

Control Of Fusarium Wilt Of Tomato Using Pgpr Isolated From Cheakpea Rhizosphere Region

Preeti Mehta¹ and Ashish saraf²

^{1,2} School of Sciences, MATS University, Raipur, C.G

Fusarium oxysporum f. sp. *lycopersici* is the causative agent of fusarium wilt ,a disease that affect tomato crops. The wilting significantly affect the yield and production of tomato . In this study the biocontrol potential of plant growth-promoting rhizobacteria *Enterobacter bugandensis* strain 247BMC(CPR2) and *Pseudomonas aeruginosa* strain DSM 50071 (CPR16) were tested against *Fusarium oxysporum* f. sp. *lycopersici*. Both the strain were isolated from the root region of chickpea plant . The results point out the ability of both rhizobacteria as safe substitutes for chemical fungicides in sustainable agriculture by demonstrating their effectiveness in lowering disease incidence and improving plant growth .

Key word –Wilt, Fusarium, Tomato, PGPR, Rhizosphere

Introduction

In India the tomato (*Lycopersicum esculentum* Mill.) is one of the most extensively cultivated and commercially significant vegetable crops is. It is an essential element of the agriculture industry due of its extensive utilization as both fresh fruit and a processed food product. Nonetheless, several hurdles impede However tomato production , mostly due to soil- and seed-borne illness that adversely affect quality and yield.[1,2].

Fusarium wilt, mostly induced by the fungal pathogen *Fusarium oxysporum*, is one of the most severe illness among them. *Fusarium* is a saprophytic fungus ubiquitous globally and pose significant health risk to human ,animal and plants.[3] Fungi produces mycotoxins including trichothecenes and fumonisins can contaminate food supplies and animal feed. These can result in diseases including cancer and suppression of immune system[4,5,6]. The global soil fungus *Fusarium oxysporum* is particularly well-known for its detrimental effect, affecting several crops through more than 120 different specialised forms[7,8]. Tomato wilt is caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol) which initially colonize on one side of the pedicel and in later stage the plant leaves turn to yellow margin and wilted further symptoms manifest as a brown discoloration of the xylem vessel. Its ability to thrive in warm, sandy, acidic soils renders it a significant danger to global tomato production.[9,10,11,12]

The rhizosphere harbors beneficial microorganisms known as plant growth-promoting rhizobacteria (PGPR), which have contributed to the understanding of biological management in plant diseases. They enhance nutrient absorption, nitrogen fixation, and disease resistance,

hence facilitating plant development[13,14]. A potential and sustainable method of controlling Fusarium wilt in tomatoes, which is caused by *Fusarium oxysporum* f. sp. *lycopersici*, is the application of plant growth-promoting rhizobacteria (PGPR). Some of the PGPR species which are commonly used to combat Fusarium wilt are *Rhizobium*, *Bacillus*, *Enterobacter*, and *Pseudomonas*[15,16,17,18]. *Pseudomonas* and *Enterobacter* species are best examples of rhizobacteria that able to diminish Fusarium wilt in tomatoes. In the rhizosphere, *Pseudomonas* and *Enterobacter* compete with the *Fusarium* pathogen for nutrients and space and preventing the pathogen from fully infecting the plant[19,20,21]. *Pseudomonas* species particularly *Pseudomonas aeruginosa*, generate bioactive substances such as siderophores, lytic enzymes (glucanase and chitinase), hydrogen cyanide (HCN), and antibiotics (phenazines, pyoluteorin). These bioactive chemicals directly inhibit the growth of *Fusarium oxysporum*. *Fusarium*'s fungal cell walls are broken down by enzymes produced by *Enterobacter* species called chitinases, which prevents *Fusarium* from colonising and spreading disease. In tomato plants, *Pseudomonas* and *Enterobacter* may both induce systemic resistance mechanism which activates the plant's defense system against pathogen invasion[22,23,24,25,26]. The aim of the current investigation was to determine the antifungal activity of *Pseudomonas aeruginosa* and *Enterobacter bugandensis* isolated from the rhizospheric soil of chickpea plants against *Fusarium oxysporum* f. sp. *lycopersici*, cause of tomato wilt. The objective was to evaluate their potential as biocontrol agents to inhibit the pathogen that causes Fusarium wilt in tomato plants.

Material and Method

Isolation of PGPR – Sample were collected from the different region of Raipur. The bacteria was isolated by serial dilution technique on LB agar media, fully grown bacterial colonies were subculture at 36 ± 1 °C for 24 hours. The culture are named as CPR 1,2,3..n (based on number of isolates). Morphological and molecular identification of isolates was done by available literature and 16s r-RNA sequencing respectively, the selected strain were send to Biokart India Pvt. Ltd., Bangluru, Karnataka for molecular identification.

Isolation of *Fusarium oxysporum*- The infected plant samples were collected from different areas within the Raipur district and after being cleaned and surface sterilization, the roots of contaminated plants samples were transferred on PDA media and incubated in the dark at 28 ± 1 °C. After the desired period of incubation (five days) the fungal colonies were develop and were transferred to new PDA plates and identified on the basis of available literature.[27]

Evaluation of PGPR traits of Rhizobacteria:

Qualitative assay for phosphate solubilization

The ability of the rhizobacteria to solubilize phosphate was assessed on Pikovskaya's Agar. The agar was inoculated with the isolates and incubated at 36 ± 2 °C for five days. Phosphate solubilization was indicated by the formation of a halo around the colonies.[28]

Determination of Indole Acetic Acid production

IAA production was tested using a modified method from Fischer et al. (2007). For this test, PGPR isolates were inoculated into 10 mL of nutrient broth supplemented with 0.2% (v/v)

L-tryptophan and incubated at 28°C for 10 days. Following incubation, the cultures were centrifuged at 3,000 rpm for 20 minutes, and the supernatant was tested for the presence of indole acetic acid (IAA). To screen for IAA, 1 mL of the supernatant was mixed with 2 mL of Salkowski reagent (35% perchloric acid, 0.5 M ferric chloride), and the tubes were incubated in the dark for 30 minutes. The development of a red color indicated a positive result, with uninoculated broth serving as the negative control. [29].

Ammonia production

For this, the isolates were inoculated into peptone water and incubated for 2 days at room temperature. After incubation, 2-3 drops of Nessler's reagent was added to the culture. Formation of a brown to yellow color was considered as positive result. Untreated growth medium was used as negative control[30].

HCN production

Nutrient agar was amended with 4.4 g glycine/L and the culture was streaked on modified agar plate. A Whatman filter paper (No. 1) soaked in 2% Sodium carbonate (in 0.5% picric acid) was placed at the top of the each isolated strain separately. Plates were sealed with parafilm and incubated at 36±1°C for 4 days. Development of orange to red colour on the paper indicated HCN production.[31]

Siderophore production

Siderophore production was tested using the Chrome Azurol S (CAS) assay. To carry out the test, isolates were carefully inoculated on the plates containing the CAS dye. The plates were then incubated at 37±1°C for a period of 7 days. After incubation, an orange halo or zone developed around the bacterial colonies indicated a positive result for siderophore production. The absence of such a zone signifies a negative result for siderophore production.[32]

Antifungal activity of *Enterobacter bugandensis* strain 247BMC and *Pseudomonas aeruginosa* strain DSM 50071 against *Fusarium oxysporum* f. sp. *lycopersici*

A dual culture assay was used to evaluate the antagonistic potential of bacterial isolates against the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* on Potato Dextrose Agar (PDA). A fungal mycelial disc was placed on one side of the plate, while the bacterial isolate was placed on the opposite side. The plates were incubated at 28°C for 7 days, and a control was included with only fungal growth. The formation of a clear inhibition zone around the bacterial colonies indicated antifungal activity. The size of the inhibition zone was recorded as a measure of the bacteria's antagonistic effect [33].

Pot experiment-Seeds of tomato were surface sterilized with 2.5% sodium and treated with the 48-h-old culture of the selected PGPR isolate. Fungi-infested soil was prepared. The bacteriatreated tomato seedlings were placed in the fungi-infested soil. Observations were recorded on root length, shoot length and wet weight of the seedlings ever two weeks interval of sowing by Three weeks after pathogen inoculation, the fresh weight, shoot length, and root length were measured and the wilting score was evaluated. Wilting score was evaluated based on leaf symptoms of wilting as follows: 0 = no wilt symptoms; 1 = <25% of wilting leaves; 2

= 26–50% of wilting leaves; 3 = 51–75% of wilting leaves; 4 = 76–100% of wilting leaves [34].

Result

Isolation and identification of Rhizobacteria

Isolation and identification of rhizobacteria- In this investigation, bacterial isolates from the chickpea rhizosphere were designated CPR. These designations were utilized during 16S rRNA-based phylogenetic analysis, to identify and contrast them with known sequences. Among all the isolates the strain CPR 2 and CPR 16 show maximum similarity to *Pseudomonas* and *Enterobacter*. For molecular identification, the isolate CPR 2 and CPR 16 that was extracted from the chickpea rhizosphere was sequenced using the 16S rRNA gene. The NCBI BLAST n tool was used to evaluate the 1378 bp (CPR 2) and 1211 (CPR 16) bp sequence that was obtained. CPR 2 had the highest sequence similarity of 99.56% with *Enterobacter bugandensis* strain 247BMC (Accession No. NR_148649.1) CPR 16 *Pseudomonas aeruginosa* strain DSM 50071 (Accession No. NR_117678.1) had the highest similarity (97.52%). Both of the strain are variants of already existed rhizobacteria from the chickpea root region and have been previously studied for antifungal activity against *F. oxysporum* f.sp. *lycopersici*.

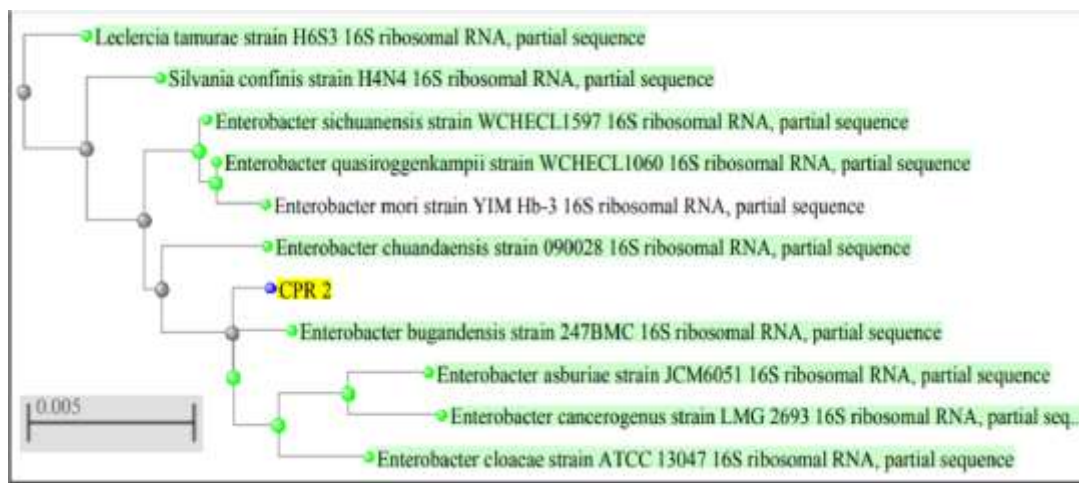


Fig. 1: Phylogenetic tree based on 16S rRNA sequences showing the relationship of CPR2 with reference strains

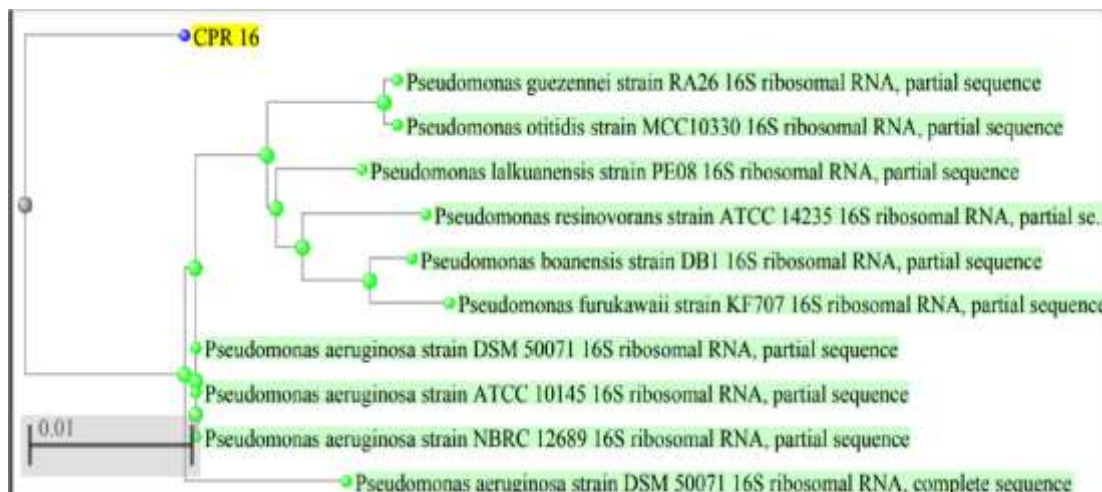


Fig. 2 : Phylogenetic tree based on 16S rRNA sequences showing the relationship CPR16 with reference strains

In the phylogenetic tree constructed using 16S rRNA sequences, the scale bar represents genetic distance between similar species. Value of 0.01 and 0.005 indicates 1% and 0.5 sequence difference. The close grouping of CPR2 and CPR16 with the reference strains of *Pseudomonas aeruginosa* and *Enterobacter bugandensis*, respectively, showed high genetic similarity and support for their identification.

Identification of Fusarium Pathogen

F. oxysporum f. sp. *lycopersici* isolated from the tomato plant and identified on the basis of morphological and colony characteristic. *Fusarium oxysporum* generates fuzzy or cottony colonies with pigmentation on the reverse side that range in from white to light pink. The fungus produces macroconidia and microconidia. The macroconidia are curved, larger, and feature three to five septa. The microconidia are smaller, oval to elliptical, typically single-celled, and they sporadically have one or two septa. The fungus also produces spherical, thick-walled chlamydospores, which can be isolated or found in clusters. These chlamydospores provide the fungus the ability to withstand extreme conditions, which aids in its survival in the soil.[27]

Characterization of isolates for plant growth promotional (PGP) traits- *Enterobacter bugandensis* strain 247BMC and *Pseudomonas aeruginosa* strain DSM 50071 were further analyzed for their plant growth promoting trait such as Qualitative assay for phosphate solubilization, Determination of Indole Acetic Acid production, Ammonia production, HCN production, and siderophore production. The result are shown in table 1.

Table 1 : Plant growth promoting Trait of Rhizobacteria

Isolates	Phosphate solubilization	IAA production	Ammonia Production	HCN production	Siderophore production
Enterobacter bugandensis strain 247BMC	+	+	+	+	+
Pseudomonas aeruginosa strain DSM 50071	+	+	+	+	+

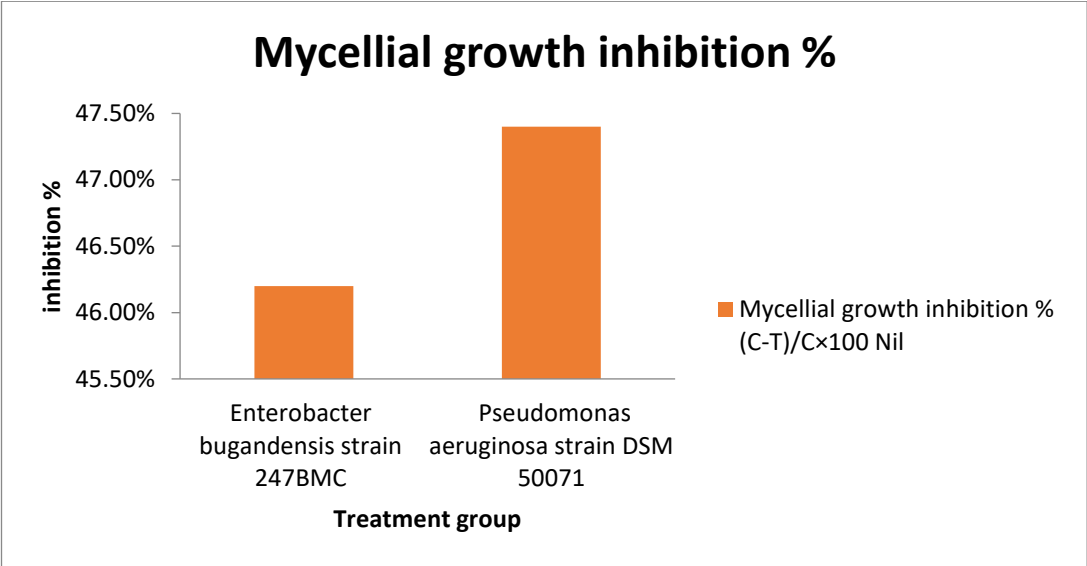
Both *Enterobacter bugandensis* strain 247BMC and *Pseudomonas aeruginosa* strain DSM 50071 can solubilize phosphate and makes phosphate available to promotes plant growth (Table 1). Both of the isolates produce plant hormone IAA. Both PGPR produce ammonia, which increase nitrogen availability and promote plant growth. HCN is a secondary metabolite that is useful in suppressing root pathogens and providing a biocontrol mechanism, is produced by both isolates. Siderophores that chelate iron from the environment and increase plant availability while depriving pathogens of iron and preventing their development, are produced by both isolates. So both *Enterobacter bugandensis* strain 247BMC and *Pseudomonas aeruginosa* strain DSM 50071 show a full range of plant growth-promoting traits, making them promising for improving plant health and defense against pathogens.

Antagonistic activity

The antagonistic activity of *Enterobacter bugandensis* strain 247BMC and *Pseudomonas aeruginosa* strain DSM 50071 were tested against *F. oxysporum* f. sp. *lycopersici* by dual culture technique. Both the strain inhibit the growth of fungal pathogen. The percentage growth inhibition of *Enterobacter bugandensis* strain 247BMC is 46.2% and *Pseudomonas aeruginosa* strain DSM 50071 is 47.4%.

Table 2: Antifungal activity of PGPR isolates against *F. oxysporum* f.sp. *lycopersici* in dual culture assay

S.NO	Treatment	Mycellial growth inhibition % (C-T)/C×100
1	Control	Nil
2	<i>Enterobacter bugandensis</i> strain 247BMC	46.2%
5	<i>Pseudomonas aeruginosa</i> strain DSM 50071	47.4%



Graph1: Growth Inhibition of Fusarium oxysporum by Selected PGPR Strains

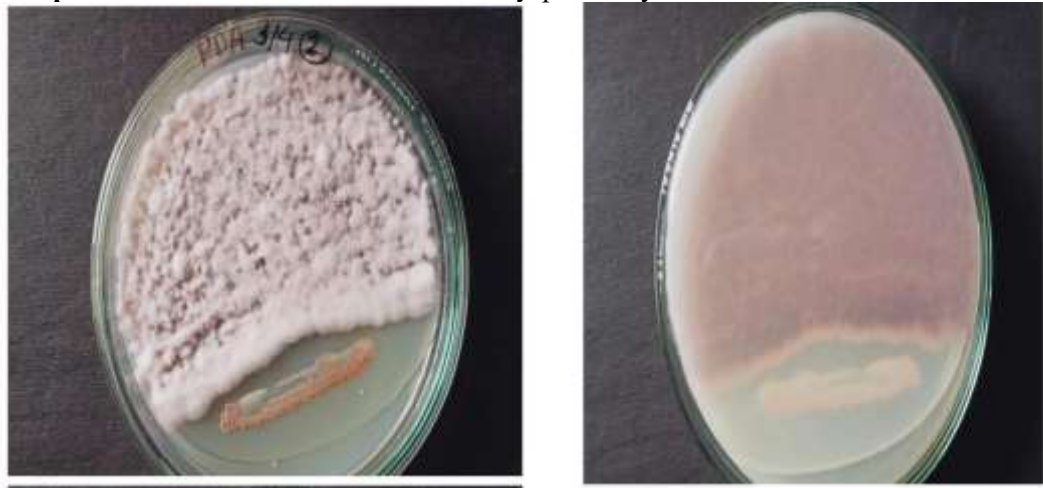


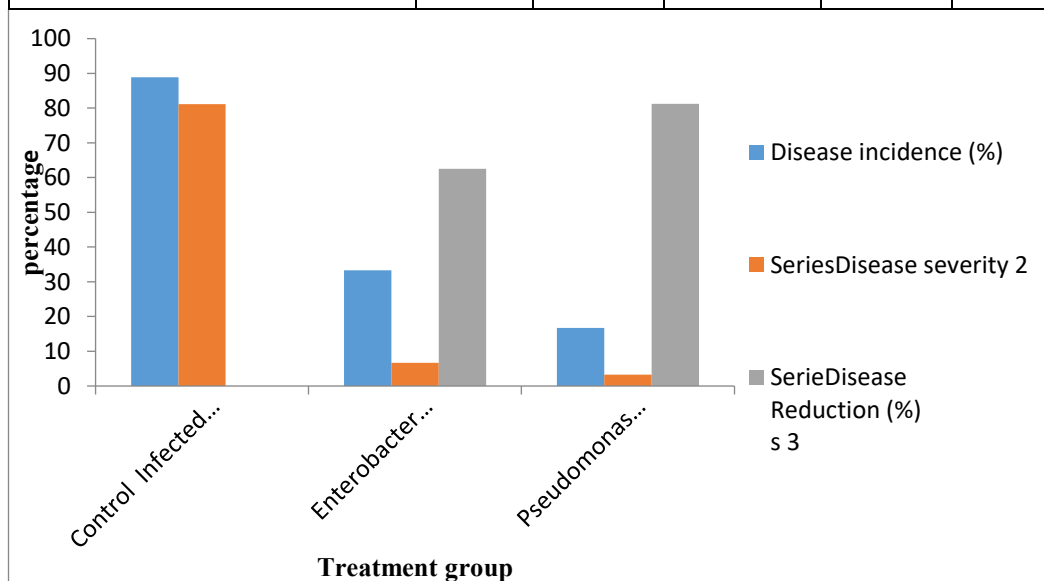
Fig 1.1 Dual culture technique

Pot Experiment –

Table 3: Comparison of disease incidence (%) and severity among tomato plants treated with PGPR isolates CPR2 and CPR16 under F.oxysporum f. sp. lycopersici

Group	Toatal plant	Infected plant	Disease incidence (%)	Disease severity	Disease Reduction (%)

Control Infected (F.oxysporum f. sp. lycopersici)	18	16	88.9	81.1	
Enterobacter bugandensis strain247BMC+F.oxysporum f. sp. lycopersici	18	6	33.3	6.7	62.5
Pseudomonas aeruginosa strain DSM 50071 + F. oxysporum f. sp. lycopersici	18	3	16.7	3.3	81.2



Graph 2: graph showing the effectiveness of Rhizospheric Bacterial Isolates in Reducing fusarium wilt severity

The table 3 elucidates the impact of several bacterial treatments on tomato plants afflicted with *Fusarium oxysporum* f. sp. *lycopersici*. The presents the quantity of infected plants, total plants, illness incidence (%), disease severity, and disease decrease (%) across three distinct groups. In the Control Infected group plant was only treated with *F. oxysporum* f. sp. *lycopersici*. Out of 18 plants, 16 were infected and recorded disease incidence is 88.9%. The severity of the illness is at peaked, measured at 81.1%. A separate treatment including plants treated with *Enterobacter bugandensis* strain 247BMC and *F. oxysporum* resulted in a disease incidence of 33.3% and disease severity of 6.7%, indicating a disease decrease of 62.5% relative to the control. The subsequent treatment of plant with *Pseudomonas aeruginosa* strain DSM 50071 and *F. oxysporum*. Only 3 out of 18 plants were infected,

yielding a disease rate of 16.7%.The illness severity was just 3.3% and this therapy attained achieved the maximum disease decrease of 81.2%



Fig.1.2. Plant treated with *F. oxysporum f. sp. lycopersici*



Fig. 1.3. Plant without any treatment



Fig 1.4. Plant treated with *F. oxysporum f. sp. lycopersici*



Fig 1.5: Plant treated with *F. oxysporum f. sp. lycopersici* and *Enterobacter bugandensis lycopersici* and *Pseudomonas aeruginosa* strain 247BMC strain DSM 50071

Both of the PGPR treatments significantly reduced the disease incidence and severity as compared to the control plant .*Pseudomonas aeruginosa* DSM 50071 showing the best results in controlling the disease.

Discussion

The rhizosphere region is an ecosystem that supports microbial interaction with plant, animal and other microbes. In the present study 2 plant growth-promoting rhizobacteria *Pseudomonas aeruginosa* strain DSM 50071 and *Enterobacter bugandensis* strain 247BMC isolated from cheakpea root region are effective in enhancing plant health and controlling the

pathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici*. The bacterial species belonging to genus *Pseudomonas* have multiple plant-growth promoting properties and used as biofertilizers and also used as biocontrol agent [35,36,37,38]. Both of the strain shows multiple plant growth-promoting traits. The identified bacterial strains are capable of solubilizing insoluble phosphate and make it available for plant phosphate. By converting insoluble forms of phosphate into soluble forms, they make this essential element readily available to plants, thereby promoting their growth and development. The isolates are also able to produce the plant growth hormone indole-3-acetic acid (IAA) and improve the nitrogen availability in soil by production of ammonia. Previous work done by many scientist reported the similar result [39,40,41,42,43].

Enterobacter bugandensis WRS7 isolated from the rhizospheric region have to reduce drought stress in wheat and also improve the growth by fixing Nitrogen, phosphate solubilisation, siderophore production, phytohormone like indole acetic acid and gibberellic acid production, exopolysaccharide secretion and ACC deaminase activity [44].

Apart from their plant growth promoting character, both the strain have potential to control the development of wilt in plant. *Enterobacter bugandensis* strain 247BMC and *Pseudomonas aeruginosa* strain DSM 50071 release hydrogen cyanide (HCN) and siderophore. Both the compound play a pivotal role in controlling which cause disease in plants [45]. Siderophores are the low molecular weight Fe-binding compounds, which binds Fe^{3+} and make it available for bacteria and unavailable to pathogen. HCN is toxin which directly inhibit the growth of plant pathogen [45,46]. The pot experiment clearly indicate that the *Pseudomonas aeruginosa* strain DSM 50071 is effectively reduce the disease severity in tomato plant as compare the *Enterobacter bugandensis* strain 247BMC. *P. aeruginosa* P11, P12, P66 and P112 isolated from the rhizosphere of chickpea plant were tested for biocontrol activity against fusarium wilt and plant growth promotion character evaluated under greenhouse conditions, in which *P. aeruginosa* (P10 and P12) provided better control than untreated control in seed treatment and soil-inoculation [47,48].

Conclusion

The research focused on the rhizospheric bacterial isolated from chickpea plants are plays an important role in controlling Fusarium wilt and enhance the growth of plants. Fungicides are currently widely used in to control plant disease. The study on replacing pesticides with another method which is cheaper and environment safer are currently being done at many scientist. The application of bio control agent replace the utilization of chemical pesticides, which is major cause of environmental pollution. A novel biotechnological technique to increase tomato yield by reducing the disease severity by the inoculation of PGPR may also be applied to other crops.

Acknowledgement

The authors are thankful to the MATS School of Sciences and Management, MATS University, Raipur for providing central laboratory facilities and their support throughout the study.

Conflict of Interests: The authors declare that they have no competing interest.

Reference

1. Hussein, H. Z., & Al-Dulaimi, S. I. (2020). Biological management of fusarium wilt on tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by some plant growth-promoting bacteria. *bioRxiv*.
2. Moe, M. Z., Yin, Z. Z., & Aung, S. S. (2018). In Vitro Antagonistic Activity of Soil Bacteria Against *Fusarium* in Tomato (Doctoral dissertation, MERAL Portal).
3. McGovern, R. J. (2015). Management of tomato diseases caused by *Fusarium oxysporum*. *Crop protection*, 73, 78-92.
4. Okungbowa, F. I., & Shittu, H. O. (2012). *Fusarium* wilts: An overview. *Environ. Res. J*, 6(2), 83-102.
5. Ploetz, R. C. (2015). *Fusarium* wilt of banana. *Phytopathology*, 105(12), 1512-1521.
6. Jiménez-Díaz, R. M., Castillo, P., del Mar Jiménez-Gasco, M., Landa, B. B., & Navas-Cortés, J. A. (2015). *Fusarium* wilt of chickpeas: Biology, ecology and management. *Crop Protection*, 73, 16-27.
7. Smith, S. N. (2007). An overview of ecological and habitat aspects in the genus *Fusarium* with special emphasis on the soil-borne pathogenic forms. *Plant Pathol Bull*, 16, 97-120.
8. Sampaio, A. M., Araujo, S. D. S., Rubiales, D., & Vaz Pato, M. C. (2020). *Fusarium* wilt management in legume crops. *Agronomy*, 10(8), 1073.
9. Ajilogba, C. F., & Babalola, O. O. (2013). Integrated management strategies for tomato *Fusarium* wilt. *Biocontrol science*, 18(3), 117-127.
10. Srinivas, C., Devi, D. N., Murthy, K. N., Mohan, C. D., Lakshmeesha, T. R., Singh, B., ... & Srivastava, R. K. (2019). *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity—A review. *Saudi journal of biological sciences*, 26(7), 1315-1324.
11. Gleason, M. L., & Edmunds, B. A. (2005). *Tomato diseases and disorders*. Ames, IA: Iowa State University, University Extension.
12. Attia, M. S., Abdelaziz, A. M., Al-Askar, A. A., Arishi, A. A., Abdelhakim, A. M., & Hashem, A. H. (2022). Plant growth-promoting fungi as biocontrol tool against *fusarium* wilt disease of tomato plant. *Journal of Fungi*, 8(8), 775.
13. Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 28, 1327-1350.
14. Jha, C. K., & Saraf, M. (2015). Plant growth promoting rhizobacteria (PGPR): a review. *Journal of Agricultural Research and Development*, 5(2), 108-119.
15. Shahzad, R., Tayade, R., Shahid, M., Hussain, A., Ali, M. W., & Yun, B. W. (2021). Evaluation potential of PGPR to protect tomato against *Fusarium* wilt and promote plant growth. *PeerJ*, 9, e11194
16. Recep, K., Fikretin, S., Erkol, D., & Cafer, E. (2009). Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. *Biological control*, 50(2), 194-198.
17. Loganathan, M., Garg, R., Venkataravanappa, V., Saha, S., & Rai, A. B. (2014). Plant growth promoting rhizobacteria (PGPR) induces resistance against *Fusarium* wilt and improves lycopene content and texture in tomato. *Afr J Microbiol Res*, 8(11), 1105-1111.

18. Zaim, S., Belabid, L., Bayaa, B., & Bekkar, A. A. (2016). Biological control of chickpea Fusarium wilts using rhizobacteria "PGPR". Microbial-mediated induced systemic resistance in plants, 147-162.
19. David, B. V., Chandrasehar, G., & Selvam, P. N. (2018). *Pseudomonas fluorescens*: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In *Crop improvement through microbial biotechnology* (pp. 221-243). Elsevier.
20. Lemanceau, P., & Alabouvette, C. (1993). Suppression of Fusarium wilts by fluorescent pseudomonads: mechanisms and applications. *Biocontrol Science and Technology*, 3(3), 219-234.
21. Wang, J., Deng, Z., Gao, X., Long, J., Wang, Y., Wang, W., ... & Wu, Z. (2024). Combined control of plant diseases by *Bacillus subtilis* SL44 and *Enterobacter hormaechei* Wu15. *Science of The Total Environment*, 934, 173297.
22. Mohammed, B. L., & Toama, F. N. (2019). Biological control of Fusarium wilt in tomato by endophytic rhizobacteria. *Energy Procedia*, 157, 171-179.
23. Ersöz, G., & Boyraz, N. (2022). Determination of Antagonistic Effect Between Some Fusarium Species and Root Bacteria Isolated from Eggplant Roots. *Selcuk Journal of Agriculture and Food Sciences*, 36(4), 48-52.
24. Abdulmugheth, O. M., & AL-Maliky, B. S. A. (2022). Detection of Salinity Tolerance *Pseudomonas Fluorescence* Isolates and their Effect on Control Fusarium Wilt Disease Caused by *Fusarium Oxysporum* F. Sp. *Lycopersici* on Tomato. *NeuroQuantology*, 20(1), 390.
25. Shanthi, A. T., & Vittal, R. R. (2013). Biocontrol potentials of plant growth promoting rhizobacteria against Fusarium wilt disease of cucurbit. *International Journal of Phytopathology*, 2(3), 155-161.
26. Mozumder, A. B., Chanda, K., Chorei, R., & Prasad, H. K. (2022). An evaluation of aluminum tolerant *Pseudomonas aeruginosa* A7 for in vivo suppression of Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* and growth promotion of chickpea. *Microorganisms*, 10(3), 568.
27. Aneja, K.R., 2003. *Experiments in Microbiology, Plant pathology and Biotechnology*, fourth ed. New Age International Publishers, Daryaganj, New Delhi.
28. Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* 17, 362–370.
29. Fischer, S.E., Fischer, S.I., Magris, S., and Mori, G.B. 2007. Isolation and characterization of bacteria from the rhizosphere of wheat. *World J. Microbiol. Biotechnol.* 23, 895–903.
30. Verma, J.P., Yadav, J., Tiwari, K.N., Lavakush, Singh, V., 2010b. Impact of plant growth promoting rhizobacteria on crop production. *Int. J. Agric. Res.* 5, 954–983.
31. Kesaulya, H., Zakaria, B., & Syaiful, S. A. (2015). Isolation and physiological characterization of PGPR from potato plant rhizosphere in medium land of Buru Island. *Procedia Food Science*, 3, 190-199.
32. Alexander, D. B., & Zuberer, D. A. (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biology and Fertility of soils*, 12, 39-45.
33. Ganesan, S., & Sekar, R. (2012). Fluorescent *Pseudomonas* as plant growth promoting rhizobacteria and biocontrol agents in groundnut crop (*Arachis hypogaea* L.). *International Journal of Applied BioResearch*, 12, 1-6.

34. G.Shobha ,BS Kumudini.2020,Antagonistic effect of the newly isolated PGPR Bacillus spp. on Fusarium Oxysporum, Species of International Journal of Applied Science and Engineering Research Vol. 5 No.
35. Dasgupta, D., Sengupta, C., & Paul, G. (2015). Screening and identification of best three phosphate solubilizing and IAA producing PGPR inhabiting the rhizosphere of Sesbania bispinosa. Screening, 4(6), 3968-3979.
36. Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. Annual review of microbiology, 63(1), 541-556.