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Isolation and characterization of bacterial strains for biocontrol activity in chilli against *Fusarium* sps.

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Abstract

Chilli (*Capsicum annum* L.) is one of the most important vegetable and spice crop belongs to family Solanaceae and genus *Capsicum*. It is susceptible to various diseases and pests. Among all of them, family Solanaceae mostly affected by fungal diseases. This study focuses on biocontrol activity of chilli bacterial wilt caused by *Fusarium* sps. Here, with 43 isolates obtained from soil, seed and aerial parts of chilli. Out of all 43 isolates, potent isolates were taken based on *in vitro* activities like Siderophore, HCN, *In vitro* antagonism, Dual culture. W₁RC₁ (74%), MB₂RC₄ (76%), MB₁ENC₁ (77%), MB₂ENC₁ (79%), W₁EPC₂ (76%), MB₁EPC₁ (79%) were different inhibition percentage against *Fusarium* sps. These potent bacterial isolates were compatible with each other through dual culture and taken for preparation of consortium for ongoing pot culture experiment of chilli with seven treatments involving T₁(Absolute Control), T₂ (100% RDF (Negative Control)), T₃(100% RDF + Seed treatment with consortia), T₄(100%RDF + Soil application with consortium), T₅ (100% RDF + Seed treatment + Soil application with consortium), T₆ (100% RDF + Application of fungicide Mancozeb @ 2.5g/ lit), T₇ (100% RDF + Seed treatment with consortium + Fungicidal spray). Out of all the treatments T₅ (100% RDF + Seed treatment + Soil application with consortium) treatment plants shown better results in terms of plant growth and defense enzyme activities (PO, PPO, PAL).

Keywords: Siderophore, consortium, PO-peroxidase, PPO-polyphenol oxidase, PAL- phenyl alanine

1. Introduction

Chillies are one of the most widely cultivated and consumed spice crops globally. They belong to the *Capsicum* genus and are a crucial part of various cuisines, particularly in Asian and Latin American cultures. Chillies originated in the Americas over 6,000 years ago and were introduced to Asia by Portuguese traders in the 16th century. They quickly became an integral part of Indian, Chinese, and Southeast Asian cuisine.

Microorganisms play a vital role in biocontrol activity, helping to manage chilli losses caused by diseases and pests. Screening and biocontrol potential of rhizobacteria native to plains and hilly regions to induce systemic resistance and promote plant growth in chilli against bacterial wilt disease by (Kashyap *et al.*, 2021) ^[4] *in vitro* antagonist activity of rhizobacteria against chilli wilt. Out of the 63 isolates of rhizobacteria isolated from rhizospheric soil of chilli plants, 38.09% rhizobacteria were found to have antagonistic activity against *R. solanacearum*. A marked inhibition in the growth of *R. solanacearum* culture was observed in the presence of rhizobacterial isolates. The maximum inhibition zone (22.4 mm diameter) of *R. solanacearum* was found by the isolate PDS1 isolated from the Middle Gangetic Plain region, followed by KA9 isolate (20.06 mm diameter). The use of glucose as a carbon source was found in 29.42% of rhizobacteria.

Isolation and characterization of native antagonistic rhizobacteria against *Fusarium* wilt of chilli to promote plant growth by (shiva *et al.*, 2024) ^[6] *F. oxysporum* f. sp. *capsici*, a soil borne pathogen, was found in wilted chilli plant samples during a survey. The fungus showed colonies with varying colors and velvety surfaces, changing color upon spore generation. Its hyphae were filaments, septate, hyaline, and branched at acute or right angles. The isolate's pathogenicity was evaluated *in vitro* using seedlings, causing symptoms like collapse and wilting of petiole, rachis, and leaflets 15 days post-inoculation. The signs of plant wilting in infected prostrays resembled those in the main field.

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2. Materials and Methods

2.1 Collection and Isolation of microbial isolates

Soil, seed, aerial parts of chilli samples were taken from warangal district of Telangana. Total 43 isolates were obtained from chilli samples using Nutrient Agar (NA) medium. These obtained isolates were purified by pure culture technique for ongoing further activities

2.2 Siderophore Production

Production of siderophores estimated qualitatively on aqueous ferric chloride solution for siderophores detection. Orange color zone around the colonies observed and recorded and modified by Alexander and Zuberer (1991) [1]. Siderophore production confirmed by the qualitative CAS agar test. The log-phase bacterial culture spotted on nutrient agar plates amended with the CAS solution. The plates incubated at 28°C under dark conditions for 5 days. The appearance of orange halo zones confirms siderophore production.

2.3 Hydrogen cyanide (HCN) production

Qualitative assay (Bakker and schippers, 1987) [2] for pure isolates tested by inoculating on agar plates supplemented with 4.4g glycine L-1 using alkaline picric acid as a reagent, incubated for 4 days and change to brown colour of the filter paper indicates positive for HCN production.

2.4 In vitro antagonist activity of the bacterial isolates

For *in vitro* antagonist activity both bacterial isolates and *Fusarium sps* pathogen were cocultured on Potato dextrose agar (PDA) medium according to the method described by Sijam and Dikin (2005) [7]. The bacteria which inhibiting the mycelial growth of fungi pathogen taken into consideration for biocontrol activity. The percentage reduction in growth was calculated following the formula. Where, C-diameter of fungal growth in control plate, T-diameter of fungal growth in test plate

$$\text{Percentage of inhibition (\%)} = \frac{C-T}{C} \times 100$$

2.5 Molecular characterization of potential bacterial isolates

Genomic DNA extraction done for potential and unique characters of bacterial isolates by genomic DNA isolation kit (ZR Fungal/Bacterial DNA MiniPrepTM). Genomic DNA subjected to PCR (polymerase chain reaction) for amplification of 16s rRNA gene with universal primers (Forward & Reverse) and sequencing done through outsourcing. Identification of bacterial isolates based on 16s rRNA gene sequence by BLAST-n in NCBI database.

2.6 Pot culture experiment

The experiment conducted for the evaluation of potential strains against wilt of chilli under pot culture in the Dept. of Food Safety and Quality Assurance, College of Food Science & Technology, Rudrur with seven treatments in chilli involving T₁(Absolute Control), T₂ (100% RDF (Negative Control)), T₃(100% RDF + Seed treatment with consortia), T₄(100%RDF + Soil application with consortium), T₅ (100% RDF + Seed treatment + Soil application with consortium), T₆ (100% RDF + Application of fungicide Mancozeb @ 2.5g/ lit), T₇ (100% RDF + Seed treatment with consortium + Fungicidal spray).

2.7 Peroxidase assay

Peroxidase activity was estimated quantitatively by the method described by Chen *et al.* (2000) [2]. 100 µl of the extract was taken into a spectrophotometer cuvette into which 7.5 µl of guaiacol of 50 mM concentration and 792 µl of 0.05 mM Tris

HCl buffer of pH 6.0 was added. The reaction was started by adding 100 µl of 0.6 M Hydrogen peroxide. Optical density (OD) values were taken at 470 nm. A blank was prepared which comprises guaiacol, Tris HCl buffer and Hydrogen peroxide to set the spectrophotometer at 100% absorbance. One unit of the enzyme was defined as the change in absorbance (in units) min⁻¹ g⁻¹ fresh tissue.

2.8 Polyphenol oxidase assay

Chen *et al.* (2000) [2] have described the method for the estimation of PPO activity with slight modifications. The reaction mixture for PPO comprises 1.5 ml of 0.1 M citrate phosphate buffer of pH 6.0, 5 mg/ml of proline, 500 µl of 0.01 M of catechol and 500 µl of enzyme extract. Moreover, the reaction mixture was aerated before the addition of catechol, which serves as a substrate. Enzyme activity was analysed by taking the optical density value at 546 nm. One unit of the enzyme was defined as the change in absorbance (in units) min⁻¹ g⁻¹ fresh tissue.

2.9 Phenylalanine ammonia lyase assay

PAL assay was determined quantitatively by the following method which was given by Ross and Sederoff (1992). The reaction mixture comprises 600 µl of L- phenylalanine in 500 µl of 50 mM Tris HCl of pH 8.8 and 100 µl of enzyme extract, which was incubated for 60 min at 40 °C. The reaction was stopped by adding 1 ml of 2 N HCl and 1.5 ml of toluene, vortexed for 30 sec, centrifuged at 4,000 rpm for 5 min and the toluene fraction containing trans-cinnamic acid(lower face) was separated. The absorbance was measured at 290 nm, against the toluene blank.

3. Results and Discussion

3.1 Siderophore production

Production of siderophores estimated qualitatively on aqueous ferric chloride solution for siderophores detection. Orange color zone around the colonies observed and recorded (Fig. 1). Out of 43 isolates 26 isolates showed positive in which all epiphytic bacterial isolates showed positive. Among all the isolates MB₂RC₄ (45mm) produced highest with a great difference from other isolates. Whereas MB₁RC₃ (7mm), MB₂RC₁ (8mm), MB₂RC₃ (5mm), MB₃RC₂ (6mm), W₁ENC₁ (8mm), W₂ENC₄ (5mm), MB₁ENC₃(8mm), MB₃ENC₃ (6mm), W₁EPC₂ (5mm), W₂EPC₃ (5mm), W₃EPC₂ (3mm), MB₁EPC₂ (8mm), MB₃EPC₁ (6mm). Among all the isolates W₃EPC₂ showed least siderophore production and rest all isolates were moderate as shown in (Table 1)

The study by Ghazy and El-Nahrawy (2021) [9]. found that *B. subtilis* MF497446 and *P. korensis* MG209738 are the most effective PGPRs by qualitative and quantitative assays for siderophore production and individually or in combination inoculation condition, leading to induce the disease resistance of late wilt caused by *C. maydis* in maize plant and improvement of growth promotion and yield.

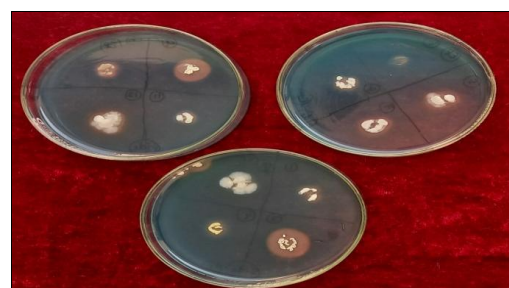


Fig 1: Siderophore production

Table 1: Qualitative production of siderophore

S. No	Isolates	Siderophore	HCN
1	W ₁ RC ₁	-	+++
2	W ₁ RC ₂	-	+
3	W ₁ RC ₃	-	++
4	W ₂ RC ₂	-	+++
5	W ₂ RC ₃	++	+++
6	W ₂ RC ₄	+++	++
7	W ₃ RC ₁	++	+++
8	W ₃ RC ₂	++	+++
9	W ₃ RC ₃	-	++
10	MB ₁ RC ₁	-	++
11	MB ₁ RC ₂	-	++
12	MB ₁ RC ₃	+	++
13	MB ₂ RC ₁	+	+++
14	MB ₂ RC ₃	+	+++
15	MB ₂ RC ₄	+++	++
16	MB ₃ RC ₁	-	++
17	MB ₃ RC ₂	+	++
18	W ₁ ENC ₁	+	+++
19	W ₁ ENC ₂	-	+
20	W ₂ ENC ₂	+++	+++
21	W ₂ ENC ₄	+	++
22	W ₃ ENC ₂	-	+
23	MB ₁ ENC ₁	-	++
24	MB ₁ ENC ₂	-	+++
25	MB ₁ ENC ₃	+	+++
26	MB ₂ ENC ₁	-	+++
27	MB ₂ ENC ₂	-	++
28	MB ₂ ENC ₃	-	+++
29	MB ₃ ENC ₁	-	+
30	MB ₃ ENC ₂	-	++
31	MB ₃ ENC ₃	+	++
32	W ₁ EPC ₁	++	+++
33	W ₁ EPC ₂	+	++
34	W ₂ EPC ₁	+++	++
35	W ₂ EPC ₃	+	++
36	W ₂ EPC ₄	++	+
37	W ₃ EPC ₂	+	+++
38	MB ₁ EPC ₁	++	++
39	MB ₁ EPC ₂	+	++
40	MB ₂ EPC ₁	+++	++
41	MB ₂ EPC ₂	++	++
42	MB ₃ EPC ₁	+	+++
43	MB ₃ EPC ₂	+++	-

3.2 HCN production

Pure isolates tested qualitatively by inoculating on agar plates supplemented with 4.4g glycine L-1 using alkaline picric acid as a reagent, incubated for 4 days and change to orange brown colour of the filter paper indicated positive for HCN production (Fig. 2) All isolates showed positive for HCN production except MB₃EPC₂. Based on amount of colour change intensity, classification of HCN producer isolates were done as strong, medium and slow producers. (Table 2)

De Vrieze *et al.* (2020) [10] verified the involvement of this known particular biocontrol trait and quantified its relative contribution to the overall anti-oomycete activity of two cyanogenic *Pseudomonas* using *in vitro* and leaf disk experiments. Findings by Anand showed the indication of higher activity or altered phenotype observed in the cyanide deficient mutant of strain suggests that the presence of cyanogenic strains.

**Fig 2:** Qualitative production of HCN

3.3 In vitro antagonist activity- Dual culture

With *Fusarium* sps W₁RC₁, MB₂RC₄, MB₁ENC₁, MB₂ENC₁, W₁EPC₂, MB₁EPC₁ showed positive for antagonistic activity in which all had very strong percent inhibition. Among them MB₁ENC₁ (77%), MB₂ENC₁ (79%) highest percentage than other isolates. (Fig. 3)

Table 2: Inhibition percentage of strains which showing antagonism

Isolates	F- PI(%)
W ₁ RC ₁	74
MB ₂ RC ₄	76
MB ₁ ENC ₁	77
MB ₂ ENC ₁	79
W ₁ EPC ₂	76
MB ₁ EPC ₁	69

**Fig 3:** Antagonism effect of bacterial isolate against pathogen *Fusarium* sps

3.4 Molecular characterization of potential bacterial isolates

Based on 16s rRNA sequence bacterial isolates identified by BLAST-n in NCBI database after creating contig using chromas software. The type of organism identified were *Bacillus cereus*, *Bacillus amyloliquifaciens*, *Bacillus thuringiensis* which all belonging to *Bacillus* spp showing biocontrol activity.

3.5 Pot culture experiment

Out of seven treatments carried out with T₁(Absolute Control), T₂ (100% RDF (Negative Control)), T₃(100% RDF + Seed treatment with consortia), T₄(100% RDF + Soil application with consortium), T₅ (100% RDF + Seed treatment + Soil application with consortium), T₆ (100% RDF + Application of fungicide

Mancozeb @ 2.5g/ lit), T₇ (100% RDF + Seed treatment with consortium + Fungicidal spray). Symptoms of wilting observed more in Negative control after application of inoculum. The other treatments which involving consortium remained healthy even after application of inoculum. (Fig 4)



Fig 4: wilting symptoms in negative control treatment(T₂)

3.6 Peroxidase assay

This activity carried by taking samples of root and shoot from all seven treatments. The results showed that peroxidase activity was in the increasing order of T₅ (100% RDF + Seed treatment + Soil application with consortium) > T₇ (100% RDF + Seed

treatment with consortium + Fungicidal spray) > T₃ (100% RDF + Seed treatment with consortia) > T₄ (100% RDF + Soil application with consortium) > T₆ (100% RDF + Application of fungicide Mancozeb @ 2.5g/ lit) > T₁ (Absolute Control) > T₂ (100% RDF (Negative Control)) for *Fusarium* sps in both root and shoot extract (Fig 5)

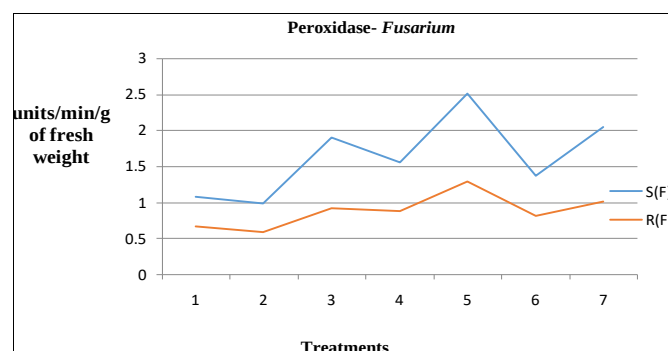


Fig 5: Peroxidase assay- Shoot (S(F)), Root (S(F))

3.7 Polyphenol oxidase- Shoot(S(F)), Root(S(F))

In this activity T₅ (100% RDF + Seed treatment + Soil application with consortium) showed highest 1.183 and 1.891 units/min/g fresh weight of root and shoot respectively in *Fusarium* sps. The lowest was observed in T₂ (100% RDF (Negative Control)) 0.485, 1.007 units/min/g fresh weight of root and shoot for *Fusarium* sps (Fig 6)

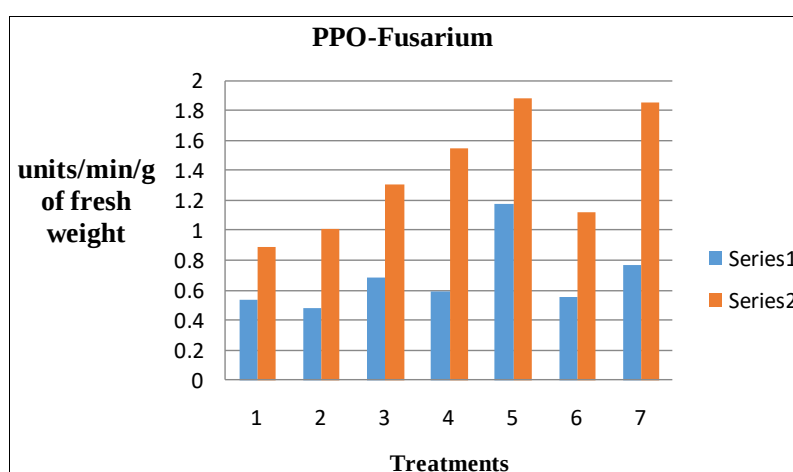


Fig 6: Polyphenol oxidase (PPO)-Series1(Root), Series2(Shoot)

3.8 Phenylalanine ammonia lyase assay

Here, In this activity for *Fusarium* sps amount of phenyl alanine produced highest in T₅ (100% RDF + Seed treatment + Soil application with consortium) > T₇ (100% RDF + Seed treatment with consortium + Fungicidal spray) > T₄ (100% RDF + Soil application with consortium) > T₃ (100% RDF + Seed treatment with consortia) > T₆ (100% RDF + Application of fungicide Mancozeb @ 2.5g/ lit) > T₁ (Absolute Control) > T₂ (100% RDF (Negative Control)) in root. In shoot highest amount were noticed in T₅ (100% RDF + Seed treatment + Soil application with consortium) > T₇ (100% RDF + Seed treatment with consortium + Fungicidal spray) and followed by T₄ (100% RDF + Soil application with consortium), T₃ (100% RDF + Seed treatment with consortia) which are on par > T₃ (100% RDF + Seed treatment with consortia) > T₁ (Absolute Control) > T₆ (100% RDF + Application of fungicide Mancozeb @ 2.5g/ lit) > T₂ (100% RDF (Negative Control)). (Fig 7)

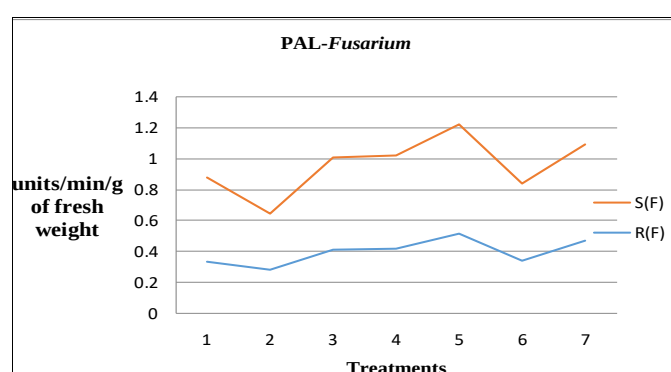


Fig 7: Phenylalanine ammonia lyase (PAL)

A study by Singh *et al.* (2024) supported application of three bacterial consortia including *P. fluorescens* 131B, *B. licheniformis* 223B and *B. subtilis* 236B showed an impressive

89.5% increase in total phenols, 91.7% in flavonoids, 134.6% in peroxidase (POX), 102% in polyphenol oxidase (PPOX), 51.9% increase in phenylalanine ammonia lyase (PAL). The four microbe consortium treatment, which included *T. afroharzianum* 5F, *P. fluorescens* 131B, *B. licheniformis* 223B, and *B. subtilis* 236B, resulted in the highest enhancements, with a 92.1% increase in total phenols, 141.6% in flavonoids, a 188.5% increase in POX activity, a 116.3% increase in PPOX, a 71.2% increase in PAL.

4. Conclusion

The effect of potent isolates played an important role in biocontrol activity against *Fusarium* spp. Out of all the treatments carried out T₅ (100% RDF + Seed treatment + Soil application with consortium) and T₇ (100% RDF + Seed treatment with consortium + Fungicidal spray) showed better results in terms of root, shoot growth, disease suppression compared to least performing treatment T₂ (Negative control). This study concluded bacteria isolates showing biocontrol activity.

5. References

1. Alexander DB, Zuberer DA. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol Fertil Soils*. 1991;12(1):39-45.
2. Bakker AW, Schippers B. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. *Soil Biol Biochem*. 1987;19(4):451-457.
3. Chen C, Belanger RR, Benhamou N, Paulitz TC. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. *Physiol Mol Plant Pathol*. 2000;56(1):13-23.
4. Kashyap AS, Manzar N, Rajawat MVS, *et al*. Screening and biocontrol potential of rhizobacteria native to gangetic plains and hilly regions to induce systemic resistance and promote plant growth in chilli against bacterial wilt disease. *Plants*. 2021;10(10):2125.
5. Whetten WR, Sederoff RR. Phenylalanine ammonia-lyase from loblolly pine: purification of the enzyme and isolation of complementary DNA clones. *Plant Physiol*. 1992;98(1):380-386.
6. Shiva B, Srinivas P, Khulbe D, *et al*. Isolation and characterization of native antagonistic rhizobacteria against *Fusarium* wilt of chilli to promote plant growth. *PeerJ*. 2024;12:e17578.
7. Sijam KA, Dikin A. Biochemical and physiological characterization of *Burkholderia cepacia* as biological control agent. *Int J Agric Biol*. 2005;7(3):385-388.
8. Singh D, Geat N, Jadon KS, *et al*. Isolation and characterization of biocontrol microbes for development of effective microbial consortia for managing *Rhizoctonia bataticola* root rot of cluster bean under hot arid climatic conditions. *Microorganisms*. 2024;12(11):2331.
9. Ghazy N, El-Nahrawy S. Siderophore production by *Bacillus subtilis* MF497446 and *Pseudomonas koreensis* MG209738 and their efficacy in controlling *Cephalosporium maydis* in maize plant. *Arch Microbiol*. 2021;203(3):1195-1209.
10. De Vrieze M, Varadarajan AR, Schneeberger K, *et al*. Linking comparative genomics of nine potato-associated *Pseudomonas* isolates with their differing biocontrol potential against late blight. *Front Microbiol*. 2020;11:857.