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Characterization and bioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04

Zhengqi Liu,* Zhihong Zhang,* Liang Qiu,† Fen Zhang,‡ Xiongpeng Xu,* Hua Wei,* and Xueying Tao*[1]
*State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China
†School of Life Sciences, Jiangxi University of Traditional Chinese Medicine, Nanchang, 330004, China
‡School of Life Sciences, Nanchang University, Nanchang 330031, China

ABSTRACT

Exopolysaccharide (EPS) was extracted and purified from *Lactobacillus plantarum* WLPL04, which has been confirmed previously as a potential probiotic for its antagonistic and immune-modulating activity. It has a molecular weight of 6.61×10^4 Da, consisting of xylose, glucose, and galactose in an approximate molar ratio of 3.4: 1.8: 1. Microstructural studies demonstrated that the EPS appeared as a smooth sheet structure with many homogeneous rod-shaped lumps. The preliminary in vitro assays indicated that the EPS could significantly inhibit the adhesion of *Escherichia coli* O157:H7 to HT-29 cells in competition, replacement, and inhibition assays at a dose of 1.0 mg/mL, with an inhibition rate of 20.24 ± 2.23, 29.71 ± 1.21, and 30.57 ± 1.73%, respectively. Additionally, the EPS exhibited a strong inhibition against the biofilm forming of pathogenic bacteria, including *Pseudomonas aeruginosa* CMCC10104, *E. coli* O157:H7, *Salmonella* Typhimurium ATCC13311, and *Staphylococcus aureus* CMCC26003. Furthermore, the EPS showed good inhibitory activity for the proliferation of HT-29 cells. The characteristics and bioactivities of this EPS may provide a promising candidate in developing functional food.

Key words: *Lactobacillus plantarum* WLPL04, exopolysaccharide, characterization, bioactivities

INTRODUCTION

Exopolysaccharide (**EPS**) is a large group of biopolymers that are produced during the metabolic process of microorganisms (Amjres et al., 2015) and can be classified as homo- or heteropolysaccharides, according to whether they are composed of one or more type of sugar. Among the variety of EPS-producing microorganisms, lactic acid bacteria (**LAB**) have been recognized as having safe status, and have attracted more attention due to their potential probiotic properties (Badel et al., 2011).

The EPS produced by LAB has received a great deal of interest in the fermented food industry (Liu et al., 2010; Serafini et al., 2013) because it is used as a natural additive of in situ fermentation to promote the physicochemical properties, such as stabilizing, emulsifying, and providing texture and mouth-feel, of cheese, yogurt, and so on (Badel et al., 2011; Patel et al., 2012; Ahmed et al., 2013). In addition to the application in industry, EPS of LAB has several potential applications due to its biological activities [e.g., antioxidant activity (Li et al., 2014a), antitumor activity (Wang et al., 2014), antibiofilm forming of pathogens (Li et al., 2014b, 2015), cholesterol-lowering activity (London et al., 2014), and immunomodulation (Wu et al., 2010; Li and Shah, 2014)]. Furthermore, EPS was shown to be important for LAB on its potential roles in stress resistance, adhesion, colonization, and host-bacteria interactions (Fanning et al., 2012; Dertli et al., 2013; Lee et al., 2016).

Recently, *Lactobacillus plantarum* attracted plenty of scientific interest due to its broad distribution in ecological niches (pickles, sausage, sour dough, and so on), beneficial effects on the host, and high commercial value for dairy products (da Silva Sabo et al., 2014; Kwak et al., 2014). The EPS-producing *L. plantarum* strains were considered beneficial microorganisms and widely used as starters for various fermented dairy products (da Silva Sabo et al., 2014; Kwak et al., 2014; Caggianiello et al., 2016), and the structures and bioactivities of EPS have been disclosed in some publications (Li et al., 2013; Fontana et al., 2015; Zhou et al., 2016). However, the molecular weight (**Mw**) and monosaccharide composition are strain dependent, and so are the biological functions (e.g., antioxidant, antitumor, and antibiofilm activity).

In our previous study, an EPS-producing *L. plantarum* WLPL04 from health human breast milk has been found with antibacterial and antiinflammatory ability, and exhibited antiadhesion capability against patho-

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[1]Corresponding author: 1027txy@163.com

gens to the human intestinal epithelial cells (Jiang et al., 2016). Its genome data have also been reported (Tao et al., 2015). The objectives of the present work were to isolate and purify the EPS from *L. plantarum* WLPL04 and identify its Mw and monosaccharide composition using gel-permeation chromatography (**GPC**) and GC-MS, respectively. Then the physicochemical properties of EPS were investigated using UV-visible spectrophotometer, Fourier-transform infrared (**FT-IR**) spectroscopy, scanning electron microscopy, and atomic force microscopy (**AFM**). Furthermore, the probiotic properties of EPS were evaluated in vitro, including antiadhesion and antibiofilm forming against pathogens, and its antitumor activities.

Due to the increasing incidence of colon cancer and the side effects of current chemical antitumor drugs, developing natural antitumor compounds is becoming critically important to prevent colon cancer (Zhang et al., 2012). Our work provided a pre-fundamental basis for reasonable use of EPS for human health.

MATERIALS AND METHODS

Bacterial Growth, Cell Culture, and Culture Conditions

The EPS-producing strain *L. plantarum* WLPL04 was previously isolated from healthy female breast milk (Jiang et al., 2016), and cultured in de Man, Rogosa, and Sharpe broth (Beijing Solarbio Science and Technology Co. Ltd., Beijing, China) at 37°C under anaerobic condition. Indicator strains of pathogens (*Pseudomonas aeruginosa* CMCC10104, *Escherichia coli* O157:H7, *Salmonella* Typhimurium ATCC13311, and *Staphylococcus aureus* CMCC26003) were cultured in Luria-Bertani medium at 37°C overnight in a shaker incubator.

Human colon cancer HT-29 cells, purchased from Cell Bank of the Chinese Academy of Sciences (Beijing, China), were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (ExCell Bio Co., Ltd., Shanghai, China) and a standard mixture of antibiotics (100 U/mL of penicillin, 100 μg/mL of streptomycin; Beijing Solarbio Science and Technology Co. Ltd., Beijing, China) in an atmosphere of 5% (vol/vol) CO_2 at 37°C, in the 75-cm^2 cell culture flasks, and the medium was replaced every 2 d.

EPS Isolation and Purification

The EPS was isolated and purified as described previously (Zhang et al., 2016). Namely, after incubation at 37°C for 24 h, culture of *L. plantarum* WLPL04 was centrifuged at 9,000 × *g* for 5 min at 4°C to remove the bacteria, and then added with 2 volumes of chilled absolute ethyl alcohol. The solution was centrifuged at 10,000 × *g* for 20 min at 4°C. The precipitates were collected and dissolved in Milli-Q water (Millipore, Shanghai, China) to dialyze against Milli-Q water for 3 d at 4°C, which was changed twice a day. Crude EPS was obtained by lyophilizing, which was additionally purified to reduce the DNA and protein content. Briefly, the crude EPS powder dissolved in 50 m*M* Tris-HCl, 10 m*M* $MgSO_4 \cdot 7H_2O$ (pH 7.5) at a final concentration of 5 mg/mL and treated with DNase I (final concentration 2.5 μg/mL, Sigma, St. Louis, MO) at 37°C for 6 h, followed by Pronase E (final concentration 50 μg/mL, Sigma) treatment at 37°C for 18 h. Afterward, trichloroacetic acid was added (12% final concentration) to precipitate enzymes and residual AA or peptides, and the mixture was stirred for 30 min at room temperature. The solution was centrifuged at 10,000 × *g* for 20 min at 4°C to collect supernatant, which was adjusted to pH 4.0 to 5.0 with 10 *M* NaOH. After dialysis at 4°C for 3 d against Milli-Q water (water was changed twice daily), the dialyzed retentate was finally freeze-dried to get pure EPS powder for subsequent experiments.

The EPS was suspended in distilled water (1 mg/L) and the concentration was determined by the phenol-sulfuric acid method (DuBois et al., 1956) using glucose as standard, and the purity of EPS was analyzed by a UV-visible spectrophotometer (TU-1901, Persee, China) in the wavelength range of 200 to 600 nm.

Molecular Weight Analysis of EPS

The Mw of the purified EPS was determined by GPC equipped with a TSK G-5000 PWXL column (7.8 × 300 mm, TOSOH, Tokyo, Japan) and TSK G-3000 PWXL column (7.8 × 300 mm, TOSOH) in conjunction with a refractive index detector (Waters 2414, Milford, MA). The samples (2.0 mg/mL, 20 μL) were injected and eluted with 0.02 mol/L KH_2PO_4 solutions at 35°C with a flow rate of 0.6 mL/min. Data were collected and processed using Breeze 1 GPC software (Waters). Molecular weights were calculated according to the relative molecular mass of dextran standards (Sigma).

Determination of Monosaccharide Composition of EPS

A total of 5 mg of the purified EPS was hydrolyzed with 2 mL of 2.0 *M* trifluoroacetic acid at 100°C for 30 min. The hydrolysates were then repeatedly co-concentrated with methanol to dryness, and converted to its trimethylsilyl derivative by adding 0.2 mL of trimethylchlorosilane, 0.4 mL of hexam-ethyldisilazane,