Research Article

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THE ISOLATION AND IDENTIFICATION OF L -ASPARAGINASE ENDOPHYTIC BACTERIA FROM *AEGLE MARMELOS*

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ABSTRACT

Virtually all plants are inhabited by diverse bacteria known as Endophytes. Endophytic bacteria are referred to as those which can be detected at a particular moment within the tissues of apparently healthy plant hosts. L-Asparaginase is a well-recognized as amino acid degrading enzyme. L-Asparaginase (EC 3.5.1.1.) has grown considerably since this enzyme was found to have antitumor activity. Most bacterial endophytes belong to mainly four phyla, but they encompass many genera and species. Their functions cannot be assigned clearly to taxonomy and seem to depend on the host and environmental parameters. Endophytic bacteria have been isolated from a large diversity of plant was reported as many as 46 different bacterial species from 27 plant species. Freshly collected leaves of *Aegle marmelos* were selected for the isolation of endophytic bacteria. The isolated bacterial strain tested by gram staining and identification done in a lab care pathology laboratory. The present study enlightens the medicinal plants like *A. marmelos* as potential sources of endophytic bacteria with the L-asparaginase activity. The optimization studies indicated the maximum enzyme was produced at 30°C for 72 hours in 120 rpm rotatory shaker incubator and pH: 8 presence of glucose as most suitable carbon source.

Keywords: *Aegle marmelos*, L –Asparaginase, Endophytes, Endophytic bacteria

INTRODUCTION

The bacteria those are detected at a particular moment within the tissues of apparently healthy plant hosts are referred endophytic bacteria[1]. The majority of such bacteria are found different compartments of the plant apoplast, including the intercellular spaces of the cell walls and xylem vessels. Some of them are able to colonize reproductive organs of plants, e.g. flowers, fruits and seeds. These bacteria do not normally cause any substantial morphological changes like root-nodules. They do not show any disease symptoms, in contrast to phytopathogens. Most endophytic bacteria have a number of plant-beneficial traits *in vitro*; some of those exhibit them in planta and only a fever of endophytes proved to be very beneficial plant-growth promoting and biocontrol material[2,3].

As the history shows more than 200 bacterial genera from 16 phyla have been reported as endophytes are isolated from sterilized surface of plats. These are both culturable and unculturable bacteria *i.e. Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Cholorobi, Chloroflexi, Cyanobacteria, Deinococcus, Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria, Spirochaetes* and *Verrucomicrobiae*[3-8].

L-Asparaginase is well-known as amino acid degrading enzyme. L-Asparaginase (EC 3.5.1.1.) have antitumor activity so it grown considerably. Asparaginase (L- Asparagine amino hydrolase) is responsible for conversion of L- asparagine to aspartic acid and ammonia. (Verma *et al*., 2007). There was also application of L-Asparaginase enzyme in many other clinical trials of tumor therapy in combination with chemotherapy. As the extraction of L-asparaginase from mammalian cells is difficult, microorganisms have proved to be a better alternative for L-asparaginase extraction[9].

Aegle marmelos (Linn.) have great mythological significance and medicinal importance in ancient system of medicine. Number of study on photochemistry of the plant advise number of bioactive chemical entity like, γ -sitosterol, aegelin, lupeol, rutin, marmesinin, β-sitosterol, flavone, glycoside, O-isopentenyl halfordiol, marmeline and phenylethyl cinnamamides. A rich source of bioactive molecule of the plant shows its medicinal properties such as antidiabetic, antiulcer, antioxidant, antimalarial, anti- inflammatory, anticancer, antiburn and radioprotective, antihyperlipidaemic, antifungal, antibacterial and antiviral, antihistaminic, anti-asthamatic, immune modulatory, wound healing, kidney problems, smallpox antidandruff and antidepressant activities[10].

MATERIALS AND METHODS

Plant Collection

The plant sample of *Aegle marmelos* was collected from the Gangeshwar shiv mandir, Adajan, Surat (Gujarat) India. The plant material was identified using Flora of Dhule and Nandurbar District[11], India at Post Graduate Department of Botany, L.K.Dr.P.R. Ghogrey Science College, Deopur, Dhule (MS) India and herbarium was also preserved. The voucher specimen number is SCD/H.No. 781.

Selection of plants and isolation of endophytic Bacteria

Freshly collected leaves of *Aegle marmelos* were selected for the isolation of endophytic bacteria were collected from each mature healthy plant and were immediately processed. The plant samples were initially subjected for surface sterilization along with some modifications. The surface sterilized plant parts *viz*., leaves were further ground with 6ml 0.9% NaCl solution using sterile pestle and mortar and kept aseptically for 15-20 min. for the release of

endophytic bacteria from host tissue. The tissue extract was diluted with 0.9% NaCl solution and plated on tryptic soy agar medium plates. All the plates were incubated at 30˚C for 3-5 days. After incubation, various colonies were selected showing different morphological and growth characters.

Identification of endophytic bacteria

The isolated bacterial strain tested by gram staining and identification done in a lab care pathology laboratory.

Qualitative plate assay for screening of L-asparaginase production

Screening method was based on the assumption where the pH indicator phenol red is incorporated in medium contain asparagines (as so le nitrogen source). Phenol red have yellow at acidic pH and turns pink at alkaline pH, in this way a pink zone formed around microbial colonies producing L-asparaginase. For screening Petri plates and M9 medium supplemented with 2.5% phenol red was prepared and autoclaved. Media was poured into sterile petri plates under sterile conditions in laminar air flow. Media was allowed to solidify. After solidification the purified cultures were streaked on the solidified plates. Plates were incubated for 48 hours at 37°C. Plates were observed for formation of pink zone around colonies. Screening for maximum L-asparaginase was done by primary screening technique. The primary screening was done by streak plate method on M9 medium, shows production of pink zone around L-asparaginase producing bacterial colony[12].

Screening (quantitative) of microbial cultures for L –asparaginase activity

The 2 microbial cultures (including consortia) were initially grown in shake flasks (20 mL media held in 100 mL EM flasks) using respective culture media (Tryptic soya broth for bacteria) Cultures were maintained at 120 rpm and at 30ºC. The fermentation samples were harvested after 72 hours of growth and the cells were removed by centrifugation (5000 rpm, 10 min, 10ºC). The cell free broths were tested for their ability to utilize asparagine as substrate in the asparaginase assay.

Determination of L-asparaginase activity

For this, mixture of 0.1 ml of enzyme extract, 0.2 ml of 0.05M Tris-HCl buffer (pH 8.6), and 1.7 ml of 0.01M L-asparagine was incubated for 10 min at 37ºC. The reaction was stopped by the addition of 0.5 ml of 1.5M trichloroacetic acid. After centrifugation at 10000 rpm, 0.5 ml of the supernatant was diluted to 7 ml with distilled water and treated with 1 ml of Nessler's reagent. The color reaction was allowed to develop for 10 min and the absorbance was read at 480 nm (against a reagent blank) using spectrophotometer (Shimadzu, Japan). The ammonia liberated was extrapolated from the standard curve derived with different concentrations of ammonium sulphate. One unit (IU) of L-asparaginase was defined as that amount of enzyme which liberates 1 µ mole of ammonia per hour under the assay conditions[13].

RESULT AND DISCUSSION

Microorganisms are prosperous sources of metabolites and products with unusual properties. They have various choices of catalytic enzymatic activity of various biochemical reactions. The enzyme, L-asparaginase has been intensively investigated more than the past few decades owing to its value as anti-neoplastic agents. Like bacteria, actinomycetes source for the production of L-asparaginase.

This study was conducted with a view to isolate bacteria associated with the leaves of *Aegle marmelos* and to assess their maximum production of L-Asparginase enzyme activity. 4 different bacterial strain isolates were obtained from surface sterilized of healthy leaves of *Aegle marmelos* and prepared extract in 0.9% NaCl. The bacterial were tested for Gram staining and identification done in a lab care pathology laboratory, Surat, Gujrat. The result showed that strain 3: *Klebsiella pneumonia* and strain 4: *Staphylococcus aureus.*

The qualitative plate assay was devised using this principle by incorporating the pH indicator phenol red in medium containing asparagine (sole nitrogen source). Phenol red at acidic pH is yellow and at alkaline pH turns pink, thus a pink zone is formed around microbial colonies producing L-asparaginase. The plate assay is advantageous as the method is quick and L-asparaginase production can be visualized directly from the plates without performing time consuming assays. And only 2 bacterial strain produced L-Asparaginase enzyme. like strain 3: *Klebsiella pneumonia* and strain 4: *Staphylococcus aureus*. So, strain 3: *Klebsiella pneumonia* are given a maximum production of L-Asparaginase enzyme then strain 4: *Staphylococcus aureus.*

The quantitative assays are done by fermentation and strain 3: *Klebsiella pneumonia* are given a maximum production of L-Asparaginase enzyme 2.75 (IU/ml) in 24 hours and reduced in 48 to 72 hours. And strain 4: *Staphylococcus aureus* are given a maximum production of L-Asparaginase enzyme 2.68 (IU/ml) in 48 hours and reduced in 72 hours. So here only 2 strains of bacterial isolate produced L-Asparaginase enzyme by fermentation.

CONCLUSION

The present study enlightens the medicinal plants like *Aegle marmelos* as potential sources of endophytic bacteria with the L-asparaginase activity. The optimization studies indicated the maximum enzyme was produced at 30°C for 72 hours in 120 rpm rotatory shaker incubator and pH: 8 presence of glucose as most suitable carbon source. The L-Asparagine substrate concentration showed direct proportional relation with an enzyme activity. The study indicates the bacterial endophytes as a possible source of L-asparaginase as the production strain. So, here 2 bacterial strain produced L-Asparaginase enzyme. like strain 3: *Klebsiella pneumonia* and strain 4: *Staphylococcus aureus*. Hence this organism may possibly be exploited for industrial production of L-asparaginase which ultimately can be supplied to cancer drug making sectors.

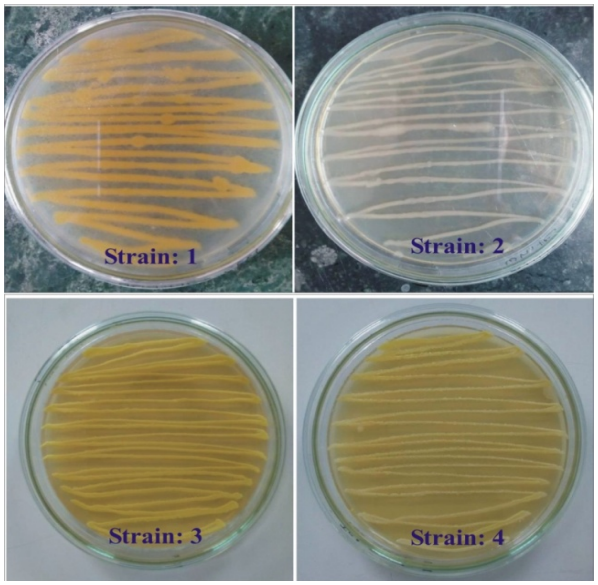

Figure 1: Isolation of L-Asparaginase Endophytic Bacteria

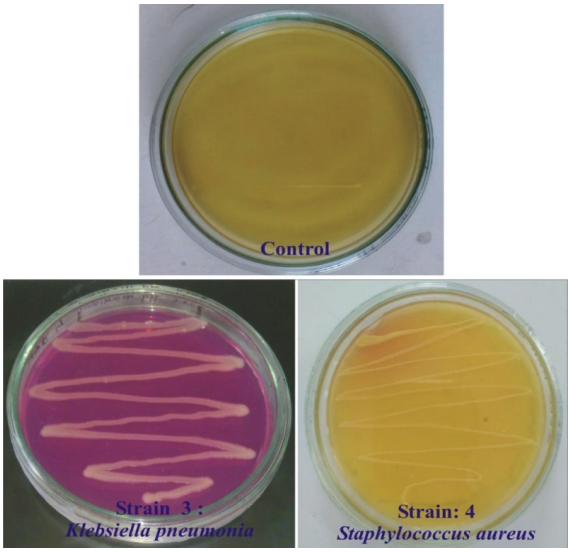

Figure 2: Qualitative plate Assay for screening of L-Asparginase production

Table 1: L-Asparginase enzyme activity of microbial isolate and tested through quantification assay after 72 hours

Sl. No.	Microbial isolates	Enzyme activity(U/ml/h)
1st day	Strain – 3: *Klebsiella pneumoniae*	0.645
2nd day	Strain – 3: *Klebsiella pneumoniae*	0.616
3rd day	Strain – 3: *Klebsiella pneumoniae*	0.600
1st day	Strain – 4*: Staphylococcus aureus*	0.348
2nd day	Strain – 4*: Staphylococcus aureus*	0.632
3rd day	Strain – 4*: Staphylococcus aureus*	0.580

Table 2: Comparison of L-Asparaginase endophytic Bacteria

Endophytic bacteria			Activity (IU/ml)
Strain	3	: *Klebsiella pneumoniae*	2.75 (IU/ml)
Strain	4	: *Staphylococcus aureus*	2.68 (IU/ml)

REFERENCES

1. Hallmann J, Quadt-Hallmann A, Mahafee WF, Kloeppe JW. Bacterial endophytes in agricultural crops, Can. J. Microbiol 1997; 43: 895–914.
2. Scherwinski K, Grosch R, Berg G. Effect of bacterial antagonists on lettuce: active biocontrol of *Rhizoctonia solani* and negligible, short-term effects on non-target microorganisms. FEMS Microbiol. Ecol. 2008; 64: 106-16
3. Berg G, Hallmann J. Control of plant pathogenic fungi with bacterial endophytes. In: Microbial root endophytes. *Springer,* Berlin. 2006; p. 53–67.
4. Samish Z, Dimant D. Bacterial population in fresh, healthy cucumbers". Food Manuf. 1959; 34: 17-20
5. Mundt JO, Hinkle NF. Bacteria within ovules and seeds. Appl. Environ. Microbiol. (;1976; 32: 694–698.
6. Mengoni A, Pini F, Huang L-N, Shu W-S, Bazzicalupo M. Plant-by-plant variations of bacterial communities associated with leaves of the nickel hyper accumulator *Alyssum bertolonii* Desv. Microb. Ecol. 2009; 58: 660-667.
7. Manter DK, Delgado J, Holm DG, Stong R. Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microb. Ecol. 2010; 60: 157-166.
8. Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol. Plant-Microbe Interact. 2012; 25: 28-36.
9. Savitri, Asthana Neeta and Wamik Azmi. Microbial L-asparaginase: A chemotherapeutic agent. Crit. Rev. Biotechnol. 2003; 27: 45-62.
10. Dhale DA and Kalme RK. Pharmacognostic Characterization of Stem and Root of *Adhatoda zeylanica* Medicus, International Journal of Pharmaceutical Sciences and Research, 2012; Vol. 3(11) 4264-4269,
11. Patil DA. Flora of Dhule and Nandurbar District, Bishen Singh Mahendrapalsingh, Dehra Dun (India), 2003.
12. Gulati R, Saxena RK and Gupta R. A rapid plate assay for screening L-asparaginase producing microorganisms". Lett Appl Microbiolog. 1997; 24: 23-26.
13. Mashburn LT, Wriston JJC. Tumor inhibitory effect of L-Asparaginase from *Escheriachia coli*. Arch Biochem Biophys. 1964; 105: 450-452.

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