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The influence of the cell free solution of lactic acid bacteria on tyramine production by food borne-pathogens in tyrosine decarboxylase broth

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ABSTRACT

The function of cell-free solutions (CFSs) of lactic acid bacteria (LAB) on tyramine and other biogenic amine production by different food borne-pathogens (FBPs) was investigated in tyrosine decarboxylase broth (TDB) using HPLC. Cell free solutions were prepared from four LAB strains. Two different concentrations which were 50% (5 ml CFS + 5 ml medium/1:1) and 25% (2.5 ml CFS + 7.5 ml medium/1:3) CFS and the control without CFS were prepared. Both concentration of CFS of *Streptococcus thermophilus* and 50% CFS of *Pediococcus acidophilus* inhibited tyramine production up to 98% by *Salmonella paratyphi* A. Tyramine production by *Escherichia coli* was also inhibited by 50% CFS of *Lactococcus lactis* subsp. *lactis* and 25% CFS of *Leuconostoc lactis*. subsp. *cremoris*. The inhibitor effect of 50% CFS of *P. acidophilus* was the highest on tyramine production (55%) by *Listeria monocytogenes*, following *Lc. lactis* subsp. *lactis* and *Leuconostoc mesenteroides* subsp. *cremoris* (20%) whilst 25% CFS of *Leu. mes.* subsp. *cremoris* and *Lc. lactis* subsp. *lactis* showed stimulator effects (160%). The stimulation effects of 50% CFS of *S. thermophilus* and *Lc. lactis* subsp. *lactis* were more than 70% by *Staphylococcus aureus* comparing to the control. CFS of LAB strains showed statistically inhibitor effect since lactic acid inhibited microbial growth, decreased pH quickly and reduced the formation of AMN and BAs. Consequently, in order to avoid the formation of high concentrations of biogenic amines in fermented food by bacteria, it is advisable to use CFS for food and food products.

1. Introduction

Biogenic amines (BAs) are organic substances formed mainly by decarboxylation of amino acids such as histidine, tyrosine, ornithine and lysine, which result in formation of the corresponding BAs that are histamine (HIS), tyramine (TYR), putrescine (PUT) and cadaverine (CAD), respectively. They are the most frequently encountered BAs in the fermented foods like cheese, meat, fish products, wine, beer, etc. (Silla-Santos, 1996). The formation of BAs in food depends on the quantities of free amino acids and the presence of microorganisms with decarboxylase activities. Microbial strains with high proteolytic enzyme activity also potentially increase the risk of BA formation in food and food products. BAs in foods are capable of adverse effect in humans. For instance, TYR, CAD, PUT, HIS, tryptamine (TRPT) may cause blood pressure, and excessive quantities can trigger migraines, gastric and intestinal problems and allergic responses in sensitive people. Consumption of food containing high levels of TYR causes migraine, headaches and a sudden rise in blood pressure (Joosten, 1988). It is also reported that high level of (100–800 mg/kg) TYR in foods is known to be toxic (Brink, Damink, Joosten, & Huis, 1990).

The roles of LAB and some FBP in the formation of BAs in different types of food and under experimental conditions have been well documented (Chang, Kung, Chen, Lin, & Tsai, 2008; Marino, Maifreni, Moret, & Rondinini, 2000; Özogul & Özogul, 2007). Although amino acid decarboxylases are not widely distributed amongst bacteria, species of many genera such as *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Shigella*, *Photobacterium* are capable of decarboxylating one or more amino acids (Silla-Santos, 1996). The amino acid-decarboxylating activity was reported for some LAB strains like *Lactococcus*, *Lactobacillus*, *Streptococci*, *Pediococcus*, *Enterococcus*, *Oenococcus* and *Leuconostoc* (Coïsson, Ceruttti, Travaglia, & Arlorio, 2004; Landete, Arena, Pardo, Manca de Nadra, & Ferrer, 2008). Buňková et al. (2009) reported that *Lactococcus lactis* subsp. *cremoris*, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp.

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bulgaricus produce TYR, whereas the other tested BAs such as HIS, PUT, CAD, agmatine (AGM), spermidine (SPD) and spermine (SPM), were not formed.

Several LAB species possess amino acid-decarboxylase enzymatic activity and may contribute to the formation of BAs in fermented foods. Zhang, Zhang, and XiaoKui (2009) reported that 13 strains (6 *Streptococcus*, 3 *Enterococcus*, 3 *Lactococcus* and 1 *Lactobacillus*) which were isolated from different sources showed strong abilities to produce BAs, especially TYR and SPM. Omafuvbe and Enyioha (2011) found that some of the LAB strains belonging to *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and *Enterococcus* genera can decarboxylate tyrosine. La Gioia et al. (2011) reported that *S. thermophilus* 1TT45 was able to produce up to 370 mg/L of TYR in milk containing tyrosine. González De Llano, Cuesta, and Rodríguez (1998) found that wild dairy Lactococcal and *Leuconostoc* strains produced TYR ranging from 370 to 807 mg/L according to HPLC analysis confirmation. Lorencova et al. (2012) stated that most of the *Lactobacillus* strains isolated from dairy products formed TYR less than 10 mg/L, whereas *Lactobacillus curvatus* DCT 106 accumulated the highest amounts of TYR (>100 mg/L). The dominant BA found in the culture supernatant of *Lactobacillus brevis* was TYR, which was formed by 96% of the strains (Sebastian, Herr, Fischer, & König, 2011). Gezginc, Akyol, Kuley, and Özogul (2012) reported that most of the *S. thermophilus* isolates which isolated from home-made yogurt have an ability to form BAs, especially TYR and HIS.

The bacteriocins from lactic acid bacteria (LAB) which generally recognised as safe (GRAS) have been paid a great deal of attention as a novel biotechnological approach to control pathogens in foodstuffs. The LAB strains can produce substances such as hydrogen peroxide, weak organic acids, reuterin, diacetyl, bacteriocins, and low-molecular-weight metabolites that inhibit pathogenic organisms (Brashears, Amezquita, & Jaroni, 2005) and also BA production (Mah & Hwang, 2009). So far, most of the studies have been focused on BA formation of selected food or single bacteria isolates. However, there are no data regarding to stimulating or inhibiting effects of CFSs from specific LAB on TYR production by common FBP *in vitro* conditions. Thus, the possible participation of the CFSs of LAB on TYR and other BAs production by several FBPs including *Salmonella paratyphi* A, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* in TDB was investigated.

2. Materials and methods

2.1. Bacterial strains

Lactic acid bacteria strains which are *Lactococcus lactis* subsp. *lactis* IL 1403 (*Lc. lactis*. subs. *lactis*), *Leuconostoc mesenteroides* subsp. *cremoris* DSMZ 20346 (*Leu. mes*. subs. *cremoris*), *Pediococcus acidophilus* ATCC 25741 (*P. acidophilus*), and *S. thermophilus* NCFB 2392 (*S. thermophilus*) were obtained from Sutcu Imam University, (Kahramanmaraş, Turkey) in BGML stock culture.

The selected 4 FBPs were *S. aureus* ATCC 29213 (*S. aureus*), *E. coli* ATCC 25922 (*E. coli*), *L. monocytogenes* ATCC 7677 (*L. monocytogenes*), which were purchased from American Type Culture Collection (Rockville, MD, USA) and *S. paratyphi* A NCTC 13 (*S. paratyphi* A) which was obtained from National Collections of Industrial Food and Marine Bacteria (Aberdeen, UK).

2.2. Preparation of CFS from lactic acid bacteria

The cells of the lactic acid bacteria strains were pre-grown in 10 ml MRS (De Man, Rogosa and Sharpe, Merck) medium (1%, v/v) at overnight incubation at 37 °C, and then 7.5 ml of the culture was added aseptically to 750 ml MRS medium in a 1000 ml serum bottle. Samples were incubated for 16 h at 37 °C without shaking. A cell-free solution was obtained by centrifuging at 9000 rpm for 20 min under refrigeration (at 4 °C) from bacterial suspension. The cell-free solution was sterilized by membrane filter (0.22 μm pore size).

2.3. The pH measurements

The pH value was determined by a digital pH metre (315i: WTW Measurement Systems, Weilheim, Germany) for homogeneous mixtures of CFS.

2.4. Determination of antimicrobial assay of LAB strains

The antimicrobial activity of CFS from the LAB against the pathogens bacteria (*E. coli*, *S. aureus*, *L. monocytogenes*, *S. paratyphi* A) was performed by using the well diffusion assay (Bonadè, Murelli, Vescovo, & Scolari, 2001). The pathogenic test bacteria were incubated in nutrient broth at appropriate temperature for 24 h. Broth culture of pathogenic bacteria (0.1 ml) was inoculated to petri dishes containing 20 ml of Muller Hinton for 24 h. Allowing the media to harden at room temperature for 15 min, wells of 5 mm diameter were made with a sterile cork borer and 50 μl of the cell-free supernatant was placed into each well. After 24 h incubation at 30 °C, the inhibition zones were subsequently examined and the diameter of the inhibition zone was measured with calipers in mm. The antimicrobial activity was determined by measuring the clear zone around the wells (Rammelsberg, 1990).

2.5. Culture media and bacterial extraction

Tyramine production from all FBP strains in this work was monitored using tyrosine decarboxylase broth (TDB). The composition of the broth is 2 g peptone, 1 g Lab-Lemco powder (Oxoid CM0017, Hampshire, England), 5 g NaCl (Merck1.06404.1000, Darmstadt, Germany), 8.02 g L-tyrosine (Sigma T3754, Steinheim, Germany) and 5 mg pyridoxal HCl (Sigma P9130, Steinheim, Germany) in per litre of water. The pH was adjusted according to their optimum growth pH with 1 M KOH (Riedel-de Haen 06005, Seelze, Germany) or 6% trichloroacetic acid (TCA) (Riedel-de Haen 27242, Seelze, Germany). The TDB was pipetted in 10, 7.5 and 5 ml bottles and then autoclaved at 121 °C in 15 min prior to use. Two different concentrations which were 50% (5 ml CFS + 5 ml TDB/1:1) and 25% (2.5 ml CFS + 7.5 ml TDB/1:3) of cell-free solutions (CFS) were prepared and the control was only TDB without CFS.

Nutrient broth (Merck 1.05443.0500, Darmstadt, Germany) was used for propagation of FBP strains and they were incubated according to their optimum growth temperature for 2 or 3 days. Production of biogenic amines was tested by 0.5 ml inoculating each food-borne pathogen strain in TDB for each CFS concentration. All of them were incubated at their optimum growth temperature for 72 h. After that, 5 ml of the broth culture containing FBP strains were removed to separate bottles and then, 2 ml trichloroacetic acid was added. They were centrifuged at 3000×*g* for 10 min and then filtered through a filter paper (Millipore). Finally, a 4 ml bacterial filter from centrifugation was taken for derivatisation stage.

2.6. Derivatisation of bacterial supernatant

A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance reaction with amines. For derivatisation of standard amine solutions, 100 μl was taken (4 ml bacterial supernatant) from each free base standard solution (10 mg/ml). Sodium hydroxide (2 M) was added, followed by 1 ml of 2% benzoyl chloride (dissolved in acetonitrile) and the solution was mixed