Evaluating the antibacterial activity of Symprove™ against common pathogens

Cornelius Dodoo. UCL School of Pharmacy. London, August 2017

Introduction

This report details an evaluation of the antibacterial activity of Symprove™ against two common intestinal pathogens – *Escherichia coli* (NCTC 10418) and *Shigella sonnei* (ATCC 25931) – and methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is an organism of great public health concern that has also been implicated in food poisoning. Symprove™ is a water-based formulation containing multi-strain, live and active bacteria. The probiotic organisms in this formulation are *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Enterococcus faecium.*

Method

Verifying the bacterial population of Symprove™

100 μl of Symprove™ was added to 900 μl of phosphate-buffered saline and vortexed for about 10 seconds. This was then serially diluted (1 in 10) and plated onto de Man, Rogosa, and Sharpe (MRS) agar. The agar plates were then incubated at 37 ^{o}C under anaerobic conditions for 48 hours after which colonies were counted.

Evaluating the antibacterial property of Symprove™

Isothermal calorimetry was used to investigate the antibacterial activity of Symprove™ against *E. coli*, MRSA, and *S. sonnei*.

Power-time curves for the pathogenic species, Symprove™, and pathogenic species co-incubated with Symprove™ were obtained using a 2277 Thermal Activity Monitor (TAM, TA Instruments Ltd., UK) operated at 37 ± 0.1 °C. 3 ml sterile calorimetric ampoules were used and cooked meat medium supplemented with 2% glucose was used as the growth medium. Frozen vials of the pathogenic species (*E. coli* (10^6 CFU/ml), MRSA (10^5 CFU/ml), *S. sonnei* (10^6 CFU/ml)) were used. Each ampoule consisted of sterilised cooked meat pellets (0.3 g)

suspended in 2 % glucose solution. The volumes used for inoculation were as follows: 30 µl of Symprove™, *E. coli,* and *S. sonnei*, and 300 µl of MRSA.

Plate counts for both Symprove™ and pathogenic species were conducted at 24 and 48 hours. *E. coli* and MRSA enumeration were conducted on nutrient agar plates whilst *S. sonnei* enumeration was conducted on MacConkey agar. Symprove™ enumeration was conducted on MRS agar plates in all instances.

Results and Discussion

A triplicate enumeration of Symprove™ yielded an average probiotic population of 4.08 x 10^8 CFU/ml. With the recommended dose of Symprove™ being 70 ml, the probiotic population per dose was computed as 2.86 x 10^{10} as shown in Table 1.

Table 1: Average probiotic enumeration per dose (70ml) after triplicate enumeration.

Test (CFU/ml)			**Average (CFU/ml)**	**Average (CFU/dose)**
A	B	C		
5.19 x 10^8	4.77 x 10^8	2.29 x 10^8	4.08 x 10^8	2.86 x 10^{10}

Thermograms obtained in all three instances showed a common scenario; one whereby the exponential growth for the pathogenic species occurred rapidly (less than an hour) as compared to that for Symprove™ in which case significant exponential growth was observed approximately 5 hours after initiating experiment (Figures 1, 3, and 5).

Plate counting data when co-incubated, revealed no *viable E. coli* colonies, a reduction of about 4 log cycles for *S. sonnei* whilst MRSA numbers were down to 10 CFU/ml at 48 hours (Figures 2, 4, and 6).

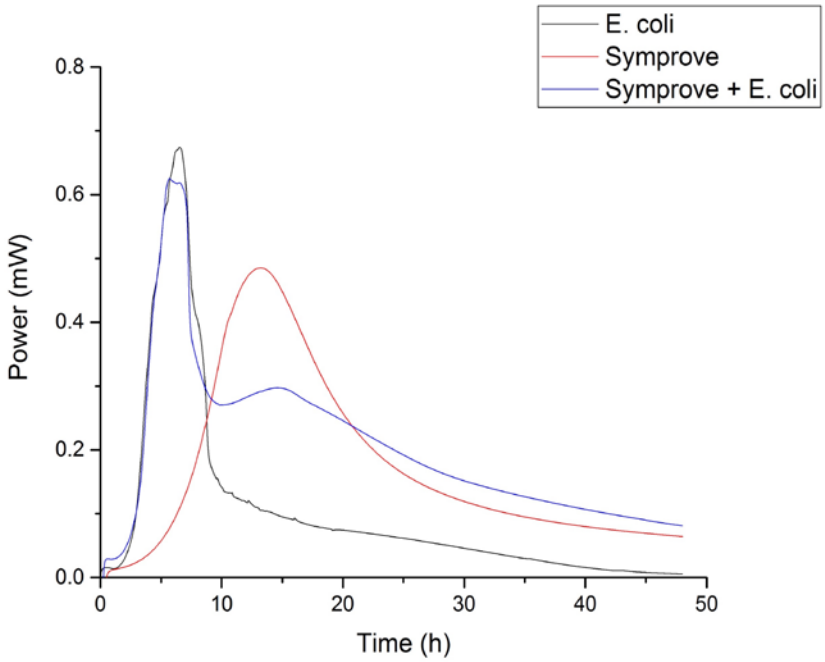

Figure 1: Thermograms obtained after the co-incubation of Symprove™ and *E. coli*, and their respective controls.

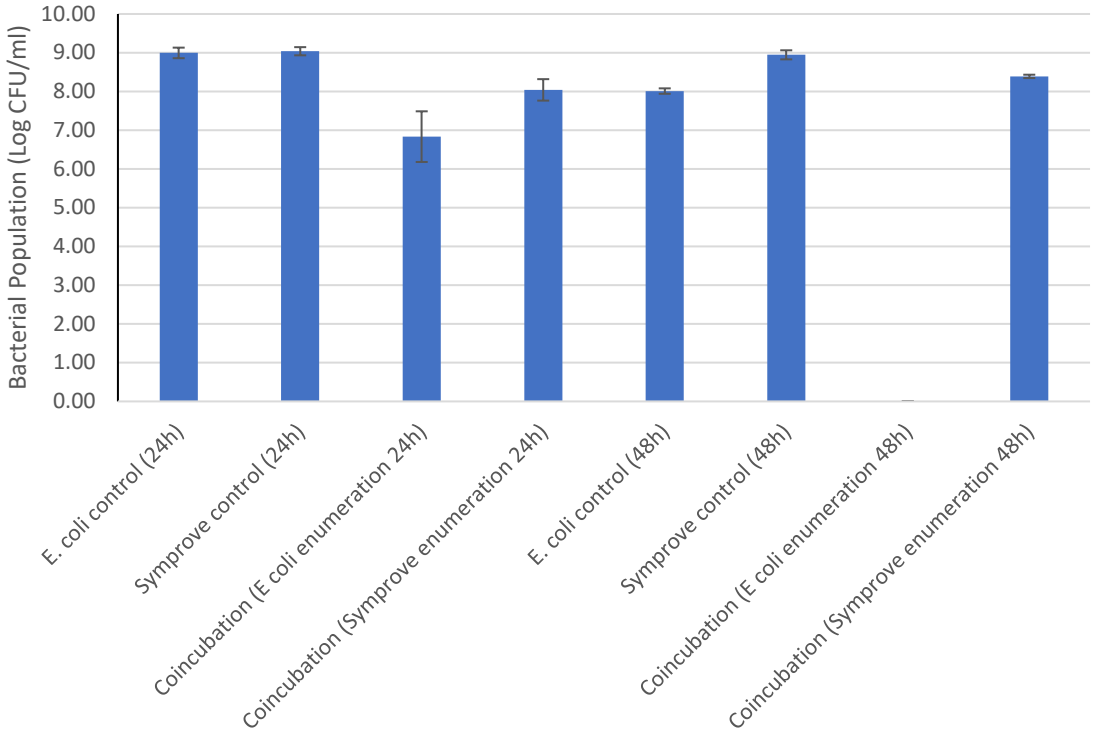

Figure 2: Plate counts at 24 and 48 hours after the co-incubation of Symprove™ and *E. coli*, and their respective controls.

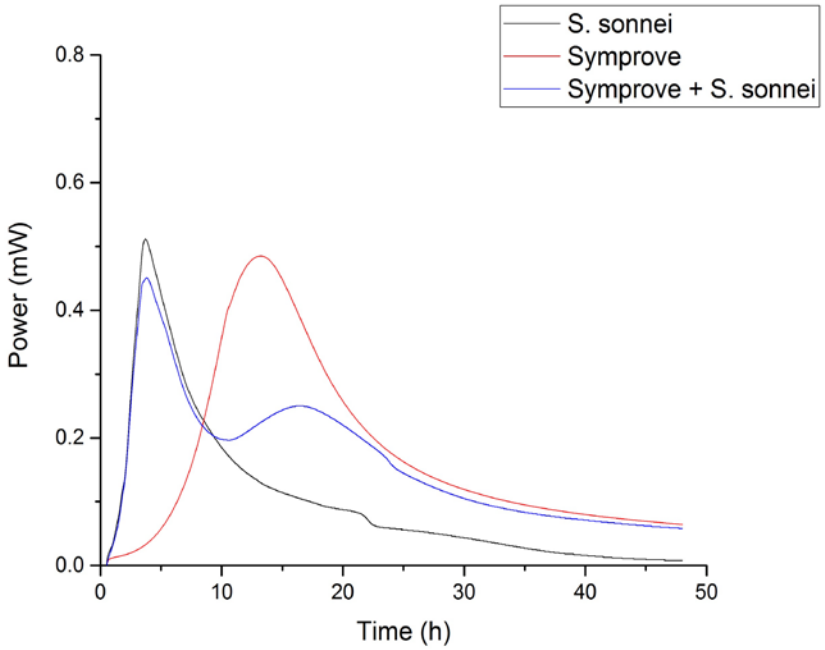

Figure 3: Thermograms obtained after the co-incubation of Symprove™ and *S. sonnei*, and their respective controls.

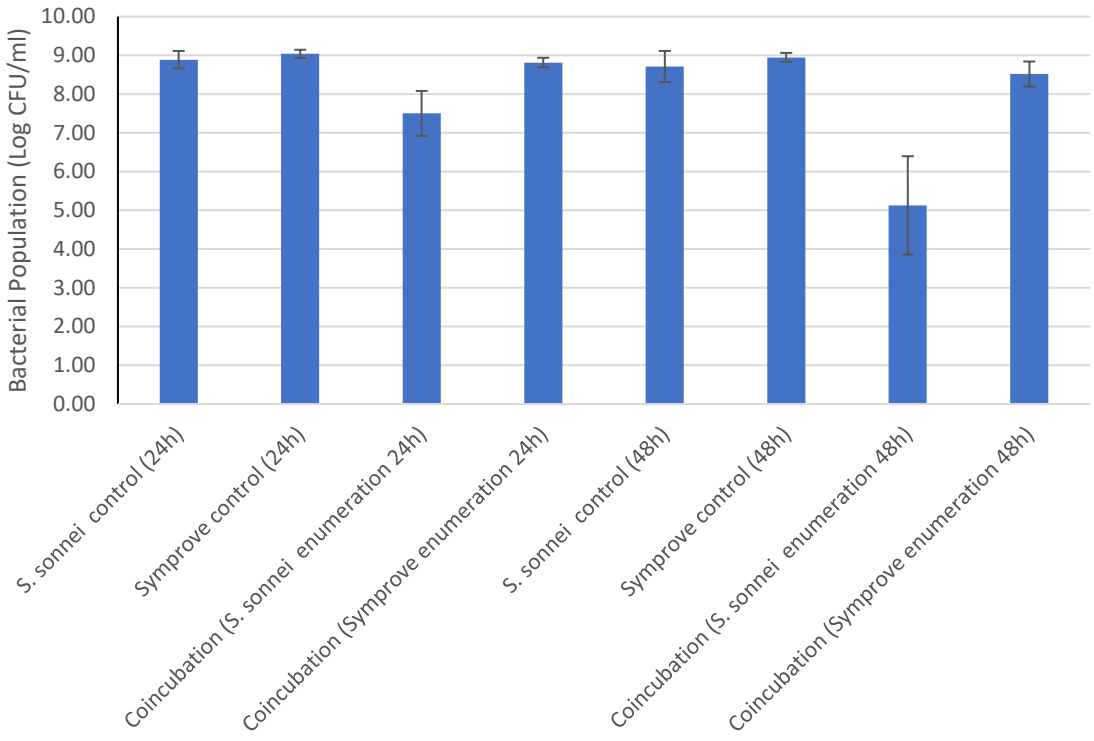

Figure 4: Plate counts at 24 and 48 hours after the co-incubation of Symprove™ and *S. sonnei*, and their respective controls.

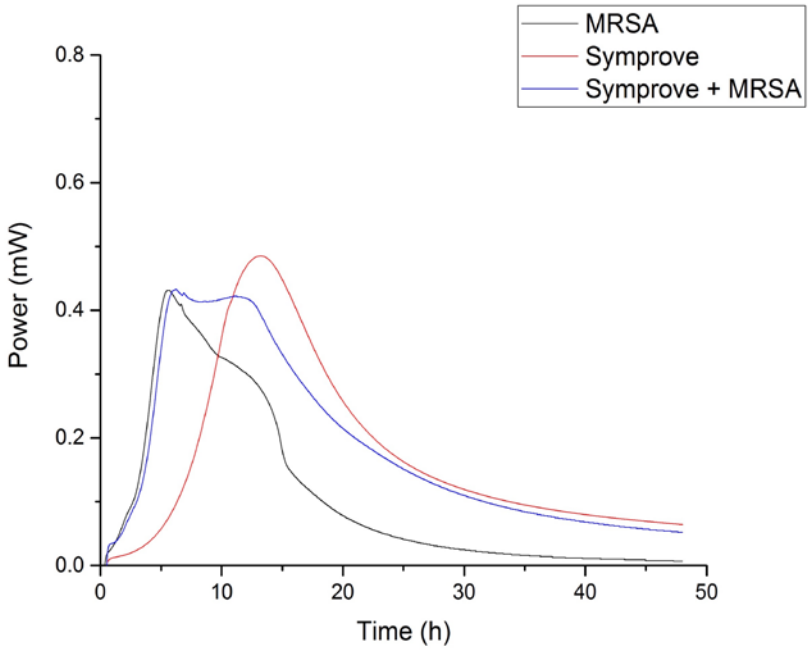

Figure 5: Thermograms obtained after the co-incubation of Symprove™ and MRSA, and their respective controls.

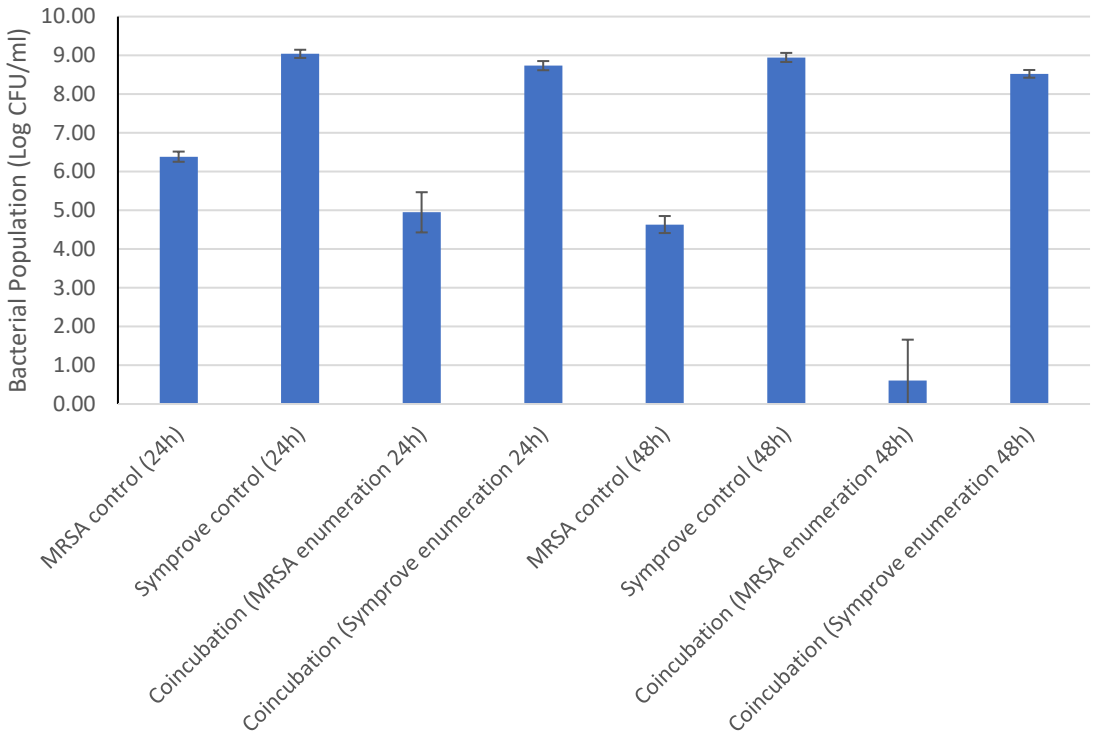

Figure 6: Plate counts at 24 and 48 hours after the co-incubation of Symprove™ and MRSA and their respective controls.

The growth rate and the generation time are often used to indicate how fast a bacterial culture grows. A change in these two parameters can indicate a positive or negative effect of a treatment on cultures and can be useful when investigating new antimicrobial compounds (Braissant et al., 2013). Antibacterial activity of probiotic strains is generally obtained as a result of their metabolic activity, however, the probiotic strains in questions here achieved exponential growth after the pathogenic species in all instances. This is the main reason why the signals obtained during co-incubation of the pathogens and Symprove™ initially resemble the pathogenic species controls since its initial growth characteristics are unaffected by the presence of probiotic strains.

The difference in peak height for Symprove™ in the co-incubated experiments could be due to the reduced nutrient availability from initial metabolism by pathogens. It was, therefore, interesting when enumeration of the pathogenic species at 48 hours upon co-incubation yielded significant differences in pathogenic numbers compared to controls despite the nutrient advantage these pathogenic organisms had over the probiotic strains. At 48 hours, the probiotic strains would have reached their stationary phase of growth and would have released any potential metabolites in the process hence, a good time for enumeration. Microorganisms are known to stay in the stationary for a very long time even without nutrients (Juturu and Wu, 2015). It was, therefore, expected that *E. coli*, *S. sonnei* and MRSA numbers would be maintained over the 48-hour period as obtained with plate counts for the corresponding controls. This was, however, not the case when the probiotic strains had grown completely.

Among the proposed mechanisms of probiotic activity include the release of chemicals or substances with antibacterial activity, competition for adhesion sites and available nutrients, and production of acids which make the milieu unfavourable for pathogenic bacterial growth (Sanders, 2009, Govender et al., 2014, Verna and Lucak, 2010).

Of these proposals, the release of antibacterial substances and a conversion of the growth medium into an acidic environment seem to be viable reasons for the reductions in pathogenic numbers. *E. coli*, however, generally adapts well to acidic environments; as such, the release of acids by probiotic organisms may not be solely responsible for the complete elimination of viable *E. coli* cells. The release of antibacterial metabolites may therefore be the potential reason for results obtained (Benjamin and Datta, 1995).

When *S. sonnei* was co-incubated, a reduction over 4 log cycles was observed. In evaluating antimicrobial effects, a 3-log reduction is considered to be a significant for antimicrobial activity (Usacheva et al., 2014, Heffernan et al., 2013, Koh et al., 2013, Sun et al., 2014). *S. sonnei* is a very infective organism with as little as 10 organisms capable of causing disease (Hochstein, 2013). Considering, a single probiotic inoculation was used in this instance, continuous dosing could possibly result in complete elimination of *S. sonnei* viability.
S. sonnei, however, has been reported to be very susceptible to acidic environments; the antibacterial property of probiotics culture supernatants against *S. sonnei* have been nullified when the pH of the media has been restored to a near neutral pH (Zhang et al., 2011, Zhang et al., 2012). The antagonistic effect obtained here could, therefore, be because of the change in pH.
L. rhamnosus, *L. acidophilus* and *L. plantarum* have been reported to demonstrate varying degrees of antibacterial activity against *S. sonnei* and *E. coli* (Tuo et al., 2013, Hutt et al., 2006, Apella et al., 1992). It was hence, not surprising for a formulation containing such probiotic organisms to demonstrate antagonistic activity against these pathogens.
Enumeration of MRSA population at 48 hours was less than 10 viable cells in comparison to the control (ca. 10^5 CFU/ml). Antagonistic effect of probiotics on MRSA has been mainly attributed to bacteriocin-like inhibitors and/or production of organic acids (Sikorska and Smoragiewicz, 2013, Brachkova et al., 2010, Jabbar et al., 2011). Jabbar et al. (2011) also reported the potential of culture supernatants of *L. acidophilus* in preventing MRSA biofilm formation.

Conclusion

It has been demonstrated here that Symprove™ has an antagonistic effect on the pathogenic species tested. This brings a new dimension to probiotic activity in that, most of the current recommendations of probiotics are as preventive therapies, however, in instances where an infection is already established, there could be a potential of using probiotics as effective means of eliminating some pathogenic species as demonstrated here with Symprove™. It is interesting to note that this study is a single dose response and that the potential of using Symprove as a multi dose technology is something to be explored further.

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