www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 249-255

www.thepharmajournal.com
Received: 14-07-2023
Accepted: 27-08-2023

Satyam
Ph.D. Scholar, Department of Plant Pathology and Agricultural Microbiology, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

AM Navale
Head, Department of Plant Pathology and Agricultural Microbiology, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

SV Kolase
Professor (CAS) Department of Plant Pathology and Agricultural Microbiology, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

TK Narute
Ex Head, Department of Plant Pathology and Agricultural Microbiology, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

AA Kale
Head Department of Biochemistry, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

AV Chandanshive
Assistant Professor and Officer In-charge, Tomato Improvement Scheme, Department of Horticulture, MPKV, Rahuri, Maharashtra, India

AG Durgude
Assistant Professor Department of Soil Science, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

MS Barge
Ph.D. Scholar, Department of Plant Pathology and Agricultural Microbiology, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

Corresponding Author:
Satyam
Ph.D. Scholar, Department of Plant Pathology and Agricultural Microbiology, Post Graduate institute, MPKV, Rahuri, Maharashtra, India

Salt and temperature tolerant rhizobacteria: A comprehensive study on biochemical profiling and biocontrol potential

Satyam, AM Navale, SV Kolase, TK Narute, AA Kale, AV Chandanshive, AG Durgude and MS Barge

Abstract
This research investigates the biocontrol potential of salt and temperature-tolerant plant growth-promoting rhizobacteria isolated from the rhizosphere soil of tomato plants, aiming to combat various plant pathogens and enhance crop resilience against diseases like vascular wilt, crown gall, root rot, leaf wilt, and curling disease. Among 138 soil samples, 12 isolates were selected for comprehensive assessment through biochemical tests and biocontrol activity evaluations. Standard methods were employed to analyse indole production, urease activity, oxidase activity, starch hydrolysis, gelatin liquefaction, methyl red test, Voges-Proskauer test, H_2S production, and citrate utilization. Biocontrol activity was determined using a dual-culture plate method, wherein actively growing phytopathogenic fungi of 5 mm diameter was placed at the center of Petri plates and Bacterial isolates were streaked approximately 2.2 cm away from the phytopathogen in a circular manner, while control plates lacked bacterial streaks. Incubation was performed at 30 °C in a BOD incubator. After 4 days, the percentage of mycelial growth inhibition was calculated. Key findings revealed that: *Pseudomonas furukawaii* STT-A8 exhibited positive results for the oxidase test, gelatin liquefaction and citrate utilization. *Achromobacter* sp. STT-A12 displayed positive results for the oxidase test and citrate utilization. Agrobacterium pusense STT-A24 and STT-A39 showed positive results for indole production, urease activity, starch hydrolysis, methyl red test, and citrate utilization. *Brevibacterium epidermidis* STT-K2 and STT-N28 tested positive for gelatin liquefaction and citrate utilization. Priestia flexa STT-K13 and Bacillus sp. STT-K24 exhibited positive results for urease activity, oxidase activity, starch hydrolysis, gelatin liquefaction, and citrate utilization. *Pusillimonas* sp. STT-K15 demonstrated positive oxidase activity. Among the 12 extremely salt and temperature-tolerant rhizobacteria isolates, the highest inhibitory effects against *Fusarium oxysporum* were observed in *Pseudomonas furukawaii* STT-A8 (87.6%), *Agrobacterium pusense* STT-A39 (78.75%), *Achromobacter* sp. STT-A12 (77.5%), *Pusillimonas* sp. STT-K15 (72.5%), *Agrobacterium pusense* STT-A24 (71.25%), *Priestia flexa* STT-K13 (71.25%), *Bacillus badius* STT-N22 (70%), *Bacillus badius* STT-N33 (68.75%), and *Bacillus badius* STT-N26 (68.42%). *Brevibacterium epidermidis* STT-K2 (55%), *Brevibacterium epidermidis* STT-N28 (50%), and *Bacillus* sp. STT-K24 (50%) also exhibited significant inhibition against *Fusarium oxysporum*. This research offers valuable insights into the biocontrol capabilities of salt and temperature-tolerant rhizobacteria, positioning them as essential inoculants for disease management and the promotion of tomato plant growth in saline environments.

Keywords: Biocontrol agent, selection, Inhibition, salt and temperature tolerant, plant growth promoting rhizobacteria

Introduction
Biochemical tests are essential tools for characterizing and identifying biocontrol agents, particularly plant growth-promoting rhizobacteria (PGPR) and other beneficial microorganisms. These tests help researchers and farmers assess the biochemical properties of these agents, which are closely linked to their effectiveness as biocontrol agents. Here are some common biochemical tests and their relationships with biocontrol agents: In the realm of sustainable agriculture, the critical role played by beneficial microbes, particularly Plant Growth-Promoting Rhizobacteria (PGPR) has garnered significant attention. As early as 2004, Kloepper and colleagues highlighted the remarkable potential of PGPR in not only promoting plant growth but also in serving as a protective shield against a diverse array of plant pathogens. Subsequently, scientists like Adesemoye *et al*. (2009) [2] have underscored the diverse applications of PGPR, ranging from biocontrol to biofertilization and bolstering plant resistance against abiotic stress.

PGPR, an invaluable class of bacteria, have proven to be vital allies for numerous plant species. Their colonization of the rhizosphere creates a symbiotic relationship that confer manifold advantages upon their host plants. These benefits encompass improved plant growth and heightened resilience against the serious and imminent dangers posed by nematodes, fungi, bacteria, and viruses, as evidenced in the studies of Kloepper *et al*. (2004) [16]. Within the PGPR community, taxa such as *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Bacillus*, and *Pseudomonas* have emerged as key players, leveraging their diverse metabolic capabilities to support plant health and combat pathogens. These bacteria employ an arsenal of mechanisms, such as antibiotic production, enzymatic degradation of fungal cell walls, sequestration of iron in the rhizosphere, induction of systemic resistance, and competition for resources in the rhizosphere, as elucidated by Parani *et al*. (2012) [25]. Notably, the synthesis of antibiotics by PGPR stands as one of the paramount mechanisms underpinning their efficacy in biocontrol. Additionally, the ability to produce antifungal enzymes, like chitinases and glucanases, further enhances their capacity to combat fungal infections. Furthermore, the production of low-molecular-mass siderophores capable of chelating iron in the soil significantly impedes the growth of fungal pathogens, as demonstrated by Köhl *et al*. (2019) [17]. For instance, *Pseudomonas fluorescens* has been recognized for its role in creating siderophores like pyochelin and pyoverdine (Bora *et al*., 2017) [5], while Pseudomonas trivalis strain BIHB 745 has been identified as a siderophore producer (Parani *et al*., 2012) [25]. In addition to antibiotics, many *Pseudomonas* species also generate lytic enzymes and other bioactive compounds that further enhance their biocontrol capabilities. The practical implications of harnessing PGPR in agriculture are underscored by the development of biocontrol products containing specialized strains of *Pseudomonas*, *Bacillus*, and *Streptomyces*. These products have been tailored to combat specific plant diseases, exemplified by *Pseudomonas fluorescens* A506's effectiveness against *Erwinia amylovora*-induced fire blight in pome fruits (Cabrefiga *et al*., 2007) [6] and *Pseudomonas chlororaphis* efficacy against diseases caused by *Drechslera* species and *Fusarium* (Johnsson *et al*., 1998; Puopolo *et al*., 2011) [5, 27]. Moreover, *Bacillus subtilis* has shown promise in promoting plant growth and salt tolerance (Han *et al*., 2014) [12], while *B. amyloliquefaciens* has been instrumental in inhibiting *Monilinia vaccinii-corymbosi*, a pathogen affecting blueberries (Ngugi *et al*., 2005) [23], and preventing bottom rot in lettuce (Chowdhury *et al*., 2013) [8]. The efficacy of PGPR extends beyond individual species, encompassing protection against bacterial and fungal pathogens, as documented for genera like *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, and *Rhizobium.* These bacteria employ a spectrum of strategies, including colonization, competition, and the release of potent antimicrobial substances, such as antibiotics and cell wall lytic enzymes, to thwart plant diseases. Additionally, they facilitate the establishment of plant resistance mechanisms (Montesinos *et al*., 2009; Lugtenberg and Kamilova, 2009; Berendsen *et al*., 2012) [22, 19, 4]. Intriguingly, microbial biological control agents shield crops from disease damage through various modes of action, which can be either direct or indirect. These agents may bolster plant resistance or directly target pathogens within plant tissues without direct antagonistic contact. Competition for resources and space is another indirect but effective strategy against pathogens (Spadaro and Droby, 2016) [32]. Moreover, the involvement of hyperparasites that infiltrate and eliminate pathogen cells, mycelium, spores, and latent structures adds another layer of complexity to this biological warfare (Ghorbanpour *et al*., 2018) [11]. The significance of PGPR and their multifaceted mechanisms in the realm of agricultural biocontrol cannot be overstated. This paper delves into the intricate web of interactions between these beneficial microbes and plants, elucidating their roles in enhancing plant health, productivity, and resilience against pathogens.

Materials and Methods

Biochemical test

The chosen salt-tolerant isolates were submitted to biochemical characterisation using the methods described by Seeley *et al*., (1991) and Cappuccino and Sherman (1987), which are considered industry standards. The many biochemical tests that were run and the techniques that were employed are briefly described here.

In-dole test

Getting ready the Tryptone Broth and transferring a little amount of a brand-new, 18–24-hour bacterial culture. Place the inoculated Tryptone Broth in an incubator that has been set to a 35–37 °C temperature range. Give it a 24-hour period to incubate. Reagent of Kovac is added to the culture. Introduce a few drops (about 0.5 ml) of Kovac's reagent carefully down the inside wall of the test tube using a sterile pipette or dropper. Over the next 2 to 5 minutes, keep an eye on the tube for any colour changes. If in-dole is present in the culture, the alcohol layer of the medium will develop a unique pink to red colour (commonly referred to as cherry-red). This colour shift is advantageous. If the only noticeable colour change is a slight yellow hue, this is considered a negative result.

Oxidase test

The trypticase soy agar plates were spotted with overnight-grown cultures of the test isolates, and the plates were incubated for 24 hours at 28 2 °C. Two to three drops of tetramethyl phenylenediamine dihydrochloride (TPD) were added to the test isolate's development after incubation. Blue coloration was seen as an indicator of an active oxidase.

Gelatin liquefaction

The test cultures were inoculated into the pre-sterilized nutritional gelatin deep tubes, and the tubes were cultured at 28 20C for 24 hours. The tubes were then put in a freezer at -40C for 30 minutes. For the test, the culture-containing tubes that didn't solidify after being refrigerated were considered positive, while those that did were considered negative.

Starch hydrolysis

We looked studied the isolates' capacity to hydrolyze starch. The test culture was added to three identical starch agar plates, which were then incubated for three days at 30 °C. Following incubation, the plates were flooded with Lugol's iodine solution and left to stand for 15 to 30 minutes. The colonies were then checked for a clear zone that would indicate starch hydrolysis.

Methyl Red – *Voges Proskauer* (MR-VP)

Test tubes containing 5 ml of MR-VP media were inoculated with the overnight cultures and incubated for 48 hours at 370C. After that, around one-fourth of the cultures that had been incubating were decanted into a clean test tube and 0.5 ml (8–10 drops) of the alpha napthanol solution (5% solution in alcohol) was added. Following the addition of decanted cultures, 0.5 ml of 40% KOH solution containing 0.3% creatine was added, vigorously shaken, and left to stand for 5–30 minutes. The presence of acetylmethylcarbinol was indicated by a pink to red tint, which was reported as a positive VP test.

Methyl red test

By incorporating a few drops of an alcoholic solution of methyl red, the test culture was examined for acidity. A prominent red colour was regarded a positive test result, whereas the formation of a yellow tint was viewed as a negative test result.

Hydrogen sulfide production

The semisolid SIM agar tubes holding the bacterial isolates were punctured. The tubes were incubated at 50 °C for 48 hours. Following incubation, the stab line developing a black colour was identified as a sign that H2S was being produced. The control was an uninoculated semisolid SIM agar tube.

Urease Activity

Test tubes containing sterilised urea broth and the overnight cultures were inoculated, and the test tubes were then incubated at 50 2 °C for 48 hours. Pink colour fading was considered a positive test result, and if the colour remained unchanged, the test was deemed negative. As a control, pre-sterilized urea broth was not infected.

Citrate utilization

The overnight cultures were added to test tubes with Simmons citrate agar slants and cultured for 18 to 24 hours at 350° to 370 °C. Because they develop slowly on citrate medium, certain organisms may need up to 7 days of incubation. For a positive test, the growth will be evident on the slant surface and the medium will be a deep Prussian blue. For a negative test, there will be no growth apparent, no colour change, or only a small amount of growth.

Biocontrol activity

The bioconrol activity of various bacterial strains, including *Pseudomonas furukawaii* STT-A8, *Achromobacter* sp. STT-A12, *Agrobacterium pusense* STT-A24, *Agrobacterium pusense* STT-A39, *Brevibacterium epidermidis* STT-K2, *Priestia flexa* STT-K13, *Pusillimonas* sp. STT-K15, *Bacillus* sp. STT-K24, *Bacillus badius* STT-N22, *Bacillus badius* STT-N26, *Brevibacterium epidermidis* STT-N28, and *Bacillus badius* STT-N33, was assessed using a dual culture plate technique (Jasim *et al*., 2016) [13]. The experiment involved the following steps:

1. **Preparation of Potato Dextrose Agar (PDA) Plates:** 30 mL of potato dextrose agar (PDA) was sterilized by autoclaving at 15 psi for 15-20 minutes. The sterilized PDA was then poured into Petri plates and allowed to solidify.
2. **Inoculation of Fungal Pathogens:** Small amounts of fungal isolates, specifically *Fusarium oxysporum* was placed at the center of the Petri plates using a sterile loop. These fungal cultures served as controls.
3. **Inoculation of Bacterial Isolates:** A 5 mm diameter mycelial disc of the respective fungal isolates was cut out using a sterile cork borer and placed at the center of the Petri plates. The bacterial isolates (*Pseudomonas furukawaii* STT-A8, *Achromobacter* sp. STT-A12, *Agrobacterium pusense* STT-A24, *Agrobacterium pusense* STT-A39, *Brevibacterium epidermidis* STT-K2, *Priestia flexa* STT-K13, *Pusillimonas* sp. STT-K15, *Bacillus* sp. STT-K24, *Bacillus badius* STT-N22, *Bacillus badius* STT-N26, *Brevibacterium epidermidis* STT-N28, and *Bacillus badius* STT-N33) were streaked 2.2 cm away from the fungal pathogens in a circular fashion using sterile toothpicks.
4. **Control Plates:** Plates without bacterial streaks were used as controls to observe the growth of fungal pathogens in the absence of bacterial intervention.
5. **Incubation:** All the Petri plates, including those with bacterial streaks and control plates, were incubated in a BOD Incubator at a temperature of 30 °C. Observations were made at 24-hour intervals.
6. **Calculation of Percentage Inhibition:** After 4 days of incubation, the percentage inhibition of mycelial growth was calculated using the following formula:

$$I = 100 \times (C - T) / C$$

Where,

I = Percentage inhibition of mycelial growth

C = Growth of the fungal pathogen (in millimeters) in the control plate

T = Growth of the fungal pathogen (in millimeters) in dual cultures under the tested conditions.

Results and Discussion

Table 1: Biochemical characteristics of salt and temperatuire tolerant Rhizobacteria.

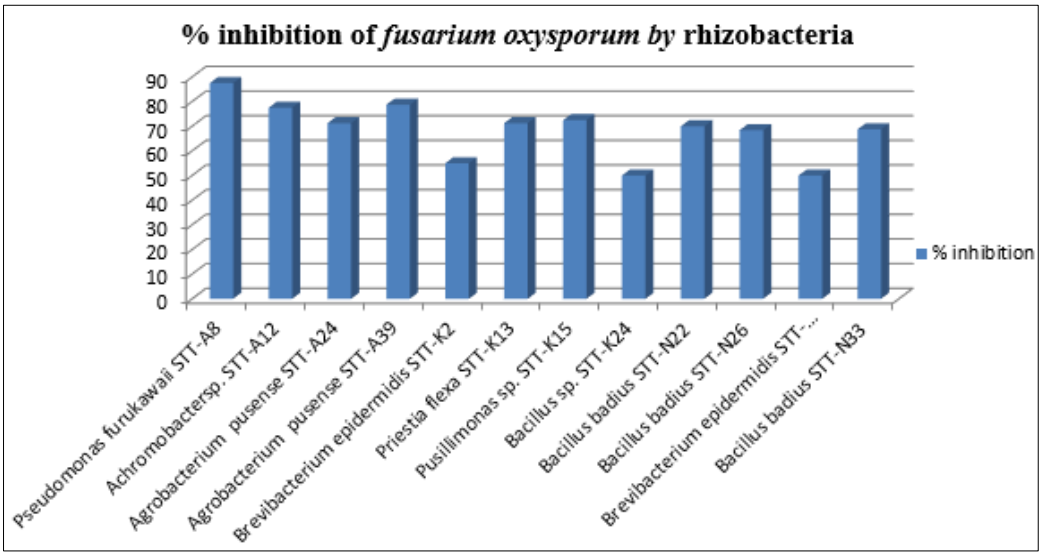
Sr. No.	Rhizobacteria	Characteristics								
		Indole	Urease	Oxidase	Hydrolysis of Starch	Gelatin Liquefication	Methyl red	Voges-proskaur	Production of H_2S	Citrate Utilization
1	*Pseudomonas furukawaii* STT-A8	-	-	+	-	+	-	-	-	+
2	*Achromobacter sp.* STT-A12	-	-	+	-	-	-	-	-	+
3	*Agrobacterium pusense* STT-A24.	+	+	-	+	-	+	-	-	+
4	*Agrobacterium pusense* STT-A39	+	+	-	+	-	+	-	-	+
5	*Brevibacterium epidermidis* STT-K2	-	-	-	-	+	-	-	-	+
6	*Priestia flexa* STT-K13	-	+	+	+	+	-	-	-	+
7	*Pusillimonas sp.* STT-K15	-	-	+	-	-	-	+	-	+
8	*Bacillus sp.* STT-K24	-	+	+	+	+	-	-	-	+

9	*Bacillus badius* STT-N22	-	-	-	-	+	-	-	-	-
10	*Bacillus badius* STT-N26	-	-	-	-	+	-	-	-	-
11	*Brevibacterium epidermidis* STT-N28	-	-	-	-	+	-	-	-	+
12	*Bacillus badius* STT-N33	-	-	-	-	+	-	-	-	-

The results of this study revealed that *Pseudomonas furukawaii* STT-A8 was shown positive test for Oxidase, Gelatin liquefaction and Citrate utilization test and negative for Indole, Urease, Starch hydrolysis, Methyl red, Voges-proskaur and H_2S production. *Achromobacter* sp. STT-A12 was shown positive test for Oxidase and Citrate utilization test and negative for Indole, Urease, Starch hydrolysis, Gelatin liquefaction, Methyl red, Voges-proskaur and H_2S production. *Agrobacterium pusense* STT-A24 and STT-A39 ware shown positive test for Indole, Urease, Starch hydrolysis, Methyl red, Citrate utilization test and negative for Oxidase, Gelatin liquefaction, Voges-proskaur and H_2S production. *Brevibacterium epidermidis* STT-K2 and STT-N28 ware shown positive test for Gelatin liquefaction and Citrate utilization test and negative for Oxidase, Indole, Urease, Starch hydrolysis, Methyl red, Voges-proskaur and H_2S production. *Priestia flexa* STT-K13 and *Bacillus* sp. STT-K24 ware shown positive test for Urease, Oxidase, Starch hydrolysis, Gelatin liquefaction and Citrate utilization test and negative for Indole, Methyl red, Voges-proskaur and H_2S production. *Pusillimonas* sp. STT-K15 was shown positive test for Oxidase, Voges-proskaur, Citrate utilization test and negative for Indole, Urease, Starch hydrolysis, Gelatin liquefaction, Methyl red, and H_2S production. Jianyang *et al.*, (2020) [14] reported similar results for *pusillimonas* Oxidase, Voges-proskaur and Citrate utilization test positive. *Bacillus badius* STT-N22, STT-N26 and STT-N33 ware shown positive test for Gelatin liquefaction and negative for Citrate utilization test Oxidase, Indole, Urease, Starch hydrolysis, Methyl red, Voges-proskaur and H_2S production. Dye, (1979) [10] have given similar biochemical characteristics with the help of which one can differentiate Rhizobium and Agrobacterium from each other. *Agrobacterium pusense* was Gram-negative, rod-shaped bacteria and it tested positive for catalase, methyl red, indole, and motility assays reported by Kaur *et al*., (2022) [18].

Table 2: Assessment of salt and temperature tolerant Rhizobacteria as biocontrol agent

Treatment	*Fusarium oxysporum*	
	Diameter±SE (cm)	% inhibition
Pseudomonas furukawaii STT-A8	1±0.03	87.5
Achromobacter sp. STT-A12	1.8±0.12	77.5
Agrobacterium pusense STT-A24	2.3±0.06	71.25
Agrobacterium pusense STT-A39	1.7±0.06	78.75
Brevibacterium epidermidis STT-K2	3.6±0.06	55
Priestia flexa STT-K13	2.3±0.06	71.25
Pusillimonas sp. STT-K15	2.2±0.06	72.5
Bacillus sp. STT-K24	4±0.14	50
Bacillus badius STT-N22	2.4±0.06	70
Bacillus badius STT-N26	2.5±0.06	68.42
Brevibacterium epidermidis STT-N28	4±0.12	50
Bacillus badius STT-N33	2.5±0.06	68.75
control	8±0.06	0
C.D.	0.23	1.478
S.E(m)	0.08	0.506
S.E(d)	0.11	0.715
C.V.	4.56	1.387



Graph 1: Showing % inhibition of *fusarium oxysporum* by different salt and temperature tolerant rhizobacterial isolates.

Out of total 12 extremely salt and temperature tolerant Rhizobacteria, the highest inhibition percentage showed (Table 2, Graph 1, Figure 1 and 2) by *Pseudomonas furukawaii* STT-A8 (87.6%), *Agrobacterium pusense* STT-A39(78.75%), *Achromobacter* sp. STT-A12(77.5%), *Pusillimonas* sp. STT-K15(72.5%) and *Agrobacterium pusense* STT-A24 (71.25%), *Priestia flexa* STT-K13(71.25%), *Bacillus badius* STT-N22(70%), *Bacillus badius* STT-N33(68.75%), *Bacillus badius* STT-N26(68.42%) followed by *Brevibacterium epidermidis* STT-K2(55%), *Brevibacterium epidermidis* STT-N28(50%), *Bacillus* sp. STT-K24(50%) against *Fusarium oxysporum*. It might be due to completely or partially destroyed by enzymes during Endolysis and exolysis lysis produced by rhizobacteria. In endolysis, destruction of a cell's cytoplasm by its own enzymes after death, which can be brought on by food deprivation, antibiosis, or other toxins and in exolysis breakdown of a cell by the enzymes of a different organism like chitinases, cellulases, etc., and it frequently culminates in the death of the cell that was attacked. Similar results reported in both leguminous and non-leguminous crops, rhizobacteria inhibits the growth of pathogenic fungi such *F. solani* and *M. phaseolina* (Omar and Abd-Alla 1998) [24]. Sorghum seeds were treated with biocontrol agents to control the *Fusarium moniliforme* infection reported by Raju *et al*., (1999) [28]. The sorghum seeds had varying degrees of *Fusarium moniliforme* infection. Pure cultures of *Pseudomonas fluorescens*, *Trichoderma harzianum*, and *Chaetomium globosum* at the rate of 1 $\times 10^8$ cfu g^{-1} were employed, as well as talc-based formulations of 28 $\times 10^7$ cfu g^{-1}, 19 $\times 10^7$ cfu g^{-1}, and 4 $\times 10^6$ cfu g^{-1} at the rate of 6 g kg and 10 g kg of seeds, respectively. Rhizobacteria considerably improve seed germination, plant growth, and yields by reducing soil-borne infections, according to a number of earlier researches (Sheikh *et al*., 2006; Mazen *et al*., 2008) [31, 20]. The current discovery is consistent with the study conducted by Al-Ani *et al*. (2012) [3], which sought to safeguard soybean plants from soil-borne diseases (*F. solani* and *M. phaseolina*). By using the agar well diffusion method, it was discovered that the two pathogenic fungi had reductions in radial growth of 33.84, 46.46, 59.27, and 65.58 percent and 39.61, 47.12, 57.06, and 64.04 percent, respectively. On the other hand, the reduction in fungal growth by the filtrate was discovered to be 54.11, 63.33, 67.11, and 72.66 percent for *F. solani.* Safin *et al*., (2020) [29] found similar results for Endophytic bacteria (*Bacillus subtilis*) and rhizospheric bacteria (*Pseudomonas fluorescens*) were used as bioagents of biofungicides. Djaenuddin *et al*., (2020) [9] found similar results against Maydis Leaf Blight by application of *Bacillus subtilis* TM4 formulation. Meena *et al*., (2022) [21] showed the similar type of biocontrol activity results of *Bacillus cereus*, *Bacillus anthracis*, *Bacillus velezensis* and *Serratia marcescens* against *Colletotrichum falcatum, Fusarium oxysporum f sp. ciceri, Helminthosporium maydis, F. oxysporum f. sp. lycopersici, Aspergillus niger, Mucor sp., Helminthosporium oryzae and Rhizoctonia solani* by using dual culture method and reported that *Bacillus velezensis* inhibites *F. oxysporium* f sp. ciceri 88.15%. *Trichoderma harzianum* showed a best biocontrol agent against *Fusarium wilt* of Chickpea reported by Abdulle *et al*., (2022) [1].

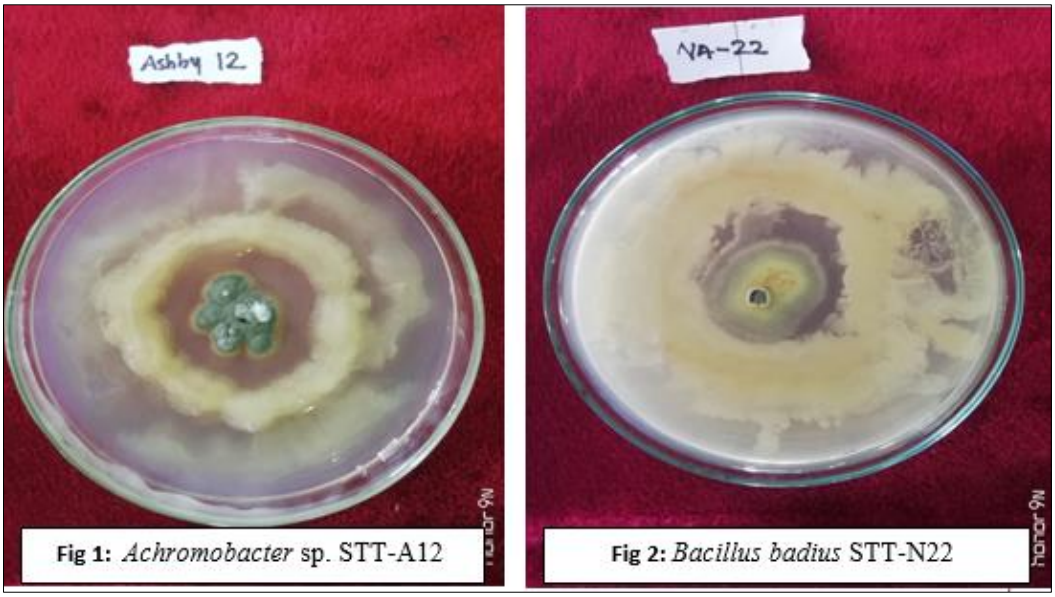


Fig 1-2: Inhibition of *fusarium oxysporium* by *Achromobacter* sp. STT-A12 and *Bacillus badius* STT-N22 respectively.

Conclusion

Among the 12 extremely salt and temperature-tolerant rhizobacteria isolates, the highest inhibitory effects against *Fusarium oxysporum* were observed in *Pseudomonas furukawaii* STT-A8 (87.6%), *Agrobacterium pusense* STT-A39 (78.75%), *Achromobacter* sp. STT-A12 (77.5%), *Pusillimonas* sp. STT-K15 (72.5%), *Agrobacterium pusense* STT-A24 (71.25%), *Priestia flexa* STT-K13 (71.25%), *Bacillus badius* STT-N22 (70%), *Bacillus badius* STT-N33 (68.75%), and *Bacillus badius* STT-N26 (68.42%). *Brevibacterium epidermidis* STT-K2 (55%), *Brevibacterium epidermidis* STT-N28 (50%), and *Bacillus* sp. STT-K24 (50%) also exhibited significant inhibition against *Fusarium oxysporum*. Among the 12 extremely salt and temperature-tolerant rhizobacteria isolates Nine (*Pseudomonas furukawaii* STT-A8, *Agrobacterium pusense* STT-A39, *Achromobacter* sp. STT-A12, *Pusillimonas* sp. STT-K15, *Agrobacterium pusense* STT-A24, *Priestia flexa* STT-K13, *Bacillus badius* STT-N22, *Bacillus badius* STT-N33, and *Bacillus badius* STT-N26 can be used as a best biocontrol agent against

Fusarium oxysporum.

References

1. Abdulle YA, Osman AA, Awale MA, Heile AO, Bilal M, Subhani MN. Efficacy of Biocontrol Agents, Plant Extracts and Fungicides on *Fusarium Oxysporum* f. sp. Ciceris. International Journal of Plant, Animal and Environmental Sciences. 2022;12(1):34-43.
2. Adesemoye AO, Kloepper JW. Plant–microbes interactions in enhanced fertilizer-use efficiency. Applied microbiology and biotechnology. 2009;85:1-12.
3. Al-Ani RA, Adhab MA, Mahdi MH, Abood HM. Rhizobium japonicum as a biocontrol agent of soybean root rot disease caused by *Fusarium solani* and *Macrophomina phaseolina*. Plant Protection Science. 2012;48(4):149-155.
4. Berendsen RL, Pieterse CM, Bakker PA. The rhizosphere microbiome and plant health. Trends in Plant Science. 2012;17(8):478-486.
5. Bora PSL, Baruah KPA, Talukdar RBK, Kataky L, Phukan A. Review on bacterial blight of rice caused by *Xanthomonas oryzae* pv. oryzae: different management approaches and role of *Pseudomonas fluorescens* as a potential biocontrol agent. International Journal of Current Microbiology and Applied Sciences. 2017;6(3):982-1005.
6. Cabrefiga J, Bonaterra A, Montesinos E. Mechanisms of antagonism of *Pseudomonas fluorescens* EPS62e against *Erwinia amylovora*, the causal agent of fire blight. International Microbiology. 2007;10(2):123.
7. Cappucino JG, Sherman N. Microbiology: a Laboratory Manual. The Benyamin/Cummings Publ. Co. Inc., New York; c1992. p. 125-179.
8. Chowdhury SP, Dietel K, Rändler M, Schmid M, Junge H, Borriss R, *et al*. Effects of *Bacillus amyloliquefaciens* FZB42 on Lettuce Growth and Health under Pathogen Pressure and Its Impact on the Rhizosphere Bacterial Community. Plos. One. 2013;8(7):e68818.
9. Djaenuddin N, Muis A. Effectiveness of *Bacillus subtilis* TM4 biopesticide formulation as biocontrol agent against maydis leaf blight disease on corn. In IOP Conference Series: Earth and Environmental Science. 2020;484(1):012096.
10. Dye M. Function and maintenance of a *Rhizobium* collection. In N.S. Subba Rao (Ed.), Recent Advances in Biological Nitrogen Fixation, Oxford and IBH Publ. Co. New Delhi; c1979. p. 435-447.
11. Ghorbanpour M, Omidvari M, Abbaszadeh-Dahaji P, Omidvar R, Kariman K. Mechanisms underlying the protective effects of beneficial fungi against plant diseases. Biological Control. 2018;117:147-157.
12. Han QQ, Lü XP, Bai JP, Qiao Y, Paré PW, Wang SM, *et al*. Beneficial soil bacterium *Bacillus subtilis* (GB03) augments salt tolerance of white clover. Frontiers in Plant Science. 2014;5:525.
13. Jasim B, Sreelakshmi KS, Mathew J, Radhakrishnan EK. Surfactin, iturin, and fengycin biosynthesis by endophytic *Bacillus* sp. from *Bacopa monnieri*. Microbial Ecology. 2016;72(1):106-11.
14. Jianyang LI, Mingming QI, Qiliang Lai, Chunming Dong, Xiupian Liu, *et al*. *Pusillimonas maritima* sp. nov., isolated from surface seawater. International Journal of Systematic and Evolutionary Microbiology. 2020;70(5):3483-3490.
15. Johnsson L, Hökeberg M, Gerhardson B. Performance of the *Pseudomonas chlororaphis* biocontrol agent MA 342 against cereal seed-borne diseases in field experiments. European Journal of Plant Pathology. 1998;104:701-711.
16. Kloepper JW, Ryu CM, Zhang S. Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology. 2004;94(11):1259-1266.
17. Köhl J, Kolnaar R, Ravensberg WJ. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Frontiers in Plant Science; c2019. p. 845.
18. Kaur J, Mudgal G, Chand K, Singh GB, Perveen K, Bukhari NA, *et al*. An exopolysaccharide-producing novel *Agrobacterium pusense* strain JAS1 isolated from snake plant enhances plant growth and soil water retention. Scientific Reports. 2022;12(1):21330.
19. Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology. 2009;63:541-556.
20. Mazen MM, El-Batanony NH, Abd El-Monium MM, Massoud ON. Cultural filtrate of Rhizobium spp. and arbuscular mycorrhiza are potential biological control agents against root rot fungal diseases of faba bean. Global Journal of Biotechnology & Biochemistry. 2008;3(1):32-41.
21. Meena KR, Satyam S, Singh A, Jaiswal A, Rai D. Benchmarking of different microbes for their biosurfactants antifungal action against plant pathogens Indian Journal of Experimental Biology (IJEB). 2022;60(12):931-938.
22. Montesinos E, Bonaterra A. Microbial Pesticides. In Encyclopedia of Microbiology, 3rd ed., Schaechter, M., Ed., Elsevier: Amsterdam, The Netherlands; c2009. p. 110-120.
23. Ngugi HK, Dedej S, Delaplane KS, Savelle AT, Scherm H. Effect of flower-applied Serenade biofungicide (*Bacillus subtilis*) on pollination-related variables in rabbiteye blueberry. Biological Control. 2005;33(1):32-38.
24. Omar SA, Abd-Alla MH. Biocontrol of fungal root rot diseases of crop plants by the use of rhizobia and bradyrhizobia. Folia Microbiologica. 1998;43:431-437.
25. Parani K, Saha BK. Prospects of using phosphate solubilizing Pseudomonas as bio fertilizer. European Journal of Biological Sciences. 2012;4(2):40-44.
26. Paterson J, Jahanshah G, Li Y, Wang Q, Mehnaz S, Gross H. The contribution of genome mining strategies to the understanding of active principles of PGPR strains. FEMS Microbiology Ecology. 2017;93(3):249.
27. Puopolo G, Raio A, Pierson LS, Zoina A. Selection of a new *Pseudomonas chlororaphis* strain for the biological control of *Fusarium oxysporum* f. sp. radicis-lycopersici. Phytopathologia Mediterranea. 2011;50(2):228-235.
28. Raju NS, Niranjana SR, Janardhana GR, Prakash HS, Shetty HS, Mathur SB. Improvement of seed quality and field emergence of *Fusarium moniliforme* infected sorghum seeds using biological agents. Journal of the Science of Food and Agriculture. 1999;79(2):206-212.
29. Safin R, Karimova L, Nizhegorodtseva L, Stepankova D, Shaimullina G, Nazarov R. Effect of various biological control agents (BCAs) on drought resistance and spring barley productivity. In BIO Web of Conferences. EDP

Sciences. 2020;17:00063.
30. Seeley HW, Van Demark PJ, Lee JJ. Microbes In Action, a Laboratory Manual of Microbiology. W.H. Freeman and Company Publishing Co., New York; c1991. p. 1-450.
31. Sheikh LI, Dawar SHAHNAZ, Zaki MJ, Ghaffar A. Efficacy of Bacillus thuringiensis and Rhizobium meliloti with nursery fertilizers in the control of root infecting fungi on mungbean and okra plants. Pakistan Journal of Botany. 2006;38(2):465.
32. Spadaro D, Droby S. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. Trends in Food Science & Technology. 2016;47:39-49.