



Isolation, Characterization and Identification of L-Glutaminase, an Anticancer agent Producing Bacteria Occurring in Soil

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Abstract

Microbes are the sources of various enzymes which are commercially important. Some enzymes, particularly L-glutaminase and L-asparaginase are medically important as they can be used for the treatment of leukemia. Many bacteria reported to produce L-glutaminase which can be used as an anticancer agent. L-glutaminase is used in the preparation of baked foods to increase the flavor of the foods. In the present paper five L-glutaminase producing bacteria were isolated from garden, market area and agricultural soils using minimal L-glutamine medium supplemented with phenol red indicator. Based on microscopic examination and biochemical characterization the bacteria were identified as Pseudomonas, Serratia, Proteus, Staphylococcus and Bacillus species. These bacteria can be improved and exploited for the commercial production of L-glutaminase.

Keywords: L-glutaminase, leukemia, anticancer, L-glutaminase producing bacteria.

Introduction

The L-glutaminase is a hydrolytic cleavage enzyme which cleaves L-glutamine into L-glutamic acid and ammonia. It is produced by a wide range of microbes. It is used in the treatment of acute cancer mainly lymphocytic leukemia. During the treatment it can be used in combination with other drugs or L-asparaginase. L-glutamine is one of the important amino acids required for living cells. It is essential for the preparation of purine and pyrimidine derivatives required for the biosynthesis of nucleotides.

Lymphocytic cancer cells lack the enzymatic machinery for the synthesis of L-Glutamine whereas healthy cells are capable of synthesis of L-glutamine. Hence, cancer cells depend upon blood serum for L-glutamine. When L-glutaminase is administered into patient's body it degrades L-glutamine present in serum and makes unavailable to cancer cells. This leads to the death of cancer cells. In addition to medical applications, L-glutaminase is used during the preparation of soy sauce and other fermented foods to increase their flavor. It increases flavor in

fermented foods by producing L-glutamic acid. It is also used in the estimation of L-glutamine concentration in the microbial culture media and to determine the rate of reaction during threonine synthesis^[1,2,3]. So far many microbial strains have been reported to produce L-glutaminase. Some of the microbes which are efficient producers of L-glutaminase include *E.coli*, *Pseudomonas* sp., *Brevibacterium* sp., *Vibrio costicola*, *Streptomyces rimosus*, *Streptomyces avermitilis* and *Streptomyces griseus*⁴. In the present paper L-glutaminase producing bacteria occurring in market area, garden and agricultural soils were isolated and identified till genus level based on microscopic observation and biochemical characterization.

Materials and Methods

Soil samples

Soil samples from garden, market area (located in Karimangar town, Telangana) and agricultural lands (nearby karimnagar town) were collected in sterile polythene bags and stored at 4⁰C until further use.

Medium for the isolation of L-glutaminase producing bacteria

Minimal L-glutamine medium was used for the isolation of L-glutaminase producing bacteria. The composition of minimal L-glutamine medium is L-glutamine – 10 g, KCl – 0.5 g, MgSO₄ - 0.5 g, KH₂PO₄ – 1.0 g, FeSO₄ – 0.1 g, ZnSO₄ – 0.1 g, NaCl – 0.5 g, Phenol red – 0.012 g and distilled water – 1000 ml. The pH of the medium should be adjusted to 6.8. To suppress the growth of fungi 50 µg/ml of nystatin was added. For the preparation of solid medium (minimal L-glutamine agar medium) 20 g of agar is added. L-glutamine serves as sole carbon and nitrogen source in the medium. Phenol red functions as pH indicator. In the agar medium L-glutaminase producing bacteria form pink zones around their colonies. In the liquid medium (Minimal L-glutamine broth) L-glutaminase positive bacteria turn the medium colour from yellow to pink.

Serial dilutions of soil samples were prepared from 10⁻¹ to 10⁻⁷. The minimal L-glutamine agar plates were inoculated with 0.1 ml of 10⁻⁷ dilution of each soil sample and incubated at 37⁰C for 48 hours. The bacterial colonies positive for L-glutaminase production (colonies forming pink zone around them) were isolated and grown as pure cultures. Each bacterial isolate was inoculated into 10 ml of minimal L-glutamine broth and incubated for 48 hours. Uninoculated broth was maintained as control^[4, 5, 6].

Detection of L-glutaminase producing bacteria

Phenol red is an indicator which exhibits yellow colour in medium at and below 7.0 (in acidic conditions) and pink or red colour in medium with pH above 7.0 (alkaline conditions). When soil samples are plated on to minimal L-glutamine agar medium the pH of the medium is 6.8 and medium appears yellow. The bacteria positive L-glutaminase production degrades glutamine and produce L-glutamic acid and ammonia (alkali). This turns medium around their colonies yellow to pink (pink zones)^[4]. When L-glutaminase positive bacteria are grown in minimal L-glutamine broth the colour of the broth changes from yellow to pink due to accumulation of ammonia (alkali).

Identification of bacteria positive for L-glutaminase production

Microscopic examination of bacterial isolates was performed after gram staining and endospore staining. In addition, motility test was performed for each bacterial isolate under microscope. Further Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Phenyl alanine test, Hydrogen sulfide test, Mannitol salt test, Urease test, Oxidase test, Catalase test, Starch hydrolysis, Gelatin hydrolysis and Casein hydrolysis were performed for all the bacterial isolates positive for L-glutaminase production. Based on microscopic examination and biochemical characterization bacteria were identified till their genus level.

Results

Soils samples of garden, market area and agricultural lands were processed using minimal L-glutamine medium supplemented with phenol red indicator to isolate L-glutaminase producing bacteria. Five bacterial isolates were found to be positive for L-glutaminase production. Pink colour zones were formed around their colonies in minimal L-glutamine agar plates. They were designated as X1 & X2 (bacteria isolated from garden soil), Y1 (bacterium isolated from market area soil) and Z1 & Z2 (bacteria isolated from

agricultural soils). When these bacteria were grown in minimal L-glutamine broth supplemented with phenol red indicator the broth colour changed from yellow to pink (Figure -1). The bacteria positive for L-glutaminase production were identified based on microscopic examination (Table-1) and biochemical tests (Table-2) till genus level. X1, X2, Y1, Z1 and Z2 were identified as *Pseudomonas*, *Serratia*, *Proteus*, *Staphylococcus* and *Bacillus* species respectively.



Figure-1: Minimal L-glutamine broth control (yellow) and culture broths positive for L-glutaminase production (pink)

Table-1: Microscopic examination of bacterial isolates

S.No.	Bacterial isolate	Gram staining	Endospore staining	Motility test
1.	X1	Gram positive rod	Negative	Positive
2.	X2	Gram negative rod	Negative	Positive
3.	Y1	Gram negative rod	Negative	Positive
4.	Z1	Gram positive coccus	Negative	Negative
5.	Z2	Gram positive rod	Positive	Positive

Table-2: Biochemical Tests of bacterial isolates

S.No.	Bacterial Isolate	Indole test	Methyl red test	Voges Proskauer test	Citrate test	Phenylalanine test	Hydrogen sulfide test	Mannitol test	Urease test	Oxidase test	Catalase test	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Bacterium identified
1.	X1	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
2.	X2	-	-	+	+	-	-	+	+	-	+	-	+	+	<i>Serratia</i>
3.	Y1	-	+	-	+	+	+	+	+	-	+	-	+	-	<i>Proteus</i>
4.	Z2	-	+	+	+	-	-	+	+	-	+	-	+	-	<i>Staphylococcus</i>
5.	Z3	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>

Discussion

Many of the microbial enzymes are exploited commercially. Certain enzymes especially L-glutaminase and L-asparaginase are medically important and significant in the treatment of some cancers. In the present work three soil samples viz., garden, market area and agricultural land are serially diluted and 10^{-7} dilution of each soil sample was used as inoculum and plated on minimal L-glutamine agar plates and colonies positive for L-glutaminase production formed pink zones after incubation period. Total five bacterial colonies were positive for L-glutaminase production. Further their ability to produce L-glutaminase was confirmed by growing each bacterial isolate in minimal L-glutamine broth. Five bacterial isolates were identified till their genus level. The bacteria positive for L-glutaminase production were identified as *Pseudomonas*, *Serratia*, *Proteus*, *Staphylococcus* and *Bacillus*. Gulbahar and Karkaz isolated L-glutaminase producing bacteria from wound infections and identified them as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli* and *Citrobacter diversus*^[7]. Suresh *et al.* performed experiments with L-glutaminase producing *Serratia marcescens* to determine the effect of physical parameters and different nutrients on L-glutaminase production^[8]. Wade *et al.*, reported L-glutaminase producing *Proteus vulgaris*^[9]. Sathish and Prakasham isolated L-glutaminase producing bacterium from Godavari river bank soils of Andhra Pradesh state and identified it as

Bacillus subtilis^[10]. Similar results were obtained for many researchers.

Conclusion

In the present study five L-glutaminase producing bacteria were isolated from soil samples of garden, market area and agricultural lands and identified till genus level. Further they can be identified till species level. They can be used to produce L-glutaminase. The conditions for the production of L-glutaminase can be optimized. These bacteria can be genetically improved to enhance the production of L-glutaminase which can be used to treat leukemia.

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