Screening of Dairy Yeast Strains for Probiotic Applications

H. Kumura, Y. Tanoue, M. Tsukahara, T. Tanaka, and K. Shimazaki

Laboratory of Dairy Science, Research Group of Animal Product Science,
Division of Bioresources and Product Science,
Graduate School of Agriculture, Hokkaido University, Sapporo-shi 060-8589, Japan

ABSTRACT

To evaluate the potential of yeasts of dairy origin as probiotics, we tested 8 species including *Candida humilis*, *Debaryomyces hansenii*, *Debaryomyces occidentalis*, *Kluyveromyces lactis*, *Kluyveromyces lodderae*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, and *Yarrowia lipolytica*, isolated from commercial blue cheese and kefir. Strains were randomly selected from each species and tested for their ability to adhere to human enterocyte-like Caco-2 cells in culture. Among the 8 species, *K. lactis* showed higher adhesive ability than *K. marxianus*, *K. lodderae*, and *D. hansenii*. The other 4 species were poorly adhesive. All species other than *K. marxianus* and *C. humilis* were resistant to acidic conditions. In the presence of bile acid, growth inhibition was undetectable when incubation was carried out at 27°C; however, it was evident for *C. humilis* and a strain of *D. occidentalis* when incubated at 37°C. Moreover, the influence of proteinase treatment of living cells of *K. lactis* and *K. lodderae* on their adhesion to Caco-2 cells was evaluated. Although a slight reduction was recognized when *K. lactis* was treated with proteinase K, the influence of intestinal protease treatments of pepsin followed by trypsin was negligible. These results indicated that a proteinaceous factor was unlikely to be involved in adhesion of *K. lactis* and *K. lodderae* to Caco-2 cells. No stimulation of IL-8 synthesis by Caco-2 cells was recognized in the presence of *K. lactis*. In conclusion, *K. lactis* was the most attractive to continue study for use as probiotic microorganisms.

(**Key words:** dairy yeast, probiotics, Caco-2 cell)

Abbreviation key: YPD = yeast-peptone-dextrose.

INTRODUCTION

In recent years, much attention has been paid to the design of functional foods that contain probiotic microbial strains responsible for health benefits in the host.

Such benefits include immune modulation, improvement of the intestinal flora, prevention or shortening of diarrhea, control of serum cholesterol, and reduction of occurrence of inflammatory bowel disease (Kaur et al., 2002; Ouwehand et al., 2002; Tuohy et al., 2003). A probiotic has been defined as “a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance,” (Fuller, 1989). However, on the basis of recent advances of research in this field, the following revised definition has been proposed: “Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well being of the host” (Salminen et al., 1999).

Lactobacillus and *Bifidobacterium* strains have been extensively studied as probiotic agents (Borriello et al., 2003; Tuohy et al., 2003) because they are normal inhabitants of the intestinal tracts of humans and other vertebrates. In addition, these strains have been frequently used to produce fermented dairy products. Even if a strain of interest belongs to the same bacterial species, its probiotic effect may vary according to the properties of individual strain. Consequently, preferable strains are selected using an in vitro model system that reflects specific effects. However, before screening, strains should be checked for acid and bile tolerance to ensure survival during passage through the gastrointestinal tract. In addition, adhesion to the intestinal mucosa is considered important for exclusion of pathogens and undesirable bacteria. Furthermore, growth of the probiotic microorganism should also lead to acceptable flavor of the functional food.

Some fermented milks such as kefir and koumiss contain lactic acid bacteria and lactose-fermenting yeasts (Seiler, 2003). Yeasts can also be found in some traditional cheeses. *Debaryomyces hansenii*, *Kluyveromyces lactis*, and *Yarrowia lipolytica* have frequently been found (De Boer and Kuik 1987; Lopez-Diaz et al., 1995) as predominant species, although they have not been adopted for deliberate use. It is not unusual to find a yeast count of 10^5 to 10^7 cells per gram of cheese (Fleet, 1990), with beneficial effects such as interaction between starter cultures, production of aroma components, and inhibitory effects against spoilage microor-

Received May 6, 2004.

Accepted August 15, 2004.

Corresponding author: H. Kumura; e-mail: kumura@anim.agr.hokudai.ac.jp.

Table 1. Isolation and identification of yeast strains from commercial blue-veined cheeses.

Source	Identified species	Number of strains
Roquefort	<i>Debaryomyces hansenii</i>	26
	<i>Debaryomyces occidentalis</i>	2
Blue des Causses	<i>Debaryomyces hansenii</i>	6
Gorgonzola	<i>Saccharomyces cerevisiae</i>	10
Stilton	<i>Kluyveromyces lactis</i>	30
	<i>Debaryomyces hansenii</i>	17
	<i>Yarrowia lipolytica</i>	4

ganisms (Jakobsen and Narvhus, 1996). However, studies of yeasts from a probiotic standpoint have conducted in a limited manner using other species (Guslandi et al., 2003; Mansour-Ghanaei et al., 2003).

In this study, yeasts isolated from commercial blue-veined cheese and kefir were tested as potential probiotics. The ability of the yeasts to adhere to human intestinal cells was observed using the enterocyte-like Caco-2 cell culture system, and tolerance to acid and bile was evaluated. Factors responsible for adhesion of yeast strains to Caco-2 cells were also discussed.

MATERIALS AND METHODS

Yeast Strains and Growth Media

The 95 strains of yeast isolated from commercial blue-veined cheese are listed in Table 1. These strains were identified by nucleotide sequence analysis of the specific amplification of the intertranscribed spacer region (White et al., 1990). Four yeast strains isolated from kefir were also investigated; they have been identified as *Saccharomyces cerevisiae* K1, *K. lodderae* K2, *K. marxianus* K3, and *Candida humilis* K4. Organisms were cultured in yeast-peptone-dextrose (YPD) medium containing 1% yeast extract (Difco, Detroit, MI), 2% polypeptone (Nihon Seiyaku, Tokyo, Japan), and 2% glucose, at 27°C for 48 h.

Cell Culture

The Caco-2 cell line was routinely grown at 37°C in 5% CO₂-95% air atmosphere in Dulbecco modified Eagle's minimal essential medium (Sigma Chemical Co., St. Louis, MO) containing L-glutamine, with 10% inactivated (56°C, 30 min) fetal calf serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin.

Adhesion Assay

The adhesion assay was performed by the method of Chauvière et al. (1992). Caco-2 cells (passages 51 to 71) were seeded at a concentration of $4 \times 10^4/\text{cm}^2$ on plastic

coverslips placed in 24-well tissue culture plates. The culture medium was changed every other day. The Caco-2 monolayers at postconfluence after 10 d were washed 3 times with PBS. Yeast strains, randomly selected from each species identified, were cultured in YPD medium at 27°C for 2 d. Subsequently, they were harvested and washed twice with basal medium by centrifugation. After cell density was counted in the suspension dispersed in the basal medium (Packard and Ginn, 1985), yeast cells were seeded at a concentration of 10^6 in 24-well culture plates and incubated at 37°C for 90 min in 5% CO₂-95% air. After incubation, the monolayers were washed 4 times with PBS, fixed with methanol, and stained with Giemsa solution followed by microscopic examination under oil immersion. Each adhesion assay was conducted in triplicate with cells from 3 successive passages. For each glass coverslip monolayer, the number of adherent yeast and Caco-2 cells was counted in 20 random microscopic areas. Adhesion ability was expressed as the number of yeast cells adhering to 100 Caco-2 cells.

Effects of Low pH and Bile on Viability and Growth of Yeast Strains

Effect of exposure to low pH was qualitatively evaluated by transferring one portion of the subcultured yeast medium to 0.5% NaCl containing 1 M HCl, pH 2.0 (10 mL), and incubating at 37°C for 3 h. Subsequently, a portion of the solution was inoculated into YPD medium and incubated at 27°C for 72 h. As an index of the growth, turbidity was visually monitored. Saline was used in place of NaCl/HCl as a control.

Growth of the strains in the presence of bile was examined using YPD medium containing 0.1% cholic acid (Wako Pure Chemical Co., Osaka, Japan). Incubation was carried out at 27 or 37°C for 24 h. If necessary, anaerobic incubation was performed using YPD medium containing 0, 0.3, or 0.5% cholic acid, with the surface of the medium in the test tube covered by liquid paraffin oil.

Proteinase Treatments

The influence of proteinase treatment on adhesion ability of *K. lactis* S1 and S25 and *K. lodderae* K2 was evaluated by the method of Sarem et al. (1996), with some modifications. The strain cultured in YPD medium at 27°C for 2 d was harvested and washed 3 times with PBS. The cells were dispersed in 0.05 M Tris-HCl buffer (pH 7.5) in the presence or absence of 0.1% trypsin (Sigma Chemical Co.) or proteinase K (Invitrogen, Tokyo, Japan). When the cells were treated with 0.1% pepsin (Sigma Chemical Co.), the buffer sys-

tem was replaced by 0.05 M glycine-HCl buffer (pH 2.5). After incubation at 37°C for 1 h, cells were harvested, washed 3 times with PBS, and suspended in basal medium. During the enzyme treatments, cell density was approximately 10⁷/mL. Then, yeast cells were counted and seeded at a concentration of 10⁶ in 24-well culture plates to perform the adhesion assay. Dual treatment, consisting of incubation with pepsin followed by tryptic digestion, was also performed; the reactions were carried out at 37°C for 1 h for each enzyme.

Immunological Analysis

Effect of yeast strains on proinflammatory cytokine of IL-8 by Caco-2 cells was evaluated by the method of Morita et al. (2002). The Caco-2 monolayers at postconfluence after 10 d were incubated with *K. lactis* S1 or S25, whose viable cell count was 10⁶ in 24-well culture plates as described above (adhesion assay). After incubation at 37°C for 24 h, the culture medium was recovered to quantify IL-8 using a commercial ELISA kit (Quantikine, R&D Systems, Inc., MN). For comparison, culture medium containing LPS (0.1 mg/mL) prepared from *E. coli* O111:B4 (Sigma Chemical Co.) was also investigated.

Statistical Analysis

Significant differences among means were analyzed by Tukey-Kramer's test. A value of $P < 0.05$ was considered significant. All statistical calculations were carried out with JMP software (SAS Institute, Inc., Cary, NC).

RESULTS

As shown in Table 1, 95 yeast strains were isolated from 4 types of commercial blue cheese; the isolates were classified into 5 species. *Debaryomyces hansenii* was frequently isolated from 3 types of cheese; in contrast, the other species were found only in one type of cheese each. For the subsequent adhesion assay, 2 to 5 yeast strains were randomly selected from each species identified.

Figure 1 shows the number of yeast cells adhesive to 100 Caco-2 cells. The genus *Kluyveromyces* possessed adhesion ability to Caco-2 cells; this was particularly evident for *K. lactis* (Figure 2). *Debaryomyces hansenii* showed adhesion ability comparable to that of *K. marxianus* and *K. lodderae*; however, the adhesion ability of *D. occidentalis* was much lower. Other yeasts, including *S. cerevisiae*, *Y. lipolytica*, and *C. humilis*, were poorly adhesive to Caco-2 cells.

Because probiotic organisms should be acid and bile tolerant, qualitative analyses were carried out using

the selected strains. After exposure to acidic conditions, all strains other than *K. marxianus* and *C. humilis* were able to grow in YPD medium (data not shown). Irrespective of the presence of bile acid, growth was observed in all strains tested when incubation was carried out at 27°C (data not shown). When incubation was performed at 37°C, limited growth was observed for *D. hansenii* and *K. lodderae*, irrespective of the presence of bile acid (Table 2). Growth inhibition due to bile acid at 37°C incubation was evident for *C. humilis* and *D. occidentalis* S2.

These results indicate that *K. lactis* has the potential for use as a probiotic microorganism. Consequently, growth of the 5 strains of *K. lactis* under anaerobic condition in the presence of elevated concentration of bile acid (up to 0.5%) was checked to simulate the intestinal environment. When incubation was carried out at 27°C, substantial growth was confirmed in the presence of bile acid; however, anaerobic incubation led to its moderate growth. Growth rate was reduced at 37°C, irrespective of oxygen availability or concentration of bile acid, which suggested that probiotic application of *K. lactis* should be expected to result in immune modulation rather than improve the intestinal flora.

The component located on the surface of microorganisms could affect mucosal immune modulation. It has been reported that proteins expressed on the surface of the cell walls in lactobacilli are responsible for bacterial adhesion to intestinal epithelial cells (Vidgrén et al., 1992; Toba et al., 1995; Roos et al., 1996; Sarem et al., 1996; Sillanpää et al., 2000; Åvall-Jääskeläinen et al., 2003; Fernández et al., 2003). Consequently, correlation between adhesion ability and expression of cell surface proteins of the yeast strains was evaluated by pretreatment of the yeast cells with proteinases, including intestinal enzymes such as pepsin and trypsin. Among 5 strains of *K. lactis*, strains S1 and S25 were selected because significant differences in adhesion ability were found. *Kluyveromyces lodderae* was investigated for comparison.

Table 3 shows the percentage of yeast cells adherent to Caco-2 cells after each treatment. Apparently, adhesion ability was partially impaired when the 3 strains were incubated with pepsin; however, the value was comparable to the pepsin-free buffer solution (pH 2.5) used as a control. The results suggested that the reduction of adhesion ability was due to low pH rather than the peptic effect. The adhesion ability of the 3 strains was maintained following trypsin treatment; however, significant reduction due to proteinase K was recognized in *K. lactis* S1 and S25, but not in *K. lodderae*. Dual treatment with pepsin and trypsin led to an insignificant decrease of adhesion ability compared with the

Figure 1. Adhesion to Caco-2 cells of representative yeast strains isolated from dairy products. Number of yeast adhering to the cell monolayer per 100 cells is shown. *Kluyveromyces lactis*: S1, S2, S10, S15, and S25. *Yarrowia lipolytica*: S8 and S12. *Debaryomyces occidentalis*: R1, and R2. *Debaryomyces hansenii*: R4, R7, R10, and 212. *Saccharomyces cerevisiae*: G1, G3, G9, and K1. *Kluyveromyces lodderae*: K2. *Kluyveromyces marxianus*: K3. *Candida humilis*: K4. Strains were isolated from commercial blue cheese except the 4 strains of *S. cerevisiae* K1, *K. lodderae*, *K. marxianus*, and *C. humilis*, which were isolated from kefir. Standard error is shown. ^{a-f}Means without common letters are significantly different ($P < 0.5$).

control. In general, adhesion ability was maintained at more than 50%.

Finally, we examined potential for induction of proinflammatory cytokine (IL-8) by Caco-2 cells using *K. lactis* S1, S25, and LPS. Because limited growth at 37°C

was monitored (Table 2), *K. lodderae* was excluded. Table 4 shows that IL-8 secretion of Caco-2 cells was stimulated when LPS was present in the medium. In contrast, no significant increase of IL-8 concentration was recognized after exposure to the yeasts.

DISCUSSION

The isolated yeast profile from commercial blue cheese was similar to that previously reported (De Boer

Figure 2. Light microscopic analysis of adherence of *Kluyveromyces lactis* to differentiated Caco-2 cells using Giemsa stain.

Table 2. Growth of the yeast strains in the presence or absence of bile acid.¹

Strain	Control (no bile acid)	Bile acid (0.1%)
<i>Kluyveromyces lactis</i> ²	+	+
<i>Yarrowia lipolytica</i> ³	+	+
<i>Debaryomyces occidentalis</i> R1	+	+
<i>Debaryomyces occidentalis</i> R2	+	–
<i>Debaryomyces hansenii</i> ⁴	–	–
<i>Saccharomyces cerevisiae</i> ⁵	+	+
<i>Kluyveromyces lodderae</i> K2	±	±
<i>Kluyveromyces marxianus</i> K3	+	+
<i>Candida humilis</i> K4	+	–

¹+ = Growth; ± = faint growth; – = no growth in yeast-peptone-dextrose medium at 37°C.

²Strains S1, S2, S10, S15, and S25 were tested.

³Strains S8 and S12 were tested.

⁴Strains R4, R7, R10, and 212 were tested.

⁵Strains G1, G3, G9, and K1 were tested.

Table 3. Effects of proteinase treatment of *Kluyveromyces lactis* S1, *K. lactis* S25, and *K. lodderae* K2 on their ability to adhere to Caco-2 cells.¹

Treatment	Strain		
	<i>K. lactis</i> S1	<i>K. lactis</i> S25	<i>K. lodderae</i> K2
No treatment	100	100	100
Control ² (pH 2.5)	66.6	54.7	72.1
Pepsin	66.1	53.7	73.7
Control (pH 7.5)	95.1	85.4	96.4
Trypsin	98.0	88.9	93.2
Proteinase K	77.0	69.3	84.8
Control (pH 2.5 and 7.5)	60.9	53.5	72.6
Pepsin + trypsin	55.7	51.0	64.4

¹Expressed as a percentage of yeast cells adherent to monolayer Caco-2 cells relative to that of no treatment.

²Control = incubation of the strain in 0.05 M Glycine-HCl, pH 2.5 and/or 0.05 M Tris-HCl, pH 7.5, in the absence of enzyme.

and Kuik, 1987; López-Díaz et al., 1995). In this study, strains of *S. cerevisiae* were isolated from cheese and kefir. *Kluyveromyces lactis* was also isolated from kefir grains (Marquina et al., 2002; Simova et al., 2002); however, we used this species only from cheeses in this study.

Saccharomyces cerevisiae has been widely applied in industry, and beneficial effects such as promotion of iron absorption (Mai et al., 2002) and improvement of intestinal conditions (Takasaki and Saitoh, 1997) have been reported. Our study demonstrated that *S. cerevisiae* showed negligible adhesion ability; however, it could survive acidic conditions and grow in the presence of bile.

Among the yeasts tested in this study, *K. lactis* was the most adhesive to Caco-2 cells, although a significant difference was observed depending on the strain. When the same parameter was adopted, some strains of bifidobacterium showed a value of >350 (Crociani et al., 1995), and *Lactobacillus rhamnosus* GG gave the highest value of >1500 (Lee et al., 2000; Bernardeau et al., 2001). However, these were interesting results because values less than 250 were frequently obtained in most adhesive lactobacilli and bifidobacteria (Chauvière et al., 1992; Crociani et al., 1995; Lee et al., 2000; Gopal et al., 2001). In the case of *K. lactis*, the value was apparently intermediate; however, it should be consid-

ered that a single yeast cell needs a larger space to adhere to the surface of an intestinal cell than a bacterium does because of the size difference between bacteria and yeasts.

Kluyveromyces lactis proliferated under anaerobic conditions, and showed acid and bile tolerance; however, growth was moderate at 37°C. Thus, we concluded that *K. lactis* as a probiotic agent could be expected to enhance immune modulation rather than improve the intestinal flora. Thus, we examined the component expressed on the surface of the cell wall that related to adhesion ability.

Proteins responsible for adhesion have been characterized and cloned (Vidgrén et al., 1992; Toba et al., 1995; Roos et al., 1996; Mukai et al., 1997; Sillanpää et al., 2000; Åvall-Jääskeläinen et al., 2003). However, the involvement of a proteinaceous factor in adhesion seems to depend on the strain; the adhesion ability of *Lactobacillus gasseri* and *L. delbrueckii* ssp. *lactis* to Caco-2 cells was impaired when the bacterial cells were treated with trypsin, whereas no reduction was observed in the case of *L. acidophilus* (Sarem et al. 1996; Fernández et al., 2003). In our study, proteinase K caused negligible reduction of adhesion ability for *K. lodderae* and significant reduction of adhesion ability for 2 strains of *K. lactis*. The results implied involvement of a proteinaceous factor for the adhesion in *K. lactis*; however, the function was unaffected by single or dual treatment with pepsin and trypsin. Consequently, it can be concluded that a protein-mediated adhesion system had a minor role in these yeast cells and that the adhesion properties would be maintained in the presence of intestinal proteinases.

For *K. lactis*, exposure to acidic conditions produced a more significant effect than proteinase treatment did with respect to subsequent adhesion ability. Because incubation of *K. lactis* in an acidic buffer led to more than 50% reduction of viability (data not shown), de-

Table 4. Interleukin-8 secretion by Caco-2 cells in the presence or absence of *Kluyveromyces lactis*.

	IL-8 secretion (pg/mL)	
	Average	SE
None	28.5	6.8
LPS (0.1 mg/mL)	180.2*	6.4
<i>K. lactis</i> S1	21.1	6.5
<i>K. lactis</i> S25	26.1	5.7

* $P < 0.05$.

crease of adhesion activity after incubation in acidic control buffer (Table 3) could be ascribed to decreased living cells.

Treatment with sodium metaperiodate led to reduction of adhesion ability to Caco-2 cells of some probiotic strains including *L. gasseri* and *L. acidophilus* (Fernández et al., 2003), which implied that carbohydrates influence the adherence of the strain to intestinal epithelial cells. Consequently, we tried to examine adhesion of *K. lodderae* and 2 strains of *K. lactis* that had been incubated in 1% sodium metaperiodate solution. However, the treatment resulted in considerable reduction of the cell viability, probably due to the sensitivity of the cell wall of the yeast to oxidation. Thus, the contribution of carbohydrates in adherence of the yeast strains to Caco-2 cells remains unclear.

Finally, production of IL-8 by Caco-2 cells was investigated after their exposure to the yeast strains. Interleukin-8 is well known as the representative proinflammatory cytokine and its synthesis by enterocytes can be induced in response to bacterial enteric pathogens. Even some strains of bifidobacteria were demonstrated to be able to promote IL-8 secretion by Caco-2 cells (Morita et al., 2002); however, the 2 strains of *K. lactis* tested in this study showed little effect on its production.

Further studies are required to consider the physiological effects of yeasts from dairy products as probiotic microorganisms, in particular, the mucosal immunological modulation induced by oral administration of the yeast. Among the strains used in this study, *K. lactis* was of interest for further investigation for this purpose. Although this species is a lactose-fermenting organism and could cause a blowing problem such as expansion of the container by the production of CO₂ gas under certain delivery conditions, it might be possible to incorporate it into some dairy products such as mold-type cheeses and frozen desserts.

ACKNOWLEDGMENTS

This work was supported by a grant from the Urakami foundation and Yotsuba Dairy Industry.

We are very grateful to Hidemasa Motoshima, Yotsuba Dairy Industry, for the gift of yeast strains isolated from kefir, and we thank Koutaroh Ishikawa for technical assistance. We express our appreciation to Satoshi Ishizuka for his valuable suggestions for statistical analysis.

REFERENCES

Åvall-Jääskeläinen, S., A. Lindholm, and A. Palva. 2003. Surface display of the receptor-binding region of the *Lactobacillus brevis* S-layer protein in *Lactococcus lactis* provides nonadhesive lacto-

- cocci with the ability to adhere to intestinal epithelial cells. *Appl. Environ. Microbiol.* 69:2230–2236.
- Bernardeau, M., J. P. Vernoux, and M. Gueguen. 2001. Probiotic properties of two lactobacillus strains in vitro. *Milchwissenschaft* 56:663–667.
- Borriello, S. P., W. P. Hammes, W. Holzapfel, P. Marteau, J. Schrezenmeir, M. Vaara, and V. Valtonen. 2003. Safety of probiotics that contain lactobacilli or bifidobacteria. *Clin. Infect. Dis.* 36:775–780.
- Chauvière, G., M. H. Coconnier, S. Kerneis, J. Fourniat, and A. L. Servin. 1992. Adhesion of human *Lactobacillus acidophilus* strain LB to human enterocyte-like Caco-2 cells. *J. Gen. Microbiol.* 138:1689–1696.
- Crociani, J., J. P. Grill, M. Huppert, and J. Ballongue. 1995. Adhesion of different bifidobacteria strains to human enterocyte-like Caco-2 cells and comparison with in vivo study. *Lett. Appl. Microbiol.* 21:146–148.
- De Boer, E., and D. Kuik. 1987. A survey of the microbiological quality of blue-veined cheeses. *Neth. Milk Dairy J.* 41:227–237.
- Del Re, B., B. Sgorbati, M. Miglioli, and D. Palenzona. 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. *Lett. Appl. Microbiol.* 31:438–442.
- Fernández, M. F., S. Boris, and C. Barbes. 2003. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J. Appl. Microbiol.* 94:449–455.
- Fleet, G. H. 1990. Yeasts in dairy products. *J. Appl. Bacteriol.* 68:199–211.
- Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bacteriol.* 66:365–378.
- Gopal, P. K., J. Prasad, J. Smart, and H. S. Gill. 2001. In vitro adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. *Int. J. Food Microbiol.* 67:207–216.
- Guslandi, M., P. Ghollo, and P. A. Testoni. 2003. A pilot trial of *Saccharomyces boulardii* in ulcerative colitis. *Eur. J. Gastroenterol. Hepatol.* 15:697–698.
- Jakobsen, M., and J. Narvhus. 1996. Yeasts and their possible beneficial and negative effects on the quality of dairy products. *Int. Dairy J.* 6:755–768.
- Kaur, I. P., K. Chopra, and A. Saini. 2002. Probiotics: Potential pharmaceutical applications. *Eur. J. Pharm. Sci.* 15:1–9.
- Lee, Y. K., C. Y. Lim, W. L. Teng, A. C. Ouwehand, E. M. Tuomola, and S. Salminen. 2000. Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. *Appl. Environ. Microbiol.* 66:3692–3697.
- López-Díaz, T. M., J. A. Santos, M. Prieto, M. L. García-López, and A. Otero. 1995. Mycoflora of a traditional Spanish blue cheese. *Neth. Milk Dairy J.* 49:191–199.
- Mai, T. T., K. Igarashi, R. Hirunuma, S. Takasaki, M. Yasue, S. Enomoto, S. Kimura, and C. V. Nguyen. 2002. Iron absorption in rats increased by yeast glucan. *Biosci. Biotechnol. Biochem.* 66:1744–1747.
- Mansour-Ghanaei, F., N. Dehbashi, K. Yazdanparast, and A. Shafahi. 2003. Efficacy of *Saccharomyces boulardii* with antibiotics in acute amoebiasis. *World J. Gastroenterol.* 9:1832–1833.
- Marquina, D., A. Santos, I. Corpas, J. Munoz, J. Zazo, and J. M. Peinado. 2002. Dietary influence of kefir on microbial activities in the mouse bowel. *Lett. Appl. Microbiol.* 35:136–140.
- Morita, H., F. He, T. Fuse, A. C. Ouwehand, H. Hashimoto, M. Hosoda, K. Mizumachi, and J. Kurisaki. 2002. Adhesion of lactic acid bacteria to Caco-2 cells and their effect on cytokine secretion. *Microbiol. Immunol.* 46:293–297.
- Mukai, T., T. Toba, and H. Ohori. 1997. Collagen binding of *Bifidobacterium adolescentis*. *Curr. Microbiol.* 34:326–331.
- Ouwehand, A. C., S. Salminen, and E. Isolauri. 2002. Probiotics: An overview of beneficial effects. *Antonie Van Leeuwenhoek* 82:279–289.
- Packard, V. S., Jr., and R. E. Ginn. 1985. Direct microscopic methods for bacteria or somatic cells. Pages 219–237 in *Standard Methods for the Examination of Dairy Products*. G. H. Richardson, ed. American Public Health Association, Washington, DC.

- Roos, S., P. Aleljung, N. Robert, B. Lee, T. Wadström, M. Lindberg, and H. Jonsson. 1996. A collagen binding protein from *Lactobacillus reuteri* is part of an ABC transporter system. *FEMS Microbiol. Lett.* 144:33–38.
- Salminen, S., A. Ouwenhand, Y. Benno, and Y. K. Lee. 1999. Probiotics: How should they be defined? *Trends Food Sci. Technol.* 10:107–110.
- Sarem, F., L. O. Sarem-Damerdj, and J. P. Nicolas. 1996. Comparison of the adherence of three *Lactobacillus* strains to Caco-2 and Int-407 human intestinal cell lines. *Lett. Appl. Microbiol.* 22:439–442.
- Seiler, H. A. 2003. Review: Yeasts in kefir and kumiss. *Milchwissenschaft* 58:392–396.
- Sillanpää, J., B. Martínez, J. Antikainen, T. Toba, N. Kalkkinen, S. Tankka, K. Lounatmaa, J. Keränen, M. Höök, B. Westerlund-Wikström, P. H. Pouwels, and T. K. Korhonen. 2000. Characterization of the collagen-binding S-layer protein CbsA of *Lactobacillus crispatus*. *J. Bacteriol.* 182:6440–6450.
- Simova, E., D. Beshkova, A. Angelov, Ts. Hristozova, G. Frengova, and Z. Spasov. 2002. Lactic acid bacteria and yeasts in kefir grains and kefir made from them. *J. Ind. Microbiol. Biotechnol.* 28:1–6.
- Takasaki, S., and S. Saitoh. 1997. Effect of dried yeast on fecal bacterial metabolites in rats. *J. Japan Soc. Nutr. Food Sci.* 50:175–179.
- Toba, T., R. Virkola, B. Westerlund, Y. Bjorkman, J. Sillanpää, T. Vartio, N. Kalkkinen, and T. K. Korhonen. 1995. A collagen-binding S-layer protein in *Lactobacillus crispatus*. *Appl. Environ. Microbiol.* 61:2467–2471.
- Tuohy, K. M., H. M. Probert, C. W. Smejkal, and G. R. Gibson. 2003. Using probiotics and prebiotics to improve gut health. *Drug Discov. Today* 8:692–700.
- Vidgrén, G., I. Palva, R. Pakkanen, K. Lounatmaa, and A. Palva. 1992. S-layer protein gene of *Lactobacillus brevis*: Cloning by polymerase chain reaction and determination of the nucleotide sequence. *J. Bacteriol.* 174:7419–7427.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequence of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, ed. Academic Press Inc., San Diego, CA.