

**COLONIZATION OF TOMATO ROOT BY ANTAGONISTIC BACTERIAL STRAINS TO FUSARIUM WILT OF TOMATO**

**KEMAMPUAN STRAIN BAKTERI ANTAGONIS TERHADAP FUSARIUM PENYEBAB LAYU PADA TOMAT DALAM KOLONISASI PERAKARAN TOMAT**

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**INTISARI**

Penyakit layu fusarium yang disebabkan oleh *Fusarium oxysporum f.sp. lycopersici* (Fol) merupakan penyakit penting pada tanaman tomat yang mengakibatkan kerusakan yang besar pada berbagai daerah penghasil tomat di dunia. Penelitian ini bertujuan untuk mempelajari kemampuan strain bakteri antagonis (H5, H22, H63, H71, *Burkholderia cepacia* strain 65 and 526) terhadap *Fusarium oxysporum f.sp. lycopersici* dalam mengkolonisasi perakaran tomat. Selain itu penelitian ini juga mempelajari pengaruh kerapatan inokulum bakteri antagonis serta suhu udara terhadap kolonisasi serta penyebaran bakteri antagonis tersebut pada perakaran tomat. Hasil penelitian menunjukkan bahwa dari 6 strain bakteri yang diteliti, 4 di antaranya populasinya pada suhu 28°/23° C (siang/ malam), 14 hari setelah tanam lebih besar dibandingkan pada suhu 23°/18° C. Meskipun pengaruh suhu udara terhadap populasi bakteri tidak berbeda secara signifikan, akan tetapi kemampuan tiap-tiap strain bakteri untuk mengkolonisasi akar berbeda secara signifikan. Tiga strain bakteri antagonis (H5, *B. cepacia* strain 65 dan 256) dapat bertahan dengan baik pada rhizosfer dan setelah 4 minggu setelah tanam, populasinya tidak berbeda secara signifikan dengan pada pengamatan 2 minggu setelah tanam. Adapun 3 strain bakteri antagonis yang lain (H5, H22 dan H63) populasinya menurun 4 minggu setelah tanam. Populasi bakteri paling besar terdapat pada daerah pangkal akar tanaman tomat yang besarnya bervariasi tergantung dari strain bakteri antagonisnya dimana selisihnya mencapai  $\log_{10} 2.7 \text{ cfu cm}^{-1}$  akar. Populasi bakteri pada daerah perakaran yang lain besarnya juga tergantung dari strain bakteri antagonisnya. Sebagai contoh, strain H71 mampu untuk mengkolonisasi akar pada populasi yang tinggi, sedangkan strain H63 tidak.

Kata kunci : Kolonisasi akar tomat, strain bakteri antagonis, layu *Fusarium* pada tomat

**ABSTRACT**

Fusarium wilt of tomato caused by *Fusarium oxysporum f.sp. lycopersici* (Fol) is an important disease in tomato which cause a significant loss of yield in major growing regions of the world. This study examined the ability of bacterial strains antagonistic to *F. oxysporum f.sp. lycopersici* (H5, H22, H63, H71, *Burkholderia cepacia* strain 65 and 526) to colonize tomato seedlings and the effect of plant growth. The effect of bacterial population size and air temperature on the bacterial colonization and their spread along the root systems was also assessed. The results

of this study showed that the bacterial population at 28°/23° C day/night temperature 14 days after planting was significantly greater than at 23°/18° C for 4 of 6 strains tested. Although there was no significant effect of temperature on bacterial population observed in this study, the ability of the bacterial strains to colonize the rhizosphere was significantly different. Three strains (H5, *B. cepacia* strains 65 and 526) survived well in the rhizosphere and at 4 weeks after planting rhizosphere populations g<sup>-1</sup> fresh root were not significantly different from those recovered 2 weeks after planting. However strains H5, H22 and H63 decreased in population size between 2 and 4 weeks after planting. The largest population of the bacterial inoculants developed in the basal region of the roots and this differed between strains by log<sub>10</sub> 2.7 cfu cm<sup>-1</sup> root. The bacterial populations in other parts of the root were also strain dependent. Strain H71, for example, was able to colonize the root segments at a high population level. However strain H63 was recovered only in a small number in all root segments.

Key words : Colonization of Tomato root, Bacterial strain antagonistic, Fusarium wilt tomato

## INTRODUCTION

Fusarium wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) is an important disease in tomato which cause a significant loss of yield in major growing regions of the world (Lemanceau and Alabouvette, 1991). The fungus occurs as three races, a third race of Fol causing similar vascular wilt symptoms on tomato cultivars resistant to race 2 occurred in Queensland in 1982 (Gratidge and O'Brien, 1982).

Fungicides do not give adequate protection against fusarium wilt. Therefore alternative ways to suppress fusarium wilt of tomato such as biological control need to be developed. Wibowo *et al.*, (1999) showed that of 88 antagonistic bacterial strains isolated from lucerne, 27 strains could inhibited the growth of Fol *in vitro*. Of 27 antagonistic bacterial strains, only 9 strains could inhibit the germination of the fungal conidium *in vitro*. In the glasshouse trial only bacterial strain H5, H22, H63 and H71 reduced the Fol stem infection of tomato seedlings. The percentage of Fol stem infected of tomato seedling treated with these antagonistic bacterial strains varied from 25 to 34% whereas the percentage of Fol stem infected at control treatment was 61%.

The establishment of beneficial bacteria on root systems through seed inoculation has long been studied. Microorganisms that can grow well in the rhizosphere are well suited to act as biocontrol agents. Rhizobacteria mainly depend on root exudates as substrates for their nutrition and growth. Root exudates play an important role in establishment and maintenance of rhizosphere populations of young plants. Both the composition and the amount of exudates are influenced by environmental conditions, but are mainly under genetic control. (Parke, 1991).

Colonization of the plant root is also important for the plant beneficial effect of plant growth promoting rhizobacteria (PGPR). Several factors contribute to the differences in rhizosphere competence among rhizobacteria. Species that have a capacity to grow quickly are likely to become dominant (Simons *et al.*, 1996). The rates of utilization of root exudates may determine different degrees of competence (Jjemba and Alexander, 1999).

Differences in the extent of bacterial attachment to the root surface may also affect the rhizosphere competence. Bacterial characteristics involved in the early stage of adherence to roots include the presence of

pili (Vesper, 1987), of root adhesive protein and surface charge protein (Glandorf *et al.*, 1994). Certain saprophytic pseudomonads and other rhizobacteria interact with root agglutinins present in the root exudates (Chao *et al.*, 1988). To monitor the bacterial population size in soil and root systems, naturally occurring rifampicin resistant (Rif) mutants were selected from the population of the wild type strains selected in the bioassay

This study examined the ability of bacterial strains antagonistic to Fol to colonize tomato seedlings and the effect of plant growth. The effect of bacterial population size and air temperature on the bacterial colonization and their spread along the root systems was also assessed.

## MATERIALS AND METHODS

**A. Selecting rifampicin (Rif) resistant mutants from the antagonistic bacterial strains.** The Rif mutants of wild type strains (H5, H22, H63, H71, *Burkholderia cepacia* strain 65 and 526) were obtained by a dilution plating method on NA plates containing 50 or 100  $\mu\text{g}$  rifampicin  $\text{mL}^{-1}$ . The wild type strains cultured on NA slopes were suspended into 5 mL of sterile deionised water. Ten fold serial dilutions were spread (0.1 mL of the suspension) onto NA plates containing rifampicin. Single colonies of Rif mutants were isolated from the plates after incubation for 48 h at 28°C and assessed for their antibiosis towards Fol *in vitro*. The wild types and Rif mutants were assessed separately with 4 replications. The genetic stability of these mutants was assessed by sub-culturing the mutants on NA slopes 10 times, and then streaking them on NA slopes containing rifampicin.

**B. Effect of temperature on root colonization by the bacterial strains.** Immediately after planting the seeds, 1 mL of bacterial suspension at  $10^{10}$  cells  $\text{mL}^{-1}$  was pipetted into each planting

hole after which 30 mL of bacterial suspension was poured evenly onto the pot. The pots were maintained in controlled environment glass houses at 23°/18° and 28°/23° C day/night temperatures. Pots were watered daily by adding 50 mL of deionised water to saucers placed beneath pots. Two weeks after planting, seedlings were harvested and shoot weight and the bacterial population size in the rhizosphere measured.

**C. Bacterial inoculum level and root colonization.** After planting the seed, 1 mL of bacterial suspension at  $10^6$ ,  $10^8$  or  $10^{10}$  cells  $\text{mL}^{-1}$  was pipetted into each planting hole after which 30 mL of bacterial suspension was poured evenly onto the pot. The pots were maintained in a controlled environment glass house at 28°/23° C day/night temperature. Pots were watered every day by adding 50 mL deionised water to saucers placed beneath pots. Two weeks after planting, seedlings were harvested and the shoot weight and the bacterial population size in the rhizosphere measured.

**D. Bacterial spread on the root systems.** After planting the seed, 1 mL of bacterial suspension at a concentration of  $10^{10}$  cells  $\text{mL}^{-1}$  was pipetted into each planting hole after which 30 mL of bacterial suspension was poured evenly onto the pot. The pots were maintained in a controlled environment glass house at 28°/23° C day/night temperature. Pots were watered every day by adding 50 mL deionised water through the saucers placed beneath pots. Four weeks after planting, seedlings were harvested and the bacterial population size along root segments measured.

**E. Quantification of rhizosphere soil and root bacterial population**

### 1. Rhizosphere soil

Tomato seedlings were carefully

taken out from the pots and the roots with soil loosely attached were then transferred into a 250 mL Erlenmeyer flask filled with 100 ml of sterile deionised water with ten 2 mm diameter glass beads. The flask was shaken for 30 min at 250 rpm with a rotary shaker. Ten fold serial dilutions were prepared by adding 1 mL of the suspension into 9 mL of sterile deionised water after which 0.03 mL of the dilution was pipetted onto nutrient agar plates and incubated for 24 h at 28° C. The number of viable cells was calculated as  $(1/0.03) \times$  number of colonies  $\times$  dilution factor.

## 2. Root segments

The tomato root was washed, weighed and the main root cut into 4 cm segments. Each segment was macerated separately in sterile deionised water using a sterile mortar and pestle. Ten fold serial dilutions were prepared by adding 1 mL of the suspension into 9 mL of sterile deionised water after which 0.03 mL of the dilution was pipetted onto nutrient agar plates and incubated for 24 h at 28° C. The number of viable cells was calculated as  $(1/0.03) \times$  number of colonies  $\times$  dilution factor.

## RESULTS

**A. Selecting rifampicin (Rif) resistant mutants from the antagonistic bacterial strains.** The Rif mutant was picked up from a single colony from the 10 dilution and only one single colony of the Rif mutant per strain was assessed in this test. The Rif mutants selected were genetically stable through 10 sub-cultures on NA slopes. Although the Rif mutants inhibited the fungal growth *in vitro*, some of them were less effective compared with the wild type strains. For example, after 7 days of incubation, the Rif mutant of strain

H4 inhibited the radial mycelial growth of Fol on PDA plates to 2 cm and this was significantly more growth than that of the wild type which inhibited radial mycelial growth of the fungus to 1.4 cm. Fungal growth in the absence of the bacteria was 4 cm (Fig. 1).

**B. Effect temperature on the bacterial root colonization.** At 28°/23° C day/night temperature the bacterial strains colonized roots better than at 23°/18° C. The statistical analysis conducted on log values showed that the population size of bacterial strains at 28°/23° and 23°/18° C were significantly different ( $p = 0.05$ ). At 28°/23° C the bacterial populations ranged from  $\log_{10}$  5.3 to 6.6 cfu g<sup>-1</sup> fresh root, whereas populations of the strains at 23°/18° C ranged from  $\log_{10}$  4 to 5.4 cfu g<sup>-1</sup> fresh root.

Table 1 shows that of 6 strains tested at 23°/18° C, strains H5, H22, and H71 had similar population size (mean of  $\log_{10}$  4.9 cfu g<sup>-1</sup> fresh root). *B. cepacia* strains 65 and 526 also colonized the rhizosphere well. The population size of *B. cepacia* strain 65 was  $\log_{10}$  5.4 cfu g<sup>-1</sup> fresh root and strain 526 was  $\log_{10}$  4.7 cfu g<sup>-1</sup> fresh root. Strain H63 had the smallest population size of  $\log_{10}$  4 cfu g<sup>-1</sup> fresh root.

At 28°/23° C, similarly large population sizes were developed by strains H71 ( $\log_{10}$  cfu 6.6 g<sup>-1</sup> fresh root), strain H22 ( $\log_{10}$  6.5 cfu g<sup>-1</sup> fresh root), *B. cepacia* strain 65 ( $\log_{10}$  6.6 cfu g<sup>-1</sup> fresh root) and strain 526 ( $\log_{10}$  6.4 cfu g<sup>-1</sup> fresh root). At this temperature strain H63 was the poorest colonizer ( $\log_{10}$  5.4 cfu g<sup>-1</sup> fresh root), but this was one log value greater than at 23°/18° C for this strain.

**C. Bacterial inoculum level and root colonization.** Table 2 shows the effect of initial inoculum population on the population of the bacterial strain in the rhizosphere after 2 weeks growth. Statistical analysis showed that there was no interaction between initial inoculum level and the bacterial populations developed in the rhizosphere. All the strains multiplied in

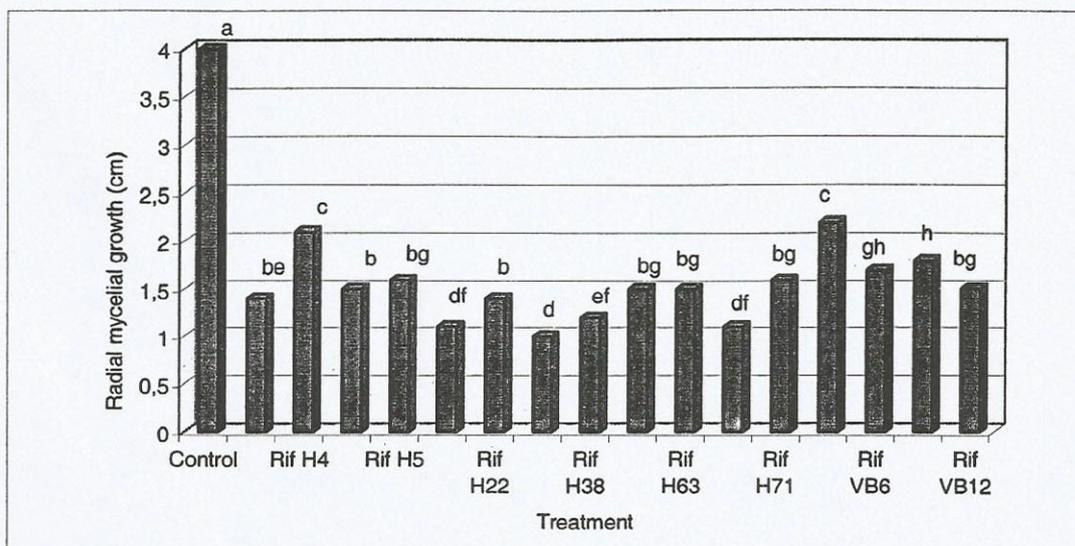


Fig. 1. In vitro antagonism against *F. oxysporum* f.sp. *lycopersici* of bacterial strains selected for rifampicin resistance. H5 was the wild type strain, Rif H5 was the mutant. Bars with the same letters were not significantly different at  $P=0.05$

Table 1. Effect of temperature on the population size of bacterial strains on 2 weeks old tomato root system ( $\log_{10}$  cfu  $g^{-1}$  fresh weight). Rif H5 was the mutant of strain H5

Bacterial Strains	Log 10 cfu $g^{-1}$ fresh weight		Mean (strain)
	Day and night temperature ( $^{\circ}C$ )		
	23/18	28/23	
Rif H5	5.1	5.9	5.5
Rif H22	4.6	6.5	5.5
Rif H63	4.0	5.3	4.7
Rif H71	5.1	6.6	5.9
65	5.4	6.6	6.1
526	4.7	6.4	5.6
Mean (temperature)	4.9	6.3	5.5
LSD for strain	0.65 ( $P = 0.05$ )		
LSD for temperature	0.73 ( $P = 0.05$ )		
LSD for strain x temperature interaction	Not significant		

the rhizosphere to a similar population even though the initial inoculum population differed by 10,000 fold. There was a slight increase in number with an inoculum of  $10^{10}$  cells  $\text{mL}^{-1}$  compared with  $10^6$  cells  $\text{mL}^{-1}$ . However the ability of the bacterial strains to colonize the rhizosphere was significantly different ( $p = 0.05$ ). At an initial inoculum level of  $10^6$  cells  $\text{mL}^{-1}$  the population size of the bacterial strains in the rhizosphere ranged from  $\log_{10}$  5.5 to 6.3  $\text{cfu g}^{-1}$  fresh root. Strain H71 ( $\log_{10}$  6.3  $\text{cfu g}^{-1}$  fresh root), strain H5 ( $\log_{10}$  6.1  $\text{cfu g}^{-1}$  fresh root), and strain H22 ( $\log_{10}$  6.1  $\text{cfu g}^{-1}$  fresh root) developed the largest populations. Strain H63 as well as *B. cepacia* strain 65 and 526 had the smallest population size of less than  $\log_{10}$  5.8  $\text{cfu g}^{-1}$  fresh root.

**D. The survival of the bacterial strains in the rhizosphere.** The bacterial inoculant strains were recovered from the rhizosphere 4 weeks after planting in large numbers, but differed in their survival. Figure 2 shows that 3 of the 6 strains tested, strain H71, *B. cepacia* strains 65 and 526, colonized the rhizosphere in large numbers, varying from  $\log_{10}$  6.3  $\text{cfu g}^{-1}$  fresh root for strain H71 to  $\log_{10}$  7  $\text{cfu g}^{-1}$  fresh root for

strain 65. The population sizes of strains 65 and 526 were not significantly different ( $p = 0.05$ ). The rhizosphere populations of strains H5, H22, and H63 were smaller compared to those at 2 weeks at  $28^{\circ}/23^{\circ}$  C (refer to table 1). At 4 weeks the population of strain H5 was  $\log_{10}$  5  $\text{cfu g}^{-1}$  fresh root,  $\log_{10}$  5.5  $\text{cfu g}^{-1}$  fresh root for strain H22, and  $\log_{10}$  4.5  $\text{cfu g}^{-1}$  fresh root for strain H63.

**E. Spread of the bacterial strains along the root systems.** Table 3 shows the spread of the bacterial strains along the root system after plants were grown for 4 weeks in the controlled environmental glass house at  $28^{\circ}/23^{\circ}$  C day/night temperature. The spread of the bacteria along the roots was significantly different between strains and their abilities to spread were influenced by age/position of the root segments. The largest populations were at the base of the root (0-4 cm from the seed). The ability of the strains to colonize root segments varied considerably from  $\log_{10}$  3.8 to 6.5  $\text{cfu cm}^{-1}$  root in the basal region, from  $\log_{10}$  3 to 6  $\text{cfu cm}^{-1}$  root in the middle region, and from  $\log_{10}$  2.4 to 5.4  $\text{cfu cm}^{-1}$  root at the tip region of the roots.

Table 2. Effect of initial inoculum level of the bacterial strains on their population size in the rhizosphere of 2 weeks old tomato seedlings grown at  $28^{\circ}/23^{\circ}$  C day and night temperature. Rif H5 was the mutant of strain H5

Bacterial Strains	Log 10 $\text{cfu g}^{-1}$ fresh root			Mean (strain)
	Initial inoculum level (cells $\text{mL}^{-1}$ )			
	$10^6$	$10^8$	$10^{10}$	
Rif H5	6.1	6.2	6.3	6.2
Rif H22	6.1	5.8	6.1	6.0
Rif H63	5.6	6.0	6.1	5.9
Rif H71	6.3	5.8	6.5	6.2
65	5.5	6.1	6.1	5.9
526	5.7	5.7	6.0	5.8
Mean (initial inoculum level)	5.9	5.9	6.2	
LSD for strain				0.45 ( $P = 0.05$ )
LSD for initial inoculum level				0.58 ( $P = 0.05$ )
LSD for strain x initial inoculum level interaction				Not significant

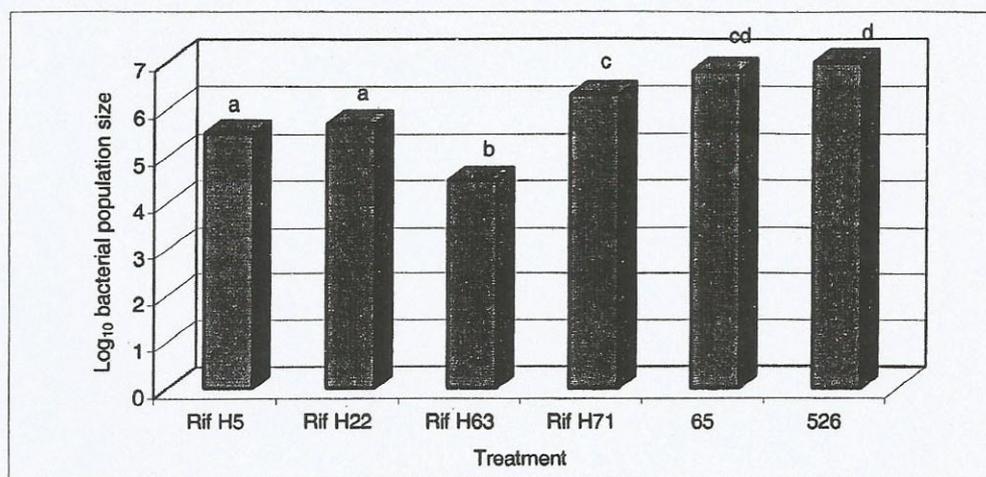


Fig. 2. The population size of the bacterial strains in the rhizosphere 4 weeks after planting at 28°/23° C day and night temperature. Rif H5 was the mutant of strain H5. Bars followed by the same letters were not significantly different at P=0.05

Table. 3. The population size of the bacterial strains in different root segments 4 weeks after planting at 28°/23° C day and night temperature. Rif H5 was the mutant of strain H5

Bacterial strains	Log 10 cfu cm <sup>-1</sup> root Root segments			Mean (strain)
	Base	Middle	Tip	
Rif H5	5.5	5.2	4.5	5.0
Rif H22	5.9	5.6	3.6	5.1
Rif H63	3.8	3.0	2.4	3.1
Rif H71	6.5	6.0	5.4	6.0
65	5.8	5.1	3.6	4.8
526	5.5	5.2	3.5	4.1
Mean (initial inoculum level)	5.5	5.0	3.8	
LSD for strain			0.26 (P = 0.05)	
LSD for root segments			0.41 (P = 0.05)	
LSD for strain x root segments interaction			0.18 (P = 0.05)	

The ability of the strain to colonize root segments varied for each strain. At the basal root region, the largest populations were formed by strain H71 ( $\log_{10}$  6.5 cfu cm<sup>-1</sup> root), followed by strain H22 ( $\log_{10}$  5.9 cfu cm<sup>-1</sup> root), strain H5 ( $\log_{10}$  5.5 cfu cm<sup>-1</sup> root), and the poorest colonizer at this region was strain H63 ( $\log_{10}$  3.8 cfu cm<sup>-1</sup> root). The populations of these strains were significantly different ( $p = 0.05$ ).

A similar result was observed for the middle root region. The population of strain H71 was against the largest ( $\log_{10}$  6 cfu cm<sup>-1</sup> root), followed by strain H22 ( $\log_{10}$  5.6 cfu cm<sup>-1</sup> root), strain H5 ( $\log_{10}$  5.2 cfu cm<sup>-1</sup> root), and strain H63 ( $\log_{10}$  3 cfu cm<sup>-1</sup> root).

At the tip region of the root, strain H71 colonized the root segment with only 1 log unit decline from the basal region at  $\log_{10}$  5.4 cfu

cm<sup>-1</sup> root. This population size was significantly larger than that of strain H5 (log<sub>10</sub> 4.5 cfu cm<sup>-1</sup> root), strain H22 (log<sub>10</sub> 3.6 cfu cm<sup>-1</sup> root), strain 65 (log<sub>10</sub> 3.6 cfu cm<sup>-1</sup> root), and strain 526 (log<sub>10</sub> 3.5 cfu cm<sup>-1</sup> root). The root length colonized was approximately 12 cm.

## DISCUSSION

The Rif mutants selected for this study were genetically stable and had similar effect on Fol growth *in vitro* as the wild type parental cultures. This indicated that the behavior of the mutants was comparable to the wild types and thus they could be used to indicate how the parental strains might behave as inoculants in populating the rhizosphere. Rifampicin (Rif) resistance is the most commonly used marker to study population dynamics and survival of plant growth promoting and disease suppressing rhizobacteria after their introduction in the rhizosphere (Bull *et al.*, 1991). However there is a possibility that the Rif mutants may be unstable under field condition after application as seed inoculants (Glandorf *et al.*, 1992).

The competitive ability of the mutants should be tested in the future. This can be conducted by co-inoculating both the mutant and the wild type strain onto the seeds and then observing the populations of the mutant in the rhizosphere in comparison with mutant alone inoculation.

The results of this study showed that the bacterial population at 28°/23° C day/night temperature 14 days after planting was significantly greater than at 23°/18° C for 4 of 6 strains tested. Temperature can modify the amount and kind of root exudates which regulate soil microbial populations (Curl and Truelove, 1986). Janes *et al.* (1988) observed that the optimal temperature for *in vivo* growth of tomato seedlings is 28° C. The growth stimulation of the plant at this temperature may enhance root exudations and larger bacterial populations.

Although there was no significant effect of temperature on bacterial population observed in this study, the ability of the bacterial strains to colonize the rhizosphere was significantly different. For example, at 28°/23° C, 14 days after planting, the largest bacterial populations in the rhizosphere were for strain H71 and *B. cepacia* strain 65 (log<sub>10</sub> 6.6 cfu g<sup>-1</sup> fresh weight root), and the smallest bacterial population was for strain H63 (log<sub>10</sub> 5.3 cfu g<sup>-1</sup> fresh weight root). At 23°/18° C, the largest bacterial population on the rhizosphere was *B. cepacia* strain 65 (log<sub>10</sub> 5.4 cfu g<sup>-1</sup> fresh weight root), whereas the smallest bacterial population was strain H63 (log<sub>10</sub> 4 cfu g<sup>-1</sup> fresh weight root). This experiment supports the concept of rhizosphere competence being strain specific with strains coming to a unique equilibrium population in the rhizosphere.

Seong *et al.* (1991) studied the effect of temperature on the population of pseudomonads on maize planted in non-sterile soil and found that high temperature increased the population of pseudomonads on the root systems. At 30° C, *P. fluorescens* colonized maize roots at log<sub>10</sub> 6 cfu g<sup>-1</sup> fresh weight root 7 days after seed germination. At 12 days after seed germination the population of *P. fluorescens* increased 10 fold. However at 18° C, *P. fluorescens* colonized maize root at log<sub>10</sub> 5 cfu g<sup>-1</sup> root fresh weight 7 days after seed germination and only log<sub>10</sub> 5.5 cfu g<sup>-1</sup> fresh root 12 days after seed germination.

The effect of the initial inoculum size on the rhizosphere population of the tomato root was strain specific. For example, at 14 days after planting strain H5 populations were not affected by initial inoculum level, whereas *B. cepacia* strain 65 developed smaller populations when applied at a smaller initial inoculum size. For *Pseudomonas* sp., *Mycoplasma* sp., *Curtobacterium* sp. in the rhizosphere of barley planted in sterile coarse sand, the inoculum population did not influence the colonization potential. Inoculation of barley seedlings at-

levels of  $\log_{10} 3$ ,  $\log_{10} 5$  and  $\log_{10} 7$  viable cells  $\text{mL}^{-1}$  resulted in rapid root colonization, with the mean over all bacterial population size of  $\log_{10} 7.7$  viable cells  $\text{mg}^{-1}$  dry weight root (Bennett and Lynch, 1981).

Hebbar *et al.* (1992b), however, who studied the effect of different inoculum densities on the ability of *B. cepacia* strain 526 to colonize and multiply in the rhizosphere of maize planted in non-sterile soil, observed that there were significant effects of initial inoculum size on subsequent rhizosphere populations. At initial inoculum level of  $\log_{10} 7$  cfu  $\text{mL}^{-1}$ , *B. cepacia* colonized the rhizosphere at  $\log_{10} 6.2$  cfu  $\text{g}^{-1}$  dry weight root two weeks after seed germination. This bacterial population was significantly larger than those recovered from smaller initial inoculum level treatments. For example at inoculum level  $\log_{10} 5$  cfu  $\text{mL}^{-1}$  the population size of this strain recovered from the rhizosphere was  $\log_{10} 5.1$  cfu  $\text{g}^{-1}$  dry weight root.

Three strains (H5, *B. cepacia* strains 65 and 526) survived well in the rhizosphere and at 4 weeks after planting rhizosphere populations  $\text{g}^{-1}$  fresh root were not significantly different from those recovered 2 weeks after planting. However strains H5, H22 and H63 decreased in population size between 2 and 4 weeks after planting. The decrease of the bacterial inoculant populations might involve antagonistic microorganisms and root secretion such as polyphenols and gallotannins or the failure of the inoculants to grow with the new root (Brown, 1974).

De Freitas and Germida (1992) studied the growth promotion by fluorescent pseudomonads on winter wheat planted in non-sterile soil under growth chamber conditions. After 10 days growth, the roots were harvested, washed, and blended. Inoculant populations growing on King's B medium containing nalidixic acid rifampicin were similar for inoculants. Populations on the roots ranged from  $\log_{10} 6$  to  $8$  cfu  $\text{g}^{-1}$  fresh weight root. However

at 90 days after planting, the bacterial populations on the roots had declined to  $\log_{10} 3$  to  $4$  cfu  $\text{g}^{-1}$  fresh weight root. Nautiyal (1997), however, observed that the population size of *P. fluorescens* NBR19926 on the roots of chickpea planted in field soil apparent be remained at a high level for 40 days after planting. Root populations were dilution plated on tryptone glucose yeast extract agar media containing rifampicin. The initial inoculum level was  $\log_{10} 6.6$  seed $^{-1}$  and at the 40 days population size was  $\log_{10} 8$  cfu  $\text{g}^{-1}$  dry weight root.

The largest population of the bacterial inoculants developed in the basal region of the roots and this differed between strains by  $\log_{10} 2.7$  cfu  $\text{cm}^{-1}$  root. The bacterial populations in other parts of the root were also strain dependent. Strain H71, for example, was able to colonize the root segments at a high population level. However strain H63 was recovered only in a small number in all root segments.

Hebbar *et al.* (1992a) showed that the seed inoculated *B. cepacia* strain 526 spread rapidly to the newly formed root surfaces of the maize seedling. The basal part of the root was colonized to a greater extent than the root tip region. Ten days after seed germination at initial inoculum level of  $\log_{10} 5.8$  cfu seed $^{-1}$ , the bacterial population size recovered from the basal region of the primary root was  $\log_{10} 5.9$  root $^{-1}$ , from the middle region of the primary root was  $\log_{10} 4.9$  root $^{-1}$ , and from the tip region of the root was  $\log_{10} 4.5$  root $^{-1}$ . Differences in populations between root segments might be due to the differences in the surface areas. The basal root region which was thicker than the other regions might support a larger population of the bacterial inoculants. The other possibility is that the younger root tip region might be able to support a more competitive community of strains from the soil, thus resulting in a smaller population of the inoculant. Another factor may also be the inability of the bacterial inoculant to

move along with the root tip as it grows.

## CONCLUSION

1. Although there was no significant effect of temperature on bacterial population observed in this study, the ability of the bacterial strains to colonize the rhizosphere was significantly different.
2. Three strains (H5, *B. cepacia* strains 65 and 526) survived well in the rhizosphere and at 4 weeks after planting bacterial populations g<sup>-1</sup> fresh root were not significantly different from those recovered 2 weeks after planting.
3. The largest population of the bacterial inoculants developed in the basal region of the roots. The bacterial populations in other parts of the root were strain dependent.

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