

Comparison of the Growth of *Lactobacillus delbrueckii*, *L. paracasei* and *L. plantarum* on Inulin in Co-culture Systems

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Lactobacillus delbrueckii TU-1, which apparently takes intact inulin into its cells and then degrades it intracellularly, was co-cultured *in vitro* with *L. paracasei* KTN-5, an extracellular inulin degrader; or *L. plantarum* 22A-3, a strain that is able to utilize fructose but not inulin; or both in order to prequalify inulin as a prebiotic agent *in vivo*. When *L. delbrueckii* TU-1 was co-cultured with *L. paracasei* KTN-5 on fructose or inulin, the growth of *L. delbrueckii* TU-1 on inulin was markedly higher than that of *L. paracasei* KTN-5, whereas the growth of *L. delbrueckii* TU-1 on fructose was much lower than that of *L. paracasei* KTN-5. These results suggest that *L. delbrueckii* TU-1 and *L. paracasei* KTN-5 were efficient at utilizing inulin and fructose, respectively. When *L. plantarum* 22A-3 was co-cultured with *L. delbrueckii* TU-1 on inulin, the growth of *L. plantarum* 22A-3 was enhanced by *L. paracasei* KTN-5 but not by *L. delbrueckii* TU-1, suggesting that the fructose moiety that *L. paracasei* KTN-5 released temporarily into the medium was “scavenged” by *L. plantarum* 22A-3. Thus, *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 were then cultured altogether on inulin. The growth of *L. delbrueckii* TU-1 was unaffected but that of *L. paracasei* KTN-5 was markedly suppressed. This evidence suggests that prebiotic use of inulin supported the selective growth of intracellular inulin degraders such as *L. delbrueckii* rather than extracellular inulin degraders such as *L. paracasei* in the host microbiota.

Key words: *Lactobacillus delbrueckii*, *Lactobacillus paracasei*, inulin, co-culture system

INTRODUCTION

The *Lactobacillus* species are among the industrially important lactic acid bacteria, which are found in fermented food products and can provide many beneficial effects to their hosts as probiotic bacteria [1, 2]. For example, strains of *L. delbrueckii* that are generally found in dairy products such as yogurt have been shown to have probiotic effects including antioxidative effects, immunostimulatory activity, and antitumor activity in both *in vitro* and *in vivo* studies [3–5]. However, these beneficial effects may be transient because up to 100 trillion intestinal commensal microbes usually prevent probiotic bacteria from establishing themselves in the intestinal environment [6, 7]. To overcome this limitation, the intake of prebiotics in addition to probiotic bacteria

has been reported to be effective [8].

Prebiotics are nondigestible food ingredients that are not hydrolyzed by human digestive enzymes but are utilized by a limited number of bacteria in the colon and beneficially affect the host by improving the consistency of gastrointestinal microbiota [9, 10]. The majority of candidate prebiotics are nondigestible oligosaccharides such as lactulose, raffinose, fructo-oligosaccharides, lactosucrose, xylo-oligosaccharides, and isomalto-oligosaccharides, but they also include highly polymerized saccharides such as resistant starch, pectin, and inulin [10]. In particular, the effects of prebiotics on inulin have been well studied. Inulin is a linear D-fructose polymer linked by beta-(2-1)-glycosidic bonds, with a terminal glucose moiety that is linked by an alpha-(1-2)-glycosidic bond [11]. The beta-(2-1)-glycosidic bond of inulin, including the first glucose-fructose bond, is not hydrolyzed to a great extent by any mammalian digestive enzymes [12]. Inulin extracted from chicory, which is a root vegetable, varies in the number of D-fructose units from a minimum of 2 to a maximum of 60. This relatively unprocessed inulin has a degree of polymerization (DP) ranging from 2 to approximately 60, and the average DP

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Table 1. Bacterial strains used in this study

Strain	Origin
<i>Lactobacillus delbrueckii</i> TU-1	Yogurt commercially available in Japan
<i>Lactobacillus paracasei</i> KTN-5	Fermented milk product commercially available in Japan
<i>Lactobacillus plantarum</i> 22A-3	Nukaduke (rice bran pickling) of eggplants

is approximately 12 [11].

Meanwhile, certain *Lactobacillus* strains, including strains of *L. paracasei* and *L. delbrueckii*, are able to grow in *in vitro* inulin monoculture systems [13–15]. Recently, Tsujikawa et al. [16] provided evidence to suggest that *L. paracasei* strains degrade inulin extracellularly to release free molecules of fructose and sucrose that can be subsequently transported into cells to be metabolized, whereas *L. delbrueckii* strains take up intact inulin into cells and then degrade it intracellularly. If the above hypothesis is accurate, it would be interesting to see which species has an ecological advantage when both species are grown on inulin together. Furthermore, the growth of *L. paracasei* rather than *L. delbrueckii* may be suppressed by the presence of any fructose-utilizing concomitant bacteria. Therefore, in this study, we examined the ability of *L. delbrueckii* to compete for inulin with *L. paracasei*, an avid fructose-utilizing but not inulin-utilizing strain of *L. plantarum* or both in co-culture systems.

MATERIALS AND METHODS

Bacterial strains used

The bacterial strains used in this study were *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 (Table 1). It should be noted that these 3 *Lactobacillus* strains were isolated in our laboratory from dietary fermented food products that were commercially available in Japan and that their taxonomic identities were confirmed by our species-specific quantitative PCR assay, which will be described later. All bacterial isolates were stored in de Man-Rogosa-Sharpe (MRS) broth (Oxoid, Basingstoke, United Kingdom) at -80°C until used.

Media and substrates used

Modified MRS medium [17], which was without glucose and supplemented with 0.5 g/L L-cysteine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan), hereafter referred to as the mMRS medium, was used as the basal fermentation medium throughout this study. The pH of the medium was adjusted to 6.5 before sterilization (121°C for 15 min).

Glucose, fructose, or inulin was added to mMRS as the

sole carbon source (2%, wt/vol). In all cases, these sugars were sterilized through membrane filtration using Millex[®] syringe filter units (pore size, 0.45 μm ; Merck Millipore, Darmstadt, Germany) and were added aseptically to the sterile mMRS medium. All of these sugars were purchased from Wako (Osaka, Japan). The DP of inulin (Wako) is reported to vary between 3 and 60 according to the information supplied by the manufacturer.

Fermentation experiments

To perform mono- and co-culture fermentations, the strains were anaerobically subcultured twice in MRS broth, and the cultures obtained after 6 hr of anaerobic growth at 37°C were centrifuged at 4,000 rpm for 15 min. After centrifugation, the bacterial pellet was washed once with phosphate-buffered saline (PBS) [0.8% NaCl, 0.02% KH_2PO_4 , 0.115% Na_2HPO_4 (pH 7.4)] and resuspended in PBS until its copy number of the 16S rRNA gene in the bacterial genome reached 10^8 copies/ml, which was followed by the spotting of 50 μL of this suspension on 5 mL of MRS or mMRS containing glucose, fructose, or inulin (2%, wt/vol at final concentration). Incubations of the media were performed anaerobically at 37°C in an anaerobic jar (Mitsubishi Gas Chemical, Tokyo, Japan) for up to 48 hr during which approximately 500 μL of the culture was withdrawn at 0, 3, 6, 12, 18, 24, and 48 hr after incubation. It should be noted that it was difficult to adjust the cell numbers of the initial inoculum of the *L. delbrueckii* strain by the plate count method because its cells were tightly bound in a chain formation and could not be separated to single cells even by a rigorous vortexing to be spread on a plate. We thus adjusted the initial cell numbers of the strains to be equivalent to each other in terms of the copy number of the 16S rRNA genes.

DNA preparations

Whole genomic DNA from each culture was prepared essentially following the method of Marmur et al. [18]. Briefly, a 200 μL aliquot of each culture was transferred to sterile bead-beating tubes containing 300 mg of glass beads (0.1 mm). This was added to approximately 500 μL of TE-saturated phenol, 250 μL of lysis buffer, and 50 μL of 10% sodium dodecyl sulfate. After

Table 2. Primers, amplicon sizes and standard bacterium used in quantitative PCR detection of target bacteria

Target bacteria	Primer	Amplicon size (bp)	Standard bacterium	Reference
<i>Lactobacillus delbrueckii</i>	F: 5'-GGRTGATTTGTTGGACGCTAG-3' R: 5'-GCCGCCTTTCAAACCTGAATC-3'	138	<i>Lactobacillus delbrueckii</i> TU-1	[19]
<i>Lactobacillus paracasei</i>	F: 5'-GCACCGAGATTCAACATGG-3' R: 5'-GGTTCTTGGATYATGCGGTATTAG-3'	117	<i>Lactobacillus paracasei</i> KTN-5	[19]
<i>Lactobacillus plantarum</i>	F: 5'-TTTGAGTGAGTGGCGAACTG-3' R: 5'-CCAAAAGTGATAGCCGAAGC-3'	145	<i>Lactobacillus plantarum</i> 22A-3	This study

centrifugation at 15,000 rpm for 5 min, the upper layer was removed to a new tube. Four hundred microliters of phenol:chloroform:isoamyl alcohol (25:24:1) was then added to the tube and centrifuged at 15,000 rpm for 5 min. The upper aqueous layer was carefully collected in a new tube. This sample was shaken in a FastPrep-24 Instrument (MP Biomedicals SARL, Illkirch, France) for 30 s at maximum speed. DNA was precipitated by adding 275 μ L of isopropyl alcohol and a 1/10 volume of 3 M sodium acetate, which had been placed in the tube at -20°C for 10–15 min. The pellet was washed with 70% ice-cold ethanol by centrifugation at 15,000 rpm for 5 min, and DNA was dried under vacuum. DNA was subsequently dissolved again in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Quantitative PCR (qPCR)

In order to quantify the growth of each strain in the co-cultures, we initially used the plate count method on our co-cultures but we found it extremely difficult to assign any colonies formed on the plates of mixed species co-cultures to the species by their appearance. Quantification of the *L. delbrueckii*, *L. paracasei*, and *L. plantarum* 16S rRNA genes was therefore performed by quantitative PCR using a TP700 Thermal Cycler Dice Real Time System Lite (Takara, Ohtsu, Japan). Primer pairs targeting a partial 16S rRNA gene for *L. delbrueckii*, *L. paracasei* [19] and *L. plantarum* were designed in this study and used for the qPCR assay (Table 2). The PCR conditions for *L. delbrueckii* and *L. paracasei* were as follows: 95°C for 10 min and 40 cycles of 95°C for 15 sec and 60°C for 1 min; those for *L. plantarum* were as follows: 95°C for 1 min and 40 cycles of 95°C for 5 sec and 60°C for 30 sec. To check the specificity of the amplifications, a melting curve was obtained by performing the following cycle: a denaturation step at 95°C for 15 sec, a 1°C increase in temperature every 20 sec starting at 60°C and ending at 95°C and a final step at 95°C for 15 sec. Standard curves for absolute quantification in the cultures were prepared using 10^2 – 10^6 copies of the PCR fragment

of the 16S rRNA gene. The correlation coefficients for all the standard curves were above 0.99. For each assay, 1 μ L of DNA solution was added to 9 μ L of a PCR mixture containing 5 μ L of THUNDERBIRDTM SYBR[®] qPCR Mix (Toyobo, Osaka, Japan), 3.6 μ L of distilled water, and 200 nM of each primer.

Sugar degradation analysis by thin-layer chromatography (TLC)

To substantiate the observed difference in fructose and inulin utilization by the strains, TLC (Merck silica gel 60 plate) was performed on the spent cultures (approximately 2 μ L each) of *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 grown in mMRS containing 2% fructose or inulin for 24 hr, which were spotted along with solutions of fructose and inulin standard (2%, wt/vol) onto different lanes of a TLC plate. The plates were developed in 1-butanol:2-propanol:ethanol:water (3:2:3:4) solvent. The spots were visualized by spraying the plates with *p*-anisaldehyde (containing acetic acid and H_2SO_4) ethanol solution (Tokyo Chemical Industry, Tokyo, Japan) and heating them at 160°C for several minutes.

RESULTS

Monocultures of *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 in mMRS supplemented with glucose, fructose, or inulin

All 3 strains grew well on glucose or fructose (Fig. 1A, 1B); in particular, the growth levels of *L. plantarum* 22A-3 on both the sugars were higher than those of the other 2 strains. In addition, *L. paracasei* KTN-5 and *L. plantarum* 22A-3 reached a stationary phase in approximately 12 hr, whereas *L. delbrueckii* TU-1 reached a stationary phase in approximately 24 hr (Fig. 1A, 1B). *L. delbrueckii* TU-1 grew faster on inulin (Fig. 1C) than on glucose or fructose (Fig. 1A, 1B), and the growth reached a stationary phase in approximately 6 hr; on the other hand, *L. paracasei* KTN-5 (Fig. 1C) showed a comparable growth pattern

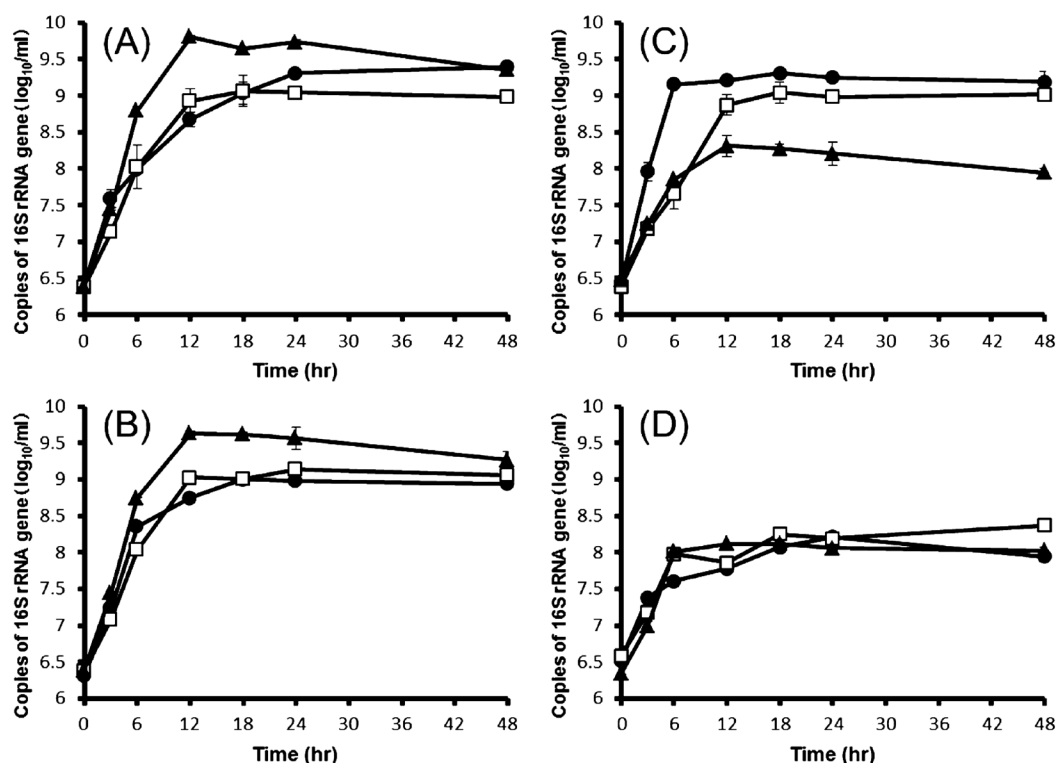


Fig. 1. Growth curves of the mMRS medium supplemented with a 2% (wt/vol) concentration of glucose (A), fructose (B), or inulin (C) and without the sugars (D). ●, Growth of *L. delbrueckii* TU-1; □, growth of *L. paracasei* KTN-5; ▲, growth of *L. plantarum* 22A-3. Each experiment was performed in triplicate.

on glucose or fructose (Fig. 1A, 1B). The growth level of *L. plantarum* 22A-3 on inulin (Fig. 1C) decreased to less than 1/10-fold of its level of growth on glucose or fructose (Fig. 1A, 1B) and was comparable to that of its growth without the sugars (Fig. 1D).

In the TLC analysis, a dark oval-shaped fructose spot was seen in the upper part of the chromatograph in mMRS with fructose that was not incubated with any strain (Fig. 2A and 2B, lane 1). The spent culture of each of the 3 strains grown in mMRS containing 2% fructose for 24 hr (Fig. 2A, lane 2) showed fructose spots. However, the darkness intensity of *L. delbrueckii* TU-1 was slightly less than that of the reference fructose spot, whereas those of *L. paracasei* KTN-5 and *L. plantarum* 22A-3 (Fig. 2A, lanes 3 and 4) were much less than that of the reference fructose spot (Fig. 2A, lane 1). mMRS with inulin that was not incubated with any strain (Fig. 2B, lane 5) showed a markedly extended spot covering a wide area and a dark oval-shaped non-migrating inulin spot far below the fructose spot. The spent culture of *L. delbrueckii* TU-1 grown in mMRS containing 2% inulin for 24 hr (Fig. 2B, lanes 2) showed an inulin spot,

but the darkness intensity decreased. The spent culture of *L. paracasei* KTN-5 grown in mMRS containing 2% inulin for 24 hr (Fig. 2B, lane 3) showed an inulin spot with a decreased darkness intensity as well as a dark oval-shaped spot in the upper part of the chromatograph. However, the spent culture of *L. plantarum* 22A-3 inoculated in mMRS containing 2% inulin for 24 hr (Fig. 2B, lane 4) showed an intact inulin spot.

Co-culture of *L. delbrueckii* TU-1 and *L. paracasei* KTN-5 in mMRS supplemented with fructose or inulin

In the co-culture of *L. delbrueckii* TU-1 and *L. paracasei* KTN-5 on fructose (Fig. 3A), *L. delbrueckii* TU-1 did not grow well, and the growth was suppressed compared with that in the monoculture on fructose (Fig. 1B) but was comparable with that without the sugars (Fig. 1D); on the other hand, *L. paracasei* KTN-5 showed a growth pattern comparable with that in the monoculture on fructose (Fig. 1B). In contrast, in the co-culture on inulin (Fig. 3B), *L. delbrueckii* TU-1 showed a comparable growth pattern to that of its monoculture on inulin (Fig. 1C), whereas *L. paracasei* KTN-5 did not grow well; its growth was

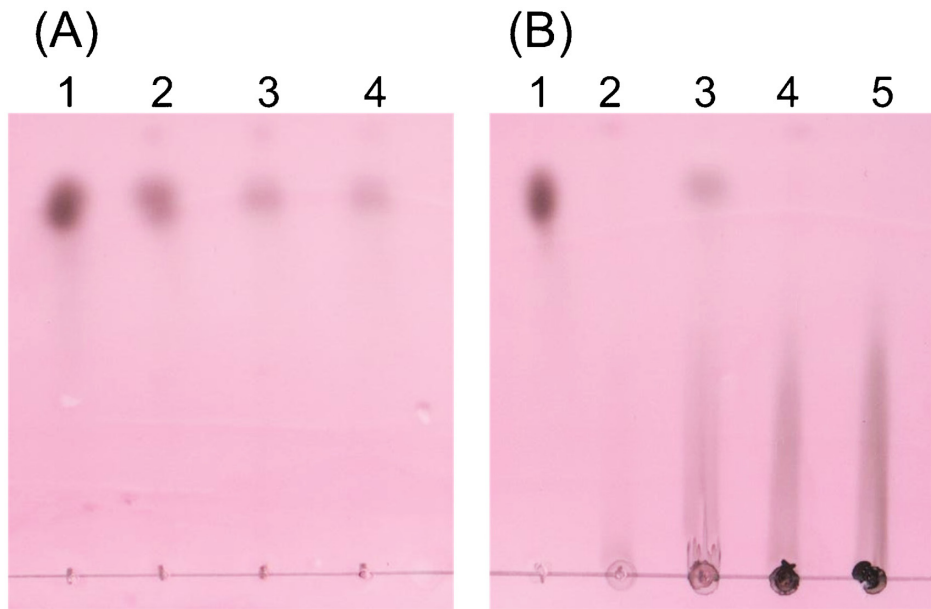


Fig. 2. Thin-layer chromatography analysis of the sugar compositions of the spent cultures of the *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 in the mMRS medium supplemented with a 2% (wt/vol) concentration of fructose (A) or inulin (B) after 24 hr of incubation. Strains were grown and harvested as follows: (A) Lane 1, fructose (2%, wt/vol); lane 2, *L. delbrueckii* TU-1; lane 3, *L. paracasei* KTN-5; and lane 4, *L. plantarum* 22A-3. (B) Lane 1, fructose (2%, wt/vol); lane 2, *L. delbrueckii* TU-1; lane 3, *L. paracasei* KTN-5; lane 4, *L. plantarum* 22A-3; and lane 5, inulin (2%, wt/vol).

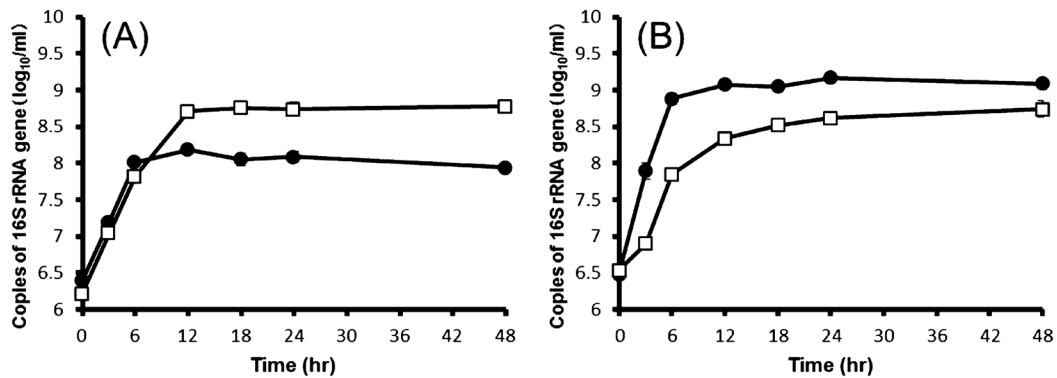


Fig. 3. Growth curves of a co-culture of *L. delbrueckii* TU-1 and *L. paracasei* KTN-5 in the mMRS medium with a 2% (wt/vol) concentration of fructose (A) or inulin (B). ●, Growth of *L. delbrueckii* TU-1; □, growth of *L. paracasei* KTN-5. Each experiment was performed in triplicate.

slower than that in monoculture on inulin (Fig. 1C).

Co-cultures of *L. plantarum* 22A-3 with *L. delbrueckii* TU-1 or with *L. paracasei* KTN-5 in mMRS supplemented with inulin

In the co-culture of *L. plantarum* 22A-3 and *L. delbrueckii* TU-1 on inulin (Fig. 4A), *L. plantarum* 22A-3 did not grow well, and the growth level was comparable with that of monoculture on inulin (Fig. 1C)

as well as without the sugars (Fig. 1D). *L. delbrueckii* TU-1 grew well and showed a growth almost comparable with that in monoculture on inulin (Fig. 1C). In the co-culture of *L. plantarum* 22A-3 and *L. paracasei* KTN-5 (Fig. 4B), the growth level of *L. plantarum* 22A-3 was much higher than in monoculture on inulin (Fig. 1C) and was comparable with that of monoculture on fructose (Fig. 1B); on the other hand, the growth level of *L. paracasei* KTN-5 decreased to less than 1/10-fold its

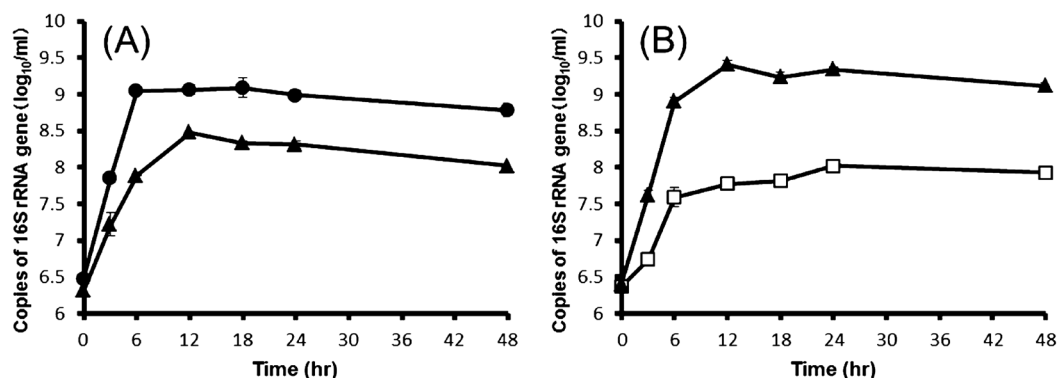


Fig. 4. Growth curves of a co-culture of *L. plantarum* 22A-3 with *L. delbrueckii* TU-1 (A) or with *L. paracasei* KTN-5 (B) in the mMRS medium with a 2% (wt/vol) concentration of inulin.

●, Growth of *L. delbrueckii* TU-1; □, growth of *L. paracasei* KTN-5; ▲, growth of *L. plantarum* 22A-3. Each experiment was performed in triplicate.

growth when monocultured on inulin (Fig. 1C).

Co-culture of *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 in mMRS supplemented with inulin

In the triple co-culture of *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 on inulin (Fig. 5), *L. delbrueckii* TU-1 grew well, but the growth level was slightly decreased compared with its growth when monocultured on inulin (Fig. 1C) as well as when co-cultured with *L. paracasei* KTN-5 or *L. plantarum* 22A-3 on inulin (Fig. 3B, 4A). The growth of *L. paracasei* KTN-5 (Fig. 5) decreased markedly to less than 1/10-fold of its growth in the monoculture on inulin (Fig. 1C), and it showed a growth pattern comparable with that when co-cultured with *L. plantarum* 22A-3 on inulin (Fig. 4B).

DISCUSSION

Our TLC analysis confirmed that *L. paracasei* KTN-5 degraded inulin to release free molecules of fructose extracellularly as reported by Tsujikawa et al. [16]. By contrast, *L. delbrueckii* TU-1 did not release any fructose molecules in the culture medium, suggesting that the strain is capable of taking up intact inulin into its cells and subsequently degrading it intracellularly. Therefore, we speculated that *L. paracasei* had to take 2 steps to utilize inulin, an initial step of degrading inulin to fructose and a subsequent step of transporting the released fructose molecules inside of its cells, while *L. delbrueckii* only took one step to transport the entire inulin molecule into its cells. This in turn suggested that *L. delbrueckii* could utilize inulin more readily than *L. paracasei*. This was realized in our co-culture experiments of *L. delbrueckii*

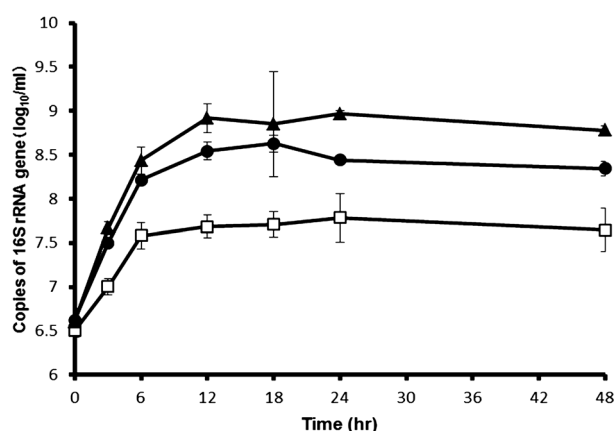


Fig. 5. Growth curve of a co-culture of *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 in the mMRS medium with a 2% (wt/vol) concentration of inulin.

●, Growth of *L. delbrueckii* TU-1; □, growth of *L. paracasei* KTN-5; ▲, growth of *L. plantarum* 22A-3. Each experiment was performed in triplicate.

TU-1 and *L. paracasei* KTN-5, in which the growth of *L. delbrueckii* TU-1 on inulin was markedly higher than that of *L. paracasei* KTN-5, whereas that on fructose was much lower than that of *L. paracasei* KTN-5, suggesting that *L. delbrueckii* TU-1 was more efficient at utilizing inulin and that *L. paracasei* KTN-5 was more efficient at utilizing fructose. This was also confirmed by our TLC analysis on fructose and inulin. Meanwhile, our TLC analysis also indicated that *L. plantarum* 22A-3 could utilize fructose but not inulin.

To investigate the influence of *L. plantarum* 22A-3 on the growth of *L. paracasei* KTN-5 or *L. delbrueckii* TU-1, we co-cultured *L. plantarum* 22A-3 with

L. paracasei KTN-5 or *L. delbrueckii* TU-1. In the co-culture experiment of *L. plantarum* 22A-3 with *L. paracasei* KTN-5 on inulin, *L. plantarum* 22A-3 grew as well as in monoculture on fructose. However, *L. plantarum* 22A-3 was unable to utilize inulin, and the growth of *L. paracasei* KTN-5 greatly decreased when compared with monoculture on inulin or co-culture with *L. delbrueckii* TU-1 on inulin. This suggests that *L. paracasei* KTN-5 released fructose into the medium following extracellular inulin degradation and that *L. plantarum* 22A-3 then used the released fructose; *L. plantarum* 22A-3 was “scavenging” the free fructose molecules that were produced by *L. paracasei* KTN-5, which were accumulating temporarily outside its cells. A similar finding was reported in a co-culture study in which *Bacteroides thetaiotaomicron* degraded inulin and released free fructose into the medium, thereby promoting concomitant bifidobacteria that were not able to degrade inulin themselves [20]. Meanwhile, in the co-culture experiment of *L. plantarum* 22A-3 with *L. delbrueckii* TU-1 on inulin, the growth of *L. delbrueckii* TU-1 was comparable to its growth when monocultured on inulin, and that of *L. plantarum* 22A-3 was comparable to its growth when monocultured without the sugars. This evidence suggests that *L. delbrueckii* TU-1 did not produce extracellular fructose from inulin; thus, *L. plantarum* 22A-3 could not “exploit” *L. delbrueckii* TU-1 for free fructose. A similar case was observed in a co-culture study of *Roseburia inulinivorans* and *Bifidobacterium longum* on inulin in which an inulin-degrading *R. inulinivorans* strain did not promote the growth of a non-inulin-degrading *B. longum* strain [21].

In the triple co-culture experiment of *L. delbrueckii* TU-1, *L. paracasei* KTN-5, and *L. plantarum* 22A-3 on inulin, the growth of *L. delbrueckii* TU-1 was relatively unaffected by the presence of *L. plantarum* 22A-3, but that of *L. paracasei* KTN-5 was markedly suppressed. These results suggest that even in the presence of fructose-utilizing concomitant bacteria, *L. delbrueckii* TU-1 had an ecological advantage as compared with *L. paracasei* KTN-5 on inulin. In an actual intestinal environment, monosaccharides such as glucose and fructose are subject to fermentation by a large number of intestinal bacteria [22], and the competition with concomitant intestinal bacteria for such sugars would be therefore more pronounced than what we observed in the co-culture experiment. Based on this, we can speculate that the prebiotic use of inulin supports the selective growth of intracellular inulin degraders such as *L. delbrueckii* but not that of extracellular inulin degraders such as *L. paracasei*.

In various studies that have used *in vitro* gut simulation models and clinical *in vivo* trials with human volunteers, it has been demonstrated that inulin has the ability to selectively increase the number of *Bifidobacterium* spp. and *Lactobacillus* spp. [23–25], including species of *B. adolescentis* and *L. gasseri* [26, 27]. In addition, it is well known that inulin utilization by lactic acid bacteria is species- and even strain-dependent [28]. One of the explanations for this species specificity may be that these strains of the species, the growth of which is promoted by inulin have an ability to degrade inulin intracellularly rather than extracellularly. To evaluate the possibility that the growth of intracellular inulin-degrading bacteria such as *L. delbrueckii* rather than extracellular inulin-degrading bacteria such as *L. paracasei* is promoted when inulin is administered as a prebiotic supplement, further studies that use *in vitro* gut simulation models and *in vivo* trials are needed.

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