JASEM ISSN 1119-8362

Full-text Available Online at www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. December, 2007
Vol. 11(4) 63 - 66

Antagonism of dominant bacteria in tea rhizosphere of Indian Himalayan regions

ANCHAL SOOD; SHIVESH SHARMA; VIVEK KUMAR*; RAM LAL THAKUR

Department of Microbiology, Division of Life Sciences, SBS (PG) Institute of Biomedical Sciences and Research, Balawala, Dehradun. 248161. Uttaranchal (India).
*Corresponding author: Department of Soil and Water, Public Authority of Agriculture Affairs & Fish Resources, PO Box 21422, Safat, 13075 Kuwait; email: vivekbps@yahoo.com

ABSTRACT: Some parts of the Indian Himalayan region are covered by established and abandoned tea bushes. Rhizospheric soils of these plants were studied for bacterial dominance and antagonism. Amongst bacteria, *Bacillus* (up to 45%) and *Pseudomonas* (up to 85%) were found to dominate the rhizosphere of established and abandoned tea bushes, respectively. Amongst the species, *B. subtilis* and *B. mycoides* appeared to be closely associated with roots of established tea bushes, and the rhizosphere of abandoned tea bushes was dominated by *Pseudomonas putida*. Four bacterial strains each of *B. subtilis* and *P. putida* were selected on the basis of maximum antibacterial activity. Incubation of indicator strains with different concentrations of bacteriocin exhibited bactericidal activity. Various species of *Bacillus* and *Pseudomonas* behaved antagonistically amongst themselves due to the production of bacteriocins under *in vitro* conditions. @ JASEM

The rhizosphere of established tea bushes have some specific characteristics, which are associated with long lived nature of tea plants viz. negative rhizospheric effect, lowering of soil pH, antagonistic activities among microbial communities and dominance of certain species (Lynch, 1987; Sood *et al*, 2007). The overall interactions amongst tea roots, microbes and environmental conditions prevailing in the tea rhizosphere seems to favor the growth of microbes, which are known to produce strong antibiotics with potential biocontrol agents. In rhizospheric niches, bacteria are constantly competing for nutrients and ecological space. As a consequence, bacteria have devised various offensive tools for intra and interspecies competition, such as antibiotic substances, bacteriolytic enzymes, and bacteriocins. In all likelihood, the bacteriocins constitute the most abundant and diverse class of antimicrobial agents (Parret *et al,* 2003). The purpose of this study was to establish whether or not tea rhizosphere *Bacillus* and *Pseudomonas* species produce bacteriocins like compounds and, if so, investigate their role in rhizosphere population dynamics of established and abandoned tea bushes.

MATERIALS AND METHODS

Isolation, enumeration and selection of dominant bacteria

A number of soil samples were taken at 0-15 cm depth from different tea gardens to isolate and enumeration of bacterial population, the counts were calculated by spread plate technique using Nutrient agar (HiMedia). These bacteria were identified up to genus level on the basis of their colony morphology and biochemical tests. *Bacillus subtilis* and *P. putida* strains due to their higher % age of occurrence have been studied for bacteriocin production. Four strains each of *B. subtilis* (HPAB7, HPAB12, HPAB13 and HPAB41) and *P. putida* (UAAP1, UAAP14, UAAP17 and UAAP122) were selected on the basis of maximum zone of antagonistic activities against indicator strains.

Detection of antimicrobial activity by the deferred agar spot test (DAS)

This method was carried out on tryptic soya agar or broth (TS) (pH 7.3 ± 0.2) (HiMedia) and Brain Heart Infusion Agar or broth (HiMedia) with 0.1 % glucose (BHIG) (pH 7.4 ± 0.2). Ten µl of culture of *Bacillus* and *Pseudomonas* test strains grown for 7 to 8 h in TS and BHIG broth were spot inoculated on the surface of TS and BHIG agar, respectively. After 18-24 h of incubation at 30°C, a soft overlay of media TS, BHIG, MRS or YDC (5 ml, 0.75 % agar), suitable for indicator strains, inoculated with 100 µl of indicator culture in the stationary phase (approximately 10^5 cells ml^{-1}), were pored over the surface of spot inoculated TS or BHIG agar. Inhibition zones were observed after 24-48 h of incubation under appropriate conditions. Clear zones of inhibition with sharp edges around spots were considered as possible results.

Effect of bacteriocin on indicator strain

An exponentially growing culture of the indicator strain of *B. mycoides* and *P. syringae* (10^7 cells/ml) was suspended in 50 mM phosphate buffer (pH 6.0) and exposed for a maximum of 140 min to various concentrations of bacteriocin (0, 25, 40 and 75 µg/ml). At different times the survival count of bacteria (cfu/ml) was determined using the AWD plate count method.

* Corresponding author: Anchal Sood

RESULTS AND DISCUSSION:

Various species belonging to the genus *Bacillus* were observed and *B. subtilis* and *B. mycoides* dominated the rhizosphere in established tea bushes. Apart from these two species, a number of other species of *Bacillus* were also observed, *B. polymyxa* 15.1 % and *B. cereus* 14.8 % (Table 1).

Table 1. Dominance of bacterial isolates in tea rhizosphere of Himalayan regions as indicated by occurrence (%)

S. No.	Isolates	Occurrence (%)
	Himachal Himalayas	
1	*B. subtilis*	45.3
2	*B. mycoides*	17.3
3	*B. polymyxa*	15.1
4	*B. cereus*	14.8
	Uttaranchal Himalayas	
1	*Pseudomonas putida*	85.6
2	*P. fluorescens*	41.3
3	*P. cepacia*	26.8
4	*P. syringae*	13.9
5.	*B. subtilis*	7.8
6.	*B. mycoides*	5.0
7.	*B. polymyxa*	4.5
8.	*B. cereus*	3.3

Values are mean of three replications

Tea bushes exhibit several remarkable features, e.g. the negative rhizosphere effect, strong antagonistic activities amongst microbial communities in the rhizosphere (Pandey and Palni, 1996; Pandey *et al,* 1997). These factors collectively, may have helped in the development of a particular bacterial population which is well adapted in the tea rhizosphere. Apart from these dominant isolates a number of other unidentified isolates besides species of *Azotobacter*, *Agrobacterium*, Phosphate solublizers etc. were also isolated but were not used for further studies, as their occurrence percentage was very low. These observations are indicative of microbial diversity in tea rhizosphere.

To determine whether bacteriocin had a bactericidal or bacteriostatic effect, various concentrations of the bacteriocin (produced by selected dominant bacterial strains) was added to the indicator *B. mycoides* and *P. syringae* strains. The survival count of the bacteria was determined using the DAS method at different time intervals of bacteriocin addition. The incubation of *B. mycoides* cells with bacteriocin decreased the cfu count; therefore, it indicates the bactericidal activity of bacteriocin (Fig. 1 and 2). With the increase in bacteriocin concentration and incubation time the bactericidal effect increased. The proteinaceous nature of bacteriocin seems to be responsible for this activity, since there was loss of bacteriocin activity after treating with proteolytic enzymes (Ward and Somkuti, 1995).

Most strains of *B. subtilis* were antagonistic to *B. mycoides* and its growth was often inhibited. *Bacillus subtilis,* which was the most dominant bacteria of the tea rhizosphere exhibited best antagonistic activity against *B. mycoides* and inhibited its growth on dilution plates as well as after obtaining pure cultures. Two strains of *B. subtilis* also inhibited the *B. polymyxa* and *B. cereus*. *Pseudomonas putida* also behaved antagonistically towards each other. The species *P. syringae* was maximum inhibited by *P. cepacia* and *P. fluorescens* (Table 2). This antagonism by *B. subtilis* and *P. putida* was due to the production of bacteriocin (Riley and Gordon, 1999). In some other cases specific inhibitions have been observed due to various non specific compounds including hydrogen peroxide, lactic acid and ammonia (Vidaver, 1983). In this way, in established tea bushes, the best adapted species of *Bacillus* (due to spore formation) is also the most dominant species (due to antagonistic activities) in tea rhizosphere compared to the other *Bacillus* species. In abandoned tea bushes *Pseudomonas putida* due to its antagonistic behavior side lined other *Pseudomonas* species. It is interesting to note that dominant microbial species were those which are known to produce antimicrobial metabolites like bacteriocin. This may be a result of the constant interaction between the root exudates, soil microorganisms and triggered by different environmental signals.

Table 2: Antibacterial activity of *B. subtilis* and *P. putida* strains against other species of *Bacillus* and *Pseudomonas*.

	Bacillus subtilis				*Pseudomonas putida*			
	HPAB7	HPAB12	HPAB13	HPAB41	UAAP1	UAAP4	UAAP7	UAAP22
B. mycoides	+	+	+	-	-	-	-	-
B. polymyxa	+	-	+	-	-	-	-	-
B. cereus	-	+	+	-	-	-	-	-
P. fluorescens	-	-	-	-	-	-	+	+
P. cepacia	-	-	-	-	+	-	+	-
P. syringae	-	-	-	-	+	+	-	+

+ Antibacterial activity present
- Antibacterial activity absent

* Corresponding author: Anchal Sood

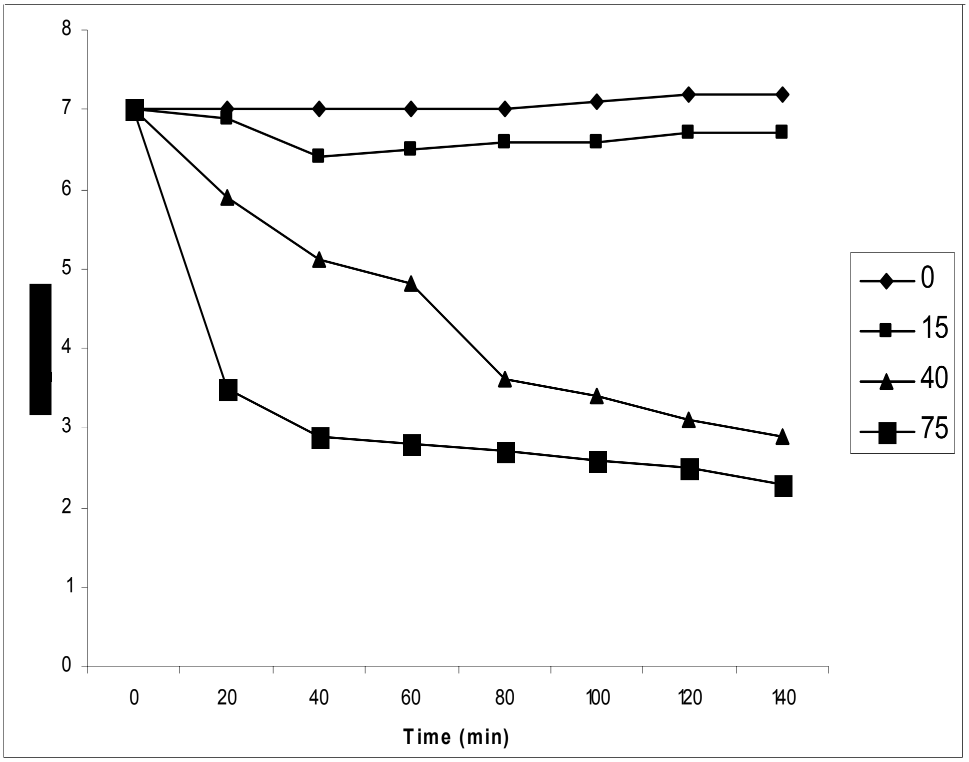

Fig. 1 Different bacteriocin concentrations (μg/ml) of *B.subtilis* strain HPAB12 affecting the growth of *B. mycoides*

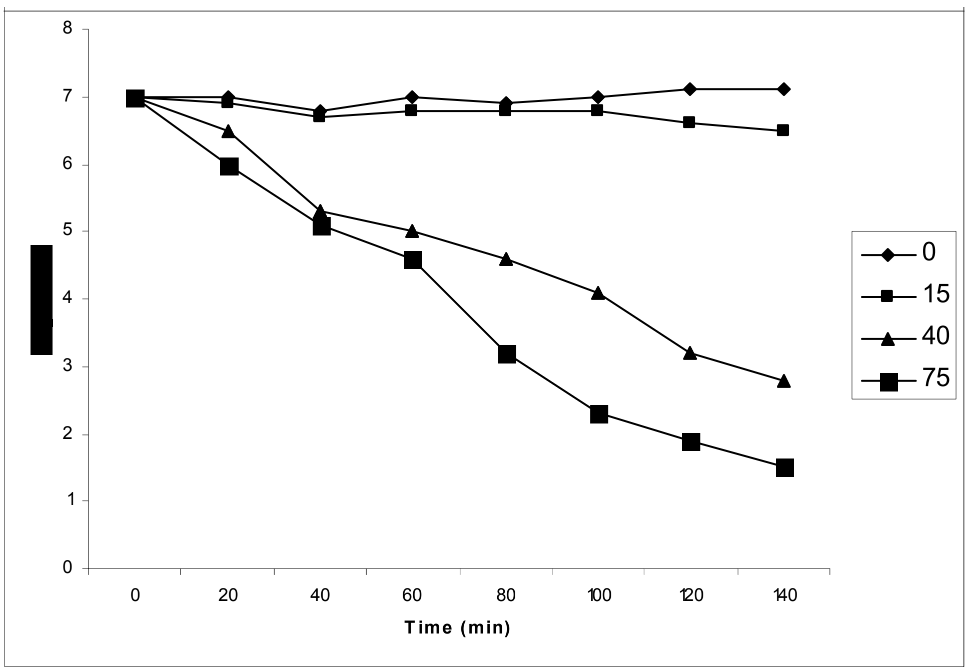

Fig. 2 Different bacteriocin concentrations (μg/ml) of *P.putida* strain UAAP1 affecting the growth of *P. syringae*

* Corresponding author: Anchal Sood

REFERENCES

Lynch, J M (1987). Biological control with microbial communities of the rhizosphere. In: Fletcher M and Gray TRG (eds) Ecology of microbial communities. Cambridge University Press: Cambridge; 55-82.

Pandey, A and Palni, L M S (1996). The rhizosphere effect of tea on soil microbes in a Himalayan monsoonal location. Biol. Fertl. Soil. 21: 131-137.

Pandey, A; Palni, L M S and Coulomb N (1997). Antifungal activity of bacteria isolated from the rhizosphere of established tea bushes. Microbiol Res. 152: 105-112.

Parret, A H A; Schoofs, G; Proost, P and Mot, R D (2003). Plant lectin-like bacteriocin from a rhizosphere-colonizing *Pseudomonas* isolate. J. Bacteriol. 185: 897-908.

Riley, M A and Gordon, D M (1999). The ecological role of bacteriocin in bacterial competition. Trends Microbiol. 7: 129-133.

Sood, A ; Sharma S and Kumar V (2007). Comparative efficacy of diffusible and volatile compounds of tea rhizospheric isolates and their use in biocontrol. Int. J. Biol. Chem. Sci. 1: 28-34.

Vidaver A K (1983). Bacteriocins: the lure and the reality. Plant Dis. 67: 471-475.

Ward D J and Somkuti G A (1995). Characterization of a bacteriocin produced by *Stereptococcus thermophilus* ST134. Appl. Microbiol. Biotechnol. 43: 330-335.

* Corresponding author: Anchal Sood