

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 al. [14]. The primer pair 16SF-16SR was used to amplify the DNA 16S region [15]. According to Sanger et al. [16], 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 μm 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-μm 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

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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 al instructions [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 nematocidal activity. This finding suggests that nematocidal 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 nematocidal 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 nematocidal 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 nematocidal 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 nematocide 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 al findings [36]. 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 nematocidal 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 nematocidal 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.

References

1. Kiewnick S, Sikora R A (2006) Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biological Control* 38: 179-187.
2. Pakeerathan K, Mountain G, Thrashing N (2009) Eco-Friendly Management of Root-knot Nematode *Meloidogyne incognita* (Kofid and White) Chitwood Using Different Green Leaf Manures on Tomato under Field Conditions. *American Eurasian J. Agric. & Environ. Sci* 6: 494-497.
3. Dong L Q, Zahng K Q (2006) Microbial control of plant-

- parasitic nematodes: a five-party interaction. *Plant and Soil* 288:31-45.
4. Barker KR, Koenning SR (1998) Developing sustainable systems for nematode management. *Annual Review of Phytopathology* 36: 165-205.
 5. Santos MAD, Ferraz S, Muchovej J (1992) Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* race 3. *Nematropca* 22:183-192.
 6. Kerry BR (2000) Rhizosphere interactions and exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annu Rev Phytopathol* 38: 423-441.
 7. Tikhonov VE, Lopez-Llorca LV, Salinas J, Jansson HB (2002) Purification and characterization of chitinases from the nematophagous fungi *Verticillium chlamyosporium* and *V. suchlasporium*. *Fungal Genetic and Biology* 35; 67-78.
 8. Atibalentja N, Noel GR, Domier LL (2000) Phylogenetic position of the North American isolate of *Pasteuria* that parasitizes the soybean cyst nematode, *Heterodera glycines*, as inferred from 16S rDNA sequence analysis. *International Journal of Systematic and Evolutionary Microbiology* 50:605-613.
 9. Kloepper JW, Rodríguez-kábana R, Mcínroy JA, Collins DJ (1991) Analysis of populations and physiological characterization of microorganisms in rhizospheres of plants with antagonistic properties to phytopathogenic nematodes. *Plant and Soil* 136: 95-102.
 10. Siddiqui IA, Ehetshamul-Haque S, Shaukat S (2001) Use of Rhizobacteria in the control of root rot-root knot disease complex of mungbean. *Journal of Phytopathology* 149: 337-346.
 11. Burkett-Cadena M, Kokalis-Burelle N, Kathy SL, van Santen E, Kloepper JW (2008) Suppressiveness of root-knot nematodes mediated by Rhizobacteria. *Biological control* 47:55-59.
 12. Harish S, Kavino M, Kumar N, Balasubramanian P, Samiyappan R (2009) Induction of defense-related proteins by mixtures of plant growth promoting endophytic bacteria against Banana bunchy top virus. *Biological Control* 51:16-25.
 13. Gamal-Eldin H, Elbadry M, Abdelaziz SA (2008) Screening of fluorescent pseudomonads bacteria isolated from rhizosphere of cultivated and wild plants in vitro for plant growth promoting traits. *J Agric Sci Mansura Univ* 33: 3365-3383.
 14. Johnsen K, Andersen S, Jacobsen CS (1996) Phenotypic and genotypic characterization of phenanthrene-degrading fluorescent *Pseudomonas* biovars. *Appl Environ Microbiol* 62: 3818-3825.
 15. Rainey FA, Rainey NW, Kroppenstedt RM, Stackebrandt E (1996) The genus *Nocardiopsis* represents a phylogenetically coherent taxon and distinct actinomycete lineage: proposal of *Nocardiopaceae* fam nov *Int J Syst Bacteriol* 46: 1088-1092.
 16. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463-5467.
 17. Thompson JD, Gibson TJ, Plewinak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Res* 25: 4867-4882.
 18. Page RD (1996) Tree View: An application to display phylogenetic trees on personal computers, *Comput. Appl. Biosci.* 12: 357-358.
 19. Sayan D, Darleen A. DeMason, Jeffrey D. Ehlers, Timothy J. Close, Philip A. Robert (2008) Histological characterization of root-knot nematode resistance in cowpea and its relation to reactive oxygen species modulation. *Journal of Experimental Botany* 59:1305-1313.
 20. Saitou N, Nei M (1987) the neighbour-joining method: a new method for reconstructing phylogenetic trees, *Mol. Biol. Evol* 4: 406-425.
 21. Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* 57:1025- 1028.
 22. Larsen P, Harbo A, Klungso S, Asheim TC (1962) On the biosynthesis of some indole compounds in the *Acetobacter xylinum*. *Physio Plant* 15: 552-562.
 23. Graan T, Ort DR (1984) Quantitation of the rapid electron donors to P700 the functional plastoquinone pool and the ratio of the photosystems in spinach chloroplast, *J Biol Chem* 295:14003-14010. (article online)
 24. Riley MB, Agudelo P (2008) A case study concerning the extraction and identification of plant-parasitic nematodes. *The Plant Health Instructor* DOI: 10.1094/PHI-T-2008-0625-01.
 25. Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
 26. Ross WW, Sederoff RR (1992) Phenylalanine Ammonia Lyase from Loblolly Pine: Purification of the Enzyme and Isolation of Complementary DNA clone. *Plant*

- Physiol. 98: 380-386.
27. Hammerschmidt R, Nuckles E, Kuc J (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol Plant Pathol* 20:73-80.
 28. Mayer AM, Harel E, Shaul RB (1965) Assay of Catechol Oxidase, a Critical Comparison of Methods. *Phytochem* 5: 783-789.
 29. Zieslin N, Ben-Zaken R (1993) Peroxidase activity and presence of phenolic substances in peduncles of rose flower. *Plant Physiol and Biochem* 31: 333- 339.
 30. Snedecor GW, Cochran W, *Statistical methods*, 7th ed. Low state Univ. Press, Ames, USA (1980).
 31. Abu-Zaed EO (2002) Molecular characterization of root-knot nematodes in middle Egypt and resistance of some tomato cultivars. Ph.D. thesis, Faculty of Agric., Fayoum, Cairo University, Egypt.
 32. Garcia KS (2007) Dissecting rhizobacteria-induced systemic resistance in tomato against *Meloidogyne incognita*. The first step using molecular tools. Dissertation, Hohen Landwirtschaftlichen Fakultät der Rheinischen FriedrichWilhelms- Universität Bonn, Germany.
 33. Ali NI, Siddiqui IA, Shaikat SS, Zaki MJ (2002) Nematicidal activity of some strains of *Pseudomonas* spp. *Soil Biol Biochem* 34:1051-1058.
 34. Kumar T, Kang SC, Maheshwari DK (2005) Nematicidal activity of some fluorescent pseudomonads on cyst forming nematode, *Heterodera cajani* and growth of *Sesamum indicum* var. RT1. *Agric Chem Biotechnol* 48:161-166
 35. Bin L, Xie G, Soad A, Coosemans J (2005) Suppression of *Meloidogyne javanica* by antagonistic and plant growth-promoting rhizobacteria. *J Zhejiang Univ Sci* 6: 496-501.
 36. Khan A, Williams KL, Nevalainen H (2004) Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. *Biological Control* 31: 346-352. (article online)
 37. Kavitha PG, Varadarajan PG, Jonathan EI (2006) Induced systemic resistance of *Pseudomonas fluorescens* against root-Knot nematode, *Meloidogyne incognita* in tomato. *Resistant Pest Management Newsletter* 15:50-52.
 38. M'Piga P, Belanger RR, Paulitz TC, Benhamou N (1997) Increased resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. *Physiol Mol Plant Pathol* 50: 301-320.
 39. Bakker, P A H M, Pieterse , CMJ , Loon, et al. (2007) Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* 97: 239-243.
 40. Tian B, Yang J, Zhang K (2007) Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action and future prospects. *FEMS Microbiol Ecol* 61: 197-213.
 41. Han SH, Lee SJ, Moon JH, Park KH, Yang KY, et al. (2006) GacS-dependent production of 2R, 3R butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. *Molecular Plant-Microbe Interaction* 19: 924-930.
 42. Remans R, Beebe S, Blair M, Manrique G, Tovar E, et al. (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean. *Plant and Soil* 302: 149-161.
 43. Franzetti L, Scarpellini M (2007) Characterization of *Pseudomonas* spp. Isolated from foods. *Annals of Microbiol* 57: 39-47.
 44. Costa R, Gomes NCM , Peixoto R S, Rumjanek N, Berg G, et al. (2006) Diversity and antagonistic potential of *Pseudomonas* spp. associated to the rhizosphere of maize grown in a subtropical organic farm. *Soil Biol Biochem* 38: 2434-2447.
 45. Gerhardt P, Murry RGE, Wood WA, Krieg NR (1994) *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology