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Evaluation of functional properties of lactobacilli isolated from Korean white kimchi

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ABSTRACT

Kimchi, probably Korea's most famous traditional fermented food, is well known for its beneficial properties. Among several hundred different types of kimchi in Korea, white (*baek*) kimchi is prepared without chilli and is widely appreciated also by non-Koreans because of its unique mild flavour. In an approach to identify the bacteriological basis for proposed health benefits, we isolated 11 *Lactobacillus* strains from six samples of white kimchi, and investigated their safety and functional features. These strains represented the species *Lactobacillus brevis, Lactobacillus plantarum* and *Lactobacillus sakei* that dominated the populations within a range of 3×10^6 to 4×10^8 CFU/mL. Following safety assessment based on antibiotic resistance and biogenic amine production, 7 different strains were selected for further studies including evaluation of their adaptation to cabbage juice and resistance to phenol. Growth in and adaptation to the cabbage juice was favourably influenced by addition of 2% salt. Final selection was based on *in vitro* passage of simulated stomach duodenum conditions (SSDP model). The strains *L. plantarum* HAC01 and *L. sakei* HAC10 were administered to a diet-induced obese (DIO) mouse model receiving a high-fat (HF) diet to assess their functionality *in-vivo*. Animal groups receiving the viable strains showed significantly lower body weight and total weight gain during 8 weeks compared to the high-fat control group. This study provides preliminary information on the use of *in-vitro* and *in-vivo* features for safety and functionality evaluation of *Lactobacillus* strains from white kimchi. These "first-level" criteria for strain selection may serve as model, thereby facilitating potentially new probiotic developments.

1. Introduction

Fermentation is one of the most widely used practices of preserving staple foods around the world. Over the centuries diverse developments in food fermentations were driven by the need for safe and wholesome foods, environmental factors, and the availability of raw materials and condiments, and, in addition, were influenced by consumer preferences and cultural traditions. Fermentation is not only a means of increasing the shelf life of a food raw material, but is also known to influence quality and functionality of foods in a positive way, e.g., by improving taste and flavour, and beneficially impacting host health. Positive perceptions of microbes are thus associated with desired changes in the food raw material during fermentation. Traditionally, fermented foods have been valued by many cultures for their health benefits and even therapeutic properties (Holzapfel, 2002; Mathara, Schillinger, Kutima, Mbugua, & Holzapfel, 2004). Beneficial health effects of fermented foods are closely related to specific bacteria, in particular lactic acid bacteria (LAB). Studying the interactions of these bacteria within the gastro-intestinal ecosystem has become a major challenge towards clarifying the complex mechanisms basic to the claimed health effects. This area represents a challenging and exciting field of multidisciplinary research, both for gastroenterologists, molecular biologists, microbiologists, food scientists and human physiologists. In spite of developments in food processing and preservation techniques, allowing us to enjoy fresh and safe dishes daily, an increasing number of scientific publications are

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revisiting benefits of functional microorganisms involved in traditional fermentation. Thus far, fermented dairy products such as yogurt have been considered both as potential source of beneficial (probiotic) bacterial strains and an ideal matrix for delivering such functional strains (Rivera-Espinoza & Gallardo-Navarro, 2010). However, part of the focus has now been shifted to a range of non-dairy fermented fruits and vegetables, typical of Asia (Swain, Anandharaj, Ray, & Parveen Rani, 2014) and fermented plant raw materials, in particular cereals, of Africa (Franz et al., 2014) and Europe (Todorov et al., 2008) as ecosystems of potentially beneficial stains.

Beneficial microorganisms can be grouped according to definitions suggested by the *European Food and Feed Cultures Association* (EFFCA, 2015). Microbial Food Cultures (MFC) are defined as preparations or formulations "consisting of concentrates of one or more microbial species and/or strains including unavoidable media components carried over from the fermentation and components, which are necessary for their survival, storage, standardisation and to facilitate their application in the food production process". Starter cultures comprise "MFC preparations used as food ingredients in one or more stages in the food manufacturing process, which develop the desired metabolic activity during the fermentation or ripening process. They contribute to one or multiple unique properties of food stuff especially in regard to taste, flavour, colour, texture, safety, preservation, nutritional value, wholesomeness and/or health benefits" (EFFCA, 2015; Herody, Soyeux, Hansen, & Gillies, 2010). Strains exerting health benefits are collectively grouped as probiotic cultures based on a "consensus definition" by WHO/FAO, (2001) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host".

Kimchi is probably the most famous traditional fermented food of Korea. It is prepared mainly with Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), but other raw materials such as radish and leek are also being used in conjunction with various condiments such as garlic, ginger and red pepper (chilli), resulting in several hundreds of different recipes. The type and combination of raw materials used may decisively influence the diversity and domination of LAB involved in the fermentation. Among the different types of kimchi "*baek kimchi*" is rather special because of its white colour and mildness, contrasting most types of kimchi characterised by a red colour by addition of chilli, the most common condiment. *Leuconostoc* spp. typically dominate the early phases of kimchi fermentation, and are soon succeeded by *Lactobacillus* spp., bringing the total population up to $>10^8$ CFU/mL after 6 days at 15 °C, and a pH around 4.2, with a subsequent steady decrease of the population (Kim, Ban, Beuchat, Kim, & Ryu, 2012).

A clinical study has shown that consumption of fermented kimchi for three months resulted in reduced body fat mass in obese and overweight patients compared to the group consuming fresh (unfermented) kimchi. Moreover, other health indicators such as blood pressure, glucose, insulin and cholesterol levels were also significantly reduced in the group consuming fermented kimchi (Kim et al., 2011). When comparing fresh (unfermented) kimchi with the fermented product, the results imply that lactic acid bacteria (LAB), representing the dominating microorganisms in the fermented product, play a beneficial role towards improving the obese-related status of the host. Several other studies have shown an effect of LAB strains against metabolic disease. However, results are still controversial and the mechanisms for explaining the effect still require further in depth studies (Kadooka et al., 2010; Lee, Jenner, Low, & Lee, 2006; Wang et al., 2015). We therefore considered it a major challenge to link postulated anti-obesity properties of kimchi with specific LAB strains.

The purpose of this study was to isolate LAB strains typical of Korean white (baek) kimchi and to select potentially probiotic strains based on their functional properties. Our focus has been on strains of *Lactobacillus* spp., representing the major putatively probiotic group within the LAB. In this study we isolated several *Lactobacillus* strains and, after confirming their safety and identity, evaluated their functional characteristics under both in-vitro and in-vivo conditions with the view on their potential application as beneficial probiotic strains.

2. Materials and methods

2.1. Isolation and identification of LAB strains from white kimchi

Samples of white kimchi were obtained in a state of active fermentation from local Korean markets and restaurants and analysed for the viable bacterial population. Not knowing the exact former history, fermentation was continued for a period of 10–15 days at 4 °C after purchase. The pH of each sample was measured using an Orion 2-Star pH meter (Thermo Scientific, USA) directly before plating of a sample. Each kimchi sample was blended with 90 mL of NaCl (0.85% m/v) in a sterile plastic bag for 5 min at 200 rpm using the Stomacher®400 Circulator (Seward, UK) and, after serial dilution in 0.85% NaCl, spread plated onto an MRS agar plate (5.5% of Lactobacillus MRS broth, BD Difco, USA, and 1.5% of Bacteriological Agar, Affymetrix, USA). After incubation at 37 °C for 24–48 h, colonies from the highest dilutions (10^{-6} and 10^{-7}) were selected for isolation and purification. Comparative colony morphology was used as a first step for quantifying presumptive strain diversity on a plate, and was followed by microscopy (Imager.A2, ZEISS, Germany) for determining the cell morphology, with rod-shapes varying from short rods (single and in pairs) with rounded ends (presumptively *Lactobacillus brevis*), to "plump" short to medium short rods (presumptively *Lactobacillus plantarum*), to coccoid rods (presumptively *Lactobacillus sakei*). Catalase activity was determined from colony growth on MRS agar (BD Difco, USA) at 37 °C for 24–48 h by using 3% H_2O_2. Catalase negative strains were identified by bi-directional 16S rDNA sequencing performed by Solgent (Korea), and further analysed using BLAST database with a sequence-matching program.

2.2. Bile salt deconjugation

To assess bile salt hydrolytic (BSH) activity of each strain, the BSH screening medium of Dashkevicz and Feighner (1989) was used and 10 μL of each strain were inoculated onto a 5 mm paper disc on the plate. A noticeable precipitation zone after 24–48 h incubation at 37 °C was considered as indication of bile salt deconjugation.

2.3. Haemolysis and gelatine hydrolysis

Bacterial strains grown at 37 °C for 16–18 h were used for both experiments. Ability to produce hemolysins was determined by streaking each strain onto a blood agar plate containing 5% of sheep blood (Hanil Komed, South Korea), followed by incubation at 37 °C for 24–48 h. The gelatine hydrolysis test was performed in Nutrient Gelatine medium, which contained 5 g/L of peptone, 3 g/L of beef extract and 120 g/L of gelatine. The medium was distributed in test tubes and strains were stab-inoculated into it. After incubation at 37 °C for 24, 48 and 72 h, the media were placed in ice for 15 min and then checked for liquefaction (Dela Cruz & Torres, 2013).

2.4. Biogenic amine formation

Strains grown in MRS broth at 37 °C for 16 h were cultured in a