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Purification, Characterization and Application of a Novel Extracellular Agarase from a Marine *Bacillus megaterium*

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Abstract A marine, gram positive, aerobic, spore forming, and non flagellated bacterium which degrades low melting point (LMP) – agarose was isolated from the west coast of India and identified as *Bacillus megaterium* based on its morphological, biochemical, and molecular characterization. This bacterium produced clear haloes or zone of clearance on agar containing plates which was a clear indication of its agarolytic property. The extracellular agarase thus obtained was purified 8.8 and 78 fold from the culture supernatant by ammonium sulfate precipitation and gel filtration, respectively. Molecular mass by gel filtration and SDS-PAGE gave values of 15 and 12 kDa, respectively. The optimum temperature and pH for maximum agarase activity were 40°C and 6.6. The activity of agarase was drastically reduced by addition of metal ions in the assay system. This agarase, gave a K_m and V_{max} value of 4 mg/mL and 2.75 μmol/min/mg. The isolation of protoplast from agarophyte like *Gelidiella acerosa* using indegenous agarase is reported for the first time. © KSBB

Keywords: agarase, Bacillus megaterium, Gelidiella acerosa, *low melting point agarose, red algal protoplast*

INTRODUCTION

Agar is an abundant biopolymer found in red algae as a cell wall component and is used as a food additive and in bacteriological media [1]. It is a polysaccharide consisting of agarose and agaropectin. Agarose has a linear chain structure composed of alternating residues [*O*-3, 6-α-anhydro-L-galactopyranosyl (1→3) O-β-D-galactopyranose] linked by β-1,4 bonds [2]. Agar oligosaccharide can be obtained by chemical as well as enzymatic hydrolysis.

Agarase (E.C.3.2.1.81) has been reported from certain marine mollusks and from several bacteria including *Actinomyces, Agarivorans, Alterococcus, Alteromonas, Bacillus, Cellulophaga, Cytophaga, Microbulbifer, Pseudoalteromonas, Pseudomonas, Saccharophagus, Streptomyces, Thalassomonas, Vibrio,* and *Zobellia* [3]. Most of these bacteria were isolated from marine environments, while a few species were isolated from river [4], hot spring [5], soil [6], brackish water and salt marshes [7], and sewage [8]. Agarase producing bacteria can be classified into two groups according to the mode of action on agar: α-agarases cleave the α-1, 3 linkage of agarose and β-agarases cleave the β-1, 4 linkage of agarose [9].

Hydrolysis of agar or agarose by agarase produces neoagarooligosaccharides, which have various chemical properties and biological activities. They inhibit the growth of bacteria, slow down the degradation of starch, reduce the calorific value of food, and present anticancer and antioxidation activities [10]. Isolation of algal protoplasts using bacterial enzymes have also been reported from agarophytes like *Gracilaria* where the objective was to develop high yield protoplast system that could be used to study production of agar and regeneration of the cell wall [11-13].

Agarase is a costly enzyme with limited supply. In light of this, we report the isolation of a potential agarase producing marine bacterium, *Bacillus megaterium*, from the west coast of India. Optimization of environmental parameters including medium components for the maximum agarase production is also described. The purification and characterization of agarase has also been demonstrated, which contributed to tremendous enhancement in agarase activity, followed by its molecular weight determination. This is the first report on agarase production by a marine *B. megaterium*. Application of isolated agarase for protoplast isolation from red algae

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Table 1. Screening of medium components for agarase production by *Bacillus megaterium*

Chemical components (g/L)	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9
$CaCl_2$, $2H_2O$-0.14	+	–	–	–	+	–	+	–	–
KCl-0.74	+	–	–	–	–	+	+	–	–
K_2HPO_4-4.20	+	–	–	+	–	–	+	–	–
KH_2PO_4-0.40	+	–	–	+	–	–	+	–	–
$MgSO_4$, $7H_2O$-5.0	+	+	–	–	+	–	+	–	–
NaCl-30.0	+	+	+	+	+	+	+	+	+
$(NH_4)_2SO_4$-0.13	+	–	–	–	–	+	+	–	–
Peptone-5.0	+	+	+	+	+	+	+	+	+
Tris base-6.10	+	+	–	–	–	+	+	–	–
Yeast extract-1.0	+	+	+	–	–	–	–	–	–
Agar	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.3

–, Absent; +, present.

Gelidiella acerosa and *Gracilaria corticata* has also been established. To the best knowledge of the authors, this is the first report on protoplast isolation from *G. acerosa* [14].

MATERIALS AND METHODS

Sample Collection

Seawater, sediment, and decayed agarophytes were collected from Okha (22°34′N 69°04′E), Dwarka (21°12′N 68°53′E), and Diu (20°43′N 70° 47′E), west coast of India in sterile containers and transferred in cold condition to the laboratory for further analysis.

Screening of Microorganism

Screening of agarase producing bacteria was carried out on Zobell Marine Agar 2216 (High Media M384) and Artificial Seawater Medium containing agar as sole carbon source. The plates were incubated at 37 ± 2°C for 48 h. Colonies that formed deep holes or clearing zones around themselves, on agar containing plates were further purified.

Identification of the Microorganism

The most promising bacterium was identified on the basis of morphological, physiological, and biochemical characteristics following standard procedures. The identification was further confirmed by 16S rDNA method. Genomic DNA was isolated from purified culture pellet. Using rDNA sequence specific consensus primers, ~1.5 kb fragment was amplified using high fidelity PCR Polymerase (GeNei Hot-Start Taq DNA Polymerase). The PCR product was cloned and bidirectionally sequenced using forward and reverse primers and one internal primer. The 16S rDNA gene sequence was compared with sequences in nucleotide database by using the BLAST algorithm at the NCBI site (http://www.ncbi.nlm.nih.gov/BLAST/) [15].

Screening of Medium Components for Agarase Production

Primarily, media reported in literature were used for the cultivation of agarolytic bacteria [16-20]. These media did not support agarase production and hence, a new medium having only three components *i.e.* sodium chloride, peptone, and agar was defined for the production of agarase, by screening conducted as shown in Table 1. Effect of salt concentration in the cultivation medium was also studied by varying the salt concentration from 3 to 20% to ascertain the halophilic nature of the isolated agarolytic bacterium.

Production of Extracellular Agarase

B. megaterium was inoculated in 50 mL of liquid medium containing sodium chloride 30.0 g/L, peptone 5.0 g/L and agar 0.3 g/L and incubated at 37 ± 2ºC for 96 h on rotary shaker at 180 rpm. The cell free medium was collected by centrifuging the culture broth at 8,000 rpm for 15 min at 4ºC and was used as crude extracellular agarase [14].

Agarase Assay

Agarase assay system consisted of 2.0 mL substrate [0.25% LMP agarose in 20 mM Tris-HCl buffer (pH 7.5)] and 1.0 mL of enzyme in a total volume of 3.0 mL. The system was incubated at 37 ± 2°C for 2 h. The reaction was stopped by heating the tubes in a boiling water bath for 15 min [21] and reducing sugar generated was determined by Nelson reagent using D-galactose as a standard. Units of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol D-galactose per minute where as specific activity of agarase was calculated as units of enzyme produced per mg of enzyme protein. Protein concentration was determined by the method of Lowry using bovine serum albumin as the calibration standard.

Purification of Agarase

All the purification procedures were carried out at 4°C. Culture fluid was centrifuged at 8,000 × g for 15 min, and the cell-free supernatant was collected and subjected to 40~60% ammonium sulphate precipitation followed by dialysis against buffer (20 mM Tris-HCl buffer, pH 8.0) overnight. Partially purified agarase thus obtained was loaded on Sepharose CL-4B column containing 0.02% sodium azide and eluted with 20 mM Tris-HCl buffer. The relative protein content of column fractions was estimated using Bradford method as well as by absorbance measurement at 280 nm. The purified and pooled fraction was also evaluated for agarase activity.

Molecular Weight Determination

Molecular weight (MW) of agarase was determined by gel filtration technique using blue dextran to decide the void volume (Vo) of the column. Immediately after applying the sample, fractions (3.0 mL each) were collected at a flow rate of 25 mL/h. Standard protein markers having MW ranging from 12.4 to 200 kDa (kilo Dalton) were individually loaded onto the column at a concentration of approximately 2~3 mg/mL and Ve for each standard was determined. The molecular weight of purified agarase fraction was determined by noting its elution volume (Ve), calculating its Ve/Vo and comparing it with a plot of log of molecular weight vs. Ve/Vo of standard molecular weight proteins markers.

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of agarase was conducted in 12% gel as per method described by Sambrook and Russel along with standard molecular weight protein markers [22].

Effects of Temperature and pH on Agarase activity

The optimum temperature for agarase activity was determined under the standard assay condition by varying the incubation temperature from 10~60°C and incubating the enzyme along with the substrate at the respective temperatures. These studies were carried out in sealed Eppendorf tubes for 2 h by incubating the tubes in water bath at the required temperatures.

The effect of pH on agarase activity was assayed by using different buffers of varying pH ranging from 4.5 to 9.0 (acetate buffer for pH 4.1 to 5.8, phosphate buffer for pH 6.0 to 7.6, and Tris-HCl buffer for pH 7.7 to 9.0). Substrate solution (0.25% LMP agarose) was prepared using these buffers. The residual enzyme activity was measured following the procedure described above.

Effect of Metal Ions

The effects of metal ions, like Al^{+3}, Ba^{+2}, Ca^{+2}, Fe^{+3}, Mg^{+2}, Mn^{+2}, Hg^{+2}, and K^{+} on the reducing sugar releasing activity of agarase was studied by using respective chlorides at 1 mM concentrations in the reaction mixture. The relative activity was defined as the percentage of activity determined with respect to that measured under the standard condition described previously.

Substrate Specificity and Storage Stability

Various polysaccharides including κ-, λ-, and ι-carrageenan (Sigma) were used instead of LMP agarose in the enzyme assay system to identify the substrate specificity of agarase.

The storage stability of agarase with respect to temperature was confirmed by incubating the enzyme at 37, 20, and –20°C and then measuring its activity at regular intervals. The effect of repeated freezing and thawing on enzyme activity was also studied.

Test for Liquefaction

The liquefaction test was performed to confirm that agarolysis occurred due to agarase. For this, 3.0 mL of 0.7% LMP agarose in 20 mM Tris-HCl buffer (pH 7.0) was taken in test tube. To this solution, 0.3% KCl (KCl helps in gel formation) was added and slightly warmed. Further, 1.0 mL of crude agarase was added. Reaction mixture was quickly frozen. After this, incubation was carried out at 30ºC for 2 h. After the incubation period was over, the samples were again frozen [14].

Estimation of Kinetic Parameters

K_m and V_{max} values for the agarase acting on LMP agarose (different concentrations of substrates varying from 1~5 mg/mL prepared in 20 mM Tris-HCl buffer were mixed with 0.986 mg of enzyme protein and incubated at 40°C for 2 h) were calculated by linear regression analysis of Lineweaver-Burk, double-reciprocal, plots of initial velocity data obtained under the condition described above.

Application of Agarase for the Isolation of Protoplasts

Fronds of *G. acerosa* and *G. corticata* were cleaned by washing with sterile seawater, 2~3 times. These fronds were sonicated gently in sterile seawater, which cleaned the tissue from epiphytes but did not dissociate or damage the cells. Protoplasts were isolated using the method described by Khambhaty *et al.* [23].

RESULTS AND DISCUSSION

A total of 5 strains producing extracellular agar decomposing enzyme were isolated from seawater, sediment, and seaweed surface, out of which the strain exhibiting significant agarase production was selected for further studies. Microscopic examination indicated that isolated agarase producer was gram positive and endospore forming.

Table 2. Morphological and biochemical characteristics of *Bacillus megaterium*

Test	Result
Form	Rods
Gram staining	Positive
Spores	+
Polar flagellum	+
Production of pigments	+ (Reddish White)
Indole production	+
Nitrate reduction	–
Voges Proskaver	–
H_2S production	+
Utilization of citrate	+
Gelatin liquefaction	+
Arabinose, Dextrose, Fructose, Galactose, Inositol, Lactose, Mannose, Maltose, Raffinose, Rhamnose, Sorbitol, Sucrose, Xylose	+
Oxidase	–

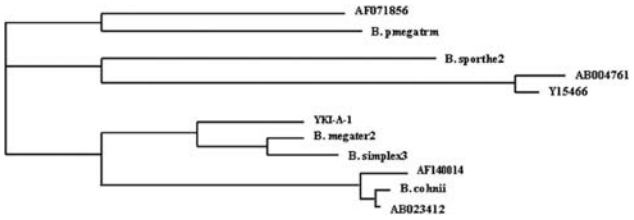

Fig. 1. Phylogenetic tree based on 16S rDNA sequence analysis showing the position of *Bacillus megaterium*. B. megater2, *Bacillus megaterium* IAM 13418 (T); B. simplex3, *Bacillus simplex* DSM 1321 (T); (B. cohnii, *Bacillus cohnii* DSM 6307 (T); AF140014, *Bacillus cohnii* str. ML-A10; AB023412, *Bacillus cohnii* str. YN-2000; B. pmegatrm, *Bacillus pseudomegaterium* ATCC 49866; AF071856, *Bacillus* 171544 str. 171544; Y15466, *Bacillus* sp. str. JJ#1; B. sporthe2, *Bacillus sporothermodurans* str. M215 DSM 10599 (T); AB004761, unnamed organism).

Further identification based on morphological and biochemical cha-acteristics signified it as *Bacillus* according to *Bergey's Manual of Systematic Bacteriology* (Table 2) which was further confirmed to be *B. megaterium* by 16S rDNA studies. The 16S rDNA sequence of this strain exhibited 99% homology with *B. megaterium.* The next closest homology was found to be *Bacillus simplex.* Information about other close homologs for the microbe can be found from the phylogenetic tree (Fig. 1). Only three species of *Bacillus i.e. Bacillus agarexedens* [24], *Bacillus cereus* [25], and *Bacillus* MK03 [26,27] have been reported as agarase producers, in addition to *Pseudomonas, Cytophaga, Alteromonas, Streptomyces, Vibrio, Thallasomonas,* and *Agarivorans* to date. This is the first report on *B. megaterium* producing agarase. The production of agarase was inducible as it was produced only in the presence of agar [14].

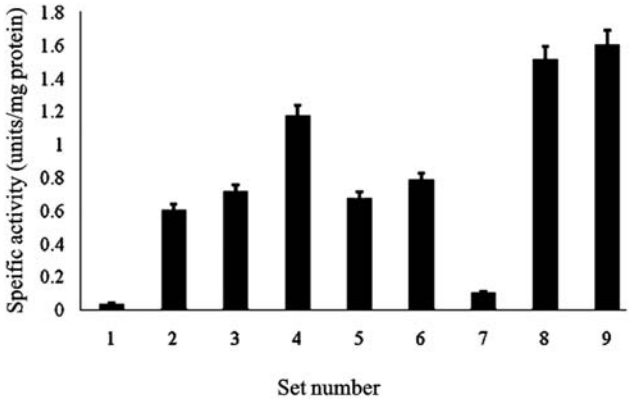

Fig. 2. Effect of various medium components on agarase production by *Bacillus megaterium.*

Medium Components for Agarase Production

In order to find a suitable medium for agarase production, various media reported in the literature were used. The outcome indicated discouraging results with respect to agarase production. As a result, thorough screening of medium components was conducted. It was observed that set No. 8 and set No. 9 which had only three components, yielded maximum agarase activity as compared to other seven sets containing 4 to 11 components. However, set No. 9 proved to be the most suitable medium for agarase production with significantly lower concentration of agar. These results concluded that components other than sodium chloride, peptone, and agar, were either inhibitory or not necessary for the production of agarase. It was also observed that agar concentration as low as 0.03% was sufficient enough for the induction of agarase production (Fig. 2). This medium yielded an enzyme having specific activity of around 1.60 units/mg protein. Hence a new medium comprising only three components was defined containing sodium chloride 30.0, peptone 5.0, and agar 0.3 (g/L) which is economically quite feasible as compared to other medium compositions reported in the literature where the authors have mentioned the use of 10~11 components. These results were further confirmed by repeating the experiment (data not shown). This is the first report on a medium, used for agarase production, containing only three components with significantly high amount of agarase activity. It was also proved that more number of components adversely affected (inhibited) agarase production. These findings proved that apart from cultural conditions like pH, temperature, incubation time, and aeration, medium components also play a noteworthy role in the production of agarase [14]. The cultivation medium used for *Bacillus* MK03 also consisted of only four medium components having peptone, NaCl, agar, and beef extract resulting in specific activity of only 0.11 and 0.45 units/mg protein [26,27].

Halotolerant nature of isolated culture was confirmed by the requirement of 3~5% sodium chloride in the culture

Table 3. Purification of agarase produced by *Bacillus megaterium*

Sr. No.	Step	Total volume (mL)	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Recovery (%)	Fold purification
1	Crude enzyme (Unoptimized medium)	100	104.1	156.20	0.66	100	1.0
2	Crude enzyme (Optimized medium)	100	286.3	172.6	0.60	110	2.42
3	Ammonium sulfate precipitation	25	67.5	11.6	5.81	6.74	8.8
4	Gel filtration	1.5	3.939	0.0765	51.49	0.044	78.0

medium used for agarase production. Shieh and Jean have reported isolation of agar-degrading bacteria that grew optimally at 2~2.5% NaCl (ca. 0.34~0.43 M) [5]. An increase in agarolytic activity of *PjaA* gene by addition of NaCl, postulating that either Na^+ or Cl^- ion was involved in the enzyme activation [28]. Recent report on the salt dependency of bacteria revealed that growth was observed over a range of 0.5 to 6% NaCl with significant growth between 1.5 and 3% NaCl [29]. The results of the present investigation gave similar observation where 3~5% salt concentration in the medium yielded maximum growth as well as agarase production.

Agarase Purification

Agarase was purified from the culture supernatant of *B. megaterium* by ammonium sulfate precipitation and gel filtration chromatography. In the present study, fractional ammonium sulfate precipitation revealed that highest agarase activity was achieved with 40~60% of ammonium sulphate precipitation. Table 3 summarizes the results of each step of agarase purification. During the process, 8.8 and 78 fold purification was achieved with specific activity of 5.81 and 51.4 units/mg protein by following only two simple steps of purification [14]. Agarase from *Bacillus* MK03 was purified 49.7 fold with specific activity of 22.2 units/mg protein and 129 fold with specific activity of 14.2 units/mg protein following four complex steps of purification [26,27].

Molecular Weight Determination

Based on the standard graph, the molecular weight of agarase was calculated using 'y' equation (y = −1.0481x + 6.482). Further, by calculating the antilog, the molecular weight was found to be 15 kDa by gel filtration. The purified agarase was once again subjected to 12% SDS-PAGE in order to check the homogeneity as well as to confirm its MW. On the gel, the protein band of agarase appeared as a single band, exhibiting a MW of 12 kDa (Fig. 3); hence the MW of agarase determined by gel filtration (15 kDa) was in concurrence with that obtained by SDS-PAGE. However, the molecular weight of present agarase was much lower as compared to molecular masses of agarase reported from *B. cereus* (90 kDa by SDS-PAGE) [25], *Bacillus* MK03-320 kDa and 42 kDa [26], and *Bacillus* MK03-113 kDa and 92 kDa [27] by gel filtration and SDS-PAGE, respectively.

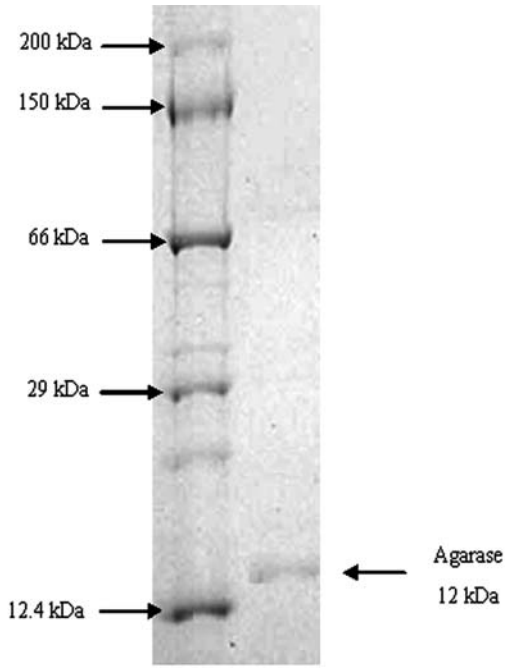

Fig. 3. Purification of agarase isolated from *Bacillus megaterium* on 12% SDS gel electrophoresis.

Effects of Temperature and pH

Temperature and pH are considered to be decisive parameters for enzyme activity. The effect of temperature and pH on agarase activity is depicted in Figs. 4 and 5. It was observed that the activity consistently increased from 10~40°C, the optimum being 40°C. But, a drastic decrease of around 50% was observed when incubated beyond 40°C. An optimum temperature of 30ºC for agarase from *Bacillus* sp. MK03 has been reported [26,27]. Several reports where agarase retained its activity at temperatures as high as 55ºC and also in a range of 30~40ºC are available [1,2,16,18,30-32]. In contrast to this, agarase of the present investigation exhibited a very narrow temperature stability indicating it susceptible to temperature.

Likewise, the agarase activity was low at pH 4.2 but in-

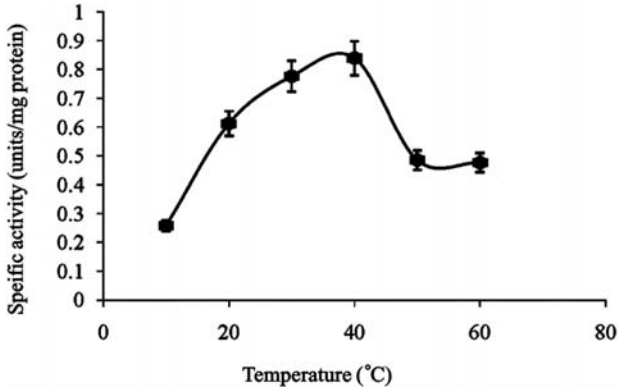

Fig. 4. Effect of temperature on agarase activity isolated from *Bacillus megaterium.*

creased sharply from pH 4.6 to 7.0 after which the activity declined sharply, however, maximum agarase activity was observed at pH 6.6. The optimum pH for agarase activity from *B. megaterium* was slightly acidic *i.e.* 6.6, whereas, the optimum pH for agarase reported from *B. cereus* was 7.0, *Bacillus* sp. MK03 was acidic, 6.1 as well as slightly alkaline, 7.6 [26,27]. Agarase from *Alteromonas* was also reported to have optimum pH of 6.5 [33]. Several authors have reported agarases having activity in a broad pH ranging from 4.0~9.0 [1,2,16,19,30,32]. Agarase active in alkaline pH have also been reported [31]. In the present study, the optimum pH range for agarase activity was considerably narrower as compared to some other reports. Thus, this agarase is pH sensitive. Most of the reports have concluded that agarase is active in acidic condition.

Effect of Metal Ions

Effect of cations like Al^{3+}, Ba^{2+}, Ca^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, and K^{+} on the activity of agarase was studied. None of them exhibited activation in activity. On the contrary there was a reduction in activity by 65~75% in the presence of cations like Al^{3+}, Ba^{2+}, Hg^{2+}, Mg^{2+}, and 25~50% with cations like Ca^{2+}, Fe^{3+}, K^{+}, and Mn^{2+} (Table 4). Hence, agarase isolated in the present study was found to be cation independent; since presence of cations adversely affected agarase activity. Similar results were obtained by Araki *et al.* where none of the metals were found to exhibit positive effect on agarase activity [1]. Report exhibiting a strong inhibition in enzyme activity by bivalent metal cations such as Mn^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, and Zn^{2+} are available but at the same time other reports on a slight activation in the activity with EDTA, K^{+} and Na^{+} are also available, drawing a conclusion that metal ions may or may not be essential for the activity of enzymes [19]. Suzuki *et al.* have also reported inhibition of agarase activity by Mg^{2+}, Zn^{2+}, Al^{2+}, Ag^{2+}, Pb^{2+}, Ni^{+}, and Fe^{2+} [26,27].

Effect of Storage Stability

Storage stability is an important criterion for making a particular enzyme commercially valuable. Agarase, which

Table 4. Effect of metal ions on agarase activity from *Bacillus megaterium*

Metal	Relative activity (%)	Reduction in relative activity (%)
Blank	100	–
$AlCl_2$	32.33	67.67
$BaCl_2$	32.94	67.06
$CaCl_2$	63.88	36.12
$FeCl_3$	74.58	25.42
$HgCl_2$	26.62	73.38
KCl	73.41	26.59
$MgCl_2$	32.36	67.64
$MnCl_2$	50.96	49.04

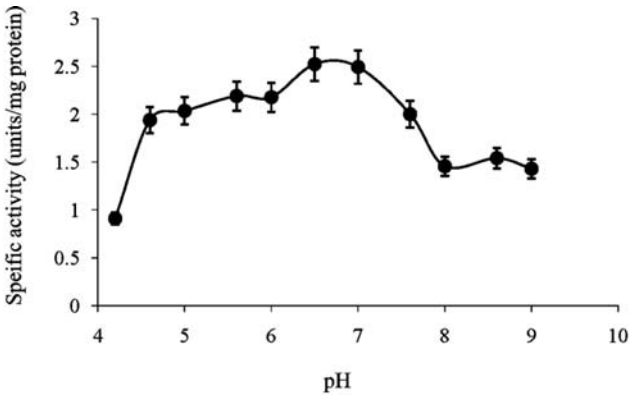

Fig. 5. Studies on effect of pH on agarase activity from *Bacillus megaterium.*

was stored at 35°C, almost lost 70% of its activity within seven days while the enzyme stored at 20°C had a loss of about 30% and the one in frozen condition did not have significant loss in the activity. However, after seven and eleven days, there was a significant decline in the activity of the enzyme that was stored at 20°C and in frozen condition, respectively (Fig. 6). It was also observed that repeated freeze thaw adversely affected agarase activity [14].

Substrate Specificity

Various polysaccharides from red algae such as κ-, ι-, and λ-carrageenan as well as LMP agarose were used to determine the substrate specificity. Apart from excellent activity towards LMP agarose, the agarase exhibited marginal activity against κ-carrageenan and no activity towards ι- and λ-carrageenan. Hence, it can be concluded that agarase is highly agarose specific since reducing sugar was not detected in the reaction mixture after incubation for up to 2 h. Insignificant activity towards κ-carrageenan may be due to negligible contamination of κ-carrageenase along with agarase during the experimental procedures.

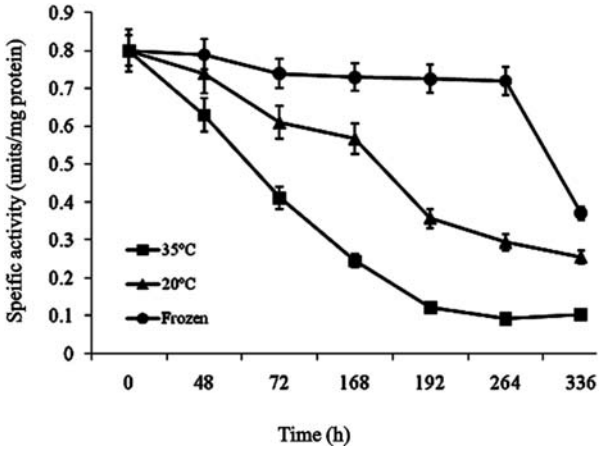

Fig. 6. Storage stability of agarase obtained from *Bacillus megaterium.*

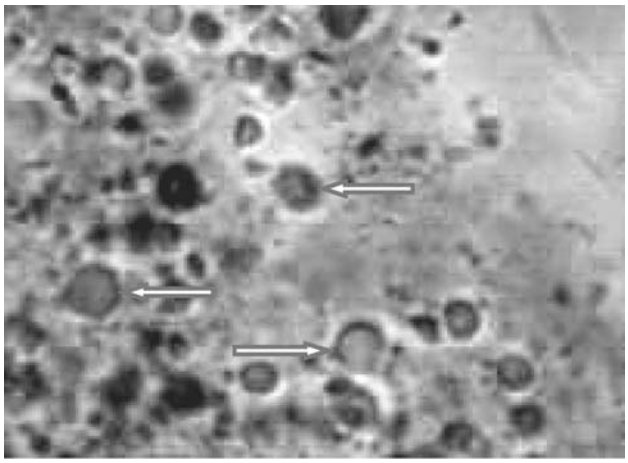

Fig. 7. Application of indigenous agarase for protoplast isolation from *Gelidiella acerosa.*

Test for Liquefaction

This test was used to confirm the agarase activity. When the enzyme substrate system, was immediately placed in frozen condition after mixing, it solidified within 4 h. After this, the system was brought to room temperature and incubated at 30ºC for 2 h. The same system was once again kept in frozen condition to check its behavior. The sample did not solidify even in the frozen condition on its incubation for 24 h. This was considered to be indicative of irreversible liquefaction of agarase due to agarolysis [14].

Kinetic Parameters

The K_m and V_{max} values were obtained by Lineweaver-Bruk plots of agarase activity at 40°C using various concentrations of LMP agarose as substrate. The K_m of agarase was 4 mg/mL, and the V_{max} was 2.75 μmol/min/mg. However, K_m and V_{max} values of 16.67 mg/mL and 3.77 unit/mL·min have been reported by Ho *et al.* [34].

Protoplast Isolation

The isolated agarase, in combination with commercially available Cellulase and Macerozyme yielded significantly large amount of protoplasts of *G. acerosa* and *G. corticata*, following relatively a very simple protocol. These freshly isolated protoplasts were circular in shape and ranged from 10~30 μm in diameter (Fig. 7). It is also important to note that to the best knowledge of the authors, this is the first report on protoplast isolation from *G. acerosa.* However, reports on protoplast isolation from *Gracilaria* species are available [11-13]. The production of such hydrolases in marine environment would have an ecological consequence as the drift seaweed would be degraded by such enzymes and release soluble organics which would influence microclimatic conditions of coastal areas. The present study will enable the utilization of these cultures as indigenous sources of phycocolloid degrading enzymes, such as agarase, which in turn will be useful as a biotechnological tool for the development of superior quality algal strains and also for other applications like isolation of DNA fragments from agarose gels, for preparing simple oligosaccharides from complex polysaccharides, for softening the agar media for culturing plant cells *etc.* [14].

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