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Identification of Egyptian Fluorescent Rhizosphere Pseudomonad Isolates Possessing High Nematicidal Activity Against the Plant Parasitic Nematode Meloidogyne Incognita

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Abstract

The in vitro nematicidal activity of 52 Egyptian rhizosphere fluorescent pseudomonad isolates (ERFP) was examined. The screening results revealed a 57% to 100% hatching inhibition for Meloidogyne incognita eggs. Cell-free supernatants (CFS) of the ERFP isolates showed total suppression of egg hatching and killed 100% of the J2, similar to the chemical nematicide Videt in vitro assay. Cultures of these isolates, CFS, and the chemical nematicide demonstrated reductions in root galling range from 81.0-95.4, 61.3-84.5, and 97.3%, respectively, in glasshouse pot studies. Additionally, as compared to the positive control, the reduction in nematode multiplication in soil for cultures, CFS, and the chemical nematicide was 91.9–95.7, 83.–84.5, and 96.5%, respectively (nematode only).

Additionally, these isolates demonstrated considerable increases in plant growth characteristics, phenolic content, and activity of the enzymes involved in plant defence. Two significant clusters were found using 16S rDNA sequences and API NE kits; strains Ps 36 and 54 appeared to be within the variability range of P. putida, whereas Ps 21, Ps 22, and Ps 14 are probably strains of P. aeruginosa. Their physiological traits, such as Ps 14 and Ps 21 and Ps 22's capacity to utilise mannitol, N-acetyl-glucosamine, and adipate, supported the distinctness of Ps 36 and Ps 54 from Ps 14, Ps 21, and Ps 22.

Keywords
Plant growth promotion; Rhizosphere fluorescent pseudomonad; Meloidogyne incognita; Biological control; Nematicidal action; rDNA 16s sequence

Introduction

One of the most economically significant pests, root-knot nematodes severely harm and destroy a range of crops globally [1]. Nematode damage to tomato crop estimates varied from 28 to 68% globally. Crop rotation and chemical nematicides are still the mainstays in the fight against plant parasitic nematodes. Chemical nematicides are unfortunately expensive, highly poisonous substances that endanger both human health and the environment. Safe and environmentally sustainable solutions are therefore becoming more and more crucial [2,3,4]. Realistic alternatives to chemical nematicides include microorganisms that can repel nematodes. A few fungi have been suggested for the biocontrol of nematodes that parasitize plants [3, 5]. Unfortunately, these mushrooms frequently struggle to successfully compete with the local soil microbes. Likewise a fungus growth Additionally, bacterial control is preferred over fungal control since bacterial development is slower than that of fungi [6,8]. The obligatory parasitic bacterium Pasteuria penetrans can prevent the spread of root knot nematodes in soil, but it is difficult to cultivate in a lab and does not fare well in soil [9]. Recent studies have concentrated on different microorganisms for the management of plant parasitic nematodes. Pseudomonads have drawn a lot of attention because of their diverse catabolic capabilities, good root colonisation abilities, and ability to create a variety of antiphytopathogenic compounds [1,9,10,11,12].

However, there is little information available on the nematicidal activity of Egyptian bacterial isolates, in particular rhizofluorescent pseudomonads. Therefore, the main goal of this work was to identify isolates from a collection of Egyptian rhizosphere fluorescence pseudomonad (ERFP) isolates that had strong nematicidal activity. In order to accomplish this goal, I 52 ERFP isolates were tested in vitro for nematicidal activity, (ii) the best isolates' cultures and cell-free culture supernatants were tested for nematicidal activity and their effects on plant growth in a glasshouse pot experiment were assessed in comparison with a chemical nematicide, and (iii) phylogenetic relationships of effective ERFP isolates based on phenotypic

Resources and Procedures:

Characterization of the Pseudomonad isolates' phenotypes:

In a recent investigation, 52 fluorescent pseudomonad isolates were examined by Gamal-Eldin et al. [13] for their ability to produce indoleacetic acid (IAA), siderophores, cyanide, proteinase, chitinase, be antagonistic to plant-pathogenic fungi, and solubilize zinc and phosphate.

The top five isolates were identified in the current investigation by utilising the API 20 NE identification kit (Bio Mérieux, France) and matching the results with the recognised species listed in the API database. All of these isolates were tested for their nematicidal activity.

16S rDNA gene PCR amplification and sequencing analysis

Each Pseudomonas isolate was cultivated in liquid Kings B medium at 30°C with 100 rpm shaking in order to isolate the bacterial genomic DNA based on Johnsen et alresearch .'s [14]. The primer pair 16SF-16SR was used to amplify the DNA 16S region [15]. According to Sanger et al[16] .'s description, the 16S rDNA was sequenced. The National Center for Biotechnology Information's Blast tool was initially used to evaluate the 16S rDNA sequences. Using the CAP tool, sequencing data received from various primers were combined (Contig Assembly and Genomic Expression programs).

Using the computer application ClustalX [17], the consensus sequences from the isolates, sequences of strains belonging to the same phylogenetic group, and sequences of additional Pseudomonas strain representatives were aligned. Tree View [18] was used to display the generated trees. Acinetobacter calcoaceticus, E. coli, and the neighbor-joining method [19] were used to generate the phylogenetic tree.

preparation of cell-free culture supernatants and bacterial inocula (CFS)

The isolated pseudomonads were cultured separately for 36 hours at 28 °C on a rotary shaker at 150 rpm in King's B medium broth. For use at 4°C, a portion of the cultures were retained without centrifugation. The bacterial cultures mixture was created by combining equal amounts of each isolate's cell suspension. The remaining portion of the bacterial cultures were centrifuged at 5,000 x g for 30 min to prepare the cell-free culture filtrate; the pellets were discarded, and the supernatants were filtered through a bacterial filter (0.22 m pore size) and stored for use at 4°C.

Making the nematode inocula

Root-knot nematode Meloidogyne incognita population was routinely kept on the susceptible tomato cultivar Castle Rock in a box with sandy loam soil at a temperature of 27°C 5°C.

The Hussey and Barker[20] extraction method was used to remove nematode eggs from highly affected tomato roots. With sterile tap water, the eggs were transferred from the 25-m sieve into a 300-ml Erlenmeyer flask. The nematode eggs/water suspension was kept in darkness at 24°C and was kept aerated with an aquarium pump to encourage egg growth and J2 hatching.

Meloidogyne incognita J2 mortality and egg hatching bioassay

Three egg masses containing roughly 300 eggs each were combined with 1 ml of the bacterial culture (2.5x108 cfu/ml) or their supernatant in 1.5 ml Eppendorf tubes in three replicates to study the effect of the bacterial isolates cultures or their supernatants on the egg hatching. The egg masses were then incubated at 28°C for 48 hours.

As a control, sterile half-strength King's B medium was utilised with the same quantity of egg masses. After 48 hours, the numbers of J2 hatchlings were counted. Using a Hawksley counting slide under a microscope, eggs and J2 larvae were counted.

500 ml of sterile King's B medium containing 1000 J2 larvae were mixed with 1 ml of each individual bacterial culture, supernatant, or mixture in 1.5 ml Eppendorf tubes in three replicates to study the impact of the bacterial isolate cultures or their supernatants on the viability of J2 larvae. These mixtures were then incubated at 28 °C for 48 h. The control was sterile half-strength King's B medium with the same quantity of J2 larvae. After 48 hours, the number of dead J2 larvae was counted under a microscope.

pot experiment

In a glasshouse at the Fayoum University Faculty of Agriculture, pot experiments were conducted. 21-day-old tomato seedlings of the variety Castle Rock (Lycopersicon esculentum Mill.) were submerged in bacterial cultures or supernatants containing 10% gum arabic. The tests were carried out in 11 cm diameter plastic pots that held one kilogramme of a sterilised 2:1 combination of sand and clay. One week after transplanting, the soil around the roots was carefully removed without causing damage to the roots, and before the soil was replaced, 1000 J2 larvae were poured into 5 cc of tap water. 5 ml of King's B medium at half strength were administered to the control plants. Four replicate pots in a randomised complete block arrangement with treatments were used. Plants were watered and fertilised according to the required amounts.

The pots were kept in a glasshouse with natural light and 26/15°C day/night temps. Plants from three replicates were harvested three weeks after infection, and the following parameters were recorded: total nitrogen content (mg/g dry weight), shoot height (cm), shoot dry weight plant-1 (g), root size plant-1 (cm3), leaves number plant-1, leaves area plant-1 (cm2), and shoot dry weight plant-1 (g). According to Larson et al. [21], total indoles (mg g-1) were measured in new shoots using the P-dimethylaminobenzaldehyde reagent. According to Graan and Ort [22], the chlorophyll content of leaves (mg/g fresh weight) was calculated.

Under a low power (x10) microscope, nematode galls on plant roots were counted, and larvae were removed from 100 cm3

of soil using a centrifugal flotation technique [23]. Nematodes extracted were counted.

Using a Hawksley counting slide and a microscope, extracted nematodes were counted. In order to monitor the progression of root galling, the fourth clone was harvested 60 days after planting. The root slices were processed for histological examination in accordance with Sayan et alinstructions .'s [24], and digital micrographs were obtained using a JVC Camera (model No. TK 890E) connected to an OLYMPUS microscope (OLYMPUS BH2, OLYMPUS OPTICAL Co. LTD No.106105 Japan). The enormous cells were discovered to be a syncytium made up of many nuclei that had developed around the head of the female worm as a result of esophageal gland secretions.

Discussion

Tomato plants, the plant parasitic nematode M. incognita, and In this study, pseudomonads were employed. M. incognita is the most prevalent species of Meloidogyne spp., accounting for around 64% of the population, according to studies on the distribution of plant-parasitic nematodes in soil [31]. The tomato was selected as the test plant for the pot experiment because it makes a good host for M. incognita and is a significant vegetable crop with wide appeal [32]. The most common bacteria found in the rhizosphere are pseudomonads, especially fluorescent pseudomonads. 52 ERFP isolates were screened in the current investigation, and a large percentage of them had nematicidal activity. This finding suggests that nematicidal activity is apparently common among ERFP isolates. The percentage of rhizosphere bacteria reported in the literature The five isolates (Ps 54, Ps 36, Ps 22, Ps 21, and Ps 14) with the highest nematicidal activity were chosen for further study based on the screening results. The five isolates' cultures and cell-free supernatants in the in vitro experiment revealed a significantly lower number of eggs hatching and a significantly higher mortality rate for J2 of M. incognita. Similar findings were made by Bin et al. [35], who discovered that various rhizobacteria's entire cultures and culture filtrates both exhibited nematicidal effects on M. javanica's J2 that ranged from 62–64% and 62–70%, respectively. This discovery shows that the five selected ERFP isolates produce nematicidal substances that could be employed to manage M. incognita.

A surprising finding from the unhatched eggs' microscopic analysis was that a significant portion of them had severe damage. Unhatched eggs treated with nematicide did not exhibit this effect, either. The lytic enzymes released by the tested isolates are thought to be the cause of this phenomena. The chitin layer of M. javanica eggs treated with chitinase or protease in a liquid culture of Paecilomyces lilacinus showed huge vacuoles, and the vitelline layer was divided and had lost its integrity, according to Khan et alfindings[36] .'s in this regard. The investigated ERFP isolates in the current study produced chitinase and proteases. Nevertheless, the cultures' potential was more pronounced than that of their cell-free supernatants, indicating that there may be additional mechanisms in play.In addition to producing nematicidal metabolites, these isolates may also be controlling M. incognita. The possibility exists that the increased resistance to M. incognita through the induction of systemic resistance (ISR) in plants caused by the presence of live bacteria.

In tomato plants that had been inoculated with ERFP isolates as opposed to noninoculated plants, whether in the presence or absence of nematodes, it was discovered that the activities of the plant-defense related enzymes PO, PPO, and PAL were significantly higher in the leaf tissues of inoculated plants than in noninoculated plants.

These findings concur with those of Kavitha and Jonathan [37], who discovered that inoculating tomato plants with FP strains led to a noticeable increase in PO, PPO, and PAL. Recent studies into the processes of biological control by plant-growth-promoting fluorescent pseudomonads have shown that PGPR strains increase the plant's defence mechanisms against pathogen attack by activating defence genes encoding chitinase, PO, PPO, and PLA as well as enzymes involved in the synthesis of phytoalexins [12], as well as by depositing newly formed barriers beyond infection sites, such as callose, lignin, and phenolics [38].

PLA is crucial for the production of phenolics, which function as powerful chemical barriers against pathogen invasion. The final stage of the production of lignin and other oxidative phenols is catalysed by PO and PPO [38]. There has been minimal investigation into whether biological control bacteria can cause induced systemic resistance (ISR) against nematodes, despite the fact that plant diseases can be controlled biologically utilising introduced bacteria, particularly rhizosphere pseudomonads [39,40]. To demonstrate the systemic resistance induced by Ps in the management of nematodes, however, more research is required. RFP also actively invade plant roots, as is widely known [41]. This may help to prevent or at least lessen nematode attacks on humans.As a result, the findings of this study strongly suggest that the ERFP isolates tested produce nematicidal metabolites, while also implying but not proving that competition for root colonisation and induction of ISR against nematodes are mechanisms for controlling nematodes by inoculation with those ERFP isolates.

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