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CULTIVATION OF THE RED MICROALGA *PORPHYRIDIUM CRUENTUM* UNDER NATURAL OUTDOOR CONDITIONS

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Growth and production of extracellular polysaccharides (EPs) by the unicellular red alga *Porphyridium cruentum* were studied in open vessels of different depths under outdoor natural climatic conditions of Pakistan and compared with cultures grown under indoor controlled conditions. In 2 cm deep cultures both growth and EPs production were found to be higher in indoor cultures than the outdoor cultures whereas in 8 cm deep cultures, both growth and EPs production were found to be higher in outdoor cultures. In this case, an increase of 58% in biomass and 163% in EPs production was observed in the outdoor cultures as compared to indoor cultures. During outdoor cultures, the depth of 8 cm was noted to be most optimum for both biomass and EPs production. Also, polysaccharides produced under outdoor conditions compared well with a number of currently used biopolymers regarding rheological properties.

Key words: Outdoor cultivation, *Porphyridium cruentum,* Extracellular polysaccharides.

Introduction

Numerous species of microalgae are known to yield significant amounts of valuable biochemicals that have potential for use in food, feed and pharmaceutical industries (Cohen 1986; Borowitzka and Borowitzka 1988; Chapman and Gellenbeck 1989). Of special interest is the unicellular red alga *Porphyridium cruentum* which produces a variety of compounds of commercial interest *viz.,* polysaccharides (Gudin and Thomas 1981; Walwyn 1992; Iqbal and Zafar 1993a&b), polyunsaturated fatty acids (Ahern *et al* 1983) and proteinaceous pigments (Gantt and Lipschultz 1972; Iqbal and Zafar 1995).

The basic inputs for microalgal mass culture are sunlight, water, CO_2, NP and some micro-nutrients. The use of artificial light is expensive and the rationale for its use cannot be made in most cases, even for high value products where economics is not the overwhelming factor. Many researchers have developed "closed" system, consisting of plastic or glass covered ponds or tubular or flat-sided reactors (Juttner 1977; Pirt *et al* 1983; Gudin and Thepenier 1986; Iqbal *et al* 1993a&b). Such methods, however, often result in prohibitive inputs rendering algal culture cost-ineffective. Economics of production is, therefore, the major limiting factor in large-scale cultivation of algae for any commercial application. Microalgal cultivation in an "open" outdoor system, on the other hand, can help to appreciably reduce energy-linked culture inputs and thus also the cost of production. With this objective in view, the present study reports the cultivation of *P. cruentum* in outdoor natural conditions and compares it in respect of extracellular polysaccharides (EPs) and biomass yield to that under artificial light and temperature conditions. Advantages likely to accrue from such an outdoor culture system are discussed.

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Materials and Methods

Microalga and growth. The red alga used in this study, *Porphyridium cruentum* strain IAM-R-I, was obtained from the Culture Collection of Algae and Microorganisms, Institute of Applied Microbiology, University of Tokyo, Japan. Cultures were maintained axenically in 250 ml Erlenmeyer flasks containing artificial sea water (ASW) medium (Jones *et al* 1963) at photon flux density of 50 $uEm^{-2}s^{-1}$ and temperature of 25±2°C.

Culture conditions. Both outdoor and indoor cultivation was done in white plastic tubs having exposed surface area of 28 cm^2 containing 1.25 and 5 litre of ASW culture medium, and having depths of 2 and 8 cm respectively plastic tubs having exposed surface area of 35 cm^2 were also used to investigate the effect of culture depth on the biomass and EPs production. For this purpose, tubs were filled with culture medium to 4, 8, 10 and 12 cm depth. Each culture tub was inoculated with uniform inoculum (approx. $1x10^6$ cells ml^{-1}) taken from two-week old cultures in exponential phase of growth. Indoor cultures were incubated under 24 h continuous illumination at a photon flux density of 50 $\mu Em^{-2}s^{-1}$ and temperature of 25±2°C. Outdoor cultures were simultaneously placed in natural environmental conditions under shadow to protect them from direct sun light and rain. Day light on the surface of the vessels during this period varied from 100 to 500 $\mu Em^{-2}s^{-1}$. Daily

maximum/minimum temperature, day/night length and precipitation during the experimental period are shown in Fig 1. Both inside and outside cultures were manually agitated twice a day.

Analytical procedures. Growth of *P. cruentum* indoor and outdoor was monitored by cell count in a WSI counting chamber (Weber Scientific International Ltd., Lancing, England) as well as by spectrophotometric measurement of absorbance at 760 nm. For dry weight determinations, 30 ml algal culture aliquots were taken from culture troughs and centrifuged for 15 min at 20,000 xg. The pellet was subsequently washed with distilled water to remove ions and bound polysaccharides from the algal cells. The process of centrifugation was repeated and cells were dried at 80°C for 48h and weighed. Viscosity of the cell-free medium and EPs solution was measured by Contraves Rheomat 115 (Contraves AG, Schaffauserstrasse 580, Zurich, Switzerland). The level of polysaccharides in the medium was measured by Alcian blue reagent (Ramus 1977), using carrageneen as the standard. For the measurement of dissolved polysaccharides (DPs), cells were removed from the medium by centrifugation, whereas for cell-wall bound polysaccharides (BPs) centrifuged cell pellet was suspended in known volume of distilled water and autoclaved for 10 min at 121°C followed by centrifugation. The supernatant was used to measure the content of BPs. The sugar component of polysaccharides was determined by the method of Albersheim *et al* 1967.

Results and Discussion

Investigations on microalgal cultivation, both under indoor controlled and outdoor natural conditions, were carried out in March and April, 1995. The average day length and the maximum/minimum temperature during the study period, respectively, was 12.29 h and 302±0.55/17.7±0.47°C (Fig 1).

Growth and EPs production by *P. cruentum* was determined under indoor and outdoor conditions in 2 cm and 8 cm deep cultures, respectively containing 1.25 and 5 litres of medium. The studies were continued for 27 days.

In the 2 cm deep cultures, the maximum cell number was found to be 30.05 x 10^9 cells l^{-1} in indoor and 21.9 x 10^9 cells l^{-1} in outdoor cultivation conditions (Fig 2a). The EPs production by cells grown indoor, was 586 mg l^{-1} while it was 311 mg l^{-1} under outdoor natural conditions (Fig 2b). A comparison of the two culture conditions indicates a 27% decline in biomass and 47% in EPs production in outdoor cultures as compared to those grown indoor. Low productivity of both biomass and EPs in the 2 cm deep cultures under outdoor natural conditions may be related to the high incidence of light. It is known that high light intensity has a growth inhibition effect on algal cultures (Iqbal and Zafar 1993a&b).

In the 8 cm deep cultures, on the other hand, biomass and EPs production in indoor cultures were 19.78 x 10^9 cells l^{-1} and 216 mg l^{-1} respectively, as compared to 31.36 x 10^9 l^{-1}cells and 570 mg l^{-1} EPs in outdoor cultures (Fig 3a & b). This indicates an increase of 58% in biomass and 163% in EPs production in outdoor cultures in comparison to those grown indoor. These yields are also significantly higher than those obtained in the 2 cm deep outdoor cultures (p=0.5 Duncan's multiple range test). The major factor attributable to this higher rate of productivity may be the availability of light to cells, which appears to be a function of culture depth. evidently, the intensity of light available to cells depends on the depth of

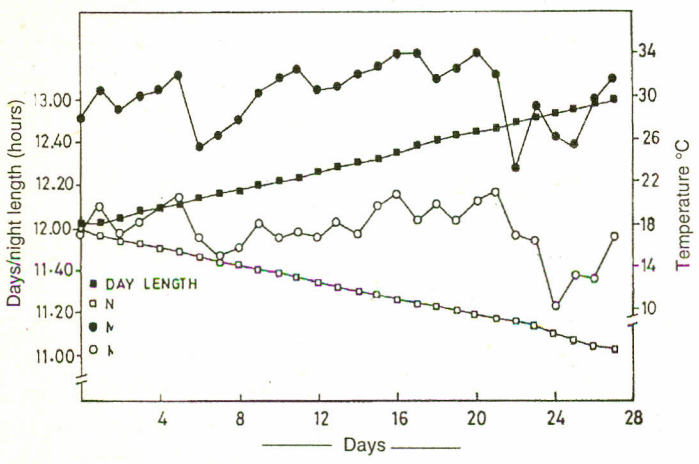

Fig 1. Day (■) and night (□) length and maximum (●) and minimum (○) temperature at Lahore, Pakistan (31°35'N, 74°18'E) in March and April, 1995.

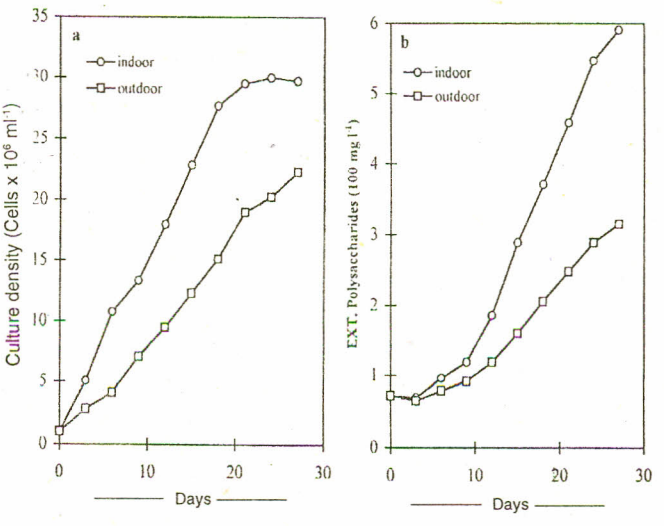

Fig 2. Growth (a) and extracellular polysaccharide production (b) by *Porphyridium cruentum* cultures grown in 2 cm deep plastic pots under indoor artificial and outdoor natural conditions.

culture indicating that the level of incident light intensity was better suited for 8 cm culture conditions than for those in the 2 cm outdoor cultures. Reports in literature are already available on the different light intensity optima reported to range from 15 μ E m^{-2} s^{-1} (Arad *et al* 1985) 60μE m^{-2} s^{-1}(Minkova *et al* 1987) to 75 μE m^{-2} s^{-1} (Iqbal and Zafar 1993 a&b) for the maximum growth and EPs production by *P. cruentum*. The wide variation in these optimum levels of light intensity for the same alga may be due to the differences in culture conditions wherein these studies were carried out. With the increase of culture depth the availability of light to cells present at the bottom of the vessel has been known to become limiting (Vonshak and Richmond 1985). This is also likely to be true under the experimental conditions reported here. Light intensity of 50μE m^{-2} s^{-1} is uptimum only for the cultures growing in 2 cm depth. However, when the culture depth increased to 8 cm the same level of light for indoor cultivation became limiting thus resulting in decreased biomass and polysaccharide productivity. On the other hand, cells grown outdoor under natural climatic cnditions received high solar energy capable of penetrating deeper into the cultures. It is of further interest to point out that no significant difference was noted in the bio-mass and EPs yields of 2 cm indoor cultures and in 8 cm outdoor cultures. It seems the conditions available to the 2 cm indoor cultures are comparable to those of 8 cm outdoor cultures thus eliminating the need to use artificial light resulting in a saving on the cost of energy input necessary for indoor algal cultivation.

In order to determine the optimum depth for the algal culture, outdoor cultivation of *P. cruentum* was done of 27 days under natural climatic conditions in culture vessels 4, 8, 10 and 12 cm deep. A significant difference, both in growth and EPs production, under different culture depths was observed (Fig 4a & b). It was, however, noted that maximum growth and EPs production occurred in 8 cm deep tubs. At this depth, the cell number and the level of EPs increased by 19% and 40% respectively, as compared to the cells grown at 4 cm depth. Beyond 8 cm, however, increase in culture depth showed an inhibitory effect which resulted, respectively, in 10% and 25% reduction in growth, and 5% and 29% decrease to EPs production in cultures grown at 10ml and 12 cm depth. These observations support the above conclusion of the direct relationship between the incident light and the depth to which it can optimally penetrate. It may be concluded that as the culture depth increased the light availability beyond 8 cm decreased progressively thus also resulting in reduced culture growth in the deeper sections of the culture vessels.

Previous studies on *P. cruentum* polysaccharides (Anderson and Eakin 1985, Cohen and Arad 1989; Cohen *et al* 1991) reveal that yield of BPs is much higher than that of DPs. It seems to be also true under the present investigation. In the 8 cm deep cultures in outdoor culture conditions the amount of total polysaccharides (DPs + BPs) after 27 days of growth was 3105 mg l^{-1} comprising 2454 mg l^{-1} and 651 mg l^{-1} as BPs and DPs, respectively (Fig 5 a&b).

Rheological studies on the *P. cruentum* polysaccharides are important in view of their possible uses in the food as well as pharmaceutical industries. It also provides indirect information on the quality of the polysaccharides as any chemical or structural change in the polysaccharides composition may alter viscosity of the polysaccharides. Such information is

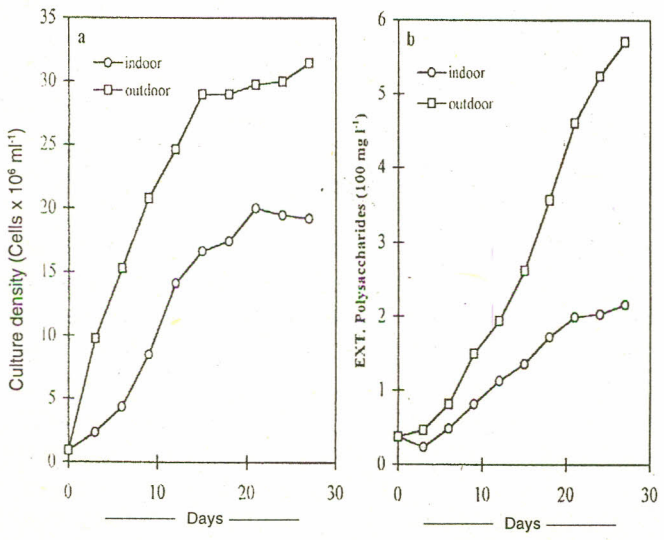

Fig 3. Growth (a) and extracellular polysaccharide production (b) by *Porphyridium cruentum* cultures grown in 8 cm deep plastic pots under indoor artificial and outdoor natural conditions.

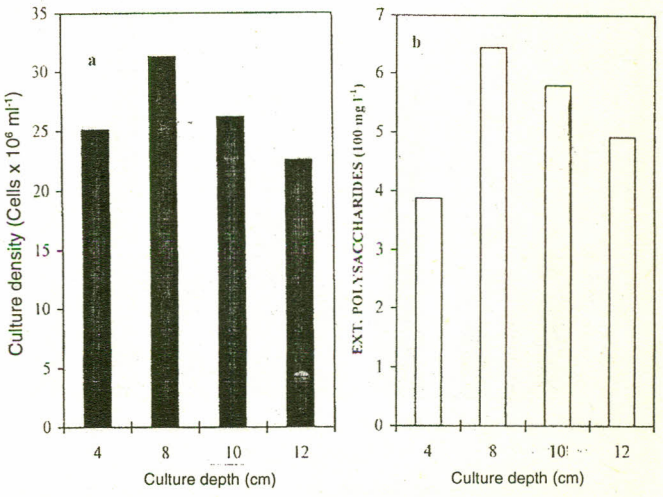

Fig 4. Effect of culture depth on growth (a) and extracellular polysaccharide production (b) by *Porphyridium cruentum* cultures grown under outdoor natural conditions.

further important since some bacterial and algal contaminations have been reported that may change the quality of the polysaccharides by using any available sugar (Jones 1982; Iqbal *et al* 1993 a&b). For the measurements of viscosity, the EPs were dissolved in a known volume of distilled water. No significant difference was observed in the viscosity of polysaccharides produced under indoor controlled or outdoor natural conditions (Fig 6). This suggests that there occurred no change in the rheological behaviour of polysaccharides when the alga was cultured under the two different conditions. This was further supported by the analysis of hydrolysates of the two polysaccharides that showed no major difference in their sugar composition (Table 1). For the purpose of determining the quality of the *P. cruentum* polysaccharides, their viscosities were compared with those of the other commercially available biopolymers such as kappa-carrageenan, lambda-carrageenan and xanthan gum. At 25°C the viscosity of the *P. cruentum* polysaccharides throughout the selected range of concentrations was considerably higher than all the commercial biopolymers investigated (Fig 6). These observations are also in agreement with Savins (1978) who in his patent has reported superiority of the *P.aerugineum* polysaccharides over xanthan gum.

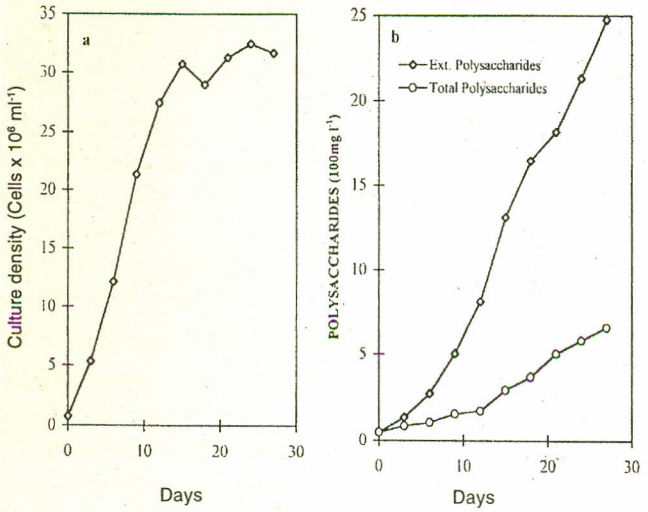

Fig 5. Growth (a) and extracellular polysaccharide production (b) by *Porphyridium cruentum* cultures grown in 8 cm deep plastic pots under outdoor natural conditions.

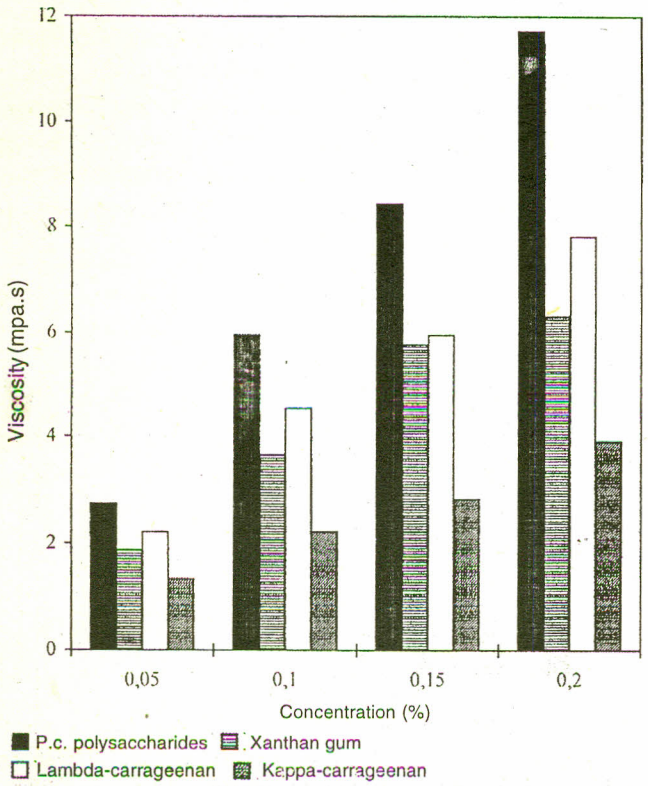

Fig 6. Viscosities of the polysaccharides from *Porphyridium cruentum* (P.c) cultures and commercially available biopolymers.

Table 1
Composition of extracellular polysaccharide of *P. cruentum* grown under indoor and outdoor culture conditions.

Culture Conditons	Sugars*		
	Rhamnose	Xylose	Glucose + Galactose
Indoor	18.47±1.6	35.44±1.2	46.09±0.8
Outdoor	19.35±1.4	36.06±1.4	44.49±1.7

*Amounts of sugars are presented as a percentage of total sugars.

Conclusion

The observations reported in the present study indicate the potential of *P. cruentum* as an EP producer under uncontrolled outdoor natural environmental conditions. Comparative studies under indoor controlled and outdoor natural conditions reveal the possibility of production of the biopolymer under natural conditions in a cheaper manner than under indoor artificial conditions. The quality of the polysaccharides produced under outdoor conditions was also found to be similar to that obtained under indoor conditions (Table 1). Large areas in Pakistan are beset with the twin problem of salinity/aridity and waterlogging. This results in denudation of soils and thus further deterioration. Microalgae, especially *P. cruentum,* have the abiiity to utilize high salinity waters (Sommerfeld and Nichols 1970) that are otherwise unsuitable for conventional agriculture. Therefore, saline water bodies in waterlogged areas of Pakistan could be used for cultivation of the red alga *P. cruentum* for the production of polysaccharides and other biochemicals such as arachidonic acid and the red pigments phycocyanin and phycoerythrin.

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