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A Study on Optimization of Exopolysaccharides from a Potential *Lactobacillus casei* KL14 KX774469

Kanika Sharma*, Nivedita Sharma and Ranjana Sharma

Microbiology Research Laboratory, Department of Basic Sciences, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (India)-173230, HP, India

*Corresponding author

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In this study, an excellent exopolysaccharide producing in house isolate i.e. *Lactobacillus casei* KL14 with accession number KX774469 was used to optimize various independent factors i.e. incubation temperature, pH, carbon source, surfactant source and nitrogen concentration for enhanced exopolysaccharide production by One Factor at a Time (OFAT) approach. The best parameters optimized from OFAT for pH, temperature, incubation time, carbon source, nitrogen concentration, surfactant and carbon concentration were 6.50, 35°C, 28 h, sucrose, 0.3%, triton-X100 and 2%. Under optimized conditions, an overall increase of 42.10% EPS production was observed with KL14, respectively. Thus, *L. casei* KL14 can be used in the speedup production of exopolysaccharides in large scale.

Introduction

Exopolysaccharides (EPS) play an extensive role as a biopolymer in the food products by replacing synthetic polymer as they are viscosifiers, texturizers, syneresis-lowering agents and for their pseudoplastic rheological behaviour and water holding capacity. They are high molecular weight, long chain of polysaccharides generally consisting of monosaccharides (repeating sugars residues) and some noncarbohydrate substitutes such as acetates, pyruvates, succinates and phosphates

(Liu *et al.*, 2016). Microbes release polysaccharides extracellularly in the environment in the form of capsules or slime. The EPS of microbial origin have started capturing market currently due to their unique rheological properties, their capability of forming very viscous solutions at low concentrations and their pseudoplastic nature (Becker *et al.*, 1998). Among different microorganisms, exopolysaccharides produced by lactic acid bacteria (LAB) from dairy products are receiving an increased attention because of their ‘food-grade’ status (Hansen

2002; Czerucka *et al.*, 2007; Galle and Arendt, 2013). The EPS from LAB have wide industrial applications as jelling agents, stabilizers, thickening agents, adhesives, flocculants, emulsifiers and flushing agents (Jayadev *et al.*, 2016).

The economic value of commercial exopolysaccharides depends on the ease of production, composition of the polysaccharide, the produced quality from microorganisms, the harvesting mode and the higher yield of product. Optimization is an important criterion for every laboratory and industry to maximize the production of desirable products. By realizing the importance of EPS and its application in industries, the present study was conducted to optimize exopolysaccharide production from lactic acid bacteria various process parameters *i.e.* temperature, pH, incubation time, carbon source and nitrogen source have been evaluated.

Materials and Methods

Source of culture collected and its characteristics

Inhouse isolated culture of *L. casei* KL14 from lassi- fermented milk with National Centre for Biotechnology Information (NCBI) accession number KX774469 was selected for optimization of exopolysaccharide by using conventional approach *i.e.* One Factor at a Time approach (OFAT). The cultures were sub-cultured periodically and were further preserved on slants and 40% glycerol in deep freezer (-20°C).

Optimization of factors enhanced for EPS production by using one factor at a one time (OFAT) approach

Various growth conditions viz. effect of incubation temperature, pH, carbon source,

surfactant source and nitrogen conc. were studied to monitor their effect on EPS production.

Effect of different temperatures

Bacterial isolate *i.e.* *L. casei* KL14 was incubated in orbital shaker at different temperatures ranging from 25, 30, 35,..., 50 °C for EPS production. The temperature showing highest EPS production was optimized for further studies. Total EPS (expressed as µg/ml) were estimated in each sample by Phenol-Sulphuric acid method of Dubois *et al.*, (1956) using glucose as standard.

Effect of different pH

L. casei KL14 was incubated at different pH ranging from 5.5 to 8 for EPS production. The pH showing maximum EPS production was optimized for further studies.

Effect of different carbon sources

L. casei KL14 was grown in MRS media (de Man, Rogosa and Sharpe) containing different carbon sources viz. glucose, sucrose, xylose, lactose, mannose and galactose. The best carbon source that resulted in maximum EPS production was selected for next step of optimization.

Effect of different surfactant sources

Six sets of MRS media flasks containing different surfactant sources viz. SDS, EDTA, Triton X100, Tween 20, Tween 80 and CTAB was grown with *L. casei* KL14 culture for optimization of enhanced EPS production.

Effect of different concentration of best carbon source

The *L. casei* KL14 isolate was incubated at respective standard condition of MRS media

with different carbon conc. *i.e.* ranging from 0.50, 1.00, 1.50,..., 3.00%. The concentration with highest EPS production was optimized for further studies.

Effect of different concentrations of ammonium citrate (N-source)

L. casei was inoculated in six flasks with standard condition of MRS media but varying nitrogen concentration ranging from 0.1, 0.2, 0.3,..., 0.6%.

The best nitrogen concentration with maximum EPS production was selected for next step of optimization.

Results and Discussion

Optimization by One Factor at a Time (OFAT) approach

Effect of different temperatures

The highest EPS production observed was 12.24 mg/ml at 35°C which is significantly higher than the production seen at other temperatures *i.e.* 25, 30, 40, 45 and 50°C tested in the present study as shown in Figure 1 and 3.

Effect of different pH

The highest exopolysaccharide production was observed at pH 6.5, *i.e.* 12.92 mg/ml for *L. casei* KL14 which is significantly higher than production at other pH values as shown in Figure 2 and 3.

Effect of carbon source

L. casei KL14 utilized all the six carbon substrates but significant maximum exopolysaccharide production *i.e.* 17.85 mg/ml was observed with sucrose as shown in

Figure 3 and 3.

Effect of surfactants

Surfactants in the fermentation medium are known to increase the secretion of exopolysaccharides by increasing cell membrane permeability (Hashem *et al.*, 2010).

L. casei KL14 utilized all the six surfactants (1%) *i.e.* SDS, EDTA, Triton X100, Tween 20, Tween 80 and CTAB for exopolysaccharide production, but maximum exopolysaccharide production *i.e.* 17.86 mg/ml with Triton X100 (Fig. 4 and 3).

Effect of carbon concentration

Sucrose, a disaccharide, upon hydrolysis produces glucose and fructose. Higher yield is obtained, since sucrose apparently acted as precursor of EPS synthesis.

Very low substrate concentration failed below (2%) to trigger exopolysaccharide production to desirable level probably due to the reason that remained without carbon and hence resulting in minimum secretion of exopolysaccharide. The maximum yield of EPS production by *L. casei* KL14 *i.e.* 28.86 mg/ml was observed at 2% of sucrose (Fig. 3).

Effect of nitrogen concentration

The maximum yield of EPS production in *L. casei* KL14 was observed at 0.3% (Fig. 3). Nitrogen sources are the most important secondary energy compounds for the growth and metabolism of microorganisms.

The nature of these compounds and the concentration used may stimulate or down regulate the production of exopolysaccharide (Sharma and Singh, 2012).

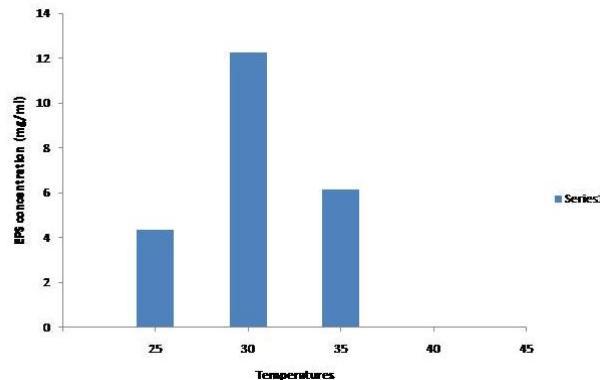


Fig.1 Effect of different temperature on exopolysaccharide production from *L. casei* KL14

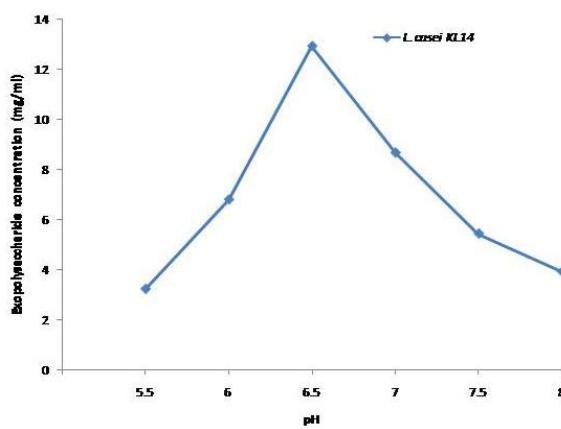


Fig.2 Effect of different pH on exopolysaccharide production from *L. casei* KL14

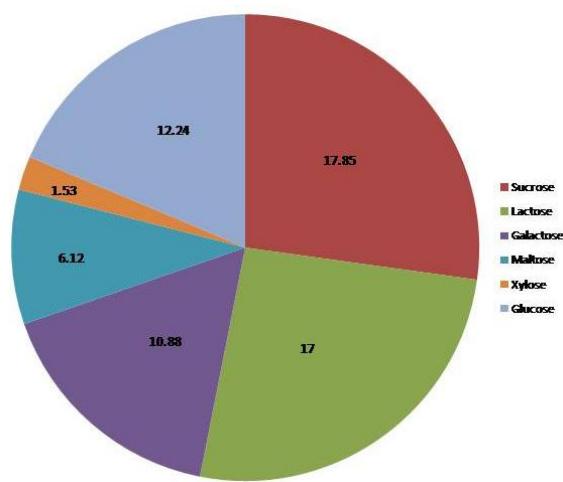


Fig.3 Effect of different carbon source on exopolysaccharide production from *L. casei* KL14

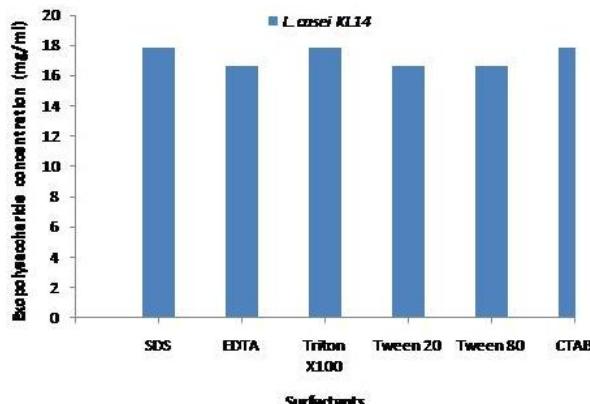


Fig.4 Effect of different surfactants on exopolysaccharide production from *L. casei* KL14

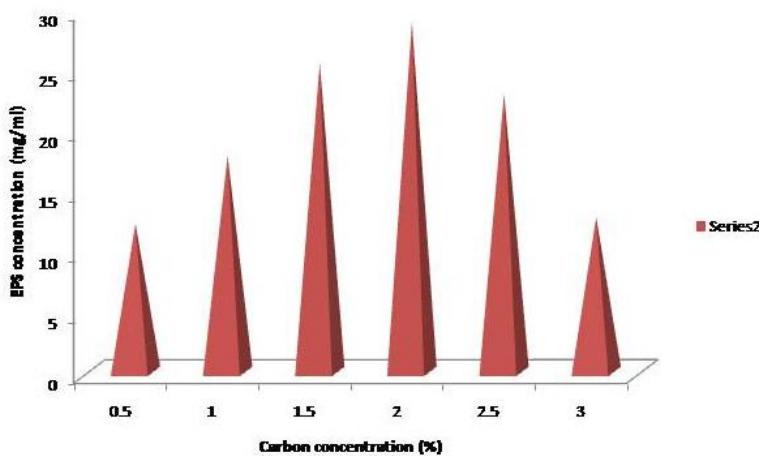


Fig.5 Effect of different carbon concentrations on exopolysaccharide production from *L. casei* KL14

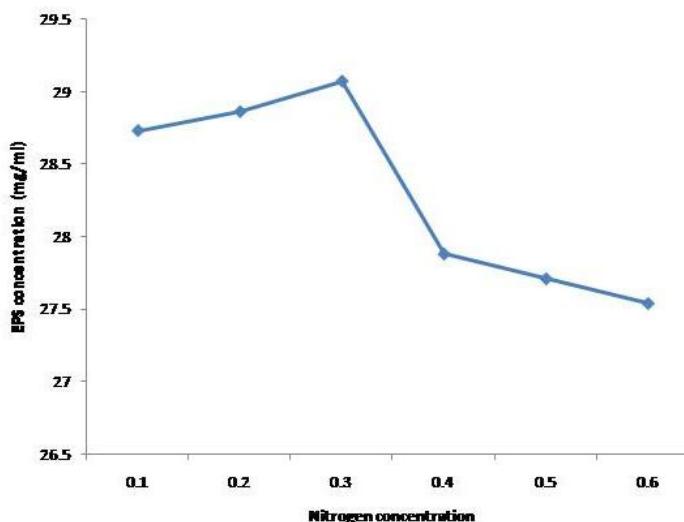


Fig.6 Effect of different nitrogen concentrations on exopolysaccharide production from *L. casei* KL14

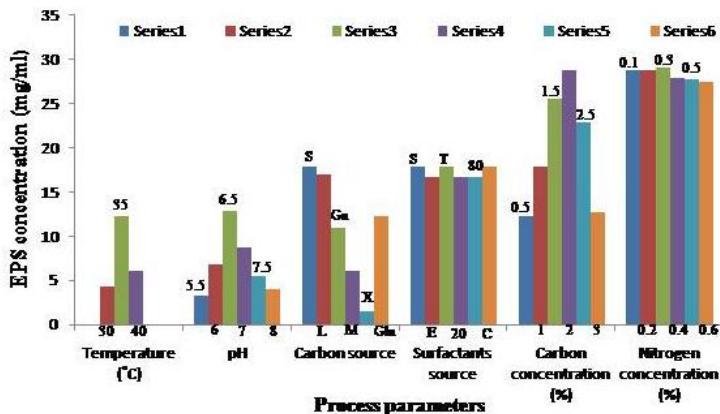


Fig.7 Optimization of various process parameters *i.e.* temperature, pH, carbon sources, surfactants, carbon concentrations (%) and nitrogen concentrations (%). Where, in carbon source S depicts sucrose, L=lactose, Ga=galactose, M=maltose, X=xylene and Glu=glucose, in case of surfactants, S=SDS, E=EDTA, T=triton X100, 20=tween 20, 80=tween 80 and C=CTAB.

It is well known that microbes are more tolerant to environmental conditions than other organisms. However, each species generally has its own characteristics and particular range of temperature (optimum) in which it grows and reproduces best. Incubation temperature is the most important physical factor which affects structure and function of macromolecules. A consistent increase in temperature not only leads to an increase in activity reaction kinetics but also accelerates the denaturation induced by higher physiological temperatures. The reduction in exopolysaccharide activity at higher temperature could be due to damaged and deformed exopolysaccharide structure. Any temperature beyond the optimum range is found to have some adverse effects on the metabolic activities of the microorganisms and it is also reported by various researchers that the metabolic activities of the microbes become slow at lower or higher temperatures. These results are in concurrence with the findings of exopolysaccharide production by Vijayabaskar *et al.*, (2011) who also observed highest production of EPS at 37°C from different bacteria. Same temperature range was used by Habibi *et al.*, (2011) and Zhang *et al.*, (2011) for examining the

exopolysaccharide production. Beyond the temperature 37°C the production declined drastically which may be due to the reason that these organisms are mesophilic in nature and may not be in a position to produce secondary metabolites at the same rate at which they produced at 37°C.

Just like all other living organisms, bacteria need appropriate physiological pH inside their cells. One way of affecting the growth and production of metabolites is by changing pH levels of the media. Bacteria prefer a certain pH balance so as to achieve maximum growth and to maintain beneficial traits such as production of metabolites (Tong and Rajendera, 1992; Selvakumar *et al.*, 2008). It also plays an important role in inducing morphological changes in microbes and in secretion of exopolysaccharide (Gupta *et al.*, 2003). Zhou *et al.*, (2014) showed that favorable pH level for EPS production was found to be 6.8 because EPS which was produced in pH 6.8, 7.0 and 7.5 was significant. The highest production was 14.3 mg/ml after 24 h at pH 6.8.

The choice of carbon substrate influences both the quantity of exopolysaccharides

produced as well as the extent of acylation of exopolysaccharides (Datta and Basu, 1999). It is widely accepted that EPS yields vary based upon carbon substrate utilized. Carbon source in the form of either monosaccharides or polysaccharides influence the production of exopolysaccharides. Many workers also reported the stimulatory effect of lactose on exopolysaccharide production from different organisms (Peiris *et al.*, 1998; Habibet *et al.*, 2011) and also reported the effect of sucrose on exopolysaccharides production from *Lactobacillus* sp. (Liu *et al.*, 2011).

Organisms are variably stimulated to increased exopolysaccharide production; however, exopolysaccharide production by some bacteria is only slightly affected in the presence of surfactants. In general, the percentage increases is greatest for organisms which produce small amounts of exopolysaccharide in the control lacking surfactant. Some workers also reported the stimulatory effect of Tween 80 on exopolysaccharide production from different organisms (Peiris *et al.*, 1998; Habibi *et al.*, 2011) and also reported the effect of Triton X100 on exopolysaccharides production from *Lactobacillus* sp.

Himanshu *et al.*, (2015) reported that carbon concentration played the most important role in cellular growth and exo-biopolymer production. Sucrose at a concentration of 100 g/l was also found to be the best source for EPS production from *B. licheniformis* 221a, at 13.57 mg/ml of medium.

Nitrogenous compounds are utilized by the microbial cells for the synthesis of nucleotides, amino acids and other metabolites. The nature and relative concentration of different nitrogenous sources in the growth medium are important for the synthesis of exopolysaccharide. Lower levels as well as excess of nitrogen are equally

detrimental causing exopolysaccharide inhibition (Sharma and Singh, 2012). When compared to use of other inorganic compounds because it was suggested that by changing the concentration of ammonium citrate EPS increases, would have synthesized inorganic source particular concentration because of bacterial cells grow vigorously and produce EPS. Ruchi *et al.*, (2008) showed maximum ammonium citrate the most suitable inorganic nitrogen source for *Lactobacillus* sp. and the exopolysaccharide production observed was 12 mg/ml.

Overall significance a step up increase of 42.10% was seen in exopolysaccharides production from 12.24 mg/ml to 29.07 mg/ml after optimization of different factors for *L. casei* KL14 is a major achievement of the present study. The present communication explains the enhanced yield of exopolysaccharides obtained after thoroughly examining different influencing parameters from single disciplinary approach (OFAT) after significant statistical analysis. The isolate *L. casei* KL14 increases in its activity to 40% through optimizing the experimental conditions.

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