release of toxins. In the present work, the potential of the strain Botryococcus braunii UC 58 as an exopolysaccharide producer and the involvement of the contaminating bacteria in the process is appreciated.

MATERIALS AND METHODS

Microalgae strains - Botryococcus braunii Kützing UC 58 from the Culture Collection of Algae of the Department of Botany of the University of Coimbra, Portugal, was used. This strain was isolated from a small lake and was non-axenic. For comparison, the axenic Botryococcus braunii strain UTEX 572 from the Austin University Culture Collection, a good producer of hydrocarbons, was also used.

Culture conditions - The cells were batch cultured in Chu 13 modified medium (Largeau et al., 1980) in 1 l cylindrical glass vessels (φ = 6.5 cm) at 25°C ± 0.1°C, under sterile conditions. Cultures were continuously illuminated with fluorescent cool-white lamps (250 µE m⁻² s⁻¹). The medium was aerated (12 l/h) with filter sterilised air containing 1% CO₂. Inoculations were carried out with dense, one week old, precultures (initial algal concentration: 0.25 g dry biomass/l).

Analysis - During growth, 40 ml samples were withdrawn aseptically. Algal growth was followed based on the dry weight. Cell samples were diluted in 50 ml of acidified distilled water (pH = 3) to avoid the interference of the polysaccharide bound fraction, filtered through a preweighed AP 25 Millipore filter and dried overnight at 80°C. Growth of the contaminating bacteria was followed by plating 0.1 ml samples on agar plates with LB (Gibco) + 1% glucose incubated at 30°C for a minimum of 5 days. The hydrocarbon content (external pool) was analysed on hexane extracts of 10-20 ml samples of algal culture dried under Vacuum (Largeau et al., 1980) by GL Chromatography in a Varian 3300 Chromatograph equipped with a column (2 m x 1/8” packed with 5% OV-101 on 100/120 Chromosorb W-AW) and a ionisation detector. The temperatures of the injector and the detector were 300 and 330°C, respectively, and the oven was operated isothermally at 280°C, n-dotriacontane being used as the internal standard. For the quantification of the extracellular polysaccharide (EPS P₁) produced during growth the phenol-sulfuric method (Dubois et al., 1956) was used (using the purified exopolysaccharide EPS P₁ under study, as standard). This method was used on samples obtained after EPS P₁ precipitation, with 2 volumes of 2-propanol from the growth medium free of cells either by centrifugation or by filtration through a GF/B Whatman filter, when the samples were too viscous. In addition, the dry weight of the alcohol precipitable material from the supernatant was also estimated. The values reported for the EPS concentration represent a medium value obtained by the two methods. For the chemical characterization of the EPS P₁ 15 mg were dissolved in 5 ml of N H₂SO₄ and hydrolyzed at 100°C for 18 h, and neutralized with saturated barium hydroxide solution. The monosaccharides were fractioned on a column of Amberlite AG 1-X2 (formate) resin. The neutral and the acidic fractions were obtained after elution with water and N formic acid respectively (Allard et al., 1987). Neutral sugars were separated by HPLC, using a Perkin Elmer Series 10 Liquid Chromatograph with a LC-25 RI Detector and a LCI-100 Laboratory Integrator equipped with a HPX 87H column and using 0.01N sulphuric acid as mobile phase (Kennedy and Sutherland, 1987). Carbazole method was used to search for the presence of uronic acid residues (Knutson and Jeanes, 1968). The relative proportion of neutral sugars was determined on the basis of peak height using a calibration with maltose as internal standard. For the studies of the bacteria-alga interaction cultures of B. braunii UTEX 572 were infected with the bacteria associated with B. braunii UC 58 strain, present in the filtrate obtained by filtration through a 8 µm pore size Sartorius membrane filter.

RESULTS AND DISCUSSION

The air-lift batch growth of B. braunii UC 58 in modified CHU 13 medium (25°C continuous illumination) began with no lag phase after inoculation with an exponential population (fig. 1). The