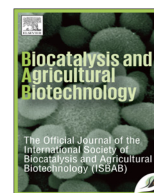
Biocatalysis and Agricultural Biotechnology 6 (2016) 219–226

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab



Characterization of extracellular agarase production by *Acinetobacter junii* PS12B, isolated from marine sediments

T. Leema Roseline, N.M Sachindra*

Department of Meat and Marine Sciences, CSIR-Central Food Technological Research Institute, Mysore 570020, India

ARTICLE INFO

Article history:
Received 8 February 2016
Received in revised form
21 April 2016
Accepted 23 April 2016
Available online 25 April 2016

Keywords:
Agar
Agarase
Acinetobacter junii
Response surface methodology

ABSTRACT

A marine, gram negative, rod shaped bacterium that degrades agar was isolated from the east coast of India and was identified to be *Acinetobacter junii* PS12B based on 16S rRNA gene sequencing. The effect of different culture conditions, namely pH of the medium, time and temperature of incubation and the agar concentration in the medium, on the agarase production by the strain, was evaluated. The agarase production was significantly ($p<0.05$) affected by the culture conditions. The optimum conditions as determined by response surface methodology were found to be a temperature of 35 °C, pH of 7.0 and time of fermentation of 33 h and agar concentration of 0.5%. Under the optimum conditions, the isolate produced 0.17 units of agarase per ml of the medium. Ammonium nitrate and sodium nitrate were the best nitrogen source in the medium for agarase production by the isolate. Supplementing the agar containing minimal media with simple sugars like glucose and galactose was found to enhance agarase production significantly by two fold. The isolate was also able to degrade carrageenan, which indicates its role in utilization of marine polysaccharides for the production of bioactive oligosaccharides.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Most of the marine microbes have evolved degradation enzymes to use agar or agarose as an energy and carbon source (Pluvinage et al., 2013) Marine bacteria secrete a specific glycoside hydrolases (GH) enzyme agarase to utilize algal cell wall polysaccharides as a carbon and energy source. Agarases are classified based on the mode of action, into α-agarase (EC 3.2.1.158), which cleaves α-1, 3 linkages to produce agaro-oligosaccharides and β-agarase (EC 3.2.1.81) which cleaves β-1, 4 linkages to produce neoagaro-oligosaccharides. β-Porphyranases hydrolyze the β-(1, 4) glycosidic bonds of the porphyran moieties (G-L6S) in agar and produces oligosaccharides with reducing ends at G residue (Hehemann et al., 2010). Based on the amino acid sequence similarity, β-agarases are found in four distinct glycoside hydrolases (GH) families in the CAZy database includes GH16, GH50, GH86, and GH118, whereas α-agarase belong to GH96. To date, a vast number of agarolytic micro-organisms from taxonomically diverse genera, as well as agarases and their encoding genes have been well reported and summarized (Chi et al., 2012; Fu et al., 2010; Michel et al., 2006). The approaches to increase the productivity of agarases by agarolytic organisms would be to isolate hyper-producers or mutants for agarases or by cloning the genes encoding for agarases and expression of these genes by molecular genetic techniques.

Agarase producing bacteria has been isolated from different environments. Most of the agarase producing isolates were found to be of marine origin. However, Feng et al. (2012) isolated an agarolytic *Rhodococcus* sp. from printing and dyeing wastewater. Agarolytic bacteria have also been isolated from terrestrial soil (Suzuki et al., 2003; Hosoda et al., 2006; Lakshmikanth et al., 2006b). In recent years, several novel agarase producing strains were isolated from marine environments. Agarolytic *Aliagarivorans marinus and Aliagarivorans taiwanensis* (Jean et al., 2009), *Simiduia agarivorans* (Shieh et al., 2008) were isolated from seawater. *Flammeovirga* sp. MY04 (Han et al., 2012), *Agarivorans* sp. (Hu et al., 2009) isolated from marine sediments were found to produce β–agarase. Seaweeds were found to be one of the primary sources of agarolytic bacteria in the marine environment (Lee et al., 2013; Oh et al., 2010). Agarolytic bacteria have also been isolated from the gut of mollusks (Fu et al., 2008; Jung et al., 2012).

Applications of agarases are well-known in the areas of food, pharmaceuticals, cosmeceuticals, and biotechnology. The neoagarooligosaccharides have been considered to have high economic value, because of their physiological and biological activity without toxicity, as GRAS. These oligosaccharides especially neoagarotetraose and neoagarohexaose exhibit antioxidative activity, scavenging hydroxyl free radicals and superoxide anion radicals and inhibiting lipid peroxidation (Wang et al., 2004; Wu et al., 2005). Additionally, neoagarooligosaccharides acts as a low-calorie

* Corresponding author.
E-mail address: nmsachindra@cftri.res.in (N. Sachindra).

http://dx.doi.org/10.1016/j.bcab.2016.04.007
1878-8181/© 2016 Elsevier Ltd. All rights reserved.

additive to improve food quality, inhibit the growth of bacteria, and slow down the degradation of starch (Giordano et al., 2006). They also have a moisturizing effect on skin and a whitening impact on melanoma cells (Kobayashi et al., 1997; Ohta et al., 2004). Because of these functional implications, agar-derived oligosaccharides have broad applications in the health-food, pharmaceutical, and cosmetic industries potentially.

Single-factor optimization can be eliminated by using response surface methodology (RSM) which is used to explain the combined effects of all the factors in a biotechnological process (Anil kumar and Suresh, 2014). Therefore, the use of RSM in biotechnological processes is gaining much importance for the optimization of enzyme production (Beg et al., 2003; Kumar and Satyanarayana, 2004; Rao et al., 2006). Some statistical approaches have been employed for agarase production by *Agarivorans albus* YKW-34 (Fu et al., 2009), *Pseudoalteromonas* sp. JYBCL1 (Jung et al., 2012), and *Streptomyces lividans* (Park et al., 2014).

In the present work, a marine bacterial isolate identified as *Acinetobacter junii* PS12B was evaluated for agarase production under different culture conditions. The strain was characterized by investigating the growth and production of agarase in the presence of various parameters to exploit the organism for production of agarase. Further, the combined effect of different culture conditions was evaluated using RSM for optimization of enzyme production.

2. Materials and methods

2.1. Sampling, isolation, screening of agarolytic marine bacteria

Marine samples such as seaweeds, seawater, and sediment collected from the east coast of India (Rameshwaram, Mandapam and Tuticorin) were suspended in the sterilized minimal mineral salts medium (MMS) (Lakshmikanth et al., 2006b) containing (g/L) K_2HPO_4 (1.2), KH_2PO_4 (0.3), $MgSO_4$ (0.1), $FeCl_3$ (0.1), NH_4NO_3 (1.0), $CaCl_2$ (0.1) and agar (1.0) (pH 7.0). After incubation at 37 °C for 72 h, a loop full of the media was streaked on MMS media plates containing 1.5% agar. Following incubation, the colonies exhibiting an obvious clear zone around the colony or pit formation, indicative of agar degradation were selected as agarolytic bacteria and purified by repeated streaking. Further confirmation of agarolytic activity was carried out by spot inoculation of purified culture on MS agar plates and overlaying with Lugol's iodine after incubation for 24 h at 37 °C. The clear zone around the colony indicates the agarolytic activity.

2.2. Agarase activity measurement

The isolated and purified cultures were inoculated to the enzyme production medium (Lakshmikanth et al., 2006b) containing (g/L) K_2HPO_4 (0.38), $MgSO_4$ (0.20), $FeCl_3$ (0.05), NH_4NO_3 (1.0) supplemented with 0.3% agar as a sole source of carbon. The pH was adjusted to 7.0 before sterilization at 121 °C for 15 min. The culture was incubated at 37 °C on the orbital shaker at 180 rpm. After 24 h of incubation, the production media was centrifuged at 8000 rpm for 15 min at 4 °C and the supernatant collected was taken as a crude extracellular enzyme.

Agarase activity was measured by the release of reducing sugars according to the 3, 5-dinitro salicylic acid (DNS) method (Miller, 1959). Briefly, 1 ml of the crude enzyme solution was mixed with 1 ml of substrate (0.25% agar in 20 mM Tris-Cl buffer, pH 7.5), vortexed and incubated for 60 min at 37 °C. After incubation, 1 ml of DNS solution was added to the mixture and heated in boiling water bath for 10 mins, cooled, and absorbance was measured at 540 nm (Hitachi U 2900). One unit (U) of activity was defined as the amount of enzyme that released 1 μmol of galactose equivalents from the substrate per minute under the specified assay condition. The specific activity was expressed in one μmol galactose equivalents/min/mg protein. Protein concentration was determined by Lowry's method using bovine serum (BSA) as the standard (Lowry et al., 1951). Galactose was used as a reference for preparing the standard curve.

2.3. Random amplification of polymorphic DNA (RAPD) analysis of agarolytic isolates

In order to determine the similarity between the strains, Random Amplification of Polymorphic DNA (RAPD) was performed using a universal M13 primer (5′-GAGGGTGGCGGTTCT-3′) for microbial typing (Schillinger et al., 2003). The amplified product was run on 1.8% agarose gel. The RAPD banding pattern was further analyzed using GeneSys® software (SYNGENE, UK) and the similarity of the band profiles and the grouping of the RAPD-PCR patterns were calculated based on the Pearson's coefficient and agglomerative clustering with unweighted pairs group matching algorithm (UPGMA), and the dendrogram was constructed using GeneSys® software.

2.4. Biochemical and molecular characterization of the selected agarolytic isolate

The strain selected based on screening for the agarolytic activity was characterized by performing various biochemical tests such as gram staining, motility test, oxidase activity, catalase production, Methyl red-Voges Proskauer (MR-VP), oxidation and fermentation of sugars. Utilization of glucose, mannitol, inositol, sucrose and 31 other sugars was assessed using Hi-carbo kit (Hi-Media Laboratories, India). Hydrolysis of esculin, gelatin and starch were also performed using standard techniques. Growth in mineral salts media at different temperatures of 10 °C, 25 °C, 37 °C and 50 °C, at different pH of 4.0, 7.0 and 10.0 were performed to characterize the growth of organisms. Hemolytic activity of the cultures was tested using blood agar. Morphological analysis of the culture isolate was carried out by scanning electron microscopy (SEM) according to the method of McDougall et al. (1994). Briefly, selected strain grown in Tryptone soya broth were centrifuged, washed thrice with phosphate buffer saline (pH 7.0) to remove salts, fixed with glutaraldehyde (2%) and subjected to gradual alcoholic dehydration. The processed samples were then analyzed by SEM (Leo-435 VP, Leo Electron Microscope, Zeiss Ltd., and Cambridge, UK).

For molecular characterization of the agarolytic isolates, genomic DNA was extracted from the bacterial strain using the standard DNA isolation protocol (Sambrook and Russell, 2001). For 16S rRNA gene sequencing and phylogeny analysis, 16S rRNA gene was amplified using the universal bacterial forward and reverse primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (51-GGTTACCTTGTTACGACTT-31) respectively. Each PCR mixture of 25 μl contained template DNA (20 ng/μl), 0.2 μM of each primer, 0.25 mM of each deoxynucleoside triphosphate (dNTP), and 2.5 U of *Taq* DNA polymerase in a final concentration of 10 mM Tris–HCl, 50 mM KCl, and 1.5 mM $MgCl_2$. PCR was performed under the following cycle conditions: an initial denaturation step at 94 °C for 5 min and 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 2 min, and extension at 72 °C for 2 mins, with a final extension step at 72 °C for 10 min (Primus 25 Thermal cycler). The PCR product of was purified and sequenced at Amnion Biosciences, Bangalore, India. The 16S rRNA gene sequences obtained was subjected to nBLAST for similarity identification. Multiple alignment and sequence similarity with that of available sequences of reference strains from GenBank database were analyzed using

Download English Version:

https://daneshyari.com/en/article/2075402

Download Persian Version:

https://daneshyari.com/article/2075402

Daneshyari.com