INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

MULTIPLE ENZYME PRODUCER *GORDONIA TERRAE* FROM LONAR LAKE

MR. Bhat*, SU. Dubey and RY. Badi

Department of Biotechnology and Bioinformatics, D.Y. Patil University, CBD Belapur, Navi Mumbai - 400614, Maharashtra, India.

ABSTRACT

Enzymes are protenic in nature, which catalyses the formation of product from specific substrate. Enzymes plays important role in many bioconversion processes. Hydrolytic enzymes are the enzymes which catalyze the hydrolysis of a chemical bond of the substrate. L-asparaginase is an antileuckemic enzyme which specifically targets cancerous cells in the human body. It converts L-asparagine into L-aspartic acid and releases ammonia. In the current study L-asparaginase producing isolates were screened from Water samples of Lonar Lake, Buldhana, Maharashtra. The maximum L-asparaginase producing isolate was found to be *Gordonia terrae* strain 5-Sj-4-3-2-M by 16sRNA sequencing. Along with L-asparaginase production the isolates potential to produce other hydrolytic extracellular enzymes like protease, amylase, esterase, lipase, inulinase, L-glutaminase was checked qualitatively. *Gordonia terrae* strain 5-Sj-4-3-2-M was found positive for all the enzymes except esterase.

Keywords: L-asparaginase, *Gordonia terrae,* Lonar lake, enzymes.

INTRODUCTION

Enzymes act as biocatalysts and target specific substrates to convert them into specific products. Enzymes form integral part of all the metabolic reactions of living cells. Microbial enzymes are in practice since ancient times. Microbial cells acts as tiny factories for various bioconversions [2]. Role of microorganisms in production of various chemicals, antibiotics and enzymes is well known. The enzymes thereof find applications in industries like pulp and paper, leather, detergents and textiles, pharmaceuticals, chemical, food and beverages, biofuels, animal feed and personal care etc [1]. Increasing interest and demand for enzymes having novel properties has become the need of hour of advanced biotechnology [3]. L-asparaginase is an enzyme had been isolated from almost all types of microorganisms like bacteria, fungi, actinomycetes, protozoa, algae, yeast and from plants as well [9]. It is used as chemotherapeutic agent against acute lymphoblastic leukemia (ALL). Currently used enzyme is from E.coli and Erwinia species. Screening of L-asparaginase from different sources is become trivial as the other available sources have shown toxicity and immunological reactions [10].

Lonar lake is located in Bludhana district of Maharashtra. It has basalt rocks within and in the surroundings. This lake is a unique ecosystem for halophilc and alkalophilic microorganisms. It had been formed 50,000 yrs ago by due to high velocity meteoritic impact [4]. The genus Gordonia was proposed by Tsukamura (1971), and strains of this genus belong to the mycolic acid-containing group of the actinomycetes and to the suborder Corynebacterineae [5]. Gordonia strains are widely distributed in soil and aquatic habitats, including marine sediments (Goodfellow & Maldonado, 2006). There are more than 20 species of geus Gordonia studied so far. Some of them are *Gordonia bronchialis, G. sputi ,G. terrae, G. aichiensis ,G. amarae, G. soli , G. defluvii* etc. [5]. Gordonia have been isolated from various environmental sources and some of them

reported to be involved in the degradation of xenobiotic compounds.

In the current study L-asparaginase producing isolates were screened from water samples of Lonar Lake, Buldhana, Maharashtra. The isolate giving high extracellular L-asparaginase production was found to be *Gordonia terrae* strain 5-Sj-4-3-2-M by 16sRNA sequencing. Along with L-asparaginase the isolates potential to produce other enzymes like protease, amylase, esterase, lipase, inulinase, L-glutaminase was checked qualitatively.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study investigation were of analytical grade and procured from Hi – media (India) and Merck (India).

Isolation of L asparaginase producing bacteria

Water samples were collected from various sites of Lonar lake,Buldhana Maharashtra State, India, in sterile plastic tubes, transported to the laboratory aseptically and stored at 4°C for further use. 10 ml of the water sample was inoculated in 100ml of Sterile M9 broth containing 1% L-asparagine and few drops of Phenol red as pH indicator, incubated at 30^{0}C for 72 hours. The enriched samples were spread on Sterile Modified M9 agar plate containing 1% L-asparagine and few drops of phenol red. Enzyme production was accompanied by an increase in pH of the medium, which results in the formation of pink zone [7]. The selected colonies were maintained on asparagine rich agar slant at 4^{o}C.

Enzyme assay

The assay of L-asparaginase was carried with the help of nesselerization method [6]. One unit of L-asparaginase activity (IU) is defined as the amount of enzyme which liberates l µmol of ammonia per min at 30 ^{0}C and pH 7.4 [6,11]. The potential isolate was grown in Sterile modified M9 medium for 24 hrs and centrifuged at 5000 rpm for 10 minutes at 4^0 C . Enzyme assay was carried out as follows [11]

1. 0.25 ml of Crude enzyme (Supernatent) + 1.25 ml of 0.2 M Borate buffer (pH8) +0.5 ml of 0.004 M L asparagines in borate buffer.
2. Incubation at 37^{0}C for 30 minutes
3. Add 0.5 ml of 15% TCA and centrifuge at 4000 rpm for 20 min
4. 1ml of supernatant +4ml of D/W +1ml of Nesslor's regent

Identification of bacterial isolate

The potential isolate S2 was further characterized by standard biochemical test according to Bergey's manual of systematic bacteriology. The results were then compared with standard biochemical chart of the isolate. Maximum L-asparaginase producing isolate was identified by using 16SrRNA sequencing technique.

Qualitative analysis of various enzymes

To check the presence of different enzymes following methods were carried out[8].

1. Extracellular protease activity
   Proteolytic activity of the culture was screened qualitatively in a medium containing 10% skim milk, and 2% NaCl. The medium was solidified by adding 2% of agar.
2. Extracellular amylase activity
   The presence of amylolytic activity on plate was determined by using standard nutrient agar medium with 1%starch. The plates were incubated for 48 hours and later were flooded with KI solution.
3. Extracellular lipase activity
   To determine lipase production, the strain was cultured on nutrient agar plate containing 2.5% olive oil and bromophenol blue (0.4 mg/L).
4. Extracellular l-glutaminase activity
   The presence of L-glutaminase activity on plate was determined using 0.5% of L-glutamine in nutrient agar medium and few drops of phenol red was added to 100ml of the medium. Phenol red was used as pH indicator.
5. Extracellular inulinase activity
   To detect the presence of inclines, the culture was grown on the medium containing 2g/L of inulin, 10 g/L of yeast extract, 20 g/L of MgSO4.7H2O, 2 g/L of KCl, 10% of NaCl, 20 g/L of agar. Inulin was the only source of carbon in the medium. Extracellular esterase activity: To see the presence of Esterase activity of the isolate, the organism was grown on the medium containing 10 g/L of yeast extract, 20 g/L of MgSO4.7H2O, 2 g/L of KCl, 0.1 g/L of CaCl2.H2O, 2% of NaCl, 20 g/L of agar, supplemented with 0.1% Tween-80

RESULTS

Out of total 3 samples only 4 isolates gave pink zone around them. Hence they were selected for enzyme assay. S2 gave maximum enzyme activity of 29.18 IU /ml. (Table no. 1)

Isolate	O.D. at 480 nm	enzyme activity in IU/ml
S1	0.233	19.88
S2	0.248	21.18
S3	0.161	13.73
S4	0.134	11.48

The isolate S2 was characterized biochemically and morphologically (Results not shown). The 16S rRNA sequencing result showed that the isolate was *Gordonia terrae strain 5-Sj-4-3-2-M*;
Potential of the isolate to produce various other hydrolytic enzymes was also investigated. Qualitative enzyme assay was carried out for L-glutaminase, protease, lipase, esterase, inulinase, amylase. (Figure a,b,c,d,e,f)

DISCUSSION

Water sample was collected from three different sites of Lonar lake. Sample was enriched in modified M9 broth. The enriched sample was spread on sterile modified M9 plates. Only 4 colonies gave pink color zone around them. Pink zone was obtained due to change in the pH of the media after release of ammonia from degraded L-asparagine the substrate by the action of extracellular enzyme L-asparaginase. Those 4 colonies were selected for further analysis and their L-asparaginase production was checked by nesselerization method [6]. Isolate number S2 gave maximum L-asparaginase production of 21.18 IU/ml. Hence that was selected for further analysis.
Isolate S2 was found to be an orange pigment producer. It was further found to be *Gordonia terrae strain 5-Sj-4-3-2-M* by 16 sRNA sequencing method.
Ahead the potential isolates ability to produce other extracellular enzyme was carried out. Isolate showed precipitation of paracasein around the colonies after 48 hours indicating the enzyme protease is being produced by the isolate (Fig. a).
Potential to produce amylase was checked on 1% starch agar plates. Upon addition of iodine colonies showed zone of clearance around (Fig. b). Extracellular production of lipase was observed by blue zone of clearance around colonies on nutrient agar inoculated with olive oil and bromothphenol blue (Fig. c). Extracellular L-Asparaginase and L-Glutaminase activity was also observed for isolate along with pink zone on plate around colonies. The plates were nutrient agar with 1% asparagine and glutamine respectively along with phenol red (Fig. d,e). The isolate found negative for production of esterase as no zone of hydrolysis was observed around the colonies on agar upto 48 hours (Fig. f). Lastly extracellular inulinase production ability of isolate was checked by adding inluine as the only carbon sourse in the nutrient medium and growth was seen. Growth indicated that the organism could produce inulinase and utilise inuline for its growth on the agar (Fig. g).

CONCLUSION

Thus our study reveals the isolation of a novel L-asparaginase producer *Gordonia terrae* strain 5 SJM-3-2-M from Lonar Lake. The isolate was found to have potential of extracellular production of enzymes like L-Glutaminase, lipase, protease, inulinase and amylase. Thus there is no doubt that further study on same isolate could reveal its encrypted potential.
This is the very first report of its kind on L-asparaginase producing *Gordonia terrae* from Lonar Lake and qualitative study of other enzymes.

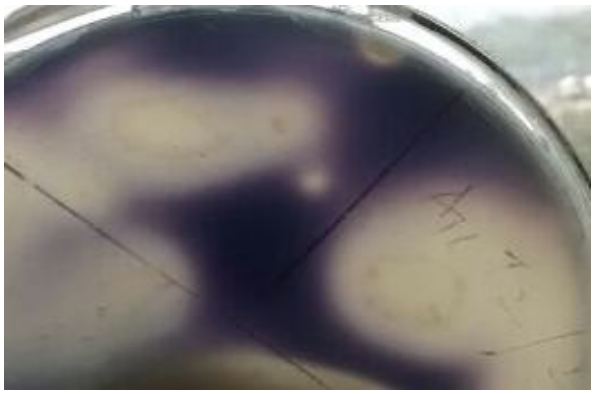

Fig. a: Positive protease activity

Fig. b: Positive amylase activity

Fig. c: Positive Lipase activity

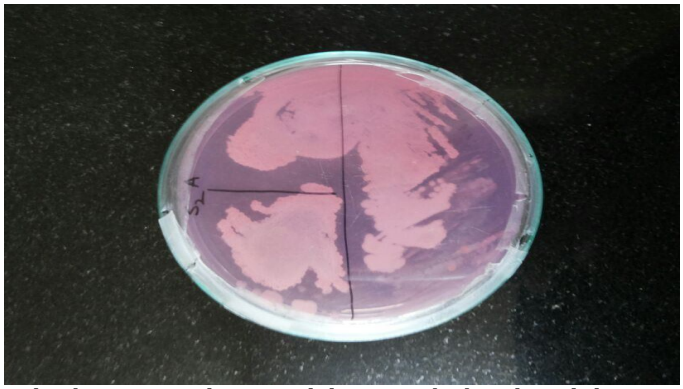

Fig. d: L-asparaginase activity growth showing pink zone

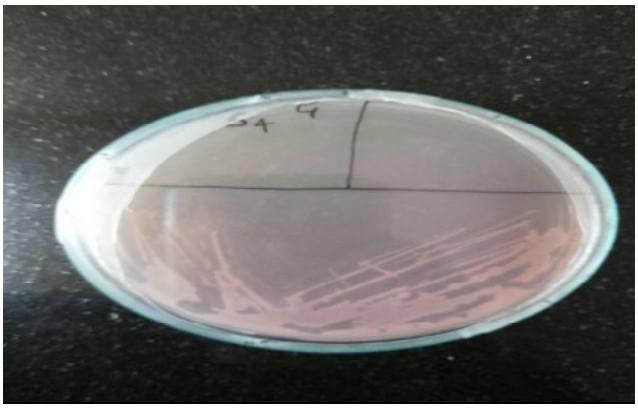

Fig. e: L-glutamianse activity growth showing pink zone

Fig. f: No hydrolysis around colonies , esterase negative

Fig. g: Growth on plate with Inulune as sole source of Carbon Photoplates showing the enzyme activity

REFERENCES

1. Adrio JL and Demain AL. Microbial Enzymes. Tools for Biotechnological Processes. Biomolecules. 2014;4:117-139.
2. Bhat MR and Microbial L. asparaginase an enzyme with encrypted potential. Journal of Pure and Applied Microbiology. 2012;6(2):895-898.
3. Chen Zhang and Se-Kwon Kim. Research and Application of Marine Microbial Enzymes. Status and Prospects. Marine Drugs. 2010;8:1920-1934.
4. Tambekar DH. Studies on biosurfactant production from lonar lake's achromobacter xylosoxidans bacterium. International journal of advances in

pharmacy. Biology and chemistry. 2012;1(3):415-419.
5. Goodfellow M, Yashwant K and Maldonado L. Bergys manual of systematic bacteriology. 2012;5:419-435.
6. Imada S, Igarasi K, Nakahama and Ison M. Asparaginase and Glutaminase activities of microorganism. Journal of General Microbiology. 1973;23:1163-1164.
7. Gulati R, Saxena RK and Gupta R. A rapid plate assay for screening of L-asparaginase producing microorganisms. Letters in Applied Microbiology. 1997;24:23-26.
8. Anil kumar S. Screening of extracellular hydrolytic enzymes from marinobacter hydrocarbonoclasticus strain ak5. The Bioscan. 2010;5(1):97-99.
9. Savitri, Asthana N and Azmi W. Microbial L-asparginase a potent antitumor enzyme. Indian journal of Biotechnology. 2003;2:184-194.
10. Verma N, Kumar K, Kaur G and Anand S. L-asparaginase a promising chemotherapeutic agent Critical review in Biotechnology. 2007;27:45-62.
11. Wriston JC and Yellin TOL. Asparaginase. A Review, Advanced Enzymes. 1973;39:185-248.