



Mice Clinical Trials

PATHOGENIC *E. COLI* STRAINS

The clinically isolated enterotoxigenic *Escherichia coli* strain, H10407 (serotype O78:H11) was used to represent the major serotypes isolated worldwide from major microbial imbalances. This ETEC strain H10407 was originally isolated in Bangladesh from a patient with severe, cholera-like diarrheal illness³. Marcia Wolfe provided the H10407 isolate used in this study. It was derived from good manufacturing practice (GMP) lots of H10407 produced at Walter Reed Army Institute of Research. This strain is fully virulent in human volunteer clinical challenge studies².

PHAGE PURIFICATION AND SOURCE

Phage isolation and purification was performed by Deerland Enzymes.

EXPERIMENTAL ANIMALS

INTESTINAL INFECTION OF MICE WITH ETEC H10407

Mice were infected orally with ETEC strain H10407 as previously described by Allen et al.¹. Briefly, strain H10407 was grown to mid-logarithmic phase in Luria broth, pH 7.4, and resuspended in sterile PBS such that the final concentration of bacteria was approximately 5×10^7 colony forming units (CFU) per dose plus 2.5×10^7 CFU per dose of *Bifidobacterium longum* in a final volume of 300:1. This amount was then administered by gavage to 12 ETEC-naïve ICR mice that had been pretreated with streptomycin to eliminate native flora and cimetidine to reduce stomach acidity prior to challenge. This procedure was repeated with the addition of 1×10^5 plaque forming units (PFU) per dose of the *E.coli* phage cocktail **PreforPro**[®]. Fecal matter was taken 2 times at 6 and 24 hours after inoculation and mice were subsequently sacrificed at 24 hours. The ileum and large intestine were harvested and plated for *E.coli* counts, *B. longum* counts and phage counts.

RESULTS

Comparing to the control, the phage cocktail resulted in the following results; *E.coli* decreased in the ileum ~10 fold (50170 to 3135 with **PreforPro**), the large intestine ~100 fold (11180 to 49 with **PreforPro**) and in the fecal matter (10525 to 67) at 24 hours. The *B. longum* counts increased ~100 fold in the ileum (40423 to 73), ~100 fold in the large intestine (1001 to 12) and ~40 fold in the 24 hour fecal sample (18050 to 505). Phage counts went up in the ileum from 897 with *B. longum* to 51150 with *E.coli* and *B.longum*. Then in the large intestines

phage counts went up from 695 to 91500. In the 24 hour fecal count, the phage counts increased from 582 to 87000. Mice with *E.coli* and with *E.coli* and *B.longum* were constipated and the ileum, cecal valve and large intestine were swollen, red and leaking when compared to the control mice with no inoculation. The mice that were infected with *E.coli* and **PreforPro** exhibited normal bowel movements and experienced no change in color or size of the various compartments of the intestine when compared with control mice.

DISCUSSION

An oral phage cocktail decreased intestinal pathogenic *E.coli* populations from 10-1000 fold while simultaneously increasing probiotic populations by 10-100 fold. The increase in probiotic counts reflects the decrease in competition and release of nutrients from the pathogenic *E.coli* bacteria. These *in vivo* results strongly support the application of phage as a prebiotic to regulate and enhance the intestinal microflora, providing support for good bacteria, helping promote and maintain a healthy digestive tract.

References

1. Allen, K. P., M. M. Randolph, and J. M. Fleckenstein. 2006. Importance of heat-labile enterotoxin in colonization of the adult mouse small intestine by human enterotoxigenic *Escherichia coli* strains. *Infect. Immun.* 74:869-875.
2. Barrow, P. A., and J. S. Soothill. 1997. Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. *Trends Microbiol.* 7:268-271.
3. Bruynoghe, R., and J. Maisin. 1921. Essais de thérapeutique au moyen du bacteriophage. *C. R. Soc. Biol.* 85:1120-1121.