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Tyramine production among lactic acid bacteria and other species isolated from kimchi

Min-Ju Kim, Keun-Sung Kim*

Department of Food Science and Technology, Chung-Ang University, Ansung, Kyungki-do 456-756, South Korea

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ABSTRACT

Lactic acid bacteria (LAB) are naturally found in fermented vegetable products. The ability of 230 kimchi bacterial isolates was investigated to produce tyramine by biochemical and genetic methods. The production of tyramine was determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The presence of the gene encoding the corresponding tyrosine decarboxylase was also determined by PCR assay. After the production of tyramine was confirmed by chromatographic and molecular methods, the bacterial isolates producing the amine were identified by 16S rRNA gene sequence and species-specific PCR analyses. Only a small proportion of the bacterial isolates (14/230 isolates) decarboxylated tyrosine *in vitro*. All of the 14 bacterial isolates that produced tyramine were shown to possess the *tdc* gene, indicating that a positive correlation existed between the production of tyramine and the presence of the corresponding decarboxylase gene. The 14 isolates included three LAB species and one other species: *Lactobacillus brevis* (six), *Lactobacillus curvatus* (four), *Leuconostoc mesenteroides* (two), and *Staphylococcus hominis* (two). This study demonstrated that only a small proportion of LAB and other microbiota growing in kimchi had the ability to produce tyramine.

1. Introduction

All around the world fermented foods and beverages are one of the indispensable components of the dietary culture of every community. In Korea, kimchi is one of the most famous traditional fermented foods. Korean napa cabbage is generally used for kimchi and it is fermented with various additional ingredients including vegetables, radish, salted fish, and spices like red pepper, garlic, and ginger at an appropriate temperature (Kwon & Kim, 2007). There are more than 200 kinds of kimchi prepared that differ depending on the raw vegetables used, processing methods, geographical locations, and seasons, etc. The most important microorganisms implicated in kimchi fermentation are various lactic acid bacteria (LAB) such as *Lactobacillus*, *Leuconostoc, Pediococcus*, and *Streptococcus* (Choi et al., 2002; Mheen & Kwon, 1984). It is known that growth of microbes is affected by the kind of cabbage used, fermentation temperature, and salt content. The unique taste of kimchi is from the various spices used and lactic acid produced by LAB during fermentation.

LAB play important favorable roles in kimchi fermentation, but some of them may produce unfavorable biogenic amines (BAs). BAs are organic bases with low molecular weights that are commonly detected in raw and processed foods (Landete, de Las Rivas, Marcobal, & Muñoz, 2007). BAs in foods are mainly formed by decarboxylation of corresponding amino acids through substrate-specific decarboxylase enzymes derived from microbes present in the foods (Silla, 1996). They are found in a wide range of foods and drinks, including dairy products, fish products, meat products, fermented vegetables, soy products, fruits, nuts, chocolates, and alcoholic beverages such as wine and beer (Ten Brink, Damink, Joosten, & Huis in't Veld, 1990). Consumption of foods containing high amounts of BAs can cause toxicological effects and has been involved in food poisoning incidents. The symptoms caused by consumption of BAs have been reported to be headaches, respiratory distress, heart palpitation, hyper- or hypotension, and several allergenic disorders.

BAs can be grouped into three classes based on their chemical structures, such as aromatic (tyramine and phenylethylamine), heterocyclic (histamine and tryptamine), and aliphatic (putrescine, cadaverine, spermine, and spermidine). Among them, tyramine is the most frequently found in fermented foods. It is formed by decarboxylation of the precursor amino acid, tyrosine, catalyzed by bacterial decarboxylase. A number of bacteria have been characterized as producing excessive amounts of BAs in fermented foods and most attention has been paid to LAB due to their significant roles in the fermentation process. Early investigations revealed

* Corresponding author. Tel.: +82 31 670 3032; fax: +82 31 675 4853.
E-mail address: keunsung@cau.ac.kr (K.-S. Kim).

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Enterococcus faecalis and *Lactobacillus* spp. as organisms with high amino acid decarboxylase activities (Rodwell, 1953). Several other LAB species with similar potentials have been described since, and the roles of such bacteria for BA production in foods have been studied in some cases. *Lactobacillus buchneri* was shown to produce extraordinary amounts of histamine in cheese, even when present in very low numbers in the inoculum (Sumner, Roche, & Taylor, 1990). Tyramine can be produced by *Lactobacillus brevis* in cheese and beer (Zee, Simard, Vaillancourt, & Boudreau, 1981) and by *En. faecalis* in cheese and sausage (Eitenmiller, Koehler, & Reagan, 1978). In addition to these rather undesirable strains in food fermentation, a number of more useful LAB strains, which may even be applied as starter cultures, were shown to produce BAs, including strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Leuconostoc mesenteroides*, and *Lactobacillus curvatus* (Chander, Batish, Babu, & Singh, 1989; Straub, Tichaczek, Kicherer, & Hammes, 1994).

Up until now, tyramine-producing LAB isolates have not been extensively screened from kimchi products even though BAs, including tyramine, have been detected in these products (Mah, Kim, No, & Hwang, 2004). Therefore, the objectives of this study were to extensively screen and identify tyramine-producing LAB isolates from kimchi and to examine by biochemical and genetic methods their capacity to decarboxylate tyrosine to tyramine *in vitro*.

2. Strains and methods

2.1. Strains and growth conditions

A total of 1100 bacterial colonies were isolated from kimchi. Among the isolates, 230 were randomly screened in this study. *Lb. brevis* ATCC 8287, *Lb. curvatus* ATCC 25601, *Le. mesenteroides* ATCC 10830, and *Staphylococcus hominis* ATCC 27844 were used as reference strains while *Bacillus cereus* ATCC 13061 was used as a negative control strain. The LAB strains were grown in de Man Rogosa Sharpe (MRS) broth (Difco, Becton Dickinson Co., Sparks, MD, USA), and the non-LAB strain was grown in nutrient broth (Difco) at 37 °C for 24 h.

2.2. Isolation of LAB from kimchi

Several types of homemade or commercially produced kimchi were collected from various sources. Kimchi samples KC-A through KC-L, KC-Q, and KC-T were collected from 14 different households, and kimchi samples KC-M through KC-P, KC-R, and KC-S were purchased at six different local markets in Ansung, Gyunggi, South Korea.

The LAB strains were isolated from kimchi samples by homogenization, ten-fold serial dilutions with saline solution, and plating on MRS agar containing 2% (w/v) $CaCO_3$. After a 2- to 3-day incubation at 37 °C, 50–100 colonies were randomly picked from each sample and transferred to new MRS agar plates. After incubating for 24 h at 37 °C, the colonies were differentiated and counted based on morphology. At least 10 colonies from each kimchi sample, possibly with different morphologies, were isolated from the highest plate dilution. All isolates considered for further analyses were able to acidify the culture medium. Presumptive LAB colonies were selected, and each colony was purified by three consecutive single colony isolations (Kim & Kim, 2012). Purified colonies were grown in 5 ml MRS broth at 37 °C for 24 h. They were harvested and kept at −70 °C after 80% glycerol was added.

2.3. Screening of tyramine-producing bacterial isolates using a decarboxylase medium

A total of 230 selected isolates and 2 reference strains were grown in MRS broth at 37 °C for 48 h. *Lb. brevis* ATCC 8287 was used as a positive control and *B. cereus* ATCC 13061 as a negative control. Production of tyramine from each isolate and reference strain was determined by using the modified decarboxylase base Moeller medium (pH 5.3) containing L-tyrosine (Difco, Becton Dickinson Co., Sparks, MD, USA) as described previously (Moreno-Arribas, Polo, Jorganes, & Muñoz, 2003). Pyridoxal-5-phosphate was included in the medium at 0.005% since its presence as a cofactor for the decarboxylation reaction has a strong enhancing effect on the amino acid decarboxylase activity (Recsei, Moore, & Snell, 1983). Then, 0.2 mL of pre-grown culture was inoculated into 3 mL of the modified decarboxylase base Moeller medium with or without the amino acid. In a positive reaction, the modified decarboxylase base Moeller medium changed color from yellow to purple. After incubation at 37 °C for 1–7 days under anaerobic conditions, variations in the broth culture from yellow to purple were observed as positive reactions.

2.4. Qualitative analysis of tyramine from tyramine-producing strains using thin layer chromatography (TLC)

After the decarboxylation reaction, the broth culture was centrifuged and filtered through a 0.45 μm syringe filter. The filtered supernatant was analyzed for tyramine on TLC plates as described previously (García-Moruno, Carrascosa, & Muñoz, 2005). Briefly, tyramine was converted to its fluorescent dansyl derivative by adding one volume of the filtered supernatant to one volume of 250 mM disodium phosphate (pH 9.0), 0.1 volume of 4 N sodium hydroxide solution, and two volumes of dansyl chloride solution (5 mg/ml of dansyl chloride in acetone). The reaction mixture was thoroughly mixed and incubated in the dark at 55 °C for 1 h. The samples were then cooled and kept at 4 °C until use. Five μl of each supernatant was spotted on a silica TLC plate (Aluminum Sheets Silica gel 60 F254, Merck, Darmstadt, Germany). The dansylated compounds were separated using a solvent mixture of chloroform:triethylamine (4:1 v/v). The fluorescent dansyl derivative spots were visualized with the aid of a transilluminator with a suitable UV-light source (312 nm).

2.5. Determination of tyramine-producing ability using HPLC

The tyramine produced by the tyramine-producing LAB isolates was quantified by HPLC, according to the procedure developed previously (Mah, Ahn, Park, Sung, & Hwang, 2003).

A stock solution of tyramine was prepared by adding tyramine to distilled water at a concentration of 10,000 ppm and was stored at 4 °C for a month. A working solution of tyramine at the concentration of 100 or 1000 ppm was prepared by diluting 100 μl or 1000 μl of each stock solution in distilled water to bring the solution to a final volume of 10 ml. Internal standard solution was prepared by dissolving 50 mg of 1,7-diaminoheptane (Sigma–Aldrich, St. Louis, MO, USA) in 50 ml of twice-distilled water, and was stored at 4 °C for 1 month.

Tyramine preparations were obtained by adding 9 ml of 0.4 M perchloric acid to 1 ml of the filtered broth culture and homogenizing the mixture by vortexing. After centrifugation at 3000 × *g* for 10 min, the supernatant was filtered through a 0.2 μm syringe filter.

One ml of each tyramine preparation was mixed with 200 μl of 2 N sodium hydroxide and 300 μl of saturated sodium bicarbonate. Then, 2 ml of dansyl chloride (Sigma, St. Louis, MO, USA) solution (10 mg/ml) dissolved in acetone and 200 μl of internal standard solution were added to the mixture and incubated at 40 °C for 45 min. Residual dansyl chloride was removed by adding 100 μl of 25% ammonium hydroxide. After 30 min of incubation at room temperature, the volume of tyramine solution was adjusted with