Isolation of Endophytes From Roots of Aloe Vera

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Abstract- *The aim of this study was to isolate endophytic microorganisms from the roots of Aloe vera from ten locations of Shrirampur, Ahmadnagar district Maharashtra, India. A total 44 endophytes were isolated. Among this 28 were endophytic bacteria and 16 were endophytic fungi from location number 7th at rate of isolation frequencies 65% and endophytic fungi was 35% respectively. Based on morphological characteristics of this isolates they were representing Ascomycetes at rate of isolation frequencies 79% for fungi and Bacillus at rate 21%. Above all endophytes were reported first time from Shrirampur, Ahmadnagar district Maharashtra, India therefore present study may represent a unique source of one or more interesting bioactive compounds.*

Keywords- Endophytes, Isolation, Interaction.

I. INTRODUCTION

The world Health Organisation (WHO) estimates that in low and middle income countries 30% of total 70 million annual deaths occur due to increased multiple drug resistance of microbes. Multidrug resistance occurs when pathogens undergo mutations [1]. Hence these conditions demand immediate action to understand clearly the epidemiology of the resistant pathogens, mechanisms of resistance and treatments available [2]. To overcome this problem, we can use and aim for purified bioactive compounds from plants and microbes. An endophyte is an organism which lives inside a plant without affecting them [3].

This is contrasted to epiphyte, which refer to the organism on the outside of the plant. They often protect their host plants against a wide range of insect and mammalian herbivores, through the production of toxic alkaloids. Endophytes are the chemical synthesizers inside plants [4,5]. Many of them are capable of synthesizing bioactive compounds that can be used by plants for defence against pathogens and some of these compounds have been proved useful for novel drug discovery [6,]. Till now merely *Aloe vera* is studied by researchers for the endophytes therefore present study was mainly focused on *Aloe vera* plant and their endophytic symbiosis. *Aloe vera* is a succulent plant which belongs to the Asphodelaceae family [7].

II. MATERIALS AND METHODS

A. Collection of Samples

The fresh and healthy roots of *Aloe vera* were selected and collected from ten locations of Shrirampur, Ahmadnagar district Maharashtra, India in month of November, 2018. The locations were named as Loc 1, Loc 2…..Loc 10. The root samples were identified and authenticated by Prof. Kukreja G., Department of Microbiology, NACSC, Ahmadnagar. A voucher specimen (N0-01/2018) was deposited at the Department of Microbiology, NACSC, Ahmadnagar. All samples were immediately brought to the laboratory and processed for isolation of endophytic microorganisms.

B. Isolation of Endophytes

Root samples of *Aloe vera* was selected and washed with the running tap water to remove soil particles. Further root samples were immersed in 70% ethanol for 2 minutes and 4% aqueous solution of sodium hypochlorite for 2 minutes and 1 minute in 0.1% $HgCl_2$. finally washed with distilled water. Then samples were cut with sterilized knife and forceps and blotted with blotting paper. To confirm the sterility of disinfection of roots sample, 1 ml. sterile distilled water that was used in final rinsed surface sterilization process were spreaded on sterile nutrient agar plates and incubate it at $37^{O}C$ for 48 to 72 hours.

Nutrient agar media and potato dextrose agar media were prepared and autoclaved at $121^{O}C$ for 15 min at 15 lbs after autoclave 50 mg/L ampicillin was added only in PDA. Sterilized both media were poured off into sterilized petri plates and kept for solidification. After solidification these plates were used for implantation of surface sterilized samples for the isolation of endophytic microorganisms.

Sterilized root samples were inoculated in sterile both media plates, sealed with parafilm and incubated at 37°C and at 25^0C ± 2°C for 15 to 20 days.

C. Identification of Endophytes

The identification of endophytic microorganisms was carried out by using standard protocols [2].

D. Purification and Preservation of Endophytes

Purification and preservation of endophytes was carried out by as per standard methods [3].

E. Statistical Analysis

Colonization frequency (CF) was described by Hata et al., 1995. Colonization frequency (%) of an endophyte species was equal to the number of segments of colonized by single endophyte divided by the total number of segments observed × 100.

$$\% \text{ percentage of frequency} = \frac{\text{Number of endophytes}}{\text{Total number of segments}} \times 100$$

III. RESULTS

Present study was conducted with a view to isolate and characterizes the endophytic microbial diversity from the roots of *Aloe vera*, collected from ten locations of Shrirampur, Ahmadnagar district Maharashtra, India in month of November, 2018. Total 50 roots samples of *Aloe vera* were collected from above said region and brought to laboratory and subjected to surface sterilization by using sterilants. After surface sterilization of 100 pieces of 50 root samples, were implanted on nutrient agar media and potato dextrose agar media plates and observed for the growth of endophytic microorganisms up to 15 days. Total 28 endophytic bacterial species and 16 endophytic fungal species were isolated from the roots of *Aloe vera*. After 15 days 44 explant pieces showed endophytic microbial growth, 16 pieces showed Actinomycetes growth and 40 pieces not showed any growth (Table 2, Figure 1).

Amongst isolated endophytic microorganisms, highest growth showed by the endophytic bacteria at rate of 65% and endophytic fungi was 35%. Among ten locations, only Location 7^{th} showed effective isolates of endophytic microorganisms while other locations failed to induce isolation on PDA and NA (Table 1). In case of endophytic bacteria location 7^{th} was dominated on other locations while in case of endophytic fungi same location was dominated. Mostly isolated endophytic fungi belong to the Ascomycetes. These fungi belong to four Ascomycota classes which includes *Aspergillus*, *Trichophyton*, *Coccidioides* species belong to Eurotiomycetes, *Curvularia*, *Alternaria*, *Aureobasidium*, *Exserohilum*, *Bipolaris* species belong to Dothideomycetes , *Geotrichum*, *Candida* species belong to Saccharomycetes and *Fusarium* species belong to Sordariomycetes class (Figure 2). As far as bacterial endophytes are concerned bacillus species were dominated from roots of *Aloe vera*.

IV. DISCUSSION

Aloe vera is the succulent, evergreen perennial plant. It originates from Arabian Peninsula but mainly found in tropical climates around the world. It is cultivated for agricultural and widely for medicinal purposes. *Aloe vera* is a stem less or short-stemmed herb. It grows to 100cm by spraying offsets [7]. The leaves are thick and fleshy, green to grey – green. The flowers are produced in summer on the spike up to 80cm. they forms arbuscular mycorrhiza. *Aloe vera* leaves contains phytochemicals which are mostly active against different pathogens. Phytochemical are acetylated mannans, poly mannans, anthrones, and other compounds. Aloe vera extracts are widely used in cosmetic industries and in medicinal industries because of their effective properties proved by scientists. They are used in dietary supplements, in traditional medicines, and in commodities [7].

The plant tissues, especially roots are excellent reservoirs for endophytic microorganisms. Environmental factors such as temperature, rainfall and atmospheric humidity and their effect on host plant made the variations in occurrence of endophytic microorganisms and their colonization frequency [1]. Microorganisms isolated from up till now uncharted areas and from extreme environments is the understandable choice for development of potential novel bioactive metabolites. It has become clear that an enormous and relatively untapped source of biological assortment is represented by microbial endophytes which are a talented source of novel natural products for use in medicine, agriculture and industry [2]. Endophytes are the important component of biodiversity. These endophytes are isolated from medicinal plants are the best source of secondary metabolites play important role in plants life. The characteristics features of endophytic microbes are that, they are tissue specific, and one or more species found in single tissue.Therefore, in present study total 10 locations were selected from Shrirampur, Ahmadnagar district Maharashtra, India in month of November, 2018. Shrirampur is situated at 19.62 N, 74.66 E in western Maharashtra [3].

Shrirampur has a tropical dry and wet climate and temperature ranges from 20^{O}C to 40^{O}C. typical in the month of March and may summer was high with 40^{O}C temperature and January and April was the coolest month. *Aloe vera* plant roots were collected from the 10 locations of Shrirampur. The soil was blackish in color with alkaline pH. After surface sterilization explant were inoculated on media plates and observed for the growth of endophytes up to 15 days. The highest endophytes were isolated from the 7th location while others were failed. 7th location was nearer to lake because of this continuous water supply was there due to which moisture were maintained during whole life cycle of *Aloe vera* plant. When we withdrawn roots of *Aloe vera* plant, we observed moisture nearer to roots and healthy roots too [4].

The present report recovered four endophytic fungal classes at rate 93%, namely Deuteromycetes, Ascomycetes, Zygomycetes and Basidiomycetes with different isolation frequencies and different *Bacillus* and *Pseudomonas* species in bacterial case [5]. Mane et al., [2] investigated seasonal diversity of endophytic microorganisms. The leaf isolates mostly fell into *Cladosporium* spp., *Verticillium* spp., *Arthrinium* spp., *Colletotrichum* spp. and *Aspergillus* spp. from *Ocimum tenuiflorum* and *Calotropis procera* respectively. In our study we have isolated *Trichophyton* spp., *Bipolaris* spp., *Aspergillus* spp., *Geotrichum* spp., *Alternaria* spp. and *Candida* spp. Fungal endophytes were isolated from leaves of *Azadirachta indica* and *Piper betle* in nashik [3]. *Cladosporium* spp., *Curvularia* spp. and *Colletotrichum* spp. were obtained as endophytic fungi while we found *Fusarium* spp., *Trichophyton* spp., *Exserohilum* spp and onemycelia sterile from the same plants [4].

In contrast, Mane et al., 2018 have observed Deuteromycetes (55-72%) with high isolation frequencies and Ascomycetes (10-35%) with low isolation frequencies from *Aloe vera* and other medicinal plants [5].

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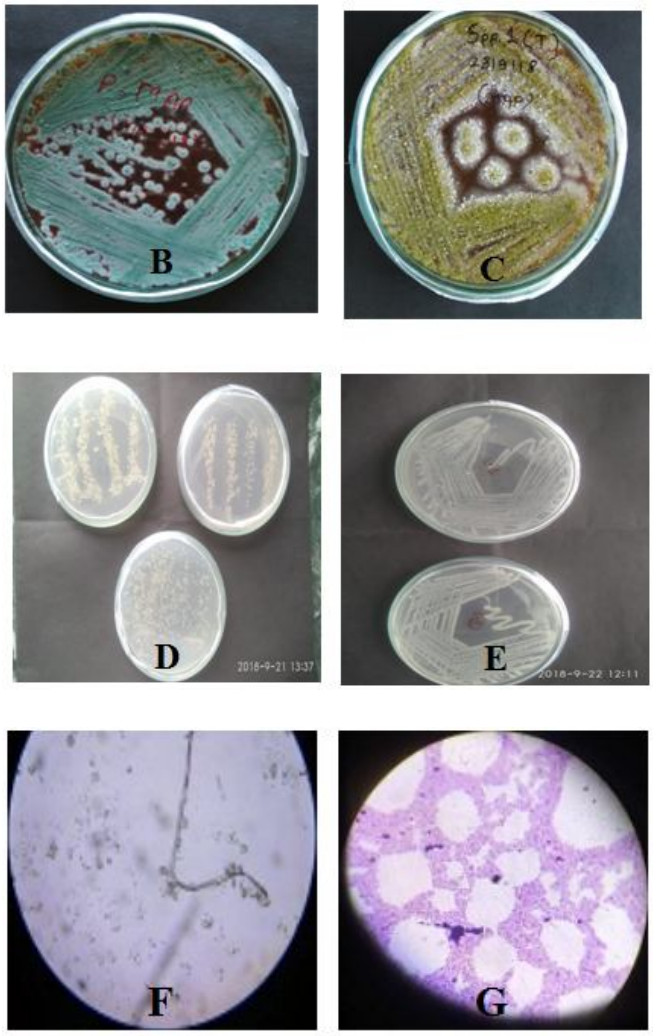

Figure 1Isolation and purification of endophytes; A Google map of Shrirampur, B & C Fungal endophytes on PDA plates, D & E Bacterial endophytes, F & G Microscopic plates of endophytic fungi and bacteria.

Table 1 Location and Total Endophytes

Locations	Endophytic bacterial growth	Endophytic fungi growth	Total endophytic microorganisms
Loc 1	-	-	-
Loc 2	2	1	3
Loc 3	4	2	6
Loc 4	-	-	-
Loc 5	1	1	2
Loc 6	4	2	6
Loc 7	9	6	15
Loc 8	6	3	9
Loc 9	-	1	1
Loc 10	2	-	2
Total	28	16	44

Table 2 Relation of samples and endophytic growth.

Explants	Total Pieces Implanted	Pieces with Endophytic Growth	Pieces with Actinomycetes growth	Pieces with No growth
Roots	100	44	16	40

Table 3 Isolation of endophytic microorganisms

Explants	Pieces with Endophytic bacterial growth	Pieces with Endophytic fungi growth	Pieces with Actinomycetes growth	Pieces with No growth
Roots	28	16	16	40

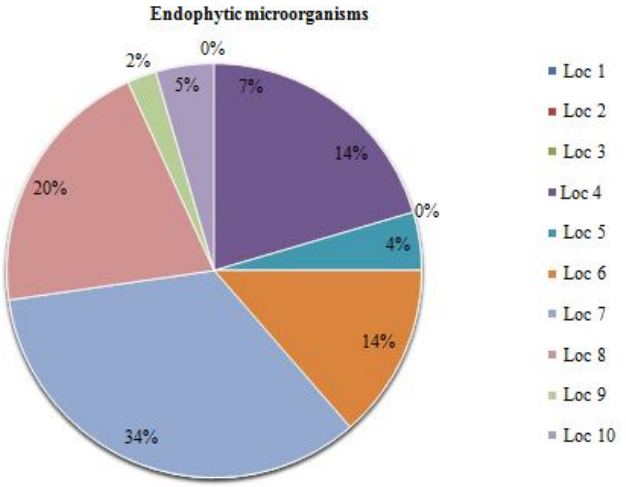

Figure 2 Isolation frequencies of endophytic microorganisms from different locations of Shrirampur

V. CONCLUSION

Our outcomes are powerfully signpost that sampling methods were satisfactory and systematic for rare species recovery. Our research demonstrated that roots of roots of *Aloe vera* were selected and collected from ten locations of Shrirampur, Ahmadnagar district Maharashtra, India have the potential to be an excellent source of endophytes and they are useful in disease management.

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