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Isolation and Screening of L-Asparaginase Producing Bacteria from Rhizosphere of Medicinal Plants in Botanical Garden, University of Ibadan, Ibadan.

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Abstract
Microorganisms are the largest of all the organisms on earth and exist almost everywhere. They contribute maximally in occurrence of nature and their functions in provisions of life essential element cannot be underestimated. Different taxa of organisms have been reported to be L-asparaginase producers, which being produce in huge amount. However, the exploration of L-asparaginase from terrestrial habitat is reducing and there is increase in the rate at which this enzyme is needed in the biomedical field. It is therefore necessary to explore the rhizosphere of some medicinal plant for isolation of L-asparaginase producers. This study aims to isolate L-asparaginase producing microorganisms from medicinal plant rhizosphere. Bacteria were isolated from rhizospheric soil of matured medicinal plant (*Azadirachta indica, Moringa oleacea, Alstona boonei* and *Khaya senegalensis*) in the botanical garden, University of Ibadan, and were screened for L-asparaginase activity using plate assay method. The best six L-asparaginase producers were identified using morphological, biochemical and molecular methods. In this present study, 145 bacterial isolates were obtained from rhizosphere of four medicinal plants and screened out for production of L- asparaginase. Out of them, sixty-seven (67) bacterial isolates showed positive L-asparaginase activity on Glycerol Asparagine Medium and modified M9 medium. Six positive L-asparaginase-producers produced high yield when subjected to secondary screening by submerged fermentation and were identified as *Amycolatopsis japonica, Stenotrophomonas pavani, Sphingobium yanoikuyae, Paenibacillus cineris, Sphingobacterium caenis* and *Actinomycetal bacterium.* Rhizospheric soil of matured plant from botanical garden, University of Ibadan is a good reservoir for diverse L-asparaginase producing microorganism, and it could be greatly explored for abundance availability of the enzyme

Keywords:Bacteria, L- asparaginase, Rhizospheric soil, Submerged fermentation.

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Introduction

Microorganisms are diverse; some are motile, while others are not; some have cell walls and photosynthesize like plants. Some cells (eukaryotic cells) have a real nucleus, while others have a fake nucleus (prokaryotic cells). Bacteria, which are single-celled and have peptidoglycan in their cell walls, are an example of a prokaryotic cell microbe. They can be found in large quantities in the air, water, animal excrement, and, most importantly, soil. Some are normal mouth, skin, and gut flora, but others thrive in harsh conditions such as high temperatures, poor sanitation, and high pH. Fungi, which are multicellular and have hyphae (a thin, threadlike structure) via which they collect nutrients from the environment as a source of energy and carbon, are examples of eukaryotic cells (Kumar *et al.,* 2013).

The importance of microorganisms cannot be overemphasized as they are widely spread in nature. They are the largest of all the organisms on earth and exist almost everywhere. They contribute maximally to the occurrence of nature and their functions in provisions of life-essential elements cannot be underestimated (Yang *et al*., 2020). Microbial metabolism yields a lot of metabolites that could be active within the milieu of production or utilizable externally as a value-added product, different bioactive compounds that are therapeutically important have been found to be produced by microorganisms from the soil, such as antibiotics, bio-pesticides and enzymes (Nikel, 2016).

Enzymes are referred to as proteins or biomolecules which speed up chemical reactions. In these reactions, 'substrate' (the molecules at the beginning of the reaction) is changed into diverse molecules, which are referred to as products. They are more important than vitamins and minerals for general health (Darwesh *et al.,* 2020). They occur naturally and are been produced by living organisms and functions as biochemical catalyst. Recently, in the medical field, different enzyme has been given much attention as they are being used as drugs, L-asparaginase is one of these enzymes and is widely present in nature. L-asparaginase is a hydrolytic enzyme, it functions by breaking down L-asparagine (an amino acid), to L-aspartic acid and ammonia, these changes cannot be reversible physiologically (Qeshmi *et al.,* 2018).

L-asparaginase is a key enzyme in the pharmaceutical, biosensor, and food industries, with anticarcinogenic potential in the treatment of acute lymphoblastic leukemia, lymphomas, and other malignancies. By exploiting weaknesses in metabolic pathways and catalyzing the degradation of L-asparagine into L-aspartic acid and ammonia, L-asparaginase specifically targets the metabolism of cancer cells, inducing nutritional starvation and cancer cell death (Radha *et al*., 2018) and in reduction of formation of acrylamide (a carcinogenic compound) in fried carbohydrate food (Ushakiranmayi *et al.,* 2017).

Various species of organisms have been identified as L-asparaginase producers, producing large amounts of the enzyme (Sarika and Naveen, 2019). Microbial source L-asparaginase is gaining popularity since it is simple to handle, easy to create in large quantities by submerged fermentation, genetically modifiable, and less time-consuming and environmentally beneficial to produce (Sarika and Naveen, 2019). Bacteria, for example, are a diverse category of bacteria. This enzyme has been found in yeast, filamentous fungus, and actinomycetes (Qeshmi *et al*., 2018). Bacteria: *Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa, Pectobacterium carotovorum, Bacillus circulans, Bacillus* sp. (Shrivastava *et al*., 2012; Subhash *et al*., 2020). Actinomycetes: *Streptomyces albidoflavus,Streptomyces gulbargensis*, *Streptomyces griseus* (Subhash *et al*., 2020). Fungi: *Fusarium* sp., *Aspergillus terreus*, *Aspergillus niger* (Mihooliya *et al.,* 2020) and *Penicillium* sp. (Subhash *et al*., 2020), Cladosporium sp. (Muneer *et al.,* 2020). Yeast: Mucor *hiemalis (*Monica *et al.,* 2013), *Trichoderma viride* (Muneer *et al.,* 2020).

The exploration of L-asparaginase from terrestrial habitats is reducing; however, there is increase in the rate at which this enzyme is needed in the biomedical field. It is therefore necessary to explore the rhizosphere of some medicinal plant for isolation of L-asparaginase producers. This study aims to isolate L-asparaginase producing microorganisms from medicinal plant rhizosphere.

Materials and Methods

Sample collection and Isolation

Soil samples were collected from rhizosphere of matured medicinal plant (*Azadirachta indica, Moringa oleacea, Alstona boonei and Khaya senegalensis*), at the depth of 15cm by ethanol and flamed sterilized cutlass, within the botanical garden, University of Ibadan, Nigeria. The sampling point were located in the coordinates 7.457481 and 3.895045 for *Azadirachta indica*,7.457481 and 3.895054 for *Alstona boonei*, 7.457970 and 3.895082 for *Moringa oleacea* and 7.458034 and 3.894965 for *Khaya senegalensis*. The samples were collected in sterile polythene

bags and brought to the laboratory for further analysis (Alzahrani *et al.*, 2020).

Ten grams of each sample were diluted into 90mL of distilled water to make 6 fold (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) dilutions, 1 mL of each dilution was measured into a Petri dish separately and 15mL sterile prepared and cooled starch casein agar (NaCl: 0.2g, $MgSO_4.7H_2O$: 0.05g, KNO_3: 2.0g, Casein: 0.3g, K_2HPO_4: 2.0g, $CaCO_3$: 0.02g, soluble starch: 10g, $FeSO_4.7H_2O$: 0.01g, agar: 15g, distilled water: 1000 mL, pH: 7.0)was poured into the plates in triplicate. This was done for each sample collected; the plates were then inverted and incubated at 27°C for 3 to 5 days. The developed colonies were observed, counted and recorded as log of cells of soil samples (Dhanam and Kannan, 2015). Each distinct pure colony obtained was stocked in the slant and stored at 4°C for further studies (Sanjotha, 2017).

Screening for L-asparaginase producers

The primary screening to check the ability of the selected colonies to produce L-asparaginase were determined on M9 medium (Na_2HPO_4; 6.0g, K_2HPO_4; 0.9g, NaCl; 0.5g, L-asparagine; 10g, 1M $MgSO_4.7H_2O$; 2mL, 0.1M solution of $CaCl_2.3H_2O$; 1mL, 20% glucose stock; 10mL, 0.005% phenol red, pH 6.5) and Glycerol Asparagine Medium (Glycerol; 1%, L- asparagine; 1%, K_2HPO_4; 0.1%, phenol red; 0.05%, pH; 6.5) both in agar plates and in broth. The plates and tubes were incubated for up to 14 days and daily observations were noted visually. Plates and tubes with change in colour of the medium from yellow to pink were selected as L-asparaginase-producing plates and tubes, and the isolates on them were selected as L-asparaginase producers (Jeyaraj *et al*., 2020).

Quantitative screening through submerged fermentation.

100 mL of Glycerol-asparagine broth in 250mL conical flask was inoculated with 1.5x 10^8 CFU/mL of fresh isolate suspension and incubated in a shaker incubator at 200rpm for 72hours. At the end of the fermentation period, the medium was centrifuged at 10,000rpm for 15 minutes and cell-free supernatant was taken as the crude enzyme (Saxena *et al.,*2015).

Determination of the L-asparaginase Activity

The activity of the produced L-asparaginase enzyme was assessed according to the method of Saxena *et al.,* (2015), in this assay, the rate of hydrolysis of L-asparaginase was determined by measuring the ammonia released during Nessler's reaction. A 0.1mL of crude extract was added to 0.2 mL of 0.05M Tris-HCl buffer (pH 8.6) and 1.7 mL of 0.01M of L-asparagine. The mixture was incubated for 10minutes at 37°C and the reaction was terminated by the addition of 0.5 mL of 1.5M Trichloroacetic acid. The mixture was centrifuged at 10,000rpm for 5 minutes and 0.5mL of the supernatant was added to 7 mL of distilled water and treated with 1 mL of Nessler's reagent. Colour reaction was allowed to develop for 10minutes and the absorbance was read at 480nm with a UV spectrophotometer. The amount of ammonia liberated was determined. One international unit of L-asparaginase enzyme was defined as the amount of enzyme that liberated 1µm of ammonia per minute under the condition of the assay (Saxena *et al.,* 2015).

$$Enzyme\ activity\ (IU) = \frac{Amount\ of Ammonia\ Liberated}{Incubation\ time\ x\ mL\ of enzyme\ used}$$

Identification of the selected isolates

Isolates that showed higher L-asparaginase activity were selected and identified based on morphological (Visual checking of their size, shape, texture, colour and odour) and molecular identification using 16SRNA primer (Feizabadi *et al.,* 2020).

Results

Isolation of Bacterial from collected samples

A total of 145 microorganisms were isolated from the eight rhizospheric soil samples (Table 1). The samples designated as RSSAI (a and b), RSSAB (a and b), RSSKS (a and b) and RSSMO (a and b). From the rhizospheric soil of the *Azadirachta indica* sample (RSSAIa), the highest log of cells was (1.8 x 10^8). The sample had 20 morphologically distinct isolates, while the RSSAI (b) sample yielded (2.4 x 10^7) with 10 morphologically distinct isolates. The

rhizospheric soil of *Alstona boonei* sample (RSSABa) log of cells was 1.2 x 10^7 with eight morphologically distinct isolates while the RSSAB (b) sample yielded (1.2 x 10^7) with 8 morphologically distinct isolates. The log of cells from the sample of rhizospheric soil from *Khaya senegalensis*(RSSKSa) was 1.3 x10^8 with 20 morphologically distinct isolates, while that from (RSSKSb) sample gave (1.3 x10^8) with 17 morphologically distinct isolates. From the rhizospheric soil of *Moringa oleacea* sample (RSSMOb), the highest log of cells was (1.5 x 10^8). The sample had 40 morphologically distinct isolates, while the RSSMO (a) sample yielded (1.3 x10^8) with 20 morphologically distinct isolates.

Table 1:Bacteria count in soil samples obtained from medicinal plants rhizosphere at the Botanical garden, University of Ibadan.

Source/Sample code	Bacteria (Log of cells)	No of morphologically distinct isolates
RSSAI(a)	1.8 x 10^8	20
RSSAI(b)	2.4 x 10^7	10
RSSAB(a)	1.2 x 10^7	8
RSSAB(b)	3.6 x 10^7	10
RSSKS(a)	1.3 x 10^8	20
RSSKS(b)	1.3 x 10^8	17
RSSMO(a)	1.3 x 10^8	20
RSSMO(b)	1.5 x 10^8	40
	Total	145

RSSAI (a): Rhizospheric soil sample from *Azadirachta indica* point A
RSSAI (b): Rhizospheric soil sample from *Azadirachta indica* point B
RSSAB (a): Rhizospheric soil sample from *Alstona boonei* point A
RSSAB (b): Rhizospheric soil sample from *Alstona boonei* point B
RSSKS (a): Rhizospheric soil sample from *Khaya senegalensis* point A
RSSKS (b): Rhizospheric soil sample from *Khaya senegalensis* point B
RSSMO (a): Rhizospheric soil sample from *Moringa oleacea* point A
RSSMO (b): Rhizospheric soil sample from *Moringa oleacea* point B

Detection of L-asparaginase production by the isolates obtained from rhizospheric soils.

The abilities of the isolates obtained to utilise L-asparagine as a sole Nitrogen source is reported in Table 2. It was observed that, out of the 145 isolates obtained, 67 isolates showed L-asparaginase producing ability on M9 medium and GA medium. This was noticed by change in colour of the acidic medium from the initial yellow colour to pink. Out of the distinct colonies obtained from RSSMO, (46%) had positive L-asparaginase production in either Glycerol-Asparagine medium or M9 Medium, of which (42% and 17%) excellent production (+++) was observed on M9 medium and Glycerol-Asparagine medium respectively. 40% of the distinct colonies from RSSKS are L-asparaginase producers, out of which 60% showed excellent production on M9 medium. Out of the distinct colonies obtained from RSSAI, (47%) had positive L-asparaginase production in either Glycerol-Asparagine medium or M9 Medium, of which (50% and 14%) excellent production (+++) was observed on M9 medium and Glycerol-Asparagine medium respectively. 40% of the distinct colonies from RSSAB are L-asparaginase producers, out of which 50% showed excellent production on M9 medium and 20% showed excellent production on Glycerol-Asparagine medium.

Positive producers were selected for further screening based on differential medium support at different days' interval on M9 medium (broth and Agar). Fifteen out of the isolates showed good consistency starting from 24 hours to 96 hours of incubation in that; there was change in colour of the medium both on agar and broth from yellow to pink at the same hour. Based on these, isolates RSSMO(b)5, RSSMO(a)4, RSSMO(b)4, RSSAB(b)3, RSSKS(b)1, RSSAB(b)2, RSSAI(a)2, RSSMO(B)8, RSSKS(b)8, RSSMO(b)37, RSSKS(a)12, RSSAI(b)3, RSSAB(a)2, RSSMO(B)2 and RSSKS(a)4 were picked as good producers of L-asparaginase as seen in Table 3.

The result after 3 days of fermentation revealed that the yield of the L-asparaginase varied with the isolates. The yield ranged from the highest value of 0.277 U/mL to the lowest value of 0.025 U/mL. Isolates RSSMO(b)5 gave 0.277U/mL followed by isolate RSSAB(b)2 and RSSKS(a)4 with yield of 0.231U/mL and 0.210U/mL respectively. The lowest yield was observed in isolate RSSKS(b)8 with the value of 0.025U/mL followed by isolated RSSAI(b)3 and RSSKS(a)12 with the value of 0.042 U/mL and 0.050 U/mL respectively (Table 4).

Table 2: Screening isolates from different medicinal plant rhizospheric soil for L-asparaginase production.

Isolates code	Screening on Glycerol Asparagine agar plate	Screening on Glycerol Asparagine broth	Screening on M9 medium agar plate	Screening on M9 medium broth
RSSMO(a)3	+	++	_	_
RSSMO(a)4	+	++	++	+++
RSSMO(a)7	+	++	_	_
RSSMO(a)11	+	_	++	++
RSSMO(a)13	+	++	_	_
RSSMO(a)13	+	+++	++	+++
RSSMO(a)14	_	++	++	+++
RSSMO(a)15	_	+	+	++
RSSMO(a)20	+	+++	++	+++
RSSMO(b)	+	++	++	+++
RSSMO(b)4	+	+	_	_
RSSMO(b)5	++	++	++	+++
RSSMO(b)6	+	_	+++	+++
RSSMO(b)7	+	+++	++	++
RSSMO(b))8	+	++	++	+++
RSSMO(b)9	_	+	++	++
RSSMO(b)11	_	++	++	+++
RSSMO(b)16	+	+	_	_
RSSMO(b)19	+	_	_	_
RSSMO(b)19	+	+	+	+
RSSMO(b)21	+	+	_	_
RSSMO(b)21	+	+	++	+++
RSSMO(b)22	+	+	++	_
RSSMO(b)25	+	+++	_	_
RSSMO(b)28	+	++	_	_
RSSMO(b)30	_	_	+	+
RSSMO(b)33	+	++	++	+++
RSSMO(b)37	+	+++	++	+++
RSSKS(a)1	+	++	++	_
RSSKS(a)4	+	++	+++	+++
RSSKS(a)5	+	_	_	_

RSSKS(a)6	+	_	++	+++
RSSKS(a)12	+	++	++	+++
RSSKS(a)15	+	++	_	_
RSSKS(a)19	+	+	_	_
RSSKS(a)20	+	_	_	_
RSSKS(b)1	+	++	++	+++
RSSKS(b)3	+	+	+++	+++
RSSKS(b)6	+	_	_	_
RSSKS(b)8	+	++	++	+++
RSSKS(b)9	+	+	+++	+++
RSSKS(b)I0	+	_	++	+++
RSSKS(b)14	+	+	++	+++
RSSAI(a)1	_	_	+	+
RSSAI(a)2	+	++	+++	+++
RSSAI(a)3	+	_	+++	+++
RSSAI(a)5	+	_	+++	+++
RSSAI(a)16	+	+++	_	_
RSSAI(a)17	+	+++	_	_
RSSAI(a)18	+	+	++	+++
RSSAI(a)19	+	+	++	+++
RSSAI(a)20	_	+	++	+
RSSAI(b)2	+	_	++	_
RSSAI(b)3	+	++	++	+++
RSSAI(b)8	+	++	+++	+++
RSSAI(b)9	+	+++	_	_
RSSAI(b)10	+	++	++	_
RSSAB(a)2	+	+	++	+++
RSSAB(a)4	+	_	++	++
RSSAB(a)5	+	+	+	+++
RSSAB(a)7	+	+++	_	_
RSSAB(a)8	+	_	-	_
RSSAB(b)1	+	_	+++	+++
RSSAB(b)2	+	+	+++	+++
RSSAB(b)3	+	+	+++	+++
RSSAB(b)4	+	+++	_	_
RSSAB(b)7	+	++	++	++

Key:
+ Good Production++ Very Good Production+++ Excellent Production

Table 3: Screening on M9 medium of isolates selected based on differential media support for L-asparaginase production.

	M9 Agar				M9 Broth			
Isolates code	**24 (hrs)**	**48 (hrs)**	**72 (hrs)**	**96(hrs)**	**24(hrs)**	**48 (hrs)**	**72 (hrs)**	**96(hrs)**
RSSMO(B)4	+	+	+	+	+	+	+	+
RSSKS(b)9	+	+	+	+	_	_	+	+
RSSMO(B)5	+	+	+	+	+	+	+	+
RSSAB(b)3	+	+	+	+	_	+	+	+
RSSAI(a)20	_	_	+	+	_	_	+	+
RSSAI(a)18	+	+	+	+	_	_	_	+
RSSKS(b)14	+	+	+	+	_	_	+	+
RSSMO(a)4	+	+	+	+	+	+	+	+
RSSAB(a)2	+	+	+	+	+	+	+	+
RSSAB(b)2	+	+	+	+	+	+	+	+
RSSAI(b)3	+	+	+	+	+	+	+	+

RSSAI(a)2	+	+	+	+	+	+	+	+
RSSMO(B)8	+	+	+	+	_	_	+	+
RSSKS(b)8	+	+	+	+	+	+	+	+
RSSMO(B)33	+	+	_	+	_	_	+	+
RSSMO(B)37	+	+	+	+	_	+	+	+
RSSMO(B)21	+	+	+	+	_	+	+	+
RSSMO(a)20	_	+	+	+	_	_	_	+
RSSKS(b)1	+	+	+	+	+	+	+	+
RSSMO(a)13	_	+	+	+	_	_	+	+
RSSKS(a)4	+	+	+	+	+	+	+	+
RSSKS(a)12	+	+	+	+	_	+	+	+
RSSKS(b)3	_	-	+	+	_	_	_	+

Keys:

\+ Positive production - Negative production

Table 4: Secondary screening of L-asparaginase by submerged fermentation.

Isolate code	Submerged fermentation (U/mL) 3 days
RSSMO(b)5	0.277±0.001
RSSMO(a)4	0.134±0.001
RSSMO(b)4	0.059 ± 0.001
RSSAB(b)3	0.042 ± 0.001
RSSKS(b)1	0.134 ± 0.001
RSSAB(a)2	0.109 ± 0.001
RSSKS(a)4	0.210 ± 0.01
RSSAB(b)2	0.237 ± 0.0001
RSSAI(a)2	0.067 ± 0.0001
RSSMO(b)8	0.060 ± 0.0001
RSSKS(b)8	0.025 ± 0.0001
RSSMO(b)37	0.085 ± 0.0001
RSSMO(b)21	0.097 ± 0.0001
RSSKS(a)12	0.050 ± 0.0001
RSSAI(b)3	0.151 ± 0.001

*Each value is a Mean of Triplicate production standard Error

Keys

\+ Positive - Negative

Identification of the Isolates

Based on the above results six isolates were selected as L-asparaginase-producers. They were RSSMO(b)5, RSSKS(a)4, RSSKS(b)1, RSSAI(b)3, RSSAB(b)2, RSSMO(a)4. Isolate RSSMO(b)5, which was found to be the best producers of L-asparaginase of all the isolates that were screened was a white small size colony with round shape (Table 5). The colony was dried, leathery and gave earthy odour. At maturity, it gave a pink pigment. RSSMO(a)4 was observed to be small dried colony with gold colour, the colony stick to the medium and turn to brown upon maturation on starch casein agar. The colony gave ginsomin smell. Isolate RSSKS(b)1 was observed to be a big, wet, round, raise colony with yellow color, but produced pigment. Isolate RSSAI(b)3 had brownish colonies which were observed to be small, dried and produce golden pigment. Isolate RSSAB(b)2 was observed to be a small dull, white colony. The colony was raised; wet, shiny but showed no pigment. Isolate RSSKS(a)4 had golden colonies, the colonies appeared to be raised, big, dry and produced yellow pigment.

The molecular identification of the six isolates (Table 6) revealed that Isolate RSSMO(b) is an Actinomycetes, which

belong to Pseudonocardioses family and the genus *Amycolatopsis* after molecular studies and blast search through the gene bank of National Centre for Biotechnology Information (NCBI). Phylogenetic analysis of the isolate using maximum likelihood comparison with other genus of *Amycolatopsis* deposited in the gene bank revealed 89.5% similarity chart with *Amycolatopsis japonica.* Isolate RSSMO(a)4, was confirmed to be *Stenotrophomonas pavani* after blast search through the gene bank of NCBI and the phylogenetic analysis revealed 94.55% similarity of the isolate to *Stenotrophomonas pavani*. The molecular analysis of isolates RSSKS(b)1 confirmed it to be *Sphingobium yanoikuyae* with 91.55% similarity. Isolate RSSAI(b)3 was identified as Actinomycetal bacterium when blasted on NCBI gene sequence. Isolate RSSAB(b)2 was confirmed to be *Paenibacillus cineris* with 94.60% similarity. Isolate RSSKS(a)4 was confirmed to be *Sphingobacterium caenis* and show 91.89% similarity.

Table 5: Morphological characterization of selected L-asparaginase producers from rhizospheric soils.

Isolates codes	Morphological Characteristics of the selected isolates
RSSMO(B)5	White, dried, round, leathering pinpoint colony with earthy odour and branched filaments that extended in form of trees.
RSSAI(b)3	Small, brown, dried colony with golden pigment
RSSMO(a)4	Gold small colony that stick to the medium with ginsomin smell
RSSKS(a)4	Gold, raised, big, dry colony that grows into the medium. The produce yellow pigment
RSSKS(b)1	Big, round, wet, raised, yellow colony
RSSAB(b)2	Dull white, small, round, raised, wet colony

Table 6: Molecular Identification of Selected L-asparaginase producing Bacteria

Isolate codes	Molecular Identification	% Similarities	Accession numbers
RSSMO(b)5	*Amycolatopsis japonica*	89.50	NA
RSSMO(a)4	*Stenotrophomonas pavanii*	94.55	MN658473
RSSAI(b)3	*Actinomycetales bacterium*	89.52	NA
RSSKS(a)4	*Sphingobacterium caeni*	91.89	MN658474
RSSAB(b)2	*Paenibacillus cineris*	94.60	MN658472
RSSKS(b)1	*Sphingobium yanoikuyae*	91.55	MN658471

Discussion

A total of One hundred and forty-five isolates were isolated from 8 samples that were collected from rhizosphere of matured medicinal plant (*Azadirachta indica*, *Alstona boonei*, *Moringa oleace* and *Khaya senegalensis*), 21% from *Azadirachta indica*, 12% from *Alstona boonei*, 26% from *Khaya senegalensis* and 41% from *Moringa oleace*. The maximum number of bacteria was observed in rhizospheric soil of *Moringa oleacea*. The number of bacteria from *Moringa oleacea* was higher than other soil samples which can be due to symbiont activity and availability of nutrient in the soil and on the species of plant. This is in accordance to the work of Khamna *et al*. (2009), who isolated different bacteria from Thai medicinal rhizospheric soil. Sharmal *et al.* (2021) suggested that, rhizosphere is a good reservoir of microorganism which may be due to high level of organic matter and type of the plant. Sixty-seven isolates showed L-asparaginase producing ability by preliminary evaluation for L-asparaginase potential; The utilisation of substrate (L-asparagine) is indicated by the accumulation of ammonia in the medium which leads to increase in pH and which in-turn turn the medium colour from yellow (slightly acidic pH) to pink due to an action of an indicator phenol red.

Six L-asparaginase producers were selected and were molecularly identified using 16s rRNA sequence to be *Amycolatopsis japonica* (an actinomycetes), *Paenibacillus cineris, Sphingobium yanoikuaye, Stenotrophomonas pavani, Shingobacteium caeni* and *Actinomycetes bacterium*. This result agrees with the result of other researchers who isolated endophyte from soil rhizosphere (Souza *et al*., 2015).

Conclusion

In conclusion, this study highlights the isolation of L-asparaginase-producing bacteria from a rhizosphere of medicinal plant. The organisms were identified as *Amycolatopsis japonica, Sphingobium yanoikuyae,Paenibacillus cineris, Sphingobacterium caeni, Actinomycetales bacterium* and*, Stenotrophomonas pavanii* by 16s rRNA gene sequence. It was confirmed that rhizospheric soil is endowned with L-asparaginase producing bacteria.

References

Abdelrazek, N.A., Elkhatib, W.A., Raafat, M.M. and Aboulwafa, M.M. (2020). Production, characterization and bioinformatics analysis of L-asparaginase from a new *Stenotrophomonas maltophilia* EMCC2297 soil isolate. *AMB Express* 10: 1-16.

Agrawal, S., and Kango, N. (2019). Development and catalytic characterization of L-asparaginase nano-bioconjugates. *International Journal of Biological Macromolecules,* 135: 142 - 1150.

Alrumman, S.A., Mostafa, Y. S., Al-Izran, K. A., Alfaifi, M.Y., Taha, T.H. and Elbehairi, S.E. (2019). Production and anticancer activity of an L-asparaginase from Bacillus licheniformis isolated from the Red Sea, Saudi Arabia. *Scientific Reports*9 (1): 1-14.

Alzahrani, N.H., Alzahrani, S.H. and Khayyat S.K.H. (2020). Isolation and Genetic Identification of L-asparaginase antitumor from *Streptomyces* sp. *Journal of Biochemical Technology* 11 (1): 6 -10

Nikel, P. I. and Lorenzo, D. V. (2018). *Pseudomonas putida* as a functionl

Chaurasia, A., Meena, B.R., Tripathi, A. N., Pandey, K.K., Rai, A.B. and Singh, B. (2018). Actinomycetes: An unexplored microorganism for plant growth promotion and biocontrol in vegetable crops. *World Journal of Microbiology and Biotechnology* 34 (9): 1-16.

Chowdhury, A.A. (2020). Study on the role of *Stenotrophomonas maltophilia* and *Stenotrophomonas pavanii* in sustainable agriculture. *Life Sciences leaflets,* 129

Darwesh, O.M., Ali, S.S., Matter, I.A., Elsamahy, T. and Mahmoud, Y.A. (2020). Enzymes immobilization onto magnetic nanoparticles to improve industrial and environmental applications. *Methods in Enzymology,*630: 481-502.

Jayam, G.D. and Kannan, S. (2014). Screening and characterization of L-asparaginase producing Streptomyces isolated from soil samples of Periyar lake, Kumily. *Bioscience Discovery,*5 (1): 50-54.

Jeyaraj, S.K., Parimelazhagan, B.P., Kalimuthu, V.P., Sivaram, V., Perumal, A. (2020). A Study on production and evaluation of L-asparaginase obtained from *Bacillus subtilis*. *Test Engineering and Management*. 82: 4413 – 4416.

Khamna S., Yokota A. and Lumyong S. (2009). L-asparaginase production by actinomycetes isolated from some Thai, medicinal plant rhizosphere soils. *International Journal of Integrative Biology* 6(1): 22-26

Meyer,S., Beuf,K., Vekeman, B., Willems, A. (2015). A large diversity of non-rhizobialendophytes found in legume root nodules in Flanders (Belgium). *Soil Biology and Biochemistry* 83: 1-11.

Moorthy, V., Ramalingam, A., Sumantha, A., Shankaranaya, R.T. (2010). Production, purification and characterisation of extracellular L-asparaginase from a soil isolate of *Bacillus* sp. *Africa Journal of Microbiology Research,*4(18): 1862-1867.

chassis for industrial biocatalysis from native biochemistry to trans-

metabolism. *Metabolic Engineering* 50: 142-155.

from Soil Samples of Akola District. *Metabolic Engineering,* 50: 142-155.

Qeshmi, F.P., Homaei, A., Fernandes, P., Javadpour, S. (2018). Marine microbial L-asparaginase: biochemistry, molecular approaches and applications in tumor therapy and in the food industry. *Microbiological Research* 208: 99 - 112.

Radha, R., Arumugam, N. and Gummadi, S. N. (2018). Glutaminase free L-asparaginase from *Vibrio cholerae*: Heterologous expression, purification and biochemical characterization. *International Journal of Biological Mac.* 111: 129 – 138.

Sanjotha G. (2017). Isolation, screening, optimization of L-asparaginase by fungi. *Research Journal of Recent Sciences* 6 (3): 1-7.

Sanjotha, G. (2016). Isolation, Characterization and Screening of Anticancerous L-asparaginase producing Microbes from Coastal region. *International Journal of Research Studies in Biosciences* 4 (8): 14-18.

Saxena, A., Upadhyay, R., Kango N. (2015). Isolation and identification of actinomycetes for production of novel extracellular glutaminase free L-asparaginase. *NISCAIR-CSIR, India.*

Sharma, P., Sharma, M.M.M., Malik, A., Vashisth, M., Singh, D., Kumar, R., Singh, B., Patra, A., Mehta, S., Pandey, V. (2021). Rhizosphere, Rhizosphere Biology, and Rhizospheric Engineering. *Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management, Springer,* Cham 577 - 624.

Nimkande, V. and Barate, D. (2014). Studies on L-asparaginase Producing Bacteria

Souza, W.R., Silva, R.E., Goodfellow, M., Busarakam, K., Figueiro, F.S., Ferreira, D., Rodrigues-Filho, E., Moraes, L.A.B., Zucchi, T.D. (2015). *Amycolatopsis rhabdoformis* sp. nov., an actinomycete isolated from a tropical forest soil. *International Journal of Systematic and Evolutionary Microbiology,*65(6): 1786-1793.

Ushakiranamayi, M., Vijayalakshmi, M., Venkat, S.R., Ganduri, K., Satish, B., Sudhakar, P. (2017). Production and optimization of L-asparaginase by an actinobacterium isolated from Nizampatnam mangrove ecosystem. *Pharmacognosy Journal* 9 (6): 19 – 24.

Wakil, S.M. and Adelegan, A.A. (2015). Screening, production and optimization of L-asparaginase from soil bacteria isolated in Ibadan, South-western Nigeria. *Journal of Basic and Applied Sciences* 11: 39-51.

Yang, Y., Liang, C., Wang, Y., Cheng, H., An, S., Chang, S. X. (2020). Soil extracellular enzyme stoichiometry reflects the shift from P-to N-limitation of microorganisms with grassland restoration. *Soil Biology and Biochemistry* 149: 107928.