Caenorhabditis elegans: A Look at Koch's Postulates and Host-Pathogen Interactions

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The interaction between host and pathogen was studied using *Caenorhabditis elegans*. *C. elegans* is a small hermaphroditic nematode, which is a useful model organism due to simple growth requirements, fast generation time, and a completely mapped genome. Koch's postulates, a set of criteria used to show the causal relationship between a microorganism and a specific disease, was demonstrated using *C. elegans* and *Pseudomonas aeruginosa*. *C. elegans* was allowed to "feed" on the bacteria. The killing effect the bacteria on *C. elegans* was monitored and analyzed. The bacteria were then used to infect freshly grown *C. elegans* to ensure that the microbe was the cause of death. Once again, the bacteria were isolated from the *C. elegans* and identified. Research involving *C. elegans* and bacteria is very helpful in understanding infectious agents and host defense mechanisms.

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INTRODUCTION

Caenorhabditis elegans is a commonly used nematode for studying bacterial pathogenesis. *C. elegans* is a host of choice due to simple growth requirements and rapid generation time (Ewbank, 2002). Another advantage of using *C. elegans* is the entire genome is essentially sequenced (Tan, 1999). Having the genome sequence is very useful when doing genetic or molecular studies of pathogenesis.

Pseudomonas aeruginosa, a human and nematode pathogen, kills *C. elegans* in two different ways. Slow-killing happens over several days when low-nutrient media is used to grow *P. aeruginosa* (ATCC 27853). Fast-killing occurs when high-osmolarity media is used to grow the bacterial lawn of *P. aeruginosa* and occurs over several hours. (Tan, 1999). In this experiment, *P. aeruginosa* was used to demonstrate Koch's postulates. Koch's postulates are the criteria used to prove the causal relationship between a specific disease and a microorganism. This criteria is that "the microorganism must be present in every case of the disease but absent from healthy organisms ... the suspected microorganism must be isolated and grown in a pure culture... the same disease must result when the isolated microorganism is inoculated into a healthy host ... [and] the same microorganism must be isolated again from the diseased host" (Prescott, 2002).

MATERIALS AND METHODS

The *C. elegans* strain N2 Bristol was obtained from Carolina Biological Supply Company. *C. elegans* was handled and prepared according to Carolina Biological Supply

Company protocol (Anonymous, 2000). *P. aeruginosa* (ATCC 27583) was obtained from Difco and *Escherichia coli* was obtained from Carolina Biological Supply Company. All bacterial strains were sterilely transferred to a test tubes containing CTA agar and stored at room temperature for future use.

Nematode Growth Agar was obtained from Carolina Biological Supply Company and plates were poured according to their protocol (Anonymous, 2000). High-osmolarity PGS (peptone/glucose/sorbitol) agar (Tan, 1999) was prepared as described and plates were poured. All plates were stored in a refrigerator.

NGM plates were swabbed with *E. coli,* using a sterile cotton-tipped swab, and incubated at 37°C overnight to allow growth of a bacterial lawn. The same procedure was used to grow a bacterial lawn of *P. aeruginosa.* Each plate was then seeded with approximately 10 to 15 *C. elegans* (N2). *E. coli* plates were used as a control. Plates were then incubated at room temperature (~ 25 °C) and examined for live worms after 24 h. Examinations were done daily until almost all the *C. elegans* had been killed. *C. elegans* was considered dead when it no longer responded to touch.

P. aeruginosa was heat-killed, as described previously (Tan, 1999). Tryptic Soy Broth (TSB) tubes were inoculated with *P. aeruginosa* and were placed, slanted, in a shaking incubator for 24 hours at 37°C . The *P. aeruginosa* was then transferred to sterile centrifuge tubes and centrifuged for five minutes at approximately 4000 rpm. The excess broth was decanted off the top and discarded. The *P. aeruginosa* then was placed in a water bath at 65°C for 30 minutes to heat kill the bacteria. The heat-killed bacteria were then transferred to a NGM plate. This transfer was done by using an inoculating loop to remove the *P. aeruginosa* lysate from the centrifuge tube and spread it on the plate. Approximately 15 to 20 *C. elegans* were transferred to this plate and kept at room temperature. The plate was examined daily.

Plates containing PGS were swabbed (using same technique as above) with *P. aeruginosa* and *E. coli* and incubated at 37°C for 24 hours. Approximately 15 to 20 *C. elegans* were transferred to each plate. The plates were kept at room temperature and examined daily.

C. elegans killed by the *P. aeruginosa* were isolated and removed from the plates. An inoculating needle was used to transfer the *C. elegans* to plates containing agar selective for Pseudomonas. The plates were then incubated at 37°C and examined for growth.

Colonies of bacteria from the dead *C. elegans* were then used to streak a new plate for isolated colonies. This plate was incubated at 37°C overnight. Isolated colonies obtained from this plate were grown in culture tubes containing TSB. The isolated *P. aeruginosa* was then transferred to and stored in CTA tubes.

The isolated *P. aeruginosa* was also swabbed onto NGM and PGS plates. These plates were then incubated overnight at 37°C. After a bacterial lawn was formed on the plates, freshly grown *C.elegans* was transferred to each plate. These plates were kept at room temperature (~25°C) and observed.

Once again, dead *C.elegans* were isolated from the NGM and PGS plates and transferred to Pseudomonas selective agar plates. These plates were incubated at 37°C and observed for growth. Once colonies were isolated from the *P. aeruginosa* growth, the organisms were identified by the green flouescent color given off by the *P. aeruginosa* when grown on Pseudomonas selective agar.

Control plates, both NGM and PGS, were swabbed with *E.coli* and incubated at the same time plates were swabbed with *P. aeruginosa. C.elegans* were transferred and observed using the same procedure as with the *P. aeruginosa* plates.

RESULTS

P. aeruginosa kills *C. elegans* in two ways, as previously described (Tan, 1999). Slow-killing of *C. elegans* occurred on NGM within 3-5 days of being exposed to *P. aeruginosa*. Fast-killing occurred on PGS and caused *C. elegans* to die within 4-36 hours of being exposed to *P. aeruginosa*. There was no death observed on the control plates of either type of media that contained an *E. coli* bacteria lawn. The heat-killed *P. aeruginosa* on NGM plates also supported the growth of *C. elegans* and no death was observed.

Koch's postulates (Prescott, 2002) was

demonstrated by isolating *P. aeruginosa* from dead *C. elegans,* growing it in pure culture, using the isolated *P. aeruginosa* to infect "freshly" grown *C. elegans,* and then once again isolating *P. aeruginosa* from the killed *C. elegans*. *P. aeruginosa* was identified as the causative agent of the death of *C. elegans.*

DISCUSSION

P. aeruginosa is a gram-negative, rod-shaped bacterium that is capable of causing disease in plants, insects, humans, and a variety of other vertebrates (Tan, 1999). *P. aeruginosa*, a pathogen of *C. elegans,* causes intestinal infections, problems with locomotion, swelling of the intestines, and cell lysis (Ewbank, 2002). Koch's postulates were fulfilled which proved that *P. aeruginosa* is the microorganism responsible for the death of *C. elegans.* Both slow-killing and fast-killing of *C. elegans* was observed.

Slow-killing occured in 3-5 days on NGM. Slow-killing of *C. elegans* involves the consumption of live *P. aeruginosa*, which colonize in the intestines and eventually cause infection which kills the nematode (Kurz, 2000). This type of killing takes days because of this infectious process.

Fast-killing occurred on PGS (high osmolarity media) over a course of 4-36 hours. This type of killing does not require live bacteria (Tan, 1999). When *P. aeruginosa* is grown on high-osmolarity media it produces a toxin which causes lethal oxidative stress to *C. elegans* (Kurz, 2000). The toxin is produced while the bacterial lawn is growning on PGS and the consumption of bacteria in not needed, so this type of killing happens rapidly.

Heat-killed *P. aeruginosa* was used to establish that death on NGM was due to bacterial infection and not a lack of nutrients in *P. aeruginosa*. When heat-killed, *P. aeruginosa* provides the same nutrients as when alive, but the bacteria will not be able to colonize in the intestines and cause infection in *C. elegans* (Tan, 1999). No killing was observed on the heat-killed *P. aeruginosa* NGM plate, which indicated that *P. aeruginosa* is a sufficient source of food. Knowing that *P. aeruginosa* is a sufficient food source indicates that the *C. elegans* were killed by the infection caused by the bacteria and not from starvation from lack of adequate food.

The control plates containing an *E. coli* bacterial lawn showed no death of *C. elegans.* This ensured that the lab conditions or other environmental factors did not contribute to the death of *C. elegans* on the various *P. aeruginosa* plates.

The *C. elegans-P. aeruginosa* system is helpful in understanding the molecular basis of animal-pathogen interactions (Kurz, 2000). Regardless of the host, invertebrate or vertebrate, universal virulence factors exists (Ewbank, 2002). Many of the virulence factors demonstrated with the *C. elegans-P. aeruginosa* systems are universal virulence factors (Kurz, 2000). The information obtained from research involving *C. elegans* can be useful in understanding pathogenesis in other host-pathogen systems. These new understandings have the potential to lead to new ways of combatting infectious diseases (Ewbank, 2002).

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