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SCREENING AND ISOLATION OF EPS PRODUCING MARINE BACTERIA AND OPTIMIZATION OF EPS PRODUCTION

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ABSTRACT

The work was intended to screen and isolate EPS secreting bacterial strains from marine water samples. A total of twelve bacterial strains were isolated from collected water samples. These strains were screened for the secretion of EPS. Five bacterial strains showed EPS production. The effect of temperature, pH and incubation time on EPS production was identified for both protein content of EPS as well as carbohydrate content of EPS. EPS production was optimized under two different environmental condition i.e., pH (acidic neutral and alkaline (4, 7and 9) and temperature (20°C and 50°C) and at three different incubation times (24 hr, 72 hr and 120 hours). Quantification of protein was done by standard Lowry's method. The production of EPS with respect to protein content was optimized in different temperature, pH and duration of incubation. All the strains produced proteins in EPS at highest quantities at 20°C and acidic as well as alkaline pH resulted in high protein content in EPS. Tb3 showed a sharp increase in the secretion of proteins in the EPS at 120 hours. Both at high temperature as well as at low temperature, the carbohydrate content was higher. Of all the strains, strain Tb1 was found to produce high amount of carbohydrate. Alkaline pH was found to be optimum for the heightened production of carbohydrate in EPS. In the case of effect of period of incubation the carbohydrate content increased from 24 hr to 72 hours. In strain Tb3 alone carbohydrate

content of EPS increased considerably as incubation period increased. The protein content of this strain also showed a similar pattern.

KEY WORDS: Exopolysaccharides, EPS, optimization, temperature, pH, Incubation period.

INTRODUCTION

Exopolysaccharides (EPS) are a group of polymers which are of high molecular weight which forms a substantial composition of extracellular polymers of microbial cell walls. Most of the microbial cells of the marine environment produce EPS on cell surfaces. These are organic molecules with polysaccharide as the most abundant component (40% - 95%).[1] Since these form a large fraction of reduced carbon reservoir of marine environment, it is thought to have a significant effect on climatic regulation. It also influences the physico-chemical environment around the bacterial cells.[2] It also form a medium for intercellular communication. The various components of EPS include carbohydrates, proteins, and humic substances.[3] The EPS has a range of carbohydrate and non-carbohydrate constituents and the variety of linkages present in them make these molecules excellent emulsifying agents.[4] EPS also help bacteria for the development of biofilms.[5] The EPS may be either capsule EPS or slime EPS. There are reports which show that capsule EPS are produced mainly during the log phase of bacterial growth and slime EPS during stationary phase.[6]

These molecules have a wide application as they have the characteristics of stabilizers, jellying agent, adhesives, thickening agents, emulsifiers, flocculants and flushing agents[7] With all these, they are used in a range of application such as detergents, textiles, paper, pulp, food, beverage industries[8] oil recovery, reclamation of heavy metal polluted soil and water etc.[9],[10],[11] Realizing the importance of EPS and its industrial applications, this study was conducted to identify and isolate marine bacteria which produces EPS and to optimize its production in terms of pH, temperature and incubation periods.

MATERIALS AND METHODS

Sample Collection and isolation of bacteria

Marine water samples were collected from Arabian Sea following standard microbial procedures. Bacterial strains were isolated from water sample using Zobell media.

Screening of Marine Bacteria for the production of EPS

All the isolated strains of marine bacteria were subjected for the screening for EPS production. The screening was done by modifying the protocol of Sayyed *et al.*[12] Zobell marine ager medium was prepared by dissolving glucose: 2.5g, yeast extract: 0.75g, malt extract: 3g, peptone: 1.25g, monosodium glutamate 0.25g, sucrose: 7.5g along with 125mL sea water and 125mL distilled water, pH was adjusted to 7.0 and was poured in petriplates after sterilization. The bacterial strains to be tested for EPS production were streaked on the solidified medium. The plates were incubated at room temperature for 3 days, oozing out of gummy substances on the periphery of the bacterial colonies indicated the production of EPS.

Quantification of EPS

Quantification of EPS production was done by estimating the quantity of protein, modifying the protocol of El-Tayeb and Khodair,[13] as well as quantity of carbohydrates. For protein estimation, one loop full of bacterial strains was inoculated at room temperature for three days. After 3 days of incubation estimation of protein was done using Lowry's et al method using spectrophotometer at 660 nm and phenol sulphuric acid method for estimation of carbohydrate at 490 nm. The total carbohydrate content was estimated by phenol sulphuric acid method proposed by Dubois et al.[14]

Optimization of EPS in terms of protein

To study the effect of different parameters, 1% inoculum containing 5x106cells/mL of the bacterial cultures were inoculated in 100mL production medium. EPS production was optimized under two different environmental condition i.e., pH (acidic neutral and alkaline (4, 7and 9), temperature (20°C and 50°C) and at three different incubation times (24 hr, 72 hr and 120 hours).

Optimization and Quantification of Carbohydrate

The EPS materials are majorly composed of carbohydrates. They are responsible for the architecture and morphology of the matrix in which the cells live. Thus, they can be considered as the microorganism's protective sheet. Though it is reported that the actual composition of EPS depends on the extraction method, it is largely composed of polysaccharides.

RESULTS AND DISCUSSION

Twelve different bacterial strains were isolated based on colony morphology for further studies. The bacterial isolates were named as Ab1, Ab2, Ab3, Ab4, Bb1, Bb2, Bb3, Bb4, Tb1, Tb2, Tb3 and Tb4.

Screening for EPS

Out of 12 strains, 5 strains exhibit EPS production and the strains are Ab4, Tb1, Tb2, and Tb3 which produced gummy secretions either around the colony periphery or by whole colony in Zobell marine agar medium (Plate 1). There are reports that the EPS may be composed of different substances. Composition analyses indicated the presence of carbohydrates, metals, proteins, and minor quantities of DNA and lipids, although the relative concentrations of these components were different for the two EPS samples. Based on these observations, EPS was quantified by measuring the quantity of carbohydrate as well as protein in it.

Effect of Temperature on protein production in EPS

Quantification of protein was done by standard Lowry's method. The production of EPS with respect to protein content was optimized in different temperature, pH and duration of incubation. The result of estimation of proteins at various temperatures, pH and duration of incubation are shown in Table: 1, Table: 2 and Table: 3 respectively. For getting rather good idea about the relation of EPS production with respect to proteins, the graphs are also placed: Figure: 1, Figure: 2 and Figure 3.

It can be seen from the figure that all the strains produced proteins in EPS at highest quantities at 20°C. Interestingly, except in Tb2, after a dip in the production at about 37°C, most of the bacterial isolates increased the production of proteins at 50°C. Previous workers reported a higher production of EPS at much lower temperatures than 20°C. This is because of the fact that EPS are produced as a strategy of escaping from extreme conditions. This may be the result of an increased protein production at 50°C also.

Effect of pH on protein production in EPS

Regarding pH, the production of proteins in EPS was found to be high both at acidic as well as alkaline pH. Kimmel et al.,[15] found, by using a one-variable-at-a-time (OVAT) approach, that optimal EPS production occurred at 20°C and pH 5.8. Another study by Mozzi *et al.*[16]

found that optimum specific production (EPS produced per gram [dry weight] of cells) and EPS yield (grams of EPS 3 100/grams of sugar consumed) were found at pH 4.0.

Effect of Incubation time on protein production in EPS

Except Bb4 and Tb1, all others showed a slight increase in the production of protein content in EPS. Of these, Tb3 showed a sharp increase in the secretion of proteins in the EPS at 120 hours. Many marine bacteria produce exopolysaccharides (EPS) as a strategy for growth, adhering to solid surfaces, and to survive adverse conditions. Optimization of the growth environment is important to achieving maximal EPS production by organisms.

Effect of Temperature on carbohydrate production in EPS

The effect of temperature on the content of carbohydrate in all bacterial strains showed a similar general pattern. The carbohydrate content was less at room temperature. Both at high temperature as well as at low temperature, the carbohydrate content was higher. Of all the strains, strain Tb1 was found to produce high amount of carbohydrate. From the results it can be made out that stress can cause the microorganisms to produce an increased amount of EPS. Monika et al.,[17] reported that the high yield of exopolysaccharides (EPS) required a moderate temperature of 28 °C for *G. applanatum* and 20 °C *T. palustris*. In their study, Aisha and Anjum[18] showed that the content of EPS increases when bacteria is under salinity stress. Jorge-Ignacio *et al.*[19] found that as the temperature dropped, synthesis of EPS increased.

Effect of pH on carbohydrate production in EPS

Generally, alkaline pH was found to have high content of carbohydrates in the EPS when compared to acidic as well as neutral pH. All the bacterial strains followed this pattern. Of all strains, Tb2 showed maximum production.

Effect of Incubation time on carbohydrate production in EPS

In the case of effect of period of incubation the carbohydrate content increased from 24 hr to 72 hours. But thereafter, there was no considerable increase in the content of carbohydrates. In the case of bacterial strain Tb3 alone, this was not true and the EPS carbohydrate content of EPS increased considerably as incubation period increased. The protein content of this strain also showed a similar pattern.

Table: 1 Effect of temperature, pH and incubation period on carbohydrate content of EPS

Strains	Temperature (°C)	pH	Incubation time (Hrs)						
	20°C	37°C	50°C	4	7	9	24	72	120
Ab4	11.14	8.60	11.38	8.58	8.6	14.32	6.08	8.60	12.58
Bb2	9.78	6.05	8.99	5.99	6.05	20.31	6.05	6.05	6.92
Tb1	15.81	14.79	15.08	10.43	14.79	13.11	6.65	14.79	14.78
Tb2	10.81	13.21	12.34	7.93	13.21	23.41	8.33	13.21	13.99
Tb3	10.36	6.07	9.36	8.92	6.07	19.51	3.64	6.07	12.38

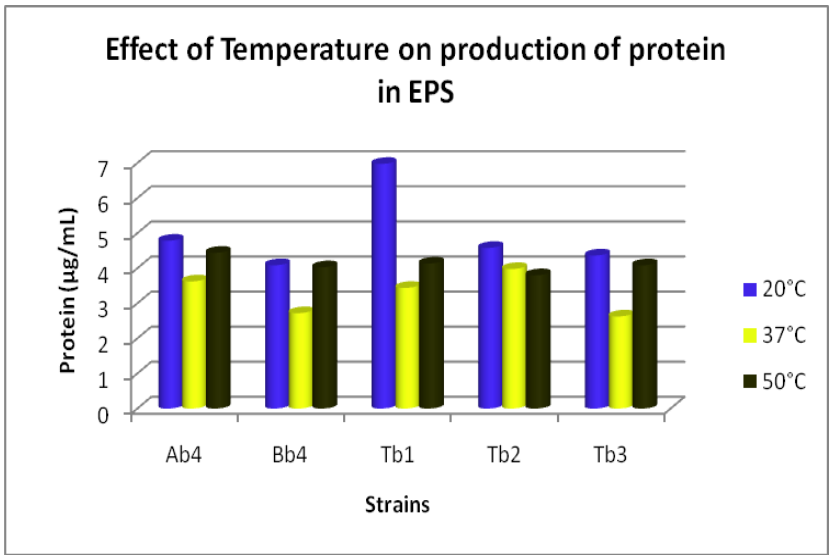


Figure: 1 Effect of temperature in EPS production

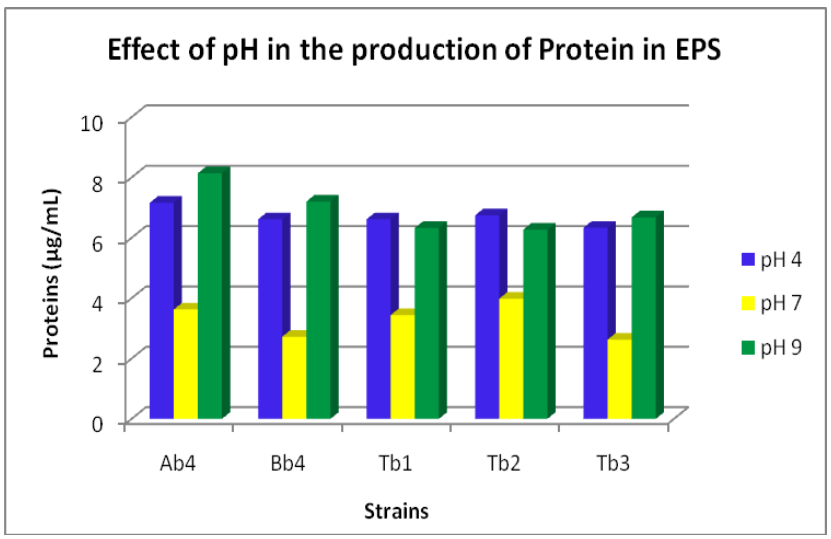


Figure: 2. Quantity of protein at different pH

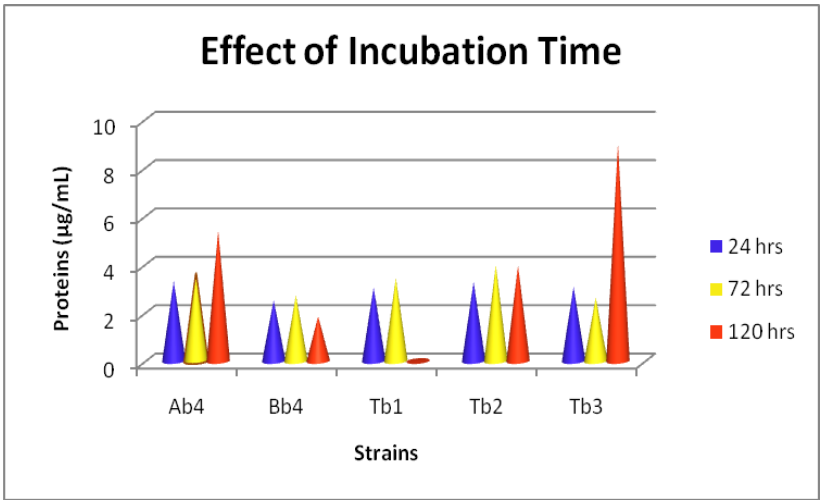
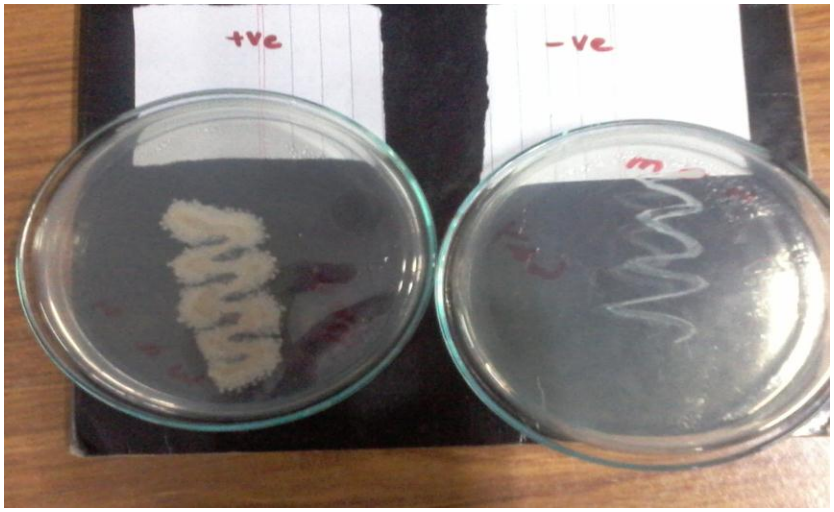


Plate 1 EPS production by bacterial strain



CONCLUSION

Production of EPS is thought to be one of the microbial survival strategies because it provides microorganisms with important advantages, including, (i) increased access to nutrients; (ii) protection against toxins and antibiotics; (iii) maintenance of extracellular enzyme activities and (iv)shelter from predation. This study showed that as stressful conditions such as high as well as low temperature, high as well as low pH showed a general trend of increase in the EPS production.

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