CELMANAX™

EFFECT OF CELMANAX™ DRY & LIQUID ON *E. COLI* AGGLUTINATION

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Introduction: CELMANAX™ is a yeast culture product which has been improved to express more of the functional carbohydrates associated with the yeast culture fermentation process. One of the functional carbohydrates associated with this production process is Mannan Oligosaccharide (MOS). MOS, or mannose, plays an important role in preventing infections by some pathogenic bacteria by agglutinating them and preventing them from binding to the host tissue. In this study, CELMANAX was tested for its ability to agglutinate some pathogenic *E. coli*.

Objective: To test the ability of CELMANAX DRY and LIQUID to agglutinate *E. coli* in an *in vitro* experiment, in-house.

Materials and Methods: The ability of CELMANAX DRY and LIQUID forms to agglutinate pathogens was determined using both a slide agglutination assay and a broth agglutination assay. For the slide agglutination experiment, CELMANAX DRY and LIQUID were tested at 2, 20 and 40 mg/mL. Seventy-five µL of log phase *E. coli* F18 (10^{10}cfu/mL) and 75 µL of CELMANAX were added to a slide and swirled for 30 seconds. Negative control had only *E. coli* and 75 µL of saline. CELMANAX control had 75 µL CELMANAX 40mg/mL and 75 µL of saline. Agglutination was observed and photographed. A separate experiment was done to enumerate the unagglutinated *E. coli* cells in the presence of CELMANAX. One mL of cell culture was centrifuged and pellet was resuspended in either sterile saline (control) or in 20 or 40 mg/mL CELMANAX. The tubes were left undisturbed and after 30 minutes unagglutinated cells from the supernatant were enumerated by plating on Tryptic Soy Agar plates. This experiment was done in quadruplicate.

Results: Light green clumps of agglutinated *E. coli* cells were seen when *E. coli* cells were mixed with CELMANAX LIQUID (Figure 1). The agglutination was directly proportional to the concentration of CELMANAX present. The agglutination was highest at 40mg/mL of CELMANAX and lowest at 2 mg/mL. No agglutination was seen in control. Similar results were obtained with CELMANAX DRY.

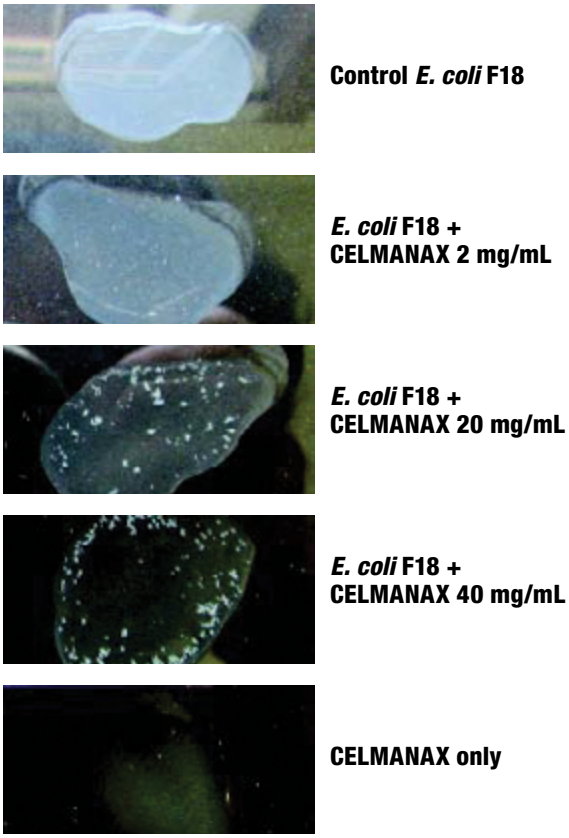
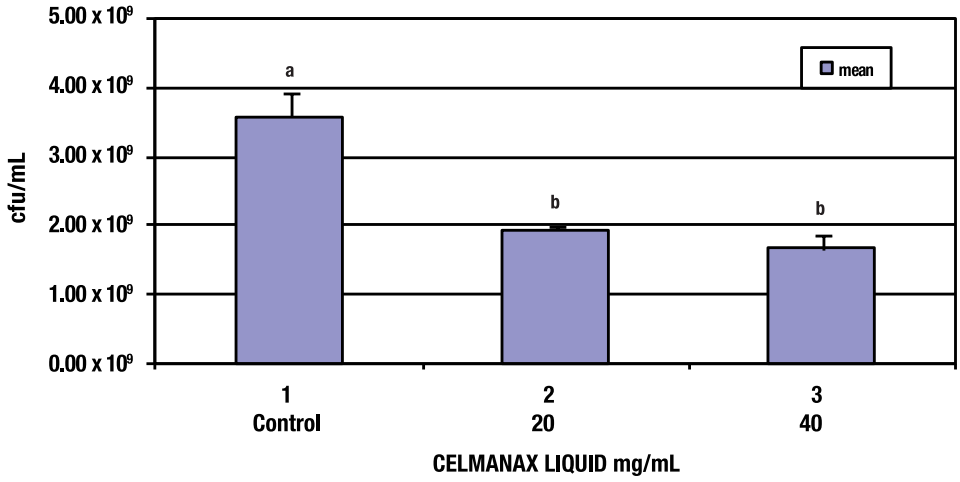


Figure 1

In the second experiment, clumps of agglutinated *E. coli* cells were seen to settle at the bottom of the test tube when cells were incubated with CELMANAX™ LIQUID. When unagglutinated *E. coli* were enumerated, 3.59 x 10^9 cfu of *E. coli* were recovered from cells suspended in saline whereas 1.95 x 10^9 (56%) and 1.68 x 10^9 (46%) cfu were recovered from cells suspended in CELMANAX LIQUID 20 and 40 mg/mL respectively (Figure 2).

Data from four replicates were analyzed by the method of analysis of variance and differences between treatment means were tested for significance by Tukey's mean separation test. The decrease in unagglutinated *E. coli* cells with CELMANAX LIQUID was found to be statistically significant at $P<0.01$ when compared to the control.

Figure 2: **CELMANAX Mediated Agglutination of *E. coli* F18**



[ab] means are different ($P<.01$) by Tukey's Mean Separation Test

Conclusions: CELMANAX DRY and LIQUID agglutinated *E. coli* at all the concentrations tested. Furthermore, CELMANAX LIQUID decreased the unagglutinated *E. coli* F18 population by >50% in an *in vitro* study.