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Selective Proliferation of Lactic Acid Bacteria and Accumulation of Lactic Acid during Open Fermentation of Kitchen Refuse with Intermittent pH Adjustment

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When minced and autoclaved model kitchen refuse was inoculated with a small amount of non-autoclaved model kitchen refuse as seed culture, incubated at 37°C for 3–5 days and intermittently pH neutralized, 27–45 g/l of lactic acid was accumulated with a small amount of acetic acid and ethanol. The highest accumulation and highest productivity levels of lactic acid were observed at an initial and adjusted pH of 7.0 and a 6 h interval of pH adjustment. After several hours of lag, the lactic acid bacteria became the dominant cell type during the incubation, while the number of coliform bacteria and clostridia decreased. Such selective and stable accumulation of lactic acid was achieved in dozens of different experiments with various refuse preparations. In contrast, with continuous pH adjustment, lactic acid once accumulated was labile and a small amount of butyric acid was produced, increasing the number of clostridia. The dominant bacteria isolated from the fermentation with intermittent pH adjustment were identified as *Lactobacillus plantarum* and *L. brevis*.

Keywords: lactic acid, kitchen refuse, open fermentation, *Lactobacillus plantarum*, *Lactobacillus brevis*, pH

Biological solid wastes such as kitchen refuse and waste from the food industry have been treated by incineration and in landfills in many countries including Japan. Although burning of biological waste materials reduces the volume and stabilizes waste, many problems with this process have been recognized recently. For example, harmful chemicals such as dioxin-related compounds are released. Incineration facilities can also be quickly damaged by temperature fluctuations after semi-continuous addition of kitchen refuse with a high water content, and the incineration process requires supplemental fuel to evaporate the water from biological material. Treatment of biological solid wastes via microbiological processes leads to the improvement of these wastes, and recycling reduces the need for landfill sites, fuel for incineration, and CO_2 emission. Aerobic biological treatment stabilizes wastes and can generate compost to fertilize soil. Anaerobic biological treatment has been used for wastewater treatment and offers an effective means of degrading the residual activated sludge, with the concurrent production of a fuel, methane. Although methane fermentation is an excellent method for converting biological solid wastes to by-product resources, the process is slow and requires large facilities.

Lactic acid has both hydroxyl and carboxyl groups with one chiral carbon in its molecule and is widely used not only in the food and pharmaceutical industries but also in the general chemical industry. It is produced via chemical synthesis or via fermentation of agricultural products using various kinds of bacteria or molds (Litchfield, 1996). Lactic acid can be polymerized to form biodegradable polyester, polylactic acid, and is considered a potential substitute for plastics manufactured from petroleum. Systematic lactic acid production, therefore, may be an advantageous strategy for the treatment and utilization of waste from the food industry, municipal solid waste, and residual activated sludge. Many studies of lactic acid production from wastes have been reported, including that from molasses (Kanwar *et al.*, 1995), bakery waste (Oda *et al.*, 1997), waste waterpaper (Schmidt & Padukone, 1997), municipal solid waste (Zhou *et al.*, 1996), and sugar-cane pressmud (Xavier & Lonsane, 1994).

However, these studies were carried out using pure culture of a lactic acid bacterium.

In attempting to develop an economical process for transforming the biological solid wastes, the authors found that certain amounts of lactic acid were produced from kitchen refuse without any sterilization. Interestingly, intermittent pH neutralization (pH swing) was effective for the stable lactic acid accumulation under open conditions. In this study we investigated the effect of intermittent pH control on the accumulation of lactic acid during open fermentation of model kitchen refuse (MKR). The change in microbial population during the fermentation was also evaluated.

Materials and Methods

Fermentation of model kitchen refuse The MKR, which was originally designed by Sankai (1999) considering data from several reports, was used with slight modification: it contained 14% (w/w) cooked meat, 40% (w/w) vegetables (peel of carrot, potato, and Chinese radish), 30% (w/w) fruits (apple, banana, and orange peel), 10% (w/w) cooked rice, and 6% (w/w) green tea residue. Composition of the model kitchen refuse is estimated to be as follows from Standard Tables of Food Composition (Kagawa, 1991): 80% water, 12% sugar, 5.6% protein, 1.8% fat, 0.3% fiber, and 0.8% ash. MKR was minced thoroughly with an equal amount of tap water, stored at −20°C and autoclaved

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before use (120°C, 15 min) to obtain reproducible results using materials from different preparations (MKR paste, pH 6.0). For seed culture, the minced kitchen refuse was divided into small aliquots, stored at −20°C, and was used without sterilization. The autoclaved MKR paste (30 ml in a 50 ml capped-centrifuge tube) was inoculated with 3 ml of seed culture (non-sterilized MKR paste) and statically incubated at 37°C. The culture pH was intermittently monitored and adjusted to appropriate pH using 10 N NaOH or 10 N HCl, mixing manually with a glass stick, unless otherwise stated. All procedures except for MKR paste preparation were done without any sterilization (open condition).

Viable cell count and isolation of lactic acid bacteria All media were purchased from Nissui Pharmaceutical, Tokyo, and were cultured at 37°C for 1–2 days, unless otherwise stated. The number of total bacterial cells was estimated by pour-plate method, culturing the saline-diluted sample statically in standard method agar. The cell number of lactic acid bacteria was estimated by pour plate method in plate count agar with bromocresol purple. The number of yeast cells was estimated using Potato Dextrose Agar with 0.1 g/*l* chloramphenicol. To estimate the cell number of *Escherichia coli* and related species (coliform bacteria), deoxycholate agar was used. The plates were incubated anaerobically (BBL GasPak Anaerobic System, Becton Dickinson). The number of clostridial cells was estimated by incubating the plates anaerobically using clostridia count agar.

To isolate microorganisms from the fermented product, colonies appearing in standard method agar were randomly transferred onto another standard method agar plate and incubated for a further 1–2 days anaerobically. DeMan Rogosa Sharpe (MRS) agar (Atlas, 1993) was also used for the separation of lactic acid bacteria, by incubating aerobically or anaerobically.

Identification of the isolated bacteria The isolated bacterial strains were initially classified by their morphological and physiological properties (Kandler & Weiss, 1989). The medium for investigating cultural properties of isolated bacteria contained 20 g glucose, 10 g peptone, 8 g meat extract, 4 g yeast extract, 2 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.05 g $MnSO_4 \cdot 4H_2O$, and 20 ml of kitchen refuge extract per liter (pH7.0, GK medium). The kitchen refuge extract, adjusted to pH 7.0, was prepared from the supernatant of minced model kitchen refuge that had been autoclaved with an equal volume of tap water at 120°C for 20 min, and centrifuged at 10,000 *g* for 20 min.

Sugar assimilation patterns of the isolated lactic acid bacteria were analyzed using an API 50 CHL identification kit (bioMerieux, France). For further identification, partial sequences of the 16S ribosomal RNA gene (16S rDNA) were analyzed: partial 16S rDNA (ca 1.3 kbp fragment) was amplified using 5′-TAACACATGCAAGTCGA-3′ and 5′-GGGAACTTATTCACCG-3′ as primers (Marchesi *et al.*, 1998). The DNA sequence was determined using an automatic DNA sequencer ABI 310 (Perkin-Elmer Japan, Tokyo). Phylogenetic relationships were analyzed using Clustal W (Talkington, *et al.*, 1981), using databases from the Ribosomal Database Project (http://rdp.life.uiuc.edu) (Thompson *et al.*, 1994) and Genbank (http://www.ncbi.nlm.nih.gov) (Benson *et al.*, 1998).

Chemical analysis Organic acids (formic acid, acetic acid, propionic acid, lactic acid, and butyric acid) in the supernatant of fermented samples (2,200 ×*g*, 20 min) were analyzed using an organic acid analysis system (Shimadzu, Tokyo): solvent delivery system, LC-10AD; column, Shim-pak SCR-102H; detector, CDD-6A; mobile phase, 5 mM *p*-toluenesulfonic acid; reaction mixture, 20 mM Bis-Tris containing 5 mM *p*-toluenesulfonic acid and 100 μM EDTA, 40°C. Selective accumulation of lactic acid was defined as the ratio of lactic acid concentration to the concentration of total organic acids (expressed as a percentage). Productivity was defined as the increase of concentration in the supernatant of the fermenting material per hour (g/*l*·h). Ethanol in the supernatant was analyzed by HPLC (Shimadzu LC-10Ai): column, Shodex Asahipak NH2P-504A (Showa Denko, Tokyo); detection, refractive index (Shimadzu RID-10A); mobile phase, water; room temperature. Optical isomers of lactic acid were analyzed using D- and L-specific lactate dehydrogenase (Boehringer Mannheim, Indianapolis, IN), according to the recommended protocol.

Results

Effect of pH adjustment on open fermentation of kitchen refuse In preliminary experiments, it was found that a certain amount of lactic acid was produced when non-sterilized kitchen refuse minced with tap water was incubated at 37°C without any other regulation. To achieve higher and more selective production of lactic acid, the effect of pH adjustment on the open fermentation of MKR paste was investigated. As shown in Table 1 (Run 1-1–1-5), the amount of accumulated lactic acid varied according to the intervals of pH adjustment, and the maximum accumulation was observed with pH adjustment intervals of 6 h or 12 h. Shorter intervals led to higher lactic acid productivity. The selective accumulation of lactic acid was over 90% when the pH was intermittently adjusted. It should be noted that when the pH was continuously adjusted to 7.0 using an online- pH monitor system (Run 1-1), lactic acid accumulated temporarily (19 g/*l*, after 36 h) but was labile and decreased rapidly, resulting in malofermentation with an unpleasant smell.

When the initial and adjusted pH of the minced kitchen refuse was varied, lactic acid accumulation was most abundant at pH 7.0 or 10.0 (Table 1, Run 2-6–2-9); however, lactic acid productivity was slightly higher at pH 7.0 than at pH 10.0. No fermentation occurred when initial pH was 3.0.

Table 1. Effect of intermittent pH adjustment on accumulation of lactic acid during the open fermentation of MKR paste.

Run[a]	adjusted pH	Interval (h)[b]	Productivity (g/*l*·h)[c]	Accumulation (g/*l*)[d]	Selectivity (%)[e]
1-1	7	0	1.05	19	83
1-2	7	6	0.70	44	92
1-3	7	12	0.58	45	94
1-4	7	24	0.40	31	94
1-5	7	—[f]	0.25	13	87
2-6	3	—[g]	0.04	2.0	—
2-7	5	6	0.42	32	96
2-8	7	6	0.65	45	94
2-9	10	6	0.58	45	92

[a]MKR samples of runs 1-1–1-5 and runs 2-6–2-9 were differently prepared.
[b]Interval of intermittent pH adjustment.
[c]Average production rate of lactic acid to reach maximum concentration.
[d]Maximum concentration of lactic acid accumulated.
[e]Ratio of accumulation of lactic acid to total organic acids.
[f]MKR paste was adjusted at pH 7.0 initially and incubated without pH adjustment.
[g]No pH change was observed.

Time course of open fermentation of model kitchen refuse To examine the fermentation process in detail, changes in concentrations of organic acids and the microbial populations were investigated. Figure 1 shows a typical time course for fermentation with a 6 h interval of adjustment to pH 7.0, although the maximum concentration of lactic acid accumulated in this case was lower than those shown in Table 1. After several hours of lag, the concentration of lactic acid increased rapidly and reached 22 g/*l* after 60 h of incubation. The lactic acid productivity was highest at 12–48 h, and once accumulated, lactic acid was constant even after 120 h (27 g/*l*). Ethanol was detected after 24 h, and accumulated to a stable 5 g/*l* after 120 h. Acetic acid accumulated after 12 h incubation and reached maximum at 36 h (2.4 g/*l*). Only a small amount of propionic acid was found, and no significant amount of formic acid or butyric acid was observed.

The number of total bacterial cells (2×10^5 cfu/ml initially) increased rapidly to 3×10^8 cfu/ml after 12 h. During the 24–120 h interval, the number was maintained at more than 10^9 cfu/ml. The number of lactic acid bacteria (initially 10^3 cfu/ml) gradually increased to reach an equivalent cell number to the total bacteria after 24 h, and remained at more than 10^9 cfu/ml even after 120 h. The number of coliform bacteria increased slightly during the first 24 h but decreased thereafter. The number of clostridia, initially estimated at 2×10^4 cfu/ml, decreased over the incubation period, and the number of yeast cells was quite low throughout the fermentation. Table 2 shows the effect of continuous pH adjustment on open fermentation of MKR paste. Compared to intermittent pH adjustment, continuous pH adjust-

Table 2. Effect of continuous pH adjustment on open fermentation of MKR paste.[a]

	Continuous pH adjustment	Intermittent pH adjustment
Organic acids accumulated (g/*l*)		
Formic acid	1.3	0
Acetic acid	3.5	2.4
Propionic acid	1.5	0.3
Lactic acid	32	27
Butyric acid	4.2	0
Selectivity (%)[b]	73	91
Cell number (cfu/ml)		
Total bacteria	2×10^9	4×10^9
Lactic acid bacteria	8×10^8	3×10^9
Coliform bacteria	8×10^3	5×10^2
Clostridia	3×10^6	4×10^2

[a]Organic acids and cell numbers were analyzed of total bacteria, lactic acid bacteria, coliform bacteria, and clostridia in the sample fermented with continuous pH adjustment (pH 7.0, 144 h) and with intermittent pH adjustment (pH 7.0, 6 h interval, 120 h).
[b]Ratio of accumulation of lactic acid to total organic acids.

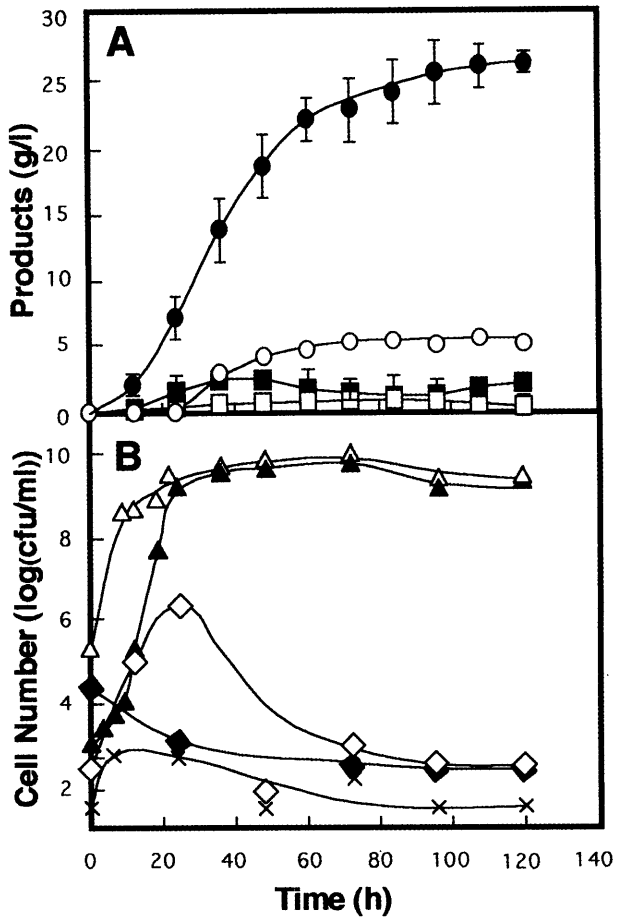

Fig. 1. Typical time course for open fermentation of MKR paste. MKR paste was incubated with the seed culture at 37°C statically, and the pH was adjusted to 7.0 once each 12 h interval. Concentrations of lactic acid (●), acetic acid (■), propionic acid (□), or ethanol (○) are shown in A. No meaningful amount of butyric acid, formic acid, or methanol was detected throughout the fermentation. Number of total viable cells (△), LAB (▲), coliform bacteria (◇), clostridia (◆), and yeast (×) is shown in B.

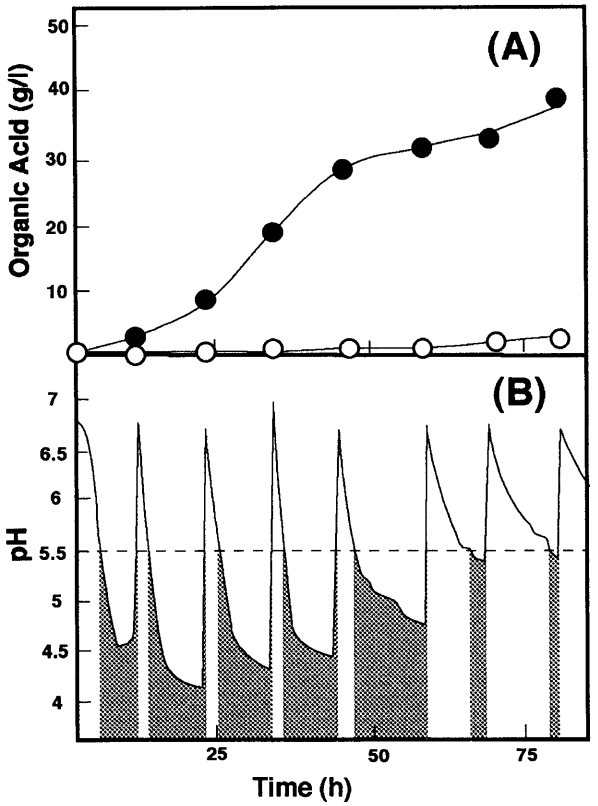

Fig. 2. Change in pH and organic acid amount during the open fermentation of MKR paste with intermittent pH adjustment. Experimental conditions are the same as in Fig. 1, and change in pH of the MKR paste was monitored continuously using a pH meter. A: amounts of lactic acid (●) and acetic acid (○) accumulated. B: change in pH, shaded area shows the period less than pH5.5.

ment produced relatively higher amounts of formic acid, propionic acid, and butyric acid, with lower selectivity of lactic acid, making the odor much worse. Higher numbers of coliform bacteria and clostridia were detected under continuous pH control than under intermittent pH control. Under continuous pH control, clostridia grew fast, reached more than 10^6 cfu/ml and maintained this number throughout the incubation period (data not shown).

The change in pH during the fermentation with intermittent pH adjustment (12 h interval) was monitored (Fig. 2). In the first interval, the culture pH decreased to 4.5 after 6 h, but it took only 3 h to reach pH 4.5 in the 3rd interval. As a result, the culture system was below pH 5.5 for 78% of the total incubation time. The concentration of lactic acid reached 39 g/*l* after 78 h in this case. These results indicated that intermittent pH adjustment caused pH swings in an acidic range, which is effective in repressing the malofermentation of MKR paste.

Identification of dominant bacteria To identify the microorganisms involved in the fermentation, 29 colonies on standard method agar plate were randomly picked from 72 h fermented MKR paste (pH 7.0, 6 h interval of pH adjustment). All isolated strains were Gram-positive, non-motile, non-spore forming, catalase negative rods, and accumulated lactate as a main organic acid in GK medium. They grew on MRS agar aerobically. These results suggested that all of these strains belong to *Lactobacillus* or a related genus. Among them, 14 strains (48% of the total isolates) showed an identical pattern of sugar assimilation: they fermented various kinds of sugars including D-galactose, D-fructose, D-mannose, L-rhamnose, L-arabinose, D-ribose, N-acetyl-D-glucosamine, maltose, sucrose, cellobiose, lactose, and raffinose. One strain (KY-1) had a cell size of 1.4–3.8 × 0.7 μm, and grew at 15°C but not at 45°C (Table 3). KY-1 produced L-lactic acid and D-lactic acid in the molar ratio of 78 : 22 in GK medium without any other organic acids, and the yield from glucose was 88%. These results suggested the 14 strains, including strain KY-1, were *L. plantarum*. Identification was confirmed by analyzing the partial sequence of amplified 16S rDNA (529 bps). The rDNA results indicated the nearest neighbor of KY-1 is *L. plantarum* ATCC 8014 (Genbank accession No. M58827, 99% homology). Another 11 strains (38% of the total isolates) showed identical sugar-assimilation patterns, and one of these isolated strains, KY-2, had a cell size of 2.0–3.2 × 0.7 μm, and grew at 15°C but not at 45°C (Table 3). KY-2 fermented glucose to L-lactic acid, D-lactic acid and ethanol in the molar ratio of 84 : 16 : 93. These results suggested the 11 strains including KY-2 were *L. brevis*. The highest similarity of partial 16S rDNA sequence of strain KY-2 (352 bp) was found in *L. brevis* ATCC 14869 (Genbank accession No.M58810, 99% homology), supporting these results. Other strains were identified as *L. pracasei* subsp. *paracasei* (2 strains), *L. delbueckii* subsp. *lactis* (1 strain), and *L. fermentum* (1 strain), from their cell morphologies and sugar-assimilation patterns.

Cultural properties of isolated strains Some cultural properties of *L. plantarum* KY-1 and *L. brevis* KY-2 were examined and compared with those of *E. coli* IFO 3301, using GK mediuum. *L. plantarum* KY-1 showed good growth over a pH range from 4.5 to 9.5 (Fig. 3). *L. brevis* KY-2 grew at pH 4.0–8.5, but good growth was observed over a narrower pH range (4.5–6.0) compared to *L. plantarum KY-1*. On the other hand, *E. coli* preferred alkaline conditions and showed quite poor growth below pH 5.5. Lactic acid production by *L. plantarum* KY-1 in GK medium was 0.66, 0.54, and 0.26 (g/*l*·h), and *L. brevis* KY-2 produced 0.069, 0.057, and 0.024 (g/*l*·h) at pH 7.0, 5.5, and 4.5, respectively. When lactic acid (10 g/*l*) was added to the medium, growth of *L. plantarum* KY-1 and *L. brevis* KY-2 was repressed at pH 4.0, but not at pH 5.5 or 7.0. For *E. coli*, lactic acid stimulated growth at pH 7.0 and 5.5 but no growth was observed at pH 4.0, with or without addition of lactic acid. The effect of acetic acid (5 g/*l*) and butyric acid (2 g/*l*) on the growth at pH 5.5 was also investigated. Acetic acid stimulated growth of *L. plantarum* KY-1 (130%, relative to that without any additive) and *L. brevis* KY-2 (360%), but slightly inhibited that of *E. coli* (80%). Growth of *L. plantarum* KY-1 and *L. brevis* KY-2 was also stimulated by butyric acid (135% and 950%, respectively), while that of *E. coli* was inhibited (40%). Addition of kitchen refuse extract

Table 3. Some properties of strains KY-1 and KY-2.

	Strain KY-1	Strain KY-2
Cell shape	rods	rods
Cell size (μm)	1.4–3.6 × 0.7	2.0–3.2 × 0.7
Spore formation	−	−
Gram-stain	+	+
Motility	−	−
Catalase activity	−	−
Growth on MRS agar		
aerobically	+	+
anaerobically	+	+
Products from glucose	D, L-lactic acid	L-lactic acid, ethanol
Growth at 15°C	+	+
45°C	−	−
Sugar assimilation	D-glucose, D-galactose, D-fructose, D-mannose, D-turanose, L-rhamnose, L-arabinose, D-ribose, N-acetyl-D-glucosamine, α-methylmannoside, amygdalin, arbutin, esculin, salicin, maltose, sucrose, trehalose, cellobiose, gentiobiose, lactose, melezitose, raffinose	D-glucose, D-fructose, D-xylose, L-arabinose, D-ribose, N-acetyl-D-glucosamine, maltose, cellobiose, melibiose, raffinose, mannitol, gluconate, 5-keto-gluconate

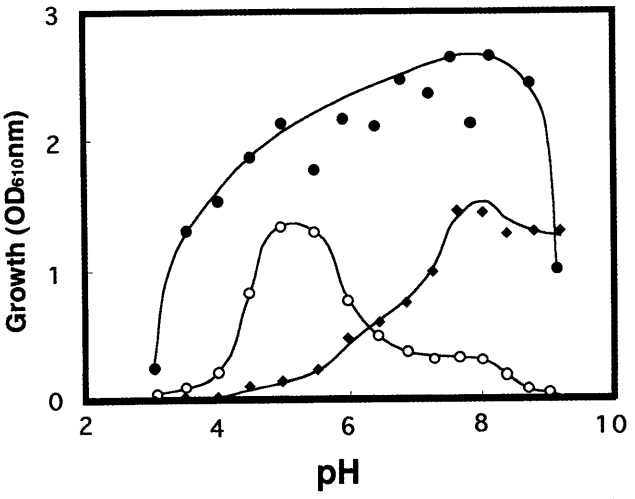

Fig. 3. Effect of pH on the growth of isolated lactic acid bacteria and *E. coli*. *L. plantarum* KY-1 (●), *L. brevis* KY-2 (○), and *E. coli* IFO 3301 (◆) were cultured in GK medium with various pH at 37°C for 24 h statically, and their turbidity was measured at 610 nm.

(0.1% (v/v)) significantly stimulated the growth of *L. brevis*. KY-2, by 25-fold at pH 7.0 and by 2.3-fold at pH 5.5. The growth of *L. plantarum* KY-1 was not affected by the addition of kitchen refuse extract.

Discussion

In this report, the authors demonstrate that a certain amount of lactic acid is stably accumulated from the minced model kitchen refuse (MKR paste) that has been incubated and intermittently pH neutralized. Selective accumulation of lactic acid was reproduced in dozens of different experiments, in spite of the open system and variation of the refuse preparation. There have been many studies on lactic acid fermentation of traditional foods (Kodama & Kumagai, 1997) and forage (Cai *et al.*, 1998) in a simple open system. But there have been few studies on the production of lactic acid in an open system using naturally-inhabiting microorganisms: D'addario *et al.* (1996) attempted to produce lactic acid from municipal solid waste under an open condition, but with little information.

This study has also shown that reproducible fermentation was made possible by the superior proliferation of lactic acid bacteria originally existing in the model kitchen refuse and maintained in the seed culture. Although the initial number of lactic acid bacteria was only 0.5% of the total bacteria, they became dominant after lag of a half day (Fig.1). Analysis of the lactic acid bacteria sugar-assimilation patterns, 16S rDNA partial sequence and cell morphology revealed that the predominant LAB types in the fermented MKR paste were *L. plantarum* and *L. brevis*. Both strains are common in nature and can be found in natural fermentation products such as silage, sauerkraut, cheese, sausage, etc. (Kandler & Weiss, 1989). *L. plantarum* is recommended as a starter culture in many cases for fermentation control of silage (Saucedo *et al.*, 1990) and food (Vaugen, 1985). *L. brevis* is heterofermentative and might be involved in the accumulation of ethanol in MKR paste. In addition, the value of optical activity of accumulated L(+)-lactic acid (67%) was explicable from the microbial population. The open fermentation of MKR paste yielded lactic acid at relatively high concentration (42% of total organic carbon in kitchen refuse at the highest), although the two major isolates showed no assimilation of the major carbon sources in kitchen refuse, starch or cellulose. This suggests the importance of the mixed culture system: some other microorganisms in the MKR might contribute to hydrolysis of polysaccharides, and the consortium in the seed culture might be constructed with some synergistic and commensurate relationships. It would be interesting to investigate the microflora in the early stage of fermentation, where a number of non-LAB could be found (Fig.1).

The ability of *L. plantarum* KY-1 to grow over a wide pH range and the ability of *L. brevis* KY-2 to grow in the acidic range seemed a likely reason for their superiority in the fermented MKR paste. Without pH adjustment, growth of these lactic acid bacteria as well as their productivity was slightly repressed. With continuous pH neutralization, various kinds of microorganisms grew, including clostridia. In this system, the accumulated lactic acid was labile, causing malofermentation. With intermittent pH neutralization, the fermentation system was periodically and momentary exposed to pH 7.0, but the pH decreased rapidly from the weak buffer action of MKR paste. Thus, the microorganisms in the MKR paste were exposed to acidic conditions for most of the fermentation period (Fig.2). This might explain why the fermentation was reproducible, and lactic acid, once produced, accumulated in a stable manner. Consequently, the pH swings from intermittent adjustment might be effective both for higher productivity of lactic acid and for growth inhibition of non-lactic acid bacteria.

There are many problems to be solved before putting the open fermentation described in this study to practical use. These include reproducibility problems caused by the variable composition of kitchen refuse, lactic acid extraction problems caused by the complexity of fermentation products, and problems relating to the production of low optical activity of lactic acid, which is disadvantageous to the production of fine chemicals. About 17% of MKR paste (50% of total carbon) still remained as the fermented residue after fluid was recovered as the crude lactate solution. In spite of this, fermentation of kitchen refuse by such an open system is not only a simple and low cost method for lactic acid production, but is also advantageous as a waste treatment. Collection and disposal of kitchen refuse is often a nasty process due to its unpleasant odor and unsanitary conditions. Increasing the concentration of lactic acid, as well as decrease in pH has been effective in improving odor by preventing the production of butyric acid, ammonia, and hydrogen sulfide(Olsen *et al.*, 1992). In addition, growth of spoilage (Deshmukh & Patterson, 1997) or pathogenic microorganisms (Masuda *et al.* 1998, Okereke & Montiville, 1991, Talkington *et al.*, 1981) might also be prevented. Lactic acid bacteria occur widely as indigenous contaminants in a variety of raw or minimally processed foods (Kandler & Weiss, 1989). We found that a variety of unsterilized waste materials from the practical field, were sufficient substrates for lactic acid production (data not shown). Such variation and flexibility in the fermentation will be reported elsewhere.

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