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From cholera to food poisoning: exploring the antidiarrheal activity of a multi-species water-based probiotic product

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Abstract

Diarrheal diseases are a leading cause of morbidity and mortality worldwide. Given the rising prevalence of antimicrobial resistance among causative diarrheal pathogens, there is an increasing imperative to explore alternative therapeutic strategies. In this study, the potential of Symprove™ (a water-based probiotic product) against diarrheal pathogens, *V. cholerae*, *E. coli*, *S. aureus*, and *L. monocytogenes* was evaluated. Investigations were carried out using isothermal microcalorimetry and colony counting. The experimental setup consisted of individual cultures and co-incubations of the pathogens and probiotic formulation. Growth curves were obtained for all setups using a thermal activity monitor, and colony counts were performed after 24–48 h for both probiotic organisms and pathogens. The growth curves obtained revealed that co-incubation of *E. coli* and *S. aureus* with the probiotic formulation showed a slightly delayed onset of growth for the pathogens although these pathogens were faster growing than the probiotic. For *L. monocytogenes*, the thermal growth curves showed dominance of the probiotic formulation. Corresponding colony counts revealed significantly reduced bacterial numbers after 48 h when the pathogens were co-incubated with the probiotic formulation. Co-incubating *V. cholerae* with the probiotic formulation showed a significant reduction in pathogen numbers after 24 h and no counts after 48 h. These findings indicate the possible inhibitory effect of the probiotic formulation and highlight the therapeutic potential of probiotics as alternative approach to conventional antibiotics.

Highlights

Antidiarrheal effects of a water-based probiotic product in real-time
Probiotics inhibit *V. cholerae*, *E. coli*, *S. aureus*, and *L. monocytogenes*
Probiotics as alternative approach to conventional antibiotics

Keywords Cholera, Diarrhea, Gut pathogens, Probiotics

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Background

Diarrheal diseases remain a major cause of morbidity and mortality worldwide, accounting for an estimated 3.6% of the global burden of disease [1]. It is the third leading cause of death in younger children, with about 1.7 billion cases globally every year [2]. In 2021, diarrheal diseases caused an estimated 1·17 million deaths globally, with children younger than 5 years disparately affected [3]. It is particularly prevalent in low- and middle-income regions, mainly due to poor living conditions resulting from inadequate water supplies, poor environmental hygiene and sanitation, and insufficient education [2].

Bacterial pathogens, such as *Vibrio cholerae*, *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*, are a leading cause of infectious diarrhoea and contribute significantly to this disease burden [1]. These pathogens exert their effects primarily through the production of toxins within the intestine or by invading and damaging the intestinal epithelium [1]. The clinical manifestations range from mild gastroenteritis to severe dehydration, bloody diarrhea, and life-threatening complications, such as haemolytic uremic syndrome or septicaemia. *Vibrio cholerae* is the causative agent of cholera, an acute diarrheal illness caused by infection with the microorganism in the intestine after ingestion of contaminated water or food. It can lead to severe dehydration and death if untreated. The importance of cholera infection or an outbreak is well-known, resulting in a significant strain on health systems' resources. It is an extremely virulent disease that can affect all age groups, with deaths occurring within hours at times due to its short incubation period [4–6]. Although the seventh pandemic of cholera was in the 1960 s [7], it is still endemic in several countries in Africa, America, and Asia. The serogroup 01 or 0139 is usually involved in epidemics [8–10]. Several pathogenic types of *Escherichia coli* can cause severe diarrhea and dehydration, including Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC) and Enterohemorrhagic or Shiga toxin-producing *E. coli* (EHEC/STEC). Although these pathotypes of *E. coli* are all diarrheagenic, they differ regarding their preferential colonization sites, virulence mechanisms, and the ensuing clinical symptoms and consequences [11]. They produce toxins that disrupt intestinal function, leading to water and electrolyte loss and causing severe diarrhea, dehydration, and, in some cases, bloody diarrhea and haemolytic uremic syndrome. They pose significant public health threats in both developing and developed countries [12]. *Listeria monocytogenes* is a major cause of gastroenteritis, which is characterised by nausea, abdominal cramps, and diarrhea [13]. Invasive infection, characterised by bacteraemia, meningitis, and encephalitis, can develop in high-risk patients [14]. Certain serotypes (notably 1/2a, 1/2b, and 4b) are frequently linked to foodborne outbreaks. In the United States of America, *L. monocytogenes* has been reported to be the third most costly foodborne pathogen [15, 16]. Similarly, pathogenic strains of *Staphylococcus aureus* can cause various infections, including foodborne illnesses. Enterotoxins produced by *S. aureus* can lead to rapid-onset nausea, vomiting, and diarrhea [17].

In light of the global burden of diarrheal diseases and the growing prevalence of antimicrobial resistance (AMR) among these diarrheal pathogens, there has been growing interest in alternative therapeutic approaches. Traditional antibiotic therapies are becoming increasingly ineffective, leading to higher treatment failure rates and prolonged disease duration. Probiotics have garnered attention as a promising non-antibiotic intervention for maintaining gut health and combating enteric infections [18, 19]. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health effect on the host [20]. They enhance the gut microbiota and maintain intestinal homeostasis [21]. These beneficial bacteria are usually members of the genera *Lactobacillus* and *Bifidobacterium*. However, some members of the genera *Streptococcus*, *Enterococcus*, *Lactococcus*, *Bacillus*, and some yeast, for example *Saccharomyces*, have also been investigated for their probiotic potential.

Symprove™ is a water-based probiotic product containing four probiotic species: *Lactobacillus acidophilus* NCIMB 30175, *Lactobacillus plantarum* NCIMB 30173, *Lactobacillus rhamnosus* NCIMB 30174, and *Enterococcus faecium* NCIMB 30176. Previous studies have shown that Symprove™ can modulate immune responses [22], improve symptoms in patients with irritable bowel syndrome [23], and chronic diverticular disease [24]. A positive effect on the symptoms of early-stage Parkinson's disease via changing microbiota composition and reducing plasma inflammatory markers in a Parkinson's disease model has been reported with the use of Symprove™ [25]. Importantly, Symprove™ has also demonstrated anti-clostridial activity and inhibitory activity against common gut pathogens suggesting it may have broad antimicrobial properties [26, 27].

Despite these findings, there is limited data on the efficacy of Symprove™ against key diarrheal pathogens. Understanding whether Symprove™ can inhibit these clinically significant bacteria may offer valuable insights into its potential therapeutic applications beyond gut health maintenance. In this study, investigations were conducted to evaluate the potential of Symprove™ in inhibiting diarrheal pathogens, *V. cholerae*, *E. coli*, *L. monocytogenes*, and *S. aureus*.

Methods

Microorganisms

The probiotic formulation used in this evaluation was Symprove™ (original flavour). The pathogenic microorganisms evaluated were *Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes. Vibrio cholerae* was obtained from the Department of Bacteriology laboratory of the Noguchi Memorial Institute for Medical Research, University of Ghana. *Escherichia coli* ATCC 25922 was purchased from the American Type Culture Collection, USA. *Staphylococcus aureus* NCIMB 9518 was obtained from ConvaTec Ltd. *Listeria monocytogenes* NCTC 10890 was purchased from the National Collection of Type Cultures (NCTC), Public Health England.

Growth media and reagents

The growth media: peptone water, thiosulfate-citrate-bile salts-sucrose (TCBS), brain heart infusion (BHI) broth and agar, de Man, Rogosa, and Sharpe (MRS) broth and agar, nutrient broth and agar, listeria selective agar base, listeria selective supplement, and cooked meat medium (CMM) were purchased from Oxoid, UK. Phosphate-buffered saline (PBS) tablets and glycerol were purchased from Fisher Scientific, UK. Glucose and Ringer's solution tablets were purchased from Sigma-Aldrich, UK.

Determination of the initial bacterial population of Symprove™

A volume of 100 µL of Symprove™ was added to 900 µL of PBS and vortexed for 10 s. This was then serially diluted (1 in 10) and plated onto MRS agar. Afterwards, the agar plates were incubated at 37 °C under anaerobic conditions for 48 h, and colonies were counted. Enumeration assay was done in triplicate.

Culture maintenance

The test microorganisms, *E. coli*, *S. aureus*, and *L. monocytogenes*, were cultivated and stored in frozen aliquots. *E. coli* and *S. aureus* were grown over 24 h on nutrient agar at 37 °C. *L. monocytogenes* was grown on BHI agar. 10 mL starter cultures of relevant bacteria were made by inoculating broth (nutrient broth for *E. coli* and *S. aureus* or BHI broth for *L. monocytogenes*) with a few colonies of bacteria from agar plates and incubating at 37 °C appropriately. The starter cultures were inoculated into a freshly made relevant broth in a 1:100 dilution. The cultures were incubated until they reached the stationary phase of growth. The cultures were then harvested by centrifuging at 3500 x *g* for 10 min at 4° C (Heraeues Stratosbiofuge Thermo Scientific, UK). The supernatant was carefully discarded, and the cells were washed twice in PBS solution by centrifuging at the same conditions. The washed cells were resuspended in ¼ strength Ringer's solution supplemented with a cryoprotectant, glycerol, in a 15% (v/v) solution to an organism density of 10^8 CFU/mL. The suspension was uniformly dispensed in 1.8 mL aliquots into 2 mL cryovials (Nunc) and sealed. The vials were frozen over liquid nitrogen in a modified bath at −90 °C [28]. The aliquots were stored under liquid nitrogen until required for experimentation. Frozen aliquots were thawed, subcultured, and checked for purity and uniformity of the stock. They were thawed by placing the vials (held in a floater) in a water bath with a temperature set at 40 °C for 3 min. They were vortexed for 1 min before use. Maintaining the cultures this way prevented natural batch-to-batch variability, which arises when fresh cultures had to be grown for each experiment. *E. coli*, *S. aureus*, and *L. monocytogenes* survived the freezing process and maintained 10^8 CFU/mL viable cells after the freezing process. However, *V. cholerae*, did not yield good recovery when cultured and stored using the same parameters. A fresh bacterial culture was prepared each day for *V. cholerae*.

V. cholerae was grown on TCBS agar and incubated overnight at 37 °C. A colony was taken to inoculate 10 mL of peptone water to create a starter culture that was incubated for 24 h. To ensure there was insignificant variation among batches on different days, an initial test was conducted whereby enumeration of freshly prepared *V. cholerae* in peptone water was conducted on different days. Additionally, all tests were conducted from the same parent culture by sub-culturing a loopful of the culture in peptone water on TCBS agar daily by streaking and using it for subsequent work.

Evaluating the activity of Symprove™ against *V. cholerae*, *E. coli*, *S. aureus* and *L. monocytogenes*

Analysis was conducted using isothermal microcalorimetry and colony counting. The experimental setup consisted of sterile ampoules containing 3 mL BHI broth for *L. monocytogenes* and its co-cultures with Symprove™. 3 mL CMM supplemented with 2% glucose was used for *E. coli a*nd *S. aureus* and their co-cultures with Symprove™. 3 mL BHI broth was used for *V. cholerae* and its co-culture with Symprove™. 30 µL each of Symprove™, *V. cholerae*, *E. coli*, *S. aureus*, and *L. monocytogenes* were used for inoculation; thus, 10^6 CFU/mL of each culture was used. Three tests comprising two controls were run (i.e., test pathogen alone in an appropriate medium and Symprove™ alone in the same medium), and a co-incubation of pathogen and Symprove™ using the same medium in which the two initial tests were performed. Tests were performed in triplicates.

Isothermal microcalorimetry using the thermal activity monitor was used to observe the effect of the probiotic formulation on the pathogenic species (*E. coli*, *S. aureus*, and *L. monocytogenes*). This was performed according to

Table 1 Average probiotic enumeration per dose (70 mL) after triplicate enumeration

Test (CFU/mL)			Average (CFU/mL)	Average (CFU/dose)	Expected population (CFU/dose)
A	B	C			
1.12×10^{8}	1.99×10^{8}	1.87×10^{8}	1.66×10^{8}	1.16×10^{10}	1×10^{10}

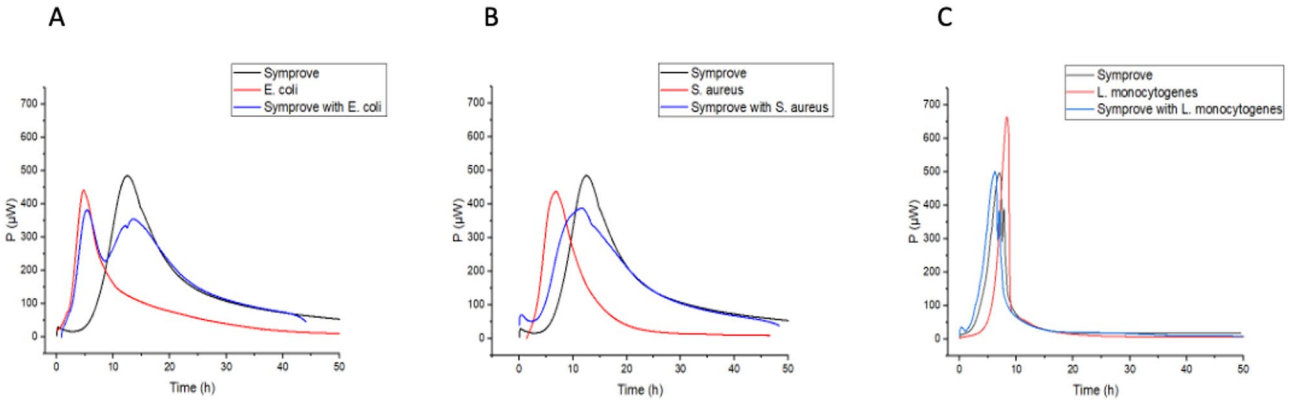

Fig. 1 Power-time (*p-t*) curves of **A** Symprove™, *E. coli* and their co-culture in CMM supplemented with 2% glucose; **B** Symprove™, *S. aureus* and their co-culture in CMM supplemented with 2% glucose and **C** Symprove™, *L. monocytogenes* and their co-culture in BHI

the methods described previously using the same media as the colony counting experiments [26, 27]. Briefly, the inoculated ampoules were allowed to equilibrate thermally at an intermediate position for 30 min in a thermometric thermal activity monitor 2277 (TAM 2277) (TA Instruments Ltd., UK) set at 37 °C (± 0.1 °C) before being lowered to the measurement position. Data were collected using the software package Digitam 4.1 and analysed using Origin Pro 8.6 (Microcal Software Inc.). The reference ampoule was loaded with 3 mL of sterile growth medium respectively, of the experiment being carried out. The instrument was calibrated at regular intervals. Experiments were performed in triplicates.

Colony counts for Symprove™, *S. aureus*, *E. coli*, and *L. monocytogenes* were conducted after 48 h. *E. coli* and *S. aureus* enumeration was conducted on nutrient agar, whilst *L. monocytogenes* was conducted on listeria selective agar base with selective supplement. Enumeration of Symprove™ was conducted on MRS agar plates in all instances. Isothermal microcalorimetry for *V. cholerae* yielded noisy signals; hence, enumeration was conducted at 24 h and 48 h for both Symprove™ and *V. cholerae* to provide more insights. To mimic the conditions as used in the thermal activity monitor, ampoules inoculated with 10^{6} CFU/mL of *V. cholerae*, Symprove™ and their co-culture in BHI broth were placed in an incubator set at 37 °C. Thus, three tests comprising of *V. cholerae* monoculture, Symprove™ monoculture and a co-incubation of *V. cholerae* and Symprove™ in BHI broth were conducted. Colony counts for both Symprove™ and *V. cholerae* were conducted at 24 h and 48 h. *V. cholerae* enumeration was conducted on TCBS agar plates whilst Symprove™ enumeration was conducted on MRS agar plates.

Results

A triplicate enumeration of Symprove™ yielded an average probiotic population of 1.66×10^{8} CFU/mL. With a recommended dose of 70 mL, the probiotic population per dose was computed as 1.16×10^{10} CFU/dose (Table 1). Previous studies on Symprove™ have confirmed the presence of the bacteria with bacterial numbers similar to the expected numbers [29].

The isothermal microcalorimeter monitors the real time metabolism of bacteria. It measures power output (in μW or $\mu J \cdot s^{-1}$) over time. As microorganisms grow, the power signal usually starts with a lag phase, then increases rapidly, showing peaks and dips. These changes reflect the heat produced by microbial activity. When the energy source runs low and waste products build up, the power signal gradually returns to its baseline level. The power-time curves (thermograms) obtained for the probiotic formulation against *E. coli*, *S. aureus*, and *L. monocytogenes* are indicated in Fig. 1. The control for the probiotic formulation had a long lag phase with an onset of growth between 5 and 10 h and a peak around 12.4 h in CMM supplemented with 2% glucose. In the same media, the pathogens under investigation (*E. coli* and *S. aureus*) had an earlier onset of growth between 0 and 5 h, with their respective peaks at approximately 4.7 h and 6.7 h (Fig. 1a and b). Co-incubation of these pathogens and the probiotic formulation showed a slightly delayed onset for the pathogens and a reduced peak height for *E. coli* and *S. aureus.* There was a 0.5 h and 4.9 h delay in peaks for *E. coli* and *S. aureus* respectively. The curve for co-incubation of *E. coli* with the probiotic product showed two peaks occurring at times that were representative of the peaks of the individual samples. However, a reduced peak

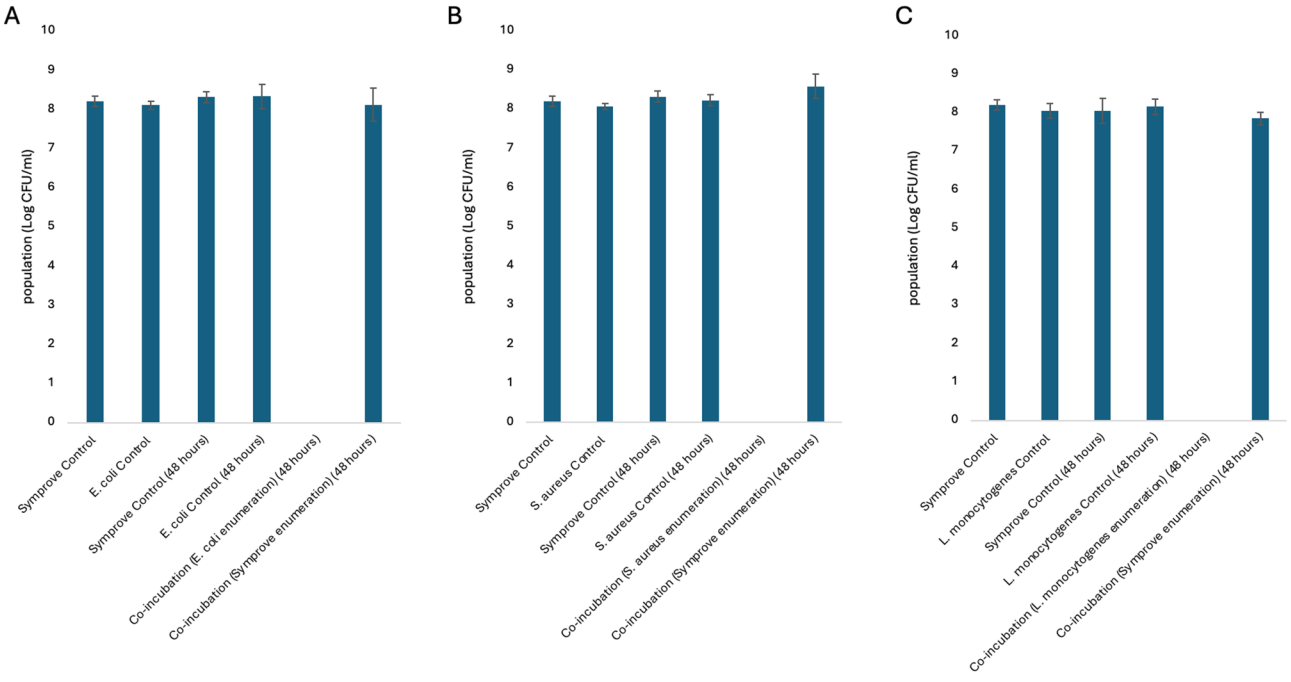

Fig. 2 Colony counts at 48 h after the co-incubation of Symprove™ and *E. coli* **A**, Symprove™ and *S. aureus* **B** and Symprove™ and *L. monocytogenes* **C** and their respective controls

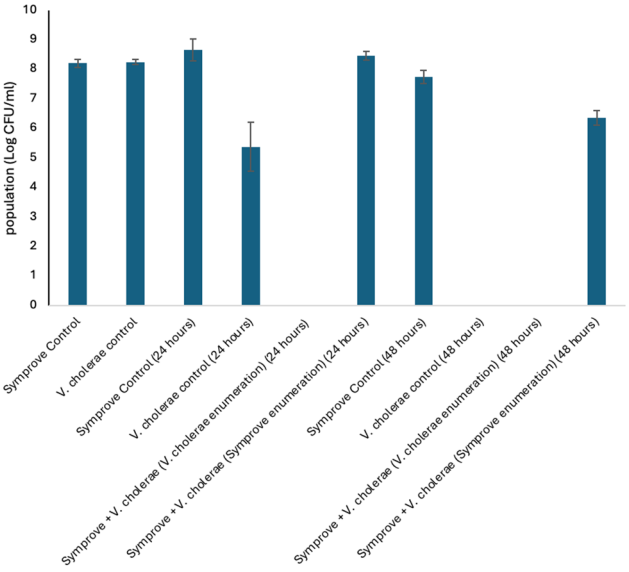

Fig. 3 Colony counts at 24 h and 48 h after the co-incubation of Symprove™ and *V. cholerae*, and their respective controls

height (60 µW decrease in peak height) was observed for the signal representative of the probiotic formulation in co-culture with *E. coli* (Fig. 1a). Co-incubation of the probiotic formulation with *S. aureus* showed a curve that is likely contributed by both samples. Individual cultures of *L. monocytogenes* and Symprove™ in BHI (Fig. 1c) show a faster onset of growth for the microorganisms in the probiotic product relative to *L. monocytogenes*. The respective peaks of the probiotic product and *L. monocytogenes* occurred at 7 h and 8.4 h. Co-cultures of the probiotic product and *L. monocytogenes* depicted a curve dominated by the probiotic, similar to the curve of the probiotic product only.

Colony counts were obtained before and after the experiment for the probiotic formulation alone, pathogens alone, and after co-incubation over 48 h (Fig. 2). It was observed that *E. coli*, *S. aureus*, and *L. monocytogenes* could maintain growth for 48 h with bacterial numbers intact upon incubation as monocultures. However, their growth was significantly inhibited by 48 h in the presence of Symprove™. No *E. coli*, *S. aureus*, or *L. monocytogenes* could be enumerated after co-incubation with Symprove™ for 48 h. The bacterial numbers obtained for the probiotic formulation at the end of the experiments were similar to those of the controls.

Figure 3 illustrates colony counts obtained after co-incubating Symprove™ and *V. cholerae* over 24 h and 48 h and the outcome of incubating these separately, i.e., controls. It is worth noting that the experiments involving *V. cholerae were* conducted in Ghana, a tropical country that has experienced outbreaks of cholera since the 1970s. The Symprove™ bacterial population obtained over the period (48 h) when incubated in pure culture was between 8.21 and 7.75 log counts. However, the control for *V. cholerae* had an initial reduction from about 8 log counts to 5 log counts by 24 h. No viable *V. cholerae* cells were found after 48 h when Symprove™ and *V. cholerae* were co-incubated. The bacteria in Symprove™ were maintained over the period; however, no numbers were

obtained for *V. cholerae* after 24 h of co-incubation and after pure incubation of *V. cholerae* for 48 h.

Discussion

Under the same media conditions, the data showed that *E. coli* and *S. aureus* had earlier onsets of growth than the probiotic product. This implied that these pathogens were well established before the microorganisms in the probiotic formulation started to thrive. In a previous study with the microcalorimeter, it was demonstrated that *S. aureus* and *E. coli* can grow concurrently with *L. acidophilus*, *B. lactis*, *and B. bifidum*, with the pathogenic species growing faster than the probiotic species because of their relatively shorter generation time [30]. This manifested as a late occurrence of the peak of the growth curves of the probiotics compared to the other species and was confirmed with plate count assay although the probiotic species consequently inhibited the pathogenic ones [30]. In the present work, it was shown that the curve produced by the co-culture of the probiotic with *E. coli* in the microcalorimeter had two peaks representing both *E. coli* and the microorganisms in the probiotic. The reduced peak height obtained for the signature curve for the probiotic could be due to reduced nutrients within the closed system. In the case of co-cultures of the probiotic product with *L. monocytogenes*, the probiotics had faster growth in the BHI medium than *L. monocytogenes*; hence, they likely produced inhibitory compounds during their growth, which killed *L. monocytogenes*. This form of growth pattern in the microcalorimeter has been previously demonstrated with mixed cultures of other probiotic species and products with *Clostridioides* (*Clostridium*) *difficile*, which had a slower growth compared to the probiotic products and species [26].

It was evident that Symprove™ exhibited significant antagonistic action on *V. cholerae* within 24 h. Standard growth curves of *V. cholerae* were previously shown to enter the stationary phase within approximately 10 h of incubation [31]. It is, therefore, highly probable that the cells entered the death phase with loss of viability within 48 h. It is important to mention that *V. cholerae* is one microorganism that, despite its high infectivity, is quite difficult to cultivate and work with under laboratory conditions. Although it can grow in various media and under laboratory conditions, *V. cholerae* cannot survive at 4 °C for extended periods [31], a reason for the difficulty in maintaining cholera batches under storage. In our present study, the viability of the cells was completely lost after 48 h.

Other studies have demonstrated the potential of specific probiotic strains to inhibit the growth of *E. coli*, *S. aureus*, *L. monocytogenes*, and *V. cholerae*. For instance, a study by Fijan et al. [32] demonstrated antagonistic activity of various probiotics against *E. coli* in vitro. Their study indicated that multi-strain probiotic formulations were more effective against *E. coli* than single-strain formulations. They reported that the most effective multi-strain probiotics contained lactobacilli, bifidobacteria, and enterococci strains, while the most effective single-strain probiotics against *E. coli* strains were *Bifidobacterium animalis* subsp. lactis BB-12 and *Lactobacillus reuteri* DSM 17,938. A clinical trial assessing the effect of *L. rhamnosus* HN001 on the carriage of *S. aureus* reported that the use of daily oral *L. rhamnosus* HN001 reduced the odds of carriage of *S. aureus* in the gastrointestinal tract; however, it did not eradicate *S. aureus* from other body sites like the skin [33]. Another clinical trial conducted in Thailand demonstrated the potential of probiotics in eliminating *S. aureus* [34]. Strains of *L. plantarum* have also been found to exhibit antagonistic activity against *L. monocytogenes*. Yang et al. [35] reported that *L. plantarum* 4–10 could disrupt the cell structure when co-cultured with *L. monocytogenes* and act as a lethal agent within 15 h.

Mao et al. [36] investigated the basis of a probiotic-based strategy to promote colonization resistance and point-of-need diagnosis of cholera using *L. lactis* against *V. cholerae* in a mouse model. They reported that the lactic acid released by the probiotic strain could be responsible for the reduction in *V. cholerae* numbers.

The findings from this study demonstrate the significant inhibitory effect of Symprove™ on diarrheal pathogens, supporting the hypothesis that it could play a crucial role in controlling diarrheal infections. The observed inhibition may be attributed to several mechanisms. Firstly, the probiotic strains in the product likely caused suppression of the growth of the pathogens through the production of antimicrobial compounds such as organic acids (e.g., lactic acid), hydrogen peroxide, or bacteriocins [35, 37]. Another potential mechanism is the modulation of the local microenvironment. The production of organic acids decreases pH, creating a hostile environment for pathogenic bacteria that prefer neutral pH conditions. Approximately 2.5 and 3 pH unit reductions in media pH were noted after incubation of Symprove ™ in BHI and CMM supplemented with 2% glucose, respectively. *S. aureus* and *E. coli* can produce acids themselves as they decreased the pH of the broth by approximately 2 pH units post-incubation. It is likely *S. aureus* and *E. coli* could have the ability to protect themselves from the self-produced acids and thereby may have the necessary factors for surviving acid stress [38, 39]. Other non-acidic metabolites may have also inhibited them or may have been synergistically hindered by the acidic and non-acidic antimicrobial metabolites of the probiotic species.

The in vitro coincubation assay provided a controlled environment to assess the direct interactions between the

probiotic and the diarrheal pathogens. However, while the results are promising, it is important to acknowledge the limitations of the model. First, the model used does not replicate the full complexity of the human gastrointestinal environment. The gut is a highly dynamic system influenced by host factors such as immune responses, mucus layers, pH variations, and interactions with other microbial communities. As a result, the inhibitory effects observed in vitro may not fully translate to real-life conditions in the human gut. Second, the pathogen strains tested were limited to selected, well-characterized reference strains. While these strains are useful for standardization and reproducibility, they may not represent the genetic and phenotypic diversity found in clinical isolates. Third, the mechanisms underlying the observed inhibitory effects remain speculative. Probiotics may act through multiple pathways such as producing antimicrobial compounds, lowering pH, competing for nutrients or binding sites, or disrupting pathogen communication systems. However, this study did not investigate these mechanisms in detail, and further molecular studies are needed to understand how Symprove™ exerts its antimicrobial effects.

Conclusions

This study provides valuable evidence that the multispecies probiotic Symprove™ exhibits significant inhibitory effects against key diarrheal pathogens such as *V. cholerae*, *E. coli*, *S. aureus*, and *L. monocytogenes*. By employing both isothermal microcalorimetry and colony counting, the study demonstrates the probiotic's potential as an alternative or complementary approach to antibiotics in the management of diarrheal diseases. However, the work is limited to in vitro experiments, with mechanistic details and host-related factors remaining largely unexplored with poor statistical analysis. Despite these limitations, the findings contribute meaningfully to the growing body of research supporting probiotics as a sustainable, non-antibiotic intervention in global diarrheal disease control. Further in vivo and clinical studies are essential to confirm the therapeutic relevance and practical application of these results.

Authors' contributions

Conceptualization: MFA; Formal analysis: MFA, CCD; Investigation: CCD, MFA, SVS, GIV; Methodology: MFA, CCD, SVS, GIV; Supervision: MFA; Writing – original draft: MFA, CCD; Writing – review and editing: MFA, CCD; Approval: MFA, CCD, SVS, GIV.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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References

1. Cissé G. Food-borne and water-borne diseases under climate change in low- and middle-income countries: further efforts needed for reducing environmental health exposure risks. Acta Trop. 2019;194:181–8.
2. WHO. Diarrhoeal diseases. 2025.
3. GBD 2021 Diarrhoeal Diseases Collaborators. Global, regional, and national age-sex-specific burden of diarrhoeal diseases, their risk factors, and aetiologies, 1990–2021, for 204 countries and territories: a systematic analysis for the Global Burden of Disease Study 2021. The Lancet Infectious Diseases. 2024.
4. Ohene-Adjei K, Kenu E, Bandoh DA, Addo PNO, Noora CL, Nortey P et al. Epidemiological link of a major cholera outbreak in Greater Accra region of Ghana, 2014. BMC Public Health (2017) 17:801. 2017;17(801):1–10.
5. Awalime DK, Davies-Teye BBK, Vanotoo LA, Owoo NS, Nketiah-Amponsah E. Economic evaluation of 2014 cholera outbreak in ghana: a household cost analysis. Health Econ Rev. 2017;7(45):1–8.
6. Noora CL, Issah K, Kenu E, Bachan EG, Nuoh RD, Nyarko KM, et al. Large cholera outbreak in brong Ahafo Region, Ghana. BMC Res Notes. 2017;10(389):1–8.
7. Harris BJ, LaRocque CR, Qadri F, Ryan TE, Calderwood BS. Cholera Lancet. 2012;379(9835):2466–76.
8. de Magny GC, Cazelles B, Guegan J-F. Cholera threat to humans in Ghana is influenced by both global and regional Climatic variability. EcoHealth. 2007;3:223–31.
9. Thompson CC, Freitas FS, Marin MA, Fonseca EL, Okeke IN, Vicente ACP. Vibrio cholerae O1 lineages driving cholera outbreaks during seventh cholera pandemic in Ghana. Infect Genet Evol. 2011;11:1951–6.
10. Paz S. Impact of temperature variability on cholera incidence in southeastern Africa, 1971–2006. EcoHealth. 2009;6(3):340–5.
11. Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, et al. Diarrheagenic Escherichia coli. Braz J Microbiol. 2016;47(Suppl 1Suppl 1):3–30.
12. Cabrera-Sosa L, Ochoa JT. Escherichia coli Diarrhea. 10th ed2020. 481-5 p.
13. Barbuddhe SB, Chakraborty T. Listeria as an enteroinvasive Gastrointestinal pathogen. Curr Top Microbiol Immunol. 2009;337:173–95.
14. Fotopoulou ET, Jenkins C, Painset A, Amar C. Listeria monocytogenes: the silent assassin. J Med Microbiol. 2024;73(3):001800.
15. Scharff RL. Economic burden from health losses due to foodborne illness in the united States. J Food Prot. 2012;75(1):123–31.
16. de Noordhout CM, Devleesschauwer B, Angulo FJ, Verbeke G, Haagsma J, Kirk M, et al. The global burden of listeriosis: a systematic review and meta-analysis. Lancet Infect Dis. 2014;14(11):1073–82.
17. Tranter HS. Foodborne Staphylococcal illness. Lancet. 1990;336(8722):1044–6.
18. Blaabjerg S, Artzi DM, Aabenhus R. Probiotics for the prevention of Antibiotic-Associated diarrhea in Outpatients-A systematic review and Meta-Analysis. Antibiot (Basel). 2017;6(4):21.
19. Guarino A, Guandalini S, Lo Vecchio A. Probiotics for prevention and treatment of diarrhea. J Clin Gastroenterol. 2015;49(Suppl 1):S37–45.
20. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506–14.
21. Yang S, Qiao J, Zhang M, Kwok LY, Matijasic BB, Zhang H, et al. Prevention and treatment of antibiotics-associated adverse effects through the use of probiotics: A review. J Adv Res. 2025;71:209–26.
22. Moens F, Van den Abbeele P, Basit AW, Dodoo C, Chatterjee R, Smith B, et al. A four-strain probiotic exerts positive Immunomodulatory effects by enhancing colonic butyrate production in vitro. Int J Pharm. 2019;555:1–10.

23. Sisson G, Ayis S, Sherwood RA, Bjarnason I. Randomised clinical trial: A liquid multi-strain probiotic vs. placebo in the irritable bowel syndrome–a 12 week double-blind study. Aliment Pharmacol Ther. 2014;40(1):51–62.
24. Kvasnovsky CL, Bjarnason I, Donaldson AN, Sherwood R, Papagrigoriadis S. Randomized Double-Blind Placebo-Controlled trial of a Multi-Strain probiotic in treatment of chronic symptoms in diverticular disease. J Am Coll Surg. 2016;223(4):S28–S.
25. Sancandi M, De Caro C, Cypaite N, Marascio N, Avagliano C, De Marco C, et al. Effects of a probiotic suspension symprove on a rat early-stage parkinson's disease model. Front Aging Neurosci. 2022;14:986127.
26. Fredua-Agyeman M, Stapleton P, Basit AW, Beezer AE, Gaisford S. In vitro Inhibition of clostridium difficile by commercial probiotics: A microcalorimetric study. Int J Pharm. 2017;517(1–2):96–103.
27. Dodoo CC, Stapleton P, Basit AW, Gaisford S. Use of a water-based probiotic to treat common gut pathogens. Int J Pharm. 2019;556:136–41.
28. Fredua-Agyeman M. Surviving process and transit: controlled freeze drying, storage and enteric coated capsules for targeted delivery of probiotic lactobacillusacidophilus. Heliyon. 2024;10(7):e28407.
29. Fredua-Agyeman M, Gaisford S. Comparative survival of commercial probiotic formulations: tests in biorelevant gastric fluids and real-time measurements using microcalorimetry. Benef Microbes. 2015;6(1):141–51.
30. Fredua-Agyeman M, Stapleton P, Gaisford S. Growth assessment of mixed cultures of probiotics and common pathogens. Anaerobe. 2023;84:102790.
31. Martinez MR, Megli JC, Taylor KR. Growth and laboratory maintenace of vibrio cholerae. Curr Protoc Microbiol. 2010;Chap. 6:Unit-6A.1.
32. Fijan S, Sulc D, Steyer A. Study of the in vitro antagonistic activity of various Single-Strain and Multi-Strain probiotics against Escherichia coli. Int J Environ Res Public Health. 2018;15(7):1539.
33. Eggers S, Barker AK, Valentine S, Hess T, Duster M, Safdar N. Effect of Lactobacillus rhamnosus HN001 on carriage of Staphylococcus aureus: results of the impact of probiotics for reducing infections in veterans (IMPROVE) study. BMC Infect Dis. 2018;18(1):129.
34. Piewngam P, Khongthong S, Roekngam N, Theapparat Y, Sunpaweravong S, Faroongsarng D, et al. Probiotic for pathogen-specific Staphylococcus aureus decolonisation in thailand: a phase 2, double-blind, randomised, placebo-controlled trial. Lancet Microbe. 2023;4(2):e75–83.
35. Yang X, Peng Z, He M, Li Z, Fu G, Li S, et al. Screening, probiotic properties, and Inhibition mechanism of a Lactobacillus antagonistic to Listeria monocytogenes. Sci Total Environ. 2024;906:167587.
36. Mao N, Cubillos-Ruiz A, Cameron DE, Collins JJ. Probiotic strains detect and suppress cholera in mice. Sci Transl Med. 2018;10(445):eaao2586.
37. Nataraj BH, Mallappa RH. Antibiotic resistance crisis: an update on antagonistic interactions between probiotics and Methicillin-Resistant Staphylococcus aureus (MRSA). Curr Microbiol. 2021;78(6):2194–211.
38. Cotter PD, Hill C. Surviving the acid test: responses of gram-positive bacteria to low pH. Microbiol Mol Biol Rev. 2003;67(3):429–.
39. Chung HJ, Bang W, Drake MA. Stress response of Escherichia coli. Compr Rev Food Sci Food Saf. 2006;5(3):52–64.

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