Glucose metabolism and polysaccharide accumulation in the marine bacterium, *Shewanella colwelliana*

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*Shewanella colwelliana*, a marine bacterium isolated in association with the oyster *Crassostrea virginica*, produces an abundant exopolysaccharide with potential commercial value as an adhesive under aqueous conditions. Its utilization of glucose was modulated by stoichiometric concentrations of yeast extract. In Brain Heart Infusion medium containing glucose, growth was diauxic with delayed glucose utilization and incorporation into exopolysaccharide. Data from radio-respirometry protocols indicate that glucose is catabolized through a combination of the hexose monophosphate and Entner-Doudoroff pathways. Exopolysaccharide production could be significantly enhanced by adjusting glucose concentrations of the growth medium.

**Key words:** Exopolysaccharide, glucose metabolism, marine bacterium, marine biotechnology, radio-respirometry, *Shewanella colwelliana*.

Secondary metabolites, including exopolysaccharides, antibiotics, surfactants and flocculants, produced by marine bacteria, microalgae and other microorganisms, have economic potential (Arad et al. 1985; Austin 1988). Marine microbial exopolysaccharides have multi-faceted industrial applications (Geesev 1982; Sandford 1985). Some exhibit anti-tumour and anti-microbial activities (Okami 1986), whereas others act as bio-attracants for invertebrates (Abu et al. 1986; Weiner et al. 1989) or as adhesives (Labare et al. 1989; Abu et al. 1991). Polymers produced by marine bacteria and microalgae offer several advantages compared with those derived from marine macroalgae. For example, the bacteria and microalgae more readily adapt to laboratory culture conditions and usually grow more rapidly than macroalgae.

Unfortunately, cultivation of marine microorganisms may not always be inexpensive or simple. The use of seawater or artificial seawater for growth of marine microorganisms can be an economical approach (Arad et al. 1985), especially if the marine microorganism is capable of utilizing a simple and inexpensive growth substrate, such as glucose. Obviously, glucose is readily available in abundance from such sources as starch and cellulose. In the present study, a marine bacterium, found to produce an exopolysaccharide with potential commercial application, was studied for its ability to metabolize glucose under conditions which enhanced exopolysaccharide production.

**Materials and Methods**

**Organism and Culture Conditions**

*Shewanella colwelliana* was originally isolated from a spat tank at the University of Delaware Mariculture Laboratories, Lewes, Delaware, USA, where it grew in association with the eastern oyster, *Crassostrea virginica*. The characteristics of the exopolysaccharide which it produces have already been described (Abu et al. 1991).

For general measurements of growth and glucose utilization in a complex medium, Brain Heart Infusion broth (BHI; Difco), supplemented with 0.2% (11.1 mM) glucose, was amended with 2.5% (w/v) NaCl and inoculated with a suspension of washed cells of *S. colwelliana* prepared from a logarithmic-phase culture (16 to 18 h). Cultures were aerobically incubated at 25°C with shaking at 150 rev/min. At intervals, samples were removed and centrifuged (10,000 × g for 10 min at 4°C) to harvest the cells. The concentration of glucose in the supernatant was measured using an immobilized glucose oxidase electrode in a glucose analyser.
were harvested and washed twice with PBS. Fresh HI was amended with Heart Infusion (HI) Broth (Difco) for 16 h at 150 rev/min. Cells overlaid with oil, was interpreted as a fermentative reaction. A yellow appearance indicated acid production and, in the tube without addition of oil. A growth (Figure 2). In the presence of 0.01% (w/v) yeast extract, growth and glucose removal depended, at least partly, on biosynthetic processes in many microorganisms (Dawes 1986). The removal of glucose from complex medium (BHI) was detected, using the glucose analyser, at about 48 h, corresponding to the late phases of growth or state of secondary metabolism (Figure 1). Growth in BHI was accompanied by increased viscosity of the spent medium (Figure 1), indicating exopolymer synthesis (Corpe 1970; Norberg & Enfors 1982; Sutherland 1983). In semi-synthetic medium, growth and glucose utilization were similar to that in minimal salts (described above), with 5 to 10 mM glucose and 0.1% (w/v) yeast extract. Both [1-14C] glucose (sp. act. 55 mCi/mM) (ICN Radiochemicals) and [3, 4-14C] glucose (sp. act. 10.3 mCi/mM) (New England Nuclear) were used at no less than 106 c.p.m./µmol glucose.

Experiments were carried out using radiorespirometers, constructed according to the method of Dobrogosz (1981), inoculated with 1 ml cell suspension (108 cells) and placed in a rocking Psychrotherm water bath (25°C). Formaldehyde-killed cells were used as controls and did not release CO2. Aliquots of the reaction mixture were counted at time zero to determine total activity. The rate of glucose mineralization was monitored by trapping and counting 14CO2 released by respiring cells in a mixture of ethanolamine/ethyl alcohol (1:2, v/v). Samples, diluted with ethanol, were counted as previously described. Background quenching was calculated by counting radioactivity of the trapping agent. The pattern of 14CO2 evolution was analysed using a SAS computer program (SAS, Cary, NC).

**Results and Discussion**

Glucose is a preferred substrate for both metabolism and biosynthetic production in many microorganisms (Dawes 1986). The removal of glucose from complex medium (BHI) was detected, using the glucose analyser, at about 48 h, corresponding to the late phases of growth or state of secondary metabolism (Figure 1). Growth in BHI was accompanied by increased viscosity of the spent medium (Figure 1), indicating exopolymer synthesis (Corpe 1970; Norberg & Enfors 1982; Sutherland 1983). In semi-synthetic medium, growth and glucose removal depended, at least partly, on the concentration of yeast extract. With 0.1% (w/v) yeast extract, growth and glucose removal were similar to that observed in BHI broth, i.e. an initial growth lag phase and depletion of medium glucose and a corresponding increase in growth (Figure 2). In the presence of 0.01% (w/v) yeast extract, the lag period was much shorter and, commensurately, glucose was removed from the medium more rapidly (Figure 3). However, the total amount of glucose removed (8%) was less than that when 0.1% yeast extract was used.