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Exopolysaccharide from *Lactobacillus plantarum* LRCC5310 offers protection against rotavirus-induced diarrhea and regulates inflammatory response

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ABSTRACT

We aimed to determine the effects of *Lactobacillus* strains against rotaviral infections. Rotaviruses are the major causative agent of acute gastroenteritis in infants and children worldwide. However, to date, no specific antiviral drugs for the treatment of rotavirus infection have been developed. We identified 263 *Lactobacillus* strains from 35 samples of the traditional Korean fermented vegetable food, kimchi. Among them, *Lactobacillus plantarum* LRCC5310, more specifically the exopolysaccharides produced by these cells, were shown to have an antiviral effect against human rotavirus Wa strain in vitro. In vivo, the oral administration of exopolysaccharides for 2 d before and 5 d after mouse infection with the murine rotavirus epidemic diarrhea of infant mice strain led to a decrease in the duration of diarrhea and viral shedding and prevented the destruction of enteric epithelium integrity in the infected mice. We demonstrated here that the exopolysaccharides extracted from *L. plantarum* LRCC5310 can be used for the effective control of rotavirus infection.

Key words: *Lactobacillus plantarum*, rotavirus, diarrhea, exopolysaccharide

INTRODUCTION

Rotavirus is the leading cause of severe diarrhea in newborns and young children worldwide, and it was estimated to be responsible for approximately over 453,000 rotavirus deaths in children annually (Tate et al., 2012). Two live, oral, attenuated rotavirus vaccines [Rotarix (GlaxoSmithKline, Brentford, UK) and RotaTeq (Merck, Kenilworth, NJ)] were recommended

by the World Health Organization in 2009 (Tate et al., 2016); these vaccines were shown to be highly efficient against severe rotavirus diarrhea, ranging from a 45 to 90% success rate (Velázquez et al., 2017). However, unusual and vaccine-derived reassortant rotavirus strains have recently been reported in the feces of vaccinated infants, and these were cases of sibling transmission or co-infection with human and animal rotavirus strains (Hemming and Vesikari, 2014; Than et al., 2015; Jeong et al., 2016). Therefore, novel approaches to the treatment and prevention of the infectious diarrhea caused by rotavirus are required.

Recently, exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) attracted much research attention in the field of probiotics, as they are natural biopolymers (Patel and Prajapat, 2013). Exopolysaccharides are large, structurally diverse polysaccharides that permeate the extracellular environment in the form of capsules or biofilms (Kleerebezem et al., 2010), and these molecules help bacteria to survive extreme environmental conditions (Nichols et al., 2005). Furthermore, they have potential health benefits, such as pathogen growth inhibition (Patten and Laws, 2015), antiviral activity (Gugliandolo et al., 2014), and immune stimulation (Ciszek-Lenda et al., 2011; Hidalgo-Cantabrana et al., 2012; Inturri et al., 2017), together with the ability to adhere to the cell surface (Caggianiello et al., 2016).

Lactobacillus plantarum is a bacterium with a generally-recognized-as-safe status, as determined by the US Food and Drug Administration, found in various habitats, such as vegetables, meat, sausages, and cheese (Tanganurat et al., 2009). This bacterium is generally used in food industry applications, including in yogurt, fermented vegetables, and beverages (Brinques and Ayub, 2011). Many potential health benefits of *L. plantarum* have been reported, such as effects on cholesterol, diarrhea, and irritable bowel syndrome (Barreto et al., 2014). After growing in glucose or sucrose, *L. plantarum* strains can produce EPS (Ismail and

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Nampoothiri, 2010), and the reported benefits include antioxidant (Zhang et al., 2013), antitumor (Shin et al., 2016), anti-inflammatory activity (Toshimitsu et al., 2017), as well as antiviral activity against herpes simplex virus (Matsusaki et al., 2016); however, no effects against rotavirus have been reported previously.

Kimchi is a Korean traditional fermented vegetable food, known for its beneficial effects on human health, including its antitumor (Kim et al., 2014b; Kwak et al., 2014), antimicrobial (Chang and Chang, 2011), antioxidative (Kim et al., 2014a; Xing et al., 2015), antiobesity (Lee et al., 2015), and immune system-stimulating activities (Lee et al., 2014). The fermentation of kimchi is induced by using plant microflora under a variety of conditions, including different bacteria, such as *Lactobacillus* and *Leuconostoc*, as well as yeast (Chang et al., 2010). Among them, *L. plantarum* is the predominantly used bacterium at the middle and later stages of fermentation due to the high acid production, resulting in the acidification of kimchi (Lee et al., 2016).

To date, many studies discussing the processing of kimchi have been conducted, but further analysis of the beneficial functions of the kimchi-isolated LAB should be performed (Khan and Kang, 2016). Therefore, we investigated the antirotavirus activity using the bacterial supernatant, lysate, and the EPS obtained from *L. plantarum* LRCC5310 isolated from the Korean traditional fermented food kimchi in vitro and in vivo.

MATERIALS AND METHODS

Screening, Isolation, and Identification of *Lactobacillus plantarum* LRCC5310

Thirty-five kimchi samples were obtained from various local markets in Jecheon, Chungcheongbuk-do, Republic of Korea. The samples were cut into pieces and blended with 10 mL of peptone water (0.85% mass/vol) in a tube. After serial dilution, the samples were spread on de Man, Rogosa, and Sharpe (MRS; Difco, Detroit, MI) agar plates and incubated at 37°C for 2 to 3 d in an aerobic incubator (Sanyo, Osaka, Japan). Representative single colonies were streaked several times to obtain pure cultures on MRS plates. The LAB isolates were identified by 16S rRNA gene sequence analysis. Amplification of the 16S rRNA was conducted by PCR as per established procedures (Lane, 1991). The 16S rRNA amplicon was sequenced using a 3730 automatic DNA sequencer (Applied Biosystems, Foster City, CA) and the obtained sequences were analyzed using the NCBI BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The biochemical characteristics were determined using the API 50 CHL kit according to the manufacturer's instructions (bioMérieux, Marcy-

l'Étoile, France; <https://apiweb.biomerieux.com>). Finally, 263 LAB were identified, and 1 of the identified LAB, *L. plantarum* LRCC5310, was selected for the evaluation of antirotavirus activity.

Preparation of Supernatants, Lysates, and EPS

Lactobacillus plantarum LRCC5310 cells were cultured in the MRS broth at 37°C for 2 d. The supernatant was prepared after centrifugation at $10,000 \times g$ for 20 min at 4°C and the bacterial lysates were obtained by dissolving bacterial colonies in 1 mL of sterile distilled water using a sonicator for 10 s. To prepare EPS, *L. plantarum* LRCC5310 cells were cultured in the MRS supplemented with 5% sucrose for 48 h at 37°C; afterward, the samples were centrifuged at $10,000 \times g$ for 20 min at 4°C. Supernatants were separated and slowly mixed with 2 volumes of cold 95% ethanol. Following a 30-min incubation at 4°C, the polysaccharides were separated by centrifugation at $10,000 \times g$ for 20 min at 4°C. The precipitated EPS samples were dried by using the vacuum at 4°C. The purified EPS samples were identified using field-emission scanning electron microscopy (SEM 515; Philips, Eindhoven, the Netherlands).

Antirotavirus Activity In Vitro

Human rotavirus (HRV) Wa strain and MA104 cells obtained from the Korean Cell Line Bank (Seoul, Korea) were used for the infection and cultivation of rotavirus. The MA104 cells were grown in the α -modified minimum essential medium (α -MEM; Gibco BRL, Grand Island, NY) containing 5% fetal bovine serum (FBS; Gibco BRL) at 37°C in presence of 5% CO₂. The HRV Wa strain (0.2 mL) at 0.01 multiplicity of infection was treated with the obtained supernatant, lysate, and EPS samples or the α -MEM together with 10 μ g/mL of trypsin at 37°C for 1 h in a monolayer of MA104 cells. The MA104 cells were washed twice with PBS and infected using the prepared solutions at 37°C for 1 h. We also included negative control samples containing nontreated cells. The unbound viruses were removed by 2 washes using fresh α -MEM and α -MEM supplemented with 5 μ g/mL of trypsin. The samples were incubated at 37°C for 24 h in a CO₂ incubator (Thermo Fisher, Waltham, MA). Antiviral activity was assessed by observing the cytopathic effect of the viruses using inverted light microscopy (DM IL; Leica, Wetzlar, Germany). Finally, viruses were harvested by 3 cycles of freezing and thawing, the samples were centrifuged at $1,000 \times g$ for 5 min at room temperature, and the supernatants were stored at -80°C until further analyses. The presence of rotaviruses in MA104 cells was determined using quantitative real-time PCR (qPCR).

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