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Research Article

Standardization of Synchronization Procedure to Collect the Similar Aged *C. elegans*

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Abstract

The selection of synchronized stage (same age group) is needed in any model organism viz worm, fly, zebrafish, those are having different developmental stages such as embryonic, larval, pupal then adult. Thus, synchronization is the key for the success of any toxicological experiments. *C. elegans* has been popularly used for the assessment of toxicity associated with pesticides and several other environmental toxicants. *C. elegans* life cycle consists of embryonic stage, four larval stages and then adult stage. Thus, obtaining the similar age group worms will be helpful for different developmental, behavioural, and neuronal toxicity studies. Though, several synchronization protocols are already available however, they are complicated, time consuming, results in lesser yield and with more animal debris but in the present study we have shown a simplified, detailed protocol to collect the synchronized worms using swinging block rotors for centrifugation purpose.

Keywords: Synchronization C; Elegans; Bleach Treatment; Gravid Adult; Centrifugation with Swinging Block Rotors

Background

Caenorhabditis elegans is one of the popular experimental model organisms because of its ease of culturing, transparency, short life cycle and homology to human genome [1]. *C. elegans* develops through various embryonic and 4 larval stages to an adult [1-3]. Culturing of worms results in presence of variable age group population in the media. Synchronous population is very much needed for eliminating the variation due to age differences. Synchronization in terms of development is to be of the same age at the same time with respect to other members of the group [4]. Several assays for toxicological assessment which are age dependent require the worms of same age group [5]. Age synchronization of worms involves lysis of the gravid animal body by using axenizing solution to liberate the eggs. Previously available age synchronization protocol requires more time and efforts, resulted in lesser yield along with more accumulation of animal debris [6,7]. The present detailed protocol has simplified the process, and has resulted in better yield along with minimum unwanted animal debris by using swinging block rotors. The current protocol will be helpful to collect the synchronized worms for conducting the studies where experimental outcome can be affected by age-differences of the animal/worms [8-10]. This will be helpful to examine the adverse effects of various pesticides/ toxicants exposure to the large population of similar aged worms.

Materials and Reagents

a. Sodium hypochlorite solution (5%, Sigma-Aldrich, CAS No. 7681-52-9)

b. 5N NaOH, (SRL chemicals, CAS No. 1310-73-2, Mumbai, India)

c. M9 buffer

Equipment

a. Refrigerated Centrifuge with swinging block rotors (Thermofisher Scientific, Model-Heraeus Megafuge 8R)

b. Laminar air flow (Thermo scientific, HERAGUARD ECO)

c. Pipettes (P1000)

d. Compound microscope (Micros, Austria)

e. 15ml flacon tube (Genaxy)

f. 90×15 mm petri plate (Genaxy)

Procedure

a) For synchronization of worm, take plate containing maximum gravid adult *C. elegans* (Figure.1).

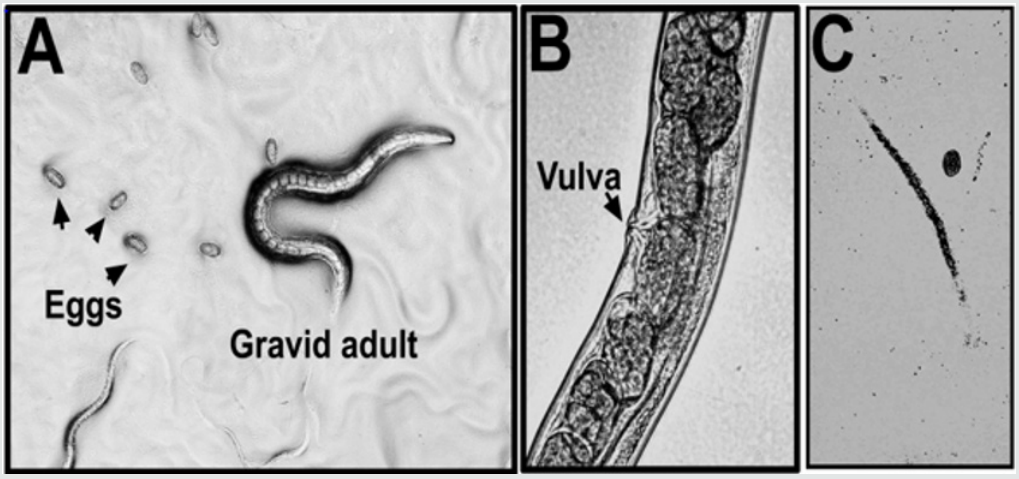

Figure 1: (A) Image showing gravid hermaphrodite *C. elegans*.
(B) Magnified image of gravid *C. elegans* showing eggs inside the body.
(C) Disintegration of *C. elegans* body after bleach treatment and release of the egg.

b) Hold the plate at an angle of 45˚, add 1 ml of M9 buffer (3gms KH_2PO_4/L, 6gms Na_2HPO_4/L, 5gms NaCl/L, 1ml of 1M $MgSO_4$/L) and swirl gently in order to dislodge worms, collect the worm wash in 15ml falcon tube by simply decanting it.

c) Add 1ml of M9 buffer on the same plate and collect the worm wash in the same 15ml falcon tube.

d) Repeat the step 3 again.

e) Pour 500µl M9 buffer, swirl gently and collect in the same 15ml falcon tube.

f) Add freshly prepared 1.5ml axenizing solution (1ml Sodium hypochlorite + 0.5ml 5 N NaOH) to the same falcon tube.

g) Tap gently with finger for exactly 7 mins.

h) Centrifuge at 3410 rpm for 30 secs at 22 ˚C using swinging block rotors.

i) Remove the supernatant carefully leaving behind the pellet in falcon tube.

j) Add 5-7 ml of M9 buffer in order to wash off axenizing solution from the surface of egg pellet.

k) Tap gently with fingers for exactly 2 mins.

l) Centrifuge at 3410 rpm, for 30 secs at 22 ˚C using swinging block rotors.

m) Discard the supernatant leaving behind the pellets containing eggs in flacon tube.

n) Pour pellets on to the unseeded plate and leave them for 10-12 hrs at 22°C.

o) Worms will get arrested at L1 stage after hatching.

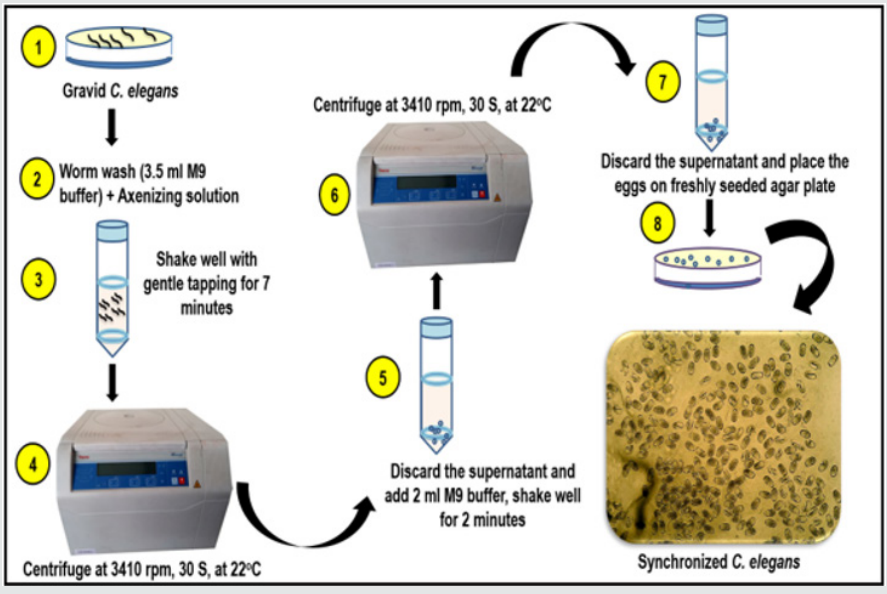

Figure 2: Age-synchronization of *C. elegans*-Schematic diagram showing steps involved in age synchronization of *C. elegans*.

p) Transfer synchronized worms onto a new freshly seeded plate either by worm picking or by taking the worm wash. Figure 2 is showing schematic diagrammatic representation of steps involed in synchronization procedure.

Note

a) Plate containing gravid adult *C. elegans*, M9 buffer, 5N NaOH, Sodium hypochlorite Solution should be at 22 ˚C at the time of synchronization.

b) Mixing of 5N NaOH (to be prepared in autoclaved distilled water) and sodium hypochlorite solution should be done freshly, just before starting the synchronization and should be mixed properly by vortexing.

c) Decreasing the rpm will give more animal debris on plate while increasing will give less number of eggs. So be careful with the time and rpm of centrifugation.

d) Animal should not be kept in axenizing solution more than 7 mins otherwise eggs will also get affected. Be quick for centrifugation and discarding the supernatant.

e) Using swinging block rotors while centrifugation gives better result as compare to fixed axis rotation.

Recipes

For NGM Plate Preparation

1. 3 gms NaCl/L
2. 2.5 gms Peptone/L
3. 17 gms Agar/L

Measure appropriate amount of chemicals and make up the final volume as 975 ml with distilled water.

4. 1M Cholesterol (1ml)
5. 1M $CaCl_2$ (1ml)
6. 1M $MgSO_4$(1ml)
7. 1M KH_2PO_4(25ml)

Note: Do not autoclave cholesterol

For M9 Buffer Preparation

1. 3 gms KH_2PO_4/L
2. 6gms Na_2HPO_4/L
3. 5gms NaCl/L
4. 1ml of 1M $MgSO_4$/L
5. Distilled water to make the final volume 1 lit.

Autoclave this at 120 ˚C for 20 mins and cool. Store in fridge at 4 ˚C temperature

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Competing interests

Authors declare that there is no competing interest.

Ethics

The study was approved by University Research Committee (No. PhD/FS/RA/005).

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