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Agarolytic activity in the enzyme extracts of *Oscillatoria* sp.

P. Kaladharan and K. Seetha

Central Marine Fisheries Research Institute, P.B. No. 1603
Kochi - 682 014.

Abstract

Oscillatoria sp. growing as epiphyte on the thallii of *Gracilaria edulis* was known to contain enzymes endowed with agarolytic properties. The alkaline PO_4 buffer(0.1 ml, pH 7.5) extracts containing protein concentration of 600 µg/ml exhibited maximum activity of agar solublization (490 µg) on 1% agar slants within the first three hours. However, at pH 6.0 the enzyme extract exhibited low rate of agarolytic activity. The *in vivo* activity of this extract was also studied on the bits of fresh thallus of *G. edulis* for cell seperation. The results are discussed in the light of possibilities of applying this cost effective marine source of enzyme on protoplast isolation and somatic hybridization of Indian agarophytes.

Microalgae have attracted much attention as economic sources of new drugs and other speciality chemicals (Metting and Pyne, 1986). Certain cyanobacteria can produce and excrete a wide variety of bio-active organic substances (Bloor and England, 1989). There have been some reports on the extracellular agarase from *Pseudomonas atlantica* (Morrice *et al.*, 1983) and *Vibrio* sp. (Aoki *et al.*, 1990). The enzymes from these microorganisms hydrolyse agar to yield neogarotetrase as a predominant product. Lovilla-Pittogo (1992) isolated agar-digesting *Vibrio* sp. from *Gracilaria* sp. showing rotton thallus syndrome. One of the marine species of blue green alga *Oscillatoria* sp., growing attached to thallus of *Gracilaria edulis* cultured in silpol lined ponds was tested for agarolytic activity as this sp. caused discoloration of the thallus in culture. This article embodies the results obtained on the agarolytic activity of the crude extract in different pH and its *in vivo* activity on the tissue bits of *G. edulis* with the view to apply this enzyme extract on cell seperation and protoplasts isolation from *G . edulis*

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Material and methods

Oscillatoria sp. found attached to seaweed *Gracilaria edulis* cultured in silpol lined shallow tanks at the Narakkal Field Station of C.M.F.R.I., was harvested and seperated from the thallus. The cleaned sample was collected in prechillied ice bucket and brought to the laboratory. The crude enzyme was extracted in alkaline phosphate buffer (0.1M, pH 7.5) by grinding in a pre-chilled mortar and pestle.

The enzyme extract was centrifuged at 8000 rpm for 10 minutes at 10-12°C and the supernatents were preserved. Protein was determined from the enzyme according to Lowry *et al.* (1951).

Agarolytic activity of the crude enzyme at pH ranging from 6.0 to 7.5 was determined on 1% agar slants with 1.0 ml each of enzyme extract taken in test tube. The activity was determined at hourly by terminating the activity at every one hour interval by boiling the content. The 0-hour incubation served as blank and the boiled enzyme extract served as controls. Total soluble sugars solublized from the agar slants were determined according to the method of Dubois *et al.* (1956).

Results and discussion

The crude enzyme extracted from *Oscillatoria* sp. having a protein concentration of 600 µg/ml at 7.5 pH on 1.0% agar slants showed agarolytic activity equivalent to 490 µg soluble sugars/ml of enzyme within three hours duration (Table 1). However, at a reduced pH (6.0) the activity was 51% less.

The blue green alga *Oscillatoria* sp. found infesting on the thallus of *G. edulis* was known to show "rotton thallus syndrome" suggesting a parasitic mode of nutrition. The enzyme produced by *Oscillatoria* sp. solublizes the agar deposited on the cell wall of *G. edulis* hence may be the decolouration of thallus. The solublization of 1.0% agar slants also showed strong possiblities of this crude enzyme capable of degrading agar. Yamaguchi *et al.* (1989) reported angiotensin-converting enzyme inhibitory activities from *Oscillatoria* spp. Agarolytic enzymes such as Agarase has been used in combination with cellulase and macerozyme to isolate protoplats from *Gracilaria thikvahiae* and *G. lemaneiformis* (Cheney *et al.* 1986). For the production of somatic hybrids from *Gracilaria* and for the isolation of viable protoplasts from *G. edulis* the enzyme extracted from *Oscillatoria* sp. is being used and this can save the cost and quanity of commercial grade enzymes as well as can accelerate the cellwall lytic process when applied in combination with commercial grade cell wall lytic enzymes either in crude or purified form.

Table 1. *Agarolytic activity of enzyme from Oscillatoria sp. at different pH on 1.0% agar slants.*

Time (hr)	Total soluble sugars formed (µg/ml/h) pH 6.0	/ pH 7.5
0 (Blank)	0	0
1	35+10	40+7
2	73+14	120+12
3	240+23	490+47
4	341+35	330+29
5	172+19	110+18

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