IJPSR (2019), Volume 10, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 February 2019; received in revised form, 20 May 2019; accepted, 01 June 2019; published 01 November 2019

ISOLATION AND CHARACTERIZATION OF L-ASPARAGINASE EXTRACELLULAR ENZYME PRODUCING BACTERIA FROM INDUSTRIAL SOIL SAMPLES

Rajesh Singh Tomar, Neha Sharma * and Shuchi Kaushik

Amity Institute of Biotechnology, Amity University, Gwalior - 474005, Madhya Pradesh, India.

Keywords:

Enzyme activity, *Salmonella* sp., *Proteus vulgaris, Bacillus subtilis,* L- Asparaginase, Algpred bioinformatics tool

Correspondence to Author:
Dr. Neha Sharma

Assistant Professor,
Amity Institute of Biotechnology,
Amity University, Gwalior - 474005,
Madhya Pradesh, India.

E-mail: nsharma2@gwa.amity.edu

ABSTRACT: Industrial soil contains a variety of L-Asparaginase producing microorganisms such as bacteria and fungi. Microorganisms can produce different types of enzymes which are frequently used in different types of industries for different purpose. In the present study, we focused on isolation of L-Asparaginase enzyme producing bacteria from industrial soil samples collected from different regions of Gwalior. These bacterial isolates were *Salmonella* sp., *Proteus vulgaris, Bacillus subtilis* which were identified based on colony morphology, Gram staining, biochemical tests and using selective and differential media. All the tested isolates were able to produce enzymes like L-Asparaginase. All the three bacteria which were isolated from soils of a different region of Gwalior able to produce L-Asparaginase enzyme. Maximum L-Asparaginase activity was shown in second (II) bacterial isolate *Salmonella* sp., which was 12.734 mg/ml. *Salmonella* sp. were not reported to show any type of allergenicity when their sequences were checked by bioinformatic tool Algpred. So, these bacterial isolates are considered as a good source of enzyme production and may be used at an industrial level. Thus from the present study, it may be concluded that microbes from soil sample can be a good source of industrially important enzymes.

INTRODUCTION: Enzymes have valuable medical and industrial applications. L-asparaginase (EC 3.5.1.1) belongs to family amidohydrolases, which catalyzes the hydrolysis of L-asparagine amino acid and makes L-aspartate and ammonia as byproducts [1]. Biochemistry of enzyme and their enzyme kinetics and the selection of proper substrate are required for enzyme assay [2]. For enzyme assays pH temperature, ionic strength, the concentration of substrate, and enzyme should be in optimum condition [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(11).4937-41

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).4937-41

It is produced by a large number of microorganisms that include *Pseudomonas stutzeri* [4], *Pseudomonas aerugenosa* [5,] and *E. coli* [6]. It is used for the treatment of acute lymphoblastic Leukemia, preliminary and advanced stages of cancer [7, 8] so L-Asparaginase is a critical extracellular enzyme of medical importance as an anti-cancer agent and an anti-microbial agent. Asparaginase is commonly used in place of heat treatment of starchy food in the industry to reduce the formation of the acrylamide, which is suspected human carcinogenic [8, 9]. Currently, L-Asparaginase has great demand in recent years at the level of food industries and pharma industries (40%) [10, 11].

Databases and bioinformatics tools can be used to find allergens, haptens, epitopes, and the presence of mast cell [12]. Algpred a bioinformatics tool is used in the prediction of allergic proteins, mast cell,

and IgE epitopes in enzyme producing bacteria by various methods incorporated in the software [13].

MATERIALS AND METHODS:

Collection of Soil Samples: Soil samples were collected from different sites of Gwalior region, Madhya Pradesh.

Isolation and Identification of Bacteria: First, the microorganisms present in soil samples were cultured on nutrient agar medium, and the morphology, colony characteristics of organisms were based on shape, size, odor, margin and surface characteristics. Gram staining was used to differentiate between gram positive and gram negative microorganism. Spore staining was also done to check spore chain morphology in all the bacterial isolates.

After phenotypical identification of bacteria, further confirmation was done based on their biochemical characterization *viz.*, annitol salt agar test, amylase production, hydrolysis of gelatin, IMViC test, catalase test, Widal test, skim milk test, carbohydrate fermentation test, Simmon citrate test and urease test by standard methods.

Enzymatic Assay: Enzyme assay was used to identify the production of a particular enzyme by the bacteria. The enzyme assay was done at the level of both qualitative and quantitative.

Qualitative Analysis: Qualitative test was done with the help of Nessler's reagent (Hans Bisswanger 2014). For this assay screening media was prepared. The constituents of this media (gm/ltr) include Na_2HPO_4 (6), NaCl (0.5), KH_2PO_4 (3), $MgSO_4$ (1 M/ltr), L-Asparaginase (5), $CaCl_2$ (0.1M/ltr), 20% glucose stock solution (10 ml), agar (2%), autoclaved at 15 psi for 15 min. Then, streaking of all five bacterial isolates was done on these media plates, but the concentration of phenol red (2.5%) which was prepared in ethanol was different in each plate. First media plate having 80 µl phenol red, the second plate contains 120 µl phenol red, the third plate contains 240 µl phenol red and the fourth plate contain 360 µl phenol red. Incubated the plates at 37 °C for 24 h to produce colonies with pink color zone around them [14].

Quantitative Analysis: Determination of specific activity.

Enzyme Preparation: All the bacterial isolates in nutrient broth media was cultivated at room temperature for 16 h on the shaker. After incubation 1 ml of all five bacterial culture was centrifuged at 10,000 rpm for 10 min at 4 °C. The Supernatant was taken, which is used in the enzyme assay.

Estimation of Protein: Amount of protein in the sample is estimated by Lowry's method. This method is used for determining the concentration of protein in sample [15].

Enzyme Activity: Enzymatic activity means the rate at which substrate molecule converts into a chemically modified product with the help of an enzyme. Activity is defined in unit/ml.

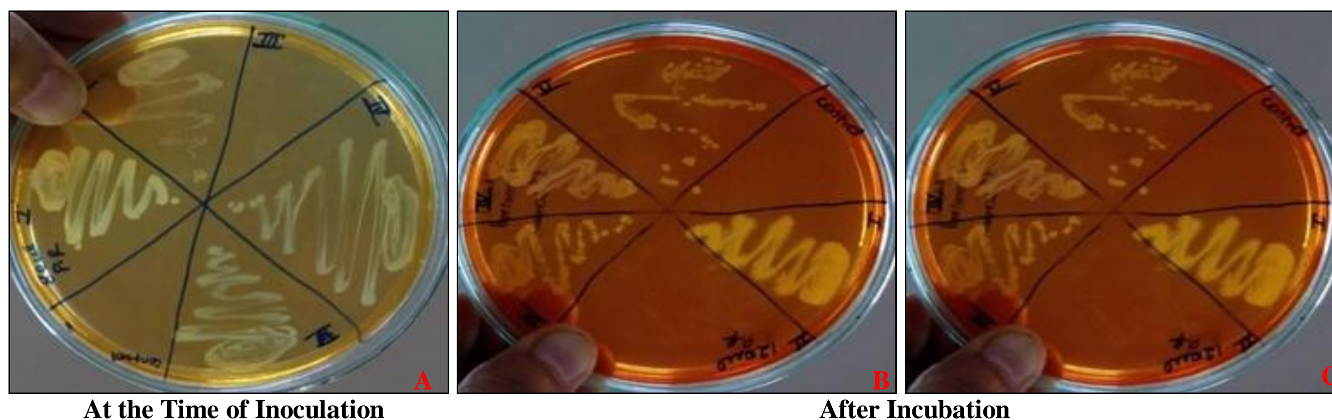
Calculation of Enzyme Activity: Activity of all five enzymes in all five bacterial isolates was calculated by the equation of standard graph of 4 mM ammonium chloride.

Calculation of Specific Activity of Enzyme: Specific enzyme activity is the number of enzyme units/ ml divided by the protein concentration in mg/ml. It is a ratio of enzyme activity to protein concentration. Its unit is µmol/min/mg or units/mg. It is used to measure the purity of an enzyme. Specific activities of each enzyme of each bacterial isolates were calculated by the following formula [16].

Specific activity = Enzyme concentration / Protein concentration

RESULTS AND DISCUSSION:

Qualitative Analysis of Asparaginase Enzyme: Primary screening of L-asparaginase producing isolates from the soil is done by the qualitative method. All the screened isolates were found to be positive for L-asparaginase production by Nessler's reagent. Upon primary screening, all the five isolates were found to be positive as they showed growth on modified M9 medium. When the concentration of phenol red dye was increased from 80 µl to 360 µl, the first bacterial isolate showed growth and the pink zone around the colonies on the medium containing the dye (240 µl). Second, fourth, fifth bacterial isolates, which showed very good L-Asparaginase enzymatic activity were not able to grow on M9 media plate when a high concentration of phenol red. The results are similar to the work done by Bhat *et al.* (2015) [17].



At the Time of Inoculation After Incubation

FIG. 1: L-ASPARAGINASE PRODUCING BACTERIA

Estimation of Concentration of Protein: It was done by Folin Lowry Method. Estimation of protein in each bacterial isolates was calculated by the following equation of standard graph **Table 1**.

Estimation of Enzyme Activity: All the five bacteria which were isolated from soils of a different region of Gwalior able to produce L-Asparaginase enzyme.

TABLE 1: ENZYME ACTIVITY, PROTEIN CONCENTRATION AND SPECIFIC ACTIVITY OF L-ASPARAGINASE

S. no.	Name of Bacterial Isolates	Enzyme activity (µmol/min)	Protein Concentration (mg/ml)	Specific activity (µmol min^{-1} mg^{-1})
1.	*Salmonella* sp. (I)	9.682	1.493	6.484
2.	*Salmonella* sp. (II)	12.734	1.029	12.375
3.	*Salmonella* sp. (III)	9.266	1.695	5.466
4.	*Proteus vulgaris* (IV)	11.932	1.513	7.886
5.	*Bacillus subtilis* (V)	10.731	1.672	6.418

Maximum L-Asparaginase activity was shown in the second (II) bacterial isolate, which was 12.734 mg/ml. Since the results are similar to the work done by Pradhan *et al.,* (2013) [18]. Hence, it can be concluded that the soil of the plant growing field and agricultural field are a good source of asparaginase producing bacteria. These fields are a good source of minerals like nitrogen which are required for the production of asparaginase producing enzyme **Table 1**.

Algpred Method for Test of Allergy: It plays an important role in the pharmaceutical industry to check the allergenicity of any drug that the particular drug can be used in the treatment of diseases. The presently available L- Asparaginase is not sufficient to meet industrial demands. Hence, there is a continuous search for new L-Asparaginase with novel characteristics for industrial application obtained from diverse bacteria isolates **Table 2**.

So, their allergenicity nature can be identified with the help of bioinformatics tool Algpred. In it, Allergenicity is identified based on reactivity with the mast cell, Mapping of IgE epitopes, Amino acid composition, BLAST and presence of dipeptides. The sequences from different organisms belonging to the same group as studied in the present work were analyzed for their antigenicity. Since the results are similar to the work done by Bucholska *et al.,* 2018. He used bioinformatics tools to find allergens, haptens, epitopes, and the presence of mast cell, which has an advantage in the field of medicine.

Mahboobi *et al.,* in 2017, [19] focused on the application of bioinformatics tools to study about *E.coli* model because it is a good source of L-asparaginase enzyme which has an anticancer ability.

Hence, it can be concluded that Databases and Associated bioinformatics tools are very useful to study Allergens, Epitopes, and Haptens present in the test organisms. Although the use of such *in-silico* tools provide us rapid and reliable results and minimize the experimental work, cost, and time but these results further need to be validated by *in-vitro* and *in-vivo* studies.

TABLE 2: ALGPRED TABLE FOR L-ASPARAGINASE PRODUCING *SALMONELLA SP.*

S. no.	Sequence	Source	Alegpred			
			MAST	**Mapping of IgE Epitope**	**SVM Method amino acid composition**	**BLAST**
1	Accession: 25330459	*Salmonella enterica* subsp. Enterica serovar Typhi (strain CT18)	GI:	*Salmonella enterica* subsp. Enterica serovar Typhi (strain CT18)	NA	NA
2	Accession: 25290114	*Salmonella enterica* subsp. Enteric aserovar Typhi (strain CT18)	GI:	*Salmonella enterica* subsp. Enteric aserovar Typhi (strain CT18)	NA	NA
3	Accession: 25325344	Salmonella enterica subsp. Enterica serovar Typhi	GI:	*Salmonella enterica* subsp. Enterica serovar Typhi (strain CT18)	NA	NA
4	Accession: OKK60401.1 GI: 1115913392	*Salmonella enterica* subsp. Enterica serovar Typhi (strain CT18)	NA	NA	NA	NA
5	Accession: OKK35851.1 GI: 1115888731	*Salmonella enterica* subsp. Entericaserovar Typhi (strain CT18)	NA	NA	NA	NA
6	Accession: OKK33792.1 GI: 1115886662	*Salmonella enterica* subsp. Enterica serovar Typhi (strain CT18)	NA	NA	NA	NA
7	Accession: CHQ60210.1 GI: 814279481	*Salmonella enterica* subsp. enterica serovar Typhi (strain CT18)	NA	NA	NA	NA
8	Accession: CGB99631.1 GI: 814216873	*Salmonella enterica* subsp. Enterica serova	NA	NA	NA	NA

CONCLUSION: This study indicates that soil can provide a good source of asparaginase producing bacteria. This study aimed to isolate and characterize the enzyme producing bacteria from soil samples collected from different regions of Gwalior. The environment where we live is the habitat for various microorganisms, mostly bacteria. They have various industrial applications like enzymes production, fabric manufacturing, bioremediation, pharmaceutics, *etc*.

Microorganisms have become increasingly important as a producer of industrial enzymes. From the results obtained in this research, it could be concluded that organism with the potential to produce enzyme can be isolated from the soil of different environmental condition. This isolates could be exploited for the commercial production of the enzyme in the industry like baking, brewery, and detergent, pharmaceutical especially L-Asparaginase can be used in the treatment of different types of cancer and textile industry. Microorganism from a soil sample is a good source of L- Asparaginase producing bacteria. These bacterial isolates were pathogenic and also showed resistance towards antibiotics might be due to the environmental condition where they present, but they were not showing any type of allergenicity when it was checked by bioinformatic tool Algpred. So, these bacterial isolates are considered as a good source of enzyme production at industrial level.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: The authors declare no competing or conflicts of interest.

REFERENCES:

1. Fernandes AI and Gregoriadis G: Polysialylated asparaginase: preparation, activity and pharmacokinetics. Biochim Biophys Acta 1997; 1341: 26-34.
2. Harold B, Sandaruwan G and Steven D, Kahl Chahrzad Montrose, Sitta Sittampalam, Michelle CS, and Jeffrey R: Weidner Basics of Enzymatic Assays for HTS NCBI Bookshelf. A service of the National Library of Medicine, National Institutes of Health 2012.
3. Bisswanger H: Enzyme assays. Perspectives in Science 2014; 1: 41-55.
4. Manna S, Sinaha A, Sadhukhan R and Chakrabarty SL: Purification, characterization and antitumor activity of L-asparaginase isolated from Pseudomonas stutzeri. MB-405. Cur Microbiol 1995; 30: 291-98.
5. Abdel-Fatteh Y and Olama ZA: L-asparaginase produced by *Pseudomonas aeruginosa* in solid state culture: evaluation and optimization of culture conditions using factorial designs. Process Biochem 2002; 38: 115-22.
6. Qin M and Zhao F: L-asparaginase release from *Escherichia coli* cells with aqueous two-phase micellar systems. Appl Biochem Biotechnol 2003; 110(1): 11-21.
7. Jain R, Zaidi KU, Verma Y and Saxena P: L-Asparaginase: A promising enzyme for treatment of acute lymphoblastic leukiemia people's. Journal of Scientific Research 2012; 5(1): 29.

8. Cachumba JJM, Antunes FAF, Peres GFD, Brumano LP, Santos JCD and Silva SSD: Current applications and different approaches for microbial L-aspqwharaginase production. Braz J Microbiol 2016; 47: 77-85.
9. Zuo S, Zhang T, Jiang B and Mu W: Recent research progress on microbial L-asparaginases. Appl Microbiol Biotechnol 2015; 99(3): 1069-79.
10. Vimal A and Kumar A: Biotechnological production and practical application of L-asparaginase enzyme. Biotechnol Genet Eng Rev 2017; 33(1): 40-61.
11. Hosamani R and Kaliwal BB: L-asparaginase an anti-tumor agent production by *Fusarium equiseti* using solid state fermentation. Inter J Drug Discovery 2011; 3(2): 88-99.
12. Bucholska J, Minkiewicz P, Darewicz M and Iwaniak A: Databases and associated bioinformatic tools in studies of food allergens, Epitopes and Haptens- a review pol. J Food Nutr Sci 2018; 68(2): 103-11.
13. Shah AJ, Karadi AV and Parekh PP: Isolation, Optimization and production of L-asparaginase from Coliform Bacteria. Asian Journal of Biotechnology 2010; 2(3): 169-77.
14. Prakasham RS, Hymavathi M, Subba Rao C, Arepalli SK, Venkateswara Rao J, Kavin Kennady P, Nasaruddin K, Vijayakumar JB and Sarma PN: Evaluation of the anti-neoplastic activity of extracellular asparaginase produced by isolated *Bacillus circulans*. Appl Biochem Biotechnol 2010; 160: 72-80.
15. Dunn RR, Fierer N, Henley JB, Jonathan W and Holly L: Menninger. Home life factors structuring the bacterial diversity found within and between homes. PLOS 2013. doi.org/10.1371/journal.pone.006413
16. Imada A, Igarasi S, Nakahama K and Isono M: Asparaginase and glutaminase activities of micro-organisms. J Gen Microbiol 1973; 76: 85-89.
17. Bhat MR, Nair JS and Marar T: Isolation and identification of l-asparaginase producing salinicoccus sp. MKJ 997975 from soil microbial flora. Int J Pharma Sci Res 2015; 6(8): 3599- 05.
18. Pradhan B, Dash SK and Sahoo S: Screening and characterization of extracellular L-asparaginase producing Bacillus subtilis strain hswx88, isolated from Taptapanihot spring of Odisha, India. Asian Pac J Trop Biomed 2013; 3(12): 936-41.
19. Mahboobi M, Sedighian H, Hedayati M, Bambai B, Soofian SE and Amani J: Applying Bioinformatic tools for modeling and modifying type II *E. coli* L-Asparaginase to present a better therapeutic agent /drug for Acute Lymphoblastic Leukemia. International Journal of Cancer Management 2017.

How to cite this article:
Tomar RS, Sharma N and Kaushik S: Isolation and characterization of L-Asparginase extracellular enzyme producing bacteria from industrial soil samples. Int J Pharm Sci & Res 2019; 10(11): 4937-41. doi: 10.13040/IJPSR.0975-8232.10(11).4937-41.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Play store)