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In vitro Screening of Temperature stress tolerance of *Rhizobial* and *Pseudomonas fluorescence* isolates

K Manasa, R Subhash Reddy and S Triveni

Abstract
A major problem in rainfed agroecosystems is predominance of abiotic stresses like high temperature, salinity, and drought where the survival of bio inoculants is a problematic issue. The variations in results from laboratory to field are more compounded due to various abiotic stresses that prevail under field conditions for a microbial inoculant to establish and to show the desired effect. Such problems can be overcome by screening programme for efficient stress tolerant isolates. This study investigates the impact of temperature on *Rhizobial* and *Pseudomonas flourescence* isolates. Fifteen *Pseudomonas fluorescence* and 15 *Rhizobium* strains were isolated from rhizospheric soils of student farm and college farm, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana state with a view to screen out temperature tolerant isolates. Selected isolates were biochemically characterized also evaluated for their plant growth promoting traits and tested for their tolerance to different temperatures such as high temperature (28 ^{0}C, 37 ^{0}C and 45 ^{0}C), using respective broth under *in vitro* conditions. After screening for above conditions result showed that two *Rhizobial* isolates RR-1, GNR-1 and *Pseudomonas flourescence* isolate i.e GGP-1 isolate tolerant to all conditions of temperature. The data obtained in the present study suggest that RR-1, GNR-1 and GGP-1 would be ideal organisms for further study in pot culture and field experiments to exploit their PGPR potential for a good biofertilizers production.

Keywords: *Rhizobium*, *Pseudomonas flourescence*, high temperatures, stress tolerant

1. Introduction
A wide range of agriculturally important microorganisms (AIMs) have been exploited for crop health management, which comprise nitrogen fixers like *Rhizobium, Bradyrhizobium, Sinorhizobium, Azotobacter, Azospirillum,* phosphate solubilisers like *Bacillus, Pseudomonas, Aspergillus, and arbuscular mycorrhizae* (AM); and fungi, bacteria, viruses and nematodes used for pest and disease management in agriculture, horticulture, and forestry. Plant growth promoting rhizo microorganisms (PGPR) is known to increase plant growth and induce host plant resistance and crop yield [Glick, 1999] [3].

As the crops are affected by abiotic stresses such as soil moisture deficit stress, high temperature, soil salinity, and so forth, microbes are also known to be affected by these conditions. Reports from Madhya Pradesh and Chhattisgarh of India indicated that the free living rhizobial population declines to lower than the minimum threshold levels required for nodulation due to high soil temperatures requiring inoculation every year [Gupta, 2001 and Rao 2000] [4].

Bacillus, Pseudomonas, and other microorganisms have been extensively studied for their ability to solubilize nutrients, their biocontrol potential, and their plant growth promoting abilities in all crop production systems. However, successful deployment of these organisms in stressed ecosystems depends on their ability to withstand and proliferate under adverse environments such as high temperatures, salt stress, mineral deficiency, heavy metal toxicity, and so forth. Inconsistency and variability in yield responses have also been attributed to adverse conditions such as interaction with other rhizospheric organisms, physical and chemical conditions of the soil (e.g., low pH), poor ability of the PGPR strain to colonize the plant roots, and environmental factors including high mean temperatures and low rainfall during the growing season [Lucy, 2004] [6].

High soil temperature in tropical regions is one of the major constrains for biological nitrogen fixation in legume crops. Temperatures in these regions average above 40 ºC (Dudeja, 1989) may affect symbiotic relationships, nitrogen content and plant production. With temperature increases, plasmid deletions (Trevors, 1986) [12] and genomic rearrangements (Soberon-Chaves, 1986) [11] may occur, resulting in alterations or in loss of symbiotic properties. Consequently, this genetic instability is compromising these *Rhizobium* strains' use in commercial inoculum production.

Total or partial plasmid deletions, under high temperature conditions, have been occurring more frequently in sensitive strains (Zurkowski, 1982) [13].

A major problem in rainfed agroecosystems is predominance of abiotic stresses like high temperature, salinity, and drought where the survival of bio inoculants is a problematic issue. The variations in results from laboratory to field are more compounded due to various abiotic stresses that prevail under field conditions for a microbial inoculant to establish and to show the desired effect. Such problems can be overcome by screening programme for efficient stress tolerant PGPRs for effective deployment of these strains to draw one or more beneficial effects.

Hence, the present study was conducted to identify strains of *Pseudomonas* and *Rhizobium* collected for their ability to withstand adverse environments such as high temperature.

2. Materials and methods

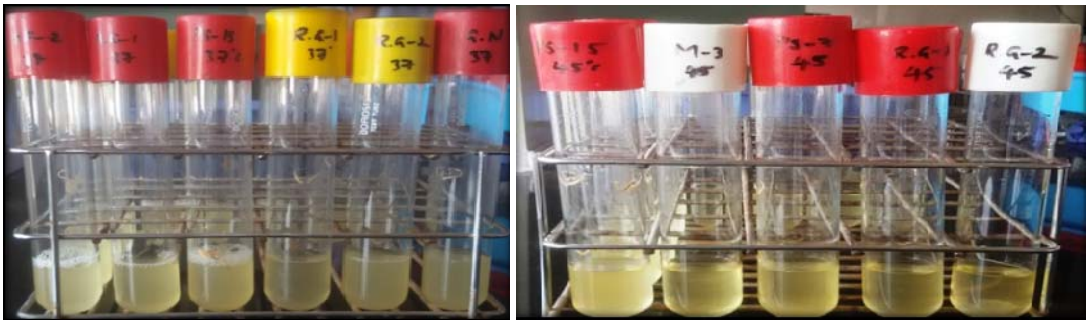
Fifteen *Pseudomonas fluorescence* and 15 *Rhizobium* isolates were isolated from different rhizospheric soils of Groundnut, Black gram, Green gram, Red gram, Soybean, Sunflower, Maize and Rice soils of student farm and college farm, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana state.

2.1 Screening of isolates for temperature stress tolerance

These 30 isolates were screened for their ability to tolerate different abiotic stresess such as high temperature (28 ^{0}C, 37 ^{0}C and 45 ^{0}C), using respective broth. YEMA broth used for *Rhizobial* isolates and Kings B broth used for *Pseudomonas flourescence.* Growth of all isolates was recorded using spectrophotometer at 600 nm with uninoculated medium as blank and O.D was recorded. (0.1 O.D contain 2x10^{5}cfu/ml)

2.2 High temperature tolerance

Ten ml of respective broth was dispensed into 30ml screw cap tubes and autoclaved. 0.1 ml of freshsly grown cultures of test strains were inoculated into broth. Inoculated tubes were incubated at different temperatures 28 ^{0}C, 37 ^{0}C and 45 ^{0}C for 24 h and O.D was recorded.



(a) Tolerance of *Rhizobium* to temperature **(b)** Tolerance of *Pseudomonas flourescence* to temperature

3. Results& Discussion

3.1 Effect of temperature on *Rhizobial* isolates

Temperature tolerance majority of the isolates exhibited luxuriant growth at the temperature ranging from 25-35°C. However, at 28 ^{0}C thirteen MR-1>GNR 2> GGR-1>GGR-2>MR-2>SYR-1>RGR-1>GNR-1>RR-2=MR-3>RR-1>MR-4isolates showed high growth while remaining two isolates showed scanty growth and at 37 ^{0}C only eleven isolates exhibited moderate growth. Further increase in temperature led to noticeable decline in growth at 45 ^{0}C, only six isolates demonstrated moderate growth. (Table. 3.1) (Fig. 3.1)

The optimum temperature for growth of root nodulating bacteria ranged from 25 °C -30 °C (Gaur, 1993; Harwani, 2006) [2, 5].

Meghvansi, 2006 [7] reported growth and survival of rhizobia in soils are adversely affected by high soil temperatures.

3.1 Tolerance of *Rhizobial* isolates to temperature

Isolates	Temp 28^0 C	Temp 37^0 C	Temp 45^0 C
RR-1	0.49	0.36	0.02
RR-2	0.50	0.38	0.03
MR-1	0.71	0.50	0.20
MR-2	0.65	0.45	0.15
MR-3	0.50	0.37	0.10
MR-4	0.43	0.30	0.04
BGR-1	0.37	0.23	0.02
GNR-1	0.58	0.42	0.11
GNR-2	0.68	0.46	0.19
GGR-1	0.67	0.45	0.18
GGR-2	0.66	0.46	0.17
SFR-1	0.38	0.24	0.03
SFR-2	0.23	0.15	0.02
RGR-1	0.60	0.41	0.12
SYR-1	0.64	0.43	0.14

Class interval OD at 28^0 C	frequency	% isolates
0.23-0.47	2	14
0.47-0.71	13	86
Class interval OD at 37^0 C	frequency	% isolates
0.15-0.33	4	26

0.33-0.50	11	74

Class interval OD at 45^0 C	frequency	% isolates
0.02-0.12	9	60
0.12-0.22	6	40

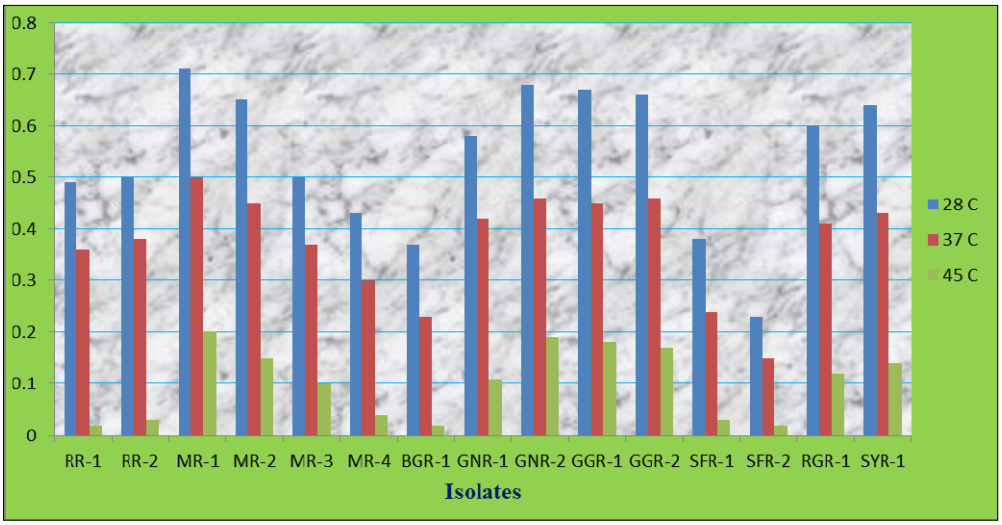


Fig 1: Temperature tolerance of *Rhizobial* isolates

Singh *et al*. (2011) [10] reported that effect of different physical parameters on the growth of *Rhizobium* was studied by keeping plates at different temperatures and preparing YEM medium of different pH. Differences in the range of growth temperatures were investigated by incubating bacterial cultures in YEM agar at 32, 34, 36, 38 and 40°C. Differences in pH tolerance were tested in YEM agar by adjusting the pH to 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. All the plates were incubated at 36°C for 72 h.

Rahmani *et al.* (2009) [8] conducted greenhouse experiment at 28 and 38ºC to study the nitrogen fixing capacity of the soybean isolates showed that ten isolates had a symbiotic index of 80% effectiveness or greater compared to nitrogen fertilized treatments at 28ºC. Some thermotolerant isolates showed good nitrogen fixing performance at 38ºC.

3.2 Effect of temperature on *Pseudomonas fluorescence* isolates

Out of 15 isolates eight isolates (53.00%) GGP-1> MP-1>RP-3>MP-3=RP-4> BGP-1 >SYP-1>RP-1 could tolerate to temperature 28 ^{0}C. Six isolates (40.00%) GGP-1> MP-1>RP-3>MP-3=RP-4> BGP-1 could be tolerant to temperature 37 ^{0}C. 6 (40.00%) isolates GGP-1> MP-1>RP-3> RP-4>MP-3>BGP-1 able to survive up to 45 ^{0}C. (Table. 3.2)(Fig 2)

Based on the Results optimum temperature for *Pseudomonas fluorescence* isolates is between 28-37 ^{0}C. 6 isolates in the order of GGP-1> MP-1>RP-3> RP-4>MP-3>BGP-1 tolerant to high temperature up to 45 ^{0}C

3.2. Tolerance of *Pseudomonas fluorescence* isolates to temperature

Isolates	Temp 28 ^{0}C	Temp 37 ^{0}C	Temp 45 ^{0}C
RGP-1	0.87	0.58	0.20
RGP-2	0.91	0.78	0.32
RP-1	0.95	0.75	0.56
RP-2	0.85	0.57	0.47
RP-3	1.02	0.88	0.49
RP-4	1.01	0.87	0.56
MP-1	1.03	0.89	0.49
MP-2	0.80	0.54	0.20
MP-3	1.01	0.87	0.48
SFP-1	0.79	0.56	0.45
SFP-2	0.86	0.57	0.46
GNP-1	0.83	0.53	0.36
GGP-1	1.12	0.90	0.50
BGP-1	0.98	0.79	0.42
SYP-1	0.95	0.75	0.38

Class interval OD at 28 ^{0} C	frequency	% isolates
0.787-0.954	7	47
0.954-1.121	8	53

Class interval OD at 37^0 C	frequency	% isolates
0.26-0.567	8	53
0.567-0.874	7	47

Class interval OD at 45^0 C	frequency	% isolates
0.15-0.355	8	60
0.355-0.56	6	40

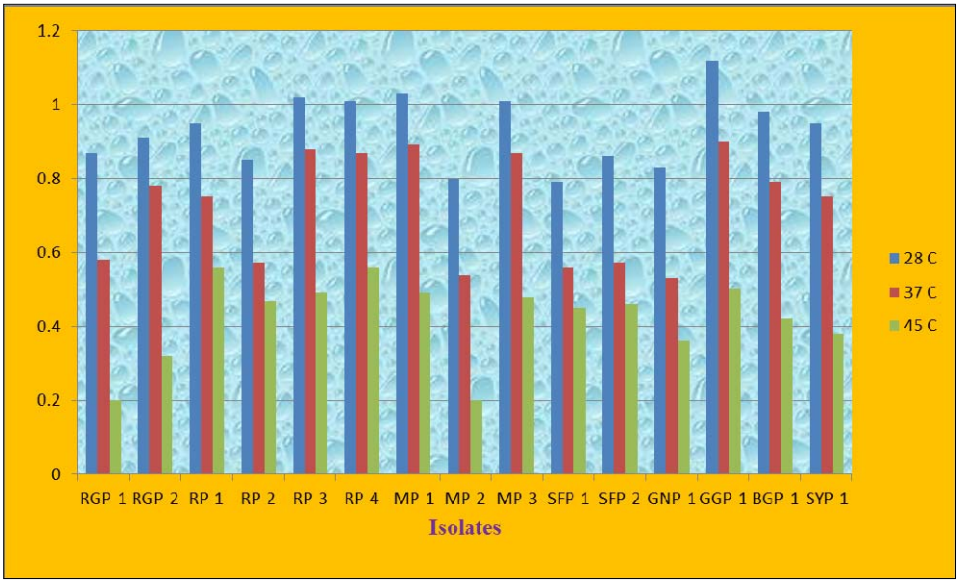


Fig 2: Temperature tolerance of *Pseudomonas fluorescence* isolates

Conclusion

The fifteen *Rhizobial* and *Pseudomonas flourescence* isolates were screened for tolerance to abiotic stress *in vitro* to different levels of temperature (28 ^{0}C, 37 ^{0}C & 45 ^{0}C).

After screening for above conditions, it was found that the two *Rhizobial* isolates RR-1, GNR-1 isolates were tolerant to almost all conditions of temperature (45 ^{0}C).

This indicates that these two isolates had good tolerance to above tested adverse conditions in addition to having multiple beneficial activity such as nitrogen fixation, phosphate solubilisation, growth promoters through IAA production.

Among the fifteen *Pseudomonas flourescence* isolates RGP-1, SFP-2, GGP-1 &RP-2 were consistent in exhibiting multiple beneficial activities such as phosphate solubilisation, siderophore production and antagonism to *Rhizoctonia solani* and *Sclerotium rolfsi*.

The data obtained in the present study suggest that *Pseudomonas flourescence* isolate GGP-1 tolerant to almost all conditions of temperature (45 ^{0}C)

RR-1, GNR-1 and GGP-1 isolates would be ideal organism for further study in pot culture and field experiments to exploit their PGPR potential for a good biofertilizers production.

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