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Evaluation of thermotolerant rhizobacteria for multiple plant growth promoting traits from pigeonpea rhizosphere

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Abstract: In pigeonpea due to the prevailing high temperature, poor nodulation have usually been observed as a limiting factor in its productivity. Plant growth promoting traits of 5 pigeonpea rhizobacterial isolates out of 32, selected on the basis of maximum growth at 30, 40 and 50°C, were evaluated for their potential application in enhancing symbiosis and plant growth of pigeonpea. The level of IAA at 30, 40 and 50°C varied from 0.95-20.96 µg/ml, 1.21-34.82 µg/ml and 0.16-17.34 µg/ml in the presence of tryptophan respectively. Maximum flavonoid production and siderophore production was recorded bacterial isolates with S12p6 (6.68 µg/ml) at 30°C and S1p1 (4.2 cm) at 40°C respectively. Isolates S1p1 and S12p6 showed relatively superior production of growth hormone, flavonoid-like compounds and siderophores can further be tested as co-inoculant with recommended *Rhizobium* for studying their efficacy under field conditions for symbiotic parameters and growth of pigeonpea.

Keywords: Pigeonpea, PGPRs, Rhizobacteria, *Rhizobium*

INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millsp) is the second most important grain legume in India. It is a valuable multipurpose legume, possessing traits for enhancing the sustainability of dry sub-tropical and tropical farmlands. It plays an important role in food and nutritional security being a rich source of proteins, minerals, vitamins and an excellent fodder with high nutrition value. In Punjab, during 2014-2015, pigeonpea occupied an area of 2.6 thousand hectares with a production of 2.39 thousand tonnes with an average yield of 9.18 quintals per hectare (Anonymous 2016).

Pigeonpea is a relatively drought-tolerant crop performs well in temperature range of 25-35°C and can survive at 45 °C. High soil temperatures in tropical areas limit nodulation and dinitrogen fixation by *Rhizobium* strains. The temperature resistant PGPR can serve as effective agents for plant survival and growth in temperature-stressed soil. The rhizobacteria facilitate plant growth and development with a wide variety of direct and indirect mechanisms under stress and non-stress conditions (Nadeem *et al.,* 2014). PGPR is known to affect the symbiotic relation between pigeonpea and its rhizobial symbiont (Tilak and Ranganayaki, 2006). Therefore inoculation with efficient strains of temperature-tolerant *Rhizobium* and PGPR would be required for exerting beneficial effects on plants with increased yields of pigeonpea crop and enhanced other biochemical functions (Singh *et al.,* 2013). Thus, the present study was undertaken to isolate and screen temperature tolerant free-living rhizobia from pigeonpea rhizospheres.

MATERIALS AND METHODS

Isolation of rhizobacteria: Rhizobacteria were isolated from pigeonpea rhizospheric soil from different locations by standard microbiological techniques on Nutrient agar medium for *Bacillus* and *Serratia,* on Yeast Extract Mannitol Agar medium for *Rhizobium* and on King's B medium (King *et al.,* 1954) for *Pseudomonas*. Ten rhizobacterial isolates were picked from King's B medium, 18 from nutrient agar medium and 4 from YEMA medium. The isolates were grown at 30° C, 40°C and 50°C in respective media and growth in terms of optical density were recorded at 600 nm using scanning spectrophotometer. Five isolates showing growth at 30°C, 40 °C and 50°C were selected for further analysis.

Biochemical characterization of rhizobacteria: Biochemical characterization of bacterial -isolates was done on the basis of Gram reaction, catalase production, starch hydrolysis, nitrate reduction and methyl red test as per the standard methods (Cappuccino and Sherman, 1992 and Holt *et al.,* 1994).

IAA production: Characterization of isolates for the production of IAA was determined as per the method given by Gordon and Weber (1951).

Flavonoid production: Total flavonoid content was

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estimated spectrophotometrically (Zhishen *et al.*, 1999). The extracted material was further processed by thin layer chromatography (TLC) by the method given by Parmar and Dadarwal (1999).

Production of siderophores: Siderophore production by bacterial isolates was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chrome azurol S (CAS).

RESULTS AND DISCUSSION

Isolation and characterization: A total of 32 of rhizobacteria were isolated from various pigeonpea rhizospheric soil samples taken from different locations of Punjab. The isolates on King's B medium showed the characteristic yellowish-green pigmentation. Two isolates S3b1 and S12b1 produced red colour pigmentation, whereas the other isolates S9p10 and S6p1 showed predominantly off-white to creamish colonies on Nutrient agar medium. The isolates Sr1 to Sr4 from YEMA medium showed gummy colonies (Plate 1) On the basis of cultural morphological and biochemical tests, they were tentatively assigned to genera *Pseudomonas, Serratia, Bacillus* and *Rhizobium* (Table 1). The growth in terms of optical density recorded at 30° C, 40°C and 50°C showed that rhizobacterial isolates attained exponential growth at both 30ºC and 40°C after 24 hrs of incubation. However, isolates S3b1, S3p1, S10b3, S1p1 and S12p6 showed higher growth at 40°C and relatively lower growth was recorded at 50°C (Fig 1). Nehra *et al.* (2007) reported four pigeonpea rhizobial strains (HR-3, HR-6, HR-10 and HR -12) which were temperature tolerant, highly efficient for all the symbiotic parameters and thus having the potential to be used as bio-inoculants in North-Western regions of India. Srivastava *et al.* (2008) isolated a thermotolerant *Pseudomonas putida* NBRI0987 from the drought-affected rhizosphere of chickpea. Kaur and Khanna (2013) reported that out of 15 rhizobial strains isolated from pigeonpea rhizosphere, four most temperature tolerant strains (LAR-2, LAR-3, LAR-4 and LAR-8) exhibited growth at 45°C. Manasa *et al.* (2017) studies suggested that the rhizobacterial isolates RR-1, GGP-1 and GNR-1 were both tolerant to high

Table 1. Cultural, morphological and biochemical characteristics of rhizobacterial isolates.

Characteristic of test organism	*Pseudomonas*	*Bacillus*	*Serratia*	*Rhizobia*
Gram's reaction	-ve	+ve	-ve	-ve
Shape	Rods	Rods	Rods	Rods
Pigment	+	-	+	+
Pigment colour	Fluorescent green	White	Red	Pink
Starch hydrolysis	+	+	+	+
Catalase production	+	+	+	+
Methyl red test	-	-	-	-
Nitrate reduction	+	+	+	+

Table 2. Effect of temperature on IAA production potential of rhizobacteria.

Rhizobacterial isolates	IAA equivalent (µg/ml) at 30°C		IAA equivalents (µg/ml) at 40°C		IAA equivalents (µg/ml) at 50°C	
	L-TRP (-)	L-TRP (+)	L-TRP (-)	L-TRP (+)	L-TRP (-)	L-TRP (+)
S3b1	9.54	20.14	9.70	26.55	4.79	6.96
S3p1	10.55	16.17	8.59	14.94	3.17	9.71
S10b3	11.98	19.54	17.90	30.01	4.58	2.43
S1p1	3.54	11.98	13.43	34.82	3.52	5.47
S12p6	6.24	20.96	18.71	29.58	3.68	17.34

Table 3. Effect of temperature on flavonoid and siderophore production by rhizobacteria.

Rhizobacterial Isolates	Flavonoid Production (µg/ml)			Siderophore production Index(cm)		
	30°C	40°C	50°C	30°C	40°C	50°C
S3b1	3.74	2.13	1.64	1.2	1.9	0.0
S3p1	4.18	3.77	1.17	0.9	1.8	0.0
S10b3	3.01	2.16	1.47	1.0	1.8	0.0
S1p1	3.77	3.44	2.48	2.0	4.2	0.0
S12p6	4.96	6.68	1.48	1.4	3.7	0.2

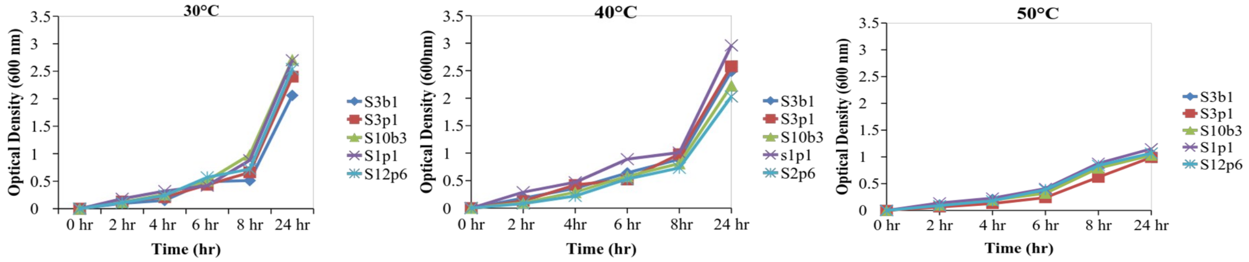

Fig.1. *Growth profile of rhizobacterial isolates at different temperatures.*

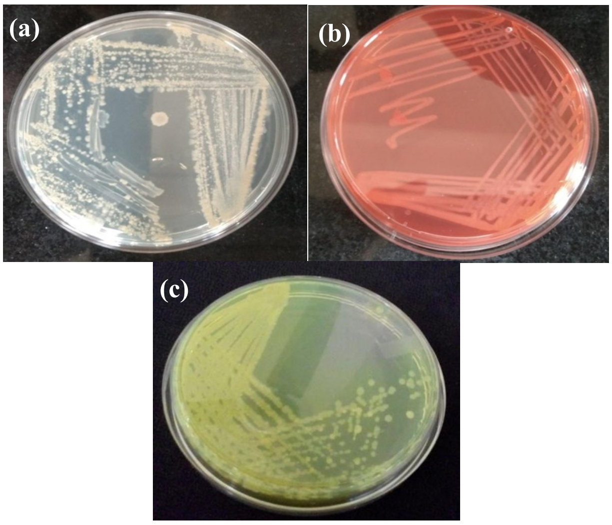

Plate 1. *Rhizobacterial diversity from pigeonpea rhizosphere, a) Bacillus isolates, b) Rhizobium isolates, c) Psuedomonas isolates.*

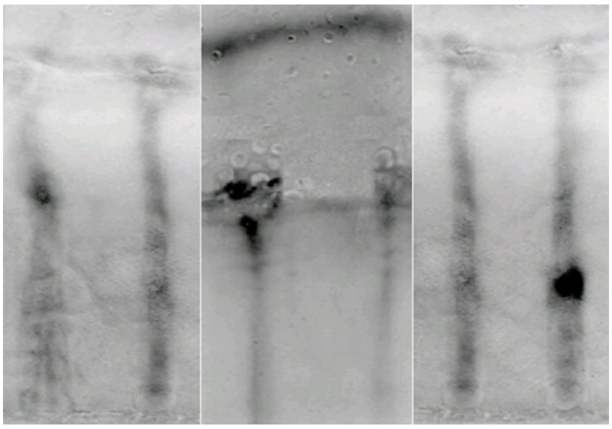

Plate 2. *TLC profile of flavonoid like compounds produced by rhizobacteria isolates by TLC.*

temperature (45°C) and also exhibit multiple beneficial plant growth promoting activities.

Indole acetic acid production: Indole-3-acetic acid (IAA) is a phytohormone produced by PGPRs that accelerates plant growth and development by improving root/shoot growth and seedling vigor. IAA production ranged from 0.28-10.08 µg/ml in the absence and 0.45-14.25 µg/ml in the presence of tryptophan after three days of incubation and increased to 0.46-11.02 µg/ml in the absence and 0.95-20.96 µg/ml in the presence of tryptophan after five days of incubation at 30° C. Surprisingly, at 40°Ca higher range was recorded (1.21-18.71 µg/ml in the absence and 1.04-34.82 µg/ml in the presence of tryptophan) after five days of incubation (Table 2). Five of the rhizobacterial isolates which showed highest IAA production at 30°C showed a further increase in IAA production at 40°C, whereas, isolates showed a sharp drop in IAA production at 50° C (0.09-4.79 (µg/ml) in absence and 0.16-17.34 (µg/ml) in the presence of tryptophan) after 5 days of incubation. IAA production by potent rhizobacterial isolates was relatively higher at 40°C as compared to 30° C and 50°C. It is imperative that rhizobacterial isolates S3b1, S3p1, S10b3, S1p1 and S12p6 which illustrated higher growth at 40°C, same showed decreased growth at 30°C and 50°C. Karnwal (2009) have reported that fluorescent *Pseudomonas* isolates produce an increased amount of indole-acetic acid in the presence of L-tryptophan.Ali *et al* (2009) reported that an isolate JGP-46 was the best producer of indole acetic acid followed by RMP-6 and AKM-P6 in sorghum under elevated temperature. Garcia *et al.* (2004) reported significant effects of three PGPR on growth parameters of *Glycine max* cv. Osumi (soybean) when co-inoculated with *S. fredii* as a result of auxins produced by the PGPR.

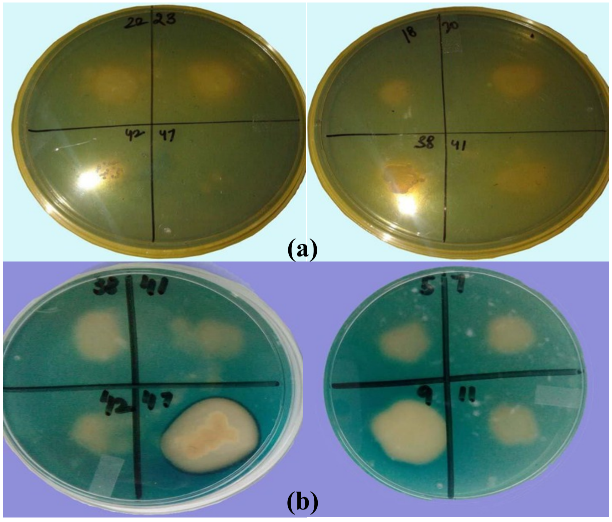

Plate 3. *Production of siderophore by rhizobacterial isolates on chrome azurol S plate, a) At 30°C, b) At 40°C*

Flavonoid production: Flavonoids exhibit a diverse spectrum of biological functions, protect plants against various biotic and abiotic stresses and play an important role in the symbiotic interaction between the plant and their microbial symbiont (Ahmad *et al.,* 2008).

Flavonoid production was recorded in the range of 0.25-6.68 µg/ml at 40°C, maximum by S12p6 (6.68 µg/ml) followed by S3p1 (3.77 µg/ ml), S1p1 (3.44 µg/ml), S10b3 (2.16 µg/ml) and S3b1 (2.13 µg/ml) (Table 3). Surprisingly, as in case of IAA-equivalents, here too the isolates showed relatively higher production at 40ºC as compared to 30ºC. At 40°C isolate S12p6 was found to be a strong producer of flavonoid followed by S3p1, S1p1, S10b3 and S3b1. The TLC plates showed a single fluorescent spot in the culture supernatant of S12p6, one spot corresponded with that of the standard naringin and other with lower mobility. However, isolate S3p1, and S1p1 exhibited production of two fluorescent spots one equivalent to naringin and other with a lower mobility. However, isolate S3b1 and S10b3 exhibited none of the fluorescent spots (Plate 2). Increased nodulation in *Vicia sativa* subsp.

nigra (vetch) following co-inoculation with *R. leguminosarum* bv. *viciae* (Rlv) and wild-type strain (Sp7) in pots, in pouches, and in a hydroponic system (Star *et al.,* 2012) have been reported to increase production of plant root flavonoids. Raghuwanshi *et al.* (1994) reported that elevated temperatures production severely affect the flavonoid production in the legume crops.

Siderophore production: Siderophores are low molecular weight compounds that are produced under iron-limiting conditions, chelate the ferric ion (Fe^{3+}) with a high specific gravity and serve as vehicles for transport of Fe(III) into a microbial cell (Gupta and Gopal 2008). Isolates were evaluated for siderophore production and showed varied results at different temperatures (Table 3). At 40°C isolates S3b1, S3p1, S10b3, S1p7 and S12p6 showed significant siderophore production, maximum being was shown by S1p1. The diameter of halo varied from 1.8-4.2 cm on CAS agar plates, maximum being produced by fluorescent *Pseudomonas* isolate S1p1 (4.2 cm) (Plate 2). However, at 50°C, none of the isolates showed siderophore production. Numerous studies of the plant growth promotion via siderophore production-mediated Fe-uptake are demonstrated with siderophore producing rhizobacterial isolates (Rajkumar *et al.,* 2010).

Conclusion

The study indicates that rhizobacterial strains offer several beneficial traits that could improve plant growth. Rhizobacterial isolates S1p1 and S12p6 were most promising for IAA production, flavonoid production and also for siderophore production at temperature 30°C, 40°C and 50°C. So, we considered the bacterial strains S1p1and S12p6 were the most feasible ones to be used as inoculant for plant growth improvement.

REFERENCES

Ahmad, F., Ahmad, I. and Khan, M.S. (2008). Screening of free-living rhizobacteria for their multiple plant growth promoting activities. *Microbiol. Res.* 163: 173-81.

Ali, S. Z., Sadhya, V., Grover, M., Kishore, N., Rao, L. V. and Venkateswarlu, B. (2009). *Pseudomonas* sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. *Biol. Fertil. Soils.* 46: 45-55.

Anonymous (2016). Package and practice for Kharif crops. Punjab Agricultural University, Ludhiana, pp 62.

Cappuccino, J. C. and Sherman, N. (1992). *Microbiology: A Laboratory Manual*: Academic distributors, New York 125-79.

Garcia, J. A. L., Probanza, A., Ramos, B., Barriuso, J. and Mañero, F. J. G. (2004). Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) and *Sinorhizobium fredii* on biological nitrogen fixation, nodulation and growth of *Glycine max* cv. Osumi. *Pl. Soil.* 267: 143–53.

Gordon, A. S. and Weber, R. P. (1951). Calorimetric estimation of Indole acetic acid. *Pl .Physiol.* 25:192-95.

Gupta, A. and Gopal, M. (2008). Sideophore producing by plant growth promoting rhizobacteria. *Indian. J. Agric. Res.* 42 (2): 153-56.

Holt, J. G. N., Krieg, R., Sneath, P. H. A., Stanley, J. T. and Williams, S. T. (ed) (1994). *Bergey's manual of Determinative Bacteriology*. The Williams and Wikins Co (Lippincoli, Williams and Wikins), Baltimore.

Karnwal, A. (2009). Production of indol acetic acid by *Fluorescent Pseudomonas* in the presence of L-tryptophan and rice root exudates. *J. Plant. Pathol.* 91(1): 61-3.

Kaur, S. and Khanna, V. (2013). Effect of temperature-tolerant rhizobial isolates as PGPR on nodulation, growth and yield of pigeonpea [*Cajanus cajan* (L) Milsp.]. *J. Food. Legumes.* 26(3 & 4): 80-83.

King, E. O., Ward, M. K. and Raney, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorecein. *J. Lab. Clin. Med.* 44:301-7.

Manasa, K., Subhash, R. R., and Trivenu, S., (2017). In *vitro* screening of temperature stress tolerance of *Rhizobial* and *Pseudomonas fluorescenc*e isolates. *J. Pharmaco. Phytochem.* 6(5): 764-67.

Nadeem, S. M., Ahmad, M., Zahir, A. Z., Javaid, A. and Ashraf, M. (2014).The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.* 32: 429-48.

Nehra, K., Yadav, A. S., Sehrawat, A. R. and Vashisha, R. K. (2007). Characterization of heat resistant mutant strains of *Rhizobium* sp. (*Cajanus cajan* L.) for growth, survival and symbiotic properties. *Ind. J. Microbiol.* 4: 329-35.

Parmar, N. and Dadarwal, K. R. (1999). Stimulation of nitrogen fixation and induction of flavonoid like compounds by rhizobacteria. *J. Appl. Microbiol.* 86: 36-44.

Rajkumar, M., Ae, N., Prasad, M.N.V., Freitas, H. (2010). Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol.* 28: 142–149.

Raghuwanshi, A., Dudeja, S. S. and Khurana, A. L. (1994). Effect of temperature on flavonoid production in pigeon pea [*Cajanus cajan* (L.) Millsp.] in relation to nodulation. *Biol. Fertil. Soils.* 17: 314-16.

Schwyn, B. and Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophore. *Anal. Biochem.*160: 47-56.

Singh, Y., Ramteke, P. W. and Shukla, P. K. (2013). Characterisation of *Rhizobium* isolates of pigeonpea rhizosphere from Allahabad soils and their potential PGPR characteristics. *Int. J.Res. Pure. Appl. Microbiol.*,3(1):4-7

Srivastava, S., Yadav, A., Seem, K., Mishra, S., Chaudhary, V. and Srivastava, C. S. (2008). Effect of high temperature on *Pseudomonas putida* NBRI0987 biofilm formation and expression of stress sigma factors RpoS. *Curr. Microbiol.* 56: 453-57.

Star, L., Matan, O., Dardanelli, M. S., Kapulnik, Y., Burdman, S. and Okon, Y. (2012). The *Vicia sativa* spp. nigra–*Rhizobium leguminosarum* bv. viciae symbiotic interaction is improved by *Azospirillum brasilense*. *Pl. Soil.* 356: 165–74.

Tilak, K. V. B. R., Ranganayaki and Manoharachari, C. (2006). Synergistic effects of plant-growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). *European. J. Soil. Science.* 57: 67–71.

Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food. Chem.* 64: 555-9.