*Journal of Applied and Natural Science* 8 (3): 1699 - 1703 (2016)

JANS

# Variable survival ability of rhizobacteria in cumin (*Cuminum cyminum* L.) rhizosphere

**Anurag Yadav[*] and Kusum Yadav[2]**

Department of Microbiology, College of Basic Science & Humanities, S.D. Agricultural University, S.K. Nagar - 385 506 (Gujarat), INDIA
[2]Department of Biochemistry, University of Lucknow, Lucknow - 226 007(Uttar Pradesh), INDIA
[*]Corresponding author. E-mail: anuragyadav123@gmail.com

*Received: December 18, 2014; Revised received: June 06, 2016; Accepted: August 28, 2016*

**Abstract**: A study was undertaken to compare the survival efficacy of two native, previously characterized bacterial biovars *viz. Bacillus subtilis* BCU5 and *Pseudomonas fluorescens* PCU17 with *Bacillus subtilis* strain MTCC1789 and *Pseudomonas fluorescens* strain MTCC4828, procured from Institute of Microbial Technology, Chandigarh, India in cumin rhizosphere and bulk soil. All the four bacterial types were made rifampicin resistant and the mutants were applied as inoculants at the dosage of 6 log, 7 log and 8 log colony forming units (cfu) $g^{-1}$ dry soil weight in pots containing cumin seedlings. The cfu of rhizosphere and bulk soil of pots was observed per week for four weeks. The results show that the initial population decline is a common feature of bioinoculants. In rhizosphere and bulk soil, the native bacterial biovars survived better than their procured counterparts. The population of *P. fluorescens* strain MTCC4828r in rhizosphere soil declined faster and reached below detection limit whereas the *P. fluorescens* biovar PCUr rhizosphere final population dropped to 3.1 log, 2.9 log and 2.13 log cfu $g^{-1}$ soil dry weight with 8 log, 7 log and 6 log cfu $g^{-1}$ soil dry weight inoculum treatment, respectively. In contrast to *P. fluorescens* strain MTCC4828r, the population of *B. subtilis* strain MTCC1789r stabilized after some decline and was comparable with *B. subtilis* biovar BCU5 population. Study concludes that the inoculant population decline in soil was the result of lower microbial load carrying capacity of soil than the provided inoculum densities. Also, the native bacteria survived better than procured ones in rhizosphere soil.

**Keywords**: *Bacillus subtilis*, Cumin, *Pseudomonas fluorescens,* Rhizobacteria, Rhizosphere, Survival

## INTRODUCTION

Purposeful microbial introduction in the soil has been in practice for decades in agriculture. Some microorganisms enhance plant growth by (i) supplying nutrients to crops (Liu *et al.*, 2012; Orhan *et al.*, 2006), (ii) controlling pathogens (Lucas *et al.*, 2009; Wei *et al.*, 2011), (iii) improving soil structure (Barea *et al.*, 2005) and by (iv) enhancing seed germination (Yadav *et al.*, 2013). Fluorescent pseudomonads and other important group of bacteria are well known for their role in plant growth promotion (PGP) in the rhizosphere (Kloepper *et al.*, 1980). Such inoculants aid in plant growth promotion and disease control. However, the success of PGP effect relies on successful survival of rhizobacteria (Bennett and Whipps, 2008). The variability in performance of plant growth promoting rhizobacteria (PGPR) in the field is probably due in part to the failure of the introduced bacteria to survive in the rhizosphere (Benizri *et al.*, 2001; Kloepper *et al.*, 1980). Population sizes of bacteria seem to decline more or less rapidly after their introduction in soil. Population decline has been noticed very often by introducing bacteria in the soil or rhizosphere and could be the result of (i) scarcity of available nutrient sources to microbes in soil (van Veen Ja Fau - van Overbeek *et al.*, 1997) and (ii) hostility of the soil environment to incoming microbes due to a myriad of adverse abiotic and biotic factors (Brimecombe *et al.*, 2001; van Veen Ja Fau - van Overbeek *et al.*, 1997). The study targets on (i) comparing the rate of survival of native bacteria from bacteria procured from culture repository, (ii) comparing the rate of survival in rhizosphere and bulk soil and (iii) application of optimum dosage that endures the survival of PGPR in soil. In comparison to pseudomonads, sporeforming bacteria survive better especially under dry conditions (Nicholson *et al.*, 2000). Hence, *B. subtilis* biovar BCU5r and *B. subtilis* strain MTCC1789r were also incorporated in the experimental design.

## MATERIALS AND METHODS

**Inoculum preparation and incorporation into pot soil:** Four bacterial types, namely *P. fluorescens* biovar PCU17, *B. subtilis* biovar BCU5, *P. fluorescens* strain MTCC4828 and *B. subtilis* strain MTCC1789 and were tested in pot experiment for their survival in

ISSN : 0974-9411 (Print), 2231-5209 (Online)  

rhizosphere and bulk soil. The bacteria survival was measured as per the method of Yan *et al.* (2003). The bacterial rifampicin resistant mutants namely, *P. fluorescens* biovar PCU17r, *P. fluorescens* strain MTCC4828r, *B. subtilis* biovar BCU5r and *B. subtilis* strain MTCC1789r were prepared in the experiment to differentiate introduced population from indigenous rhizobacteria. The mutants were prepared on trypticase soy agar (TSA) (HiMedia) supplemented with 100 µg $mL^{-1}$ Rif (Rif TSA). The persistence of rifampicin resistance was confirmed by serial culturing them on Rif TSA. The rifampicin resistant isolates were stored at 20°C in tryptic soy broth containing 20% glycerol.

One cumin seed was planted per pot containing 50 g unsterilized soil. The pots were kept in a humidity chamber for seed germination. After ten days of germination, the pots containing cumin seedlings were kept in open and watered when needed with sterile distilled water. The pots were arranged with four bacterial treatments and three replications of each treatment in the randomized block design.

Inoculum for soil treatment was prepared by streaking bacterial isolates on Petri plates containing TSA medium supplemented with rifampicin and incubating the plates at 28°C for 48 h. Bacterial cells were harvested from the Petri plates with a sterile loop and suspended in sterile distilled water to yield 11 log colony forming units (cfu) $mL^{-1}$. Bacterial suspensions were incorporated into the pot soil at the rates of 6 log, 7 log, and 8 log cfu $g^{-1}$ soil.

**Enumeration of incorporated bacteria:** Prior to enumeration, the plants were uprooted from pots and the adhering debris sticking to them was carefully removed. The rhizosphere soil sticking to roots was collected with a sterile paintbrush in a sterile Petri plate. About two grams rhizosphere and bulk soils were collected from each pot. The respective soil samples were homogenized in 100 ml sterile distilled water. The homogenates were serially diluted with sterile distilled water and 0.1 mL of selected dilutions was plated on TSA supplemented with 100 µg $L^{-1}$ rifampicin. Petri plates were incubated at 30±1°C for 24 h and counted for cfu. The observations were taken after 0, 1, 2, 3 and 4 weeks of inoculation. Three replicates were used at each sampling time for each treatment.

**Statistical analysis:** All data were analyzed with XLSTAT® software using one-way ANOVA test and standard regression analysis between inoculation duration. Least significant difference values at P = 0.05 level were used to separate treatment means when ANOVA indicated a significant F value. All experiments were conducted in three replicates.

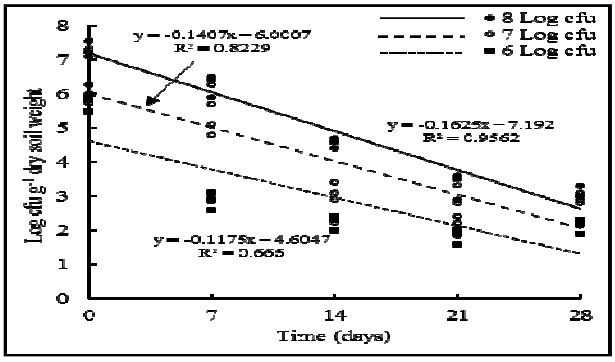


**Fig.1.** *Persistence of P. fluorescens biovar PCU17r in rhizosphere soil at three different treatment dosage. Each point represents a single observation.*

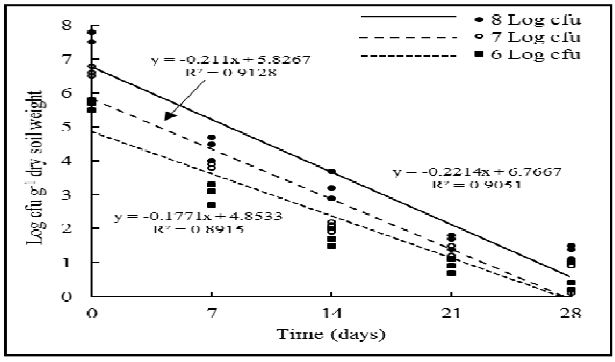


**Fig. 2.** *Persistence of P. fluorescens biovar PCU17r in bulk soil at three different treatment dosage. Each point represents a single observation.*

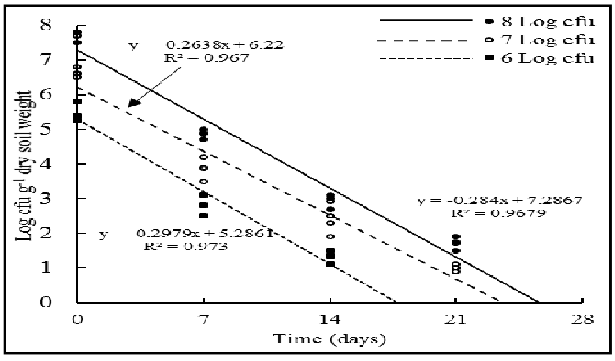


**Fig. 3.** *Persistence of P. fluorescens strain MTCC4828r in rhizosphere soil at three different treatment dosage. Each point represents a single observation.*

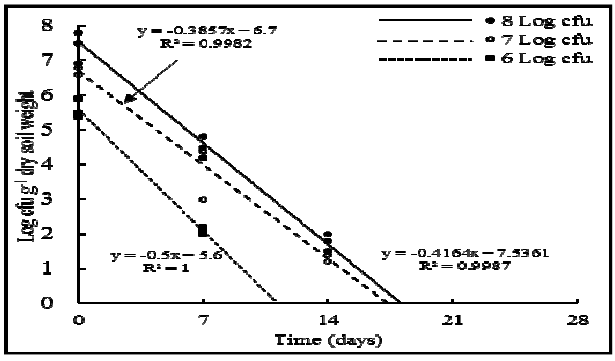


**Fig. 4.** *Persistence of P. fluorescens strain MTCC4828r in bulk soil at three different treatment dosage. Each point represents a single observation.*

## RESULTS AND DISCUSSION

The population densities of all the four bacterial inoculants decreased after the first week of inoculation, suggesting that initial decline of introduced population is a common feature of bioinoculants (Bolstridge *et al.*, 2009; Fischer *et al.*, 2010). Population decline of *P. fluorescens* and *B. subtilis* has also been reported in previous studies (Bolstridge *et al.*, 2009; Fischer *et al.*, 2010). The biovars *P. fluorescens* PCU17r and *B. subtilis* biovar BCU5r demonstrated population stabilization after the third week of inoculation, but the population of procured strain *P. fluorescens* MTCC4828r declined steadily and reached below detection limit within the observation period. The *B. subtilis* strain MTCC1789r survived for comparatively longer duration probably due to sporulation ability (Pandey and Palni, 1997). Overall, in all the observation sets the population decline of 2 log to 3 log cfu $g^{-1}$ soil was not uncommon during the first week of observation. *P. fluorescens* biovar PCU17r, *B. subtilis* biovar BCU5r and *B. subtilis* strain MTCC1789r had better survival rates in rhizosphere and bulk soil compared to *P. fluorescens* strain MTCC4828r.

**Survival of rhizobacteria in rhizosphere soil:** The microclimate of rhizosphere is complex and affects bacterial survival (Brimecombe *et al.*, 2001; van Veen Ja Fau - van Overbeek *et al.*, 1997). In our observation the rhizosphere populations of all the four bacterial treatments began to decrease after the first week of inoculation and significantly declined up to 5 log units after four weeks of inoculation (Figs. 1, 3, 5 and 7). Population of *P. fluorescens* biovar PCU17r with all the three inoculum dosage decreased till second week of inoculation, stabilized in the third week and increased to some extent in the last week of observation to reach 3.1 log, 2.9 log and 2.13 log cfu $g^{-1}$ soil dry weight with 8 log, 7 log and 6 log cfu $g^{-1}$ soil dry weight initially provided inoculum densities. The final population difference of not more than 1 log unit was observed in *P. fluorescens* biovar PCU17r strain in spite of three different initial inoculum levels. The population of *P. fluorescens* strain MTCC4828r steadily dropped per week and totally diminished in the final observation, showing the steepest negative regression slope. The population density of *B. subtilis* biovar BCU5r dropped till second week and stabilized afterwards to reach 3.45 log, 2.8 log and 2.66 log cfu g $^{-1}$ soil dry weight with 8 log 7 log and 6 log cfu $g^{-1}$ soil dry weight inoculum treatment, respectively. The final population of *B. subtilis* biovar BCU5r of three different initial inoculum dosage reached the approximately same population level, although with much lower mag-

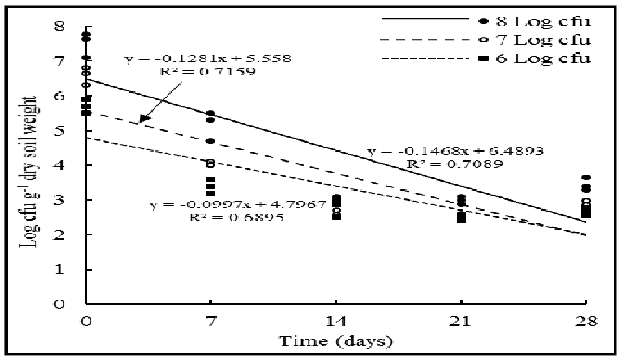


**Fig. 5.** *Persistence of B. subtilis biovar BCU5r in rhizosphere soil at three different treatment dosages. Each point represents a single observation.*

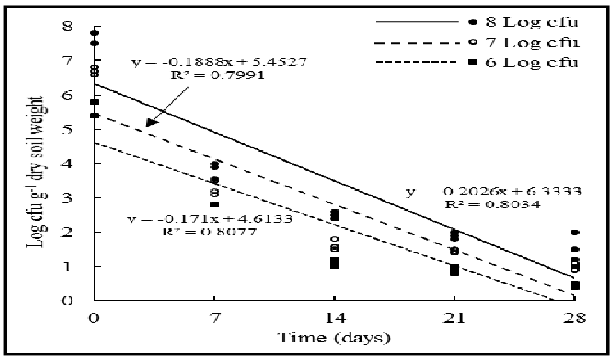


**Fig. 6.** *Persistence of B. subtilis biovar BCU5r in bulk soil at three different treatment dosages. Each point represents a single observation.*

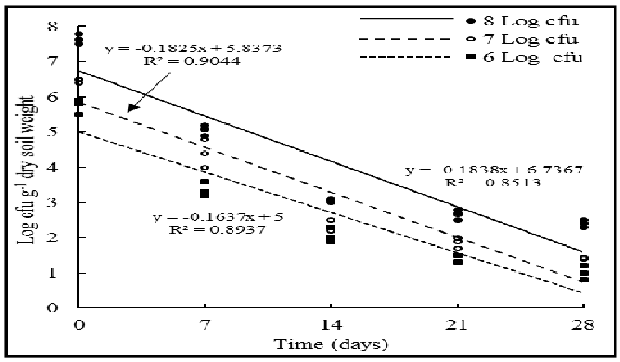


**Fig.7.** *Persistence of B. subtilis strain MTCC1789r in rhizosphere soil at three different treatment dosages. Each point represents a single observation.*

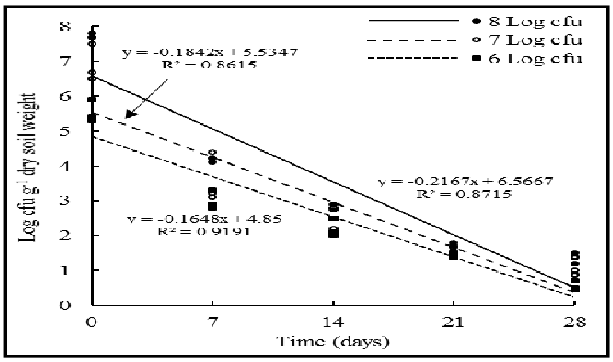


**Fig. 8.** *Persistence of B. subtilis strain MTCC1789r in bulk soil at three different treatment dosages. Each point represents a single observation.*

nitude than initially provided inoculum densities. The population of *B. subtilis* strain MTCC1789r also receded like *P. fluorescens* strain MTCC4828r but stabilized to some extent after three weeks of inoculation.

**Survival of rhizobacteria in bulk soil:** In our study the population of all the four bacterial types declined faster in bulk soil than in rhizosphere soil (Figs. 2, 4, 6 and 8); suggesting that bulk soil is not a favourable environment for their survival. Results show that *P. fluorescens* biovar PCU17r population receded fast and continued to decline till final observation to reach population of 1.33 log, 0.63 log and 0.53 log cfu $g^{-1}$ soil dry weight with 8, 7 and 6 log cfu $g^{-1}$ soil dry weight initial inoculum densities. Bulk soils are often nutritively poor and thus support lesser microbial load (van Elsas and Overbeek, 1993).

The population of *P. fluorescens* strain MTCC4828r decreased even faster and reached the undetectable limit after three weeks of inoculation. The population of native *B. subtilis* biovar BCU5r decreased sharply but stabilized from the second week of inoculation, although with comparatively lower numbers to reach 1.56 log, 1 log and 0.63 log cfu $g^{-1}$ soil dry weight with 8, 7 and 6 log cfu $g^{-1}$ soil dry weight treatments, respectively. In spite of sharp decline, the *B. subtilis* strain MTCC1789r population was still detectable in the final observation to reach 1.36 log, 0.92 log and 0.55 log cfu with 8, 7 and 6 log cfu treatments, respectively.

**Comparison of application doses on rate of bacterial survival:** At the three different initial inoculum densities the population of *P. fluorescens* biovar PCU17r was higher than *P. fluorescens* strain MTCC4828r and that of *B. subtilis* biovar BCU5r from *B. subtilis* strain MTCC1789r. Application of *P. fluorescens* biovar PCU17r at 8 log CUF $g^{-1}$ soil resulted in maximum population density of rhizosphere soil and bulk soil bacteria in the final observation ($P = 0.05$). In all the three inoculum treatments of rhizosphere and bulk soil the population of procured *P. fluorescens* strain MTCC4828r declined rapidly with the steepest negative regression slope. The population of *B. subtilis* biovar BCU5r declined till second week of inoculation but stabilized from third week with a population density difference of approximately 1 log cfu $g^{-1}$ dry soil weight. This suggests that the initial inoculum density had not much effect on the final population stabilization of *B. subtilis* biovar BCU5r. Population stabilization of BCU5r biovar was also observed in bulk soil with the same findings. *B. subtilis* strain MTCC1789r showed a survival pattern similar to *B. subtilis* biovar BCU5r. The treatment of strain MTCC1789r with initial inoculum dosage of 6 log and 7 log showed continuous population decline to become comparable in the final observation. The population of 8 log cfu $g^{-1}$ dry soil weight dose, however, stabilized with greater population number, which suggests that higher inoculum dosage helps in better bacterial population survival (Frey-Klett *et al.*, 1999).

## Conclusion

It can be concluded from results that native bacterial inoculant survive better in rhizosphere soil. Choosing spore-forming inoculants like *B. subtilis* provides additional survival advantage. To some extent, the application dose of inoculum affects rhizobacterial survival to modulate plant growth promotion and biological control activities. The observation suggests that the experimental soil had lower microbial load carrying capacity than initially provided inoculum densities which caused decline of inoculant population.

## REFERENCES


Barea, J.M., Azcón, R. and Azcón-Aguilar, C. (2005). Interactions between mycorrhizal fungi and bacteria to improve plant nutrient cycling and soil structure, Microorganisms in soils: roles in genesis and functions. Springer, pp. 195-212.

Benizri, E., Baudoin, E. and Guckert, A. (2001). Root Colonization by Inoculated Plant Growth-Promoting Rhizobacteria. *Biocontrol Science and Technology*, 11(5): 557 -574.

Bennett, A.J. and Whipps, J.M. (2008). Beneficial microorganism survival on seed, roots and in rhizosphere soil following application to seed during drum priming. *Biological Control*, 44(3): 349-361.

Bolstridge, N., Card, S., Stewart, A. and Jones, E.E. (2009). Use of rifampicin-resistant bacterial biocontrol strains for monitoring survival in soil and colonisation of pea seedling roots. *New Zealand Plant Protection*,62:34-40.

Brimecombe, M.J., De Lelj, F.A. and JM Lynch, J.M. (2001). The rhizosphere. the effect of root exudates on rhizosphere microbial populations, Pinton, R., Varanini, Z. and Nannipieri, P. (Eds.), The rhizosphere. biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York, pp. 95-140.

Fischer, S., Jofré, E., Cordero, P., Gutiérrez Mañero, F. and Mori, G. (2010). Survival of native Pseudomonas in soil and wheat rhizosphere and antagonist activity against plant pathogenic fungi. *Antonie Van Leeuwenhoek*, 97 (3): 241-251.

Frey-Klett, P., Churin, J.-L., Pierrat, J.-C. and Garbaye, J. (1999). Dose effect in the dual inoculation of an ectomycorrhizal fungus and a mycorrhiza helper bacterium in two forest nurseries. *Soil Biology and Biochemistry*, 31(11): 1555-1562.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, 286(5776): 885-886.

Liu, F., Xing, S., Ma, H., Du, Z. and Ma, B. (2012). Plant growth-promoting rhizobacteria affect the growth and nutrient uptake of Fraxinus americana container seedlings. *Appl Microbiol Biotechnol*.

Lucas, J.A., Ramos Solano, B., Montes, F., Ojeda, J., Megias, M. and Gutierrez Mañero, F.J. (2009). Use of two PGPR strains in the integrated management of blast

disease in rice (Oryza sativa) in Southern Spain. *Field Crops Research*, 114(3): 404-410.

Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J. and Setlow, P. (2000). Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews*, 64(3): 548-572.

Orhan, E., Esitken, A., Ercisli, S., Turan, M. and Sahin, F. (2006). Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Scientia Horticulturae*, 111(1): 38-43.

Pandey, A. and Palni, L.M. (1997). Bacillus species: the dominant bacteria of the rhizosphere of established tea bushes. *Microbiol Res*, 152(4): 359-365.

van Elsas, J.D. and Overbeek, L.S.v. (1993). Bacterial responses to soil stimuli, Kjelleberg, S. (Ed.), Starvation in bacteria. PlenumPress, New York, pp. 55-79.

van Veen Ja Fau - van Overbeek, L.S., van Overbeek Ls Fau - van Elsas, J.D. and van Elsas, J.D. (1997). Fate and activity of microorganisms introduced into soil, Microbiology and Molecular Biology Reviews.

Wei, Z., Yang, X., Yin, S., Shen, Q., Ran, W. and Xu, Y. (2011). Efficacy of Bacillus-fortified organic fertiliser in controlling bacterial wilt of tomato in the field. *Applied Soil Ecology*, 48(2): 152-159.

Yadav, S.K., Dave, A., Sarkar, A., Singh, H.B. and Sarma, B.K. (2013). Co-inoculated biopriming with *Trichoderma*, *Pseudomonas* and *Rhizobium* improves crop growth in *Cicer arietinum* and *Phaseolus vulgaris*. *International Journal of Agriculture, Environment & Biotechnology*, 6(2): 255-259.

Yan, Z., Reddy, M.S. and Kloepper, J.W. (2003). Survival and colonization of rhizobacteria in a tomato transplant system. *Can J Microbiol*, 49(6): 383-389.