Production and characterization of multifacet exopolysaccharide from an agricultural isolate, *Bacillus subtilis*

Bhavana V. Mohite[1*]
Sunil H. Koli[1]
Jamatsing D. Rajput[1]
Vikas S. Patil[2]
Tarun Agarwal[3]
Satish V. Patil[1*]

[1] *School of Life Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India*

[2] *University Institute of Chemical Technology, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India*

[3] *Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal, India*

Abstract

This study aims to explore the fermentative production and physicochemical properties of an exopolysaccharide (EPS) produced from agricultural isolate, *Bacillus subtilis* S1 in submerged culture. The structural characterization (Ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy, and ^{13}C Nuclear magnetic resonance spectrometry) revealed that the EPS is an acidic heteropolymer consisting of glucose, glucuronic acid, pyruvic acid, and succinic acid. The non-Newtonian shear thickening nature of EPS with a 1.55×10^7 Da molecular weight is confirmed by rheology analysis. The extracted EPS was 61.3% amorphous with partial crystallinity (38.7%) as confirmed by X-ray diffraction analysis. The EPS shows two-step decomposition and thermal stability up to 300 °C as confirmed by thermogravimetric analysis and differential scanning calorimetry analysis. The EPS has a small *Z*-average particle size (74.29 nm), high porosity (92.99%), high water holding (92.39%), and absorption capacity (1,198%). The biocompatible nature is confirmed by cytotoxic testing on the human keratinocytes cell line. The demonstrated unique characteristics of *Bacillus* EPS presents it as a choice of biomaterial for diverse applications. Volume 00, Number 0, Pages 1–14, 2019

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1. Introduction

The microbial polymers are biocompatible and biodegradable in contrast with synthetic polymers. Therefore, the interest in the production of large quantity of known microbial exopolysaccharide (henceforth EPS) by fermentation is boosted in the past few years [1]. The physiological role of EPS producing bacteria is very diverse, for instance, stress tolerance, protection against drying, grazing protozoan, bacteriophages, antimicrobial agents [2–4], and toxic metal ions [5] along with nutritional benefits like carbon source, chemoattractant, and surface active agent [6].

Bacteria have better EPS secreting ability as compared with fungi and algae [3]. Among which, in particular, Gram-positive *Bacillus* spp. are known to produce a high quantity of EPS. The *Bacillus subtilis* is adapted to the formation of biofilm, which is the intricate community of bacteria formed at air–liquid interface or solid surfaces [7]. The cells in the biofilm are held together by an extracellular matrix consists of amyloid-like fibers and an EPS [8, 9]. In aerobic organisms like *B. subtilis*, the energy created is reliant on the coupling efficacy of ATP synthesis and respiration. In the excess of carbon source, the respiration rate leads to high metabolic activity for the higher production of EPS [6]. The biofilm formation and porous nature of EPS help in the formation of stable colonies that mediate

*Address for correspondence: Bhavana V. Mohite, PhD, School of Life Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Post Box 80, Jalgaon 425001, Maharashtra, India. Tel.: +91 257 2257421; Fax: +91 257 2258403; email: mohite_bhavna@rediffmail.com; or Satish V. Patil, PhD, School of Life Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Post Box 80, Jalgaon 425001, Maharashtra, India. Tel.: +91 257 2257421; Fax: +91 257 2258403; email: satish.patil7@gmail.com.

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