

RESEARCH ARTICLE

In vitro screening of plant growth promoting rhizobacteria to control bacterial wilt (*Ralstonia solanacearum*) of tomato (*Lycopersicon esculentum*)

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SUMMARY

Ralstonia solanacearum is the causative agent of bacterial wilt that causes considerable damages in the yield of various crop plants. The intent of the study was to evaluate potential of bacterial antagonists to suppress bacterial wilt disease development and evaluate the role of the strains as plant growth-promoting rhizobacteria (PGPR) in tomato. One hundred rhizosphere bacterial isolates were screened against virulent strain of *Ralstonia solanacearum*. After *in vitro* screening 10 antagonistic strains designated PR3, PR17, PR26, 1NAB15, 1NAB20, 3NAA1, 3NAA2, 2CBA2, 2CBA4, 2CBA18 showed antagonistic effect by producing inhibition zone suppressing the growth of *R. Solanacearum*. The isolate PR3 showed the highest inhibition zone measuring 33.3mm whereas 1NAB15 showed the lowest zone measuring 10mm. The present study, therefore, suggests that the use of PGPR isolates which showed the antagonistic activity can be used as inoculants/ bioantagonists might be beneficial for the control of bacterial wilt of tomato in field studies.

Key Words : *Ralstonia solanacearum*, PGPR, Bacterial wilt, Tomato

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The most common disease bacterial wilt of tomato is caused by *Ralstonia solanacearum* earlier called as *Pseudomonas solanacearum* is

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responsible for the wilt disease which is the most destructive and widespread disease that causes high yield loss of various crop plants of different families (Hayward, 1991; Ali, 1993; Chakraborty *et al.*, 1994 and Hayward and Hartman, 1994). It has a large host range of more than 200 species in 50 families (Aliye *et al.*, 2008). Plants of solanaceae family, such as tomato (*Lycopersicon esculentum* L.), eggplant (*Solanum melongena* L.) and chilli (*Capsicum* spp.) are affected by *R. solanacearum* in various countries, including India, Egypt, Indonesia, China and Brazil (Hayward and Hartman, 1994). The pathogen infects roots of susceptible plants, usually

through wounds (Pradhanang *et al.*, 2005). Colonization by the bacterium in the xylem prevents water movement into upper portion of the plant tissue (Kelman, 1998). The symptoms of disease start as drooping of leaf which is followed by wilting of whole plants within a few days, which leads to plant death. The infected plants may recover, temporarily, in the evening, when temperatures are cooler. A few days later, a sudden and permanent wilt occurs. The roots and lower portion of the stems have a browning of their vascular system. The control measures which are being employed are field sanitation, crop rotation, use of resistant varieties, plant breeding etc but these are also not very much helpful and effective. Till date, no effective control method has been developed for bacterial wilt disease. Plant breeding, field sanitation, crop rotation, and use of bactericides have given only limited success (Ciampi-Panno *et al.*, 1989). It is well known that plant growth promoting rhizobacteria (PGPR) help plants to increase resistance against pathogens by secreting biologically active compounds and/or elicitation of induced systemic resistance (ISR) (Heil and Bostock, 2002). Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria which colonize plant roots and by a wide variety of mechanisms enhances plant growth. The use of PGPR is increasing in agriculture and offering an attractive way to replace chemical fertilizers, pesticides, and supplements which are harmful for the environment and for living beings too. Various recent studies have indicated that biological control of bacterial wilt disease could be achieved using antagonistic bacteria (McLaughlin *et al.*, 1990 and Ciampi-Panno *et al.*, 1989).

MATERIAL AND METHODS

Antagonistic isolates :

A total number of 100 isolates of plant growth promoting rhizobacteria (PGPR) were collected from the Department of Biological Sciences, SHIATS, Allahabad. The isolates were sub-cultured for further studies on nutrient agar (NA) medium and incubated at 28°C for 48h. For long term preservation, obtained isolates were stored in Eppendorftubes containing NB (nutrient Broth) with 20 per cent glycerol at -20°C.

Plant pathogen and culture conditions :

R. solanacearum strain belonging to race 1 biovar 3, which is a standard virulent strain on tomato, was provided by the Indian Agricultural Research Institute, New Delhi. It was grown on nutrient agar(NA) medium.

Preliminary screening for antagonistic activity *in vitro* :

This test was performed to screen the antagonistic activity of 100 isolates against *R. Solanacearum* according to the method described by Li *et al.* (2004). Cultures of *R. Solanacearum* were grown overnight in nutrient broth, 0.5 ml of the liquid culture was mixed with 15 ml lukewarm melted nutrient agar in sterile petri plates and allowed to solidify. Afterwards, each tested isolate was spotted on the surface of agar plates and incubated them at 28°C for 72 h. Consequently, those bacteria that displayed positive inhibition activity were considered as antagonistic isolates and selected for further research investigations.

In vitro assay for studying antagonism :

To check the antagonism ability of selected potential PGPR against *R. Solanacearum* carried out by agar-well diffusion method. NA medium was used in order to the favour the growth of *R. Solanacearum* and growth-promoting rhizobacteria. A 30ml medium was poured in sterilized Petriplates and allowed to solidify. One-hundred micro-liters of *R. Solanacearum* suspension (overnight liquid culture of *Ralstonia* centrifuged and then the cell pellets were re-suspended to final concentration ~10⁸ CFU/ml) was spread on the solidified plates and two holes of 9 mm diameter punched into the agar. In these holes 30 µl suspension of each test antagonist (10⁹ cfu/ml) was added and the plates incubated at 28 °C for 48 h. One well was filled with water used as control. Inhibition of *R. solanacearum* growth was assessed by measuring the radius of inhibition zone (mm) after incubation for 48 h at 28 °C. The trial was done three times with four replicates.

RESULTS AND DISCUSSION

After *in-vitro* screening using agar-well diffusion technique, among 100 isolates of producer bacteria, 10 isolates *viz.*, PR3, PR17, PR26, 1NAB15, 1NAB20, 3NAA1, 3NAA2, 2CBA2, 2CBA4, 2CBA18 showed antagonistic effect against *R. Solanacearum* (Table 1 and Plate 1). Distinct inhibition zones were developed around the antagonistic bacteria. Among 10 isolates PR3 displayed a high ability to suppress *R. solanacearum* growth, with inhibition zone over 33.3mm whereas 1NAB15 displayed low ability to suppress *R. solanacearum* growth with minimum zone of inhibition measuring 10 mm. PR17, PR26 AND 3NAA1 showed inhibition zone between 30-20mm. The other remaining antagonistic isolates showed inhibition zone between 20-10mm.

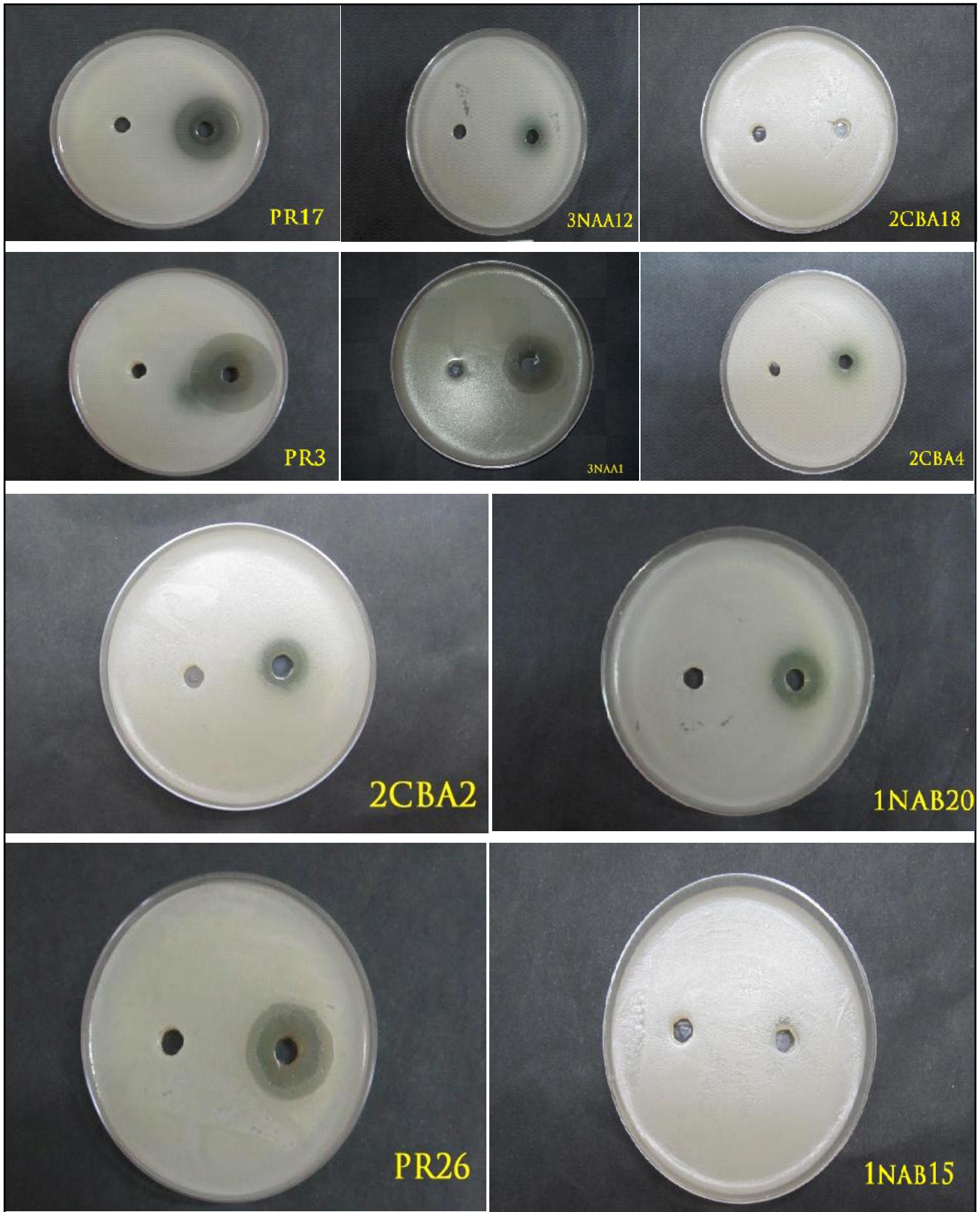


Table 1 : Antagonistic activity of five antagonistic bacterial isolates against three isolates of *R. solanacearum*

Strains	Bacterial species	Mean diameter (mm)	Degree of antagonism
PR 3	<i>Enterobactor</i> sp.	33.3	++++
PR 17	<i>Enterobactor</i> sp.	28.5	++++
PR 26	<i>Rhizobium</i> sp.	26.5	++++
1NAB15	<i>Bacillus</i> sp.	10	++
1NAB20	<i>Bacillus</i> sp.	20	+++
2CBA2	<i>Pseudomonas</i> sp.	19	+++
2CBA4	<i>Pseudomonas</i> sp.	15	+++
2CBA18	<i>Pseudomonas</i> sp.	11.5	++
3NAA1	<i>Bacillus</i> sp.	25	++++
3NAA12	<i>Bacillus</i> sp.	19.2	+++
Medium '+' (10-15 mm),		Strong '+++' (11-20 mm),	Very strong '++++' (over 20 mm)

Conclusion :

In conclusion, when 100 micro-organisms which were having three PGPR activities were screened *in-vitro* by agar-well diffusion method, it was found that 10 isolates viz., PR3, PR17, PR26, 1NAB15, 1NAB20, 3NAA1, 3NAA2, 2CBA2, 2CBA4, 2CBA18 showed impressive antagonistic effect against *R. Solanacearum*. Control of *R. Solanacearum* by these antagonists PGPR may help reducing chemical applications and their environmental impacts in agricultural systems where solanaceous crops are grown and will lower the cost of disease management. The use PGPR antagonists for increasing the yield and crop protection is a promising approach in modern systems of sustainable agriculture. Future research should be directed towards detailing the mode of action of these potential antagonists strains and field studies need to be conducted to confirm the effectiveness of these antagonists under natural conditions.

REFERENCES

- Ali, M.S. (1993). Bacterial wilt of potato in Bangladesh, 196 p. A paper presented on International Workshop on Bacterial wilt of potato held at NEW DELHI, INDIA.
- Aliye, N., Fininsa, C. and Hiskias, Y. (2008). Evaluation of rhizosphere bacterial antagonists for their potential to bioprotect potato (*Solanum tuberosum*) against bacterial wilt (*Ralstonia solanacearum*). *Biol. Control*, **47**: 282- 288.
- Ciampi-Panno, L., Fernandez, C., Bustamante, P., Andrade, N., Ojeda, S. and Conteras, A. (1989). Biological control of bacterial wilt of potatoes caused by *Pseudomonas solanacearum*. *Am. Potato J.*, **66** : 315-332.
- Chakraborty, M., Chattarjee, B., Habib, A.K.M.A., Sengupta, C., Som, M.G. and Samaddar, K.R. (1994). Occurrence of *Pseudomonas solanacearum* Biovar 3 on eggplant, tomato and potato in West Bengal. *ACLAR. Bact. Wilt News*, **1** : 10.
- Hayward, A.C. (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathol.*, **29** : 65-87.
- Hayward, A.C. and Hartman, G.I. (1994). *Bacterial wilt, the disease and its causative agent, Pseudomonas solanacearum*, 259 p. Wallingford.
- Heil, M. and Bostock, R.M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Ann. Bot.*, **89** : 503-512.
- Kelman, A. (1998). One hundred and one years of research on bacterial wilt. In bacterial wilt disease: molecular and ecological aspects. Prior, P., Allen, C., Elphinstone, J. (Eds.), Springer-Verlag, Berlin Heidelberg.
- Li, G.C., Jin, L.P., Xie, K.Y. and Qu, D.Y. (2004). Advances in research on bacterial wilt of ginger in China. *Chinese Potato*, **18** : 350-354.
- Liu, M., Zhang, M., Ji, J.C., Yin, F.Q., Zhang, Y., Tu, Y. and Ye, Y.J., (2005). Advances in research on bacterial wilt of ginger in China. *Chinese Agril. Sci. Bulletin*, **21** : 337-341.
- McLaughlin, R.J., Sequeira, L. and Weingartner, D.P. (1990). Biocontrol of bacterial wilt of potato with an avirulent strain of *Pseudomonas solanacearum*: interactions with root-knot nematodes. *American Potato J.*, **67** (2) : 93-107.
- Pradhanang, P.M., Ji, P., Momol, M.T., Olson, S.M., Mayfield, J.L. and Jones, J.B. (2005). Application of acibenzolar-S-methyl enhances host resistance in tomato against *Ralstonia solanacearum*. *Pl. Dis.*, **89** : 989-993.

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