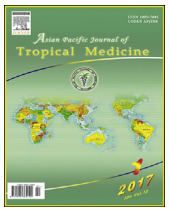
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2016.12.009

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

Walter Chingwaru[1,2,3✉], Jerneja Vidmar[2,3,4]

[1]*Department of Biological Sciences, Faculty of Science, Bindura University Science Education, P. Bag 1020, Bindura, Zimbabwe*

[2]*Maribor Institute of Biomedical Sciences, Žitna ulica 10, 2000 Maribor, Slovenia*

[3]*Institute Ceres/Zavod Ceres, Lahovna 16, 3000 Celje, Slovenia*

[4]*Department of Plastic and Reconstructive Surgery, University Medical Centre Maribor, Ljubljanska 5, 2000 Maribor, Slovenia*

ARTICLE INFO

Article history:
Received 16 Oct 2016
Received in revised form 17 Nov 2016
Accepted 16 Dec 2016
Available online 28 Dec 2016

Keywords:
Probiotic
Inhibition
Escherichia coli
Lactobacillus plantarum
Diarrhoea
Paediatric

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].

✉First and corresponding author: Dr. Walter Chingwaru, Department of Biological Sciences, Faculty of Science, Bindura University Science Education, P. Bag 1020, Bindura, Zimbabwe.
Tel: +263 7777 666 06
E-mail: wchingwaru@yahoo.co.uk
Peer review under responsibility of Hainan Medical University.

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

2.2. Statistical analysis

For the well diffusion assay, a one-way analysis of variance (ANOVA) was performed with post-hoc tests for Least Significant Difference (LSD) to determine the effect on the size of inhibition zone of the probiotic strain/product treatments ($P < 0.05$). Differences between treatments were tested using ANOVA with post-hoc tests for Least Significant Difference (LSD) to rank performances of each probiotic product or strain against the *E. coli* strains ($P < 0.05$). Calculations were performed using the SPSS software v.18 (Chicago, ILL, USA).

3. Results

CLP1, CLP2 or CLP3 exhibited significantly greater inhibition of the clinical *E. coli* strain COA6479 compared to PL and PJ (cultured in NB), and PJ (cultured in MRS) ($P < 0.05$). Only CLP2 exhibited significantly greater inhibition than LGG ($P < 0.05$) (Figure 1).

All probiotic strains and products showed significantly lower inhibition against *E. coli* (approximately 30% less) than garamycin (40 mg/mL) ($P < 0.05$) (Figure 2).

Exposure to the probiotic products (PL) or LGG had no inhibitory effect against the clinical strain of *E. coli* COA6597. However, all strains of *L. plantarum* CLP1, CLP2, CLP3 and CLP4 exhibited high inhibition against COA6507 (12–14 mm diameter of clearance). Exposure to PL (MRS) had significant greater inhibitory effect than PL or PJ (in NB) against *E. coli* COA6507 ($P < 0.001$) (Figure 3).

CLP1 and CLP4 exhibited significantly greater inhibition of the clinical *E. coli* strain COA6497 compared to PL or BG (cultured in MRS broth) ($P < 0.05$). When PL or BG was cultured in NB, they did not inhibit the growth of *E. coli* COA6497. Zones of inhibition of the *E. coli* strain by LGG or PL cultured in MRS was not significantly greater than that caused by CLP1 or CLP4 ($P > 0.60$) (Figure 4).

CLP1 and CLP2 had significantly greater inhibition of *E. coli* strain COA6961 than PJ enriched in nutrient broth or PL enriched in nutrient and MRS broth. However, PL, PJ, BG or LGG (each cultured in MRS broth) had significantly greater inhibition than PJ (cultured in NB) against strain COA6961 ($P < 0.05$). Exposure of COA6961 to CLP1 produced significant inhibition than that caused by LGG ($P < 0.05$) (Figure 5).

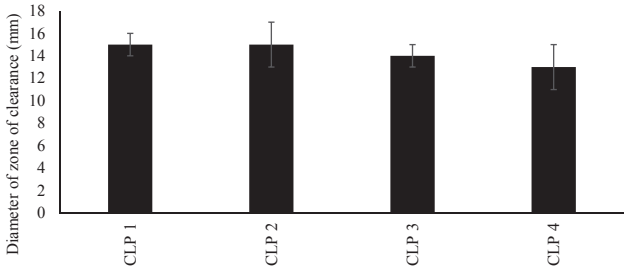


Figure 2. Inhibition (mm) of *E. coli* COA6507 using crude single-strain cultures enriched in MRS broth from Balkan cheeses (CLP1, CLP2, CLP3, CLP4 or LGG) or multi-strain paediatric antidiarrhoeal probiotic products (PL, PJ or BG) after 24 h incubation at 37 °C.
Values shown are means (bar = SE) of 3 independent experiments.

CLP1, 2 and 3 exhibited significantly greater inhibition of the clinical *E. coli* strain COA6525 than PL (cultured in NB) or PJ (cultured in NB or MRS) ($P < 0.05$). Only CLP2 exhibited significantly greater inhibition than LGG ($P < 0.05$). However, LGG (cultured in MRS) exhibited no significantly different inhibition against strain COA6525 compared to that of CLP1 and CLP4 ($P > 0.05$), but had a significantly greater inhibition compared to that of PL and PJ (cultured in NB or MRS) ($P < 0.05$) (Figure 6).

Products PJ (cultured in NB/MRS) had no effect against strain COA6488; as such strains CLP1-4 had inhibition zones that were significantly greater than that of PJ/PL.

All *L. plantarum* strains (CLP1 to 4) showed significantly greater inhibition than PJ and PL ($P < 0.05$), but not BG or LGG (Figure 7).

Generally, CLP1, 2, 3 and 4 had significantly greater antimicrobial activity against most of the clinical isolates of *E. coli* against the commercial probiotic products tested, namely Prolife, Probio Junior and Biogaia ($P < 0.05$). However, CLP1 and CLP4 showed the greatest inhibition effect against most of the clinical strains of *E. coli*, and a standard strain of *E. coli* (ATCC 11105) (Table 2).

Pellets of crude cultures of putative lactobacilli that were cultured in MRS broth from fermented Zimbabwean products, namely DSLM, KVC, PSM and COB, showed significantly greater inhibition of a clinical strain of *E. coli* (BIN7000) than probiotic products that are in use against diarrhoea in children (BG, PJ and PL) and LGG ($P < 0.05$). Furthermore, washed

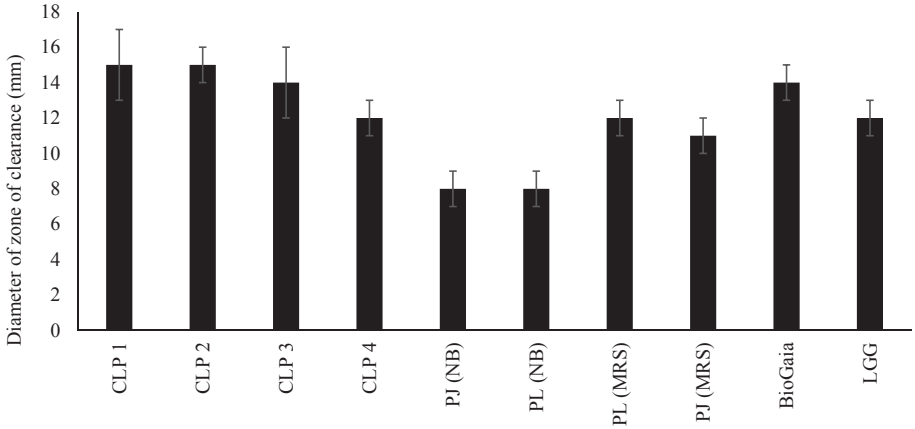


Figure 1. Inhibition (mm) of *E. coli* COA6479 using crude single-strain cultures enriched in MRS broth from Balkan cheeses (CLP1, CLP2, CLP3, CLP4 or LGG) or multi-strain paediatric antidiarrhoeal probiotic products (PL, PJ or BG) after 24 h incubation at 37 °C.
Values shown are means (bar = SE) of 3 independent experiments.

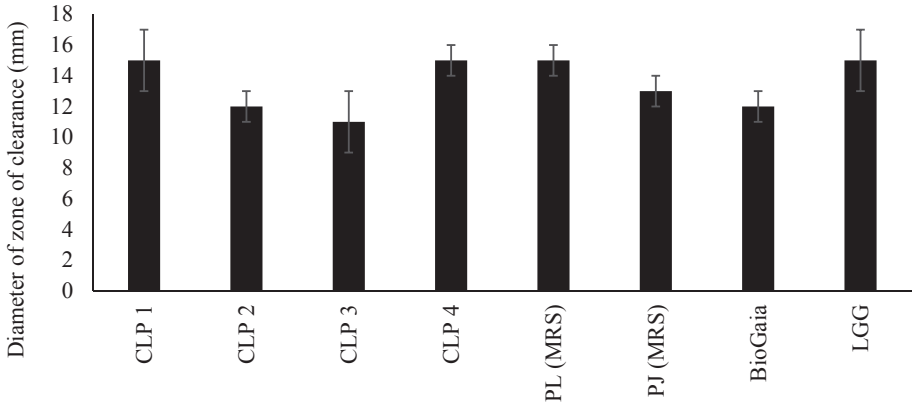


Figure 3. Inhibition (mm) of *E. coli* COA6497 using crude single-strain cultures enriched in MRS broth from Balkan cheeses (CLP1, CLP2, CLP3, CLP4 or LGG) or multi-strain paediatric antidiarrhoeal probiotic products (PL, PJ or BG) after 24 h incubation at 37 °C.
Values shown are means (bar = SE) of 3 independent experiments.

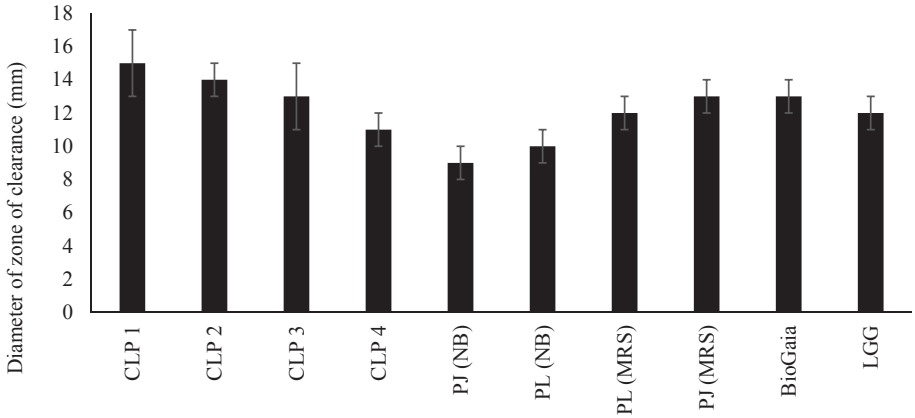


Figure 4. Inhibition (mm) of *E. coli* COA6961 using crude single-strain cultures enriched in MRS broth from Balkan cheeses (CLP1, CLP2, CLP3, CLP4 or LGG) or multi-strain paediatric antidiarrhoeal probiotic products (PL, PJ or BG) after 24 h incubation at 37 °C.
Values shown are means (bar = SE) of 3 independent experiments.

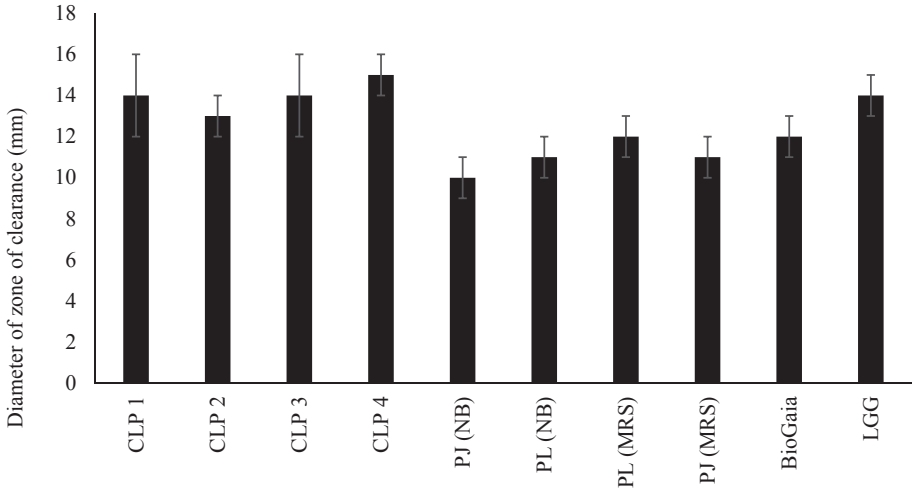


Figure 5. Inhibition (mm) of *E. coli* COA6525 using crude single-strain cultures enriched in MRS broth from Balkan cheeses (CLP1, CLP2, CLP3, CLP4 or LGG) or multi-strain paediatric antidiarrhoeal probiotic products (PL, PJ or BG) after 24 h incubation at 37 °C.
Values shown are means (bar = SE) of 3 independent experiments.

pellets from most of probiotic cultures (PSM, DSLM, PVC, COB, PJ and BG) obtained after incubation for 24 h showed significantly greater inhibition than the *E. coli* strain (BIN7000) compared to that obtained after incubation of the same cultures for 12 h (Table 2).

Extracts of crude cultures of putative lactobacilli that were cultured in MRS broth from some fermented Zimbabwean products, namely PSM, DLSM, KVC, COB, showed significantly greater inhibition of a clinical strain of *E. coli* (BIN7000) than those from probiotic products that are in use against

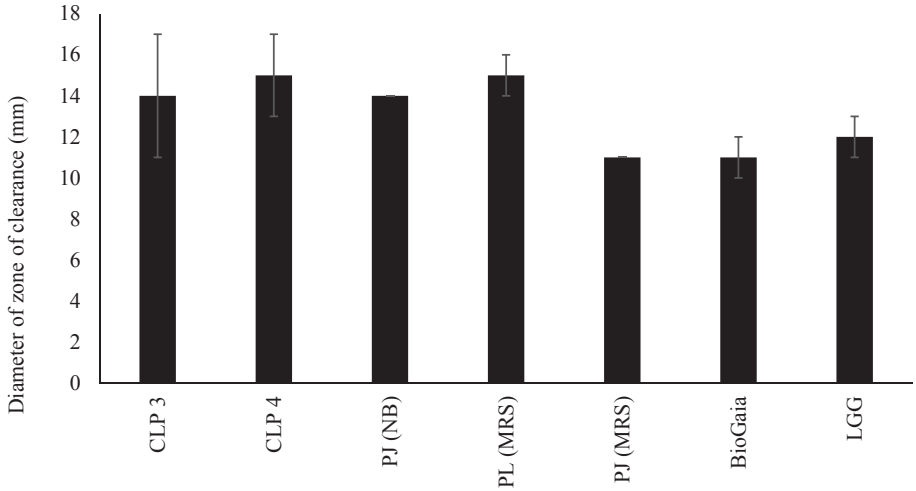


Figure 6. Inhibition (mm) of *E. coli* COA6488 using crude single-strain cultures enriched in MRS broth from Balkan cheeses (CLP1, CLP2, CLP3, CLP4 or LGG) or multi-strain paediatric antidiarrhoeal probiotic products (PL, PJ or BG) after 24 h incubation at 37 °C.
Values shown are means (bar = SE) of 3 independent experiments.

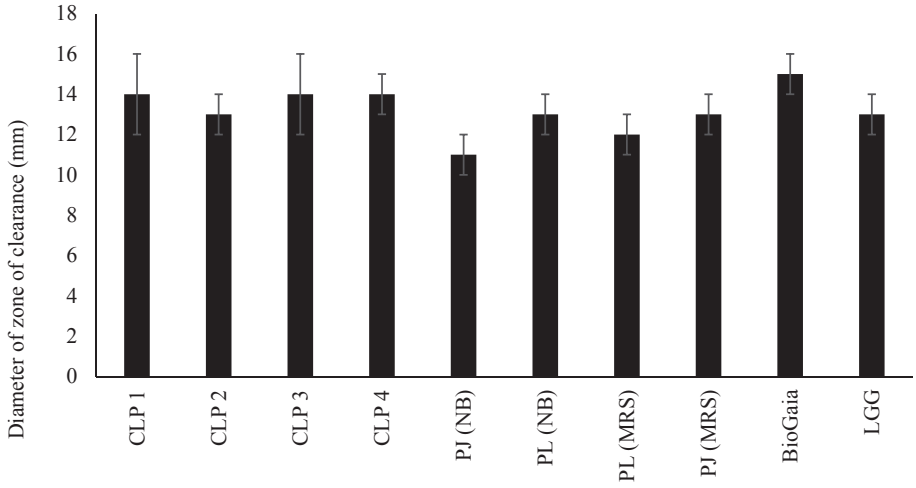


Figure 7. Inhibition (mm) of control strain of *E. coli* (ATCC 11105) using crude single-strain cultures enriched in MRS broth from Balkan cheeses (CLP1, CLP2, CLP3, CLP4 or LGG) or multi-strain paediatric antidiarrhoeal probiotic products (PL, PJ or BG) after 24 h incubation at 37 °C.
Values shown are means (bar = SE) of 3 independent experiments.

Table 2
Inhibition of a strain of *E. coli* (BIN7000) by washed cells and supernatants of putative lactobacilli cultured from Zimbabwean.

Groups	Washed cells		Supernatants	
	12 h	24 h	12 h	24 h
PSM	9.0 ± 2.0	17.5 ± 2.0	17.5 ± 4.0	15.5 ± 1.0
DLSM	11.5 ± 1.0	17.0 ± 1.0	17.0 ± 1.0	15.0 ± 1.0
KVC	0	16.5 ± 1.0	16.5 ± 1.0	15.5 ± 1.0
COB	0	17.5 ± 1.0	17.5 ± 1.0	13.5 ± 1.0
BCLP1	11.0 ± 2.0	14.0 ± 1.0	14.0 ± 2.0	15.0 ± 1.0
BCLP2	14.0 ± 2.0	16.0 ± 2.0	13.0 ± 2.0	14.0 ± 2.0
BCLP3	10.0 ± 1.0	11.0 ± 1.0	11.0 ± 1.0	10.0 ± 1.0
BCLP4	15.0 ± 1.0	17.0 ± 2.0	14.0 ± 1.0	15.0 ± 2.0
PJ (NB)	12.0 ± 2.0	13.0 ± 4.0	14.0 ± 4.0	15.0 ± 1.0
PJ (MRS)	10.0 ± 1.0	11.0 ± 1.0	12.0 ± 1.0	10.0 ± 1.0
BioGaia	9.0 ± 1.0	10.0 ± 1.0	14.0 ± 1.0	13.0 ± 1.0
LGG	14.0 ± 1.0	14.0 ± 1.0	–	–

(i) Commercial probiotic products (DSLM, KVC, PSM), (ii) single-strain cultures of putative probiotic strains from Balkan traditional cheese (CLP1, CLP2, CLP3, CLP4 or LGG), (iii) enriched multistrain commercial paediatric antidiarrhoeal probiotic products (PL, PJ or BG) and (iv) *L. rhamnosus* LGG strain. ANOVA was used to assess differences between the different mixtures and the single strains.

diarrhoea in children (PL (MRS) and BG), and LGG ($P < 0.05$). Furthermore, extracts from most of probiotic cultures (DLSM and COB) obtained after incubation for 12 h showed significantly greater inhibition than the *E. coli* strain (BIN7000) compared to those obtained after incubation of the same cultures for 24 h.

4. Discussion

We report high inhibition of clinical strains of *E. coli* associated with paediatric gastroenteritis and a standard strain of *E. coli* (ATCC 11105) by pellet, extract or crude cultures of putative lactobacilli from the fermented foods (Probrand sour milk and Kefalos Vuka vuka cheese) and beverages (Chibuku opaque beer), and from the strains of *L. plantarum* (CLP1, CLP2 and CLP3). The clinical strains of *E. coli* used in this study, having been isolated from children who visited a Slovenian clinic with symptoms of diarrhoea were implicated for the occurrence of diarrhoea in the children. Several distinct trains of *E. coli* have been shown to cause various forms of diarrhoea, particularly (i) traveller's diarrhoea (enterotoxigenic *E. coli*, or

ETEC), (ii) hemorrhagic colitis and haemolytic-uraemic syndrome (enterohemorrhagic *E. coli*, or EHEC), (iii) persistent diarrhoea (enteroaggregative *E. coli*, or EAEC), and (iv) watery diarrhoea of infants (enteropathogenic *E. coli*, or EPEC) [25]. The observed high *in vitro* inhibition of clinical paediatric strains of *E. coli* by putative lactobacilli from a selection of Zimbabwean products or three strains of *L. plantarum*, namely CLP1, CLP2 and CLP3, may imply great prophylactic or therapeutic potential of the products or the novel strains of *L. plantarum* against diarrhoea in infants. A wider selection of our strains of *Lactobacillus* spp. has been shown to harbour great gut integrity protective potential. Nissen *et al.* demonstrated the enhancement of transepithelial electrical resistance (TER) across monolayers of pig small intestinal epithelial cells (PSIc1) in an established functional intestinal cell model including pig blood monocytes (PoM2) by strains of *Lactobacillus* spp. (PCS26, PCK87 and PCK66) that were isolated from Balkan fermented foods.

While a selection of paediatric antidiarrhoeal probiotic products contain bacterial strains that are widely shown to inhibit diarrhoea in infants, namely *Bacillus coagulans*, *L. acidophilus*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (*L. bulgaricus)*, *Bifidobacterium bifidum* (Prolife), *Lactobacillus casei* (*L. casei)*, *L. rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *Bifidobacterium infantis* and *L. bulgaricus* (Probio Junior), or *L. reuteri* DSM 17938 (*L. reuteri* Protectis) (BioGaia), our new strains of *L. plantarum* and those that are borne in commercial Zimbabwean probiotic foods/products (Probrand sour milk, Dairibord Lacto sour milk, Kefalos Cheese and Chibuku opaque beer) demonstrated a greater antimicrobial effect. The fermented foods and beverages that are consumed in Zimbabwe may have prophylactic, therapeutic or both effects against diarrhoea that is caused by *E. coli*. Zimbabwe, like her neighbours in Southern Africa, is plagued with high prevalence of HIV infections, which often complicate the control of diarrhoeal infections due to the associated fall in immunity [26]. Realisation of the full potential of such products in the control of diarrhoea and other diseases may lie in further studies that will provide information on the concentrations of live bacteria that should be introduced in the products, improved storage methods and the possible use of adjuvants to potentiate the probiotic effects of fermented products. Diarrhoea is a common clinical manifestation of HIV infection affecting at least 50% of HIV patients regardless of the stage of the disease [26].

An interesting finding in this study is that inhibition of strains of *E. coli* occurred when either supernatant extracts or pellets were used. The observed antimicrobial activities of extracts can be attributed to microbial metabolites. Inhibition by washed cells could imply a possible competitive exclusion of *E. coli* by the lactobacilli, or antimicrobial activities of metabolites of the lactobacilli or both effects. Studies to determine the exact mechanisms of action and the types of metabolites that are released by lactobacilli (our new strains and those isolated a selection of fermented Zimbabwean foods/probiotic products) are underway.

It was also interesting to note that greater inhibition tended to occur with washed lactobacilli after 24 h of incubation in MRS broth than 12 h. On the contrary, the supernatant extracts were shown to have reduced effect with time of incubation (12 h *vs* 24 h). This discovery may be an important consideration if the any products would emanate from the study. *Lactobacillus* species are shown to enter into their exponential phase after about 8 h of incubation and stationary phase after 24 h (Saadatzadeh *et al.*) [27]. Furthermore, Saadatzadeh *et al.* [27] have shown a reduction in the concentration of lactic acid in extracts of *L. casei* with time (12–48) h. A reduction in the antimicrobial activity of our microbial extracts may be attributed to attrition in antimicrobial active compounds.

While *L. rhamnosus* LGG is frequently included in probiotic products that are claimed to treat diarrhoea, our cultures of putative lactobacilli and strains of *L. plantarum* (CLP1, CLP2 and CLP3) and those cultured from fermented Zimbabwean products showed significantly greater inhibition ($P < 0.05$) of diarrheagenic/clinical strains of *E. coli*. Based on this *in vitro* study, our collection of putative lactobacilli from the Zimbabwean products and the strains of *L. plantarum* (CLP1, CLP2 and CLP3) may be good candidates for use in the control of diarrhoea in infants. More studies are required to reveal the mechanisms at play and validate the current findings.

Results here-in, based on comparative data gathered, present two *L. plantarum* strains (CLP1 and CLP4) with greater high inhibition against clinical strains of *E. coli* in comparison with the widely used *L. rhamnosus* LGG strain and probiotic products. Notably, CLP4 strain showed the greatest inhibition against most strains of clinical *E. coli*, and had greater inhibition compared to that of *L. rhamnosus* LGG. However, Probio Junior (containing *L. casei*, *L. rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *Bifidobacterium infantis*, and *L. bulgaricus*) was shown to be competitive compared to our new strains. However all probiotic strains and products showed significantly lower inhibition against *E. coli* (approximately 30% less) than garamycin (40 mg/mL) ($P < 0.05$). Generally the *E. coli* strains tested showed variable susceptibility to probiotic strains or their combinations, and to antibiotics. Besides the inhibition of *E. coli in vitro*, our previous studies have shown that the new probiotics harbour immunomodulatory activities, ability to maintain gut health and antiviral activities against rotavirus. With these properties, fermented Zimbabwean products and novel strains of *L. plantarum* (CLP1, CLP2 and CLP3) have great potential for use against diarrhoea in infants and, perhaps, older persons. However, the observed characteristics need validation, and the safety of the strains remains to be studied.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work forms part of project PathogenCombat (FP6-007081), funded under the Framework 6 Programme of the European Union. We acknowledge the tireless effort of Miss Lidija Gradišnik, our laboratory technician, and other colleagues throughout the work.

References

[1] Levkovich T, Poutahidis T, Smillie C, Varian BJ, Ibrahim YM, Lakritz JR, et al. Probiotic bacteria induce a 'glow of health'. *PLoS One* 2013; **8**(1): e53867.

[2] Walsham AD, MacKenzie DA, Cook V, Wemyss-Holden S, Hews CL, Juge N, et al. *Lactobacillus reuteri* inhibition of

enteropathogenic *Escherichia coli* adherence to human intestinal epithelium. *Front Microbiol* 2016; **7**: 244.

[3] Wang Z, Wang L, Chen Z, Ma X, Yang X, Zhang J, et al. *In vitro* evaluation of swine-derived *Lactobacillus reuteri*: probiotic properties and effects on intestinal porcine epithelial cells challenged with enterotoxigenic *Escherichia coli* K88. *J Microbiol Biotechnol* 2016; **26**(6): 1018-1025.

[4] Zhou M, Zhu J, Yu H, Yin X, Sabour PM, Zhao L, et al. Investigation into *in vitro* and *in vivo* models using intestinal epithelial IPEC-J2 cells and *Caenorhabditis elegans* for selecting probiotic candidates to control porcine enterotoxigenic *Escherichia coli*. *J Appl Microbiol* 2014; **117**(1): 217-226.

[5] Mohan V. The role of probiotics in the inhibition of *Campylobacter jejuni* colonization and virulence attenuation. *Eur J Clin Microbiol Infect Dis* 2015; **34**(8): 1503-1513.

[6] Lee do K, Park JE, Kim MJ, Seo JG, Lee JH, Ha NJ. Probiotic bacteria, *B. longum* and *L. acidophilus* inhibit infection by rotavirus *in vitro* and decrease the duration of diarrhea in pediatric patients. *Clin Res Hepatol Gastroenterol* 2015; **39**(2): 237-244.

[7] Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. *Int J Environ Res Public Health* 2014; **11**(5): 4745-4767.

[8] Narayan SS, Jalgaonkar S, Shahani S, Kulkarni VN. Probiotics: current trends in the treatment of diarrhoea. *Hong Kong Med J* 2010; **6**(3): 213-218.

[9] Yonekura L, Sun H, Soukoulis C, Fisk I. Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: technological characterization, storage stability and survival after in vitro digestion. *J Funct Foods* 2014; **6**(100): 205-214.

[10] Yang X, Brisbin J, Yu H, Wang Q, Yin F, Zhang Y, et al. Selected lactic acid-producing bacterial isolates with the capacity to reduce *Salmonella* translocation and virulence gene expression in chickens. *PLoS One* 2014; **9**(4): e93022.

[11] Salerno-Goncalves R, Safavie F, Fasano A, Sztein MB. Free and complexed-secretory immunoglobulin A triggers distinct intestinal epithelial responses. *Clin Exp Immunol* 2016; **185**: 338-347.

[12] Haghshenas B, Haghshenas M, Nami Y, Yari Khosroushahi A, Abdullah N, Barzegari A, et al. Probiotic assessment of *Lactobacillus plantarum* 15HN and *Enterococcus mundtii* 50H isolated from traditional dairies microbiota. *Adv Pharm Bull* 2016; **6**(1): 37-47.

[13] Alvarez-Sieiro P, Montalban-Lopez M, Mu D, Kuipers OP. Bacteriocins of lactic acid bacteria: extending the family. *Appl Microbiol Biotechnol* 2016; **100**(7): 2939-2951.

[14] Gao Y, Li B, Li D, Zhang L. Purification and characteristics of a novel bacteriocin produced by *Enterococcus faecalis* L11 isolated from Chinese traditional fermented cucumber. *Biotechnol Lett* 2016; **38**(5): 871-876.

[15] Kommineni S, Bretl DJ, Lam V, Chakraborty R, Hayward M, Simpson P, et al. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature* 2015; **526**(7575): 719-722.

[16] Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait? *Appl Environ Microbiol* 2012; **78**(1): 1-6.

[17] Štaudova B, Micenkova L, Bosak J, Hrazdilova K, Slaninkova E, Vrba M, et al. Determinants encoding fimbriae type 1 in fecal *Escherichia coli* are associated with increased frequency of bacteriocinogeny. *BMC Microbiol* 2015; **15**: 201.

[18] Lim KB, Balolong MP, Kim SH, Oh JK, Lee JY, Kang DK. Isolation and characterization of a broad spectrum bacteriocin from *Bacillus amyloliquefaciens* RX7. *Biomed Res Int* 2016; **2016**. 8521476.

[19] Huang SH, He L, Zhou Y, Wu CH, Jong A. *Lactobacillus rhamnosus* GG Suppresses meningitic *E. coli* K1 penetration across human intestinal epithelial cells *in vitro* and protects neonatal rats against experimental hematogenous meningitis. *Int J Microbiol* 2009; http://dx.doi.org/10.1155/2009/647862.

[20] Maragkoudakis PA, Chingwaru W, Gradisnik L, Tsakalidou E, Cencic A. Lactic acid bacteria efficiently protect human and animal intestinal epithelial and immune cells from enteric virus infection. *Int J Food Microbiol* 2010; **141**(Suppl 1): S91-S97.

[21] Nissen L, Chingwaru W, Sgorbati B, Biavati B, Cencic A. Gut health promoting activity of new putative probiotic/protective *Lactobacillus* spp. strains: a functional study in the small intestinal cell model. *Int J Food Microbiol* 2009; **135**(3): 288-294.

[22] Huber EM, Heinemeyer W, Li X, Arendt CS, Hochstrasser M, Groll M. A unified mechanism for proteolysis and autocatalytic activation in the 20S proteasome. *Nat Commun* 2016; **7**: 10900.

[23] Polak-Berecka M, Wasko A, Paduch R, Skrzypek T, Sroka-Bartnicka A. The effect of cell surface components on adhesion ability of *Lactobacillus rhamnosus*. *Antonie Van Leeuwenhoek* 2014; **106**(4): 751-762.

[24] Brimacombe CA, Ding H, Beatty JT. *Rhodobacter capsulatus* DprA is essential for RecA-mediated gene transfer agent (RcGTA) recipient capability regulated by quorum-sensing and the CtrA response regulator. *Mol Microbiol* 2014; **92**(6): 1260-1278.

[25] Singh AP, Aijaz S. Enteropathogenic *E. coli*: breaking the intestinal tight junction barrier. *F1000Research* 2015; **4**: 231.

[26] Dikman AE, Schonfeld E, Srisarajivakul NC, Poles MA. Human immunodeficiency virus-associated diarrhea: still an issue in the era of antiretroviral therapy. *Dig Dis Sci* 2015; **60**(8): 2236-2245.

[27] Saadatzadeh A, Fazeli MR, Jamalifar H, Dinarvand R. Probiotic properties of lyophilized cell free extract of *Lactobacillus casei*. *Jundishapur J Nat Pharm Prod* 2013; **8**(3): 131-137.