Phagocytic behavior of Asterias rubens *blood cells or coelomic epithelia cells* in vitro

Helen Nilsson Sköld, Bodil Hernroth
SLC-Kristineberg, Fiskebäckskil, Sweden

1. By cutting the outermost edge of an arm of a sea star, coelomic fluid can be collected into a 15 ml Falcon tube when holding the animal above the tube. To avoid activation of the coelomocytes, the coelomic fluid should be collected into an equal amount of CMFSS buffer. (Tubes shall be kept on ice at all times). Phagocytosis measurements have been done using two different protocols with FITC-labelled yeast*, as described;
2. Phagocytosis measurement using a microtiter plate
   - **A.** To quantitatively analyse the phagocytic capacity of sea star coelomocytes, heat-killed yeast cells were labeled with fluorescein-5-isothiocyanate* (FITC) and diluted with PBS to a concentration of 10^8 cells/ml.
   - **B.** Coelomic fluid was sampled into CMFSS buffer and the numbers of coelomocytes were adjusted in PSB to a concentration of 10^7 cells/ml**. 50 µl of coelomocytes and yeast cells were incubated at RT in 96-well microtiter plates for 45 min before adding trypan blue solution (Sigma) for quenching extracellular FITC-yeast signal.
   - **C.** After 8 min, microplates were analyzed at 485/535 nm in a plate reader.
   - **D.** Phagocytic index (PI, %) was calculated in relation to yeast-FITC diluted and incubated in buffer without coelomocytes:
     PI = 100−((Abs yeast ×100)/Abs(yeast+coelomocytes))
     where the Abs values represented the mean value of five wells.
3. Phagocytosis analysis using confocal microscopy with time-lapse-recording
   - **A.** Morphology and phagocytic behavior of blood cells or cells migrating from dissected explants of coelomic epithelium, were recorded using a confocal microscope. Explants were transferred to either 4 ml of cell-free coelomic fluid obtained by centrifugation (2900***g***, 10 min, 4°C) and filtration (0.2 µm), or filtered sea water in plastic chambers.
   - **B.** Both cells that migrated from the explants and the coelomocytes collected from the coelomic fluid and put into the chambers were exposed to FITC-labelled yeast, (see below), and the behavior of the cells assessed by time-lapse video recording (1 image per second for up to 40 min).

* *FITC-labeling of yeast (Saccharomyces cerevisiae): Boil yeast (2 g yeast/50 ml 0.9% NaCl) in a water bath for 30 min. Wash yeast 6x (200g, 10 min, RT) and resuspend in 0.9% NaCl. Determine density of yeast and dilute to 10^9 /ml. Incubate yeast 10^9 /mL in FITC-solution (0.1 mg/ml in 0.1 M carbonate buffer, pH 9.5), 37°C, 30 min (mix every 10 min). Wash FITC-yeast 5x (120g, 10 min, RT) with PBS. Resuspend final pellet in PBS (10^9 yeast /ml), aliquot and freeze at -80°C.*

** *The total number of coelomocytes is measured by diluting the coelomic fluid 1:5 in 4% formalin dissolved in sterile filtered seawater and then microscopically determined using a Bürker chamber.*

Sea stars: Specimens of A. rubens are collected by snorkelling or scuba divers and maintained in basins with running seawater at 15°C and 33 PSU salinity.

Apparatus: Plate reader: Wallac 1420 VICTOR TM multilabel counter (EG&G Wallac). Leica SP5 confocal laser scanning microscope with time-laps imaging recording.

Solutions: CMFSS buffer (435 mM NaCl, 10.7 mM KCl, 27 mM Na_2SO_4, 16.6 mM $C_6H_{12}O_6$, 12 mM HEPES pH 7.4); Phosphate-buffered saline solution (PBS: 137 mM NaCl, 2.7 mM KCl, 15.8 mM Na_2HPO_4, 1.23 mM KH_2PO_4; pH 7.4).

Plasticware: 15 ml Falcon tubes, 96-well plates, Eppendorf tubes (all sterile). Plastic chambers diameter 34 mm (Leica).

Additional information:

Andersson RS, Mora LM *(1995) Phagocytosis: a microtiter plate assay. In Techniques in Fish Immunology 4. Immunology and Pathology of Aquatic Invertebrates. (Eds. JS Stolen et al.) pp. 109-115. Fair Haven, NJ: SOS Publications*

Oweson C, Nilsson Sköld H, Pinsino A, Matranga V, Hernroth B *(2008) Manganese effects on the haematopoiesis of Asterias rubens. Aquatic Toxicol 89:75-81*

Holm K, Dupont S, Nilsson Sköld H, Stenius A, Thorndyke M, Hernroth B *(2008) Induced cell proliferation in putative haematopoietic tissues of the sea star, Asterias rubens (Linnaeus). J Exp Biol 211:2551-2558*

ASSEMBLE
ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES