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R. C. WHATLEY[†]
SUNIL BAJPAI*[,‡]

[†]*Department of Geology, University of Wales, Aberystwyth, Cardiganshire, SW 23 DB, UK*
**Department of Earth Sciences, University of Roorkee, Roorkee 247 667, India*
[‡]*For correspondence. (e-mail: sunilfes@rurkiu.ernet.in)*

Agar depolymerizing (Agarolytic) bacteria isolated from mangrove soil samples of Andaman

The Andaman and Nicobar group of islands are covered by one lakh fifteen thousand hectares of mangrove forests, which is approximately 25% of the total mangrove area of India. Mangrove ecosystem is a resource of plants, animals and microbes which are interdependent and living in close association. Microbes play an important role in nutrient recycling in this unique ecosystem and have become an important area of study in the search for novel microbial products, including enzymes. In this article, isolation and identification of microbial source of agarase enzyme from mangroves of Andaman is described.

About 1000 soil samples collected from various mangrove inhabited areas of South Andaman were subjected to isolation of bacteria in Zobell Agar (ZA) medium containing yeast extract, 5.0 g; peptone, 1.0 g; K_2HPO_4, 0.5 g; a trace amount of ferrous sulphate; agar, 15.0 g; distilled water, 250 ml and filtered sea water, 750 ml. Diverse cultural, morphological and metabolic types of bacteria were screened simultaneously using enriched and selective media such as xylanolytic, cellulolytic, sulphur oxidizing, DNase producing, halophillic, starch – caesin, tributyrin, milk and alginate-containing media[1]. Pour-plated soil samples after 24 h of incubation showed depressions simultaneous to the bacterial growth on ZA plates (Figure 1). On subsequent subculturing in fresh ZA medium plates, depressions continued to appear around the bacterial colonies. Among six such bacterial isolates recorded, one isolate namely BR 6/3 liquefied the solid medium completely within 72 h (Figure 2). Incubation of this liquefied medium at 4°C showed complete loss of gelling property of the agar in the medium. The pure culture of the isolate was obtained by incorporating a graded concentration of agar up to 3% in Zobell Broth medium and by repeated sub-culturing in the early logarithmic growth phase. The isolate produced white, opaque, butyrious colonies on ZA within 4 to 6 h culturing. It was motile, gram-negative curved/elliptical rod-shaped bacteria having polar flagellum. The isolate produced gelatinase, amylase and lipase enzymes. The organism was positive to oxidase and methyl red and negative to catalase, citrate, Voges-Proskauer, H_2S and ammonia tests. The strain produced acid from galactose, fructose, sucrose, maltose, dextrose, arabinose, melibiose, xylose and raffinose. Based on cultural, morphological and biochemical tests, the isolate was identified as *Alteromonas* spp.

The strain was able to degrade different types of agar and agarose used for protein and nucleic acid separation. The

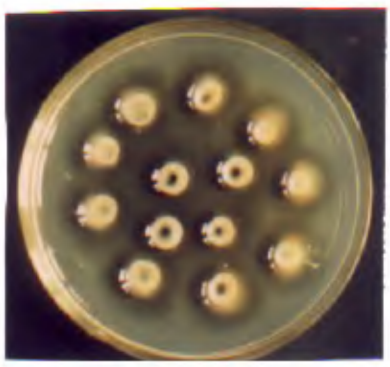

Figure 1. Circular depressions in the ZA plate containing 3% agar arising after 24 h of inoculation by agarolytic bacteria.

Figure 2. Cracks/depolymerization of agar in the ZA plate inoculated with BR 6/3 strain after 48 to 72 h of incubation.

culture filtrate free of bacteria could also depolymerize the agar and the presence of agar was found to enhance the production of agarase enzyme. The top supernatant (crude enzyme preparation, ECP) obtained after 3 cycles of slow freezing and quick thawing of bacterial cells in PBS attacked the solidified agar, indicating the presence of extra-cellular exo-acting agarase enzyme. The optimum pH and temperature for the enzyme activity was found to be 6 to 9 and 25 to 37°C, respectively. The extra-cellular production of agarase was suppressed by glucose as well as galactose when incorporated at the rate of 1% in ZA medium. The ECP when analysed by SDS-PAGE showed polypeptide bands having agarolytic activity in the molecular weight range of 45 to 52 kDa.

In the present study, the percentage of agarolytic bacteria was found to be very low (< 1%), though the soil samples were collected periodically from mangrove litter sediments where salinity percentage and other physio-chemical characters varied throughout the year. Natarajan and Venkateswaran[2], however, detected higher counts of agarolytic bacteria in sediment samples in low saline brackish waters than in open sea surface water in Porto Novo coastal waters. Optimum enzyme activity of an agar-decomposing bacteria from Abalone[3] and a Cytophaga-like bacterium from tar ball had an optimum pH and temperature of 7.4 and 37°C, respectively[4]. Leon *et al.*[5] reported higher production of agarase enzyme in the presence of agar by *Alteromonas* spp. Various other workers have also reported the isolation of agarolytic bacteria from marine environment and stressed the importance of agarase enzyme in the elution of embedded DNA fragments from agarose gels after electrophoresis. Agarolytic strain of *Agrobacterium auriantiaculum* from Cuban waters[6], agarolytic bacterial epiphytes in *Gracilaria. gracilis* observed under scanning electron microscope[7], N_2-fixing agar degrading bacteria from seawater and eel grass-bed sediments of Japan[8], a unique gene *AgarA*, responsible for agarase production from *Vibrio* spp[9,10] and marine agarolytic gliding bacteria belonging to the genus *Microscilla*[11] have been reported.

The industrial scale production and further utilization potential of the enzyme in degradation of very complex biopolymers in the control of environmental pollution and in biotechnology research need to be evaluated after fermentation studies.

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B. R. SHOME*
RAJESWARI SHOME*,‡
S. P. S. AHLAWAT†
N. D. VERMA*

†*Central Agricultural Research Institute,*
P.B. No. 181,
Port Blair 744 101, India
**Division of Animal Health,*
ICAR Research Complex for NEH Region,
Umroi Road, Umiam,
Meghalaya 793 103, India
‡*For correspondence.*
(e-mail: raji_shome@123india.com)