Biotechnol. Bioprocess Eng. **2001**, 6: 359-362

Effect of Aeration Rates on Production of Extracellular Polysaccharide, EPS-R, by Marine Bacterium *Hahella chejuensis*

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Abstract The production of an extracellular polysaccharide, EPS-R, from the marine bacterium *Hahella chejuensis* was investigated at various aeration rates in a batch culture. Higher aeration rate resulted in enhanced EPS production and increased the viscosity of the culture broth. At an aeration rate of 1.5 vvm, EPS-R (12.2 g/L) was obtained with a yield ($Y_{P/S}$) of 0.6 from the STN medium after 72 h of cultivation. The *H. chejuensis* cells changed their rod morphology to a short-rod form in the stationary growth phase.

Keywords: aeration rate, extracellular polysaccharide, *Hahella chejuensis*, marine bacterium

INTRODUCTION

Many extracellular polysaccharides (EPS) produced by microorganisms have been studied and are currently used in a wide range of industries due to their functions such as gel formation, emulsifying, film formation, and antitumor activity [1-5]. There have been many reports on physiological conditions, such as culture medium, pH, and agitation speed for enhancing the production of EPS [6-15]. The effect of aeration rates has been studied to increase the production of EPSs [16,17] and there have also been many attempts to enhance the production of EPS using agitation designs and new types of bioreactors [18,19].

Hahella chejuensis, isolated from Cheju Island, Korea, produces a novel EPS, designated as EPS-R [20]. In a previous study, a complex medium was defined for the production of EPS-R from *H. chejuensis*. As such, high production of EPS-R was obtained when glucose and tryptone were used as the carbon and nitrogen sources, respectively [21]. However, the effect of aeration on cell growth and production of EPS was not examined.

Accordingly, this study describes the effect of the aeration rate on the production of EPS-R by the marine bacterium *H. chejuensis* . Electron microscopic observation of the EPS-R production from the strain is also presented.

MATERIALS AND METHODS

Microorganism and Culture Conditions for Production of EPS-R

H. chejuensis was cultured in an STN medium (20 g sucrose, 10 g tryptone, 10 g NaCl, 5 g, $MgSO_4$, 1 g $CaCl_2$, 83 mg KH_2PO_4, 67 mg K_2HPO_4, 5 mg $FeCl_2$, 1 mg $MnCl_2$, 1 mg $ZnCl_2$, 1 mg $NaMoO_4$, per liter, pH 7.0). The cultivation was carried out in 3 L of the STN medium at 25°C and 300 rpm, using a 5-L jar fermentor.

Separation of Cell and EPS-R from Culture Broth

Due to the high viscosity of the culture broth, it (100 mL) was mixed with 0.1 g of dried diatomaceous earth (Sigma, St. Louis, USA) and 100 mL of distilled water (DW). After centrifugation (10,000 × *g*, 4°C, 20 min), the precipitate was washed with DW, dried at 80°C for 3 days, and then used for determining the dry cell weight. The pigment produced by the strain during the growth was removed from the cell free culture broth as follows. Two volumes of methanol and chloroform were added to the cell-free culture broth, and then the chloroform phase was discarded using a separating funnel. Two volumes of ethanol were added to the remaining mixture, which was then mixed vigorously and allowed to rest at 4°C overnight. The precipitate obtained by centrifugation (10,000 × *g*, 4°C, 20 min) was used as crude EPS-R after drying at 80°C for 3 days.

Analytical Methods

The amount of sucrose in the culture broth was measured by the anthrone-sulfuric acid method [22]. The apparent viscosity of the culture broth was estimated using a Vicostar-R (J. P. Selecta, Spain) fitted with a No. 2 spindle at 25°C.

Scanning Electron Microscopy

The microorganism was cultured in the STN medium at 25°C, 300 rpm, and 1.5 vvm. The culture broth was collected during the lag, exponential, and stationary

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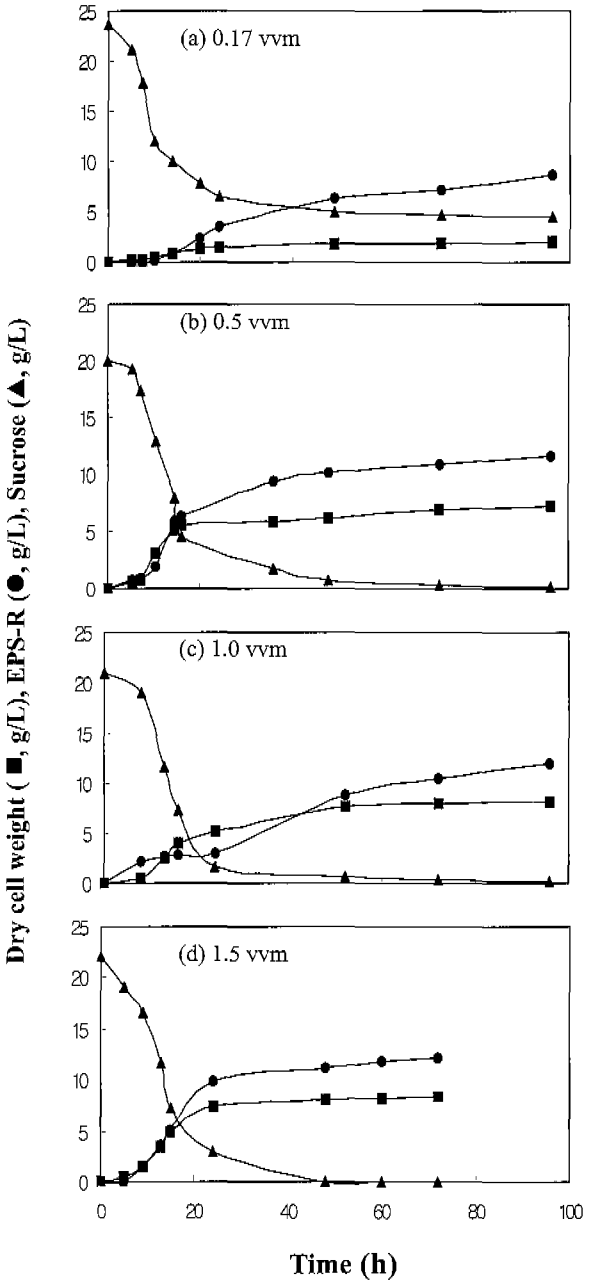

Fig. 1. Profiles of the EPS-R production, growth, and sucrose consume by *H. chejuensis* during the cultures in a 5-L fermentor under various aeration rates. A, 0.17 vvm; B, 0.5 vvm; C, 1.0 vvm; D, 1.5 vvm.

phases, respectively, and subsequently fixed with glutaldehyde (2%, v/v). After lyophilizing for 3 days, the samples were used in a scanning electron microscopy (Stereo Model 260 Cambridge, England).

RESULTS AND DISCUSSION

The EPS-R production by *H. chejuensis* was investigated with aeration rates of 0.17, 0.5, 1.0, and 1.5 vvm. As the aeration rate increased the cell growth and the production of EPS-R increased. However, no significant differences in the growth and EPS-R production were found with aeration rates of 1.0 and 1.5 vvm, and a maximal cell dry weight and EPS-R production of about 8 g/L and 12 g/L, respectively. These values were reached faster with an aeration rate of 1.5 vvm than with 1.0 vvm. The cultivation with the aeration rate of 1.5 vvm was stopped after 72 hours as the culture broth had become highly viscous and the air could not disperse well in the jar. An improved production of EPS-R by *H. chejuensis* seemed to be caused by an increase in the mass of the cells producing EPS-R under higher aeration rates. The positive effect of improved oxygen supplementation was also previously observed in the production of pullulan by *Aurobasidium pullulans* [23,24], curdlan by *Alicaligenes faecalis* [25], and EPS by *Ganoderma lucidum* [17], whereas the production of schyzophyllan by *Schyzophyllum commune* and scleroglucan by *Sclreotium glucanicum* increased under a limitation of dissolved oxygen [26]. By increasing the aeration rate, the apparent viscosity of the culture broth increased (Fig. 2). This result appeared to be due to the increased EPS-R production. A similar result was observed in the case of *Klebsiella* sp. where the apparent viscosity of the culture broth increases with the production of EPS BS-1 from *Klebsiella* sp. under improved aeration [16]. The yield ($Y_{P/S}$) of EPS-R was 0.60 from the cultivation with an aeration rate of 1.5 vvm. The yield of EPS-R was 2 to 3 times higher than the yield of EPSs when compared with other bacteria utilizing sucrose for producing EPSs. The yield of EPS from *Azotobacter vinellandii* MTCC 2460 is 0.3 when the strain was cultured in a medium containing 10 g/L of sucrose [7]. *Lactobacillus* sp. LB180 converts 100 g/L of sucrose in a medium to 20 g/L of EPS [13]. Xanthan production is not affected by aeration rates but also by agitation speed during fermentation [27]. Yang *et al.* [18] designed a novel, cetrifugal, fibrous-

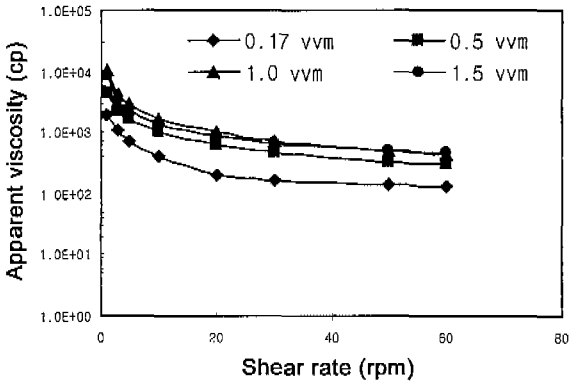

Fig. 2. The apparent viscosity of the culture broth obtained from the cultures of *H. chejuensis* at various aeration rates. The samples were collected after 96 h in the cases of 0.17, 0.5, and 1.0 vvm and the culture broth from the culture at 1.5 vvm of the aeration rate was obtained after 72 h.

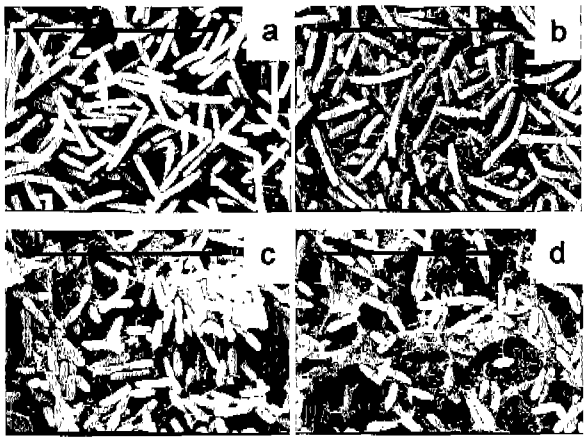

Fig. 3. The scanning electron microscopes of *H. chejuensis* grown in the STN medium at 25°C, 300 rpm, and 1.5 vvm. The bars indicate 10 μm. a, 10 h; b, 16 h; c, 41 h; d, 69 h.

bed bioreactor to produce xanthan by *Xanthomonas campestris* and they achieved higher production by improving the oxygen transfer relative to changing the agitation speed in the new bioreactor [18]. Similarly, more EPS-R was produced from *H. chejuensis* by controlling the agitation speed during the culture.

The production of EPS-R in the STN medium by *H. chejuensis* was observed relative to the incubation time by scanning electron microscopy (Fig. 3). The cells were cultured at 1.5 vvm (Fig. 1d). After a short culture time and during the exponential phages, only rod-shaped cells were observed (Fig. 3a and b). On the other hand, cells of short-rod form were observed in the stationary phase (Fig. 3c and d). As such, production of EPS-R must be a reason for this morphological change after the growth of the strain. The morphological change involved in the production of EPS was also observed in the case of *Vibrio cholerae*. *V. cholerae* O1 strain TSI-4/R, which is coccoid and produces EPS, builds a biofilm during the growth in a liquid medium. The cells in the biofilm are rod-shaped. However, the cells of *V. cholerae* O1 strain TSI-4/T, which does not produce EPS, remains as coccoid [28].

Fibrous materials were secreted from the cells of *H. chejuensis* during the exponential growth phase. The amorphous materials increased relative to the culture time. During the stationary phase, the accumulation of materials outside the cells was clearly seen in the scanning electron microscopes, although the amount of EPS-R did not increase (Fig. 1c). Accordingly, it would seem that the cells changed their morphology during the stationary phase, thereby resulting the secretion of EPS-R into the medium.

Acknowledgments This study was supported by 'Life Phenomena & Function Research Project' of the Korean Ministry of Science and Technology and by 'Development of Marine Novel Compounds Program' of the Korean Ministry of Maritime Affairs and Fisheries.

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[Received April 6, 2001; accepted September 10, 2001]