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EFFECT OF SIMULATED MICROGRAVITY ON CAENORHABDITIS ELEGANS EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT, LIFESPAN, AND MONITORING OXIDATIVE STRESS

Abstract

C. elegans gravid adult hermaphrodites were lysed using a mixed solution of sodium hypochlorite and sodium hydroxide to extract eggs. The collected eggs were suspended in M9 buffer and loaded into HARV (High Aspect Ratio Vessels) vessels to simulate microgravity; each vessel holds 10 ml of M9 buffer/egg suspension. Four vessels were attached to the bioreactor rotating at 24 rpm, and 4 other vessels were kept on a shaker to serve as control. The bioreactor and shaker were kept in an incubator at 201 C. After 48 hours the contents of all vessels were collected, each in a separate 15 ml centrifuge tubes, centrifuged and each pellet in small amount of M9 buffer was transferred into 100 mm NGM plates seeded with E. coil. Upon examination most of C. elegans were at the arrested L1 because there was no food available in M9 buffer, some were still at the egg stage especially those that were exposed to simulated microgravity. Plates were examined and counted daily and the pattern of development was observed in both treatments. When worms reached L4/young adults they were used to determine oxidative stress using the DCFH oxidation method to determine the level of reactive oxygen species that was generated in the worms during exposure to stress such as simulated microgravity. Oxidation of 2',7'-dichlorodihydro-fluorscein diacetate (DCFH-DA) by peroxides yields the DCFH which is further oxidized by ROS to the florescent derivative dichlorofluorofluorescein (DCF). Fluorescence measurements were averaged and plotted graphically. Also whole animals exposed to DCFH-DA, were examined by fluorescence microscope to show areas where ROS are mostly present. Other adults were used to determine the rate of reproduction. When the first few eggs were observed, adults were selected to study the lifespan and were transferred to NGM plates that were treated previously with FUDR to avoid overlapping of populations. Plates were observed every other day; the number of alive and dead adults was scored until the last adult was scored as dead. Results showed that exposure of C. elegans to simulated microgravity reduced the rate of hatched eggs; development of the hatched larvae were much slower than the control worms, and it took them a longer time to lay eggs. Determination of the fluorescence generated by DCF proved that ROS level was much higher in C. elegans population that was exposed to simulated microgravity than the control.