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Isolation and Screening of Bacterial Exopolysaccharide from Leguminous Soil as A Potential of Indole Acetic Acid

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Abstract

The present study emphasizes the production of Exopolysaccharide and Indole acetic acid from microorganism such as Leuconostoc mesenteroides which enhance the agricultural productivity. Soil sample from different leguminous soil was collected and culturing on the ATCC no.14 media. The bacterial culture showed growth on the media in the form of Thick, mucoid slimy creamish colour colonies with Gram positive, Non-motile, Indole –ve, VP +ve, negative catalase test and produced gases from all sugars which confirmed bacterial isolate was Leuconostoc mesenteroides. Exopolysaccharide production were done successfully by Leuconostoc mesenteroides. Highest exopolysaccharides production were seen in fifth days of incubation i.e 4.6 mg/ml. After 10 days of incubation were centrifuged at 5000 rpm for 10 mins. Supernatant was mixed with 2 drops of orthophosphoric acid and Salcowsci reagent, Pink colour showed presence of IAA i.e. Indole Acetic Acid. The production of unknown IAA was found to be 88.80 µg/ml by comparing with values of standard IAA. Application of indole acetic acid (IAA) on the growth of plant, it was found that the same concentration of indole acetic acid (IAA) apply to the seeds of Fenugreek (Trigonella foenum-graecum). The results showed that the length of growth of Fenugreek plant with standard IAA was found 15cm while the IAA_{LM} (Indole Acetic Acid produced from Leuconostoc mesenteroides) was found to be 18cm. EPS has ability to hold water helps in soil aggregation, and IAA act as growth stimulator for plants. It has wide applications in agriculture, which maintains soil quality without using chemical fertilizer that aids in plant growth ultimately contribute to Indian economy.

Keywords : *Exopolysaccharides, leguminous soil, indole acetic acid, Leuconostoc mesenteroides.*

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INTRODUCTION

India is Agriculture land. Agriculture plays a vital role in the Indian economy. Over 70 per cent of the rural households depend on agriculture. Agriculture is an important sector of Indian economy as it contributes about 17% to the total GDP and provides employment to over 60% of the population. There should maintenance of productivity to fulfill the market demand. But now days slow agriculture growth is issue of concern [1]. There are many factors affecting the agriculture productivity such as natural factors like, rainfall, temperature, soil type, soil moisture and many more, on which the crops yield is dependent. There are many problems are there associated with Agriculture Problem, that are Soil Erosion, Drought, Use of Fertilizers etc. due to which there is decrease in quality of land and ultimately affect the growth of plants. Soil Erosion refers to wearing a way of field's topsoil by physical forces of water and wind. As top soil is rich in organic matter, fertility and soil life. It leads to loss of soil structure and wash away important nutrients. Thus directly influence the land productivity, soil moisture and depletion of nutrients [2]. Another concerning problem is Drought. It is nothing but below average precipitation rate, through which there is limited water availability in soil .it can inhibited the adsortsion of nutrients by roots of plants especially legume. Afterwards use of fertilizer is today's main concern. For more productivity rate of crops there is using chemicals, fertilizers which limits the use of land up to some time for use. As we know these are chemicals which causes pollution of environment and also toxic for human health too [3].

So for all these problems effort take place to maintain the productivity of land by natural way i.e by using microbiological conservation. It includes the use of microorganism which can improve soil structure by aggregating soil through microorganism such as bacteria producing exopolysaccharide.

Exopolysaccharide is a complex mixture of macromolecular contained on outside of the bacterial cell. There are some microorganism which can form (biopolymer) exopolysaccharide *Leuconostoc* sp., *Pseudomonas* sp. *Erwinia*, *Azotobacter* etc. there is wide applications of exopolysaccharide. Exopolysaccharide protects the bacteria from variety of environmental stresses, contributes to soil aggregation as an adhesive .the basic concept of aggregation is the formation of secondary particles through the incorporation of minerals particles with organic and inorganic materials, indirectly this leads to enhance the nutrient availability to plants thus increases its productivity. EPS also protects the crop yield from desiccation and drying in case of low moisture content [4].

In Addition to it many physiological activities of plants are regulated by variety of plant growth regulators and phytohormones. Among which Indole acetic acid plays a critical role in root nodule development, formation of laterals roots, adventitious roots, primary root elongation. As we know roots are very much crucial for plant growth for absorption of water and nutrients. But in some time plants are not able to produce IAA in sufficient quantities which results in low growth and development. EPS and IAA are also contributes to signalling pathway specially in leguminous plants. They maintain symbiotic relationship between bacteria and plants which indirectly increases nitrogen uptake by nodules and supply to plants. In all the aspects : They contribute to global Nitrogen Cycle, Potential applications in Sustainable [5]. Therefore current study emphasize the production of Exopolysaccharide and Indole acetic acid from microorganism which enhance the agricultural productivity.

MATERIAL AND METHODS

Soil Sampling

Soil sampling have been taken for the purpose to isolate bacteria that producing exopolysaccharide (EPS) in Leguminous soil (groundnut) at a depth of 10-15 cm soil samples taken in moderation, homogenized and put into a sterile plastic bag. Equipment have been cleaned and sterile with a wash and then rinsed or wiped with alcohol swabs.

Serial Dilution Method

Serial dilution method was performed to get isolated colonies of *Leuconostoc mesenteroides*. Label three screw-capped tubes 1:100, 1:10000 and 1: 1000000. In scientific notation this would be 10^{-2}, 10^{-4}, and 10^{-6} followed by transferring aliquot 9.9 ml of sterile distilled water into each of tube using a sterile 10ml pipette. 1gm of soil sample was dissolved in 10 ml of distilled water to prepare an original suspension culture. After that using a 100 µl micropipette and sterile tip, 0.1 ml of soil suspension culture was transfer into the 10^{-2} tube. Cap the tube immediately. Either mix it for a few seconds on a vortex mixer or vigorously flick the tube to adequately disperse the bacteria evenly throughout the tube and break up bacterial clumps. The procedure was applied for the next tubes in the same manner. One plate is labelled as "0.1 ml of 10^{-4}" and another plate "0.1ml of 10^{-6}. Mix the dilution tube and aliquot the indicated amount from the appropriate tube onto the ATCC no.14 medium. L-shaped glass spreader was used to spread the inoculums evenly around the plate. Incubate the plates for 24 hours at 28^0C [6]. Bacteria that produce EPS characterized by colonies of bacteria that form thick slime (mucoid) subsequently selected and purified by streaking the four quadrant to obtain single colonies.

Identification of *Leuconostoc mesenteroides* from soil samples on the basis of morphological, cultural and biochemical characteristics

For the study of Gram character, a well isolated colony was selected by touching with inoculating loop and placed on clean glass slide along with the drop of distilled water. A staining procedure was performed as per the standard procedure. Shape, size, gram character was then observed.

The isolated colonies were identified on the basis of morphology by performing Gram staining and motility, biochemical by testing sugar fermentation using Glucose, Lactose, Mannitol, Maltose, Sucrose, IMViC Test, Catalase test, Oxidase test, Triple Sugar Iron (TSI) test, Urease test and cultural characteristics by inoculating bacteria on Mannitol salt agar, Baird-Parker medium, and Cysteine Lactose Electrolyte Deficient agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India). They were incubated aerobically at 37 °C for 24 hours [7].

Production of Exopolysaccharide (EPS)

Selection and screening bacterial exopolysaccharide producing potential by setting the dry weight exopolysaccharide produced by bacteria in liquid medium ATCC no. 14 (per liter of medium): 0.2g KH_2PO_4;0.8g K_2HPO_4; 0.2 g $MgSO_4.7H_2O$; 0.1 g $CaSO_4.2H_2O$; 2.0 mg $FeCl_3$; $Na_2MoO_4.2H_2O$ (trace); 0.5 g Yeast Extract, 20 g sucrose; with pH 7.2. Using sucrose as a carbon source method proposed by Colonies of bacteria that form thick slime (mucoid) on solid medium no.14 ATCC were grown in 20 ml liquid medium ATCC no. 14 and incubated at a temperature of 28^oC for three days at the top of the machine shaker with 200 rpm rotation. At the end of incubation, cells were harvested with 1 mm EDTA by adding

500µl, then shaken until homogeneous and then centrifuged at 9000 rpm for 10 min. The supernatant was separated from the bacterial cell deposition was taken, coupled with cold acetone solution with a ratio of 1: 3 Then have been performed again with the speed centrifugation 10,000 rpm for 2 times 10 minutes. Deposition of biomass in the form of exopolysaccharide then washed with distilled water and dried at 60^0C 24 hours or until dry weights obtained were fixed [8].

Confirmatory test for EPS:

Molisch test Procedure

The test solution is combined with a small amount of Molisch's reagent (alpha naphthol dissolve in ethanol) in a test tube. After mixing, a small amount of concentrated sulphuric acid is slowly added down the sides of sloping test tube.

Production of IAA

To testing Bacterial that produced IAA production from exopolysaccharide producing bacteria, NB media and the addition of L-tryptophan have been used. L-tryptophan is a precursor of IAA will provide a high production rate as reported. Bacterial isolates were grown on NB medium for 10 days at 28^0C.

Test for IAA

Bacteria that have grown up after incubation were centrifuged at 3000 rpm for 10 minutes. Taken supernatant (2 ml)was mixed with two drops of orthophosphoric acid and 4 ml of Salcowsci reagent (50 ml, 35% of the sulphuric acid (H_2SO_4), 1 ml of 0.5 M solution of $FeCl_3$). To Indicated to IAA production by Pink discoloration showed. Optical density was taken at 530 nm using a UV-Vis spectrophotometer. The concentration of IAA produced by bacteria was measured through a standard curve of pure IAA (Sigma-Aldrich) which obtained in the range of 10-100mg/ml [9].

RESULT AND DISCUSSION

Isolation of *Leuconostoc* species from soil sample

Soil sample from leguminous soil after culturing it on the ATCC no.14 media. The bacterial culture showed growth on the media in the form of Thick, mucoid slimy creamish colour colonies. The isolate was characterized through cultural, morphological, and biochemical studies and isolated species was identified as *Leuconostoc mesenteroides.*

Morphological characteristics

Gram Staining

Table No.1 Morphological Characteristics of *Leuconostoc mesenteroides*

Gram Reaction	Gram Character	Shape	Motility
Purple	Gram Positive	Short cocci chain	Non -motile

Biochemical characteristics

Table No.2 IMViC Characteristics by *Leuconostoc mesenteroides*

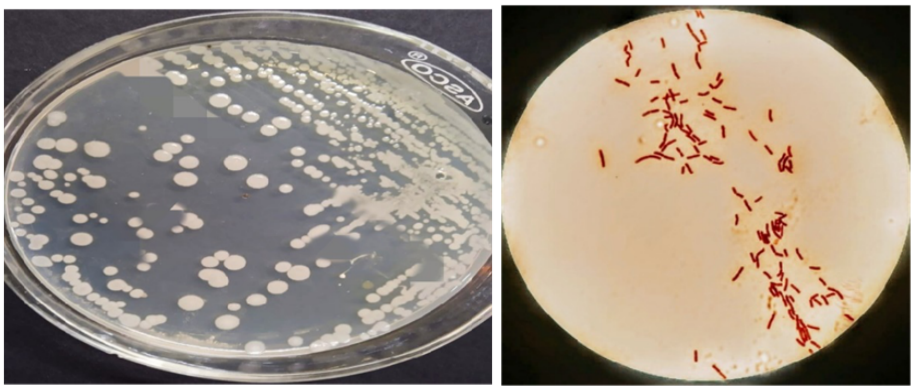
Sample	Indole	MR	VP	Citrate	Urease	Catalse	Oxidase	TSI		
								Acid	Gas	H_2S
S1	- ve	- ve	+ ve	- ve	- ve	- ve	- ve	+ ve	+ ve	+ ve

Table No. 3 Sugar fermentation characteristics *Leuconostoc mesenteroides*

Sample	Dextrose		Lactose		Sucrose		Mannitol		Maltose	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
S1	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve

Table No.4 Cultural characteristics of *Leuconostoc mesenteroides*

Sr. No.	Media	Observation
1.	ATCC no. 14	Thick slime (mucoid) colonies are observed
2.	Sheep Blood Agar	Non haemolytic colonies are observed
3.	Nutrient Agar	Round shiny slimly colonies observed



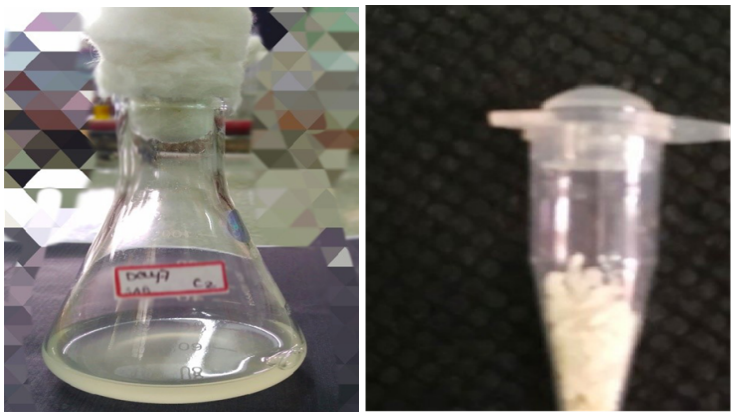
(a) (b)

Fig 1 : (a) Isolated colonies on ATCC No.14 (b) Gram Staining of *Leuconostoc mesenteroides*

The present study deals with the isolation of *Leuconostoc mesenteroides* from leguminous soil .The given isolate was found to be Gram positive, Non-motile, Indole –ve, VP +ve . On ATCC No.14 medium spherical, convex smooth, shiny colonies were observed. They showed negative catalase test and produced gases from all sugars. The similar trends of results were found in the study of Dimić Gordana R [10] work on *Leuconostoc mesenteroides*, they found two types of spherical colonies, one is compact colonies and other one is spreaded colonies along with negative catalase test and produced gases from glucose, supported the present investigation. The work done by Mu'minah *et al,* [4] showed that isolated colonies from potato rhizosphere was found to be Gram negative. They have potential to produce Exopolysaccharide on MacConkey medium on which thick, slimy colonies are observed.

Production of EPS by using *Leuconostoc mesenteroides*

After incubation, cells were harvested with 1 mm EDTA by adding 500 µl, and shaken until homogeneous and then centrifuged at 9000 rpm for 10 min and after dried it was found to be in range of 100- 130 mg/10 ml. Exopolysaccharide production were done successfully by *Leuconostoc mesenteroides*. In order to produce exopolysaccharides, *Leuconostoc mesenteroides* culture were grown in ATCC No. 14 broth in 3 different flask for three, five and seven days of incubation. Highest exopolysaccharides production were seen in fifth days of incubation i.e 4.6 mg/ml. ATCC medium supplemented with sucrose might be used by *L. mesenteroides* as a carbon source to produced EPS. It can be determined primarily on the basis of visual observation as they produced slime, thick EPS that it provides. The work done by Mu'minah *et al*., [4] showed that found isolates which produced EPS upto 2.24 mg/ml, correlates with present investigation. Study showed by B. Saritha Kumari *et al.* [11] they isolated Rhizobium strain from Root nodule with addition of different carbon source .maximum EPS upto 966 mg/g. The observation of Mu'minah *et al*., [4] revealed that isolated strain produced EPS dry weight in range of 0.10 to 2.24 mg/ml , which also agree with present work.



(a) (b)

Fig. 2 : (a) ATCC No. 14 medium (b) Dry weight of EPS

Confirmation of EPS production

Molisch Test

The broth is combined with a small amount of Molisch reagent in test tube. After mixing, a small amount of concentrated sulphuric acid is slowly added down the sides of the sloping test tube without mixing to form Purple ring indicating presence of saccharides.

Congo red agar

CRA plates were inoculated with test organism and incubates at 37°C for 24 hours. Red-black colonies were observed confirms production of exopolysaccharides.

(a) (b)

Fig. 3 : (a) presence of sugars by Molisch test (b) Confirmation of EPS by Congo-red Agar plate

Determination of Indole Acetic Acid Production by using *Leuconostoc mesenteroides*

Bacterial isolates were grown in Nutrient Broth medium with addition of L-tryptophan as precursor of IAA. After 10 days of incubation were centrifuged at 5000 rpm for 10 mins. Supernatant was mixed with 2 drops of orthophosphoric acid and Salcowsci reagent, Pink colour showed presence of IAA i.e. Indole Acetic Acid.

Quantification of IAA

Different dilutions of pure IAA having concentration 1mg/10ml were prepared. Afterward test reagents were added to it that gave it pink colour. The absorbance of each dilution was measured with the help of colorimeter. The production of unknown IAA was measured by plotting graph between Absorbance and Concentration of pure IAA, as the value in between the values of standard IAA which is obtained 88.80 µg/ml.

Table 5: Quantification of IAA

Sr. No.	Test Tube	Absorbance at 530nm	Concentration of maltose (µg/ml)
1.	Blank	0.00	00
2.	1	0.60	20
3.	2	0.62	40
4.	3	0.73	60
5.	4	0.80	80
6.	5	1.01	100
7.	**IAA_{LM}**	**0.88**	**88.80**

IAA_{LM} - Indole Acetic Acid produced from *Leuconostoc mesenteroides*

(a) (b)

Fig 4: (a) Quantative determination of unknown IAA (b) Comparative study of effect of std. IAA and exp.IAA on plant growth

While performing the application of indole acetic acid (IAA) on the growth of plant, it was found that the same concentration of indole acetic acid (IAA) apply to the seeds of Fenugreek (*Trigonella foenum-graecum*). The results showed that the length of growth of Fenugreek plant with standard IAA was found 15cm while the on the other hand the growth of length of Fenugreek plant with IAA_{LM} (Indole Acetic Acid produced from *Leuconostoc mesenteroides*) was found to be 18cm. From the above experiments it is confirmed that IAA produced by bacteria is also effectively promote the growth of plants.

Investigation was done by Susilowati et al, (2018).in ATCC medium along with sucrose, IAA produced found to be 1.0652 ppm. The study showed by Mu'minah et al., (2015) isolates produced IAA in range of 0.40-21.14 mg/l [12]. The work done by B. Saritha Kumari et al., (2009) supported the present investigation they found maximum yield of IAA upto 149.7 ug/ml. as they used different carbon sources, may be there in increased amount of IAA can be found [11]. EPS has ability to hold water helps in soil aggregation, and IAA act as growth stimulator for plant .They are non hazardous i.e economically friendly. It has wide applications in agriculture, which maintains soil quality without using chemical fertilizer that aids in plant growth ultimately contribute to Indian economy.

REFERENCES

1. Kekane Maruti Arjun (2013). Indian Agriculture- Status, Importance and Role in Indian Economy. International Journal of Agriculture and Food Science Technology. ISSN 2249-3050, Volume 4, Number 4, pp. 343-346
2. Jim Ritter, (2011). OMAFRA Factsheet, Soil Erosion – Causes and Effects, Order No. 87-040
3. Mc Garry D., Sharp G. 2000 Rapid, Immediate, Farmer-Usable Method of Assessing Soil Structure Condition to Support Conservation Agriculture Conservation Agriculture pp 375-380
4. Mu'minah, Baharuddin, Hazarin Subair, Fahruddin and Baso Darwisah. (2015).Isolation and Screening of Exopolysaccharide Producing Bacterial (EPS) from Potato Rhizosphere for Soil Aggregation. Int.J.Curr.Microbiol.App.Sci, 4(6): 341-349.
5. Pallab Kumar Ghosh, Tarun Kumar De, and Tushar Kanti Maiti.(2015). Production and Metabolism of Indole Acetic Acid in Root Nodules and Symbiont (Rhizobium undicola) Isolated from Root Nodule of Aquatic Medicinal Legume Neptunia oleracea Lour. Journal of Botany. Volume 2015, Article ID 575067, 11 pages
6. Benson HJ (2002) Microbiological applications-laboratory manual in general microbiology, 8th edn. McGraw Hill International edition, New York
7. Cheesbrough, M. (2000). Microbiological test: District Laboratory Practice in Tropical Countries. In: Cremer, A. and Evan, G. (eds). Cambridge University Press, UK. Pp: 1-226.
8. Montersino S, Prieto A, Muñoz R, de Las Rivas B.(2008). Evaluation of exopolysaccharide production by Leuconostoc mesenteroides strains isolated from wine. J Food Sci. ;73(4)
9. Sheela Chandra, Kazim Askari, and Madhumita Kumari.(2018). Optimization of indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. *J Genet Eng Biotechnol*. 16(2):581–586.
10. Dimić Gordana R (2006). Characteristics of the Leuconostoc mesenteroides subsp. mesenteroides strains from fresh vegetables. Acta Periodica technologica (37). 34-39
11. B .Saritha kumari et al. (2009). Studies on exopolysaccharides and indole acetic acid production by Rhizobium strains from indigofera. Biology. Vol 3 (1) pp.010-014.
12. Susilowati, A.; Puspita, A. A.; Yunus, A. (2018). Drought resistant of bacteria producing Exopolysaccharide and IAA in rhizosohere of soyabean plant in wonogiri regency centra java Indonesia .Earth Environ.Sci. IOP Conference Series: Earth and Environmental Science, Volume 142, Issue 1, pp. 012058 .

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