THE PRODUCTION OF LACTIC ACIDS FROM *Lactobacillus acidophilus*

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ABSTRACT

The ratio of D- and L-lactic acid produced in media by 39 strains of the *L. acidophilus* group lactic acid bacteria (A_1-A_4, B_1, and B_2 subgroups) was analyzed by high performance liquid chromatography (HPLC). *L. acidophilus* (A1) and *L. johnsonii* (B2) produced the lowest (5 strains average : 41.9%) contents of D-lactic acid in MRS broth, respectively. Generally, the most dominant lactic acid (more than 50%) produced by each strain in MRS broth was a D-type isomers, except for the A1 subgroup. In *L. gasseri* DSM 20243^T (B1), increases in the sugar content in MRS broth induced both a high production of total lactic acids and a high ratio of D-lactic acid. In skim milk, D-lactic acid decreased by 20 - 30% in 5 strain types of the *L. acidophilus* group except *L. amylovorus* JCM 1126^T (A3). By sequentially changing the medium, the content of D-lactic acid in the final MRS broth reverted to almost the same level of that in the initial MRS broth. These results indicated that the ratio of D-lactic acid produced by *L. acidophilus* group bacteria may be controlled by improving fermentative condition.

Key words : Lactic acid, D-lactic acid, *Lactobacillus acidophilus*, HPLC.

INTRODUCTION

Lactobacillus (*L.*) *acidophilus* group lactic acid bacteria have been widely used in fermented milk products such as acidophilus yoghurt and sweet acidophilus milk (10), especially in European countries. The intake of these bacteria from fermented products can have beneficial effects on human health due to the survival and habitation of the bacteria in gastrointestinal tracts (2), as is the case with bacteria of the *Bifidobac-terium* group. *L. acidophilus* group bacteria produce only lactic acids in media and are considered to be superior to *Bifidobacterium* (7) which produced acetic acids and formic acids simultaneously as a result of hetero fermentation. Moreover, *L. aci-dophilus* group bacteria have physiological function (13) which are beneficial for human health such as reducing serum cholesterol, anti tumorigenesis, anti mutagenesis and immunoactivatium (10).

The *L. acidophilus* group has been classified into 6 subgroups (*L. acido-philus* (A1 subgroup), *L. crispatus* (A2), L. *amylovorus* (A3), *L. gallinarum* (A4), *L. gasseri* (B1), and *L. johnsonii* (B2)) by DNA homology and cell wall composition analysis (6). The characteristic location of some strains are known by specifics interactions; *L. gallinarum* (A4) in the chicken intestine, and *L. acidophilus* (A1), *L. crispatus* (A2) and especially *L. gasseri* (B1) in the human intestine.

Lactic acid bacteria produce lactic acids as final metabolic products from carbohydrates and are also known to be antimicrobial components similar to antibiotics, hydrogen peroxide and bacteriocins (12). Lactic acids consist of 2 type of D- and L-isomers and are biosynthesized by D- and L-lactate dehydrogenases (LDH), respectively. Although L-lactic acid is biosynthesized and easily metabolized in the human body, D-lactic acid is difficult to metabolize and occasionally causes metabolic disorders such as acidosis. The World Health Organization (WHO) recommends that a baby less than 3 months old

should not be given more than 100 mg of D-lactic per day. Therefore, special attention must be paid to the selection of lactic acid bacteria in fermented milk products for baby foods. There have been no detail reports on compositional analysis of lactic acids produced by the *L. acidophilus* group, especially the strains of *L. gasseri* (B1), which are the most frequently detected in the human gastro-intestinal tract (11).

In this report, we describe the com-positional characteristics of lactic acids produced by the *L. acidophilus* group, focusing on the possibility of inductive changes in the D-lactic acid ratio by medium controls.

MATERIALS AND METHODS

Bacterial strains and media.

Table 1 shows the 39 bacterial strains used in this study including names, origins and other designations. Each strain was propagated successively 2 times into a Lactobacilli MRS broth (Difto Laboratories, Detroit, U.S.A.) with 1% inoculum at 37 °C for 24 h. Reconstituted skim milk (10% (w/v) in water) was autoclaved at 115 °C for 15 min. Both MRS and MRSL broth, in which lactose was substituted for glucose, were autoclaved at 121 °C for 15 min.

Determination of total lactic acids in media.

The total amount of lactic acids produced in a culture medium (MRS, MRSL or skim milk) after incubation at 37 °C for 48 h with 1% inoculum was determined by the neutralizing titration method. The quantity of lactic acids (mg/ml) was calculated from the total amount (ml) of 0.1N NaOH solution needed for returning to each initial pH value of the MRS broth (6.4), MRSL broth (7.0) or 10% (w/v) skim milk (6.6), with the formula (9 x factor of NaOH x ml of NaOH).

HPLC analysis of lactic acids.

Lactic acid samples were prepared from each culture medium by the method of Otsuka *et al.* (9) with a slight modification. After incubating MRS broth, MRSL broth or 10% (w/v) skim milk (each 5 ml) with 1% inoculum of each strain at 37 °C for 48 h, the cell-free supernatant was ob-tained from each medium by centrifugation (3,000 x g, 10 min, 20 °C). After acidification by the addition of 6N HCl (10 µl), the supernatant (1 ml) was extracted with diethylether (DE, 4 ml) by vigorous shaking (20 sec) using a vortex mixer. After evaporation of the DE extract, 2-hydroxy-2-methylbutylic acid (250 µg/50 µl of distilled water, internal standard (I.S), Wako Pure Chemical Industries, Tokyo, Japan) was added to the residue containing lactic acids, followed by HPLC analysis with 10 µl injection.

Identification and quantification of lactic acids were carried out by HPLC according to the method of Olieman and Vries (8), except for I.S. The lactic acid composition in samples was calculated from a calibration curve made from the peak area ratios of standard D- and L-lactic acid against I.S.. HPLC analysis was performed using a L-6200 Intelligent Pump, a L-3350 RI Monitor (Hitachi Ltd., Tokyo, Japan), a DS-L300 Data Station (Japan Spectroscopic Co., Hachioji, Japan), equipped with a COSMOSIL 5C18-AR column (4.6 x 150 mm, Nacalai Tesque, Kyoto, Japan). Elution was performed isocratically at 25 °C with distilled water containing both 8mM N,N-dipropyl-L-alanine and 4mM copper (II) acetate (pH 5.5) at a flow rate of 0.8 ml/min.

Total amounts of lactic acid extractable with DE were repeatedly examined. Data varied with different conditions of media. However, the difference of ratio of D- and L- lactic acid extracted were remained always within few percent in repeated experiments using the samples spiked with lactic acid. Therefore, analytical data obtained by HPLC were considered to be reliable for the determination of ratio of D- and L- types of lactic acid.

Compositional analysis of lactic acids.

Compositional changes in lactic acids produced in culture media were investigated by HPLC with sequential changes in the inoculation medium, from the MRS broth to the MRSL broth, then to skim milk, and finally returning to the MRS broth again. Each strain was inoculated in the next medium after 24 h incubation at 37 °C. For final HPLC analysis, a medium incubated for 48 h at 37 °C was used.

In sequential changes of the medium, five type strains, except the A3 subgroup (*L. amylovorus* JCM 1126^T), produced almost the same proportional of lactic acids in each initial and final MRS broth, and produced a smaller proportion of D-lactic acid in skim milk compared to MRS broth (Table 3). These results suggest that the ratio and amount of lactic acid produced by the *L. acidophilus* group vary with changes in the medium composition.

In the changes of the carbohydrate composition in MRS broth, a high amount of sugar in the media induced an increase in total lactic acid amount. The total amount of lactic acids was almost the same (ca. 14 mg/ml) in MRS broth containing Glc (1%), Gal (1%), Glc (1%) + Gal (1%), or Lac (2%). However, the ratio of D-lactic acid (%) was lowest (49.9%) in MRSL broth (containing 2% Lac) among these 4 sugar composition. The relationship between D-lactic acid production (%) and sugar composition (3) in media still remains unclear. In conclusion, these data suggest that good selection of the medium may enable control or improvement of the D-lactic acid ratio (or content) in fermented milk produced with *L. acidophilus* group lactic acid bacteria.

Lactic acid in the final product during glycolysis in the *L. acidophilus* group, and its biosynthesis in controlled by enzymatic activity of a D- and/or L-lactate dehydrogenase (EC 1.1.12.8 and 1.1.1.27) which are located in the cytosol of bacterial cells. Moreover, a *L. sake* strain is reported to have a lactate racemes (LR, EC 5.1.2.1) which play an important role in isomeric change of lactic acid (5). Hino and Kuroda (4) recently reported that activity of LR from *Megasphaera elsdenii* is induced by the addition of lactic acid to a glucose-free broth. However, the existence of LR in *L. acidophilus* group lactic acid bacteria has not been clarified. The preparation of a "knocked-out" strain by gene technology (1) which has no gene of LR, D- or L-LDH may be useful for elucidation of the relationship between the lactic acid biosynthesis and related enzymes.

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