



Isolation, selection and characterisation of salt and temperature tolerant plant growth promoting rhizobacteria in tomato (*Solanum lycopersicum*) under saline environments

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ABSTRACT

The present study was focused on isolation, selection and characterisation of salt- and temperature-tolerant plant growth promoting rhizobacteria (PGPR) from rhizosphere soil of tomato (*Solanum lycopersicum* L.). A total of 46 isolates were developed during 2020–2023 at Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra on Ashby's Mannitol agar media. The medium was supplied with various NaCl concentrations, MgCl₂ concentrations, and pH levels to examine the tolerance to salt and temperature. All the 46 isolates showed promising salt tolerance. Out of these isolates, nine isolates were extremely salt tolerant, two isolates were tolerant, nine isolates were moderately tolerant while 26 isolates were moderately sensitive to NaCl concentration. On the basis of the salt tolerance limit, rhizobacteria that promote plant growth in salt- and temperature-tolerant environments were categorised. Out of 46 isolates, eight isolates showed growth at MgCl₂ concentration of 4.0%, two isolates at 3.0%, 13 isolates at 2.0% MgCl₂, 15 isolates at 1.0% and eight isolates at 0.5% MgCl₂. In case of pH, eight showed growth at pH of 9.0, whereas the other 38 showed development at pH of 7.0. The plant growth promoting activity check was positive for four isolates (*Pseudomonas furukawaii* STT-A8, *Achromobacter* spp. STT-A12, *Agrobacterium pusense* STT-A24, and *A. pusense* STT-A39). *P. furukawaii* STT-A8 and *Achromobacter* spp. STT-A12 showed temperature tolerance up to 60°C while *A. pusense* STT-A24 and *A. pusense* STT-A39 showed temperature tolerance up to 50°C. The experiment was done to characterise salt- and temperature-tolerant PGPR that would be a crucial inoculum to enhance tomato plant growth and development in saline environment.

Keywords: Nutrient management, NaCl concentration, PGPR, Plant growth

In arid and semiarid regions, salinity presents a significant agricultural challenge (Rao and Sharma 1995), affecting approximately 40% of the Earth's land. Egamberdiyeva *et al.* (2007) highlighted the detrimental impact of soil salinity on irrigated soils, which can lead to reduced soil and water quality, hinder crop growth, and even force the abandonment of agricultural lands. In these challenging conditions, plant growth-promoting rhizobacteria (PGPR), which are large active bacterial groups, find support in the rhizosphere of soil surrounding plant roots and the soil that the roots inhabit. Rhizobacteria that promote plant growth have been the subject of study by Rangarajan *et al.* (2003). They are known to rapidly colonise the rhizosphere and reduce soil-borne diseases at the root surface. This ability to enhance plant growth is advantageous to the plant itself (Bloembergen and Lugtenberg 2001). The direct application

of microorganisms for improving plant development and preventing pests is a rapidly advancing field.

Among these beneficial microorganisms, *Pseudomonas* act as the most promising class used in both plant growth promotion and biocontrol of plant diseases. These bacteria produce metabolites such as phytohormones, siderophores, volatile hydrogen cyanide, antibiotics, organic acid and also atmospheric nitrogen fixation, phosphate solubilisation, and other unidentified mechanisms, attributed to their beneficial effects (Glick and Ibdid 1995). *Pseudomonas* spp. is often cited as prime examples of root-colonising bacteria (Lugtenberg *et al.* 2001). Enhancing the success rate of field applications relies on a better understanding of the microbial interactions responsible for PGPR (Farzana *et al.* 2009). Notably, PGPR can influence plant growth through various direct and indirect mechanisms. For example, Siddikee *et al.* (2010) found 14 halotolerant bacterial strains increased root length and dry weight in canola plants under salt stress. Three of these bacteria, *Brevibacterium epidermidis* RS15, *Micrococcus yunnanensis* RS222, and *Bacillus aryabhatai* RS341 demonstrated over 40% increases in root elongation

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and dry weight compared to uninoculated salt-stressed canola seedlings. In a separate study by Kaur *et al.* (2022), the inoculation of tomato (*Solanum lycopersicum* L.) plants with *Achromobacter* spp. EMCC1936 significantly boosted vegetative growth and yield, while *Agrobacterium pusense* was found to be a qualified plant growth-promoting bacterium capable of producing indole-3-acetic acid, gibberellic acid, and ammonia.

Environmental stressors, such as salinity, water stress, soil pH, temperature, and heavy metals, can impact nitrogen fixation in plants (Singleton *et al.* 1982, Kucuk and Kivanc 2008). Therefore to understand the response of PGPR under conditions like saline environment, the research experiment was conducted to isolate, select and characterise salt and temperature tolerant plant growth promoting rhizobacteria from rhizosphere soil of tomato.

MATERIALS AND METHODS

Collection of rhizosphere soil samples of tomato:

Forty six rhizosphere soil samples were collected from saline tract of four districts of western Maharashtra, viz. Ahmednagar, Nashik, Pune and Satara to isolate salt and temperature tolerant rhizobacteria. From each site, soil samples were randomly collected by dividing the field into different homogeneous units based on visual observation. At the sampling location, the surface trash was cleared away. The auger was driven to a plough depth of 15 cm, and the soil sample was drawn. Ten samples were collected from each field and placed in a polythene bag. The soil samples were thoroughly mixed, and roots, stones, pebbles, and debris were removed. The soil samples were air-dried by spreading them on a clean surface and allowed to dry naturally, avoiding direct sunlight and excessive heat. The bulk of the samples were reduced to 1 kg by thoroughly mixing the sample into four equal parts. Two opposite quarters were discarded, and the remaining two quarters were remixed. The method was carried out several times to get the required sample size. The final sample was collected in a clean cloth or polythene bag. The bag was labeled with relevant information such as the name of the farmer, location of the farm, survey number, previous crop grown, present crop, crop to be grown in the next season, date of collection, and the name of the sampler.

Serial dilution: Rhizosphere soil samples were sent straight to the Plant Pathology and Agricultural Microbiology laboratory, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (19.34°N, 74.64°E), Maharashtra in sterile plastic bags. There, 1 g of each soil sample was dissolved in 9 mL of sterile deionised water, and the samples were then serially diluted. A total of 100 μ L of the stock solution and 900 μ L of sterile distilled water were mixed to make a 10^{-1} dilution. The procedure was iterated until a dilution of 10^{-6} was obtained. Each dilution was then plated onto Ashby's Mannitol agar using a 100 μ L sample. The initial media's pH was adjusted to 7 prior to autoclaving. After incubation for two days at $28 \pm 2^\circ\text{C}$, the plates were examined to verify the colony's growth. A subset of isolated rhizobacteria were

cleaned, preserved, and chosen for further studies.

Assessment of salt and temperature tolerant rhizobacteria at different NaCl level: In order to verify the ability of rhizobacteria isolates to withstand salt and temperature, the isolates underwent testing using varying concentrations of NaCl salt and were categorised using the Cardoso *et al.* (2015) salt tolerance method. In Ashby's Mannitol Agar medium, 0.15, 0.3, 0.6, 0.9, 3.0, and 6.0% NaCl were added as a supplement. The rhizobacterial isolates were streaked onto Ashby's Mannitol agar medium plates that had higher than normal NaCl concentrations. The plates were then incubated at 28°C for 2–7 days. Following the incubation period, the growth of temperature- and salt-tolerant rhizobacterial isolates was detected. The isolates were graded as follows: +++ for full growth, ++ for little growth, + for growth, and - for no growth. For additional research, the rhizobacterial isolates with complete development were chosen.

Assessment of salt and temperature tolerant rhizobacteria on different MgCl_2 concentration: The ability of rhizobacterial isolates to withstand salt and temperature was also verified by testing them against varying concentrations of MgCl_2 salt. Ashby's Mannitol Agar Medium was used for this, with additions of 0.5, 1.0, 2.0, 3.0, and 4.0% MgCl_2 . Similar incubation and grading method were used as in case of NaCl.

Assessment of salt and temperature tolerant rhizobacteria on different pH level: The rhizobacterial isolates that were shown to be resistant to salt and temperature were verified by subjecting them to several pH values in the medium. Using HCl and NaOH, the pH of Ashby's Mannitol Agar Medium was changed to 7.5, 8.00, 8.50, and 9.00. Similar incubation and grading method were used as in case of NaCl.

Assessment of salt and temperature tolerant rhizobacteria on different temperature range: To demonstrate the temperature tolerance, their ability to tolerate heat stress was also examined. In brief, 1 mL 24 h old bacterial culture was poured in 10 mL of six test tubes of Ashby's Mannitol broth medium out of which one kept as a control and remaining five tested for temperature tolerance by placing the test tube in water bath for 10 min at different temperature i.e. 45, 50, 55, 60 and 65°C , consequently place that test tube incubated in incubator at $28 \pm 2^\circ\text{C}$ for 48 h. As a result of that culture colour change observed at selective temperature, after that take 1 mL of each temperature test tube broth and grow on Ashby's Mannitol agar media and observe growth by spreading each strain on Ashby's Mannitol agar medium and then plates were incubated in incubator at $28 \pm 2^\circ\text{C}$ for 48 h.

RESULTS AND DISCUSSION

Effect of NaCl concentrations on rhizobacteria for salt and temperature tolerance: Total 46 salt- and temperature-tolerant rhizobacterial pure culture isolates were obtained. Among 46 isolates, nine isolates were extremely salt tolerant ($>5.4\%$ salt tolerance limit), two isolates were tolerant (2.10–3.6%), nine isolates were moderately tolerant

Table 1 Assessment of rhizobacteria for salt and temperature tolerance on different NaCl concentrations

Isolate no.	Salt tolerance limit [NaCl concentration (%)]										Class
	0.15	0.3	0.6	0.9	3.0	6.0	9.6	10.8	16.0	25.0	
STT-A1	+++	+++	++	++	++	--	--	--	--	--	Tolerant
STT-A2	++	+	--		--	--	--	--	--	--	Moderately sensitive
STT-A3	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A4	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A5	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A6	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A7	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A8	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant
STT-A9	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A10	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant
STT-A11	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A12	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant
STT-A13	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A14	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A15	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A16	+++	+++	++	--	--	--	--	--	--	--	Moderately sensitive
STT-A17	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant
STT-A18	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A19	++	+	--		--	--	--	--	--	--	Susceptible
STT-A20	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A21	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A22	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A23	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A24	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant
STT-A25	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A26	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A27	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant
STT-A28	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant
STT-A29	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A30	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A31	+++	+++	++	--	+	--	--	--	--	--	Tolerant
STT-A32	++	+	--		--	--	--	--	--	--	Moderately sensitive
STT-A33	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A34	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A35	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A36	+++	+++	++	++	++	--	--	--	--	--	Tolerant
STT-A37	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A38	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A39	+++	+++	+++	+++	+++	++	--	--	--	--	Extremelytolerant
STT-A40	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A41	+++	+++	++	+	--	--	--	--	--	--	Moderately tolerant
STT-A42	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A43	++	+	--	--	--	--	--	--	--	--	Moderately sensitive
STT-A44	++	+	--	--		--	--	--	--	--	Moderately sensitive
STT-A45	++	+	--	--		--	--	--	--	--	Moderately sensitive
STT-A46	+++	+++	+++	+++	+++	++	--	--	--	--	Extremely tolerant

+++ , Thick colony; ++, Flattened; +, Growth; -, No growth. Extremely sensitive tolerance limit <0.15% salt; Moderately sensitive, 0.15–0.60% salt; Moderately tolerant, 0.90–1.50% salt; Tolerant, 2.10–3.60% salt; Extremely tolerant, >5.40% salt (Cardoso *et al.* 2014).

Table 2 Assessment of rhizobacteria for salt and temperature tolerance on different $MgCl_2$ concentration and pH levels

Isolate no.	Tolerance level								
	$MgCl_2$ coccentration (%)					pH			
	0.5	1.0	2.0	3.0	4.0	7.0	8.0	8.5	9.0
STT-A1	++	++	++	++	--	++	--	--	--
STT-A2	++	++	-	-	--	++	--	--	--
STT-A3	++	++	-	-	--	++	--	--	--
STT-A4	++	++	--	--	--	++	--	--	--
STT-A5	++	++	--	--	--	++	--	--	--
STT-A6	++	++	--	--	--	++	--	--	--
STT-A7	++	++	--	--	--	++	--	--	--
STT-A8	++	++	++	++	++	++	++	++	++
STT-A9	++	++	--	--	--	++	--	--	--
STT-A10	++	++	++	++	++	++	++	++	++
STT-A11	++	++	--	--	--	++	--	--	--
STT-A12	++	++	++	++	++	++	++	++	++
STT-A13	++	++	--	--	--	++	--	--	--
STT-A14	++	++	--	--	--	++	--	--	--
STT-A15	++	++	--	--	--	++	--	--	--
STT-A16	++	++	--	--	--	++	--	--	--
STT-A17	++	++	++	++	++	++	++	++	++
STT-A18	++	++	--	--	--	++	--	--	--
STT-A19	++	++	--	--	--	++	--	--	--
STT-A20	++	++	--	--	--	++	--	--	--
STT-A21	++	++	++	--	--	++	--	--	--
STT-A22	++	++	++	--	--	++	--	--	--
STT-A23	++	++	++	--	--	++	--	--	--
STT-A24	++	++	++	++	++	++	++	++	++
STT-A25	++	++	++	--	--	++	--	--	--
STT-A26	++	++	++	--	--	++	--	--	--
STT-A27	++	++	++	++	++	++	++	++	++
STT-A28	++	++	++	++	++	++	++	++	++
STT-A29	++	++	++	--	--	++	--	--	--
STT-A30	++	++	++	--	--	++	--	--	--
STT-A31	++	++	++	--	--	++	--	--	--
STT-A32	++	++	++	--	--	++	--	--	--
STT-A33	++	++	++	--	--	++	--	--	--
STT-A34	++	++	++	--	--	++	--	--	--
STT-A35	++	++	++	--	--	++	--	--	--
STT-A36	++	++	++	--	--	++	--	--	--
STT-A37	++	--	--	--	--	++	--	--	--
STT-A38	++	--	--	--	--	++	--	--	--
STT-A39	++	++	++	++	++	++	++	++	++
STT-A40	++	--	--	--	--	++	--	--	--
STT-A41	++	--	--	--	--	++	--	--	--
STT-A42	++	--	--	--	--	++	--	--	--
STT-A43	++	--	--	--	--	++	--	--	--
STT-A44	++	--	--	--	--	++	--	--	--
STT-A45	++	--	--	--	--	++	--	--	--
STT-A46	++	++	++	++	--	++	--	--	--

+++, Thick colony; ++, Flattened; +, Growth; -, No growth.



Fig. 1 0.3% NaCl tolerance limit for salt and temperature tolerance (STT).

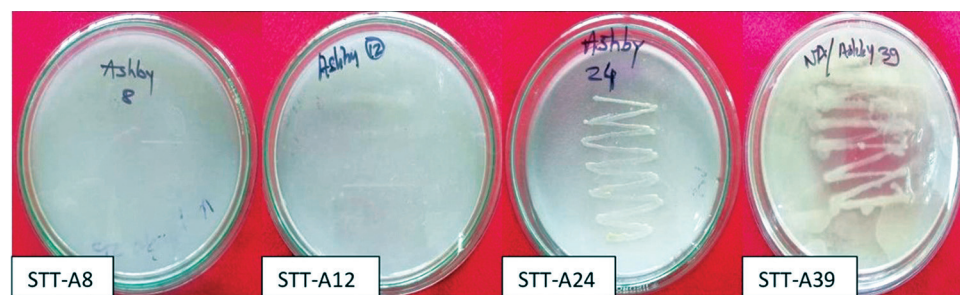


Fig. 2 0.6 NaCl tolerance limit of salt and temperature tolerance.



Fig. 3 6% NaCl tolerance limit of salt and temperature tolerance.

(0.09–1.50%) and 26 isolates were moderately sensitive to NaCl concentration (0.15–0.60%) (Table 1, Fig. 1, 2 and 3). Classification of salt and temperature tolerance of rhizobacteria was done on the basis of salt tolerance limit. Similar results were found in investigations of Kumar *et al.* (2014) where certain bacterial strains exhibited tolerance to high salinity (up to 7% NaCl). Furthermore, stress tolerance was comparatively lower in *Pseudomonas* species as opposed to *Bacillus* strains, largely due to the formation of endospores by the *Bacillus* isolates. In a related study by Patel *et al.* (2017), they found that out of the isolates tested, 29 (43.0%) were able to thrive in the presence of 15 g of NaCl/100 mL (w/v). The results are in agreement with observations of Damodarachari *et al.* (2018) who found that five isolates (BS 1, BS 3, BS 14, BS 18, BS 42)

demonstrated resilience to NaCl concentrations ranging from 1.5–20%. Similarly, Sharma *et al.* (2021) also yielded comparable results. In their study, isolates HB6P2 and HB6J2 demonstrated the highest level of salt tolerance, with a peak tolerance at 10% salt concentration. This was followed by isolates HB4A1, HB4N3, and HB8P1, which also exhibited notable tolerance to salts. Kumari *et al.* (2022) presented similar findings in their research. They identified isolates E-2, T-2, and T-1, displayed growth at salt (NaCl) concentrations of 7%, 6%, and 6%, respectively. Similar results were also found in investigations of several scientists (Rai and Prasad 1986, Zahran 1999, Kumari and Khanna 2015, Khan *et al.* 2019).

Effect of $MgCl_2$ and pH on rhizobacteria for salt and temperature tolerance: Among 46 isolates, eight rhizobacteria showed growth (Table 2) upto 4.0% $MgCl_2$ concentration. Seven isolates showed growth up to 3.0% while four isolates showed growth up to 2.0% $MgCl_2$ concentration. Seven isolates showed growth up to 1.0% and 20 showed growth up to 0.5% $MgCl_2$ concentration. The results are in agreement

with observations of Pagare *et al.* (2018) who reported that out of 33 isolates, 20 isolates showed growth at $MgCl_2$ concentration of 0.75%, four isolates showed growth at 0.50% and nine isolates showed growth at 0.40%.

Out of 46 salt and temperature tolerance rhizobacterial isolates (Table 2), 8 isolates, viz. STT-A8, STT-A10, STT-A12, STT-A17, STT-A24, STT-A27, STT-A28, and STT-A39 showed growth at pH up to 9.0 and remaining all isolates showed growth at pH 7.0. The results are in agreement with observations of Damodarachari *et al.* (2018) who found that out of 44 isolates, four isolates (BS 1, BS 3, BS 14, BS 18) were capable of growing across a pH range of 4–12. The growth of rhizobacteria on different pH has been reported by other scientists also (Tsegaye *et al.* 2019, Jianyang *et al.* 2020).

Table 3 Assessment of rhizobacteria for salt and temperature tolerance at different temperature ranges

Isolates	Temperature					Colour developing temperature
	Control	50°C	55°C	60°C	65°C	
STT-A8	+++	+++	+++	+++	---	Greenish-65°C
STT-A10	+++	+++	+++	+++	---	Colourless 65°C
STT-A12	+++	+++	+++	+++	---	Colourless 65°C
STT-A24	+++	+++	---	---	---	Light Yellow 55°C
STT-A27	+++	+++	---	---	---	Pale yellow 55°C
STT-A39	+++	+++	---	---	---	Pale yellow 55°C

Effect of temperature on rhizobacteria for salt and temperature tolerance: Out of the 46 isolates, six isolates selected for further studies related to temperature tolerance (Table 3). Out of six isolates, three isolates (STT-A8, STT-A10 and STT-A12) showed temperature tolerance up to 60°C while other three isolates (STT-A24, STT-A27 and STT-A39) showed temperature tolerance up to 50°C. Similar results were found in investigations of Kumar *et al.* (2014) whose research indicated that certain bacterial strains exhibited tolerance to high temperatures (up to 50°C). In a related study by Patel *et al.* (2017), 40 isolates (58.20%) displayed the capacity to tolerate high temperatures, withstanding up to 70°C. The results are in agreement with observations of Damodarachari *et al.* (2018) who noted that six isolates (BS 10, BS 14, BS 18, BS 27, BS 37, BS 43) exhibited tolerance to temperatures spanning from 20–50°C.

In the present study, isolation, selection and characterisation of salt and temperature tolerant rhizobacteria have been studied. Based on the results obtained from this study, four isolates STT-A8, STT-A12, STT-A24 and STT-A39 can tolerate concentrations of NaCl 6%, MgCl₂ 4% and pH 9.0. *Pseudomonas furukawaii* STT-A8 (NCBI Accession no-OR432529) and *Achromobacter* spp. STT-A12 (NCBI Accession no-OR432558) showed temperature tolerance limit at 60°C while *Agrobacterium pusense* STT-A24 (NCBI Accession no-OR428230) and *Agrobacterium pusense* STT-A39 (NCBI Accession no-OR428223) showed temperature tolerance limit at 50°C. Therefore, it can be deduced that above four plant growth promoting salt- and temperature-tolerant rhizobacteria may be able to survive in saline soil and could help in nutrient management to plants.

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