

ROUTES OF CONTAMINATION

ATTRACTION OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*, TO FOODBORNE PATHOGENIC BACTERIA, AND ITS POTENTIAL AS A VECTOR OF *SALMONELLA* POONA FOR PREHARVEST CONTAMINATION OF CANTALOUPE (K. N. Caldwell, G. L. Anderson, P. L. Williams, and L. R. Beuchat)

Soil is a source of microbial contamination of fruits and vegetables, as evidenced by the isolation of soil-residing pathogenic bacteria from produce. Free-living, microbivorous nematodes are among the primary grazers of bacteria in soils and also have potential to serve as vectors of microorganisms, including enteric pathogens, to the surface of fruits and vegetables. Most nematologists do not attach particular importance to free-living nematodes as vectors of plant pathogens. However, a critical examination of the role nematodes may play in plant and perhaps human diseases has been suggested. *Caenorhabditis elegans*, a microbivorous, free-living nematode, has been used extensively in biological studies. Feeding primarily on bacteria, the adult worm lives approximately 2 weeks under optimal environmental conditions. The worm is routinely cultured in the laboratory on *Escherichia coli* OP50, a uracil-deficient non-pathogenic strain that grows slowly on K agar but serves as a nutrient source for multiplication and reproduction. The objectives of this study were to determine the propensity of *C. elegans* to migrate toward three human enteric pathogens and cantaloupe juice, as well as its survival and reproductive behavior in the presence of these pathogens. The potential of *C. elegans* as a vector to transport *Salmonella* in soil to the surface of cantaloupe rind was also investigated.

The propensity of *C. elegans* to be attracted to seven strains of *Escherichia coli* O157:H7, eight serotypes of *Salmonella*, six strains of *Listeria monocytogenes*, and cantaloupe juice was investigated. Adult worms (20 - 30) were placed on the surface of K agar midway between a 24-h bacterial colony and 10 µl of uninoculated tryptic soy broth (TSB) or cantaloupe juice positioned 1.5 cm apart. The number of nematodes that migrated to the colony, TSB, or cantaloupe juice within 5, 10, 15, and 20 min at 21°C was determined, followed by incubating plates at 37°C for up to 7 days to determine the ability of *C. elegans* to survive and reproduce in bacterial colonies. The nematode was attracted to colonies of all test pathogens, and survived and reproduced within colonies for up to 7 days. *C. elegans* was not attracted to cantaloupe juice. The potential of *C. elegans* to serve as a vector to transport *Salmonella* Poona to cantaloupe rind was investigated. Adult worms that had been immersed in a suspension of *S. Poona* were deposited 1 or 3 cm below the surface of soil on which a piece of cantaloupe rind was placed. The rind was analyzed for the presence of *S. Poona* after 1, 3, 7, and 10 days at 21°C. The presence of *S. Poona* was evident more quickly on rind positioned on soil beneath which *C. elegans* inoculated with *S. Poona* was initially deposited compared to rind on soil beneath which *S. Poona* alone was deposited. The time required to detect *S. Poona* on rind was longer when the rind was placed 3 cm above the inoculum, compared to 1 cm. Free-living nematodes may play a role in the preharvest dispersal of incidental human pathogens in soil to the surface of raw fruits and vegetables in contact with soil during development and maturation, as evidenced by the behavior of *C. elegans* as a test model.

INTERACTION OF A FREE-LIVING SOIL NEMATODE, *CANENORHABDITIS ELEGANS*, WITH SURROGATES OF FOODBORNE PATHOGENIC BACTERIA (G. L. Anderson, K. N. Caldwell, L. R. Beuchat, and P. L. Williams)

The agricultural impacts of plant and animal parasitic nematodes have long been recognized and, by virtue of their effects on fruit and vegetable production, have been extensively studied. Comparatively little is known regarding the impact of free-living microbivorous nematodes on produce production and safety, although they are the most abundant and wide spread soil mesofauna. The association of free-living nematodes and various genera of bacteria has been studied. While it is recognized that free-living nematodes avoid certain bacteria, it is clear that they do not uniformly avoid foodborne pathogens. Two human enteric pathogens, *Salmonella* and *Shigella*, are reportedly ingested and defecated by free-living saprozoic nematodes and *Salmonella* Typhimurium is known to infect the free-living soil nematode *Caenorhabditis elegans*. From these reports, it appears that free-living

nematodes may be important as vectors of pathogenic bacteria, including some forms capable of causing human disease.

Soil is a source of microbial contamination of fruit and vegetables, as evidenced by the isolation of soil-residing pathogenic bacteria from produce. In a survey of vegetables for the presence of amoebae and *Salmonella*, nematode eggs and larvae have been recovered using a naccional-ether method. The recovery of nematodes from uncooked vegetables indicates that agronomic conditions and marketing practices may be conducive to the survival of nematodes on fresh produce. This also indicates that if free-living nematodes are present on raw produce, they may serve as vehicles for contamination with pathogenic bacteria, either by contact with their surface or via eggs or voided material from their gastrointestinal tract.

We undertook a study to evaluate the interaction of *C. elegans* with bacterial surrogates for foodborne pathogens occasionally occurring or persisting in soil. Nematode/bacterial interactions were characterized to determine the propensity of young adult worms to be attracted to bacterial colonies, to compare the feeding and development of young adult worms cultured on this diverse group of bacteria, and to examine the dispersal of bacteria by *C. elegans* following feeding on monoxenic cultures. We evaluated the association between a free-living soil nematode, *C. elegans*, with *Escherichia coli*, an avirulent strain of *S. Typhimurium*, *Listeria welshimeri*, and *Bacillus cereus*. On an agar medium, young adult worms quickly moved toward colonies of all four bacteria; over 90% of 3-day adults entered colonies within 16 min after inoculation. After 48 h, worms moved in and out of colonies of *L. welshimeri* and *B. cereus*, but remained associated with *E. coli* and *S. Typhimurium* colonies for at least 96 h. Young adult worms fed on cells of the four bacteria suspended in K medium. Worms survived and reproduced using nutrients derived from all test bacteria, as evidenced by eggs laid by second generation worms after culturing for 96 h. Development was slightly slower in worms fed on Gram-positive bacteria compared to Gram-negative bacteria. Worms fed for 24 h on bacterial lawns formed on tryptic soy agar dispersed bacteria over a 3-h period when transferred to a bacteria-free agar surface. Results suggest that *C. elegans* and, perhaps, other free-living nematodes are potential vectors for both Gram-positive and Gram-negative bacteria, including foodborne pathogens in soil.

