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Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi

Na-Kyoung Lee [a], Kyoung Jun Han [a], Sung-Ho Son [a], Su Jin Eom [a], Si-Kyung Lee [b], Hyun-Dong Paik [a, c, *]

[a] Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Republic of Korea
[b] Department of Bioresources and Food Science, Konkuk University, Seoul 143-701, Republic of Korea
[c] Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Republic of Korea

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ABSTRACT

The probiotic properties of *Lactococcus lactis* KC24 isolated from kimchi were studied. *L. lactis* KC24 retained activity in artificial gastric juice (pH 3.0, 0.1% pepsin for 2 h) and bile acid (0.1% oxgall for 24 h). This strain did not produce the carcinogenic enzyme, β-glucuronidase. *L. lactis* KC24 adhered strongly to Caco-2 cells (16.62% and 18.44% of cell adherence and high hydrophobicity, respectively). Antimicrobial effects of *L. lactis* KC24 were studied by the competition with other microorganisms to adhere to intestinal epithelial cells. *L. lactis* KC24 inhibited the adhesion of 6 pathogens (3 *Listeria monocytogenes* strains and 3 *Staphylococcus aureus* strains) to the mucus layer. The anti-inflammatory effect of *L. lactis* KC24 was demonstrated through the reduction of nitric oxide in the lipopolysaccharide-induced production. The antioxidant effect determined through ferric reducing antioxidant power (FRAP) and inhibition of β-carotene and linoleic acid oxidation was significant with a much higher FRAP value than that observed for ascorbic acid (1 mg/mL). The anticancer effect was observed against gastric carcinoma (AGS), colon carcinoma (HT-29 and LoVo), breast carcinoma (MCF-7), and lung carcinoma (SK-MES-1) cells (>50% cytotoxicity). These results indicate that *L. lactis* KC24 could potentially be used in the formulation of multifunctional probiotics products.

1. Introduction

The intestinal microbiota plays an important role in the health of the host because it is involved in nutritional, immunologic, and physiological functions. The intestinal microbiota could influence host of health (Hooper & Gordon, 2001; O'Sullivan et al., 2005). Probiotics are live microorganisms that are administered in adequate amounts to humans or animals for improving the general health conditions (Monteagudo-Mera et al., 2012). Probiotics must survive in the acidic gastric environment and colonize the gastrointestinal track. Probiotics are known to be beneficial not only for adjusting intestinal balance but also for their anti-inflammatory, antioxidant, and anticancer effects (Klayraung & Okonogi, 2009). However, individual strains have to be tested for each property because the characteristic ascribed to a probiotic are strain-specific (Lee, Kim, Han, Eom, & Paik, 2014; Monteagudo-Mera et al., 2012).

The protective effects of probiotic bacteria against gastrointestinal pathogens have been studied using various antimicrobial mechanistic techniques such as the production of organic acids and other antimicrobials or inhibition of adhesion of pathogens in *in vitro* cell culture (Alderberth, Cerquetti, Poilane, Wold, & Collignon, 2000; Alp, Aslim, Suludere, & Akca, 2010). Several studies have shown that ingestion of probiotics alleviates the pathological condition of oxidative stress-related model diseases, implying that probiotics can serve as antioxidants to animals (Forsyth et al., 2009; Ito et al., 2008). Oxidative stress has been associated with many diseases involving cancer, diabetes, heart disease, neurological disorder and aging (Ibrahim, Mustafa, & Ismail, 2014; Valko et al., 2007). In addition, probiotics have demonstrated anti-inflammatory properties in inflammatory bowel disease and have shown anticancer effects *in vivo* systems and related cell lines (Commane, Hughes, Shortt, & Rowland, 2005; Marcinkiewicz et al., 2007).

Lactococcus lactis is widely used as a starter bacteria in the manufacture of fermented dairy or meat products. *L. lactis* has been selected as a new probiotic organism because their safety when

* Corresponding author. Tel.: +82 2 2049 6011; fax: +82 2 455 0381.
E-mail address: hdpaik@konkuk.ac.kr (H.-D. Paik).

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used in various fermented foods (Salminen et al., 1998). Several *L. lactis* studies are focused on their immunomodulatory (Kimoto, Mizumachi, Okamoto, & Kurisaki, 2004), anticarcinogenic (Commane et al., 2005), and antioxidant (Zhang et al., 2011) effects.

Kimchi is a Korean vegetable fermented food, produced by various vegetables including cabbage or radish, salt-fermented seafood, ground red pepper and various seasonings. Kimchi is usually a homemade food and can be a source of probiotic bacteria. A variety of microorganisms including *Leuconostoc* sp., *Lactobacillus* sp., *Lactococcus* sp., and *Weissella* sp. are known to be present in kimchi (Lee et al., 2011). These lactic acid bacteria have been characterized according to taxonomy, probiotic characters, production of antimicrobials, and antioxidants (Lee et al., 2011, 2014).

L. lactis KC24 isolated from kimchi, demonstrated a bactericidal effect against *Listeria monocytogenes* in dairy products through the production of bacteriocin KC24 (Han, Lee, & Paik, 2013). Our study is aimed at investigating the probiotic properties of *L. lactis* KC24 including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects.

2. Materials and methods

2.1. Bacterial strains and culture

L. lactis KC24 was isolated from kimchi, and incubated in MRS broth (Becton Dickinson, San Diego, USA) at 35 °C. *L. lactis* KC24 was maintained in MRS broth containing 20% glycerol at −70 °C. *L. monocytogenes* (ATCC 15313, ScottA, and H7962) and methicillin-resistant *Staphylococcus aureus* (ATCC 33591, ATCC 33593, and ATCC 33594) incubated in tryptic soy broth (Becton Dickinson) at 35 °C were used for antimicrobial mechanism studies.

2.2. Cell cultures

RAW 264.7 cells (murine macrophage cell line, KCLB 40071), MRC-5 cells (human lung cell line, KCLB 10171), SK-MES-1 cells (human lung carcinoma cell line, KCLB 30058), Caco-2 cells (human colon adenocarcinoma, KCLB 30037), HT-29 cells (human colon adenocarcinoma cell line, KCLB 30038), LoVo cells (human colon adenocarcinoma cell line, KCLB 10229), AGS cells (human stomach adenocarcinoma cell line, KCLB 21739), HeLa cell (human cervix adenocarcinoma cell line, KCLB 1002), and MCF-7 cells (human breast adenocarcinoma cell line, KCLB 30022) were obtained from the Korean Cell Line Bank (KCLB; Seoul National University, Seoul, Korea). The cell lines were cultured in RPMI 1640 (in LoVo, AGS, and MCF-7 cells) or Dulbecco's Modified Eagle Medium (MEM; Gibco, Grand Island, NY, USA) (in RAW 264.7, MRC-5, SK-MES-1, Caco-2, HT-29, and HeLa) as strain-dependent medium containing 10% fetal bovine serum (FBS; Gibco) and 1% streptomycin/penicillin (Gibco) at 37 °C in an atmosphere of 5% CO_2 and 95% air.

2.3. Tolerance to artificial gastric juice and bile acid

The tolerance to artificial gastric juice was determined at a controlled pH 3.0 by incubation in MRS broth containing 0.1% (w/v) pepsin (Sigma–Aldrich, St. Louis, MO, USA) for 2 h at 37 °C. The tolerance to bile acid was determined by incubation in artificial bile acid consisting of MRS broth with 0.1% (w/v) oxgall (BD BBL, Franklin Lakes, NJ, USA). The numbers of viable cells were determined by incubating aliquots on MRS agar plates at 37 °C for 24 h.

2.4. Production of enzymes

Enzyme production was assessed using API ZYM kit (Bio-Merieux, Lyon, France); trypsin, α-chymotryptsin, α-galactosidase, β-glucuronidase, lipase (C14), alkaline phosphatase, α-fucosidase, α-mannosidase, cystine arylamidase, esterase (C4), β-glucosidase, valine arylamidase, β-galactosidase, esterase lipase (C8), α-glucosidase, N-acetyl-ß-glucosaminidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, and leucine arylamidase. *L. lactis* KC24 was suspended in sterile saline (0.85% NaCl) at 10^5 CFU/mL and added to each cupule. After inoculation, the cultures were incubated for 4 h at 37 °C. One drop of each of ZYM A and ZYM B reagents was serially added to each cupule. The approximate amount of substrate hydrolysis was determined as color strength.

2.5. Bacterial adhesion to solvents

The bacterial adhesion to solvents test (BATS) was performed with the method described by Kos et al. (2003) with some modifications. Bacterial cells were suspended in phosphate-buffered saline (PBS; pH 7.2) to obtain a concentration of 1×10^8 CFU/mL. The cell suspension (3 mL) was mixed with 1 mL of solvent. Xylene was used as an apolar solvent, chloroform as an electron acceptor and ethyl acetate as an electron donor. The mixture was vortexed for 1 min and allowed to stand for 20 min to separate into two phases. The absorbance of the aqueous phase was measured at 600 nm using a UV spectrophotometer. The affinities to solvents with different physicochemical properties (hydrophobicity and electron donor-electron acceptor interactions) were expressed using the Eq. (1):

$$\mathrm{BATS}(\%) = \left(1 - \frac{\mathrm{At}}{\mathrm{A0}}\right) \times 100 \quad (1)$$

where A_0 and A_t are the absorbance before and after extraction with organic solvents, respectively.

2.6. Autoaggregation and coaggregation assays

Specific cell–cell interactions were determined using autoaggregation and coaggregation assays (Collado, Meriluoto, & Salminen, 2008; Xu, Jeong, Lee, & Ahn, 2009). The log-phase-grown bacterial cells were harvested by centrifugation (5000 × *g*, 15 min). For the autoaggregation assay, each bacterial suspension (4 mL) was vortexed for 10 s and incubated for 5 h. Each hour, 0.1 mL of the upper suspension was transferred to another tube containing 3.9 mL of peptone water, and the absorbance was measured at 600 nm. The autoaggregation was expressed as Eq. (2):

$$\mathrm{Autoaggregation}(\%) = \left(1 - \frac{\mathrm{A5}}{\mathrm{A0}}\right) \times 100 \quad (2)$$

where A_0 and A_5 represent the absorbance at t = 0 h, 5 h, respectively.

For the coaggregation assay, each bacterial suspension (2 mL) was vortexed for 10 s and incubated for 5 h. Each hour, 0.1 mL of the upper suspension was transferred to another tube containing 3.9 mL of peptone water, and the absorbance was measured at 600 nm. The coaggregation was expressed as Eq. (3):

$$\mathrm{Coaggregation}(\%) = \left[(\mathrm{Aprobiotic} + \mathrm{Apathogen}) - \frac{\mathrm{Amix}}{(\mathrm{Aprobiotic} + \mathrm{Apathogen})}\right] \times 100 \quad (3)$$

where A_{mix}, $A_{probiotic}$, and $A_{pathogen}$ represent the absorbance of the mixed bacterial suspension, probiotic, and pathogens, respectively.