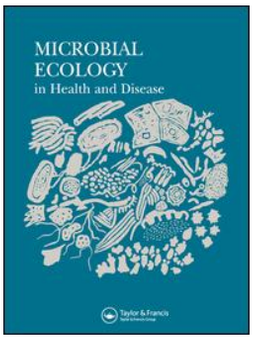

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ORIGINAL ARTICLE

The effect of probiotic drinks containing homofermentative or hetrofermentative strains of lactobacilli on the growth of *Candida albicans* and *Escherichia coli* in mixed culture

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Abstract
The effect of *Lactobacillus* species isolated from probiotic drinks on the growth of *Candida albicans* and *Escherichia coli* was investigated. Two of the isolates, *Lactobacillus casei* Immunitas® and *L. casei* Shirota, are homofermentative organisms. They produced lactic acid that lowered the pH of the culture medium, resulting in a reduction in cell numbers of *C. albicans* and *E. coli* in mixed culture over a 24 h period. The third isolate, which was listed as *L. reuteri* and a hetrofermentative organism, produced the most DL-lactic acid compared to the other two isolates that produced L(+)-lactic acid in excess of D(−)-lactic acid and also lowered the cell numbers of *C. albicans* and *E. coli* in mixed culture over a 24 h period. Identification of each probiotic isolate was confirmed by an API 50 CHL strip test. Both homofermentative isolates were positively identified as *L. casei*, whereas the hetrofermentative isolate was identified as *L. casei*, a homofermentative organism – not *L. reuteri*, which is a hetrofermentative organism.

Key words: *probiotic isolates, DL-lactate, L-lactate, homofermentative, hetrofermentative, mixed culture, antibiotics*

Introduction

For a number of decades living cultures of *Lactobacillus* species have been advocated by various authorities as a means of controlling enteric and vaginal infections caused by *Candida albicans* (1). *C. albicans* is a classic example of an opportunistic pathogen and it is found in healthy individuals growing on mucosal surfaces, albeit in small numbers (2). In this type of habitat, the proliferation of this organism is held in check by various factors including the presence of bacteria which compete with *Candida* for the available mucosal surfaces. However, if the local environmental conditions change, such as alterations in pH, prolonged exposure to antibacterial antibiotics or the presence of sugars, all of these features tend to induce the organism to spread rapidly across the mucosal surfaces and result in the development of the clinical condition known as candidiasis or thrush (3). Vaginal thrush is thought to originate from the gastrointestinal tract. *C. albicans* is reported as being able to enhance mucosal colonization (4). While various types of candidiasis can be treated with antifungal agents such as nystatin, miconazole and amphotericin B, the last 20 years has seen a marked increase in the marketing of specific probiotic products (5). These commercial formulations are aimed at improving the well-being of individuals and controlling the development of secondary *C. albicans* infections by establishing a benevolent and beneficial lactobacilli bacterial flora in the gut. This report records our investigations into the effects of three commercial preparations of *Lactobacillus* probiotic drink formulations on the growth of mixed cultures of *C. albicans* and a dominant bacterial species of the human gut, *Escherichia coli*.

Materials and methods

Microorganisms and their maintenance

The *Lactobacillus* strains used this study were isolated from the following commercially available probiotic drinks: Actimel [A] (*L. casei* Imunitass®), Orchard Maid [OM] (*L. reuteri*) and Yakult [Y] (*L. casei* Shirota). The *C. albicans* strain NCPF 3179 used in this study was obtained from the National Collection of Pathogenic Fungi. The *E. coli* strain

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used in this study is a laboratory strain from the University of East London culture collection, culture no. 57.

All strains were grown aerobically in de Man, Rogosa, Sharpe (MRS) broth (Oxoid CM 359, Basingstoke, UK) for 24 h at 37°C.

API 50 CHL identification of probiotic isolates

The identification of probiotics was determined by carrying out an API 50 CHL strip test on each of the isolates. A type culture *L. reuteri* ATCC 55730 (donated by Biogaia Biologics Inc., Raleigh, NC, USA) was also profiled using the same identification system.

Effect of lactic acid on the growth of a mixed culture of probiotic isolates and C. albicans or probiotic isolates and E. coli

The method used was described previously by Elsom et al. (6). It involves growing all the cultures aerobically in MRS broth for 24 h at 37°C and inoculating into a pre-warmed flask of MRS broth (50 ml) to give approximate cell numbers of 6×10^7 colony forming units (cfu)/ml Actimel, 1.5×10^7 cfu/ml Orchard Maid, 6.65×10^6 cfu/ml Yakult, 7.5×10^5 cfu/ml *C. albicans* and 1.25×10^7 cfu/ml *E. coli*. Inoculation size was confirmed by carrying out a surface plate count (7) on MRS agar (Oxoid CM 371). For each experiment one flask of each culture was inoculated and grown to mid log phase at 37°C, then the probiotic isolate and either *C. albicans* or *E. coli* were mixed to give a 100 ml volume of mixed culture. The mixed cultures were kept in static microaerophilic conditions at 37°C. Samples were taken at 0, 2, 4, 6 and 24 h. Cell numbers were determined by carrying out a surface plate count (7) using MRS agar when testing probiotic isolates and *C. albicans*. When testing probiotic isolates and *E. coli*, Rogosa agar (ROG; Oxoid CM 627) was used to determine the cell numbers of probiotic isolates and Eosin Methylene Blue (EMB; Oxoid CM 69) was used to enumerate numbersof *E. coli*.

When each sample was taken an extra 1 ml was removed from the mixed culture and tested to determine the concentration of D- and L-lactate produced by each of the probiotic isolates under test. The procedure used follows the method of Bergmeyer (8), which involves terminating the growth of the cultures by freezing to −20°C and storing the samples for lactate analysis. The lactate determination was carried out as follows in that 0.4 ml of 0.4 M perchloric acid was added to 0.2 ml of culture medium and left on ice for 10 min. After centrifugation at 13 000 rpm in a microfuge for 5 min, 0.4 ml of the supernatant was removed to separate microfuge tubes. Neutralization was accomplished by the addition of 50 µl of 3 M KOH. After standing on ice for 10 min, the perchlorate was removed as the insoluble potassium salt by centrifugation at 13 000 rpm for 5 min. D- and L-lactate concentrations were determined by monitoring NADH formation, by conversion of lactate to pyruvate using L-LDH and D-LDH (suspension in 3.2 M $(NH_4)_2SO_4$; Sigma Chemical Co.) using a microtitreplate assay. Then 0.2 ml of 0.5 M glycine/0.4 M hydrazine buffer, pH 9.0, was added to each well, together with 20 µl of 30 mM NAD^+ and a 10 µl sample of standard. Samples were diluted 1:5. The microtitre plate was incubated at 37°C and agitated at 11 000 rpm before an initial absorbance reading at 340 nm was taken. The reaction was started by adding 5 µl L-LDH (diluted 1:5 in water). Absorbance readings were recorded every minute for 15 min to ensure complete conversion of lactate to pyruvate. This was followed by the addition of 5 µl of D-LDH and absorbance readings were again taken for the following 15 min. The increase in absorbance after the addition of the two enzymes was calculated and compared with standard curves constructed using L- and D-lactate. After taking into account appropriate dilution factors, the L- and D-lactate concentrations in the original culture medium were calculated.

Results

API 50 CHL identification of probiotic isolates

The API 50 CHL results confirmed that *L. casei* Immunitas® (A) was identified as *L. casei* and *L. casei* Shirota (Y) was identified as *L. casei* but *L. reuteri* (OM) was also identified as *L. casei*, which is a homofermentative Lactobacillus whereas *L. reuteri* is a hetrofermentative *Lactobacillus*. The type strain of *L. reuteri* was identified as *L. fermentum*.

Effect of lactic acid on the growth of a mixed culture of probiotic isolates and C. albicans or probiotic isolates and E. coli

The mixed culture of Actimel and *C. albicans* was grown over a 24 h period and the number of *C. albicans* cells was reduced by 99.83% (Table I). When Actimel and *E. coli* were grown in a mixed culture over a 24 h period the numbers of *E. coli* cells dropped to zero after 24 h (Table I).

The mixed culture of Orchard Maid and *C. albicans* was grown over a 24 h period and the numbers of *C. albicans* cells were reduced by 99.74% (Table II). When Orchard Maid and *E. coli* were

Table I. Actimel (*L. casei* Immunitas®) versus *C. albicans* and *E. coli* in mixed culture grown statically at 37°C.

Time (h)	Viable Actimel cell count (cfu/ml)	Viable *C. albicans* cell count (cfu/ml)
0	7×10^6	4×10^5
2	2×10^7	7×10^5
4	6.67×10^7	1.83×10^6
6	1.5×10^8	3×10^6
24	2×10^9	5×10^3
Time (h)	Viable Actimel cell count (cfu/ml)	Viable *E. coli* cell count (cfu/ml)
0	2×10^7	7.33×10^6
2	6.5×10^7	1.03×10^7
4	3×10^8	1.67×10^7
6	6×10^8	1.5×10^7
24	4.5×10^8	0

Table III. Yakult (*L. casei* Shirota) versus *C. albicans* and *E. coli* in mixed culture grown statically at 37°C.

Time (h)	Viable Yakult cell count (cfu/ml)	Viable *C. albicans* cell count (cfu/ml)
0	1.67×10^7	5.5×10^5
2	6.5×10^7	7.5×10^5
4	1.5×10^8	2.83×10^6
6	5×10^8	1.61×10^5
24	1.5×10^9	3.5×10^3
Time (h)	Viable Yakult cell count (cfu/ml)	Viable *E. coli* cell count (cfu/ml)
0	4.5×10^7	1.33×10^6
2	1.67×10^8	1.67×10^6
4	5×10^8	1×10^6
6	6.5×10^8	6.67×10^5
24	4.67×10^8	0

grown in a mixed culture over a 24 h period the numbers of *E. coli* dropped to zero after 24 h.

The mixed culture of Yakult and *C. albicans* was grown over a 24 h period and the numbers of *C. albicans* cells were reduced by 99.88% (Table III). When Yakult and *E. coli* were grown in a mixed culture over a 24 h period the numbers of *E. coli* cells dropped to zero after 24 h (Table III).

All of the probiotic isolates that were grown in mixed culture over a 24 h period, with either *C. albicans* or *E. coli*, increased their cell number by a minimum of 5×10^7 cfu/ml over the incubation time (Tables I–III).

The amount of D-lactate produced by the three probiotic isolates covered the range of 0 mM to 45.3 mM (Table IV), whereas the amount of L-lactate produced by the three probiotic isolates covered the range of 5.06 mM to 90.7 mM (Table IV).

The probiotic strain that produced the most D-lactate over the 24 h incubation period was *L. reuteri* (OM), which produced 45.3 mM (Table IV).

Table II. Orchard Maid (*L. reuteri*) versus *C. albicans* and *E. coli* in mixed culture grown statically at 37°C.

Time (h)	Viable Orchard Maid cell count (cfu/ml)	Viable *C. albicans* cell count (cfu/ml)
0	5.83×10^6	1×10^6
2	1.83×10^8	1.16×10^6
4	3×10^8	1.33×10^6
6	7×10^8	1.067×10^6
24	1×10^9	3.5×10^3
Time (h)	Viable Orchard Maid cell count (cfu/ml)	Viable *E. coli* cell count (cfu/ml)
0	1.53×10^7	4×10^5
2	6.5×10^7	4.33×10^5
4	1.67×10^8	5.67×10^5
6	4×10^8	1×10^6
24	7×10^7	0

The probiotic strain that produced the least D-lactate over the 24 h incubation period was *L. casei* Shirota (Y), which produced 32.9 mM (Table IV).

The probiotic strain that produced the most L-lactate over the 24 h incubation period was *L. casei* Shirota (Y), which produced 90.7 mM (Table IV). The probiotic strain that produced the least L-lactate over the 24 h period was *L. reuteri* (OM), which produced 65.4 mM (Table IV).

Discussion

The results of this study show that all three of the *Lactobacillus* species clearly inhibited the growth of *E. coli* and *C. albicans*. None of the species completely inhibited *C. albicans* but they did reduce the cell population by 99% under the conditions of the test. With *E. coli* the cell count was reduced to zero. These observations confirm the findings of Elsom et al. (6).

Fooks and Gibson (9) have surveyed the methods by which probiotic organisms could exert their effects. These include chemical inhibition or stimulation, competition for nutrients, immune clearance and competition for adhesin receptors. Pertinent to the present investigation is the production of chemical inhibitors.

The results presented here clearly show that *L. casei* Immunitas® and *L. casei* Shirota both produced relatively large amounts of L-lactate, while *L. reuteri* produced more D-lactate then the other *Lactobacillus* species investigated. The probable explanation for this variation in lactate production lies in the fact that the former organisms are classified as homofermentative species. The latter organism is classified as a hetrofermentative species. Homofermentative species produce lactic acid as the major or sole product of glucose fermentation, whereas those

Table IV. D- and L-lactate production by three dairy health drink lactobacillus isolates grown in the presence of *E. coli* or *C. albicans* in mixed culture.

		E. coli		*C. albicans*	
Product	Time (h)	D-lactate (mM)	L-lactate (mM)	D-lactate (mM)	L-lactate (mM)
Actimel	0	ND	12.3	ND	12.1
	2	ND	21.7	ND	20.7
	4	12.2	27.6	4.3	16.8
	6	7.5	41.8	8.2	22.7
	24	38.1	75.1	42	74.9
Yakult					
	0	ND	36.3	6.8	16.8
	2	ND	30.7	ND	29.2
	4	8.6	38.3	7.0	30.3
	6	13.6	47.2	9.2	41.2
	24	37.5	86.7	32.9	90.7
Orchard Maid					
	0	7.3	5.06	4.5	23.8
	2	7.5	37.5	6.1	18.3
	4	9.7	38.6	6.8	39.5
	6	16.9	43.9	25.1	50.9

ND, not determined.

that produce equal molar amounts of lactate, carbon dioxide and ethanol from hexoses are designated as hetrofermentative species.

Although the results indicate that the inhibition of the test microorganisms was due to lactate production by the lactobacilli, there still remains the possibility that this inhibitory effect could be due to the production of antibiotics. *Lactobacillus* species are known to produce bacteriocins (10). This class of antibiotic acts primarily on Gram-positive bacteria, and as the test bacterium used in this study was Gram-negative, bacteriocin production by the *Lactobacillus* species was not a key factor in their mode of action. However, *L. reuteri* does produce an antimicrobial compound, i.e. reuterin, which is regarded as a broad-spectrum antibiotic (11) and therefore could have influenced the results in the studies involving *E. coli*. The growth of *C. albicans* was unlikely to have been affected by bacteriocins.

The significance of the results presented here is that all the isolates were successful in controlling the growth of *E. coli* and *C. albicans in vitro*, probably by lactate production. It is not known if the three isolates possessed the properties of an efficient probiotic as defined by Fooks and Gibson (9). These include the ability of the probiotic to survive gastric acidity, bile secretion and competition with the indigenous microbial flora.

Probiotic preparations have been used with varying degrees of success in treating *Candida* and *E. coli* infections. Tomoda et al. (12) demonstrated that a milk formulation containing *L. acidophilus* and *Bifidobacterium* species effectively reduced the count of *Candida* cells in the faeces of cancer patients. There is also evidence that *Lactobacillus* species can moderate traveller's diarrhoea caused by *E. coli* (13). Hilton et al. (14) achieved a 74% success rate in treating *Candida* vaginitis with *L. acidophilus* yoghurt given orally, while de Vrese and Schrezenmeir (15) suggested that the vaginosis could be treated with a probiotic preparation applied to treated tampons or in gel beads.

A recent study on the effect of *Lactobacillus* species in preventing post-antibiotic vulvovaginal candidiasis published in the *British Medical Journal* by Pirotta et al. showed that the use of *Lactobacillus* species (oral or vaginal forms) to prevent post-antibiotic vulvovaginal was not supported by their results (16).

The results of the mixed culture experiments for the three probiotic drink isolates indicate that they may be of use in controlling and redressing the balance of the normal flora when it has been disrupted. However, further investigations will be required to ascertain the usage in an *in vivo* situation.

This investigation was carried out as a model system with a limited number of *Lactobacillus* isolates tested and further investigations are planned using a range of *Lactobacillus* isolates to ascertain the therapeutic potential of the species.

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References

1. Barilla J. The balance of the bugs. Better Nutrition. 1999;61: 54.
2. Sobel JD. Pathogenesis and treatment of recurrent vulvovaginal candidiasis. Clin Infect Dis. 1992;14(Suppl):148–53.
3. Kent HL. Epidemiology of vaginitis. Am J Obstet Gynecol. 1991;165:1168–75.
4. Wellmer A. Adhesion of *Candida albicans* isolate to buccal epithelial cells. Mycoses. 1999;42:43–7.
5. Reid G. Probiotics for urogenital health. Nutr Clin Care. 2002;5:3–8.
6. Elsom GK, Freeman JA, Hide D, Salmon DM. Antibacterial and anticandidal effect of aqueous extract of garlic on the growth of mixed cultures and the anticandidal and platelet activity of commercial preparations of garlic. Microb Ecol Health Dis. 2003;15:193–9.
7. Miles AA, Misra SS. The estimation of the bactericidal power of blood. J Hyg Camb. 1938;38:732.
8. Bergmeyer UH. Methods of enzymatic analysis, 2nd English edn. Weinheim: Verlag Chemie, Academic Press; 1974. p. 582–90.
9. Fooks LJ, Gibson GR. Probiotics as modulators of the gut flora. Br J Nutr. 2002;88(Suppl 1):S39–S49.
10. Saaverda L, Castellano P, Sesma F. Purification of bacteriocins produced by lactic acid bacteria. Methods Mol Biol. 2004;268:331–6.
11. Axelsson L, Chung TC, Dobrogosz WJ, Lindgren SE. Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. Microb Ecol Health Dis. 1989;2:131–6.
12. Tomoda T, Nakano Y, Kageyama T. Variation of intestinal *Candida* of patients with leukaemia and the effect of *Lactobacillus* administration. Japanese J Med Myco. 1983; 24:356–8.
13. Hilton E, Kolakowski P, Smith M, Singer C. Efficacy of *Lactobacillus* GG as a diarrhoea preventative. Journal of Travellers Medicine. 1996;4:3–7.
14. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. Ann Intern Med. 1992; 116:353–7.
15. de Vrese M, Schrezenmeir J. Probiotics and non-intestinal infectious conditions. Br J Nutr. 2002;88(Suppl 1):S59–S66.
16. Pirotta A, Gunn J, Chondros P, Grover S, O'Malley P, Hurley S, et al. Effect of lactobacillus in preventing post-vulvovaginal candidiasis: a randomized controlled trial. BMJ. 2004;329: 548.