

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 2, February 2019

Isolation and Characterization of Endophytic Bacteria from Aloe Vera Plant and Screening of its Antibiotic Activity

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ABSTRACT: Endophytic bacterium is a species of fungus or bacterium that lives inside the plant tissue for at least a few parts of its life cycle and do not cause any type of bacterial disease in plant. These species are ubiquitous. They enhance host growth, nutrient acquisition and improve the plant's ability to tolerate a biotic stresses, such as drought, and decrease biotic stresses by enhancing plant resistance to insects, pathogens and herbivores.

Aloe Vera is a succulent plant species of the genus Aloe. It is mainly cultivated for medicinal uses. It is found in many consumer products including beverages, skin lotion, cosmetics, or ointments for minor burns and sunburns.

KEYWORDS: Endophytes, Aloe. Vera, Bioactive Compounds, Anti- microbial activity.

I. INTRODUCTION

Medicinal plants have been used as an important source of medicine for centuries ago. Till date there are many people about 80% of people are there who rely on these medicinal plants for medicine and used as herbs to cure skin disease. The natural source of medication leaves a huge impact of medication on preventing and treating the diseases. The use of these plant extracts with the characterized antibiotic assay may help in overwhelming the microbial diseases.

Aloe Vera is a stem less or very short-stemmed plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. It belongs to the kingdom -plantae, clade- Angiosperms, Order- Asparagales, Family- Asphodelaceae, Subphylum- Asphodeloideae, Genus-Aloe, and Species-A.vera. Despite its wide use as a folk remedy over a long time, the biochemical functions have not been systematically investigated. The plant has been presented as a constituent of many phytochemicals, vitamins, nutrients, and anti-nutrients found in foods.

Aloe Vera gel is used commercially as an ingredient in yogurts, beverages, and some desserts, but at certain high doses, its toxic properties could be severe when taken orally. It is a xerophytes type of plant which can survive at very harsh conditions the conditions which are not favorable for normal vegetation to grow, so it also has a property to retain high water content in its leaves its gel mainly consists of 99.3% water. The remaining 0.7% is of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give it special property.

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Endophytes are the bacterial species that live within the plant tissues for a specific period of time of its life cycle but do not cause any type of bacterial disease. Endophytes are ubiquitous with rich biodiversity, which have been found in every plant species examined to date. It is noteworthy that, of the nearly 300,000 plant species that exist on the earth, each individual plant is the host to one or more endophytes. Mutualism interaction between endophytes and host plants may result in fitness benefits for both partners.

The present study was focused on the isolation of endophytic bacteria from *A. Vera* gel which is capable of showing antibacterial activity that can be used in food and pharmaceutical industries against various bacterial pathogens.

II. OBJECTIVE

Isolation of endophytic Bacteria sp. from *Aloe Vera* gel and its antimicrobial activity. This study was conducted to determine the significance of endophytic bacteria in *A. Vera* gel for the prevention and treatment of various infections caused by bacteria.

III. LITERATURE SURVEY

This research paper is based on the laboratory test performed on aloe vera plant to isolate and characterize the endophytic bacteria isolated from the gel of these collected plant specimens of aloe vera plant. At first there is a selection of suitable plant source then these plants were surface sterilized and then the bacteria was isolated from the aloe vera gel. Then characterization was done of these isolated culture based on gram staining and biochemical test and then mass multiplication of this effectively isolated was done which was then followed on to the antibacterial assay test and from which certain results were predicted.



Fig: Aloe Vera plant (Plant source- Kolkata)

It is cultivated worldwide, primarily as a crop for "Aloe gel," which comes from the leaf. Aloe vera is widely used today in: Food - it is approved by the FDA as a flavoring. Cosmetics. Food supplements, Herbal remedies.

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IV. MATERIALS AND METHODOLOGIES

SAMPLE COLLECTION:

Aloe Vera plant samples were collected from parts of eastern Kolkata in West Bengal and central Bangalore markets.



Fig: plant source from Kolkata

The shown plant specimen is collected from Kolkata West Bengal where a vera plant is grown mainly for cosmetic products, pharmaceutical products.



Fig: plant source from Bangalore.

The shown plant specimen is collected from central markets in Bangalore (yeswanthpur) which is cultivated mainly in the form of hybrid products for mainly pharmaceutical products and cosmetics.

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SURFACE STERILIZATION

First the collected plant samples were thoroughly washed with distilled water nicely for 5 minutes then it was washed with 70% ethanol for 2 minutes then it was again washed with distilled water for 3-5 minutes then it was washed by immersing in sodium hypochlorite solution (200 ppm) for 5 minutes and then rinsed with distilled water. Plant samples were subsequently rinsed thrice in sterile de-ionized water thrice to remove contaminants.

V. ISOLATION OF ENDOPHYTIC BACTERIA FROM ALOE.VERA GEL

Endophytic bacteria were isolated from sterilized aloe Vera leaves. In sterile conditions a cut was made in the leaves to squeeze out the gel. Then 100 ml of Lureia Bertani agar media and 100 ml of Lureia Beratani broth was prepared and was sterilized. Then this sterilized media was poured onto plates and kept for solidification after solidification a cotton swab soaked in aloe Vera gel was streaked onto the solidified agar plates and was incubated for 24 hours at a temperature of 22-28⁰ c. The LB broth was also inoculated with the squeezed aloe Vera gel and was kept for incubation at a temperature for 22-28⁰c for 24 hours. When the growth comes then 50 micro liters of this LB broth was taken and streaked onto sterilized LB agar media plates and kept for incubation after which the endophytic bacterial colonies appeared.



Fig: sterilized LB agar and broth

The shown picture shows sterilized lb agar and broth which is prepared by dissolving 2.5 gm of lb media in 100ml of distilled water for broth and for agar add 2.5 gms of lb media in 100 ml of distilled water and followed by addition of 0.28 gm of agar agar.

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Fig: Inoculated LB broth with A.vera gel

This picture shows a conical flask which contains 100 ml lb broth which is prepared by 2.5gms of lb media dissolved in 100ml of distilled water and then sterilized and then inoculated with aloe vera gel and then kept for incubation at 22-24⁰c for 28 hours.



Fig: Inoculated LB agar media with 50 micro liters of inoculated lb broth.

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This picture shows inoculated lb agar media plate. This plate was prepared by pouring 20 ml of sterilized agar media onto a sterilized plate and then it was inoculated by aloe vera gel and then it was incubated at 22-24⁰c for 28 hours.

VI. CHARACTERIZATION OF EFFECTIVE ISOLATE

Characterization of effective isolate was done by doing gram staining from the inoculated plates followed by certain biochemical tests (catalase tests). Catalase test is done by making a smear of the bacterial isolate on a clean glass slide and then a drop of hydrogen peroxide was added and checked for the effervescence or bubble formation. The isolate which was tested gives positive result on catalase test.

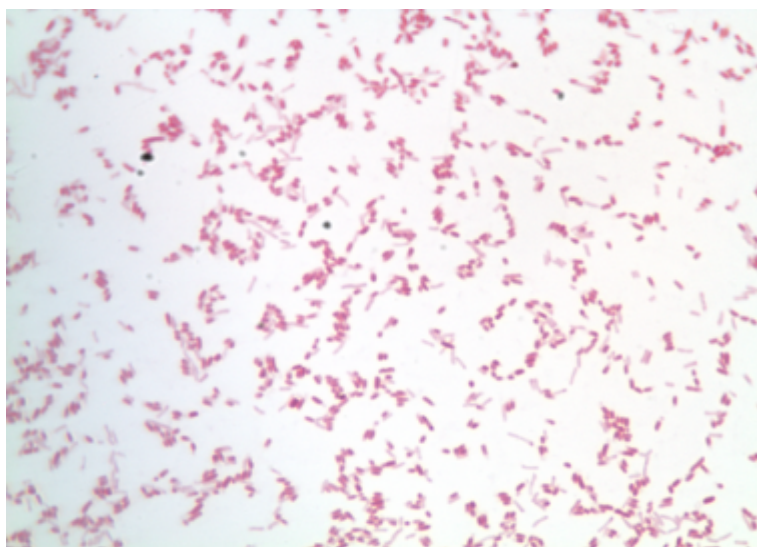


Fig: Gram staining of the effective isolate-Gram negative rods.

This figure shows the microscopic view of stained slide of the effective isolate. The slide was prepared by first making a thin smear of the effective isolate then it was flooded with drops of crystal violet stain and kept for 30 seconds then it was washed with distilled water then the smear was flooded with Gram's iodine and kept for 45 seconds then the slide was washed with distilled water then the smear was flooded with ethanol and kept for 45 seconds and then washed with distilled water and then the smear was flooded with saffranin and kept for 45 seconds and then the smear was washed with distilled water and air dried and then focused onto a microscope.

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Fig: catalase test of the isolate showing positive result for gram negative bacillus sp.

The slide shows catalase positive test slide. The slide was prepared by making smear of the effective isolate and then few drops of hydrogen peroxide was added and effervescence was seen..

VII. GROWTH KINETICS STUDY

To figure out the growth curve of the effective isolate 50 ml of the LB media was prepared in 250 ml conical flask and was inoculated with the isolate grown and was incubated at a temperature of 22-28⁰ c for 24 hours and then from that incubated media 1.5 ml of this incubated media was taken and was inoculated in 150 ml of LB broth and was again incubated for 24 hours at the same incubation temperature and the optical density readings were taken after every 2 hours. Five sets of readings were taken based on which the growth curve was prepared.

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Fig: 50 ml of LB broth inoculated with the isolated bacteria.

This figure shows that 50ml of lb broth was prepared and sterilized at 15lbs pressure at 121⁰c and then divided among 5 test tubes and inoculated with the isolated bacteria from the aloe vera gel and was kept for incubation at 22-24⁰c for 28 hours.

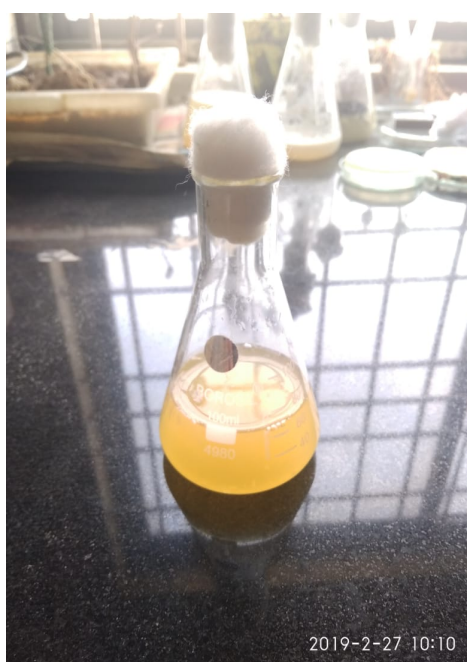


Fig: 150 ml of LB media inoculated for growth kinetics study

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This figure tells about the lb media which was inoculated with the cultured isolate from aloe vera and then was maintained for growth kinetics study, from this growth kinetics study the growth pattern of the effective isolate can be observed and we can predict after how much time interval does the log phase stationary phase and lag phase is obtained.

Optical density readings:

Time duration	OD values(600 nm)
2 hours	0.05
4 hours	0.98
6 hours	0.14
8 hours	0.22
10 hours	0.32
12 hours	0.33
14 hours	0.33
16 hours	0.24
18 hours	0.22

VIII. MASS MULTIPLICATION WAS DONE FOR THE ISOLATE

For mass multiplication studies, 350 ml of media was prepared in 500 ml flask and autoclaved at 121°C and 15 lbs pressure for 15 minutes, 6.5 ml of inoculums (24 hrs old) was inoculated onto this media by using sterilized loop and incubated again at 37°C for 6 days and the flask was kept in orbital shaker at 120 rpm.

IX. ANTI BACTERIAL ASSAY

Anti bacterial assay of the isolate was done with agar well diffusion method. In this method first the plates were filled with sterilized agar and was kept for solidification then it was inoculated with *Pseudomonas*'s and *Staphylococcus*'s and then the media was punctured with gel puncture instrument and then in this media 100 micro liters of the isolate inoculated broth was filled and was incubated for 24 hours and the zone of inhibition was measured.

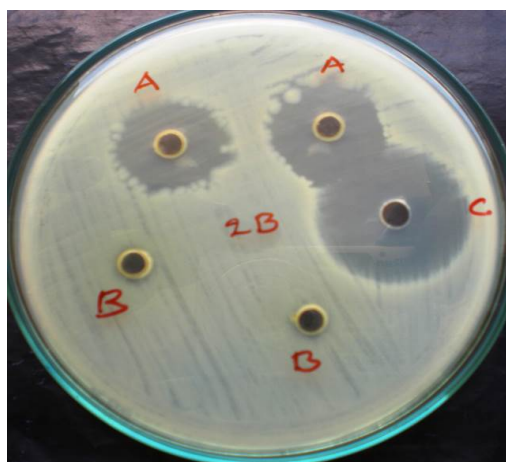


Fig: activity against. *Staphylococcus* sp.

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Plate shows the anti bacterial activity of the effective isolate upon staphylococcus species. the plate was prepared by streaking a sterilized molten nutrient agar plate with staphylococcus species and then inoculating the plate with effective isolate broth into the wells made on the plates.

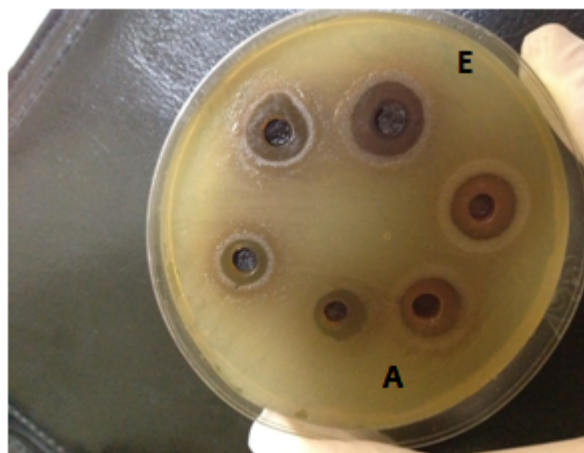


Fig: activity against pseudomonas sp.

Plate shows the anti bacterial activity of the effective isolate upon pseudomonas species. The plate was prepared by streaking a sterilized molten nutrient agar plate with pseudomonas species and then inoculating the plate with effective isolate broth into the wells made on the plates.

X. RESULTS

1. The endophytic bacteria were isolated from the taken plant samples and were maintained on the LB media.



Fig: isolated endophytic bacteria On the LB agar plate.

This plate was prepared by pour plate method where in the sterilized petriplates 50 micro liters of the effective isolate broth was poured and then followed by the addition of sterilized molten lb agar which was then kept for solidification then kept for incubation for 28 hours at a temperature of 22-24⁰c.

2. Characterization of the effective isolate: was done on the basis of gram staining which tells that the isolate obtained were gram negative rods and hence was identified as bacillus sp. And the isolates were circular in shape with opaque nature on smooth surface.

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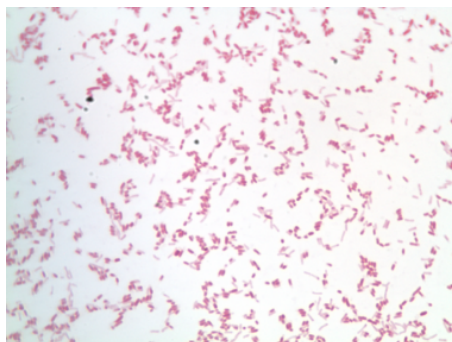


Fig: gram staining of the effective isolate-gram negative rods.

This figure shows the microscopic view of stained slide of the effective isolate. The slide was prepared by first making a thin smear of the effective isolate then it was flooded with drops of crystal violet stain and kept for 30 seconds then it was washed with distilled water then the smear was flooded with grams iodine and kept for 45 seconds then the slide was washed with distilled water then the smear was flooded with ethanol and kept for 45 seconds and then washed with distilled water and then the smear was flooded with saffranin and kept for 45 seconds and then the smear was washed with distilled water and the air dry and then focused onto a microscope.

3. Absorbance was measured at 600 nm for figuring out the growth kinetics study and it was seen that after 10 hours has passed on a stationary phase of growth was obtained which continued for further two more hours and then the growth was certainly decreasing with the advancement of time.

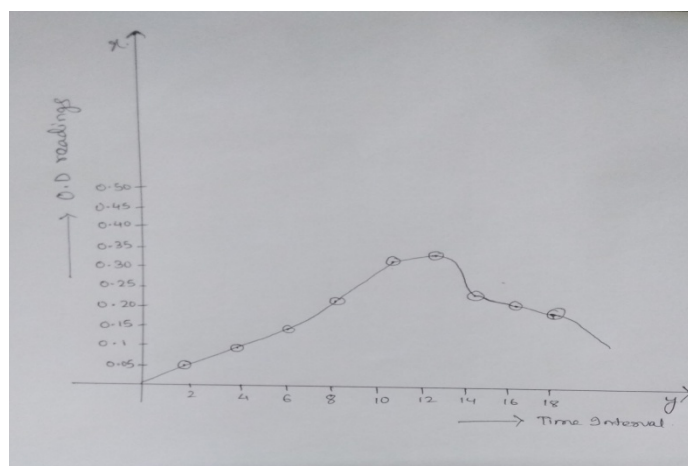


Fig: Growth kinetics curve of the effective isolate.

The following graph was made based on the values which were obtained as optical density readings of the inoculated lb broth which was prepared for the growth kinetics study. The graph shows that from time interval of 2-10 hours there is log phase that means the growth of the bacteria is increasing and then from 10-12 hours it is stationary that means the growth activity has reached its maximum level and then from 14-18 hours the growth activity is decreasing showing lag phase of the curve.

4. Antibacterial assay: Zone of inhibition showed inhibitory activity against *S. aureus* with a zone of inhibition of 24 mm and in activity against *Pseudomonas sp.* was observed giving a zone of inhibition of 24.5 mm respectively.

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XI. DISCUSSION

A. Vera has been widely used in skin cares, cosmetics and as nutraceuticals, in various skin conditions such as cuts, burns, and eczema. Microorganisms have been used for discovery of novel drug for the past few decades, but presence of endophytic bacteria in *Aloe barbadensis* was reported by Mamta, *et al.*... In (2012).

The isolation of endophytic bacteria from *A. Vera* was carried out with the idea that unusual endophytes may produce unexploited natural products.

Endophytes have proven to be rich sources of novel natural compounds with a wide-spectrum of biological activities and a high level of structural diversity. Molecular techniques have been successfully used for identifying endophytic communities in recent studies (Promputtha, *et al.* 2005, Sette, *et al.* 2006, Tedersoo, *et al.* 2006, Morakotkarn, *et al.* 2007).

The obtained isolate can be effectively used in food and pharmaceutical industries to obtain bioactive compounds for various bacterial pathogens. The study of endophytic bacteria is important, not only for understanding their ecological role in their interaction with plants but also for their possible biotechnological applications.

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