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Functionality and safety of lactic bacterial strains from Korean kimchi

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ABSTRACT

The functional characteristics and safety of five different putative probiotic *Lactobacillus plantarum* strains and one *Leuconostoc citreum* strain, isolated from kimchi, were studied. In view of their potential application in food biotechnology, this information was considered necessary, and included the determination of antibiotic resistance, haemolysis, gelatinase activity and biogenic amine production. In addition to a *Lactobacillus fermentum* strain isolated from pig faeces, two commercial probiotic strains (*Lactobacillus rhamnosus* GG, ATCC 53103, and *Lactobacillus plantarum* 299v, both originating from the human intestinal track) were included in the studies for reference purposes. With the exception of *L. fermentum*, which was resistant to a low concentration (MIC: 30 μg/ml) of tetracycline, all strains were susceptible to the 8 antibiotics tested (erythromycin, gentamicin, ampicillin, tetracycline, chloramphenicol, streptomycin, ciprofloxacin, and benzylpenicillin). All strains survived physiological conditions typical of the upper GIT, comprising a pH of 3.0 for 1 h, and a subsequent 2 h under conditions simulating the duodenum, which included the exposure to 10 % of bile salts. Moreover, all strains were resistant to 0.4 % phenol, and were unable to produce any detectable biogenic amines under the test conditions. These preliminary *in vitro* tests indicate the safety and functionality of the five selected *L. plantarum* strains and thus their potential as probiotic candidates.

1. Introduction

Kimchi is the best-known Korean traditional fermented vegetable-based food, for which around 100 different vegetable types and even more recipes may be used. Among the numerous kinds of kimchi, baechu kimchi is the most common, and is prepared with baechu cabbage or “Chinese cabbage” (*Brassica rapa* subsp. *pekinensis*). Kimchi is generally considered as one of the five healthiest foods in the world (Lee et al., 2011a). It is perceived that practically all kinds of kimchi provide beneficial effects to human health by supplying various functional components such as vitamins, minerals, fibers and phytochemicals through various raw materials. These typically include garlic, ginger, red pepper (chili). Yet, in particular the typical lactic acid bacteria (LAB) involved in the fermentation are considered to have health promoting properties. Various beneficial properties of kimchi have been reported and include anti-oxidative activity (Hwang, Song, & Cheigh, 2000; Lee et al., 2004; Ryu, Ryu, Lee, Jeon, & Moon, 2004), anti-aging effects (Kim, Kwon, & Song, 2000; Kim, Ryu, & Song, 2002), anti-mutagenic, anti-genotoxic and anti-tumor activities (Cho, Rhee, Lee, & Park, 1997; Park, 1995; Shin, Chae, Park, Hong, & Choe, 1998), anti-microbial activity (Sheo & Seo, 2003), immune stimulation (Kim, Kwon, Song, Lee, Youn, & Song, 1997), weight-controlling, lipid-lowering, and anti-atherogenic activity (Kim & Lee, 1997; Kim et al., 2004; Kwon, Chun, Song, & Song, 1999; Sheo, & Seo, 2004). A particular advantage of kimchi is that, in contrast with sauerkraut, it is being served in a raw (uncooked) state with almost every meal, thereby continuously providing live micro-organisms to the human gastro-intestinal tract (GIT). Typically, a Korean adult consumes average of 50–200 g of kimchi per day (Kim & Chun, 2005).

The kimchi fermentation is typically characterized by LAB of which species are “generally considered as safe” (so-called “GRAS” status in the USA) and is dominated by strains of the genera *Lactobacillus, Leuconostoc, Weissella* and *Pediococcus.*

The LAB genera and particularly species involved in traditional lactic fermentations, are generally considered to be safe for human

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use, both according to basic principles laid down for "Generally Recognised as Safe" ("GRAS") status, and by the "Qualitative Presumption of Safety" ("QPS") risk assessment approach. Yet, in recent years, strains of the genera *Lactobacillus, Leuconostoc, Pediococcus*, and *Bifidobacterium* have been isolated from infective lesions and clinical specimens on rare occasions. Such infrequent cases of infection are estimated to represent about 0.05% to 0.4% of cases of infective endocarditis or bacteraemia (Gasser, 1994; Saxelin et al., 1996; Borriello et al., 2003). Even when a pathogenic nature could not be established, and in general an "opportunistic" background may explain these rare cases (Salminen, von Wright, Ouwehand, & Holzapfel, 2000), a final "proof" of safety is presently either required or desired for new strains isolated from traditional fermented foods such as kimchi, before their use in food biotechnology (Franz, Hummel, & Holzapfel, 2005).

Physiological properties of LAB strains typical of kimchi may contribute in various ways to human health, and deserve more detailed investigation, because major contributions to the health benefits of kimchi are considered to be related to the viable LAB population (Park & Kim, 2010; Lee et al., 2011a, b). On the other hand, some authorities presently require proof of safety of newly isolated LAB strains before their introduction, at elevated levels, for commercial use in food biotechnology. Applications of such strains include their use as starter cultures, for bio-preservatives and as probiotics. The assumption of "GRAS" status is commonly based either on a long period of safe use of a strain or is a reflection of the wide acceptance of lactic fermentations throughout human history (Holzapfel, 2002). In the USA, the Food and Drug Administration (FDA) and its Division of Biotechnology and "GRAS Notice Review", have the responsibility of safety ("GRAS") regulating a new microbial strain under the conditions of its intended use in food production or processing (Franz, Hummel, & Holzapfel, 2005). As mentioned, the novel "decision-tree" approach of the EU was introduced for a pre-market safety assessment of microorganisms intended for application in food biotechnology. By this "Qualified Presumption of Safety" (QPS), the safety of a defined taxon (genus or group of related species) can be made on the basis of four "pillars": (a) established identity, (b) body of knowledge, (c) possible pathogenicity, and (d) end use. Exclusion or qualification of safety concerns should result in granting QPS status for a given taxonomic group (EFSA, 2007).

One particular requirement for such "new" strains is the proof of absence of transferable resistance to therapeutic antibiotics. Another primary criterion for functionality of a strain in the human gut is the ability to survive the passage of the upper GIT, and its interaction under conditions typical of the small intestine. The present work was conducted on 6 different potentially probiotic strains isolated from kimchi with the purpose of providing information on their functional characteristics and safety which were deemed necessary for their application in food biotechnology. The studies focused on determinations of antibiotic resistance, haemolysis, gelatinase activity and biogenic amine production. For reference purposes, one *Lactobacillus* strain isolated from pig faeces and two commercial probiotic strains were included in the studies.

2. Materials and Methods

2.1. Strains

In collaboration with the Technology Innovation Center, CJ Foods R&D, Seoul, Korea, six lactic acid bacterial strains, CJLP55, CJLP56, CJLP133, CJLP136, CJLP243 and CJGN34, were isolated from various baechu kimchi batches (kimchi made from Chinese cabbage as major ingredient) fermented below 10 °C, using MRS agar for plating. An additional strain, CJCS1, was isolated from pig faeces, using MRS agar for plating, and this strain was selected for reference purposes. Representative colonies were picked from plates with the highest dilution still showing growth and were subsequently purified. Preliminary phenotypic identification was based on cell morphology and arrangement, determination of homo- or hetero-fermentation mode of glucose, and the determination of the stereo-isomer of the lactic acid produced from glucose. Growth at different temperatures and pH, and tolerance towards NaCl were tested in MRS broth. Carbohydrate fermentation patterns were determined at 37°C by using API CH50 strips and the API CHL medium system according to the manufacturer's instruction (bioMérieux, Marcy l'Etoile, France). Final identification was confirmed by amplifying the 16S rRNA gene of the selected strains by PCR using primers (27F 5'- AGA GTT TGA TCC TGG CTC AG-3', 1492R 5'-GGT TAC CTT GTT ACG ACT T-3'). PCR products were sequenced bi-directionally at the SolGent company (SolGent, Korea). The partial 16S gene sequences were compared with sequences in the nucleotide database of GenBank (accession numbers: NC-007576 for *Lactobacillus sakei*, and NC-014554 for *L. plantarum*).

Lactobacillus plantarum strains CJLP55, CJLP56, CJLP133, JLP136, and CJLP243, and *Leuconostoc citreum CJGN34* were isolated from kimchi and selected for further investigation. *Lactobacillus fermentum* CJCS1 was isolated from pig faeces and served as (intestinal) control, together with the commercial probiotic strains *Lactobacillus rhamnosus* GG (ATCC 53103) and *L. plantarum* 299v, both originating from human intestinal track.

Experiments were standardized as follows. All strains were stored at -80°C in MRS broth with 20% (v/v) glycerol added. All stock cultures were propagated twice in MRS broth for 18 h before each experiment.

2.2. Growth conditions

A growth curve of each strain was established by plate counting to determine the time point of the occurrence of the late log phase at 37 °C. Every strain was tested under the same standardised conditions in MRS broth at 37 °C. Each strain was incubated for 18 h before the test, subsequently to a 1 % (v/v) inoculation. Viable numbers were determined every h by plate counting over a period of 18 h.

2.3. Studies on survival under gastrointestinal conditions

Essential for selection of bacteria as potential probiotics is their ability to survive under physiological conditions typical of the upper gastro-intestinal tract (GIT). Resistance to acid and bile were tested in MRS broth at pH 2.0 to 3.5 and in MRS broth with bile salts, using the *in vitro* gastro-intestinal model for simulated stomach duodenum-passage (SSDP) (Haberer, Du Toit, Dicks, Ahrens, & Holzapfel, 2002, modified).

A simulated stomach duodenum passage assay was designed to represent a simplified and standardised test system giving predictive values for the assumed survival of LAB in the human stomach and duodenum under 'normal' conditions (Schillinger, Guigas, & Holzapfel, 2005). MRS broth was prepared following the manufacturer's instructions. The pH was adjusted to 3.0 with 5 M HCl and the medium dispersed in the flasks containing the required volume for the test set-up, followed by sterilization at 121 °C for 15 min. Synthetic duodenum juice was prepared by completely dissolving $NaHCO_3$ (6.4 g L^{-1}), KCl (0.239 g L^{-1}), and NaCl (1.28 g L^{-1}) in distilled water. The pH was adjusted to 7.4 with 5 M HCl before sterilizing at 121 °C for 15 min. The oxgall solution was prepared by reconstituting 10 g of oxgall in 100 ml distilled water and sterilizing at 121 °C for 15 min.