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Isolation of endophytes from potato and their antagonist effect against *Fusarium oxysporum*

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Abstract

Plant endophytes may be intercellular or intracellular depending upon their location in the plant tissue because they are present inside the cells or in the intracellular space, respectively. Isolation of endophytic bacteria has been reported from both monocot and dicot plants, ranging from woody trees, such as teak and pear, to herbaceous crop plants such as mustard and maize. The aim of this study was the isolation of endophytes from potato and their antagonist effect against *Fusarium oxysporum*. Endophytic fungi were isolated from leaves, stems and roots of healthy Potato plant derived from Chak No.359/E.B Village, Tehsil Burewala. Isolation of endophytic fungi from plant parts was done according to the method described by Petrini. The media used in the present study was the Potatodextrose agar (PDA) for fungus and nutrient agar medium for maintaining bacterial stains. *F.oxysporum* was taken from the Plant pathology lab of UAF sub-campus Burewala-Vehari . The results of the experiment clearly revealed that the stems, root and leaf of the potato plants under present investigation had the maximum colonization frequency for fungal endophytes. *Fusarium oxysporum* showed rapid growth 5-7cm in5 days. *Fusarium oxysporum* was white and growing rapidly that later produced dark violet pigments in PDA. Erwinia showed light green, circular, shining, slimy, smooth characteristics. The isolate strain of Bacillus showed rodshaped, fuzzy white or slightly yellow circular and irregular characteristics.

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Introduction

Plants are one of the major and important sources of microorganisms. Different parts of growing plants including leaves, stems, lowers, buds, fruits, and roots represent a specific habitat for the microorganisms. These microorganisms include bacteria, fungi, and viruses, among them bacteria are the most common microbial resident of the plant. These microorganisms are found both as endophytes (within plant tissues) and epiphytes. The word "endophyte" is derived from two Greek words "endo" means inside and "phyton" means plant. Endophytic microbes can be deined as those microorganisms that colonize the internal tissue of the plant including vascular system without any mark of infection or harmful effect on the host plant (Adesodun *et al*., 2010).

An endophyte is an endosymbiont which includes bacteria, and viruses that usually colonize inside plant tissues. They are ubiquitous and have been reported from almost every plant studied so far. Isolation of endophytic bacteria has been reported from both monocot and dicot plants, ranging from woody trees, such as teak and pear, to herbaceous crop plants such as mustard and maize. Studies carried out suggest that majority of these microorganisms come from the soil and the main organ where endophytic bacteria get entry into plants is the root. Many candidate genes with unknown functions have been found to be differentially expressed during plant-microbe interactions (Aravind *et al.,* 2005).

Plant endophytes may be intercellular or intracellular depending upon their location in the plant tissue, i.e., they are present inside the cells or in the intracellular space, respectively. There are more than 352,000 species of plants present on the Earth. Among them, each individual plant is likely to be a host to one or more endophytic microorganisms. Every plant studied so far has been found to be associated with at least one kind of endophytic microbe. For instance, certain organic compounds including amino acids secreted by tomato roots were reported to function as chemo attractants for P. luorescens strain WCS365. Once released, bacteria sense these molecules and respond to their surrounding environment via two-component sensor systems (Cutright *et al.,* 2010).

A number of researchers have studied endophytic bacteria by using different plant parts independently. Bacterial diversity analysis of culturable endophytic bacteria from common bean leaves showed the number of endophytic bacteria in the range of 4.5 × 102–2.8 × 103 CFU g–1 of fresh tissue weight. Bacillus, Delftia, Methylobacterium, Microbacterium, Staphylococcus, Paenibacillus and Stenotrophomonas common endophytic bacterial isolates (Dhaibani *et al*., 2013).

The diversity of endophytic bacteria in branches of citrus plants analyzed under microscopic observations. The selected both healthy plants and plants infected with Xylella fastidiosa, a plant pathogenic bacterium which infects all the cultivars of Citrus sinensis and causes citrus variegated chlorosis. Additionally, above study showed that *Alcaligenes sp.; Bacillus cereus; Bacillus pumilus; Enterobacter cloacae; Burkholderia cepacia; Curtobacterium laccumfaciens; Methylobacterium sp. including M. extorquens, M. fujisawaense* (Espen *et al*., 1997).

The aim of this study was the isolation of endophytes from potato and their antagonist effect against *Fusarium oxysporum*. It also included the microscopic examination of the bacteria to check the growth of the bacteria under different conditions with supplementation of the nutrient medium such as dextrose Agar and different phases of the inoculation were observed during the study and to check the different rates of the bacteria.

Materials and methods

Sampling of Potato

Endophytic fungi were isolated from leaves, stems and roots of healthy Potato plant derived from Chak No.359/E.B Village, Tehsil Burewala. Apparently healthy looking plants were carefully chosen for sampling. The plant parts were brought to the laboratory in sterilized bags and processed within few hours after sampling.

Processing of sample

Isolation of endophytic fungi from plant parts was done according to the method described by Petrini. First the plant material was rinsed in tap water to remove the dust and debris then cut into small pieces by a sterilized blade under aseptic conditions. Sampling different part of the Potato plant, three sample were taken from roots, stems and leaves. These samples were washed in running tap water to remove soil particles and adhered debris, and finally washed with distilled water.

Disinfecting plant samples

Sample were immersed in 70% ethanol for 1-3 min and 4% aqueous solation of sodium hypochlorite for 1-2 min, I min with 70% ethanol again and finally rinsed 4-5 times with sterile distilled water (Yang *et al.*, 2008).

Disinfecting root and stem samples

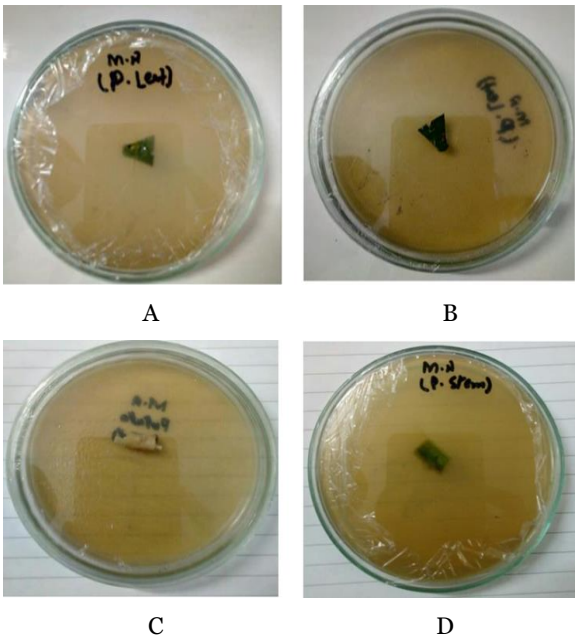
With some modification sample were treated with 70% ethanol for 3-4 min and 4% aqueous of sodium hypochlorite 2 min, 0.1%y mercury chloride for 1.5 min and rinsed with sterile distilled water . Root tissues were immersed in 70% ethanol for 1-3 min and 5% aqueous solution of sodium hypochlorite I min, 2 min with 70% ethanol, and I min with 0.1% mercury chloride and rinsed one time with sterile distilled water (Ghani *et al.*, 2019).

Potato Dextrose Agar (PDA)

The media used in the present study was the Potato dextrose agar (PDA) for fungus and nutrient agar medium for maintaining bacterial stains. The PDA contains dextrose as a carbohydrates source which serve as growth stimulant and potato infusion that provides a nutrient base for luxuriant growth for most fungi. The PDA consisted a Liter of Potato extract prepared in a laboratory from 200 grams peeled potato boiled in water and 20 g agar. The growth media was autoclaved at 1210 C at 15 PSI for 15 minutes (Naeem *et al.*, 2019).

Inoculation of Fusarium oxysporum

F. oxysporum was taken from the Plant pathology lab of UAF sub-campus Burewala-Vehari. Potato dextrose agar is a nutrient rich medium for growing a wide range of fungi. Fungus were picked with a sterilized tooth pick and transferred to fresh PDA plates and finally kept in an incubator at 27°C under dark conditions. All the procedure was carried out into laminar hood under sterilize condition (Sameeh *et al.,* 2018).



A B C D

Fig. 1. Potato sample on petri plates containing PDA, A & B are from potato leaf and C & D are samples taken from potato stem.

Inoculation of Bacteria

Different bacteria were taken from the Plant pathology lab of UAF Sub-campus Burewala-Vehari. Small amount of sample was inoculated in LB agar plate. A sterile loop is then used to spread the bacteria out in one direction from the initial site of inoculation. This is done by moving the loop from side to side, crossing several times the initial site. The loop is then sterilized by flaming it to red hot on burner and then bacteria is further spread on the plate by taking some inoculum from the previous streaked at bacteria and streak it to the remaining part of the plate in o again and the first streaks are then spread out themselves. This is repeated 2-3 times, moving around the LB agar plate (Yang *et al.,* 2018).

Results and discussion

Growth of the Endophytes

The results of the experiment clearly revealed that the stems, root and leaf of the potato plants under

present investigation had the maximum colonization frequency for fungal endophytes.

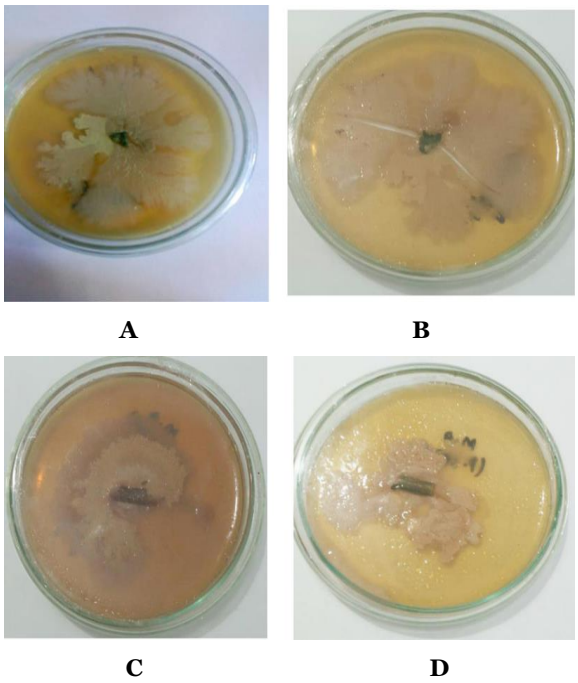


Fig. 2. Growth of Endophytes after three weeks per inoculation. Endophytic growth A & B from Potato leaf and C & D are from root and stem**.**

Growth of the F. oxysporum

Six plates were inoculated with *Fusarium oxysporum*. Plates were incubated at 27oC. *Fusarium oxysporum* showed rapid growth 5-7cm in 5 days. *Fusarium oxysporum* was white and growing rapidly that later produced dark violet pigments in PDA.

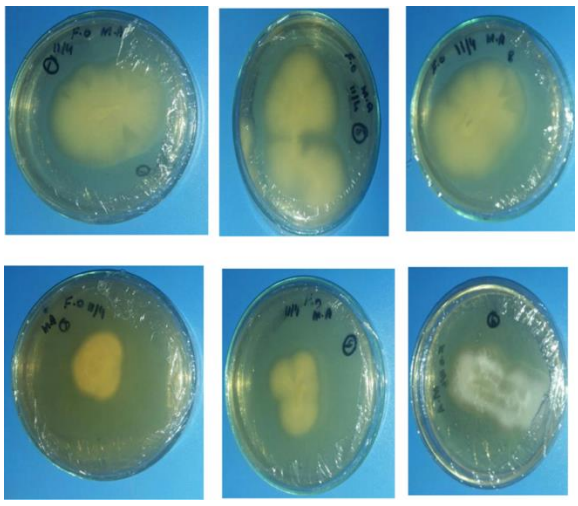


Fig. 3. Growth of F. oxysporum after four days per Inoculation.

Growth of Erwinia Bacteria

Erwinia showed light green, circular, shining, slimy, smooth characteristics. The isolate strain of Bacillus showed rod shaped, fuzzy white or slightly yellow circular and irregular characteristics.

Fig. 4. Growth of Bacillus Bacteria on LB agar after four days per inoculation.

Morphological characteristics of *Fusarium oxysporum* included oval to kidney-shaped microconidia, sickle-shaped, thin-walled and delicate macroconidia. Microconidia produced in false heads on short monophialides and a single, terminal chlamydospore. In addition to the macroscopic characters, its microscopic characters included elongated and curved macroconidia with a slightly tapered tip (Ghani *et al.,* 2019).

Trichoderma was cultured on potato dextrose agar (PDA) medium at 30°C for 48 hours. Primarily the isolate was identified by studying the colony morphology on PDA medium. The isolate grew rapidly on PDA which are white, yellow-green or bright green in colors. The conidiophores were erect and arose from short side branches. Potato dextrose agar is a nutrient rich medium for growing a wide range of fungi. Trichoderma was picked with a sterilized tooth pick and transferred to fresh PDA plates and finally kept in an incubator at 27°C under dark conditions. All the procedure was carried out into laminar flow hood under sterilize condition (Sameeh *et al.*, 2018).

Three colonies of endophytes were used as a antagonist against *Fusarium oxysporum*. The endophytes did not control the growth of *Fusarium oxysporum*. But these results showed that at least they are not pathogenic to the plant. However, the role as plant growth promoting bacteria was yet to be revealed in an independent experiment (Dhaibani *et al.,* 2013).

Conclusion

Endophytic fungi are a rich and reliable source of natural compounds with interesting biological activities, a high level of biodiversity and may also produce several compounds of pharmaceutical significance, which is currently attracting worldwide scientific investigations toward isolation and exploration of their biotechnological promise. They represent a relatively unexplored ecological source, and their secondary metabolism is particularly active because of their metabolic interactions with their hosts. In nature, plants seem to be in a close interaction with endophytic fungi.

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