

## Polychaos fasciculatum

**Note:** The culturing conditions below are not necessarily the optimal growth conditions for each strain, as much variation is found between strains, and cultures are not always kept in optimal growth conditions at CCAP for practical reasons. There may be more info in the individual strain data on the website.

On receipt of culture: cultures should be subcultured into fresh sterile medium as described below, ideally within a few days of receipt. If the culture vessel is very full on receipt and subculturing cannot be done immediately, we advise transferring half of the culture to a sterile container to provide air space.

**ACDP Hazard Gp:** 1 - Non pathogenic / non hazardous. Unlikely to cause human disease.

**Culture Medium:** MP (Chapman-Andresen's Modified Pringsheim's Solution) with boiled rice grains and food organism *Chilomonas* sp. (transferred along with the amoebae)

Media recipes can be found on our website: <a href="www.ccap.ac.uk/index.php/media-recipes/">www.ccap.ac.uk/index.php/media-recipes/</a>

Lighting: dark or low light

**Light Cycle: -**

Temperature: 20 degrees C

**Sub Interval:** 4 weeks (at CCAP, may vary depending on environment)

Culture Vessel: 50ml tissue culture flask or 5cm diameter petri dish with lid

## **Culture Method:**

Prepare fresh culture flasks or dishes, half-filled with fresh MP and boiled rice grains (3-4 grains for a tissue culture flask, 1 grain for a petri dish). Store the vessels at 20 degrees C for an hour prior to use.

Observe the cultures using an inverted microscope and choose dense cultures, the amoebae tend to accumulate in the vicinity of the rice grains.

About 4ml of liquid is transferred from a prepared fresh flask/dish to a dense culture using a sterile plastic Pasteur pipette. The pipette is used to agitate the culture and dislodge the amoebae. 4ml of culture is then transferred back to the fresh flask/dish.

Incubate, lay the tissue culture flasks flat.

Use strict aseptic techniques throughout and if possible carry out all subculturing within a laminar flow cabinet (particularly important for axenic strains).