



# An antioxidant exopolysaccharide devoid of pro-oxidant activity produced by the soil bacterium *Bordetella* sp. B4

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## HIGHLIGHTS

- ▶ An exopolysaccharide was purified from *Bordetella* sp. B4.
- ▶ The exopolysaccharide has antioxidant but no pro-oxidant activity.
- ▶ The exopolysaccharide protected human umbilical vein endothelium cells from high glucose-mediated damage.
- ▶ The production of EPS reached to 10.2 g/L when cholesterol was added to the culture medium.

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## ABSTRACT

An exopolysaccharide (EPS) with a molecular weight of 230 kDa, was isolated from *Bordetella* sp. B4. The EPS was identified as linear alpha-1,6-(6-methyl)-glucan with *N*-acetyl-D-glucosamine branches at alpha-1, 4-linkages by IR and NMR spectroscopy. The free radical scavenging capacities of EPS on 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>+</sup>), H<sub>2</sub>O<sub>2</sub>, -OH and lipid peroxidation were 2-, 86-, 134- and 18-fold higher than that of ascorbic acid, respectively. Compared with ascorbic acid, the EPS was more effective in preventing DNA and protein from free radical damage induced by 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). More significantly, the EPS did not degrade DNA and protein by the pro-oxidant effect in the presence of copper ions and H<sub>2</sub>O<sub>2</sub>. Furthermore, EPS could protect human umbilical vein endothelium cells (HUVECs) from high glucose-mediated damage. The production of EPS reached 10.2 g/L in the fermentation medium containing 3.0 g/L cholesterol, suggesting that *Bordetella* sp. B4 was a potential producer of antioxidant EPS.

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## 1. Introduction

Some exopolysaccharides from bacteria and fungi possess immunomodulatory (Mallick et al., 2010), antitumor (Lu et al., 2011), antiviral (Faccin et al., 2007), hypocholesterolemic (Vinogradov et al., 2004) and antioxidative (Vaz et al., 2010) activities. Since antioxidants can also be pro-oxidants (Rietjens et al., 2002), isolation of

antioxidant polysaccharides with no pro-oxidant activity would be beneficial for application in prevention and treatment of diseases associated with oxidative stress. *Bordetella* sp. B4CGMCC 2229, a gram-negative bacterium from soil produces exopolysaccharides, especially in the presence of cholesterol (Yanliang et al., 2010).

In the present paper, we report purification, structure characterization, antioxidant and pro-oxidant activity of the EPS. Moreover, we demonstrate that addition of purified cholesterol oxidase (COD) or H<sub>2</sub>O<sub>2</sub> to strain B4 medium increases EPS production, suggesting that EPS biosynthesis is associated with oxidative stress.

## 2. Methods

### 2.1. Reagents

Cholesterol and ascorbic acid were purchased from Klontech Industrial Sales, Inc. (USA). Beta-cyclodextrin and 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) were obtained from

**Abbreviations:** ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; COD, cholesterol oxidase; CTAB, cetyltriethylammonium bromide; DCF-DA, 2',7'-dichlorofluorescein diacetate; DMSO, dimethyl sulfoxide; EPS, exopolysaccharide; HUVECs, human umbilical vein endothelium cells; IR, infrared spectrum; MTT, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NMR, nuclear magnetic resonance; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive species; TCA, trichloroacetic acid.

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