ProDigest
Gastrointestinal Expertise

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ABSTRACT

Evaluation of the anti-pathogenic activity of Symprove™ in the human gastrointestinal tract using the SHIME® technology platform

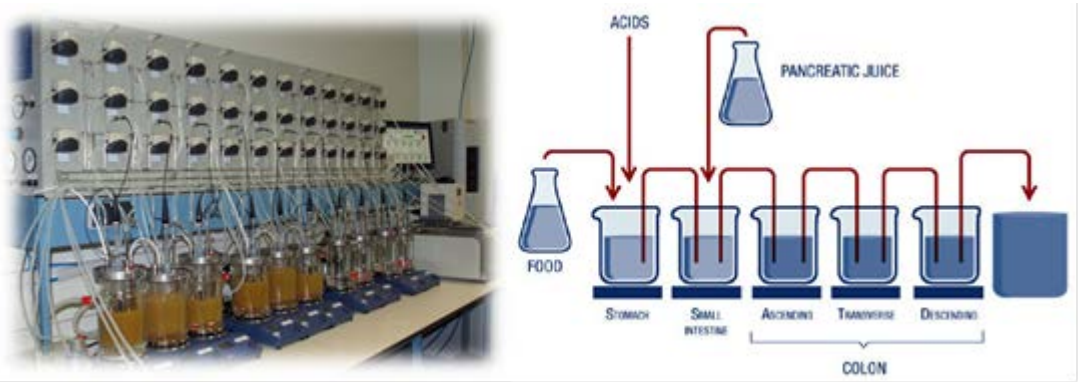
Background.
The aim of this study was to evaluate the survival of the bacterial strains present in SymproveTM under simulated conditions for the human upper gastrointestinal tract (GIT). For this purpose, a Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) was used, which allows to re-create the physiological conditions that are representative of the human GIT. The tests were conducted under specific fasted conditions that were requested by SymproveTM.
Secondly, the aim of this study was to study the potential effect of SymproveTM in terms of anti- pathogenic activity against a strain of Escherichia coli (AIEC). For this purpose, short-term colonic batch experiments were performed that simulate the colonic fermentation process that occurs in the distal colon.

To assess viability after passage in the human upper gastrointestinal tract under very controlled conditions, using the SHIME system. The main endpoint was the evaluation of the survival of the strains by quantification of the colony forming units obtained through spread plating on agar media and by flow cytometric quantification of viable and non-viable bacterial cells. Furthermore, DNA was extracted from samples that were treated with propidium mono azide allowing to quantify the viable bacterial cells through qPCR.

Then in the next stage of the SHIME® system the anti-pathogenic activity of SymproveTM on a strain of Escherichia coli (adherent-invasive E. coli = AIEC) in the colon was evaluated. This was done by performing short-term colonic incubations inoculated with AIEC together with a metabolically active colonic microbiota, and with or without the test product. Two doses of the product were tested. The extent of anti-pathogenic activity of SymproveTM against AIEC was compared versus reactors that were not inoculated with SymproveTM (negative control) and versus incubations to which ciprofloxacin was added (positive control). The main endpoint was quantification of colony forming units of AIEC through selective spread plating.

Simulator of the Human Intestinal Microbial Ecosystem SHIME

The reactor setup used in the experiments was adapted from the SHIME, representing the gastrointestinal tract (GIT) of the adult human, as described by Molly et al. (1993)1. The SHIME consists of a succession of five reactors simulating the different parts of the human gastrointestinal tract. which allows to re-create the physiological conditions that are representative of the human GIT. The first two reactors are of the fill-and-draw principle to simulate different steps in food uptake and digestion, with peristaltic pumps adding a defined amount of SHIME feed and pancreatic and bile liquid, respectively to the stomach and small intestine compartment and emptying the respective reactors after specified intervals. The last three compartments – continuously stirred reactors with constant volume and pH control – simulate the ascending, transverse and descending colon. Retention time and pH of the different vessels are chosen in order to resemble in vivo conditions in the different parts of the gastrointestinal tract.



Overview of the TWINSHIME setup, consisting of two parallel SHIME systems. Each SHIME reactor simulates the stomach, small intestine, ascending colon, transverse colon and descending colon.

1Molly, K., M. V. Woestyne, et al. (1993). "Development of a 5-step multichamber reactor as a simulation of the human intestinal microbial ecosystem." Applied Microbiology and Biotechnology 39: 254-258.

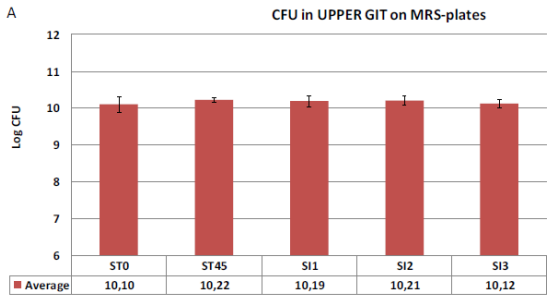
Summary results and conclusions

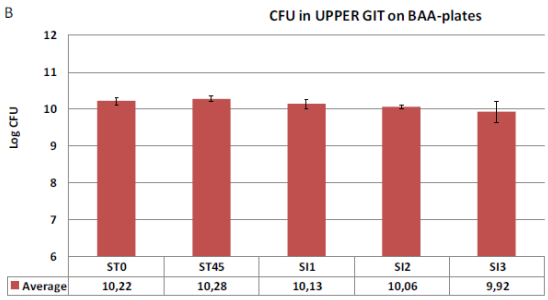
GIT Survival

Gastric phase. The background medium contained salts, mucins, pepsin and phosphatidylcholine. Symprove was added at 35mL, being half a standard dose (as the reactor simulates half a human). Incubation was done for 45 min at 37 °C, with the pH being kept constant at 3. Sampling was done at (ST0) and after 45 min of incubation (ST45). Experiments were performed in biological triplicate.
The number of CFU's were determined through spread plating on two different agar media. The number of viable and non-viable bacterial cells were determined through flow cytometry. Further, the samples were subjected to DNA extraction.

Small intestinal phase. Incubation was done for 3h at 37°C, with the pH increased till pH 7.4 over a 100- min time period. A raw animal pancreatic extract (pancreatin) containing all the relevant enzymes in a specific ratio as well as defined ratios of the different enzymes was used, in addition bovine bile extract was supplemented (bovine bile is a closer match to human than porcine in terms of tauro- and glycocholate). Sampling after 1h (SI1), 2h (SI2), and 3h (SI3) of small intestinal incubation. Experiments were performed in biological triplicate.
The number of CFU's were determined through spread plating on two different agar media and the number of viable and non-viable bacterial cells was determined through flow cytometry. Further, the samples were subjected to DNA extraction. Determination of the colony forming units (CFU) through spread plating on MRS agar and bile aesculin agar medium during these experiments demonstrated the outstanding survival of the bacteria of Symprove.

The bacterial strains of SymproveTM displayed an outstanding survivability upon passage through the upper GIT under modified fasted conditions.





Average log (CFU) ± stdev (n = 3) obtained through spread plating on MRS agar (A) and bile aesculin agar (B). Data are representative for samples collected during passage in the stomach (ST0 and ST45) and small intestine (SI1, SI2, and SI3) under modified fasted conditions. Differences in samples (ST0/ST45/SI1/SI2/SI3) as compared to their preceding sample were indicated with *. *: statistically significant change ($p < 0.05$).

Whereas the constant low pH conditions of the fasted stomach in general have a detrimental effect on bacterial cell survival and culturability, no significant decrease was observed in the number of CFU of Symprove during passage through the fasted stomach and this both on MRS agar and bile aesculin agar.
Upon entering the small intestine, the pH conditions become more favorable compared to those residing in the stomach. However, the secretion of pancreatic juice, containing pancreatic enzymes and bile salts, in the small intestinal lumen can have detrimental effects on bacterial culturability and survival. The obtained results suggested that the bacteria present in Symprove were not sensitive to these bile acids and maintained culturable throughout the small intestinal incubation.

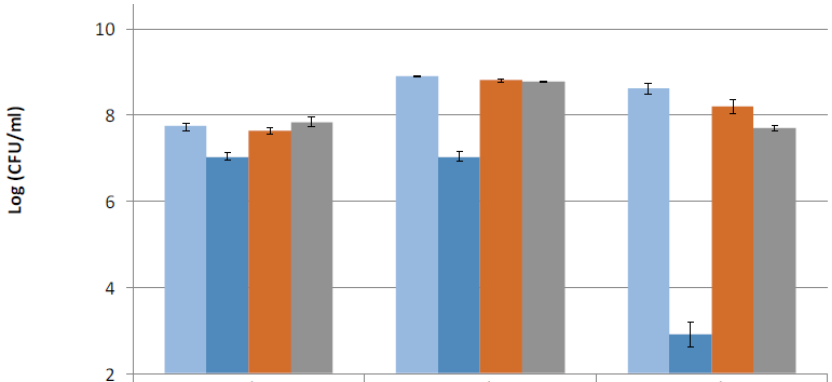
Determination of the counts of total bacteria, viable bacteria, and non-viable bacteria through flow cytometry revealed that the bacteria comprised in Symprove were capable to survive the passage of the upper GIT under modified fasted conditions. Comparing the results of viable bacteria obtained through flow cytometry and the results of CFU obtained through spread plating at the start of the experiment revealed that nearly all viable bacteria were in a culturable state. Therefore, it can be concluded that upon storage the bacteria contained in Symprove maintained their culturability and hence metabolic activity and did not transform into a viable but nonculturable (VBNC) state.

Anti-pathogenic activity of Symprove against AIEC

Short-term colonic batch incubations were performed using a representative colon medium containing host -and diet-derived compounds. These were inoculated with fresh fecal inoculum containing metabolically active colonic microbiota. An overnight grown culture of AIEC was inoculated to all incubations. The negative control contained the colon medium, inoculum, and AIEC. Positive control experiments contained in addition ciprofloxacin (antibiotic that inhibits AIEC). Further incubations which contained a colon medium, inoculum, AIEC, and SymproveTM. Two doses of the test product were tested namely 6% (vol/vol) (corresponding to the dose tested during upper GIT experiments) and 12% (vol/vol). All bottles were incubated for 48h at 37°C under anaerobic conditions. Samples were taken at the start of the incubations (0h) and after 24h and 48h of incubation.

Samples were used to determine the number of CFU's of AIEC through spread plating on a selective agar medium. Further, the samples were subjected to DNA extraction.

Selective quantification of the CFU of AIEC through spread plating on McConkey agar medium, supplemented with ampicillin and erythromycin, revealed that AIEC grew out to the highest levels during the negative control experiments. Dosing of Symprove resulted in significant lower concentrations of CFU of AIEC after 24h of incubation and even in a decrease of the CFU after 48h to levels which were significantly lower ($p < 0.05$) as compared to the negative control experiments. The highest dose resulted in the largest effect.
These results indicated that the metabolic activity of Symprove during the colonic incubations had an anti-pathogenic effect against AIEC.



	0h	24h	48h
Neg control	7,74	8,91	8,61
Pos control	7,03	7,03	2,90
Dose 1	7,63	8,81	8,21
Dose 2	7,84	8,78	7,70

Figure 11: Average Log (CFU/ml) ± stdev (n = 3) obtained through spread plating on McConkey agar supplemented with ampicillin and erythromycin. Samples were taken at the start of the incubation (0h) and after 24h and 48h during different sets of colonic incubations.

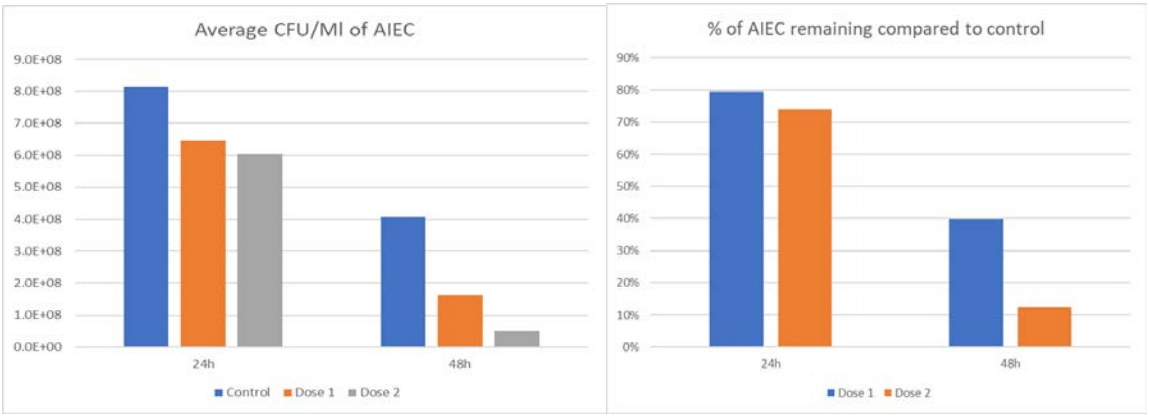


Figure 11b. Alternative representation of figure 11, showing values as CFU/mL and Figure 11c showing % of AIEC remaining compared to control.