INTERACTIONS BETWEEN FOODBORNE PATHOGENS AND PROTOZOA ISOLATED FROM LETTUCE AND SPINACH

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Previously Brandl et al. (2005) showed that protozoa from moist soil could enhance survival of *Salmonella enterica* by sequestering the bacteria in released food vacuoles (vesicles). Protozoa on fresh produce may similarly protect human pathogens associated with produce. This study examined the ability of protozoa, isolated from produce, to release vesicles containing *Escherichia coli* O157:H7, *S. enterica* (from outbreak sources), and *Listeria monocytogenes* (isolated from mint leaves). Spinach and romaine lettuce (not prepackaged) were rinsed with a saline solution, and the rinse was treated to enrich for protozoan species. Among several protozoan species observed in the rinses, two ciliate species were isolated: *Glaucoma* sp. from the lettuce, and *Colpoda steinii* from the spinach. *Glaucoma* was cultured axenically; *C. steinii* could not be axenized, but was cultured in bacterized cereal leaves medium. In addition to the ciliates, an amoeba sp. was also isolated. A strain of *Tetrahymena pyriformis* (ATCC 30202) originally isolated from moist spinach, and soilborne *Tetrahymena* sp. were also studied; however, the latter one was tested only with *E. coli* O157:H7, because it was tested previously with the other two pathogens (Brandl, et al., 2005). Protozoa were washed and suspended in buffered saline amended with washed GFP- or DsRed-labeled bacterial cells. After 24 h, expelled vesicles were enumerated with a hemacytometer, and ciliates were enumerated microscopically from fixed aliquots. Controls were unfed ciliates. All tests were run twice in duplicate. Vesicles were produced by *Glaucoma* with all bacterial strains, although *Listeria* resulted in the fewest number per ciliate (ave. = 12 and 28 compared with 81 and 220 for *Salmonella*; and 88 and 220 for *E. coli* O157:H7). Vesicle production was also observed with two *Tetrahymena* species. *T. pyriformis* produced vesicles with *E. coli* O157:H7 and *S. enterica* (ave. = 34 and 79 for *E. coli* O157:H7; and 83 and 158 for *S. enterica*) but not with *L. monocytogenes*. *Tetrahymena* sp. produced an average of 30 to 150 vesicles with *E. coli* O157:H7. All vesicles contained intact fluorescing bacteria. No vesicles were observed in controls. Protozoa increased in numbers after they fed on pathogens for 24 h. *T. pyriformis*, the soilborne *Tetrahymena* sp. and *C. steinii* increased to a greater extent than did *Glaucoma* or the amoebae. No change in numbers of *Glaucoma* sp. was observed in controls. However, numbers of *C. steinii*, amoebae and the two *Tetrahymena* species decreased slightly in controls. *C. steinii* and the amoeba species formed cysts, and no entrapment of bacteria inside cysts was observed. Results show that different protozoan species respond differently to foodborne human pathogens; however, two species from produce and one from moist soil can ingest such pathogens and release them in concentrated forms that may protect the bacteria from harmful environmental condition.

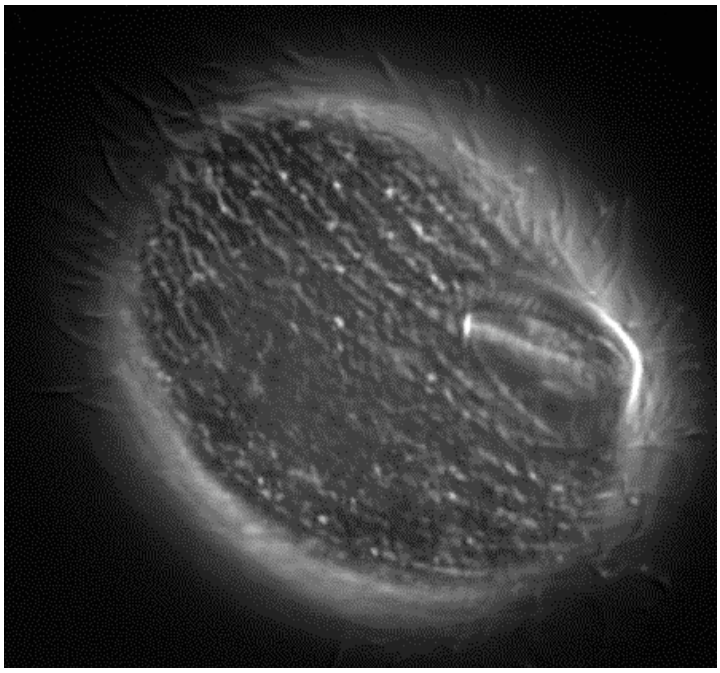

Fig. 1. ***Glaucoma* sp.,** isolated from lettuce.

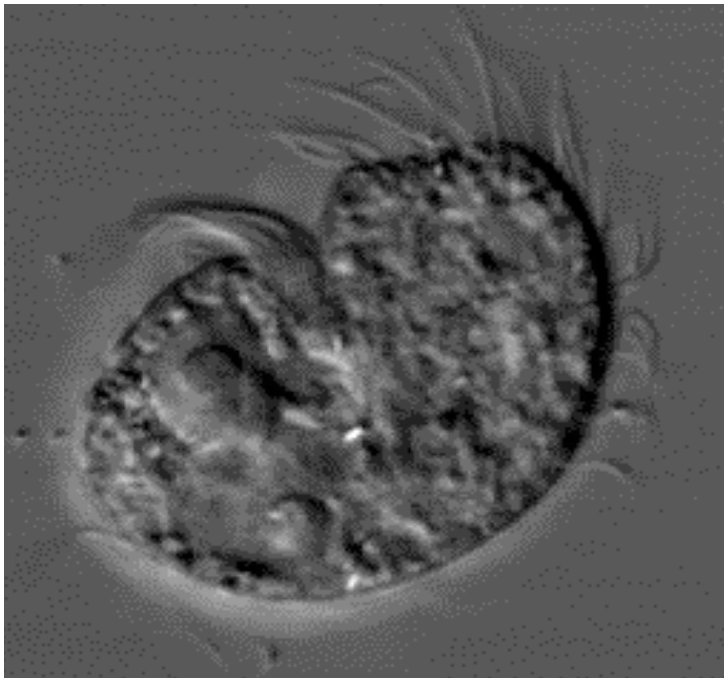

Fig. 2. ***Colpoda steinii***, isolated from spinach.

Reference: Brandl, M.T., B.M. Rosenthal, A.F. Haxo and S.G. Berk. 2005. Enhanced survival of *Salmonella enterica* in vesicles released by a soilborne *Tetrahymena* species. Appl. Environ. Microbiol. **71:**1562-1569.