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PRELIMINARY STUDIES ON EMBRYOGENESIS OF *CEPHALOBUS LITORALIS* (NEMATODA: CEPHALOBIDAE)

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Cephalobus litoralis (Akhtar, 1962) Andrassy, 1984 completes its embryogenic development in about 18.5 hr and deposites 20-25 eggs in 10 hr. Usually the first cleavage division occurs within the uterus. Development of the embryo is fast and early cleavage divisions are similar to *A. complexus*. Gastrula stage is reached within 8-11 hr from egg deposition. Lima bean stage is followed by comma stage within 11-16 hr. Tadpole stage transforms to plum stage within 12.5-18.5 hr. The first larval stage develops within 14-20.5 hr of deposition and emerges out of the egg within 14.5-22.5 hr.

Key words: Cephalobus litoralis, Embryogenesis, Egg deposition, Nematode model.

INTRODUCTION

Embryogenic development of plant parasitic nematodes has been studied due to their economic importance. However, in recent years, free living nematodes have aroused great amount of interest on account of their lately realized potential in many developmental studies [8].

The embryonic and post-embryonic (Thorne, 1925: Thorne, 1937), *Rhabditis teres* (Schneider, 1866), *Panagrellus redivivus* (Linn., 1767: T. Goodey, 1945) have also been studied.

Cephalobus litoralis (Akhtar, 1962) Andrassy, 1984 is a free living nematode which reproduces parthenogenetically, however, males are also observed very rarely. *C. litoralis* has short life span i.e. 72-90 hr, prolific fecundity, are inexpensive to maintain, desiccation and freeze tolerant and are malleable to a variety of laboratory purposes [5]. It was for this reason that embryonic development of this nematode species was studied which forms the subject of this report.

The embryogenic development of *Cephalobus litoralis* is similar to *Acrobeles complexus* in most phases, but some aspects are variable.

MATERIALS AND METHODS

Preparation of culture. Pure population of *Cephalobus litoralis* was raised from a single egg. Nematodes were cultured on Pea Meal Paste (PMP) as described by Saeed *et al.* [5]. Large number of freshly deposited eggs in water and also from dissected gravid females were collected. They were washed in 0.05% sodium hypochlorite solution (commercial bleach), for 5 min followed by rinsing with sterile distilled water to prevent micro-organisms during observation period.

Preparation of slides. A healthy looking single-celled egg was selected and placed in a drop of distilled water on a shallow cavity slide and a glass cover slip was placed which was sealed using wax.

Egg deposition and embryogenesis were observed under high power of a light microscope at (28±5°). Illustrations were made with the help of camera lucida.

RESULTS

Egg. Eggs of *C. litoralis* are hyaline, colorless, and oblong in shape (Fig. 1). Fifteen eggs were measured (length 25.6-30.0 µm; width 12.0-16.0 µm) and the average egg size was 28 x 14 µm. The egg shell consists of two main layers, an inner thin and an outer thick layer. The egg membrane is transparent and stretchable.

Egg deposition. C. litoralis has monodelphic gonad with ovary occupying about 54% of the body length. The ovary stretches towards the anterior and where it reflexes and stretches posteriorly well behind the vulva.

Eggs are deposited singly and it has been observed that 20-25 eggs are deposited in 10 hr. Vulva is a transverse slit like opening situated at the posterior region of the nematode. It has been observed that before egg-deposition, vulval lips move rapidly and the egg comes down near the vulval opening. Finally the egg exits with a little jerk.

Embryogenesis. Different phases of embryogenesis drawn with the help of a camera lucida are given in Fig. 1.

First development phase. Frequently it has been noted that the first cleavage appears immediately after maturation of the egg. The single celled stage 'P_o' (Fig. 1-A) is usually occurred in the uterus of nematode. The first cleavage is almost equatorial, dividing the egg axis transversely. This cleavage results into two blastomeres, an anterior blastomere (S_1) and a posterior blastomere (P_1). The P_1 is slightly larger than the S_1. The first cleavage occurs in about 60-90 min (Fig. 1-B). The second cleavage is parallel to the first cleavage, thus dividing the anterior blastomere (S_1) into

two sister blastomeres, A and B, resulting into three celled stage (Fig. 1-C). This division is completed in about 30-60 min. The cells were designated according to the system followed by Chitwood [1, 6]. Large, rounded nuclei were easily distinguishable from the cytoplasm because they were

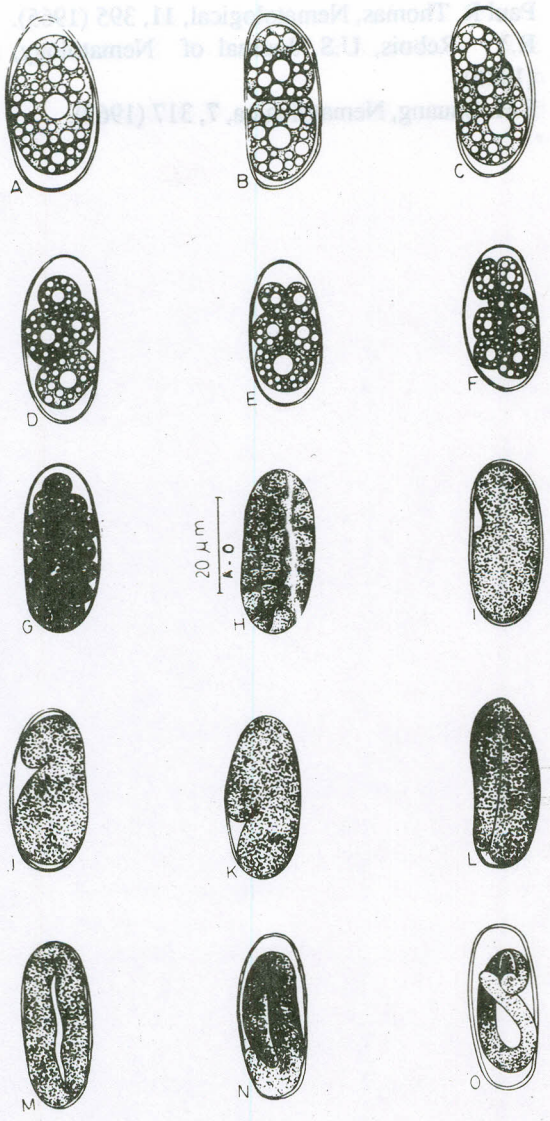

Fig. 1. *Cephalobus litoralis* (Embryogenic development): A. One-celled stage; B. Two-celled stage; C. Three-celled stage; all cells in a line; D. Four-celled stage; arranged in a zig-zag manner; E. Five-celled stage; F. Eight-celled stage; G. Multi-celled stage; H. Gastrula stage; I. Lima bean stage; J. Comma stage; K. Tadpole stage; L. Plum stage; M. Early larval stage; M. Middle larval stage; O. Late larval stage.

less dense and delimited by the dark border. After an interval of another 30-90 min, the posterior cell (P_1) divides to give rise to P_2 and EMSt. The four cells are arranged in a zig-zag manner (Fig. 1-D). Now the division starts in the anterior blastomere 'A' and in about 40-50 min, 'A' divides into 'a' and ∝ blastomeres, resulting into five celled stage (Fig. 1-E). The divisions continue and the blastomere 'B' divided into 'b' and β; EMSt into 'E' and 'MSt'; P_2' into 'P_3' and 'S_2'. Thus the eight-celled configuration is reached in about 60-90 min. (Fig. 1-F). After this stage, cell division was so rapid that it was not possible to follow the cell lineage upto multicelled stage which was observed in the following 3-3.5 hr (Fig. 1-G).

Second developmental phase. Now the second half of the embryogenesis starts. The cellular rearrangement within the embryo takes place and in about 90-120 min. gastrulation stage is completed (Fig. 1-H). Lima bean stage follows in about 60-90 min. (Fig. 1-I). After this the cell proliferation ends.

Then morphogenesis and elongation begins and embryo takes about 2-3 hr in comma stage for the rearrangement of cells and within the mouth portion invagination appears (Fig. 1-J). Then the embryo transforms to tadpole stage and acquires bluntly rounded shape (Fig. 1-K). Duration between comma to tadpole stage is about 60-90 min. In the plum stage elongation of body begins with the reduction of width and the embryo acquires the length two-fold of the egg (Fig. 1-L), in about 30-40 min. After a period of 60-120 min. larval stage arrives and the movements of the larva start (Fig. 1 M-O). The movement of larva is so rapid that is hardly stays in a posture for any length of time. As the larva continues its movement, the egg-shell becomes softened. It ruptures spontaneously at one of the polar ends eclision occurs within 60-120 min. Thus the embryogenic development completes in about 18.5 hr.

DISCUSSION

Early cleavage divisions (first developmental phase) in *A. complexus* is very similar to *C. litoralis*. As *C. elegans* is also free-living nematode and is a widely used eucaryote model for the study of aging, developmental biology and genetic engineering research, [2, 3]. Its life cycle duration is 3.5 days and embryogenesis completes in 11.5 hr. In comparison with *C. elegans*, the life cycle duration of *C. litoralis* is 3-4 days [5] and it completes its embryogenesis in about 18.5 hr. Like *C. elegans, C. litoralis* is also free-living nematode and easy to culture. Both organisms have short generation time and embryogenesis is completed in only few hours.

Present work indicates that *C. litoralis* has the embryological characteristics similar to *C. elegans*. Although *C. litoralis* is a little known species and only very small amount of work has been done so far, it could also be used as an ideal organism for many studies like toxicology, senescence, genetic engineering and as an environment pollution indicator.

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