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Agarase and β-mannanase production from *paenibacillus sp.*, MSL-9 utilizing diverse polysaccharides

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ABSTRACT

An agar utilizing bacterium, strain MSL-9 was identified as *Paenibacillus sp.* The bacterium was capable of utilizing wide range of polysaccharides such as agar, alginate, carrageenan, starch, xylan and galactomannans. It was acclimatized to mannan polymer, guar gum (GG) for mannanase production. The growth profile and extracellular enzyme production revealed optimal conditions as pH 8.0, 30°C for agarase (5.66 U/mg) and mannanase (5.73 U/mg) at 24 hrs and 48 hrs respectively. The enzymes were active at broad range of pH.

KEYWORDS

Polysaccharides;
Agarase;
Mannanase;
Paenibacillus sp.;
MSL-9.

INTRODUCTION

Hydrocolloids (Gums) are a group of hydrophilic compounds or biopolymers with diverse structure and functions. Among these include polysaccharides extracted from plants, algae (mainly seaweeds) and microbes. Agar, a structural polysaccharide in cell walls of some red algae, such as *Gelidium* and *Gracilaria* is an important jellifying agent for biochemical use and in the food industry. Agar is composed of two principal components; Agarose (70%) and agaropectin (30%). Agarose is the gelling component and comprises a linear chain of alternating 3-*O*-linked-α-D-galactopyranose and 4-*O*-linked-3, 6-anhydro-β-D-galactopyranose[1]. Similarly, galactomannans, another important group of polysaccharide produced in certain plants as cell wall and storage polysaccharide, are composed of β-1,4 linked mannopyranose units often possessing branch points from their 6-position linked to α-D galactose and are acetylated at *O*-2 and *O*-3 positions depending on origin. Several genera of bacteria that degrade and utilize agar and galactomannans have been isolated and characterized. Agarases catalyze the hydrolysis of agar. They are classified into α-agarase (E.C.3.2.1.158) and β-agarase (E.C.3.2.1.81) according to the cleavage pattern. α-Agarases cleave α-1,3 linkages to produce agarooligosaccharides of series related to agarobiose[2], while β-agarases cleave β-1,4 linkages to produce neoagarooligosaccharides of series related to neoagarobiose[3]. So far, several agarases have been isolated from different genera of bacteria found in seawater, marine sediments and other environments. Agarases have a wide variety of applications. They have been used to hydrolyze agar to produce oligosaccharides, which exhibit important physiological and biological activities beneficial to the health of human being[4]. Besides that, agarases also have other uses as tools to isolate protoplasts from seaweeds[5] and to recover DNA from agarose gels[6], and to investigate the composition and structure of cell

wall polysaccharide of seaweeds. Recent progress in cloning and sequencing of these enzymes has led to structure-function analyses of agarase[7-10]. This information will provide valuable insights into the use of this enzyme.

β-Mannanase (EC.3.2.1.78) is an enzyme capable of hydrolyzing β-1, 4 mannosidic linkages in the main chain of β-mannans, glucomannans and galactoglucomannans yielding manno-oligosaccharides and mannose[11]. β-Mannanase has wide biotechnological applications. They are used in textile, paper and pulp industry, in hydrolysis of coffee extracts, in detergent industry, in oil extraction of coconut meal, for degradation of thickening agents, as non-nutritional food additives, to improve the nutritional value of poultry feeds[12-14].

The agarases, and mannanase from various bacteria isolated from different sources are plenty and reported elsewhere whilst, reports on bacteria producing both agarase and mannanase are only few. In this context, we have selected an agar degrading bacterium; strain MSL-9 utilizing agar as sole source of carbon and energy. The strain was acclimatized for mannanase production by providing guar gum (GG) as sole source of carbon and energy. Utilization of other polysaccharides by the bacterium is also reported herewith.

MATERIALS AND METHODS

Bacterial strain and culture conditions

The microorganism was isolated from sea water, Colaba, India. The isolation of agarase producing strain was carried out as described by Lakshmikanth et al[15]. An agar degrading bacterium designated as strain MSL-9 stored at 4°C on minimal mineral salts (MMS) medium-agar slants in our laboratory was selected. The MMS medium was used for standardizing the culture conditions and optimization of production of extra cellular agarase by MSL-9. The medium contained ($g.l^{-1}$) of the following composition, K_2HPO_4, 0.38; $MgSO_4$, 0.20; $FeCl_3$, 0.05; $NaNO_3$, 0.3. The pH of the medium was adjusted to 7.0 and this was supplemented with agar (0.1%, w/v) as the sole source of carbon and energy. The bacterial cells were maintained on MMS agar plates/slants and sub cultured fortnightly.

Culture conditions for acclimatization to galactomannnan gum

The MMS medium supplemented with guar gum (0.3%) as only the source of carbon and energy was inoculated with bacterial seed culture previously grown on MMS media supplemented with agar. The cultures were maintained on MMS-agar (1.5%) and / guar gum (0.2%) plate/slants or LB agar (1.5%)/ guar gum (0.2%) slants and sub cultured fortnightly.

Cell morphology and biochemical characterization

The characterization of phenotypic features, Gram's staining, motility tests and various biochemical tests were carried out as described in Bergey's manual of systematic Bacteriology. The 16S rDNA sequence similarity was performed for molecular identification of the strain. The strain MSL-9 with agar-degrading activity was investigated for assimilation and degradation of polysaccharides ($1.5g.L^{-1}$), alginate, cellulose, CM-cellulose, carrageenan, guar gum and starch, which were added to MMS medium separately and pH was adjusted to 8.0. The culture supernatant obtained after 24 to 48 hrs of incubation at 30°C was analyzed for growth and degradation of polysaccharide.

In plate assay method for polysaccharide degradation test, the MMS basal medium supplemented with the polysaccharide was solidified using agar (1.5% w/v), inoculated with strain MSL-9 and allowed to grow for a week at 30°C. Degradation of starch and agar was detected by staining with Lugol's iodine solution whilst, that of CM-cellulose, cellulose and guar gum by staining with congo red solution.

Production of agarase and mannanase

In 250ml flasks, 50 ml aliquots of the MMS medium supplemented with either agar or guar gum (0.3%) was dispensed and pH of the medium was adjusted 8.0, and then autoclaved at 121°C for 15 min. After sterilization, the flasks were inoculated with 1ml of seed culture grown for 24 hrs and then incubated in orbital shaker maintained at 30°C, 160 rpm. The culture supernatants were analyzed for enzyme activities.

Extraction of enzyme

At the end of incubation period, the bacterial cells were separated from the culture by centrifugation at 10,000 $rev.min^{-1}$ at 4°C for 15 min in a cooling centrifuge. The clear supernatant thus obtained used as the crude enzyme source. The protein content of enzyme preparation was determined by the method of Lowry et.al[16].

Agarase assay

The agarase assay was performed as described by

Lakshmikanth et.al[15] using neocuproin method of Dygert et.al[17]. One unit of the enzyme activity was defined as the amount which liberates 1 μM galactose per minute under assay conditions.

β-Mannanase assay

Mannanase activity was determined by DNS method[18]. The mannolytic activity was defined as the amount of enzyme that liberates 1μg of mannose per minute under assay conditions

RESULTS AND DISCUSSION

Identification and biochemical characterization of strain MSL-9

The morphological and biochemical properties of strain MSL-9 are as presented in TABLE 1. The cells are rod shaped, motile, spore forming, facultative anaerobic, Gram positive bacteria. Colonies grown on MMS agar plates are non pigmented, circular, slightly convex, mucilaginous and cream colored. Positive for oxidase and urease. Citrate, succinate, acetate, fumarate, malate, and oxalate were utilized. Hydrolysis of casein, starch, carrageenan, and alginate, guar gum, locust bean gum was observed. While, cellulose and CM-Cellulose were not utilized. Growth was not inhibited by the presence of 5% NaCl. Growth occurred between 25°C and 37°C, optimal between 28°C and 30°C at an optimal pH of 8.0. Acid but no gas was produced from glucose, galactose, sucrose and mannose.

Based on 16S rDNA sequence analogy, the strain MSL-9 was identified as belonging to genus *Paenibacillus sp.* and designated as *Paenibacillus sp.* MSL-9. The nucleotide sequence was deposited in GenBank (Accession number FJ 859876).

Production of agarase and mannanase

A typical growth profile and agarase production by *Paenibacillus sp*. MSL-9 is presented in figure 1. The agarase production in extra cellular medium was observed after 6 hr of incubation of bacterium in MMS medium supplemented with agar and reached maximum at 24 hr of incubation. Whilst, the mannanase production in extracellular medium was observed after 12 hrs of incubation of bacterium with GG and reached maximum at 48 hrs of incubation (Figure 2). The production of agarase and mannanases from *Paenibacillus sp.*, MSL-9 at optimal conditions pH 8 and 30 °C is presented in TABLE 2.

TABLE 1 : Phenotypic and biochemical characteristics of *Paenibacillus sp*. MSL-9

Growth on nutrient broth	+
Growth temperature:	
5°C	-
25°C	+
30°C	+
45°C	+
pH range of growth	5.0-11.0
Relation of free oxygen	aerobic
NaCl requirement	-
Morphological features:	
Form	rods
Gram staining	Gram positive
Motility	motile
Cultural Characteristics:	
Agar cultures	Colony round,mucilaginous
Pigment production	absent
Hydrolysis of:	
Starch	+
Casein	+
Gelatin	+
Carrageenan	+
Alginate	+
Galactomannans	+
CMC	-
Cellulose	-
Urea	+
Indole	+
MR-VP	+
Assimilation of carbohydrates:	
Glucose	+
Galactose	+
Lactose	+
Sucrose	+
Maltose	+
Fructose	+
Mannitol	+
Utilization of :	
Acetate	+
Malate	+
Fumarate	+
Succinate	+
Lactate	+

The mannan degrading enzyme systems have been described in a great variety of bacteria and fungi species

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and it was found to be inducible. The reports on marine isolates producing β-mannanase are only few[19]. Many bacteria of *Paenibacillus sps* are known to degrade and assimilate agar and mannan polysaccharides. Both agarases and mannanase produced by *Paenibacillus sp.* MSL-9 were found to be active at alkaline pH and hence have lot of industrial potentials. Moreover, the bacterium is capable of utilizing diverse polysaccharides of different origin and hence can be used in the production of simple oligosaccharides, which could serve as starting material for production of biofuels using appropriate microorganisms.

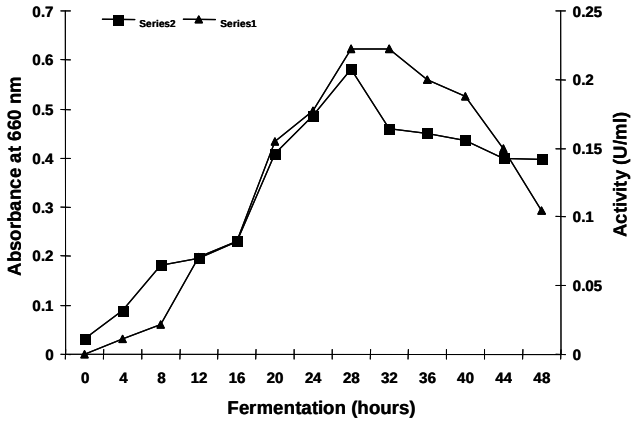

Figure 1 : The growth profile and agarase production from *Paenibacillus sp*. MSL-9.

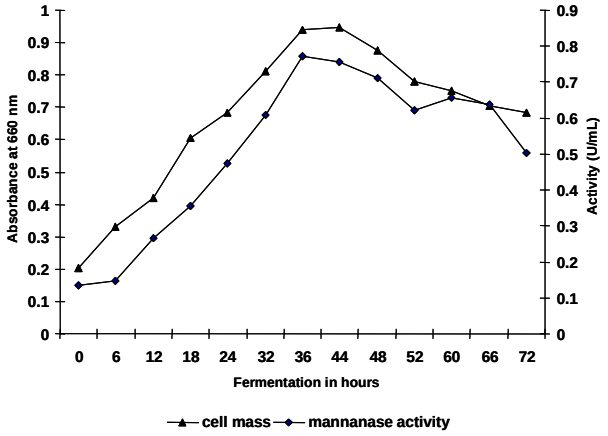

Figure 2 : The growth profile and mannanase production from *Paenibacillus sp.* MSL-9.

TABLE 2 : Agarase and mannanase activities in crude enzyme preparations of *Paenibacillus sp*. MSL-9.

Carbon Source	Enzyme	Activity (U/mg)
Agar-Agar	Agarase	5.66
	Mannanase	ND
Guar Gum	Agarase	ND
	Mannanase	5.73

ND-Not Detected.

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