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Title: A simple method for counting bacteria with active electron transport systems in water and sediment samples. [Methods section only.]

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A simple method for counting bacteria with active electron transport systems in water and sediment samples.

KIEL-MEERESFORSCH. 31, 2, 83-86

(A partial translation from German to English - "method" section only).

Translated by D. Powell

METHOD

The preparation of the electron transport systems in active cells from water and sediment samples, by the addition of INT and the formation of red formazon is given. Sodium succinate, NADH and NADPH served as electron donors.

The following were mixed with distilled water in a beaker and filtered through a membrane filter of pore size 0.2 μ m to obtain the necessary solutions:

- a) 0.4 M disodium succinate
- b) 0.88 mM reduced nicotinamide-adenine-dinucleotide (NADH)
- c) 0.25 mM reduced nicotinamide-adenine-dinucleotide phosphate (NADPH)
- d) 0.2% 2 - (Iodophenyl) - 3 - (p-Nitrophenyl) - 5 - phenyl - 2H - tetrazolium chloride (INT).

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The solutions should be fresh daily and should be preserved in the refrigerator.

10 mls of the water or sediment deposit to be investigated are mixed with the same quantity of sterilized water and the following solutions added to the mixture:

0.5 ml succinate solution, 0.5 ml NADH-NADPH solution, 1 ml. INT solution. The reaction takes place at pH 7.7. The samples are incubated for 30 mins. at a temperature of 20°C and fixed with 0.15 ml. of formaldehyde. 1 or 2 mls of these samples are filtered through a nucleopore filter (Shannon) with pore width of 0.2 µm. Crystals are washed out from the filter.

Colouring of the cell wall followed filtration. The following solutions are necessary at this point.

1. Molybdenum phosphoric acid: 1% in distilled water. The stock solution is to be used in a 1:10 dilution with distilled water.
2. Methylgreen: 10% in distilled water. The stock solution should be used diluted with formaldehyde and distilled water in the ratio of 1:5:5.

After the incubation and washing the filter with 2 ml of distilled water, 1 ml. of molybdenum phosphoric acid solution is added, the solution allowed to stand for 1 minute, then filtered at a vacuum of 0.5 ats., rinsed with 3 ml. of distilled water, 1 ml. of methylgreen solution added and allowed to stand for three minutes, filtered, rinsed with 3-4 ml. of distilled water and air-dried.

Microscopic investigation was carried out on a small piece of filter paper which had been cut out, put on a slide with immersion oil and covered with a cover slip. A blue light filter was used in the microscope in order to get better results.

Organisms with active electron transport systems are stained red in the active cell parts. The red colour of formazon stays stable in the fixed cell. The cell wall, on the other hand, stains light green. One can therefore investigate and separate with ease the red-stained bacteria

cells which have active electron transport systems, and the green-stained bacteria cells which are not active.

It is possible to see several sites of electron transport in the larger cells. Especially impressive are the plankton-algae, protozoa, and small metazoa.

Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.