

Isolation, screening and Identification of Indole Acetic Acid and Gibberellic Acid Producing Fungi from Strawberry Rhizosphere Soil

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ABSTRACT

The rhizosphere, a dynamic interface between plant roots and soil, hosts a diverse community of microorganisms essential to plant growth and development. Among these, rhizospheric bacteria and fungi play critical roles in nutrient cycling, pathogen suppression, and stress tolerance, which are vital for sustainable agriculture. Natural bioresources like rhizospheric microorganisms are increasingly being explored to enhance plant growth and productivity. Recent studies have focused on fungal isolates from the rhizospheric soil of strawberry plants in Bhilar, Mahabaleshwar, assessing their potential to synthesize the plant growth-promoting hormones indole acetic acid (IAA) and gibberellic acid (GA3). These hormones are instrumental in plant development, with broad applications in agriculture and horticulture. This study aimed to isolate, screen, and characterize fungal strains capable of producing IAA and GA3. Five fungal isolates were obtained from rhizospheric soil samples of strawberry varieties Chandler, Camarosa, and Nabila. The isolates were cultured in Potato Dextrose Broth to produce IAA and GA3, with an additional Potato Dextrose Agar medium enriched with L-tryptophan specifically for IAA production. Quantitative analysis using spectrophotometry revealed that isolate F67 *Fusarium* spp. produced the highest level of IAA (6.0734 µg/mL), while isolate F62 *Aspergillus* spp. yielded the highest GA3 (75.6406 µg/mL). These findings indicate that the fungal isolates from strawberry rhizospheres could serve as valuable bioresources for natural plant hormone production, supporting sustainable agriculture and horticulture practices.

Keywords: Rhizosphere microorganisms, Indole acetic acid, Gibberellic acid (GA3), *Aspergillus* spp., *Fusarium* spp..

1. INTRODUCTION

Soils are largely intricate systems, with numerous factors playing different functions substantially due to the exertion of soil organisms (Chiang and Soudi 1994). Soil microflora plays a vital role in evaluation of soil conditions and in stimulating factory growth (Kiran 1999). Microorganisms are desirable in adding factory growth as they're involved in mineralization conditioning in soils and several biochemical metamorphosis. Type of civilization and crop operation practices set up to have lesser influence on the exertion of soil microflora (Gill 1980). Nonstop use of chemical diseases over a long period may beget imbalance in soil microflora and thereby laterally affect natural parcels of soil leading to soil declination (Manickam, and Venkataraman, 1972). Micro fungi play a vital part in nutrient cycling by regulating soil natural exertion (Arunachalam *et al.*, 1997). The amounts of inorganic and organic essential present in the soil have a direct effect on the fungal population of the soil (Gaddeyya *et al.*, 2012).

The genus *Fragaria* is a member of the Rosaceae family, subfamily Rosoideae, which comprises 25- 26 species. *Fragaria x ananassa* Duch. is the predominant species commercially, being the most important species cultivated for strawberry product encyclopedically (FAO 2000). Strawberry (*Fragaria rosaceae*) is considerably cultivated worldwide because of its nutritive value, minerals, macronutrients owing to the antioxidants vitamins, and micronutrients it contains (Petrasch *et al.*, 2019). shops have complex connections with an array of microorganisms, particularly fungi and rhizospheric bacteria, which can lead to an increase in factory vigor, growth and development and changes in factory metabolism (Aly *et al.*, 2011). The group of rhizosphere fungi that enhance factory growth and populate factory roots is appertained to as PGPF (Hyakumachi 1994). Michel Dignand 2004 says that Strawberry cultivars are largely receptive to several destructive and economically important pathogens like *Colletotrichum acutatum*, *C. fragariae*, *C. gloeosporioides*, *Rhizopus nigricans*, *Alternaria alternata*, *Phytophthora cactonum*, *P. paracitica*, *Fusarium solani*, *Botrytis cinerea*, *Aspergillus flavus*, *Aspergillus niger*.

Rhizosphere diversity is therefore a term to decode the numerous organisms inhabiting the soil, closest and mostly influenced by plant roots. Plants grow in close association with complex microorganisms predominantly within the rhizosphere. The rhizosphere is the zone where soil, plants, and microorganisms interact, serving as a hotspot for intense microbial activity (Huang *et al.*, 2014; Nicolitch *et al.*, 2016).

Studies of microorganisms that produce auxins to support plant growth, development, and yield primarily focus on rhizosphere fungi due to their significant impact on ecosystem functioning and structure, as well as their crucial role in various ecological services (Orgiazzi *et al.*, 2012). These ecological services provided by soil fungi, which include rhizosphere fungi, encompass essential roles in disease suppression, nutrient cycling, and water regulation, all of which contribute to healthier and more robust plant growth (Jenkins, 2005). Consequently, the use of fungi in recovering nutrient content is currently a favorable method for many agricultural practices over chemical fertilizers. This preference for fungal applications is further supported by the fact that the use of chemical fertilizers and pesticides has created environmental pollution (Suhag 2016), making the use of fungi in agricultural practices a promising, profitable, and environmentally friendly technique for farmers. Moreover, excessive use of chemical fertilizers leads to reduced colonization of plant roots by symbiotic fungi, which in turn diminishes the beneficial effects of these fungi in the soil (Aziz and Zainol, 2018), further reinforcing the advantages of fungal-based approaches.

Phytohormones play a crucial role as signals and regulators of plant growth and development. Among these, auxins, particularly indole-3-acetic acid (IAA), are the most extensively studied plant growth regulators, encompassing biochemical, physiological, and genetic aspects (Elena *et al.*, 2002). IAA is a natural auxin synthesized in various non-seed plants, fungi, algae, and bacteria (Raut *et al.*, 2017). Other plant growth regulators, such as cytokinins and gibberellins, are important biotechnological products widely used in horticulture, agriculture, and other plant-related industries (Bandelier and Renaud, 1997). Gibberellins (GA) have selective effects on different parts of plants, and in addition to higher plants, some fungi and bacteria also produce gibberellins (Gutierrez *et al.*, 2001). Gibberellic acid influences processes such as seed germination, leaf expansion, and flower development (Davies, 2004; Haisel *et al.*, 2001). The ability of microbes to produce IAA is a critical characteristic for bio-fertilizer microorganisms.

In the present study we are going to the isolation, screening and identification of potent fungal strains from strawberry rhizospheric soil.

2. MATERIALS AND METHODS:

2.1 Collection of samples: Soil (rhizospheric) samples were collected near the roots and also plant part (leaves and roots) samples of 3 varieties of strawberry plants were collected from Bhilar, Mahabaleshwar, where microbial activity is most intense. The samples were separated and labelled according to plant varieties. The soil samples were collected from the depth above 0–15 cm (plow layer) of the soil.

2.2 Isolation of fungi: The isolation of fungi from rhizospheric soil was carried out in order to identify each fungus up to species level. The serial dilution plating method was used to dilute the soil sample as described by (Waksman 1922, Ben-David & Davidson 2014). 0.1 mL of the solution in each tube was pipetted into the prepared PDA plate. The solution was then spread on the plate by using a spreader and incubated at room temperature for seven days (Aziz & Zainol 2018).

2.3 Identification: Fungal propagules may appear colourless or display various colors. Hyaline spores, mycelia, conidia, and cytoplasm can be stained using cotton blue, while lactophenol cotton blue is used as a cleaning agent. The stained samples were then examined under a light microscope (Magnus MLXi Plus) for identification, and microphotographs were taken at magnifications ranging from 10X to 40X (Raja *et al.*, 2017).

2.4 Screening and Estimation of fungal isolates for IAA production: The fungal isolates were quantitatively tested for the production of indole-3-acetic acid (IAA) following the method of Brick *et al.*, (1991). Fungi were cultured in Potato dextrose broth supplemented with 1g/L tryptophan (1000 µg/mL). A loop of actively growing mycelium spore suspension from each fungal isolate was inoculated into 5 mL of Potato dextrose broth, incubated at 30°C, and agitated at 130 rpm. After 6 days, each culture was centrifuged at 13,000 rpm for 20 minutes. To detect indole derivatives, Salkowski's reagent (0.01 M FeCl₃ in 35% HClO₄) was added. 2 mL of each fungal culture were mixed with an equal volume of Salkowski's reagent and incubated in the dark for 60 minutes. The optical density was then measured at 530 nm using a UV spectrophotometer (Model Elite Auto 8). The IAA concentration produced by each fungal strain was calculated using a standard IAA concentration curve (Hussein & Joo, 2015).

2.5 Screening of Fungal Isolates for GA Production: To screen for GA3-producing fungal isolates, 15 mL of sterile Potato dextrose broth was inoculated with a loopful of fungal isolate and incubated for 7-8 days at 30°C with agitation at 130 rpm. After 7 days, the cell-free supernatant was collected and used to measure the amount of GA3 produced by the fungal isolates, as described in the method below (Desai, 2017).

2.5.2 Estimation of Gibberellins: Gibberellins were estimated calorimetrically using a standard method (Holbrook *et al.*,

1961). To 15 mL of the supernatant, 2 mL of zinc acetate reagent (21.9g of zinc acetate + 1 mL of glacial acetic acid, with the volume adjusted to 100 mL using distilled water) was added. After 3 minutes, 2 mL of potassium ferrocyanide (10.6% in distilled water) was added, and the mixture was centrifuged at 2000 rpm for 15 minutes. To 5 mL of the supernatant, 5 mL of 30% HCl was added, and the mixture was incubated at 20°C for 75 minutes. A blank was prepared using 5 mL of 5% HCl. The absorbance was measured at 254 nm, and the gibberellin concentration was determined using a standard curve prepared with gibberellic acid (GA3, Hi-media) as the standard (100-1000 µg/mL) (Sharma *et al.*, 2018).

3. RESULTS

3.1 Isolation of microorganisms: The soil sample contained a considerable population of bacteria and fungi, a total of 67 microbes were isolated. In that total 61 bacterial isolates and 6 fungal isolates were found in rhizospheric soil samples.

3.2 Isolation and screening for IAA production: In this study, about 5 different fungal colonies were obtained from the soil samples were collected along with plant debris from Bhilar, mahabaleshwar, maharashtra. All isolates were able to produce indole-3-acetic acid (IAA), as confirmed by preliminary screening. Further tentative quantification shows isolate F67 as a strong IAA producer. Morphological characteristics and lactophenol cotton blue staining identified F67 as *Fusarium* spp. Additionally, isolate F62 showed potential for gibberellic acid (GA) production, and was tentatively identified as *Aspergillus* spp. Many *Fusarium* spp. and *Aspergillus* spp. were able to produce the auxin phyto-hormone like indole-3-acetic acid, Gibberellic acid (GA3) and its production has been suggested to promote root growth (kumar *et al.*, 2017).

3.3 The making of Indole Acetic Acid (IAA) standard curve: The standard curve is used to obtain equations in calculating the Indole Acetic Acid concentration values produced by isolates. The process results of testing the standard solution obtained indicate its reaction by producing a pink color. Red density is directly proportional to the increase in IAA concentration produced (Gusmiaty 2019).

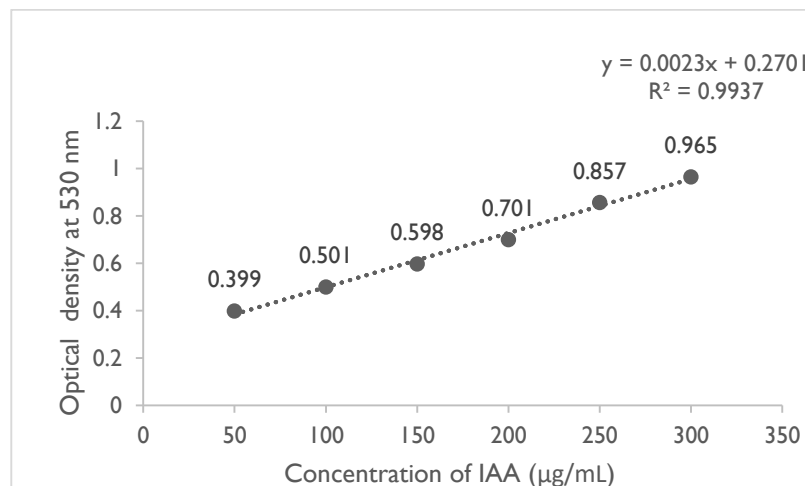


Figure 1. IAA Standard Curve

3.4 Production capability of fungi isolate IAA test: The colour observations result carried out on fungus isolates obtained all isolates that had concentrated pink colour but in that F67 having high IAA concentrations while the other fungal isolates did not have a proper pink colour formation reaction which produced a deep yellow colour which was compared with aqueduct colour and salkowski solution as control (Gusmiaty 2019).

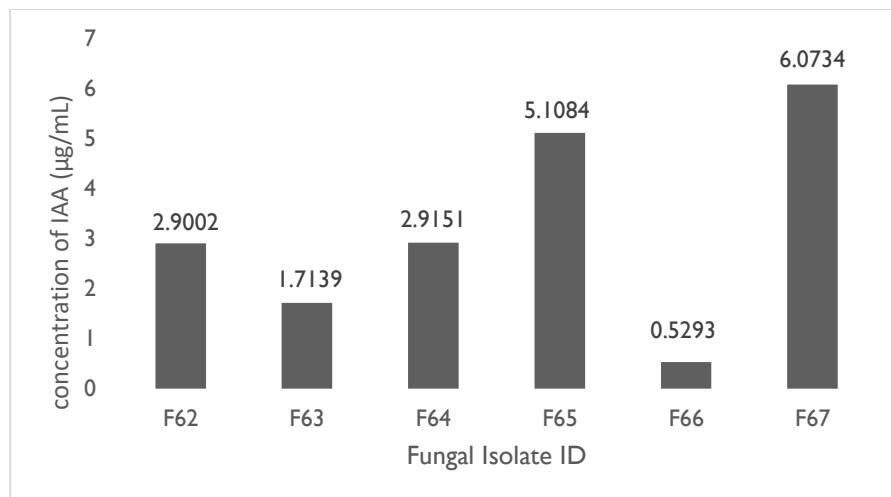


Figure 2. Amount of Indole acetic acid produced by various plant rhizofungi are shown.

The fungal isolates found in Mahabaleshwar were able to produce IAA at varying concentrations. *Fusarium spp.* exhibited the highest IAA production. As a result, these isolates can be propagated and later utilized in the formulation of biofertilizers. IAA plays a significant role in promoting plant growth by stimulating the development of longer shoots and roots. Additionally, IAA has the potential to help prevent diseases caused by soil-borne pathogens. The ability of microbes to produce IAA is a key characteristic that biofertilizer microbes must possess. The IAA-producing isolates found in the Suren community forest plants could be applied as biological fertilizers directly to the soil around plants (Gusmiaty *et al.*, 2019). According to Eglal *et al.*, (2020), the highest IAA and GA production was achieved with isolate 19, which produced 0.098 µg/mL of IAA and 0.77 µg/mL of GA. In comparison, our study identified isolate F67, which produced 6.0734 µg/mL of IAA, and isolate F62, which produced 75.6403 µg/mL of GA.

3.5 Isolation and screening for GA production:

Screening of rhizofungi for GA 5 fungal isolates from the rhizosphere of strawberry plant were tested for their activity for GA's production out of them 5 fungal were identified to produce GA's. Among the F62 i.e. *Aspergillus spp.* produce a higher amount of gibberellic acid shown after 7-8 day's incubation.

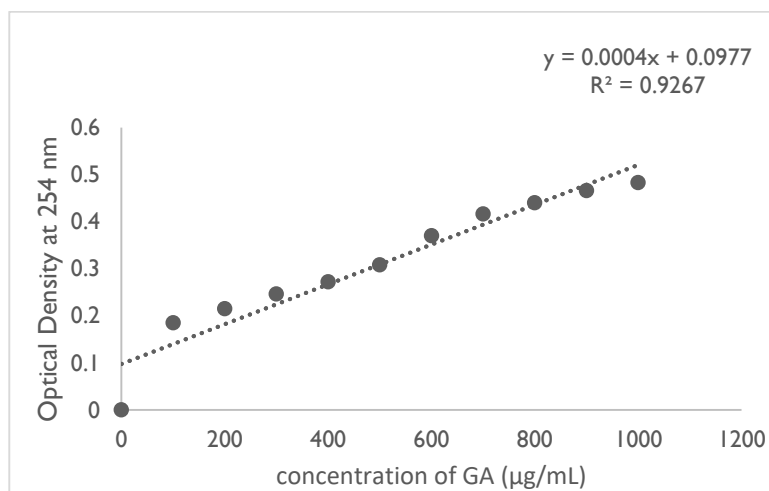


Figure 3: Determination of gibberellic acid by spectrophotometer

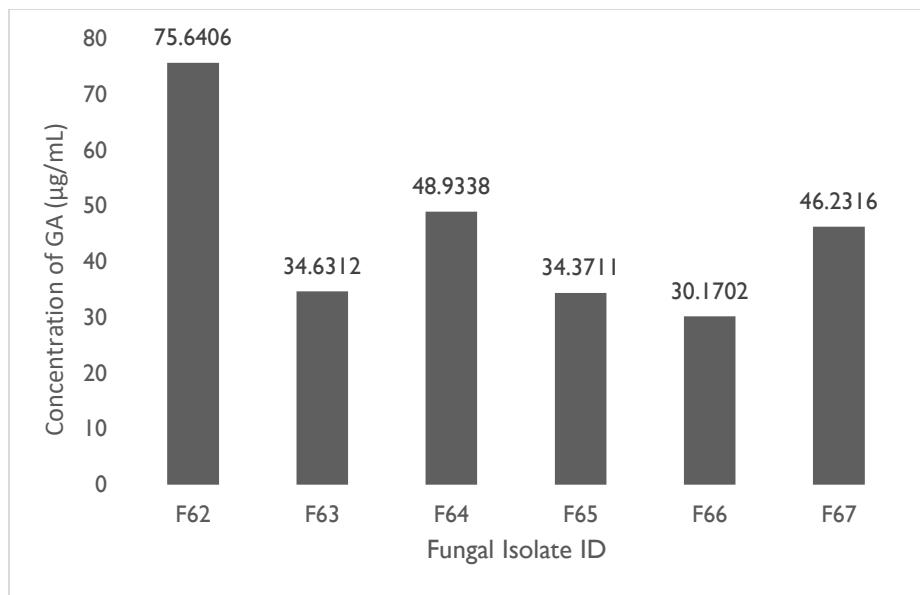


Figure 4. Amount of gibberellic acid produced by various plant rhizofungi are shown.

4. DISCUSSION

This study aimed to isolate and characterize microorganisms capable of producing plant growth-promoting substances, particularly Indole-3-acetic acid (IAA) and gibberellic acid (GA), from soil samples collected from Bhilar, Mahabaleshwar, Maharashtra. A total of 67 microbial isolates were obtained, consisting of 61 bacterial and 6 fungal isolates. These microorganisms play key roles in enhancing plant growth, nutrient uptake, and soil health.

In the screening for IAA production, five fungal isolates were tested, with all showing positive results for IAA production. Among them, the F67 isolate, identified as *Fusarium spp.*, exhibited the highest IAA production, making it a promising candidate for biofertilizer applications. The quantification of IAA using a standard curve demonstrated a direct relationship between the pink color intensity and IAA concentration. The F67 isolate produced 6.0734 µg/mL of IAA, which is significantly higher than other isolates in this study.

In addition to IAA, the study also assessed GA production. Among the five fungal isolates from the rhizosphere of strawberry plants, F62 (*Aspergillus spp.*) produced the highest amount of GA i.e 75.6406 µg/mL, indicating its potential for enhancing various plant developmental processes, such as seed germination and stem elongation.

5. CONCLUSION

In conclusion, the study highlights the potential of fungal isolates from strawberry rhizospheres in Bhilar, Mahabaleshwar, as valuable bioresources for producing plant growth-promoting hormones, specifically indole acetic acid (IAA) and gibberellic acid (GA3). The fungal isolates F67 (*Fusarium spp.*) and F62 (*Aspergillus spp.*) demonstrated high levels of IAA and GA3 production, respectively. These findings suggest that utilizing these fungal strains could support sustainable agriculture by providing natural alternatives to synthetic growth regulators, enhancing plant growth and productivity.

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