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Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria

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ABSTRACT

Organic acids (lactic, acetic, succinic, propionic, formic and butyric acid) production by lactic acid bacteria (LAB) were investigated in various fish infusion broth (anchovy, sea bass, tilapia and trout) and MRS broth (control) by using HPLC method. Significant differences were found in organic acid levels ($P < 0.05$) among bacterial strains. Succinic acid formation was the highest by LAB whilst acetic acid was produced at the lowest levels. The highest lactic acid production was observed with *Lactobacillus lactis* subsp. *lactis* and *Pediococcus acidilactici* in anchovy infusion broth, with values of 2403 and 2345 mg/L, respectively. Acetic acid production was 822 and 803 mg/L by *Lactobacto acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis* in anchovy infusion broth, respectively while succinic acid production ranged from 142 mg/L by *Lb. delbrueckii* subsp. *lactis* in sea bass infusion broth to 9231 mg/L by *Lb. lactis* subsp. *lactis* in MRS broth. Propionic acid formation by *Pc. acidilactici* was 3747 mg/L in sea bass infusion broth whereas *Lb. lactis subsp. cremoris* produced less than that in MRS broth. The result of the study indicated that LAB strains had a great ability to produce succinic acid. Also other organic acid production varied significantly depending on bacterial strains and growth medium.

1. Introduction

Lactic acid bacteria (LAB) are a group of gram positive, non-spore forming cocci or rods (Rattanachaikunsopon & Phumkhachorn, 2010). They can grow at temperatures from 5 to 45 °C and optimum pH condition is 5.5–6.5. These bacteria have complex nutritional requirements that include amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates (Vodnar, Paucean, Dulf, & Socaciu, 2010). LAB is the most important group of microorganisms used in fermented foods. They have crucial importance due to their physiological features, such as substrate utilization, metabolic capabilities and probiotic properties (Hladíková, Smetanková, Greif, & Greifová, 2012). The industrial importance of the LAB is related their acceptance as GRAS status for human consumption (Liu, Han, & Zhou, 2011; Silva, Carvalho, Teixeira, & Gibbs, 2002).

Lactococcus, Lactobacillus, Leuconostoc, Streptococcus and *Pediococcus* are the most commonly used genera as starter cultures in fermentation processes of milk, meat and vegetable products (Hladíková et al., 2012; Nieto-Lozano et al., 2010). The preservative action of starter culture in food systems is attributed to the combined action of a range of antimicrobial metabolites produced during the fermentation process (Caplice & Fitzgerald, 1999). These are end products such as some organic acids (lactic, acetic and propionic acids) (Rattanachaikunsopon & Phumkhachorn, 2010). Organic acids can, therefore, be either naturally present as constituents of the food or can later be added directly or indirectly to the products (Theron & Lues, 2010). Sugar fermentation followed by a reduction in pH due to the production of lactic and other organic acids is an important factor for the inhibition of growth of undesired microorganisms. The low pH makes organic acids liposoluble, allowing them to break through the cell membrane and reach the cytoplasm of pathogens (Djadouni & Kihal, 2012; Haller et al., 2001). Gerez Carbajo, Rollán, Torres Leal, & Font de Valdez (2010) evaluated the antifungal activity of 33 LAB strains. They found that the antifungal activity of these LAB strains was related to the production of lactic acid, acetic acid, and phenyllactic acid. Choesri, Rusmana, Suwanto, and Mubarik (2013) found that 23

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isolates of LAB isolated from an Indonesian fermented fish (bekasam) could inhibit the growth of pathogenic bacteria and LAB inhibition on the pathogenic bacteria was due to production of the organic acid.

The levels and types of organic acids produced during the fermentation process depend on the species of organisms, culture composition and growth conditions (Lindgren & Dobrogosz, 1990; Yang, 2000). Lau and Liong (2014) found that the concentrations of the acids produced by LAB species were strain-dependent and all strains studied showed higher concentration of lactic acid than acetic acid. Urbienė and Leskauskaitė (2006) investigated the formation of benzoic, sorbic and nucleic acids during fermentation of milk with different commercial lactic acid bacteria starters. The organic acids content in fermented milk was affected by the type of commercial lactic acid bacteria starters while the highest concentration of the organic acids was detected in the milk fermented by *Lactobacillus acidophilus* (Urbienė & Leskauskaitė, 2006).

Various LAB strains was isolated in raw and fermented fish products, i.e. *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus divergens*, *Lactobacillus gasseri*, *Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus alimentarius*, *Lactobacillus buchneri*, *Lactobacillus pentosus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus garvia*, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Weisella confuse*, *Aerococcus* spp. (Nair & Surendran, 2005; Paludan-Muller, Huss, & Gram, 1999; Paludan-Muller, Madsen, Sophanodora, Gram, & Moller, 2002; Rusmana, Suwanto, & Mubarik, 2013; Tanasupawat, Shida, Okada, & Komagata, 2000). Detection of organic acids produced by lactic acid bacteria has great significance to the food industry since the most suitable starter cultures have ability to improve food quality and safety. These were selected according to their technological characteristics, including their effectiveness in lactic acid production and pH reduction ability, as well as antimicrobial activity against food-borne pathogens, when used as starter cultures (Hwanhlem et al., 2011). There are various studies related to antibacterial activity of lactic acid bacteria on pathogenic bacteria. De Baere et al. (2013) developed a rapid and sensitive HPLC-UV method to quantitatively detect of formic acid, acetic acid, propionic acid, butyric acid, succinic acid and lactic acid produced during in vitro fermentation. The study suggested that the method used has been successfully approved for the in vitro screening of supernatants of bacterial cultures for the presence of butyric acid. A very limited number of experiments have been conducted on organic acid production by LAB strains (Vodnar et al., 2010; Hwanhlem et al., 2011). Thus, the aim of the study was to determine in vitro organic acid production by LAB in MRS broth and fish (anchovy, sea bass, trout and tilapia) infusion broth. To the best of our knowledge, this is the first report investigating the organic acid production of lactic acid bacteria in fish infusion broth.

2. Material and methods

2.1. Bacterial strains

The used LAB species were *Lactococcus lactis* subsp. *cremoris* (MG 1363), *Lactococcus lactis* subsp. *lactis* (IL 1403), *Lactobacillus plantarum* (FI8595) and *Streptococcus thermophilus* (NCFB2392). They were obtained from Sutcu Imam University, Kahramanmaras, Turkey in BGML stock culture. *Leuconostoc mesenteroides* subsp. *cremoris* (DSMZ 20346), *Lactobacillus acidophilus* (ATCC 11975), *Pediococcus acidilactici* (ATCC 25741) and *Lactobacillus delbrueckii* subsp. *lactis* (ATCC 10697) were purchased from Institute of Refik Saydam Hifzisihha (Ankara, Turkey).

2.2. Fish species

Fish infusion broth (FIB) was prepared using four different marine and fresh water fish species which were anchovy (*Engraulis encrasicolus*), sea bass (*Dicentrarchus labrax*), trout (*Oncorhynchus mykiss*) and tilapia (*Oreochromis* spp.).

2.3. Organic acid analysis

2.3.1. Culture media and bacterial extraction

FIB was prepared according to method of Okuzumi, Okuda, and Awano (1981) with minor modifications. Two hundred 50 g of fish flesh was homogenized with 2 vol of water (w/v), steamed at 100 °C for 1 h and filtered. The filtrate was enriched with 1% glucose and 0.5% NaCl. Protein content of fish infusion broth was determined using Lowry, Rosebrough, Farr, and Randall (1951) method. The pH values of anchovy, sea bass, trout and tilapia infusion broth were 6.40, 6.56, 6.57 and 6.61. The protein and carbohydrate content of anchovy, sea bass, trout and tilapia infusion broth were 6.45, 8.25, 7.84 and 7.5% and 1.6, 1.52, 1.41 and 1.3%, respectively.

MRS broth (Merck 1.10661) was used for propagation of LAB cultures. Lactic acid bacterial strains were incubated at 37 °C for 24 h which after 0.5 mL of these bacterial cultures was removed and put into MRS and FIB broth. The cultures were incubated at 37 °C for 4 days.

Organic acid extraction was performed according to methods of Asami, Hong, Barrett, and Mitchell (2003) with minor modifications. For extraction, 5 mL of the FIB, MRS broth containing LAB strains were removed to separate bottles and then 1 mL metaphosphoric acid added. They were vortexed for 2 min and then centrifuged at 3000 × g for 5 min. After that, bacterial supernatant was taken and filtered through a membrane filter (0.45 μm). They were transferred into a clean HPLC tube for analysis.

2.3.2. Chemical reagents

Metaphosphoric acid (04103) and organic acid standards including lactic acid (L661), acetic acid (45,725), propionic acid (P1386), butyric acid (B103500), formic acid (06419) and succinic acid (14,079) were purchased from Sigma–Aldrich (Munich, Germany).

2.3.3. Analytical method

Organic acid determination was performed using HPLC method according to method of De Baere et al. (2013) with modification. Organic acids were identified by comparison of retention times and area values with standard of lactic acid, acetic acid, propionic acid and butyric acid. The organic acid content was expressed as mg/L for fish samples.

2.3.4. HPLC apparatus and column

A Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector and two binary gradient pumps (Shimadzu LC-10AT), auto sampler (SIL 20AC), column oven (CTO-20AC), and a communication bus module (CBM-20A) with valve unit FCV-11AL was used. The column was C18 reversed phase, Chromosil (250 × 4.6 mm, 5 μm in particle size) for the organic acid analyses.

2.3.5. Chromatographic conditions

Chromatographic separation was carried out using continuous gradient elution with a mixture of 0.5% metaphosphoric acid and acetonitrile with a flow rate of 0.8 mL/min. The total separation time was less than 9 min and the injection volume was 10 μl. Detection was monitored at 210 nm.