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Isolation and identification of endophytic fungi from *Aloe vera* (L.) Burm. f. root

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Abstract

The medicinal potential of microorganisms linked to plants has been well acknowledged. Endophytic fungi are a special kind of symbiotic microorganisms that live inside plant tissues without harming their hosts in any obvious way. The isolation and identification of endophytic fungi from the roots of *Aloe vera*, a plant with therapeutic and pharmaceutical benefits, is the main goal of this work. Samples of healthy plants' roots were taken, surface-sterilized, and cultivated in controlled environments on appropriate growth media. Enzymatic assay profiling was used for biochemical identification, while colony traits, hyphal structure, and spore morphology were used for morphological characterisation. were identified from a total of 6 fungal species identifying which was *Mucor*, *Microsporum, Candida* spp., *Fusarium, Aspergillus* and *Trichoderma*, and *Mucor*. The varied endophytic fungus population in *Aloe vera* roots and its possible significance for plant health and biotechnological uses are highlighted in this work.

Key words : *Endophytic fungus, Aloe vera, Pharmaceutical, Biochemical, Fungal diversity, Morphological identification*.

Medicinal plants are traditionally used worldwide as remedies for the treatment of various diseases and the composition of bioactive compounds produced by these medicinal plants varies widely depending on the plant species and their association with microbes[6]. Microorganisms associated with plants have come out with products with high therapeutic potential. Endophytes are the group of microorganisms that colonize the intracellular part of plant tissue[10]. Many medicinal plants have microbial endophytes within their tissue without any symptoms of infection, the symptomless interaction with microbes is non-pathogenic to weakly or slightly pathogenic depending upon the type of microbial species[13]. The most common examples of the association of a microbe with a plant can be that of nitrogen-fixing bacteria in leguminous plants, or the close association

of fungi and plants in the form of mycorrhizae[9]. Recently, research on endophytic microorganisms has increased due to their intimate interaction with the host species, and it is believed that the phytochemical constituents of plants are related directly or indirectly to the interactions of endophytic organisms with their host[7].

On a basic level, the term endophyte emanates from the Greek word "endo" meaning within, and "phyton" meaning plant. Earlier endophytes were elucidated as microbes such as bacteria and fungi that inhabit the internal tissue of plants without causing any evident harm to the host plant[12].

Aloe vera (L.) Burm. f. is a medicinal plant known worldwide for traditional medicine and is of significant commercial importance. It is widely used for the production of tonics, cosmetics, and the food industry and has great medicinal potential with numerous active ingredients[4]. It is a plant that is rich in countless medicinal properties like antioxidant, anticancer, antiulcer, and wound healing properties, and it also has a skin protection effect. The pharmacologically important compounds are found in the parenchymatous tissue of the fleshy parts of the plant[11]. Endophytes are known to produce metabolites such as alkaloids, terpenoids, steroids, quinones, isocoumarin derivatives, flavonoids, phenols, phenolic acids, and peptides. *Aloe vera* can serve as a good model plant for studying the effects of fungal endophyte colonization on secondary metabolism. They have significant economic importance, and a well-documented chemical profile and some of its therapeutic chemicals are known to be affected by endophytic fungi colonization[8].

This study will mainly focus on the endophytic community isolated from the root of *Aloe vera*. *Aloe* plants are known for their nutritional and therapeutic values. Other uses include treating wounds and burns, also diabetes, and elevated blood lipids in humans.

Healthy and Mature plant was collected from the botanical garden of Bhopal (M.P.). The sample were washed with sterile distilled water to remove the soil particles and tightly packed in polythene bags under humid condition and kept in room temperature.

Isolation and identification of endophytic fungus :

Endophytic fungus was isolated by using root fragment. After removal of the soil the sample taken into the shaken in water with detergent tween 80 at 70 rpm for 10 minutes to remove the density of the Exophytic fungus. The surface of the fragment was disinfected by successive process sequentially ethanol (70%), 2.5% sodium hypochlorite (active chlorine), and ethanol (70%) for 1min, 5min, and 30s. At the end four washes performed using autoclaved distilled water. At last, the sample were air-dried, the extern al part was removed, and the inner region of the plant segment were collected. After that the segment were placed on potato dextrose agar plate and incubated at 28°C. The incubated plates were examined for endophytic fungi and fungal hyphae were visible on the sample after 3 to 4 days. Later on, the hyphal tips of morphologically distinct endophytic fungi were carefully collected and transferred to newly prepared potato dextrose agar[3].

Endophytic fungi colony preservation :

The decontaminate fungal endophytes preserved for long term use. The endophytic fungi were transferred to sterile eppendorf tubes with 1 mL of 30% (v/v) sterile glycerol solution and sterile solid media in 250 mL conical flask, incubated at 28 °C for 4 days and then maintained at –20 °C.

Identification :

Morphological identification :

Morphological assessment is usually considered as the first step of identification. The sample of isolated fungus were mounted on sterile slides after that it was stained by lacto-phenol cotton blue and investigated in 40X light microscopy. The fungal culture was identified on the basis of spore shape, phenotypic characteristics, spore type, growth colour, growth rate using standard manual.

Biochemical identification[1]:

Amylase production test :

Starch agar medium was prepared and sterilized at 15lb pressure for 15 minutes. Poured the media on sterilized Petri plate and allowed to solidify. Each of the starch agar plates were labelled with the fungal ID of the pure culture isolated from sample. A single streak was made on each plate for each pure culture into the centre of the plate. Inoculated plates were incubated for 48 hours at 37 °C in an inverted position. The surface of the plates was flooded with iodine solution with the dropper for 30 seconds. The excess iodine solution was drained off. The plates were observed for the hydrolysis around the line of growth of each organism *i.e.* the colour change of the medium.

Casein hydrolysis :

Skimmed milk agar medium was prepared and pH was adjusted to 7.2 and sterilized by autoclaving at 15 lb pressure for 15 minutes. The autoclaved media was poured into sterile Petri plates and allowed to solidify. Skimmed milk agar plates were labelled with the isolated pure culture fungal Id, to be inoculated and one as control. Streaked each culture into its labelled Petri plate across the surface of the medium. The plates (inoculated and un-inoculated) were incubated for 24-48 hours at 37°C in an inverted position. The inoculated plates were observed for clear zone around the line of growth.

Lipase production test :

The lipase production test was carried out using Tributyrin Agar Medium to detect the enzymatic activity of fungal isolates. The medium was prepared by incorporating tributyrin oil into nutrient agar and sterilized at 15 lb pressure for 10-15 minutes. The sterilized medium was then poured into sterile Petri dishes and allowed to solidify. Each plate was labelled with the fungal ID of the pure culture isolated from the sample. Using a sterile inoculating loop, a single streak of each pure culture was made in the centre of the plate. The inoculated plates were incubated at 37°C for 48 hours in an inverted position. After incubation, the plates were flooded with Gram's Iodine Solution using a dropper and left for 30 seconds before draining off the excess solution. The plates were then observed

for zones of hydrolysis, indicated by clear areas around the fungal growth, signifying lipid breakdown due to lipase activity.

Carbohydrate :

Carbohydrate broth medium containing 1% of the desired carbohydrate and suitable pH indicator was prepared. A small inverted Durham's tube was placed in each test tube to detect gas formation. About 5–10 ml of medium was dispensed into test tubes and autoclaved at 121°C for 15 minutes. After sterilization, the medium was allowed to cool and labelled with the pure isolated culture ID to be inoculated. Each tube was inoculated aseptically using a sterile inoculating loop and incubated at 37°C for 24–48 hours. After incubation, the tubes were observed for colour change and gas formation. A change in colour of the medium from red to yellow (acid production) and appearance of gas bubbles in the Durham's tube indicated a positive carbohydrate fermentation test, while no colour change and absence of gas indicated a negative result.

Urease test :

Urea agar media was prepared and pH was adjusted to 6.8. Poured 10 ml of medium in tubes and autoclaved at 121°C for 15 minutes. The medium was allowed to solidify in a slanting position to form slopes. Tubes of the urea agar medium were labelled with the pure isolated culture Id to be inoculated. Inoculated slants were incubated for 24-48 hours at 37 ^{0}C. The slants were observed as to their colour for the presence of Urease (red or cerise colour) and for no Urease (yellow colour).

Citrate utilization test :

Potato dextrose agar medium was prepared and autoclaved at 15lb pressure for 15 minutes. The medium was poured to the culture tube. And the slant was prepared. Potato dextrose agar slant inoculated with isolated pure culture by means of a stab and streak inoculation and one tube was kept as an un-inoculated comparative control. The slant was incubated for 45 hours. The slant culture was observed for the growth and coloration of the medium (Mackie and McCartnet 1996).

Pectin :

Hankins agar media were prepared and autoclaved for this process. Pour the media into the sterile plate and allow the medium to solidify. Two plates aseptically inoculated with different pH and other two plate inoculated with different pH by respective organism Incubate inoculated plates in inverted position. Flooded all the plates with 1% aqueous solution of hexadecyl trimethyl ammonium bromide. Observed the plates for the formation of clear zone around the microbial growth.

Cellulase :

Potato dextrose agar media were prepared and autoclaved for this process. Pour the autoclaved media into sterile Petri plate and allow for solidify. Inoculated the appropriately labelled plate with the respective organism and kept for incubation at 35^{0}c in at inverted position for 2-5 days. Flooded the plates with 1% aqueous solution of hexadecyl trimethyl ammonium bromide. Observed the plates for the formation of zone around the microbial growth.

Figure 1. Microscopic view of isolated fungal species from mangroves trees

Six fungal endophytes in all were separated from the *Aloe vera* plant's root and identified using conventional technique, biochemical testing, and morphological traits. *Microsporum*, *Tricoderma*, *Trichomonas*, and *Candida*, *Mucor* and *Aspergillus*—were successfully identified up to the genus level[4]. They might be distinguished from one another by their distinctive colony and microscopic characteristics. As result show in figure number 1 and table number 1 and 2.

Table-1. Identified endophytic fungal species based on macroscopic and microscopic features

Fungal isolate	Fungal Name
Isolate 1	*Mucor* spp.
Isolate 2	*Microsporum* spp.
Isolate 3	*Candida* spp.
Isolate 4	*Fusarium* spp.
Isolate 5	*Aspergillus* spp.
Isolate 6	*Trichoderma* spp.

Table-2. Biochemical estimation of endophytic fungus

Sample ID	Amylase	Case in	Lipase	Carbohy drate	Urea-se	Citrate	Pectin	Cellu lase
S1	Slightly +ve	+ve	Slightly +ve	+ve	+ve	Slightly +ve	+ve	+ve
S2	Slight.+ve	+ve	Slight.+ve	+ve	+ve	Slight.+ve	+ve	+ve
S3	Slight.+ve	+ve	+ve	+ve	+ve	Slight. +ve	Slight. +ve	+ve
S4	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
S5	Slight.+ve	+ve	+ve	+ve	+ve	Slight.+ve	+ve	+ve
S6	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Studies on endophytic fungi have demonstrated that they possess the exceptional capacity to create their own habitat inside plant cells without clearly endangering the host. According to studies, these fungi boost disease resistance, drought tolerance, and plant development[2].

The endophytic fungi *Microsporum, Trichoderma, Trichomonas, Candida, Mucor,* and *Aspergillus* were found in the roots of Alovera plants in this study. Which is new area that is getting more attention is the use of fungi as biofertilizers, which is proving its worth by enhancing plant growth and productivity through a variety of traits that promote plant growth, such as the production of phytohormones, siderophores, and hydrolytic enzymes; making different nutrients available; and protecting plants from pathogens. So, this study will be help full related to further research.

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