

Optimization of pH and temperature for exopolysaccharide production by *Lysinibacillus macroides*

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ABSTRACT

Exopolysaccharides (EPS) are the secondary metabolites secreted by some bacteria outside their cell walls. This study aims to optimize EPS production at optimum initial media pH and incubation temperature. Culture of Lysinibacillus macroides, selected for this study, was inoculated in MYE medium for EPS production and incubated. EPS was extracted by centrifugation (10000 rpm or 14534 g) and adding of chilled ethanol (3 times volume) in the supernatant. Results of the optimization study indicated that initial media pH of 7.5 and incubation temperature of 30°C were optimum for Lysinibacillus macroides to produce maximum amount of EPS.

Keywords: *Exopolysaccharide; Optimization; Initial Media pH; Incubation Temperature*

1. INTRODUCTION

Exopolysaccharides (EPS) are polymeric substances secreted by many microorganisms as a secondary metabolite, mainly composed of carbohydrates (**Chaudhari et al., 2017**). Many types of EPS produced by bacteria (xanthan, gellan, pullulan and dextran), plants (starch, cellulose and pectin) and algae (agar, carrageenan and alginate) (**Sharma, 2013**). These EPS can be classified into homopolysaccharide and heteropolysaccharide, depending on their composition (**Angel, 2015**). Now-a-days, bacterial EPS have been emerged as a novel and economical polymeric substance against synthetic polymers (**Suresh Kumar et al., 2007**).

Rhizosphere soil is one of the richest source for the EPS producing bacteria as it contains high amount of organic materials (**Amellal et al., 1998**). Many species of Gram-positive and Gram-negative bacteria like *Bacillus*, *Lactobacillus*, *Streptococcus*, *Xanthomonas*, *Pseudomonas*, *Azotobacter*, *Klebsiella*, *Rhizobium*, etc. are reported as EPS producers (**Vaishnav, 2017**).

EPS can provide protection to the living cells against osmotic stress, predation, desiccation, antibiotics and toxic metal compounds (**Vidhyalakshmi and Nachiyar, 2011**). EPS have various applications in the food, medicine and pharmaceutical industries, so its demand has been significantly increased in the recent years in the global market (**Chen et al., 2016**).

There are so many factors and growth conditions affecting cell growth and EPS production. Initial media pH and incubation temperature are among them, which are very crucial factors to control for EPS production. The present study aims to optimize initial media pH and incubation temperature for EPS production by *Lysinibacillus macroides*, an isolate obtained from rhizosphere soil.

2. MATERIALS AND METHODS

2.1 Microorganism and growth media

Lysinibacillus macroides, isolated from the rhizospheric soil, were taken for this study. Modified Yeast Extract (MYE) medium containing (g L⁻¹) sucrose, 30; yeast extract, 1; KH₂PO₄, 1; and MgSO₄, 0.5; was used for the EPS production (**Vaishnav, 2017**).

2.2 EPS production and extraction

100 mL of MYE medium was inoculated with 10% v/v inoculum and incubated at room temperature for 96 h on a rotary shaker (120 rpm) (**Pawar et al., 2013**). After incubation, 5% w/v of trichloroacetic acid (TCA) was added to the medium and kept in agitation for 30 min at room temperature to precipitate proteins (**Zhang et al., 2013; Shao et al., 2014; Fontana et al., 2015; Dave et al., 2020**). Precipitated proteins were separated by centrifugation (10,000 rpm) and recovered supernatant. Three volumes of ice-cold ethanol were added to the supernatant and kept overnight at 4°C. Precipitated EPS was collected by centrifugation as above, given three washes with ice-cold ethanol, dried (65°C) and weighed (**Vaishnav, 2017**).

2.3 Optimization of initial media pH and incubation temperature

Four flasks of 50 mL sterile MYE broth medium were prepared, adjusted to different pH (6.5, 7, 7.5, and 8, respectively), inoculated with 10% inoculum of *Lysinibacillus macroides*, and incubated at room temperature ($32\pm 2^\circ\text{C}$) for 96 h. After incubation, the EPS production was determined from all the flasks as mentioned above.

To find out the optimum incubation temperature, four flasks of 50 mL sterilized MYE broth medium was prepared, inoculated with 10% inoculum of *Lysinibacillus macroides*, and incubated under different temperatures (20, 30, 37, and 45°C , respectively) for 96 h. After completion of the incubation period, the EPS production was measured as above.

3. RESULTS AND DISCUSSION

3.1 Optimization of growth parameters

Many factors affect the growth and secondary metabolite production of microorganisms like inoculum size, incubation period, agitation speed, initial media pH, temperature, aeration rate, type and concentrations of carbon and nitrogen source, etc. Among these, pH and temperature are very important factors for EPS production. They can affect drastically the growth of *Lysinibacillus macroides* and its ability to produce EPS. The present study reveals the effect of these two crucial parameters.

3.2 Effect of initial media pH

pH is one of the very important factors which can affect the production of EPS. By increasing the initial pH of the medium, EPS production was also observed in the increasing pattern at a certain level, but then it was found to decrease. As the results are shown in Fig. 1, the production of EPS increased from 12.17 g L^{-1} to 29.23 g L^{-1} as the initial pH of the medium increased from 6.5 to 7.5 and then decreased at pH 8. This might be due to the changes in the hydrogen ion concentration in the growth medium (Shivakumar and Vijayendra, 2006). The maximum EPS production of 29.23 g L^{-1} was obtained at pH 7.5. Pawar et al. (2013) also obtained maximum EPS production at pH 7.5. Many of the researchers reported that the neutral pH (7 to 7.5) favours EPS production (Lee et al., 1997; Sonawdekar and Gupte, 2016), but some researchers found higher EPS production rates at acidic pH (5 to 6) also (Kuntiya et al., 2010; Huang and Liu, 2008).

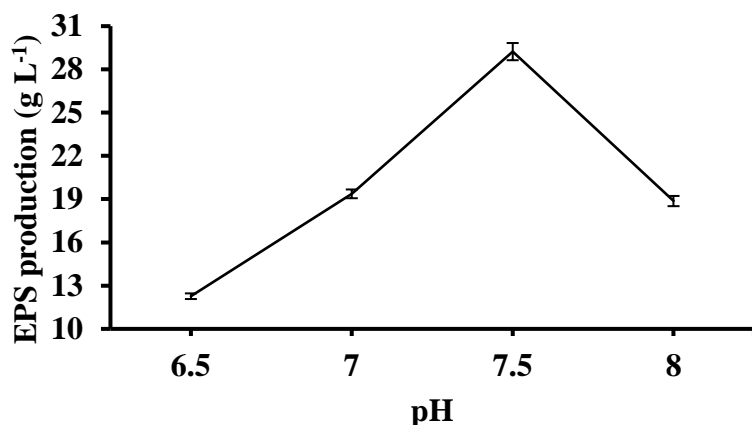


Figure 1: Effect of pH on EPS production

3.3 Effect of incubation temperature

The EPS production increased gradually by increasing incubation temperature from 20 to 30°C and reached to maximum production of 28.37 g L⁻¹ at 30°C (Fig. 2). Similar results were obtained by some researchers (Shivakuma and Vijayendra, 2006; Kaur et al., 2013; Prasertsan et al., 2008) and found optimum temperature of 30°C for EPS production. However, the production of EPS was reduced by the further increasing temperature to 37°C and 45°C. Many researchers obtained maximum EPS production at lower incubation temperature of 20°C and 25°C too (Lim et al., 2004; Mubarak and Amer, 2013).

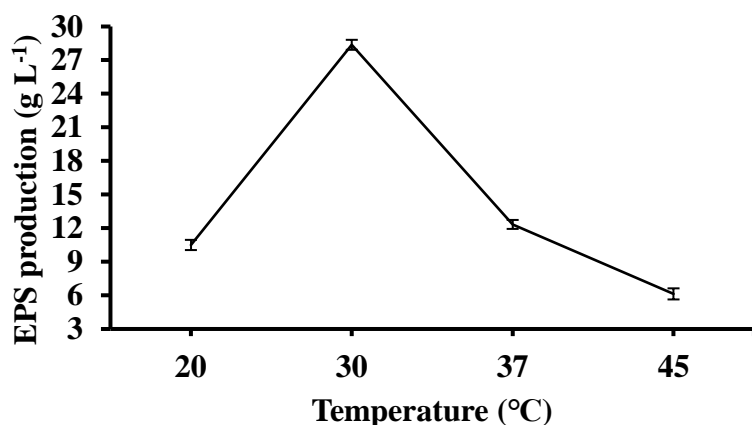


Figure 2: Effect of temperature on EPS production

4. CONCLUSION

EPS production was carried out in MYE medium by *Lysinibacillus macroides*. The EPS production was optimized under different environmental conditions like pH and temperature. The optimum condition for EPS production was found in the growth medium with the initial pH of 7.5 and the optimum incubation temperature found was 30°C for EPS production by *Lysinibacillus macroides*.

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