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Inhibitory Power of Kefir: The Role of Organic Acids

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ABSTRACT

Milk and MRS broth fermented with kefir grains from different households were examined for inhibitory activity toward gram-negative and gram-positive strains. Fermented milk obtained with 10 g per 100 ml of inoculum (final pH 3.32 to 4.25) and MRS broth fermented with 1 and 10 g per 100 ml of inocula (final pH 4.18 to 5.25) had inhibitory power demonstrated by spot test and agar well diffusion assay. This inhibitory effect could be assigned to the undissociated form of lactic and acetic acid produced during the fermentation process. Kefir supernatants inhibited the growth of *Escherichia coli* 3 in nutrient broth at 37°C for 24 h. However, supernatants of yogurt or milk artificially acidified with lactic and acetic acids allowed the growth of *E. coli* 3 in the same conditions. A bacteriostatic effect of milk fermented with kefir grains over *E. coli* 3 was also demonstrated.

Kefir is a traditional fermented milk originated many centuries ago in the Caucasian mountains and obtained by fermentative activity of kefir grains. These grains are composed mostly of proteins and polysaccharides *(1, 13)* and a complex microflora coexisting in a symbiotic association. Yeast, lactic acid bacteria, and acetic acid bacteria are generally found as kefir microflora constituents. The most frequently reported genera are *Saccharomyces*, *Candida*, *Kluyveromyces*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Acetobacter (2, 8, 15–17, 23, 24)*.

Kefir has been associated with longevity in Caucasian people. Several research studies brought to light some beneficial effects of kefir. Kefir has been used for the treatment of gastrointestinal and metabolic disorders, atherosclerosis, allergic diseases, and tuberculosis *(19)*. Several studies demonstrated antitumoral activity of kefir *(7, 11)*, stimulation of the immune system *(12)*, and both antibacterial *(25)* and antifungal activity *(19)*.

It is well known that many metabolic products from lactic acid bacteria have strong inhibitory power over the growth of saprophytic and pathogenic bacteria *(20)*. This antagonistic activity may involve different mechanisms, such as competition for available nutrients and production of inhibitory metabolites (hydrogen peroxide, organic acids, diacetyl, and bacteriocins). In many cases, a combination of these factors may be responsible for inhibition. Brialy and coworkers *(3)* demonstrated the intrinsic inhibitory power of fresh kefir toward *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*, but there was no inhibition over *Saccharomyces cerevisiae* and *Candida albicans*. *Lactococcus lactis* DPC3147, a strain isolated from Irish kefir grains, produces a bacteriocin with a broad spectrum of inhibition. Ryan et al. *(18)* showed that most gram-positive bacteria tested were sensitive to this bacteriocin, but *Salmonella* Typhi, *E. coli*, and *Pseudomonas aeruginosa* were not inhibited. Zacconi et al. *(25)* reported that kefir administration prevents colonization of chicken caecum with *Salmonella* Kedougou and ascribed this effect not only to the complexity of kefir microflora but also to its variability.

The purpose of the present study was to evaluate the inhibitory power of kefir over gram-negative microorganisms isolated from human clinical fecal specimens and gram-positive microorganisms isolated from food samples. The role of organic acids in bacterial inhibition was also analyzed.

MATERIALS AND METHODS

Strains and growth media. The Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) kefir grains collection was obtained from different private households in Argentina and designated CIDCA AGK1 and CIDCA AGK2. Stock kefir grains were maintained at −20°C and reactivated by successive subculture in milk at 20°C as described previously *(5)*.

To obtain fermented milks, active kefir grains were washed with sterile distilled water, inoculated into sterile nonfat milk (Las tres niñas, Sancor S.A, Santa Fe 2322, Argentina) or MRS *(4)* broth (Difco Laboratories, Detroit, Mich.), and incubated at 20°C. Kefir grains were separated from the fermented milk or MRS broth by filtration through a plastic sieve previously sanitized by immersion in 70% ethanol and then washed with sterile water.

E. coli 3, *E. coli* EPI 173, *E. coli* EPEC 18131, *E. coli* EHEC 18773, *Salmonella* sp. 521, *Salmonella* no Typhi 18684, *Salmonella* sp. (poly B), *Shigella flexneri*, *Shigella sonnei* 170, *S. sonnei* 171, *Bacillus cereus*, *Bacillus subtilis*, and *S. aureus* were used in inhibitory assays as target microorganisms. Gram-negative strains were isolated from human fecal specimens and provided by Dr. H. Lopardo of the Hospital de Pediatría Garrahan in Buenos Aires, Argentina. Gram-positive strains were isolated from food samples and provided by Dr. M. T. Painceira of the Cátedra de Microbiología General, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina. All strains were propagated at 37°C in nutrient broth containing 5 g/liter of meat peptone and 3

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g/liter of meat extract (pH 6.8 ± 0.2). Solid media were prepared by adding 15 g of agar per liter of broth formulation. All media components were obtained from Difco Laboratories.

Yogurt was prepared with *Lactobacillus delbruekii* subsp. *bulgaricus* CIDCA 331 and *Streptococcus thermophilus* CIDCA 324 inoculated at 1% into sterile nonfat milk, incubated at 37°C, and harvested at pH 3.57.

Culture supernatants preparation. Kefir supernatants were obtained by centrifugation of fermented milk or MRS broth at 14,000 × *g* for 15 min. Yogurt supernatants were obtained from fermented milk by the same procedure. In one set of experiments, artificially acidified milks were obtained by adding 2.35% racemic lactic acid (May and Baker, LTD, Dagenham, England) and/or 0.12% acetic acid (Merck, Darmstadt, Germany) to the milk. The pH was adjusted to 3.42 to 3.53 with 2.5 N NaOH. Milks acidified with hydrocloric acid were obtained by adding 2 N HCl to reach pH 3.47. In another set of experiments, artificially acidified milks were centrifuged as described above to obtain supernatants. The pH of supernatants was determined at 25°C with a Cole-Parmer (Chicago, Ill.) combined glass-calomel microelectrode. All supernatants were then filtered through 0.45-μm membrane filters (Millipore Corporation, Bedford, Mass.). Sterile filtrates were stored at −20°C until use.

Supernatants were obtained from three or more independent cultures. The acid content of each supernatant was determined by high-performance liquid chromatography (HPLC). The inhibitory activity of each supernatant was also assessed in liquid and solid media.

Inhibitory activity test. Inhibitory activity of supernatants toward the selected test microorganisms was determined by either the agar spot test or the agar well diffusion assay. Standard 9-cm plates containing 12 ml of nutrient agar were dried at 37°C for 10 min. A 0.5 McFarland suspension of target bacteria, made in 0.01 g/liter of tryptone (corresponding to 10^8 CFU/ml), was swabbed over the dried plate in three directions. For the spot test, 10 μl of supernatant was spotted onto the agar surface. For the agar diffusion assay, 5-mm-diameter wells were uniformly bored in the agar, and 25 μl of supernatant was dispensed into them. Plates were incubated at 37°C for 24 h. After incubation, inhibition zone diameters around the drop or surrounding each agar well were measured with a calipter (millimeter scale). Inhibition was considered positive when diameter was greater than 6 mm.

Effect of supernatants on the growth of *E. coli* 3. One milliliter of sterile nutrient broth was simultaneously inoculated with 50 μl of 10^8 CFU/ml *E. coli* 3 and 50 μl of milk supernatant, uniformly mixed, and incubated at 37°C. Sampling was made every 30 min for optical density determination at 550 nm using a Beckman DU-650 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.).

Effect of kefir grains on the growth of *E. coli* 3. Milk was simultaneously inoculated with kefir grains AGK1 in a kefir grain/milk ratio of 2 g per 100 ml and with *E. coli* 3 at 10^6 CFU/ml. After 24-h incubation at 20°C, surviving *E. coli* 3 were determined by viable counts and expressed as CFU/ml.

Organic acids determination. Organic acids were both qualitatively and quantitatively determined by HPLC according to Lombardi et al. *(9)*. Acid separation was performed with an AMINEX HPX-87H ion-exchange column (Biorad Labs, Richmond, Calif.), and organic acids were detected with a wavelength detector at 214 nm (Waters 996, Millipore). Acid identification was based on matching the retention times with standard acids. Ten-milliliter samples were added to 40 ml of 0.01 N H_2SO_4, shaken for 1 h, and centrifuged at 14,000 × *g* for 10 min. The resulting supernatants were filtered first through filter paper and then through a 0.45-μm membrane filter (Millipore). Ten microliters of the resulting filtrate was injected in the chromatograph (Waters 717, Millipore). Analyses were performed at a flow rate of 0.7 ml/min at 60°C using 0.009 N H_2SO_4 as the mobile phase. HPLC grade reagents were used as standard acids (Sigma Chemical Co., St. Louis, Mo.). Solvents were degassed under vacuum.

TABLE 1. *In vitro inhibition of gram-negative and gram-positive microorganisms by milk supernatants fermented with kefir grains*

Target microorganism	Diameter of inhibition zone (mm)[a] ± SD	
	Supernatant of AGK1[b]	Supernatant of AGK2[b]
Escherichia coli 3	11.22 ± 1.68	11.11 ± 1.02
Escherichia coli EPI 173	11.89 ± 2.71	11.33 ± 1.80
Escherichia coli EPEC 18131	12.22 ± 2.46	11.44 ± 2.44
Escherichia coli EHEC 18773	12.56 ± 1.26	12.44 ± 0.51
Salmonella spp. 521	11.89 ± 3.59	12.00 ± 0.73
Salmonella no typhi 18684	13.11 ± 2.52	12.11 ± 2.69
Salmonella spp. (poly B)	11.89 ± 2.69	12.67 ± 2.08
Shigella flexneri	13.33 ± 2.31	13.44 ± 2.37
Shigella sonnei 171	13.33 ± 0.66	12.88 ± 1.39
Shigella sonnei 170	12.67 ± 2.03	12.78 ± 2.04
Bacillus cereus	20.50 ± 2.12	19.50 ± 2.12
Bacillus subtilis	16.50 ± 3.50	14.00 ± 1.00
Staphylococcus aureus	14.00 ± 2.83	13.50 ± 2.12

[a] Diameters of inhibition zone in agar well diffusion assay. Values are expressed as mean ± SD of three independent assays.
[b] Supernatants of fermented milks were obtained from three independent milk cultures by inoculating 10 g of kefir grains in 100 ml of milk and incubating at 20°C during 48 h.

Statistical analysis. Differences in diameters of inhibition zone and in growth kinetics were tested for significance by analysis of variance to determine any significant effects at $P < 0.05$.

RESULTS AND DISCUSSION

The agar difussion assay was performed to determine the inhibitory power of culture supernatants obtained with CIDCA AGK1 and CIDCA AGK2 kefir grains. Table 1 shows that the inhibitory zone diameter was greater for gram-positive than for gram-negative microorganisms. For each target strain, the diameter of inhibition zones produced by both kefir grain supernatants did not show significant differences ($P > 0.05$).

Spot test results presented in Table 2 show that *E. coli* 3 was inhibited only by fermented milk supernatants prepared with 10 g per 100 ml of inoculum and whose pH values were between 3.32 and 4.25. However, spent culture supernatants from MRS obtained with 1 and 10 g per 100 ml of inocula (final pH 4.18 to 5.25) inhibited *E. coli* 3.

Organic acid chromatograms from all supernatants analyzed throughout the whole fermentation process showed lactic and acetic acids as the only detectable end products

TABLE 2. *In vitro inhibition of* Escherichia coli *3 by spot test assay*

Variable	1 g/100 ml			10 g/100 ml		
	6 h	24 h	48 h	6 h	24 h	48 h
pH of milk supernatant	6.20–6.28	5.12–5.77	4.06–5.27	4.26–5.06	3.59–4.25	3.32–3.84
Escherichia coli 3 inhibition (N^+/n)[a]	0/5	0/8	0/11	0/11	8/11	13/13
pH of MRS supernatant	5.84–5.91	5.25–5.73	4.60–4.78	4.62–4.70	4.20–4.53	4.18–4.34
Escherichia coli inhibition (N^+/n)[a]	0/2	1[b]/2	4/4	4/4	4/4	4/4

[a] N^+/n, number of positive spot test inhibition assay per number of total spot test inhibition assays. Each assay was performed with fermented milk or fermented MRS broth supernatants obtained from independent cultures. Inhibition zones with diameters of ≥6 mm were considered positive.

[b] Fermented MRS broth with pH 5.25 was the supernatant with inhibitory activity.

(data not shown). As expected, pH decreased with organic acid production (Fig. 1A, 1B, 1D, and 1E). After 48-h incubation, lactic acid concentration in fermented milk (pH 3.67) and in MRS (pH 4.20) was 2.21 ± 0.20% and 2.14 ± 0.62%, respectively. After 48-h incubation, acetic acid reached a concentration of 0.10 ± 0.01% in milk and 0.62 ± 0.02% in MRS. In MRS broth, the acetic acid level was high from the beginning of the incubation due to the sodium acetate content of this medium (Fig. 1E). It is known that the undissociated form of organic acids are the main species involved in the inhibitory power *(6, 14)*. The undissociated form of each acid was calculated from its dissociation constant, concentration, and pH (Fig. 1C and 1F). Lactic acid had the highest proportion of its undissociated form in milk, whereas acetic acid had the highest proportion in MRS. The increase of the undissociated acetic acid form in MRS may be due to the pH drop, since acetic acid concentration determined by HPLC during the fermentation process showed a small increase. After 6 h of fermentation, the pH of the medium is lower than acetic acid pK_a (4.75), so the undissociated form of this acid had an important increment.

To evaluate if the organic acids were the only agents responsible for the inhibitory effects, artificially acidified supernatants were prepared and assayed. Table 3 shows that kefir-fermented milk supernatants had inhibitory power. When milk was supplemented with both acids at similar concentration and pH to the kefir-fermented milk, inhibition of target strains was also observed. On the other hand, when milk was supplemented individually with each acid to reach similar pH and concentration to the fermented milk, the inhibitory effect was only observed for lactic acid. The pH per se had no inhibitory effect, since acidified milk with HCl did not inhibit target strain growth. When lactic and acetic acid were evaluated individually in supplemented milk in ranges from 0 to 3.06% and 0 to 0.50%, respectively, lactic acid showed an inhibitory effect toward the target strain if the undissociated form was more than 0.47%. On the other hand, undissociated acetic acid showed the same behavior at concentrations higher than 0.28% (data not shown). After a 48-h incubation, undissociated lactic acid concentration in fermented milk and MRS was enough to inhibit the growth of *E. coli* 3. In MRS-fermented broth, the concentration of undissociated acetic acid was higher than the concentration that showed inhibition.

Inhibition of the growth of *E. coli* 3 in nutrient broth at 37°C by different supernatants was studied (Fig. 2). Supernatants from kefir-fermented milk inhibited the growth even after 24 h of incubation. Artificially acidified milk supernatants with the same pH and organic acid concentrations as kefir-fermented milk also affected the growth of *E. coli* 3 during the first 8 h. However, these supernatants allowed the growth of *E coli* 3 after 24 h of incubation, whereas supernatants of kefir-fermented milk did not.

Inhibition of *E. coli* 3 by yogurt supernatant was not observed despite the fact that initial pHs (4.65 ± 0.06) in nutrient broth supplemented with kefir or yogurt supernatants were identical.

Figure 3 shows the influence of fermented milk supernatants from AGK1 kefir grains on the viability of *E. coli* 3. A bacteriostatic effect could be observed in every culture despite the initial level of the target strain. After a 15-h incubation, *E. coli* 3 could reach a level of $10^9 - 10^{10}$ CFU/ml in nutrient broth. The presence of kefir supernatants prevented further growth of this microorganism (Fig. 3A). This bacteriostatic effect also could be observed in experiments in which *E. coli* 3 was inoculated in milk at 10^6 CFU/ml with 2 g per 100 ml of AGK1 kefir grains (Fig. 3B). Under these experimental conditions, there may be a competition for nutrients between kefir microflora and *E. coli* 3. Otherwise, these results would suggest that substances responsible for the inhibition could appear at early stages in the milk fermentation.

There are only a few reports about inhibitory power of kefir *(3, 15, 21)*. The present work shows the ability of milk and MRS broth fermented with CIDCA AGK1 and CIDCA AGK2 kefir grains to inhibit the growth of several gram-negative and gram-positive bacteria.

Acid production by lactic acid bacteria is one of the oldest methods used to influence the growth of gram-negative bacteria *(6)*. Inhibition of the growth of a nonpathogenic laboratory *E. coli* strain by 0.09% undissociated lactic acid was reported by Presser et al. *(14)*. In our work, *E. coli* 3 was inhibited by at least 0.47% of undissociated lactic acid. This difference could be explained because in general pathogenic *E. coli* strains are significantly more acid tolerant than nonpathogenic *E. coli* strains *(10)*. Shafic and

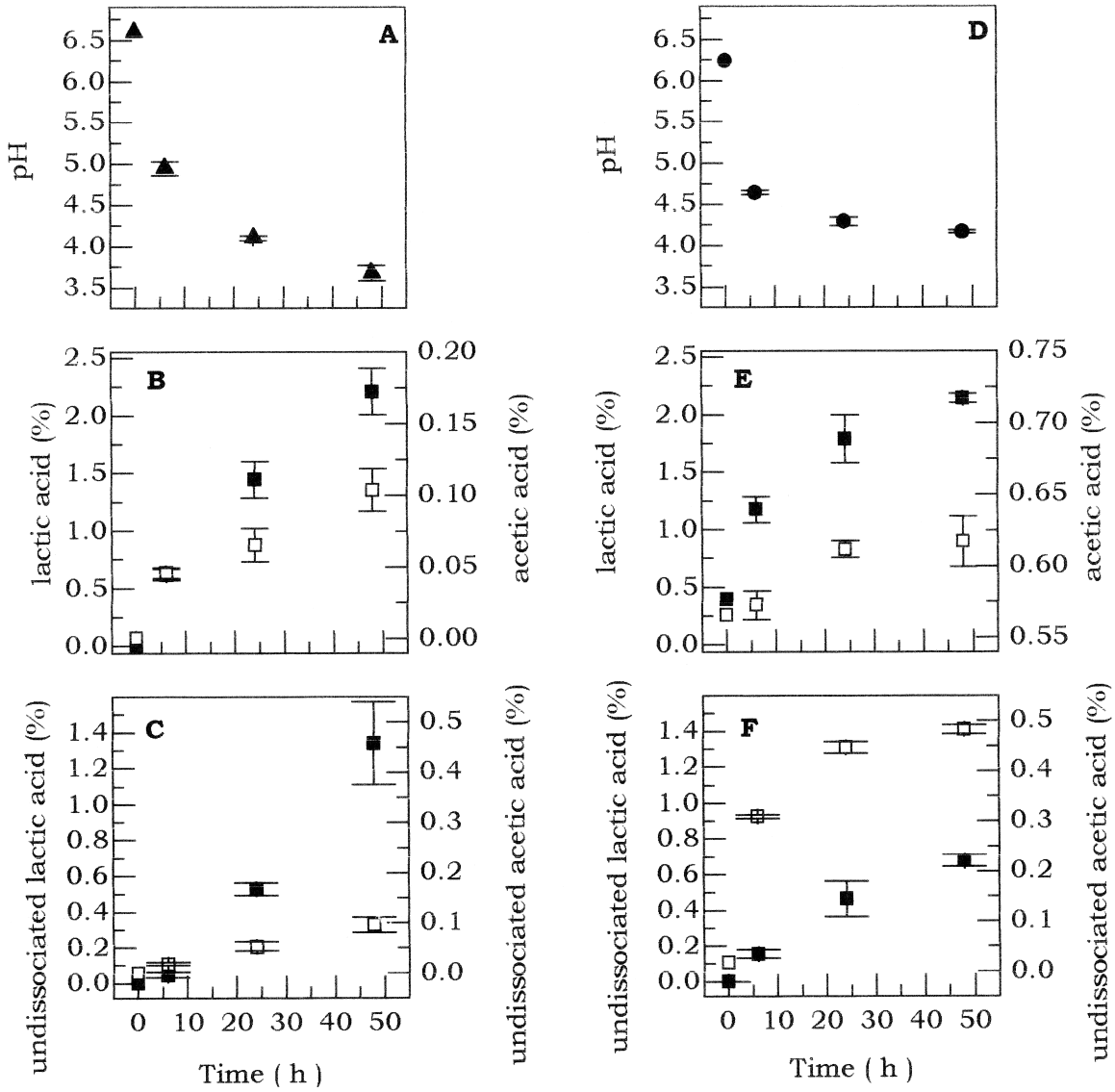

FIGURE 1. *Acidification kinetics (●) in milk (A) and MRS broth (D). Production kinetics of lactic (■) and acetic (□) acids in milk (B) and MRS broth (E) fermented with kefir grains at 10 g per 100 ml of inoculum. Undissociated lactic (■) and acetic (□) acid content in milk (C) and MRS broth (F) fermented with kefir grains at 10 g per 100 ml of inoculum.Values represent the mean of two independent assays.*

Musleh *(22)* showed that 0.10% of acetic acid inhibited various foodborne bacteria with the exception of *E. coli*, which required a concentration of at least 0.30% for its inhibition.

Our results show that the effect of kefir on *E. coli* was bacteriostatic and mainly due to organic acids produced during the fermentation process. In addition, no inhibitory effect was observed with neutralized supernatants (data not shown). Although lactic acid was important for inhibition, the presence of acetic acid improved the inhibitory effect. For instance, yogurt supernatants (which do not contain acetic acid) did not inhibit *E. coli*, whereas kefir did. The synergistic inhibitory effect of mixtures of lactic and acetic acid have been ascribed to the potentiation of acetic acid at the lower pH produced by lactic acid, shifting the equilibrium in favor of the undissociated form of the acids *(6)*.

Considering the inhibitory effect over gram-negative bacteria isolated from clinical feces, we believe that kefir could be potentially used as a probiotic. If gut colonization with kefir strains could be achieved, acid production in situ could inhibit colonization with pathogenic microorganisms. Furthermore, as a homemade product, kefir presents a low risk of contamination due to its ability to inhibit and/or to compete with spoilage microorganisms.

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REFERENCES

1. Abraham, A. G., and G. L. De Antoni. 1999. Characterization of kefir grains grown in cow's milk and in soya milk. J. Dairy Res. 66: 327–335.

TABLE 3. Escherichia coli *3 inhibition by fermented milk or artificially acidified milk supernatants*

Sample[a]	Lactic acid (%)	Acetic acid (%)	pH	Undissociated lactic acid (%)	Undissociated acetic acid (%)	Inhibition[b]
AGK1	2.07[c]	0.09[c]	3.74	1.18	0.08	+
AGK2	2.35[c]	0.11[c]	3.61	1.50	0.11	+
Milk	2.35	0.12	3.42	1.72	0.11	+
Milk	0	0.12	3.50	0	0.11	−
Milk	2.35	0	3.53	1.60	0	+
Milk	0	0	3.47	0	0	−

[a] Supernatants of fermented milks (AGK1, AGK2) were obtained by inoculating 10 g of kefir grains in 100 ml of milk and incubating at 20°C for 48 h. Acidification or alcalinization of milk was made with 2 N HCl or 10% NaOH to reach indicated pH levels.

[b] +, positive inhibition (diameters of zone inhibition ≥6 mm); −, negative inhibition (diameters of zone inhibition <6 mm).

[c] Organic acids determined by HPLC.

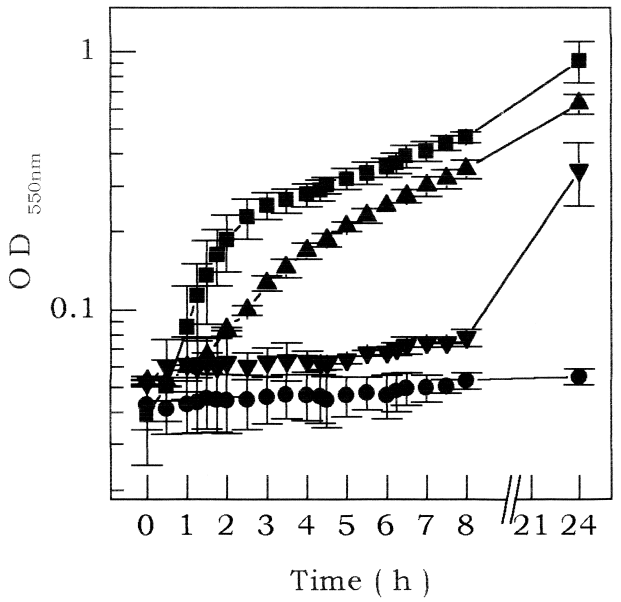

FIGURE 2. *Growth kinetic of* E. coli *3 in nutrient broth at 37°C (■) supplemented with kefir-fermented milk supernatant (●), yogurt supernatant (▲), or acidified milk supernatant (2.35% lactic acid and 0.12% acetic acid, pH 3.42) (▼). Values represent the mean of three or more independent experiments.*

2. Angulo, L., E. Lopez, and C. Lema. 1993. Microflora present in kefir grains of the Galician Region (North-West of Spain). J. Dairy Res. 60:263–267.
3. Brialy, C., P. Rivalland, L. Coiffard, and Y. De Roeck Holtzhauer. 1995. Microbiological study of lyophilized dairy kefir. Folia Microbiol. 40:198–200.
4. De Man, J. C., M. Rogosa, and M. Sharpe. 1960. A medium for cultivation of lactobacilli. J. Appl. Bacteriol. 23:130–135.
5. Garrote, G. L., A. G. Abraham, and G. L. De Antoni. 1997. Preservation of kefir grains, a comparative study. Lebensm.-Wiss. Technol. 30:77–84.
6. Helander, I. M., A. von Wright, and T.-M. Mattila-Sandholm. 1997. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends Food Sci. Technol. 8:146–150.
7. Hosono, A., T. Tanabe, and H. Otani. 1990. Binding properties of lactic acid bacteria isolated from kefir milk with mutagenic amino acid pyrolyzates. Milchwissenschaftliche 45:647–651.
8. Kosikowski, F. 1982. Cheese and fermented milk foods, 2nd ed. Kosikowski and Associates, New York.
9. Lombardi, A. M., A. E. Bevilaqua, and A. N. Califano. 1994. Variation in organic acids content during ripening of Reggianito cheese in air-tight sealed bags. Food Chem. 51:221–226.
10. Massa, S., C. Altieri, V. Quaranta, and R. De Pace. 1997. Survival of *Escherichia coli* 0157:H7 in yoghurt during preparation and storage at 4°C. Lett. Appl. Microbiol. 24:347–350.
11. Murofushi, M., M. Shiomi, and K. Aibara. 1983. Effect of orally administered polysaccharide from kefir grain on delayed-type hypersensivity and tumor growth in mice. Jpn. J. Med. Sci. Biol. 36: 49–53.
12. Osada, K., K. Nagira, K. Teruya, H. Tachibana, S. Shirahata, and H. Murakami. 1994. Enhancement of interferon-β production with sphingomyelin from fermented milk. Biotherapy 7:115–123.
13. Ottogalli, G., A. Galli, P. Resmini, and G. Volonterio. 1973. Composizione microbiologica, chimica ed ultrastruttura dei granuli di kefir. Ann. Microbiol. (Paris) 23:109–121.
14. Presser, K. A., D. A. Ratkowsky, and T. Ross. 1997. Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. Appl. Environ. Microbiol. 63:2355–2360.
15. Rosi, J. 1978. I microorganismi del kefir: I Lieviti. Sienza Tecnica Lattiero-Casearia. 29:59–67.
16. Rosi, J. 1978. I microorganismi del kefir: Gli Acetobatteri. Sienza Tecnica Lattiero-Casearia. 29:221–227.
17. Rosi, J., and J. Rossi. 1978. I microorganismi del kefir: I fermenti lattici. Sienza Tecnica Lattiero-Casearia. 29:291–305.
18. Ryan, M. P., M. C. Rea, C. Hill, and R. Paul Ross. 1996. An application in Cheddar cheese manufacture for a strain of *Lactococcus lactis* producing a novel broad-spectrum bacteriocin, lacticin 3147. Appl. Environ. Microbiol. 62:612–619.
19. Saloff-Coste, C. J. 1996. Kefir: nutritional and health benefits of yogurt and fermented milks. Danone World Newsl. 11:1–7.

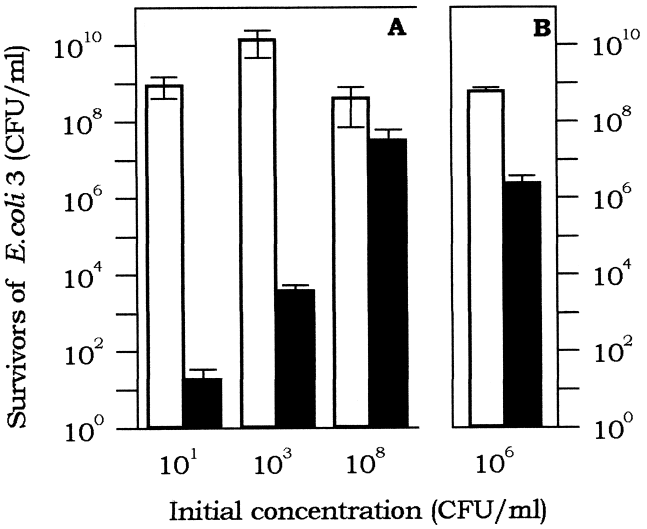

FIGURE 3. *(A) Survivors (CFU/ml) of* E. coli *3 after 15 h of incubation at 37°C inoculated at different initial concentrations in nutrient broth (□) and nutrient broth with AGK1-fermented milk supernatant (■). (B) Survivors (CFU/ml) of* E. coli *3 after 24 h of incubation at 20°C inoculated at 10^6 initial concentration in milk (□) and milk with AGK1 kefir grains at 2 g per 100 ml of inoculum (■).*

20. Schillinger, V. 1990. Bacteriocins of lactic acid bacteria, p. 55–79. *In* D. D. Bills and S. Kung (ed.), Biotechnology and Food Safety. Butterworth-Heinemann, Boston, Mass.
21. Serot, T., X. Dousset, J. Zucca, and N. Torcatis. 1990. Mise en évidence et purification partielle de substances antibactériennes produites par *Leuconostoc mesenteroides* et *Lactobacillus plantarum* isolés de grains de kéfyr. Microbiol. Aliment. Nutr. 8:71–76.
22. Shafic, M. H., and R. M. Musleh. 1988. Resistance of local strains of food bacteria to organic acids. J. Biol. Sci. Res. 19:611–622.
23. Toba, T. 1987. Fermented milks produced with mesophilic lactic acid bacteria: kefir and scandinavian ropy sour milk. Jpn. J. Dairy Food Sci. 36:235–244.
24. Vayssier, Y. 1978. Kefir: qualitative and quantitative analysis. Revue Laitiere Francais. 361:73–75.
25. Zacconi, C., M. G. Parisi, P. G. Sarra, P. Dallavalle, and V. Botazzi. 1995. Competitive exclusion of *Salmonella kedougou* in kefir fed chicks. Microbiol. Aliment. Nutr. 12:387–390.