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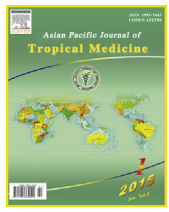

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Potential of Zimbabwean commercial probiotic products and strains of *Lactobacillus plantarum* as prophylaxis and therapy against diarrhoea caused *Escherichia coli* in children

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ABSTRACT

Objective: To evaluate the potential of commercial fermented products sold in the country, and strains of *Lactobacillus plantarum* (*L. plantarum*) as prophylaxis and therapy against diarrhoea in children.

Methods: The antimicrobial potential of cultures of lactobacilli enriched from 4 Zimbabwean commercial food/beverage products: Dairibord Lacto sour milk (DLSM), Probrand sour milk (PSM), Kefalos Vuka cheese (KVC) and Chibuku opaque beer (COB); and four strains of *L. plantarum* obtained from Balkan traditional cheeses against clinical strains of *Escherichia coli* (*E. coli*) was assayed using the well diffusion method. Three commercial paediatric antidiarrhoeal drug products: Biogaia (BG), Prolife (PL) and Probio Junior (PJ) and a mutant strain of *E. coli* [strain 11105 (ATCC) – a vitamin B-12 auxotroph and penicillin G acylase-producing strain] were used as controls. An agar diffusion assay and a competitive exclusion assay were carried out on Mueller Hinton agar.

Results: Crude cultures of putative lactobacillus strains obtained from Zimbabwean dairy products (Probrand sour milk, Kefalos Vuka vuka cheese and Chibuku opaque beer) had significantly higher antimicrobial activities against clinical strains of *E. coli* than strains of *L. plantarum* isolated from Balkan cheeses (CLP1, CLP2 or CLP3) and crude microbial cultures from commercial paediatric probiotic products (BG, PJ and PL) of a culture of *Lactobacillus rhamnosus* LGG ($P < 0.05$).

Conclusions: The putative *Lactobacilli* from four commercial Zimbabwean dairy products (Probrand sour milk, Kefalos Vuka vuka cheese and Chibuku opaque beer), and three strains of *L. plantarum* from Balkan cheeses (CLP1, CLP2 or CLP3) exhibited high antibacterial activities that can be harnessed to control paediatric diarrhoea that is caused by pathogenic strains of *E. coli*. Studies to characterise the probiotic potential of the live cultures in the products and the new strains of *L. plantarum* are underway.

1. Introduction

Probiotic bacteria are known to impart positive health effects on the host [1]. A number of probiotic microorganisms have been shown to inhibit enteropathogens including strains of *Escherichia coli* (*E. coli*) [2–4], *Campylobacter jejuni* [5] and rotavirus [6]. Strains of *Lactobacillus rhamnosus* (*L. rhamnosus*) GG are known to inhibit bacterial agents of diarrhoea, particularly *E. coli*, *Campylobacter jejuni* and *Shigella* species [7]. Additionally, a number of probiotic products have been used to treat diarrhoea, including milk products such as fermented milk and yoghurt [8], and medicines (suspensions, powders or capsules) containing live probiotic microorganisms [9].

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A number of probiotics reportedly control enteropathogens through the following mechanisms: (i) direct antimicrobial activity through production of bacteriocins or inhibitors of virulence gene expression [10]; (ii) competitive exclusion by preventing access of the pathogen to binding sites or stimulation of epithelial barrier function [2]; (iii) stimulation of immune responses through the regulated expression of secretory immunoglobulin A (sIgA), and anti-inflammatory and pro-inflammatory cytokines [11]; and (iv) inhibition of the virulence gene or protein expression in gastrointestinal pathogens [12].

Despite the availability of probiotic products that are reportedly efficacious against diarrhoea, the race is on to identify more naturally occurring probiotics or engineer new microbial strains with greater efficacies than the existing ones. Bacteriocins are frequently shown to inhibit enteropathogens in different studies. Several bacterial strains have been shown to produce bacteriocins, including *Lactobacillus plantarum* (*L. plantarum*) [13], *Lactobacillus acidophilus* (*L. acidophilus)* [14], *Enterococcus faecium* KH24 [15], nonpathogenic *E. coli* (microcins from *E. coli* strain Nissle 1917) [16,17], bacilli [16,18] and yeasts such as *Saccharomyces boulardii* [16]. Competitive exclusion was shown to occur against *E. coli* K1 by LGG on Caco-2 cells [19].

We aimed to validate the novelty of our isolates, which were shown in our previous studies to (i) inhibit a clinical strain of rotavirus [20] and *Listeria monocytogenes in vitro* (unpublished), (ii) have immunomodulatory activities [20] and (iii) maintain polarity in porcine enterocytes *in vitro* [21]. Based on preliminary studies on their bioactivities *in vitro*, we hypothesised that a selection of our collection of putative probiotics had greater inhibition against diarrheagenic strains of *E. coli* compared to the single or multi-strain commercial probiotic products. We therefore set to determine the inhibition of a standard strain of *E. coli* (ATCC 11105) and 6 clinical strains of *E. coli*, isolated from infants who visited a paediatric clinic in Slovenia and Zimbabwe presenting with diarrhoea, by probiotic strains isolated from commercial probiotic products and probiotic isolates from Balkan cheeses. *E. coli* (ATCC 11105), a mutant, is a vitamin B-12 auxotroph and a penicillin G acylase-producing strain [22]. We tested seven commercial probiotic products, namely Biogaia (BG), Prolife (PL) and Probio Junior (PJ), Dairibord Lacto sour milk (DLSM), Kefalos Vuka vuka cheese (KVC), Probrand sour milk (PSM) and *L. rhamnosus* GG (enriched for probiotic bacteria in MRS or Nutrient Agar) against the collection of strains of *E. coli*.

2. Materials and methods

The efficacy of probiotic strains to inhibit clinical isolates of *E. coli*, obtained from infants suffering from diarrhoea at a paediatric facility of the University of Maribor Hospital, Slovenia and at a hospital in Bindura, Zimbabwe, and a control *E. coli* strain (ATCC 11105), was tested using well diffusion method. Probiotic products, *L. plantarum* or *L. rhamnosus* GG (LGG) strains were introduced into MRS broth or nutrient broth (NB) depending on the strain/strains included in the product. Nutrient broth would allow microorganisms such as *Bacillus coagulans* and *Streptococcus thermophilus* to grow, while MRS broth allows *Lactobacillus* spp. and *Bifidobacterium* spp. to grow. The broth cultures were incubated at 37 °C (aerobically or anaerobically according to microorganisms contained).

2.1. Well diffusion assay

From isolated colonies on streak plates, strains of *L. plantarum* (CLP1-4) were incubated anaerobically overnight in MRS broth (Sigma–Aldrich, Missouri, USA). The single or multi-strain probiotic products were cultured in MRS broth (Sigma–Aldrich, Missouri, USA) and nutrient broth (NB) (Sigma–Aldrich, Missouri, USA) depending on the strains included in the product. One mL of each ProLife (liquid product) or 1 g of PJ or BG was inoculated into NB or MRS broth. MRS broth tubes were incubated anaerobically at 37 °C for 24 h, while NB tubes were incubated aerobically at 37 °C for 24 h. At the same time, strains of *E. coli* were inoculated in MacConkey broth and incubated aerobically at 37 °C for 24 h (Table 1).

Overnight probiotic cultures in MRS/nutrient broth were spun at 2000 rpm in a centrifuge, and the supernatants were removed and stored. The pellet of each probiotic strain was then washed once with Ringer's solution to remove the broth from the cells. The pellets were then re-suspended in 2 mL of Ringer's solution (pH 7.4) and their absorbance was adjusted to an OD of 1 at 650 nm equivalent to 2×10^8 colony forming units per millilitre (CFU/mL), as previously reported by Polak-Berecka *et al.* [23] using a Multiskan (Thermo Electron Oy, Vaanta, Finland). The *E. coli* broth cultures were also adjusted (in nutrient broth) to an OD at 650 nm of 1, which is equivalent to 5×10^8 CFU/mL as previously reported by Brimacombe *et al.* [24]. The *E. coli* cultures were then spread evenly on nutrient agar using sterile cotton tipped swabs to achieve a lawn of growth. A sterile cock-borer (4 mm in diameter) was used to drill 5 evenly spaced holes in an agar plate. A total of 60 μL of the probiotic cultures, supernatant fractions or solutions of a standard antibiotic [garamycin (40 mg/mL)] were introduced into each well, and the plates were incubated aerobically (NA) at 37 °C for 24 h.

After 24 h of further anaerobic incubation at 37 °C, the zones of inhibition for each *E. coli* strain were measured using a ruler. Mean inhibition scores for each probiotic product/strain against a particular strain of *E. coli* were recorded.

Table 1
Probiotic composition of commercial products tested.

Product	Composition
Prolife (PL) (Manufactured by Jadran-Galenski Laboratorij d.d., Rijeka, Croatia)	2.6×10^8 living bacterial cells/mL. *Bifidobacterium coagulans*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, *Bifidobacterium bifidum*.
Probio Junior (PJ) (Manufactured by Fidimed, Trzin, Slovenia)	1×10^9 CFU per bag, namely *L. casei*, *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. infantis* and *L. bulgaricus*.
BioGaia (BG) (Manufactured by BioGaia AB, Stockholm, Sweden)	2×10^9 CFU/mL, sunflower oil, medium chain triglyceride oil and *L. reuteri* DSM 17938 (*L. reuteri* Protectis).
Dairibord Lacto sour milk	*Lactobacillus* spp. (unspecified)
Kefalos Vuka vuka cheese	Cultures not specified
Zimbabwe Probrand sour milk	Cultures not specified

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