OPTIMIZATION OF NUTRITIONAL CONDITIONS FOR EXOPOLYSACCHARIDE PRODUCTION BY USING MARINE *PSUDOMONAS AEROGINOSA* ISOLATED FROM ANJERLE BEACH

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ABSTRACT: The exopolysaccharides (EPS) producing ability was evaluated by nutritional and chemically defined media by the laboratory isolated haloalkalotolernt *Psudomonas aeroginosa* which gave highest yield EPS . It was identified as *Psudomonas aeroginosa* on selective media and 16S rDNA gene sequencing. The favorable conditions for EPS yield by the microorganism growth in a chemically defined medium were tested with carbon, nitrogen, sodium chloride, metal ions which is a significant factors for EPS production . Glucose was the most favorable carbon source for EPS production and maximum production (4.6 g/L) was recorded with 1.5% glucose. Among different nitrogen sources, Ammonium sulfate at 0.7% level was proved to be the best for EPS production which enhanced the EPS production to3.98 g/L, while the metal ions (MgSo4)were also found to be favorable to growth and EPS production. An EPS production of 4.70 g/L was achieved when the growth medium was supplemented with 3.5% NaCl. The maximum EPS production was viewed at the 72hrs of incubation at submerged condition
Key words: *Psudomonas aeroginosa exopolysaccharides*, optimization.

1. 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 (Flemming *et al* .,2001) In recent years, increasing awareness is being paid to microbial exopolysaccharides (EPS) mainly because of their bioactive role and wide range of potential applications in modern biotechnology especially in medicine and pharmaceuticals as antiangiogenic or antiviral agents or even in case of targeted drug delivery, as well as in wound dressing (Vandamme *et al* 2002)The advantages of microbial exopolysaccharide over plant and micro-algal biopolymers are due to their novel functionality, easily reproducible chemical and physical properties, and stable cost and supply. Moreover, EPS from halophiles have stability towards higher temperature, salinity and even pH (Wan-Mohtar *et al* 2016) .These unique properties of EPS produced by halophiles seem to offer varied applications in various fields of industry as marine halophiles are stable to high temperature, salinity and even pH.

For some EPS-producing bacteria, such as *Xanthomonas*, *Pseudomonas* and *Rhizobium* spp., nitrogen limiting conditions result in increased EPS production. Certain commercially available and important microbial EPSs are dextrans, xanthan, gellan, pullulan, yeast glucans and bacterial alginates (Banik et al 2000)

Pseudomonads are one of the richest sources of exopolysaccharides. Extracellular slime is a characteristic of certain Pseudomonas strains and the formation of complex exocellular slime has been reported in strains of *P. aeruginosa* under various cultural conditions (Williams et al 1977)

The structure, composition and viscosity of the microbial polysaccharides depend on several factors, such as the composition of the culture medium, carbon and nitrogen source, mineral salts, trace elements, type of strain, and fermentation conditions (pH, temperature, oxygen concentration, agitation (Conti *et al*., 1994 and Duta *et al*., 2004).

Optimization of medium components is an important step for enhanced exopolysaccharide production .Nutritional components in growth media as well as physical and biochemical parameters are very important for production of exopolysaccharide .Media formulation and nutritional parameters optimization can be successfully carried out by classical approach . Classical method such as one at time factor is changing one independent variable while fixing all other at a certain level (Ahamad *et al*.,2006; Alexeeva *et al*., 2002; Patidar *et al*., 2005). Because of its easy and convenience one-factor-at-a-time method has been the most popular method for improving the fermentation medium and process condition.

The present study was conducted to optimize the medium components such as carbon source, nitrogen source and metal sources for EPS production by *Psudomonas aeroginosa* under submerged cultivation.

2. MATERIALS AND METHOD

2.1 Microrganism and Medium for fermentation

Psudomonas aeroginosa was isolated from coastal surface sea water from **Anjerle beach**, Dapoli , India on January 2014. Location of Anjerle beach was found to be 17.85˚N 73.09˚E. The bacterium was identified using the 16S rDNA gene sequence analysis and was submitted to Genbank under the accession number HM 119395. It was propagated on Zubella marine agar slants at 30°C for 3 days and sub cultured monthly. The seed medium that was also used as the basal medium consisted of 10 g/l glucose, 5 g/l peptone, 3 g/l malt extract , 3 g/l yeast extract .

2.2 Effect of various carbon source on EPS production

Optical density of overnight incubated marine isolated *Psudomonas aeroginosa* at different carbon sources at 1% concentration (glucose ,galactose ,maltose, mannitol, sucrose , arabinose ,lactose, raffinose, fructose, jaggary and molasses) is measured at 600 nm and EPS was extracted by ethanol precipitation method and measured in terms of yield (g/l)

2.3 Effect of glucose on EPS production

To determine the optimal concentration of glucose in the medium, ten different concentrations (0.5,1.0,1.5,2.0,2.5,3.0,3.5,4.0,4.5, and 5.0 (%) of glucose were used as the carbon source for production of EPS .Glucose was sterilized separately at 110 ˚ C and growth of organism at 600 nm and yield of exopolysaccharide was determined .

2.4 Effect of various nitrogen source on EPS production

The growth of selected bacteria in different nitrogen sources was investigated by inoculating into YMG broth containing different types of nitrogen sources viz yeast extract, peptone, potassium nitrate, casein, gelatin ,urea, tryptone, glycine, ammonium sulfate, ammonium nitrate. Then each of the above flasks was inoculated with freshly prepared bacterial suspension and incubated at 30˚ C for 72 hrs. After incubation exopolysaccharide yield in g/L was determined .All the experiment were carried out in triplicates .

2.5 Effect of Ammonium sulfate on EPS production

To find the appropriate of concentration ammonium sulfate for EPS production and bacterial growth of AB4 *Psudomonas aeroginosa* in submerged culture, different levels of ammonium sulfate ranging from 0.1 g/l to 1 g/l were tested.

2.6 Carbon-nitrogen ratio:

To find out the suitable effects of carbon-to nitrogen ratio (C/N) ratio on bacterial growth, exopolysaccharide of *Psudomonas aeroginosa* , the concentration of the carbon and nitrogen in g/l were varied as follows: 1:0.1, 1:0.2, 1:0.25 1:0.3, 1:0.5. and 1:1 carbon and nitrogen free media (0:0) was used as control.

2.7 Effect of metal ions on EPS production

Different metal ions at 0.02% concentration viz. Magnesium chloride, Ferrous sulfate ,Ferric chloride, Zinc sulfate , Calcium chloride , Copper sulphate, potassium chloride ,Sodium bicarbonate ,Potassium sulfate, Strontium chloride , Manganese sulfate, Ammonium sulfate ,Lead acetate ,Cobalt chloride) were introduced into the production medium individually to determine the effect of metal ions on microbial growth and EPS production.

2.8 Effect of Sodium chloride on EPS production

The effect of salt concentrations on EPS production was checked by adding NaCl at varying concentrations from 0.5% to 5.0 % at an interval 0.5 % in seed medium .the production was carried out at 30 ˚C for 72 hrs and yield of EPS was recorded .

3.RESULT AND DISCUSSION

3.1Effect of various carbon source on EPS production

The effect of carbon sources on the production of EPS were studied in the fermentation medium containing various carbon sources .The result (fig.1) showed that glucose is the best carbon source for EPS production by AB 4 *P. aeruginosa* giving 1.56 g/l production followed mannitol and arabinose which gave 1.44 g/l EPS when supplemented in seed medium . Pal *et al* in 2015 has reported that EPS production was maximum in 2% (w/v) glucose followed by sucrose and maltose. our result with accordance with Shrama *et al.* (2015) who found glucose was efficient carbon, for the production of the EPS by *Cordyceps cicadae.*

3.2Effect of glucose on EPS production

The effect of various concentration of glucose on EPS production after 72 hours of incubation period at 30^0C showed maximum amount of EPS production at 1.5 % glucose supplemented medium while minimum amount of EPS production was found to be 3.5% . As shown in Figure 2, EPS production was enhanced gradually till the glucose concentration in the medium reached at 1.5 % (w/v), producing 4.7 g/L of EPS. Further increase in glucose (4–5%) in the medium retarded the EPS production by the isolate AB4 *Psudomonas aeroginosa* . Zhang *et al* 2011 reported a maximum EPS production was 44.49 mg/L from *Lactobacillus fermentum* when the medium was supplemented with 2% glucose and 0.5% whey protein concentrate .

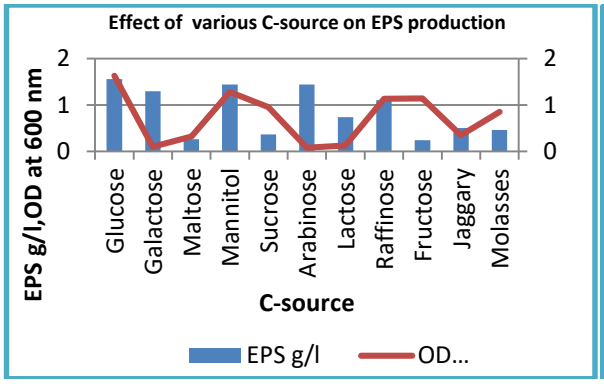


Fig. 1 Effect of various C-source on EPS production

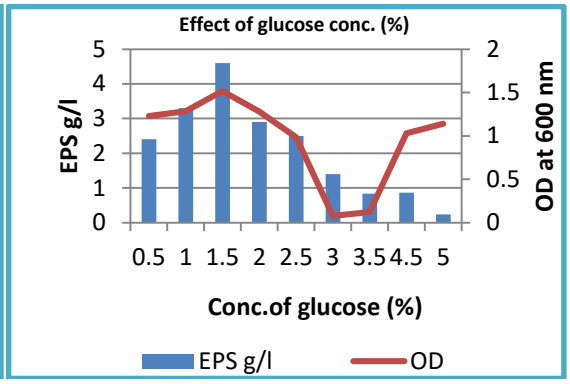


Fig . 2 Effect of glucose conc. (%) on EPS production

3.3Effect of various nitrogen source on EPS production

To investigate the effect of nitrogen sources for the EPS production, cells were cultivated in the medium containing various nitrogen sources. Each nitrogen source was added in seed medium at a concentration level of 5 g /L. Amongst nitrogen sources examined, ammonium sulfate was favorable for the EPS production which yield 3.6 g / l . (fig. 3) Sutherland (1982) confirmed that most bacteria utilize ammonium salt or amino acids as nitrogen source for polysaccharide production. NH4Cl was the most excellent nitrogen source for EPS production by *Enterobacter cloacae* (Meade *et al.*, 1994).

3.4 Effect of Ammonium sulfate on EPS production

To find out the most suitable concentration ammonium sulfate of for EPS production the isolate was grown in media containing 0.1–2 % of ammonium sulfate , other conditions of growth were same as in previous experiments. It was found that EPS production was increased up to 4.3 g/L when the medium was supplemented with 0.7% ammonium sulfate (Fig .4).

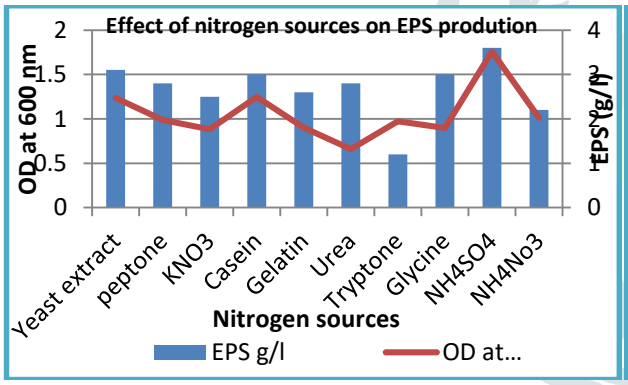


Fig.3 **Effect of nitrogen sources on EPS production**

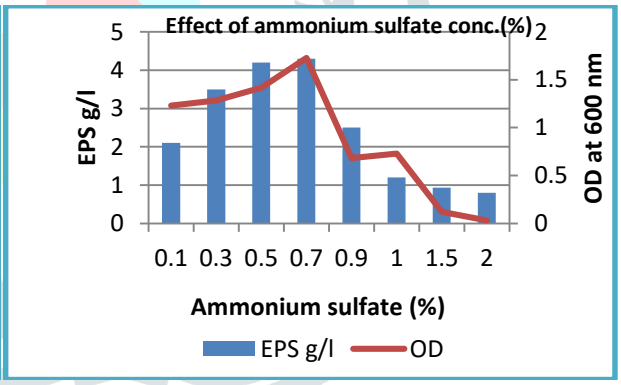


Fig.4 Effect of ammonium sulfate conc. (%)on EPS

3.5 Effect of C/N ratio on EPS production

Several reviewer reported that ratios of carbon and nitrogen sources play the most important role in cellular growth and exopolysaccharide production . In order to determine the effects of carbon nitrogen ration (C/N) on growth and exopolysaccharide production of AB 4 *P. aeruginosa* the concentration of dextrose and yeast extract in the basal medium was varied. Concentration of 1% (w/v) of each of glucose and ammonium sulfate in the basal medium was used for ratio 1:0.5. Other ratios were varied accordingly as follows: 1: 0.1, 1: 0.2, 1: 0.7 and 1: 1. Highest increase in biomass was recorded in C/N ratio 1: 0.7 (3.68 g/l) and least in 1: 0.1 (0.83 g /l) as compared to the control without carbon and nitrogen sources (0.025 g/l).

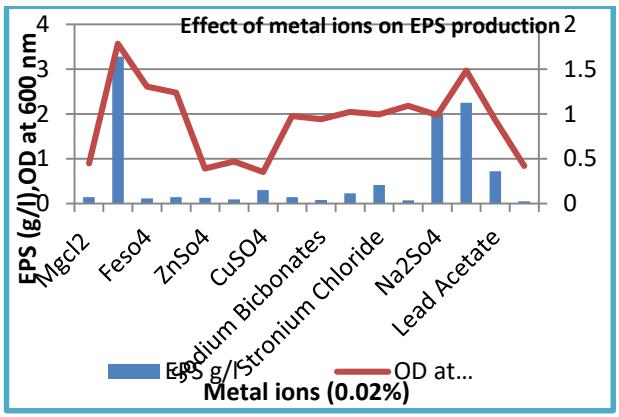
3.6Effect of metal ions on EPS production

Among the tested metal ions, the maximum amount of EPS production was observed in magnesium sulfate (3.28 g/l) supplemented medium. Followed by this, sodium hydrogen phosphate was the second best metal ions on EPS production (2.25 g/l), whereas the minimum amount of EPS production was observed in cobalt chloride (0.05 g/l)(Fig.5).

3.7 Effect of Sodium chloride on EPS production

Sodium chloride plays an important role in production of exopolysaccharide and could alter the osmolarity of the cell membrane of bacterium which favored the more extrusion of exopolysaccharide from cell to media. It was observed that the growth and production of exopolysaccahride was maximum as at 3.5 % and minimum was at 1.5 to 2 % (Fig.6) EPS production of 4.7 g/L was obtained when

the growth medium was supplemented with 3.5% NaCl. Arias *et al* in 2003 reported that optimum salt concentrations for EPS production by *H. maura* was 2.5% and same report was given by Shivakumar *et al* (2012).



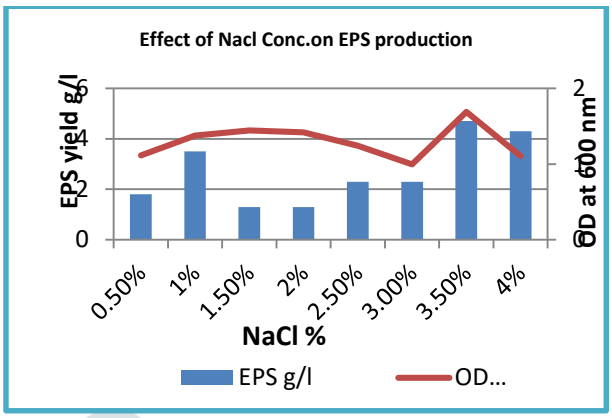


Fig.5 Effect of metal ions on EPS production **Fig 6 Effect of Nacl (%) on EPS production**

CONCLUSION

The present work studied that the marine bacterium *Psudomonas aeroginosa AB4* is capable of producing considerable amount of exopolysaccharide utilizing an array of carbon and nitrogen sources under high saline (3.5%) and high pH (10) conditions suggesting its implication in the production of the exopolysaccharide under extreme saline as well as alkali environment. Glucose and ammonium sulfate was found to be best source for EPS production The organism thereby could be a potential source of biopolymer possessing unique properties for future biotechnological and industrial applications.

ACKNOWLEDGMENT

Authors are thankful to Shivchhatrapati College, Aurangabad and Government of institute of science for their infrastructure and the teaching and non-teaching staff of the microbiology department for their constant support.

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