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Caenorhabditis elegans: A Model Organism in Biotechnological Research

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ABSTRACT

Caenorhabditis elegans is a roundworm from Nematoda phylum of smooth-skinned, un-segmented worm with a cylindrical body shape tapered at the ends; includes free-living and parasitic forms both aquatic and terrestrial. It is non-hazardous, non-infectious, non-pathogenic, non-parasitic organism. It is about as primitive an organism that exists, which shares many of the essential biological characteristics that are central problems of human biology. The worm is conceived as a single cell which undergoes a complex process of development, starting with embryonic cleavage, proceeding through morphogenesis and growth to the adult. It has a nervous system with a brain (the circumpharyngeal nerve ring). It exhibits behavior and is even capable of rudimentary learning of embryogenesis, morphogenesis, development, nerve function, behavior and aging and how they are determined by genes.

1. INTRODUCTION

It is the model organism in molecular biology and can be used to study toxicity *in-vivo*, because of characteristics having a short lifespan and grows at either 15°C or 20°C and takes about 3.5 days at 20°C for a fertile adult to develop from the one-cell embryo. At 15°C this process takes about twice as long, varying the incubation temperature (between 15°C and 20°C) is the only way to control the rate of worm growth and development. Higher temperatures (20°C to 25°C) can further accelerate the rate of development but can cause a drop in fertility and poor health, especially in some mutant strain [1]. Temperature >25°C are usually harmful and should be avoided under normal circumstances. Embryogenesis normally takes 14-16 hours at 20°C. This is followed by 4 larval stages during all growth occur. Wild type N2/Bristol strain kept at 20°C will begin producing and laying eggs 3-4 days into their complete life cycle and produce an average of 220 or more self-fertilized progeny. It is generally found as dauer juveniles always in the uppermost layer of the compost in which freshly rotting organic materials are present, prefers low oxygen concentration of 6% and avoids very low oxygen concentration (2%) and higher oxygen concentration (>12%) also [2].

The value of the organism as a model system because of the combination of two important facts: the first one is basic cellular mechanisms are conserved between *C. elegans* and higher organism. The second one is the superficial application of sophisticated molecular, genetics and cell biological techniques can be executed against the backdrop of known cellular development, defined anatomy and a sequenced well-annotated genome [3]. Its cells and organs are small as compared to their mammalian counterparts; also they are enclosed within a pressurized and comparatively impermeant cuticle, generally found in anthropogenic habitats: in compost, mushroom beds and garden soil in Europe, Asia, North Africa, North America and Australia [3].

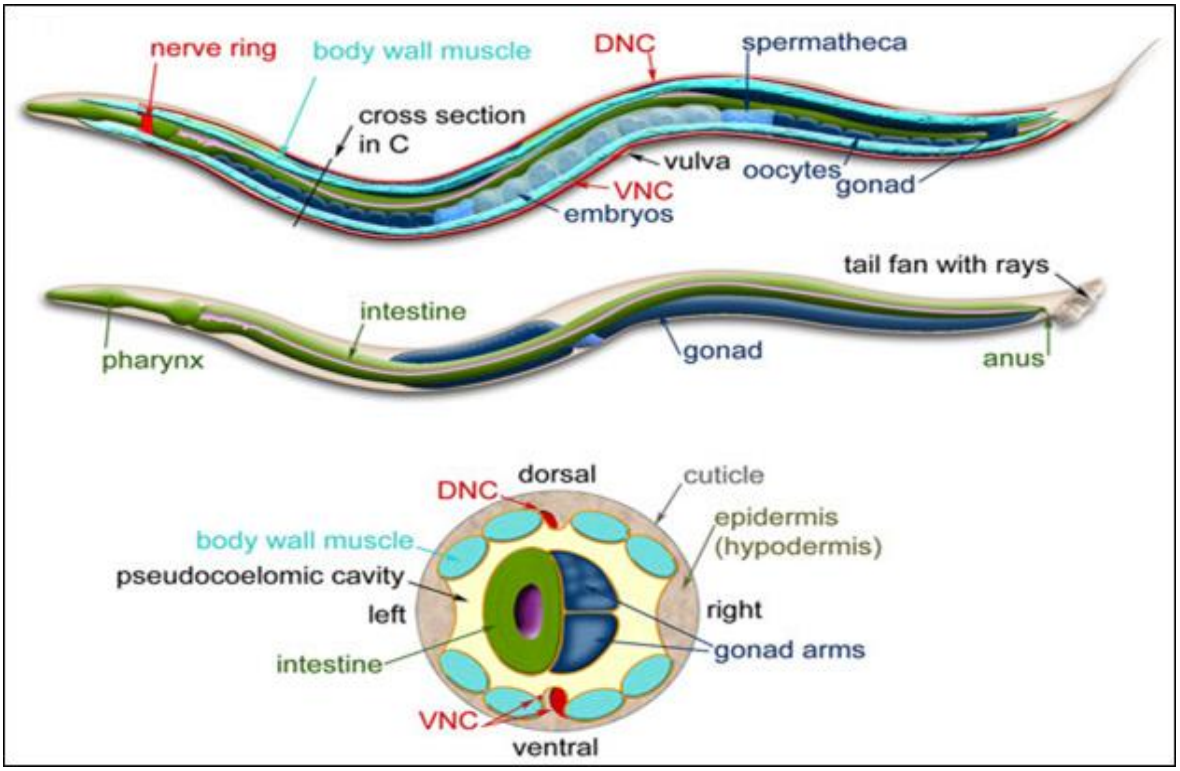


Figure No. 1: Anatomy of *C. elegans* (www.wormatlas.org)

The adult worm essentially comprises a tube, exterior cuticle, containing two smaller tubes, pharynx and gut, and reproductive system. Most of the volume of the animal is taken up by the reproductive system. From the 959 somatic cells of the hermaphrodites, some 300 are neurons [4]. Neural structures include a battery of sense organs in the head which mediate responses to taste, smell, temperature and touch, although it lacks eyes, it can respond slightly to light. Among other neural structure is an anterior nerve ring with a ventral nerve cord running back down the body (there is also a smaller dorsal nerve cord). There are 81 muscle cells. It moves through four longitudinal muscle bands paired sub-dorsally and sub-ventrally. Alternative flexing and relaxation generate dorsal-ventral waves along the body, propelling the animal along [5].

C. elegans used as an animal model to evaluate the toxicity over multiple generations. Because of short lifespan and large brood size per nematode, multigenerational studies provided a prospective screening tool to monitor its lifespan and fertility [6]. Nematodes come into contact with material waste due to its residence in every type of soil makes testing of biological effects in nematodes applicable. The development and function of this diploid organism are encoded by an estimated 19,800 distinct genes [6].

It is very unique among all major model organisms and can be found in two sexes, one is hermaphrodite (XX) and another one is male (XO) [7]. Both sexes can be distinguished by the greater size of hermaphrodites and because of differences in their sex organs. A hermaphrodite produces both eggs as well as sperms that is why they are self-fertile [7]. Hermaphrodite is a modified female that in the fourth larval stage produces and stores sperms to be used later to fertilize oocytes produced by self-fertilization were encased in an eggshell and initiate development within the uterus of the hermaphrodite and after reaching at 30 cell stage; the egg-embryos were laid by the worm. The progeny of hermaphrodites are also hermaphrodites, except in one condition when a rare nondisjunction leads to male (XO). Transparency makes it efficient for screening large populations [7].

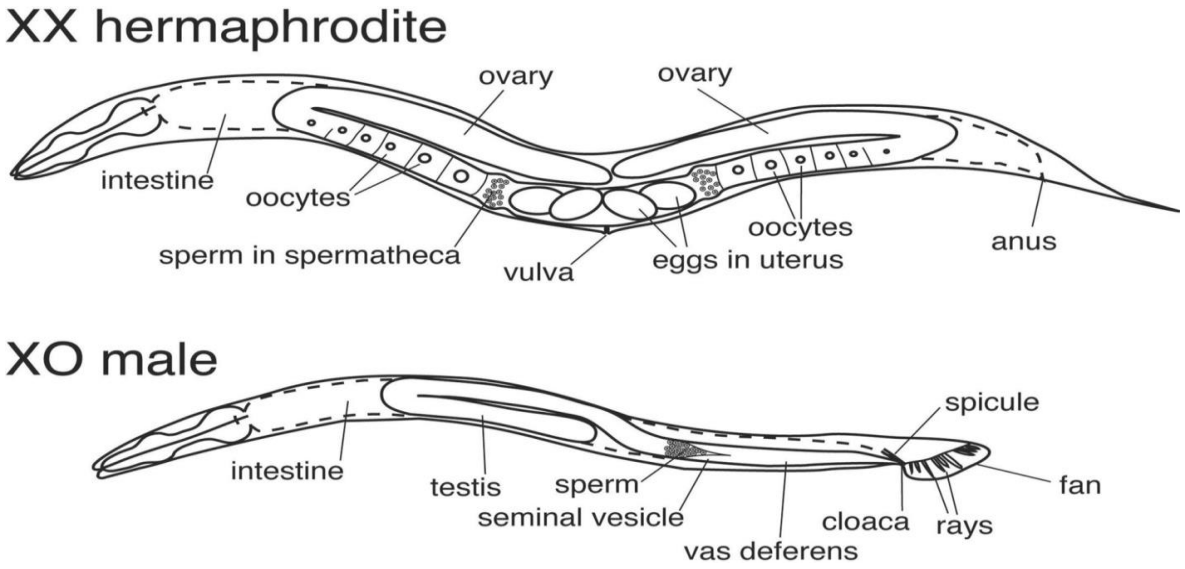


Figure No. 2: Types of *C. elegans*

Hermaphrodites are self-fertile, producing first sperm stored in spermatheca and then oocytes. Within the first day of L4/adult hermaphrodites accumulate the fertilized eggs in the uterus; a young adult hermaphrodite generally stores 10-15 eggs in the uterus at a particular time [7]. Egg-laying occurs specifically when specialized sex-specific muscle contract, opening the vulva and allowing eggs to be expelled. Nematodes are very attractive in evolutionary developmental biology as phylum is rich in species and many organisms among them can be cultured under specified laboratory conditions. Mostly functional studies of *C. elegans* have been performed by using laboratory strain N2 [8].

It is 1 mm long soil-dwelling roundworm (a nematode) is comparatively simple. This simplicity makes it a model organism, very small size, rapid growth, ability to self-fertilize,

transparency and less number of body cells have eventually made the organism an ideal choice for the study. Observation of all stages of development simply by watching the worm under a stereo microscope. Development is tightly programmed that each worm contains a remarkably small and consistent number of cells (959 cells in hermaphrodites and 1031 cells in males) [8].

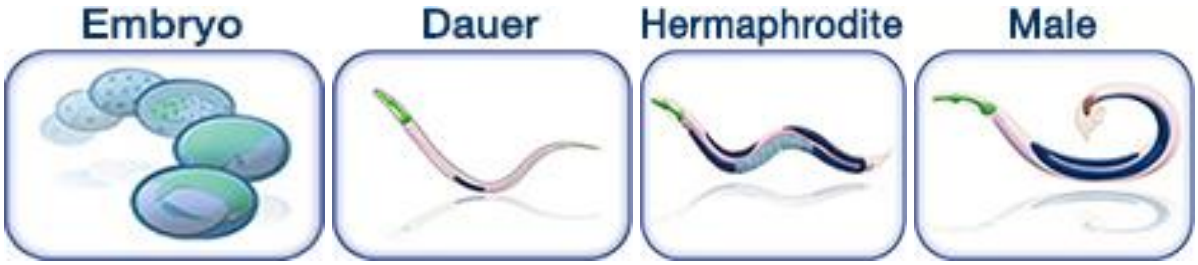


Figure No. 3: Different Types of *C. elegans* (www.wormatlas.org).

C. elegans whole genome was sequenced in 1998. Its genome size is approximately 100 Mb and the average gene size is 5 kb, 5 exons/gene. It contains 5 autosomes (2n=10) and one X chromosome. It contains approximately 19,800 protein-coding genes [8]. It possesses approximate >70% of proteome which is human homologues, easy to culture in the laboratory having a very short lifespan and large brood size [8].

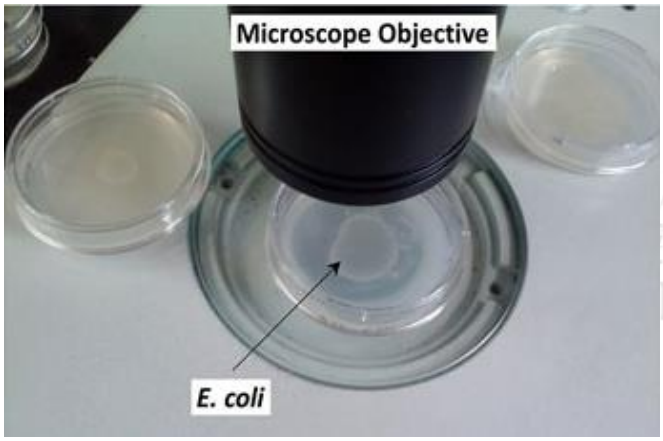
2. *Caenorhabditis elegans*:

2.1: Scientific Classification:

Kingdom	Animalia
Phylum	Nematoda
Order	Rhabditida
Family	Rhabditidae
Genus	*Caenorhabditis*
Species	*C. elegans*

2.2: Organism Description:

Caenorhabditis elegans is a free-living soil nematode that eats bacteria. It is an important model organism for studies of genetics, development and cell biology. The organism feeds on *E. coli* OP50 bacterial lawn on Nematode Growth Media Agar Plate [9]. For the successful culture, it is most important to maintain sterility all the time. Transfer a small amount of 24-hour broth culture onto the surface of a Nematode Growth Media Agar plate and spread the liquid culture evenly over the surface. Incubate the plate overnight at 37°C to produce a bacterial lawn [9]. Using a sterile scalpel, cut a small block of agar from an active plate and place the block face down on the surface of the new agar plate. So, the worms will be in contact with the new agar or transfer the worm by using platinum wire from used culture plate to the new plate. Incubate the worms at an appropriate temperature for optimal growth [9].



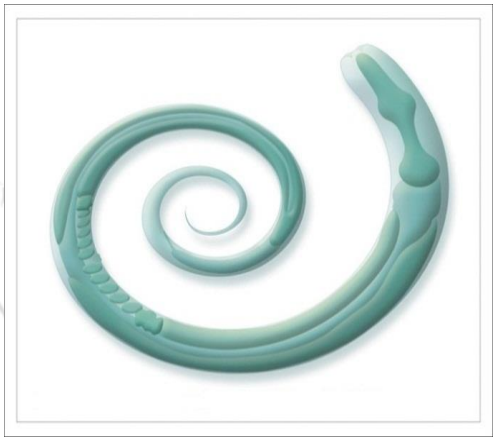


Figure No. 4: *E. coli* OP50 (www.wormbook.com) **Figure No. 5: *C. elegans***

There are two sexes of *C. elegans*: male and hermaphrodite. They are differentiating by their body length and organs. Mature male is smaller than the hermaphrodites and their tails are fan-shaped. A culture generally contains many more hermaphrodites than males. The larger hermaphrodite has a pointed tail and self-fertilize. Males can fertilize hermaphrodites, but the hermaphrodites cannot fertilize each other [9].

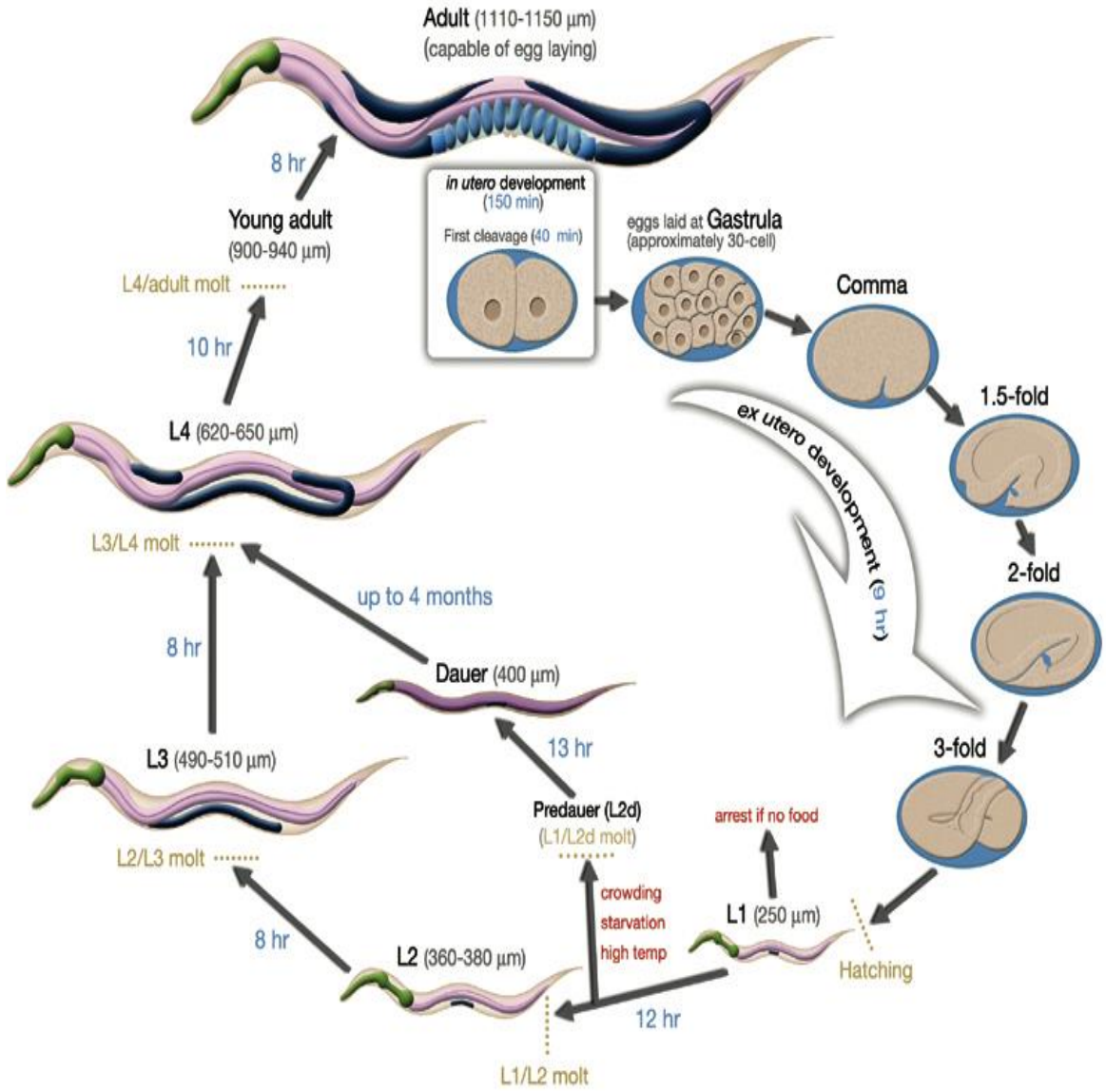


Figure No. 6: Life cycle of *C. elegans* (www.wormatlas.org).

It has six chromosomes: five autosomes and an X-chromosome. Hermaphrodites are diploid for all six, whereas males are diploid for the autosomes but are haploid for X-chromosome. The organism is present in the soil as dauer juveniles but unlikely to develop in soil without the addition of an organic matter. They are colonizers of nutrient and microorganism rich organic materials [10].

2.3: Maintenance of *C. elegans*:

2.3.1: Preparation of Bacterial Food Source:

C. elegans is usually grown on the laboratory strain *E. coli* OP50 as a food source. *E. coli* OP50 is a uracil auxotroph whose growth is limited on NGM plates. A limited bacterial lawn is most desirable, it allows for easier observation and better mating of the worms. A starter culture of *E. coli* OP50 can be obtained from the CGC or can be recovered from the worm plates. Use the starter culture to isolate single colonies on a streak plate of a rich medium such as LB agar. By using a single colony from the streak plate, aseptically inoculate a rich broth like LB broth. Allow inoculated cultures to grow overnight at 37° C. *E. coli* OP50 culture is then ready for use in seeding NGM plates. *E. coli* OP50 streak plate and liquid culture should be stored at 4°C and will remain usable for several months or cryopreserved [10].

2.3.2: Preparation of NGM Petri Plates:

C. elegans is generally maintained in the laboratory on Nematode Growth Medium (NGM) agar which has been aseptically poured into petri plates. Petri plates are available in different sizes such as 35 mm, 60 mm and 90 mm. Smaller size plates (35 mm diameter) are very useful for mating or at the time of using expensive drugs. Medium size plates (60 mm diameter) are useful for general strain maintenance and larger plates (90 mm diameter) are useful for growing larger quantities of worms such as certain mutant screens [10].

NGM agar medium can be poured into petri plates easily and aseptically using a peristaltic pump. This pump can be adjusted so that a constant amount of NGM agar is dispensed into each plate. A constant amount of agar in the plates reduces the need for re-focusing the microscope when a switch from one plate to another. Desired drugs can be added to the NGM agar just before being poured [10].

It may be desirable or advisable to use 24-well or 96-well microtiter plates when expensive drugs or screening large numbers of the individual animal is being carried out. For 24-well plates, 1.5 ml NGM agar and seeding with an *E. coli* OP50 lawn, 96-well plates filled with 50 µl of NGM liquid with *E. coli* HB101 to each well. For *E. coli* HB101 cells are suspended in 4X their volume of NGM liquid, assuming 1 gm of cells equals 1 ml. One volume of the *E. coli* suspension is then added to 24 volumes of NGM liquid. Care must be taken to prevent

worms from crawling between wells; only one strain of *C. elegans* should be used per microtiter plate. So, there is no possibility of strain mixing [10].

2.3.3: Seeding NGM Plates:

Sterile technique must be used at the time of apply approximately 0.05 ml of *E. coli* OP50 liquid culture to small or medium NGM plates or 0.1 ml to large NGM plates using a pipette. If desired, then the drop can be spread using the pipette tip or a glass rod. Spreading will create a larger lawn, which can aid in visualizing the worms. Care should be taken not to spread the lawn to the edges of the plate, keep the lawn in the centre. The worms tend to spend most of the time on the bacteria. If the lawn extends to the edges of the plate, the worms may crawl up the sides of the plate, dry out and die. Allow the *E. coli* OP50 lawn to grow overnight at room temperature or 37°C for 8 hours (cool the plates to room temperature before adding worms). Seeded plates stored in an air-tight container will remain usable for 2-3 weeks [10].

2.3.4: Culturing *C. elegans* in Petri Plates:

2.3.4.1: Transferring Worms Grown in NGM Plates:

It is a transparent organism and visualized under a dissecting stereomicroscope equipped with a transmitted light source. Standard 10X eyepieces and objectives which range from 0.6X to 5X (total magnification of 6X and 50X) are widely used. Several methods are used for transferring an organism from one petri plate to another. Quick and convenient "chunking" method where a sterilized scalpel or spatula is used to move a chunk of agar from an old plate to a new fresh plate. There will usually be hundreds of worms in the chunk of agar [10]. The worms will crawl out of the chunk and spread out onto the bacterial lawn of the new plate. This method is most appropriate for transferring worms that have burrowed into the agar or are difficult to pick individually (such as on a starved plate). The chunking method is fine for transferring homozygous stocks but it is not advisable if the population is heterozygous or if a stock must be maintained by mating [10].

Another method for transferring worms is to use strips of sterilized filter paper which have been cut to ½ or ¼ inch wide and 2-3 inches long. The sterilized filter paper is gently set upon the petri plate, where it absorbs moisture and picks up worms. The filter paper is then touched to a fresh NGM plate where the worms are deposited. Discard the filter paper after

use. This method is also fine for transferring homozygous stocks but it is not advisable for the heterozygous population of worms or must be maintained by mating [10].

The third method is to pick a single organism with a worm pick. A worm pick can be made by mounting a 1-inch piece of 32 gauge platinum wire into either the tip of a Pasteur pipette or in a bacteriological loop holder. The platinum wire heats and cools quickly and can be flamed often to avoid contaminating the worm stocks. The end of the wire used for picking up worms can be flattened slightly with a hammer and then filled with an emery cloth to remove sharp edges; sharp points can poke holes in the worms and kill them or make holes in the agar. The tip of the wire can be either flattened or slight bend that forms a hook [10].

To pick a worm identified under the dissecting microscope, slowly lower the tip of the wire and gently swipe the tip at the side of the worm and lift. Another method is to get a blob of *E. coli* OP50 at the end of the worm pick before gently touching it to the top of the chosen worm. The worm will stick to the bacteria. Several organisms at a time can be picked up by this method, although worms left too long on the pick will desiccate and die. To put a picked worm on a fresh plate, slowly lower the tip of the worm pick, gently touch the surface of the agar and hold it there to allow the worm to crawl off from the pick [11].

2.4: Collection of Organisms:

2.4.1: Procurement of *C. elegans*:

C. elegans (Bristol strain/N2), wild type hermaphrodite worms can be collected from Caenorhabditis Genetics Center (CGC, University of Minnesota, Department of GCD, Minneapolis, MN, USA, funded by National Institute of Health).

2.4.2: Procurement of *E. coli* OP50 Bacterial Food Source:

The worms can be maintained on a special strain of bacteria *Escherichia coli* OP50, it is auxotrophic for uracil. Hence, limited growth on the plates and this facilitates easy observation of the worms. The starter culture of *E. coli* OP50 obtains from the Caenorhabditis Genetics Center (CGC, University of Minnesota, Department of GCD, Minneapolis, MN, USA, funded by National Institute of Health) and subculture under controlled laboratory condition grown on “Luria-Bertani” (LB) broth.

2.5: Growth Media:

2.5.1: Nematode Growth Media (NGM) Agar:

For the growth of nematode *C. elegans*, NGM agar can be provided as a growth medium. Various components can be used to prepare NGM agar into the laboratory.

2.5.2: Worm Pick:

Worm pick is generally used to transfer the worms from the plate to another plate. It is generally made up of platinum wire and Pasteur pipette.

Figure No. 7: Worm Pick made of Platinum Wire

2.5.3: Stereo Microscope:

Stereo Microscope or dissecting microscope is an optical variant designed for low magnification observation of a sample, typically using light reflected from the surface of an object rather than transmitted through it. The instrument uses two separate optical paths with two objectives and eye-pieces to provide slightly different viewing angles to the right and left eyes.

Figure No. 8: Stereomicroscope

3: Advantages and Disadvantages of *C. elegans* as a Model System:

Advantages	Disadvantages
Easy and inexpensive growth which can apply to any laboratory	Lack of particular organs such as heart, kidneys, bone etc.
Small size (1 mm)	Lack of circulatory system
A short lifespan (2-3 days)	Lower conservation of biological pathways with humans relative to other model organisms.
Hermaphrodites available	*C. elegans* restricted to the early phase of product development
Transparent organism	
Genome sequenced	
Mutants available	
High homology with humans	
Engaged and active *C. elegans* community	
No bioethical regulations applied	

4: Culture Media:

4.1: Nematode Growth Media Agar:

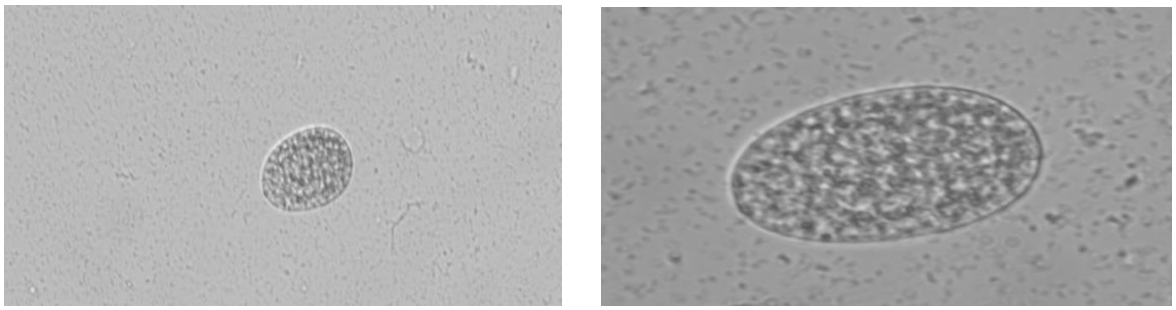
Components	Amount (gm/l)
Agar powder	17 gm
Peptone	2.5 gm
NaCl	3 gm
$MgSO_4$	1 ml
$CaCl_2$	1 ml
PPB	25 ml
Cholesterol	1 ml
Distilled water	1000 ml

4.2: Buffer:

4.2.1: M9 Buffer:

Component	Amount (gm/l)
KH_2PO_4	3 gm
Na_2HPO_4	6 gm
NaCl	5 gm
Distilled water	1000 ml

5: Nomarski Images of Developmental Stages of *C. elegans* at Different Magnification:



(a) (b)

Figure No. 9: Eggs (a) 10X (b) 40X

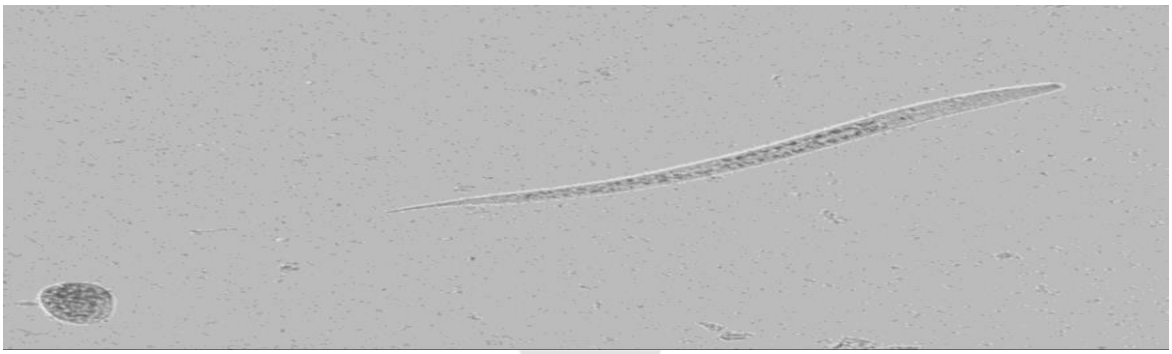


Figure No. 10: L1 Stage at 10X

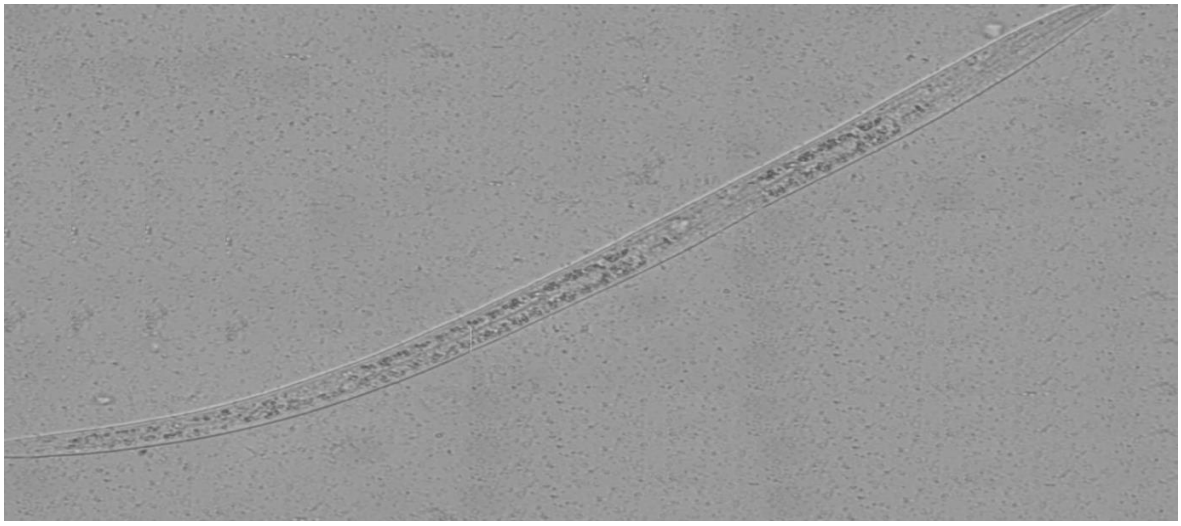


Figure No. 11: L1 Stage at 40X

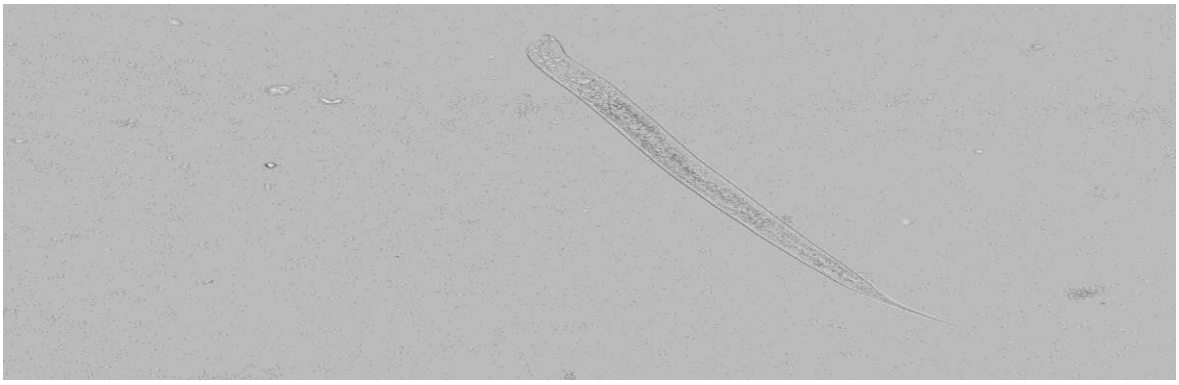


Figure No. 12: L2 Stage at 10X

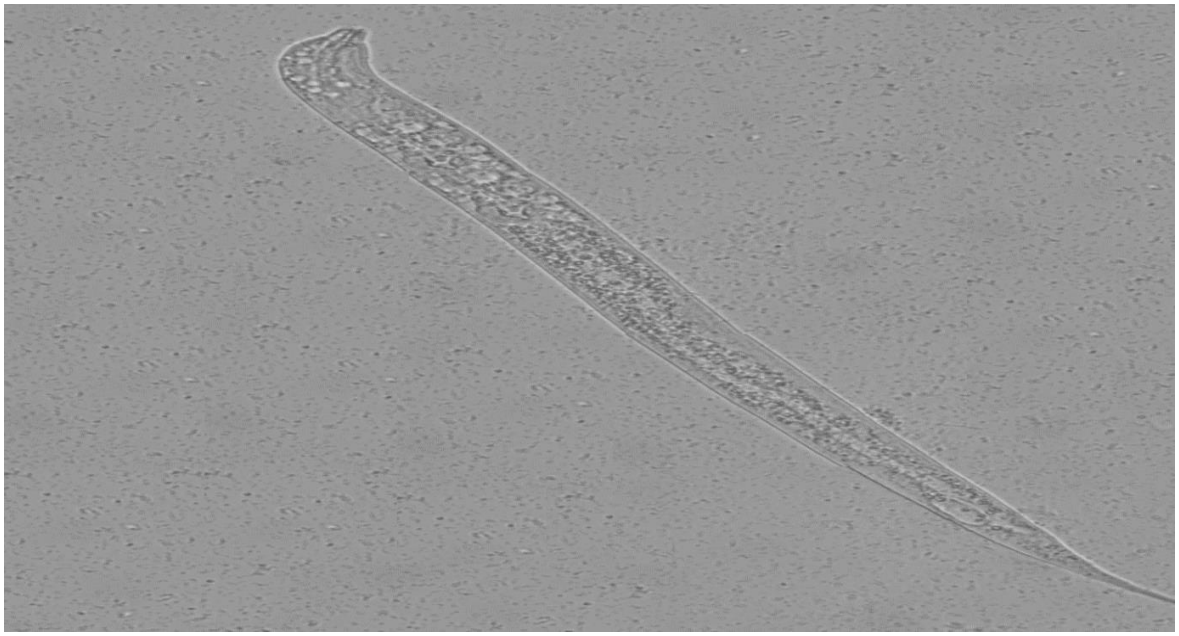


Figure No. 13: L2 Stage at 40X

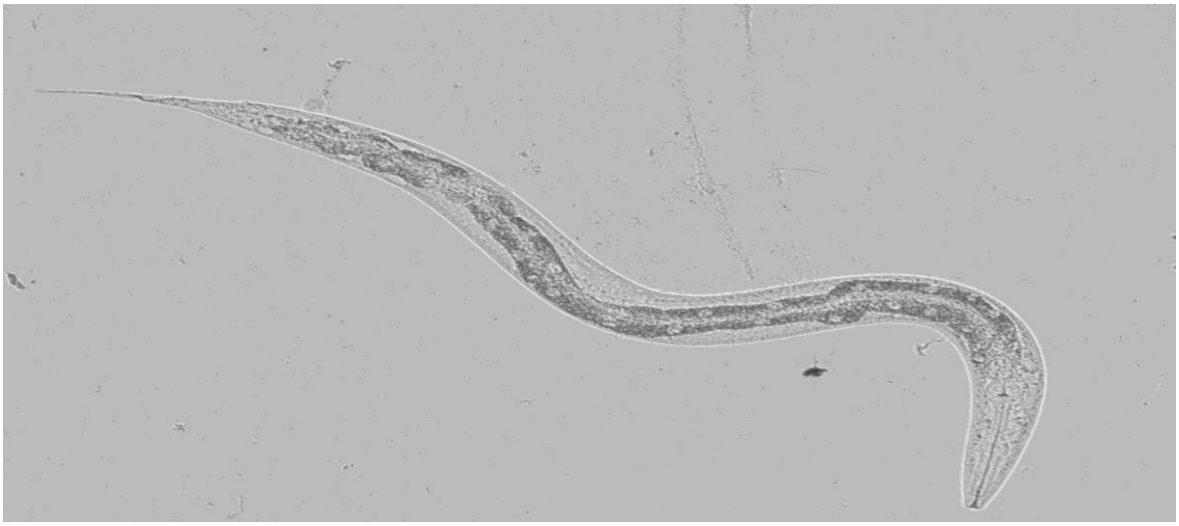


Figure No. 14: L3 Stage at 10X

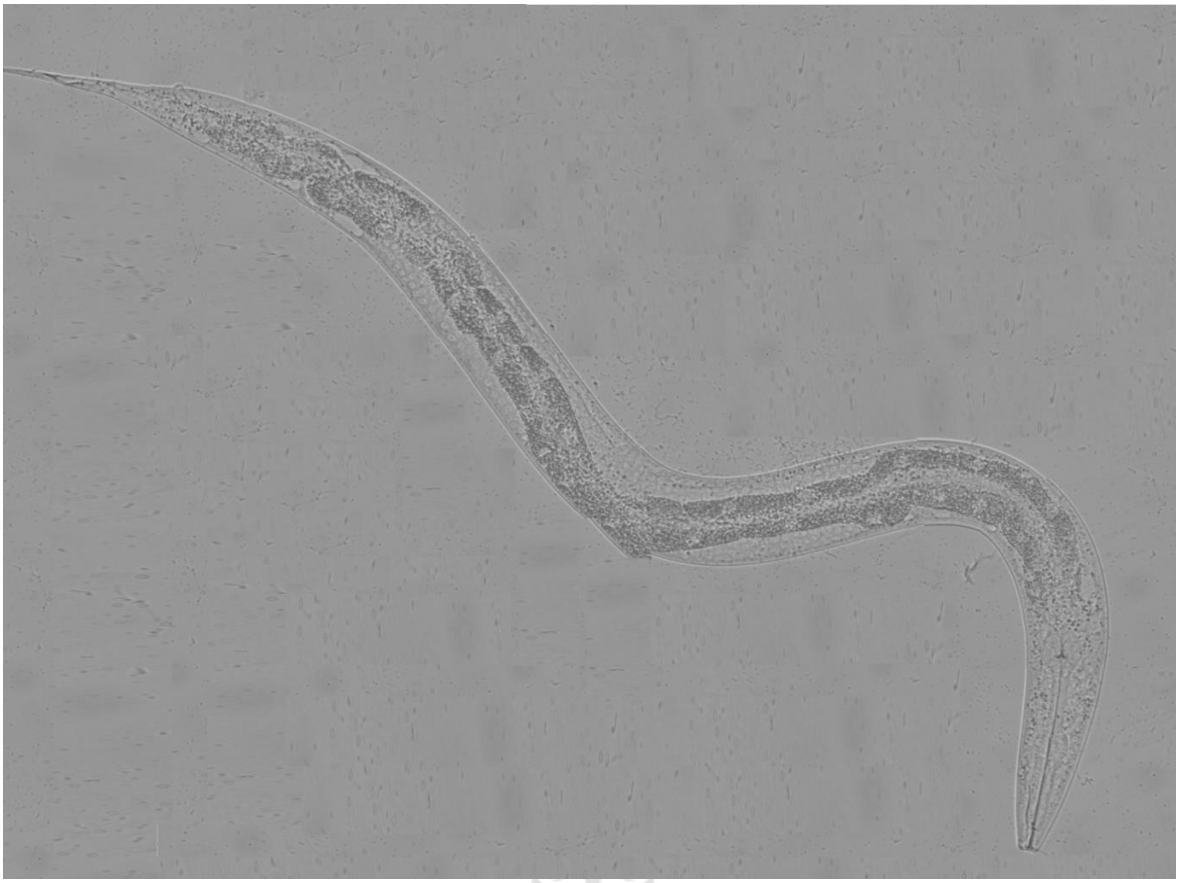


Figure No. 15: L3 Stage at 40X

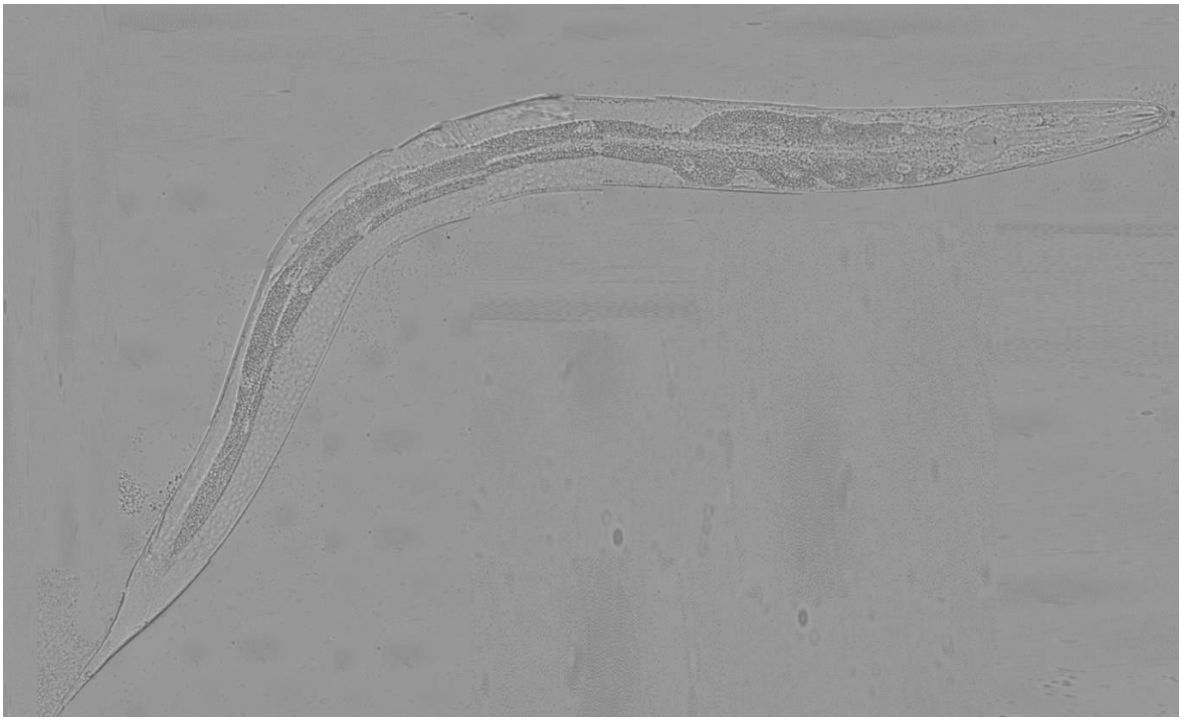


Figure No. 16: L4 Stage at 10X

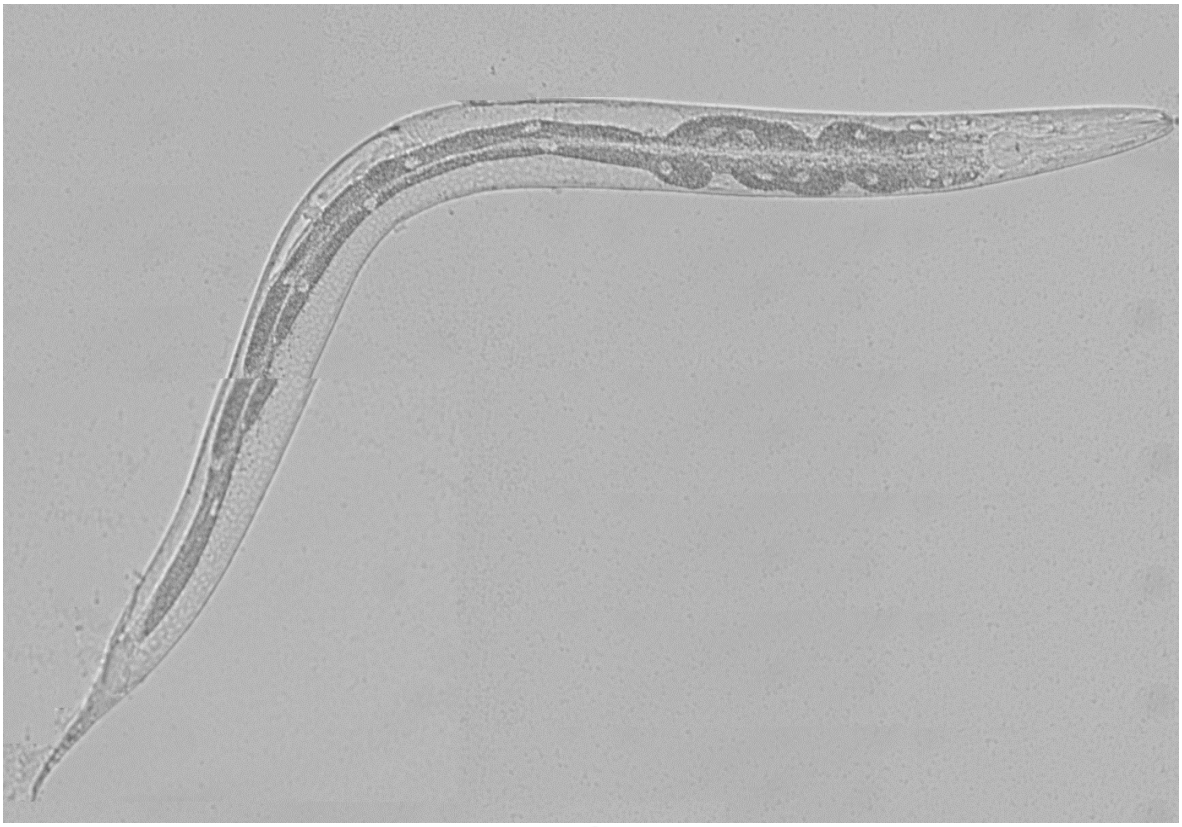


Figure No. 17: L4 Stage at 40X

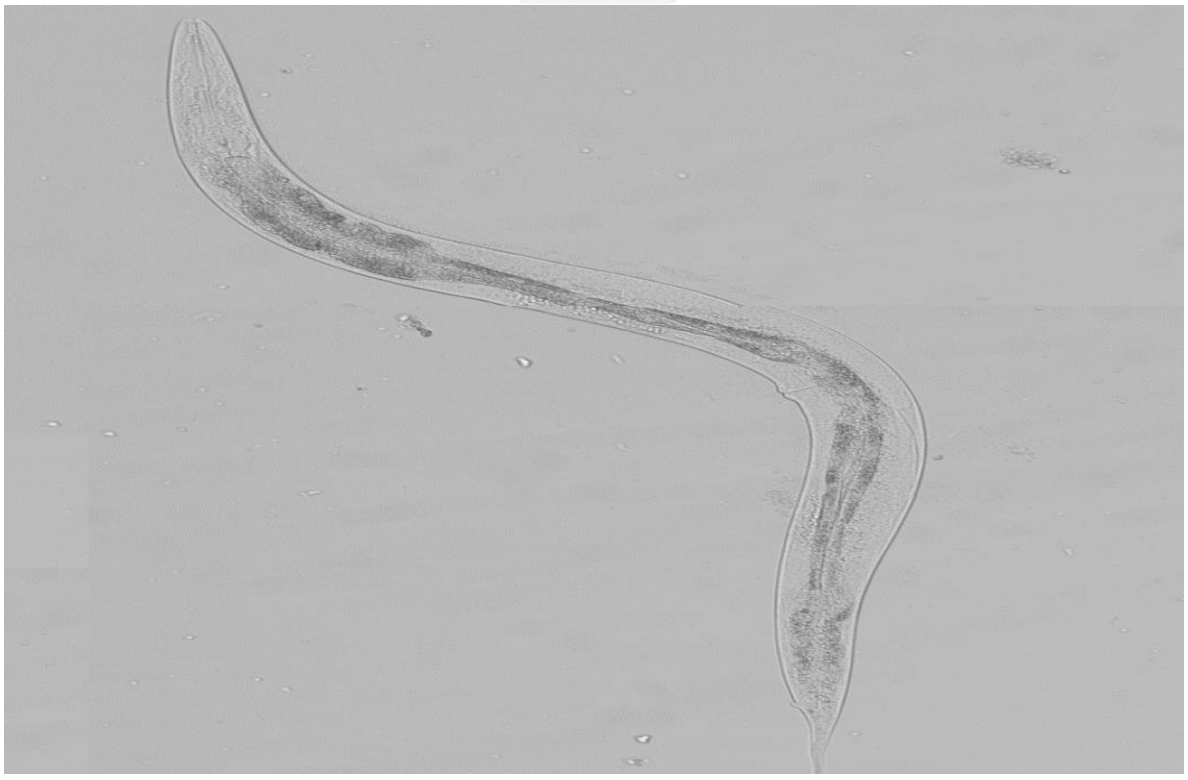


Figure No. 18: Young Adult Stage at 10X

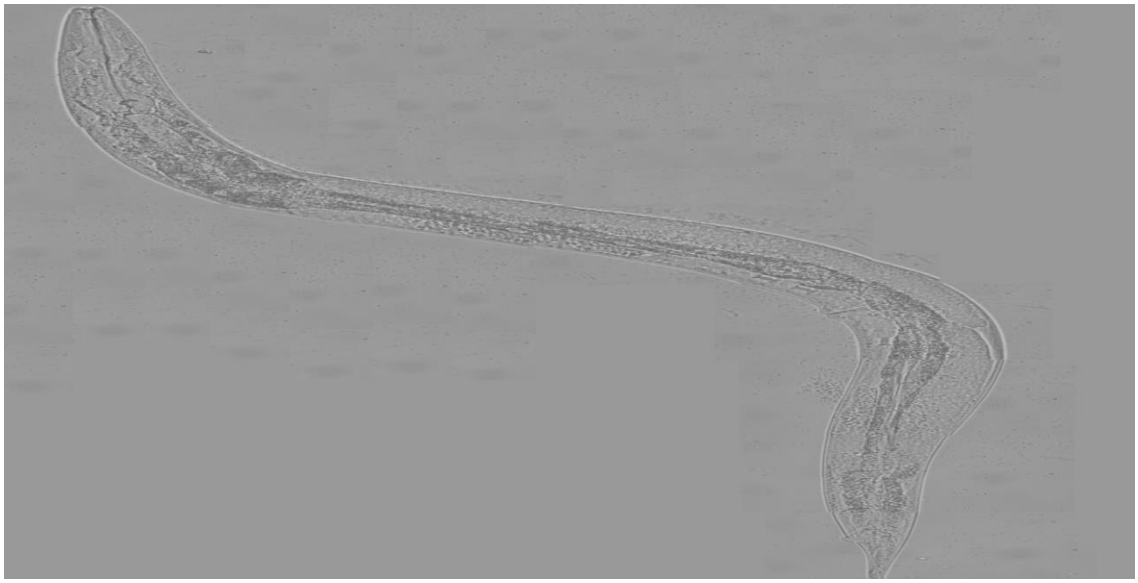


Figure No. 19: Young Adult Stage at 40X

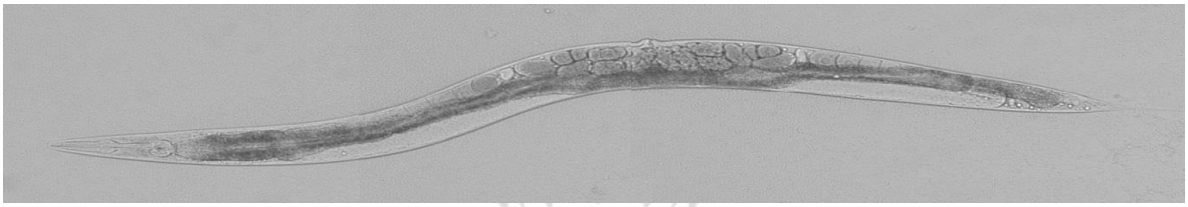


Figure No. 20: Gravid at 10X

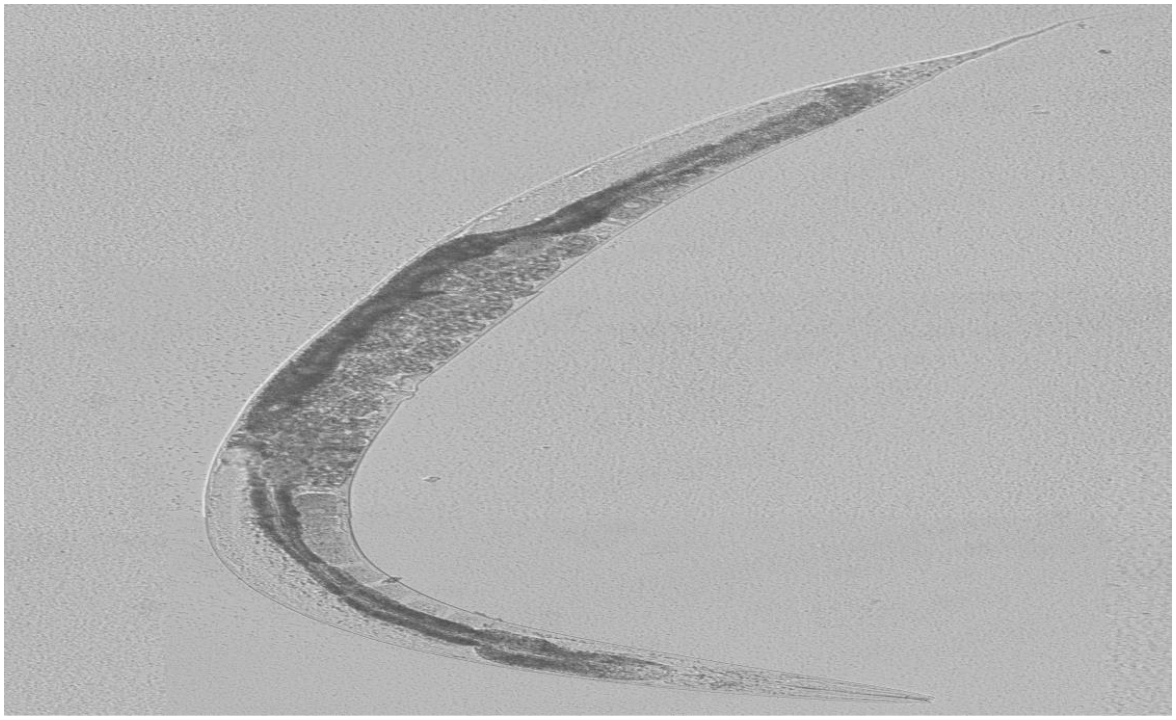


Figure No. 21: Gravid at 40X

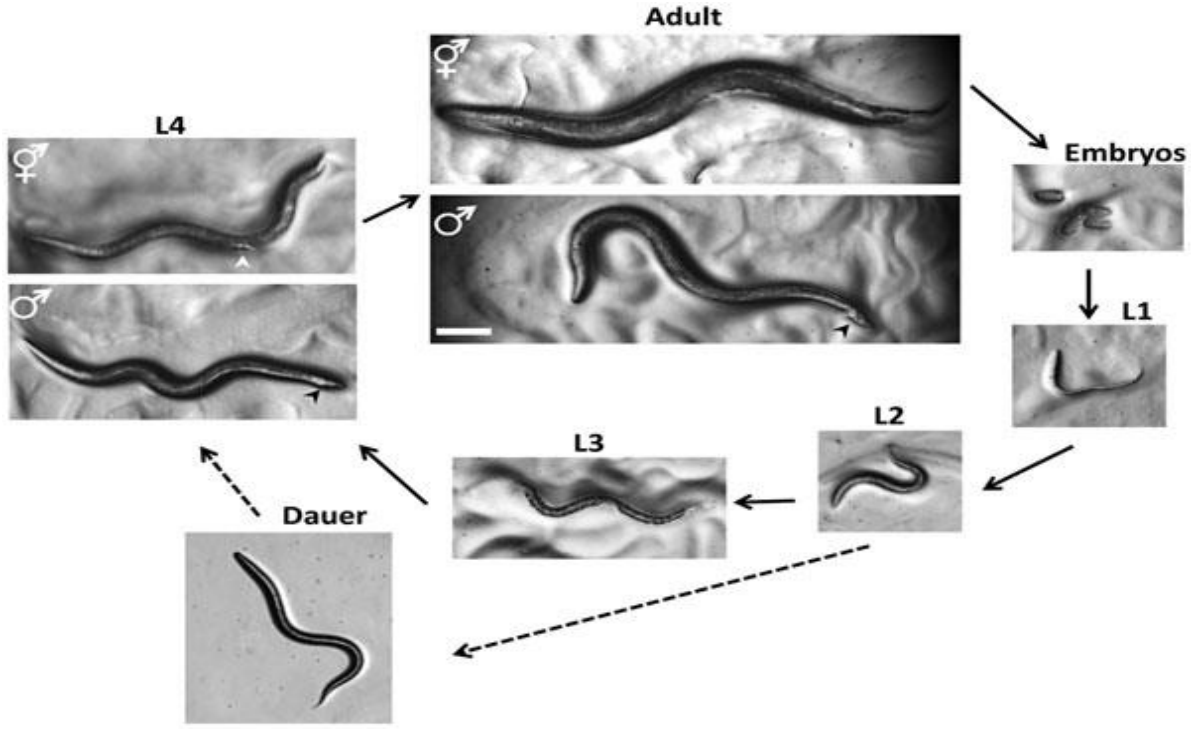


Figure No. 22: Life Cycle of *C. elegans* Under Stereomicroscope (www.wormatlas.org)

6: REFERENCES

[1] Contreras E., Cho M., Zhu H., Puppala H., Escalera M., Zhong W. and Colvin V. (2013). "Toxicity of quantum dots and cadmium salt to *Caenorhabditis elegans* after multigenerational exposure." *Environ Sci. Technol*. Vol. 47 (2), pp. 1148-1154.

[2] Contreras E., Puppala H., Escalera G., Zhong W. and Colvin V. (2014). "Size-dependent impacts of silver nanoparticles on the lifespan, fertility, growth and locomotion of *Caenorhabditis elegans." Environ Toxicol Chem.* Vol. 33 (12), pp. 2716-2723.

[3] Kim J. H., Lee S. H., Cha Y. J., Hong S. J., Chung S. K., Park T. H. and Choi S. S. (2017). "*C. elegans*-on-a-chip for *in situ* and *in vivo* Ag nanoparticles uptake and toxicity assay." *Nature/Scientific Reports*. Vol. 7.

[4] Kim S. W., Kwak J. and An Y.J. (2013). "Multigenerational study of gold nanoparticles in *Caenorhabditis elegans*: Translational effects of maternal exposure." *Environmental Science and Engineering*. Vol. 47, pp. 5393-5399.

[5] Moon J., Kwak J., Kim S. W. and An Y. J. (2017). "Multigenerational effects of gold nanoparticles in *Caenorhabditis elegans*: Continuous versus intermittent exposures." *Environmental pollution*. Vol. 220, pp. 46-52.

[6] Moragas L. G., Laromaine A. and Roig A. (2015). "*C. elegans* as a tool for *in vivo* nanoparticle assessment." *Advances in Colloid and Interface Science*. Vol. 219, pp. 10-26.

[7] Moragas L. G., Yu S. M., Carenza E., Laromaine A. and Roig A. (2015). "Protective effects of bovine serum albumin on superparamagnetic iron oxide nanoparticles evaluated in the nematode *Caenorhabditis elegans.*" *ACS Biomaterials Science and engineering*.

[8] www.cgc.umn.edu

[9] www.wormatlas.com

[10] www.wormbook.com

[11] Yu Z., Zhang J. and Yin D. (2016). "Multigenerational effects of heavy metals on feeding, growth, initial reproduction and antioxidants in *Caenorhabditis elegans.*" *PLoS One*. Vol. 11 (4), pp. 1-13.