Topical Review

Tea Rhizosphere: Characteristic Features, Microbial Diversity and Applications

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ABSTRACT

In recent years tea (Camellia sinensis) rhizosphere has been examined in some detail, particularly in relation to plant-microbe interactions. The discovery of the presence of a 'negative rhizosphere effect' in established (not young) tea bushes is considered to be an interesting and a novel characteristic of tea rhizosphere. Other important and associated features include colonizatiton of tea rhizosphere soil pH, etc. These findings have opened up newer avenues for further investigations in the field of 'rhizosphere microbiology'. As the tea bushes grow old, their rhizosphere is colonized by relatively lesser number of microbes indicating increasing selectivity with age. Dominance of a specific microbial population, belonging to the community of general antagonists, represents a fine example of mutual selection in nature. In this paper an attempt has been made to review the distinguishing features associated with the microbial activity and microbial diversity in the tea rhizosphere, and potential applications for the tea industry.

Keywords : antagonistic fungi; antibiotic; beneficial microbes; biocontrol; bush age; disease diagnostics; hardening tissue culture; ice nucleating bacteria; microbial inoculants; pH; phyllosphere; plant nutrition; rhizosphere; -ve rhizosphere.

Introduction

Lorenz Hiltner (1904) was the first to recognize the importance of microbial activity associated with the root system in plant nutrition. He used the term "rhizosphere" to describe this zone of intense microbial activity around roots of the members of leguminosae. Since Hiltner's pioneering work, there have been continuous efforts to gain an in-depth understanding of the "rhizosphere microbiology" and the progress has been reviewed from time to time (Starkey 1958, Katznelson 1965, Rovira 1965, Russell 1973, Brown 1975, Barber 1978, Newman 1978, Curl 1982, Lynch 1987, Bowen and Rovira 1989, Chanway et al. 1991, Rovira 1991, Yang and Crowley 2000, Whipps 2001). The major components influencing the rhizosphere are: (1) the plant species; (2) the microbial communities on and around the root, and (3) the environmental conditions. Most experiments related to rhizosphere microbiology have been confined to annuals; perennials, tree species in particular, have received much less attention (Ivarson and Katznelson 1960, Rangaswami and Vasanthrajan 1962, Dangerfield et al., 1975, 1978).

Tea (Camellia sinensis) rhizosphere has been investigated in recent times in relation to plant microbe interactions. Some of the specific features associated with tea plantations are: (1) it is grown in more than 50 countries around the world from Georgia (43°N latitude) to Nelson (South Island; 42°S latitude) in New Zealand from sea level to 2300 m above mean sea level with air temperature varying between -8° to 35°C and 1,150 to 8,000 mm annual precipitation; (2) it grows best in soils with pH between 4.5 and 5.6; (3) tea is a small tree in nature which is maintained as a shrub by continuous pruning cycles; and (4) it is often grown as monoculture over large areas (for details see Barua 1989, Willson and Clifford 1992, Banerjee 1993, Jain 1999). In this paper an attempt has been made to review the distinguishing features associated with the microbial activity in tea rhizosphere and possible applications in the tea industry; it has been arranged under the following heads:

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1. The rhizosphere effect

In general, the rhizosphere is a zone of greater microbiological activity than the corresponding root-free soil. Plant roots exert a strong influence on soil microorganisms resulting in the "rhizosphere effect" which is defined as the overall positive influence of plant roots on "soil microorganisms". The influence of an individual plant in the rhizosphere is reflected as the R:S (rhizosphere to non rhizosphere) ratio. For bacteria and fungi R:S values commonly range from 5 to 20; actinomycetes, somewhat less affected by the rhizosphere, typically exhibit R:S values in the range of 2 to 12 (Curl and Truelove 1986). This numerical value for the rhizosphere: soil ratio (Katznelson 1946) has been in use as an index of the rhizosphere effect on the soil microbial populations. Rovira (1965) cited the example of genge (*Astragalas sinicus* L.), a legume, for which R:S ratios between 100 and 400 have been reported; values as high as 2000 are also on record (Fitter and Hay 1981). Rouatt and Katznelson (1961) studied R:S ratios for bacteria for different annual plants and found that the rhizosphere effect varied with the plant species, for example, ratios of 24.3, 5.5, 5.9, 3.3, 3.6, and 5.9 were recorded for red clover, flax, oat, maize, barley, and wheat, respectively. Though, reports on the rhizosphere effect in relation to perennial plants are limited, the available reports also indicate a positive influence of plant roots on soil microorganisms. Rangaswami and Vasanthrajan (1962) carried out experiments using three species of six-year old full grown citrus trees during periods of active growth and dormancy; ratios of 90-100, 5-6, and 3-4 for bacteria, actinomycetes and fungi, respectively were reported. Ivarson and Katznelson (1960) have also reported stimulation of microbial population in the rhizosphere of yellow birch, with R:S ratios ranging from 8 to 10.

Experiments carried out using soil samples collected from various tea gardens in India revealed several distinguishing features (Pandey and Palni 1996, Pandey and Palni 1997, Pandey et al. 1997, Pandey and Palni 1999). The sampled tea gardens are located in the Himalayan region representing subtropical or temperate conditions, are characterized as monsoonal, some experiencing snowfall as well. Details of the study sites are given in Table 1.

Table 1. Type and age of tea bushes and general climatic data for the study sites

Study sites	Type and Age (years) of tea bushes		Elevation m amsl	Mean monthly Temperature		Total rainfall
				Max. °C	Min. °C	(mm)
1. Banuri Tea Exp. Garden	Chinery	4,8 & > 100	1309	23.2	13.5	2460.6
2. Mansambal Tea Estate	Assamica	> 30	1309	23.2	13.5	2460.6
3. Temi Tea Estate	Chinery	4 & 15-20	1697	20.9	8.5	1806.2
4. Singell Tea Estate	Chinery	32, 44 & 123	1240	19.4	14.2	2187.2
5. Pant Tea Estate	Chinery	> 100	1577	23.5	10.1	1069.8

Source: Pandey and Palni 1997

The initial experiments, which were carried out at Banuri tea experimental garden and Mansambal tea estate, both in Kangra district of Himachal Pradesh, gave interesting and thought provoking results. Investigations carried out for a period of twelve months, at monthly interval in Banuri tea experimental garden, confirmed the stimulation of microbial populations in the rhizosphere of young tea plants as expected. Contrary to this, the rhizosphere of established tea bushes was found to inhibit the microbial populations as seen in terms of R:S values (Table 2). Microbial analyses of soil samples collected from Mansambal tea estate, where the bushes were of *assamica* type and the plantations were more than thirty years old, also revealed strong inhibition of rhizosphere microbial communities. Amongst the three microbial communities, bacteria, actinomycetes and fungi, bacteria were found to be the most suppressed group in the established tea rhizosphere. Actinomycetes and fungal populations were also suppressed but to a lesser extent. The stimulation of microbes in the rhizosphere ("rhizosphere effect") due to plant roots is a well known and general phenomenon and indicates a "positive" influence of plant roots on rhizosphere microorganisms. The term "**negative rhizosphere effect**" indicating suppression of microbes around the root zone has been used for the first time to the best of our knowledge, in case of rhizosphere of established tea plants. This, we believe, is an important observation and is against the general norm (Pandey and Palni 1996).

Similar experiments were also conducted from other tea plantations to confirm the status of rhizosphere effect in established bushes. The soil samples were obtained from various locations (1) Temi tea estate, Sikkim (2) Singell tea estate, Kurseong, and (3) Pant tea estate, Bhowali. The tea bushes were of various age groups. While Temi and Singell tea estates represented the well maintained tea plantations, Pant tea estate was an abandoned tea garden. In almost all cases, the R:S ratios showed similar trend as obtained from Kangra tea plantations, except in case of estimations of bacterial populations from Temi tea estate, that showed inhibitory effect even at 4 years

Table 2.
R:S ratios for three groups of microorganisms at three depths during a 12 month cycle

Months	**Bacteria**			**Actinomycetes**			**Fungi**		
	Soil Depth (cm)								
	0-15	**15-30**	**30-45**	**0-15**	**15-30**	**30-45**	**0-15**	**15-30**	**30-45**
Nov	0.29	0.08	0.04	0.63	0.46	0.30	0.51	0.44	0.40
Dec	0.59	0.57	0.42	11.2	0.08	0.16	0.80	2.00	-
Jan	0.91	0.93	0.74	1.29	0.13	1.10	0.26	0.79	1.00
Feb	0.99	0.86	0.84	0.39	0.66	0.47	1.24	2.33	-
Mar	0.44	0.36	1.01	0.24	1.76	0.57	1.55	0.89	-
Apr	0.51	0.51	0.44	0.85	0.92	1.04	0.86	0.82	0.22
May	0.48	0.57	0.09	0.80	0.56	0.23	0.81	0.38	-
Jun	0.64	0.50	0.09	0.65	0.62	0.22	0.65	0.26	-
Jul	0.65	0.16	0.73	0.93	1.16	0.48	0.82	0.83	0.48
Aug	0.62	0.56	0.92	0.52	0.50	0.56	0.66	1.17	0.38
Sep	0.16	0.26	0.31	0.34	0.56	0.25	1.23	1.23	0.77
Oct	0.80	0.90	1.06	0.73	0.30	0.06	0.76	0.94	0.76

R : S = Rhizosphere:Soil; - = not detected
Source: Pandey and Palni 1996

Table 3.
Influence of age of tea bushes on R:S ratios in three groups of microbes at different locations

Study site	Age of tea bush (years)	R : S ratio		
		Bacteria	Actinomycetes	Fungi
Banuri Tea Exp. Garden	4 8 >100	1.5 0.8 0.5	1.8 1.1 0.8	4.9 2.1 0.9
Mansambal Tea Estate	>30	0.1	0.5	0.8
Temi Tea Estate	4 15-20	0.6 0.4	1.1 0.5	1.3 1.1
Singell Tea Estate	32 44 123	0.3 0.3 0.5	0.4 0.5 0.7	1.3 1.1 0.8
Pant tea Estate	>100	0.9	0.9	1.1

Source: Pandey and Palni 1997

of age (Table 3). It was also observed that the 'negative rhizosphere effect' was more pronounced in case of well maintained tea gardens, where tea bushes were subjected to regular cultural practices in comparison of abandoned tea gardens. This was recorded from two sites, namely (1) Banuri tea experimental garden, where soil samples were also obtained from abandoned tea bushes growing along the margins of the tea garden, and (2) Pant tea estate, where only abandoned tea plantations were available (Table 3). In a recent study conducted from a newly developed tea garden at Bhimtal, positive rhizosphere effect has been reported in case of fungi and actinomycetes (Palni et al. 1998). Various factors that may contribute to lowering of microbial populations in established tea rhizosphere have been discussed in the next few pages.

(1.1) Root exudates

A major factor influencing the biological activity in and around the rhizosphere is the amount and nature of root exudate. Root exudates are low molecular weight compounds, including mostly water-soluble and some volatile compounds. Most of these substances are simple organic compounds (Rovira 1959, 1965). The root exudates may differ considerably both qualitatively and in terms of quantity depending on various factors, namely the plant species, age of the plant, soil nutrient status and environmental factors. (Katznelson 1965, Rovira 1965, Hale et al. 1971, Brown 1975, Hale and Moore 1979).

Experiments carried out in relation to tea rhizosphere indicated that tea roots, probably after attaining a certain age, start secreting exudates that contain antimicrobial metabolites to which bacteria are the most susceptible group. The antibacterial activity of tea root exudates was demonstrated by supplementing the culture medium with the rhizosphere soil extract. The five most abundant bacterial strains, originally isolated from the tea rhizosphere, were selected as test organisms. Marked reduction in the number of bacterial colonies was observed when the medium was supplemented with the rhizosphere soil extract. This clearly indicated that at least some of the antimicrobial metabolites accumulate in the rhizosphere soil, which would appear to be water-soluble and heat tolerant (Table 4; Pandey and Palni 1996). The assessment of quality and quantity of root exudates of tea bushes of various age(s) under natural conditions remains to be carried out.

Table 4.
Effect of rhizosphere soil on counts (colony forming units x 10^4 ml^{-1}) of five most abundant bacterial strains isolated from the rhizosphere of established tea bushes

Strain No.	Colony-forming units on		Reduction over control %
	TY agar + soil extract	TY agar (control)	
2	449	516	12.9
17	19	29	34.4
21	160	334	52.0
31	31	65	52.3
32	435	467	6.8

(TY: tryptone-yeast; values are an average of three readings)
Source: Pandey and Palni 1996

(1.2) Antagonistic activity

Colonization of rhizosphere and rhizoplane with populations of antagonistic microbial communities may be a major factor in reducing the overall number of microorganisms in the rhizosphere. This may include various categories of antagonism, such as competition, antibiosis, parasitism and predation. Enumeration of soil microbes is generally carried out using the serial dilution technique. It was interesting to observe that almost every soil dilution plate (from established tea rhizosphere) exhibited inhibition zones ranging from 1-12 in number, much more in comparison to control plates from the corresponding non-rhizosphere soil (Pandey et al. 1997, Pandey and Palni 1999). A closer examination of the changes in the bacterial and fungal populations in soil samples of tea rhizosphere and non-rhizosphere are suggestive of *in situ* interaction between these two groups of microbes in nature. In general, when the bacterial counts were very high, the fungal population was found to be low in the rhizosphere samples, and vice versa (Pandey et al. 1997). It is interesting to note that while an established tea rhizosphere typically exhibits the 'negative rhizosphere effect', it also harbours larger populations of antagonistic microorganisms in comparison to the corresponding non-rhizosphere soil.

(1.3) Soil pH

Tea plants prefer acidic soils; pH between 4.5 to 5.6 (Natesan 1999) is considered favourable for the growth of tea plants. Furthermore, available data for pH values of tea rhizosphere and the corresponding non-rhizosphere soil samples across various sites also indicate that the tea plantations lower the soil pH over a period of years (Table 5). In general, the pH of rhizosphere soil was found to be lower than that of the non-rhizosphere soil, and the negative rhizosphere effect was more pronounced in soils showing lower pH. Lowering of pH seems to be a factor contributing to reduced microbial populations in the established tea rhizosphere. Lowering of pH by tea plants under in vitro conditions has also been reported by Konishi et al. (1991); transfer of tea roots to a liquid medium caused lowering of pH from 5.5 to 4.0.

Table 5.
The effect of age of tea bushes on soil pH

Study site	Age (years)	Soil pH	
		Rhizo-sphere soil	Non-rhizosphere soil
Banuri Tea Exp. Garden	4 8 >100	5.5 5.3 4.9	5.9 (0.4) 5.8 (0.5) 5.9 (1.0)
Mansambal Tea Estate	>30	4.7	5.8 (1.1)
Temi Tea Estate	4 15-20	4.6 4.4	5.1 (0.5) 5.1 (0.7)
Singell Tea Estate	32 44 123	4.9 4.3 4.6	5.4 (0.5) 5.3 (1.0) 5.4 (0.8)
Pant Tea Estate	>100	6.0	6.1 (0.1)

Values within parentheses indicate difference in the pH of rhizosphere and non- rhizosphere soil
Source: Pandey and Palni 1997

(1.4) Climate and soil depth

During a study conducted over a period of twelve consecutive months at Banuri tea experimental garden, data on various climatic parameters, viz.

soil temperature, soil moisture, soil pH, atmospheric temperature and rainfall were also recorded. The soil samples were collected from three depths, 0-15 cm, 15-30 cm and 30-45 cm. Soil pH, as a single factor, was found to play an important role in determining the soil microbial population, as also discussed in the preceeding section. High rainfall affected a number of related parameters, e.g., abrupt fluctuations in climatic parameters like atmospheric as well as soil temperature, soil moisture were recorded following a heavy rainfall or an occasional snowfall. The three groups of microbial communities, bacteria, actinomycetes and fungi were found to respond differently to environmental changes. Out of various parameters examined, the most crucial single factors which were found to affect the bacterial, actinomycetes and fungal populations were soil temperature, soil pH and soil moisture, respectively (Pandey and Palni 1999).

In general, the bacterial population decreased with an increase in soil depth in established tea bushes and the negative rhizosphere effect was found to be more pronounced with increasing depth. R:S values as low as 0.29 in soil samples from 0-15 cm depth, 0.08 in samples from 15-30 cm depth and still lower (0.04) in samples from 30-45 m depth were recorded during November (Table 2). Few exceptions were also recorded. For example, during the peak winter (January and February) the maximum bacterial population was recorded in soil samples from the lowest depth, probably because of the natural insulation available in deeper soil layers. The actinomycetes population was found to decline in extreme winter months, and the optimal period for the proliferation of actinomycetes was from March to May, and then during September and October, when the temperature was moderate. In this case also, the maximum population was recorded at 0-15 cm depth. The highest fungal population was recorded during monsoon and these were adversely affected in winters. As far as the negative rhizosphere effect in established tea rhizosphere is concerned, it was the least affected group and the suppressive effect was more pronounced at deeper depths.

(1.5) Age of the tea bush

The rhizosphere effect may be detected within a few days of plant growth in short duration plants and the effect increases with plant age; generally it diminishes at senescence. Crop plants tend to exhibit much greater rhizosphere effect than tree species (Ivarson and Katznelson 1960, Dangerfield et al. 1978). In fact, in short lived plants, all processes related to plant growth take place at a faster pace; this also affects the plant-microbe interactions including the rhizosphere effect. Since plant growth and related processes are relatively slow in long lived plants, this may also influence the soil microbial populations at a slower rate. The magnitude of various activities in the rhizosphere seems to be highly affected by the plant species, together with the age of the plant. In the case of young tea rhizosphere which showed a positive rhizosphere effect, the R:S values recorded for bacteria, actinomycetes and fungi were as high as 5.3, 28.8, and 5.4, respectively. This indicated that after a certain age, the tea roots start producing antimicrobial metabolites causing suppression of microorganisms in the rhizosphere (Table 3; Pandey and Palni 1996).

2. Microbial diversity

Based on the biotic and abiotic factors, a given rhizosphere is dominated by certain microbial species. The rhizosphere of the established tea bushes on account of long lived nature of tea plants and other characteristics, e.g., the negative rhizosphere effect, is a remarkable example of natural selection. The locations selected for this study were monsoonal and spread across the Indian Himalayan region, some experiencing snowfall. Factors associated with the tea rhizosphere, mainly the amount and chemical composition of root exudates, various edaphic and environmental factors, and the cultural practices associated with tea plantations, would appear to collectively influence the development of a specific microbial population, well adapted to tea rhizosphere. Data obtained from various locations revealed that various species of *Bacillus* amongst bacteria dominated the established tea rhizosphere (Fig. 1; Pandey and Palni 1997). It was interesting to observe that *Pseudomonas*, a common soil bacterium was isolated from the soil samples collected from young tea rhizosphere; it was not detectable in the soil samples collected from established tea rhizosphere. Both *Bacillus* and *Pseudomonas*, are common soil inhabiting bacteria; species of *Bacillus* would seem to be better adapted for survival in the tea rhizosphere. *Bacillus subtilis* and *B. mycoides* were found in all the samples collected at a monthly interval for twelve months (Banuri Tea Experimental Garden). *Bacillus subtilis* was also dominant during extreme winters, i.e., January and February. Species of *Bacillus*, due to their spore forming nature, can survive under adverse conditions, and can be activated by a variety of treatments, notably exposure to heat (Sneath 1986, Slepecky and Hemphill 1992). During the winter months (January and February) most bacterial species fail to grow due to low soil temperature, and only a couple of species of *Bacillus* namely, *B. subtilis* and *B. mycoides* could be isolated on agar plates during these months. Their presence could be related to their endospore forming ability. It should also be noted that the competition amongst various species of *Bacillus* may also contribute towards dominance of these two species of *Bacillus* in winter. Most species of *Bacillus* were found to behave antagonistically, particularly towards *B. mycoides* (Pandey and Palni 1997).

Amongst fungi, species of *Penicillium* and *Trichoderma* were most dominant in the established tea rhizosphere. In case of *Penicillium*, the species differed at different locations and the population was found to fluctuate with changes in the overall climate (Banuri Tea Experimental Garden). The dominant species of *Penicillium*, identified from different locations were: *P. erythromellis*, *P. janthinellum*, *P. lanosum*, and *P. raistrickii*. Contrary to this only two species of *Trichoderma*, namely *Trichoderma konengii* and *T. pseudokonengii*, developed more or less stable population in the established tea rhizosphere. Their population did not fluctuate much with climatic variations. The fungi were able to survive even during the extreme winters (Banuri Tea Experimental Garden; Pandey and Palni 1999, Pandey et al. 2001). The populations of the two dominant fungi (i..e., species of *Penicillium* and *Trichoderma*) were seem to be inversely correlated (statistically significant) with the two most dominant rhizosphere bacteria, namely *Bacillus subtilis* and *B. mycoides* (Pandey et al. 2001). This is an interesting example of the expression of *in situ* antagonism, in addition to and in confirmation of results of earlier *in vitro* antagonism studies carried out on petridish based assays (Pandey et al. 1997). Details regarding population and abundance of fungi in newly planted tea bushes have been reported by Palni et al. (1998).

A large number of actinomycetes were also isolated from the soils, and observations were recorded on colony morphology, biochemical tests, pigmentation and sporulation. The most dominant actinomycetes isolated from the tea rhizosphere were found to form brown to gray coloured hard pustule like colonies, secreting diffusible brown pigment in the agar. These colonies were obtained from all the locations, and were able to survive during extreme winters when the temperature was well below 0°C. Based on many morphological and microscopic characteristics, these actinomycetes were grouped under *Streptomyces* (Shirling and Gottlieb 1966, Collins and Lyne 1980). Several other actinomycetes, e.g., star shaped, lobed, in various shapes and colours, sporulating as well as non-sporulating colonies were also obtained on agar plates. Their populations were very sensitive to climatic changes and most of them could not survive during winters.

It is interesting to note that the dominant microbial species, in all the three groups, were those which are known to produce strong antimicrobial metabolites, including antibiotics. This may be a result of the constant interaction between the root exudates, soil microorganisms and climatic conditions.

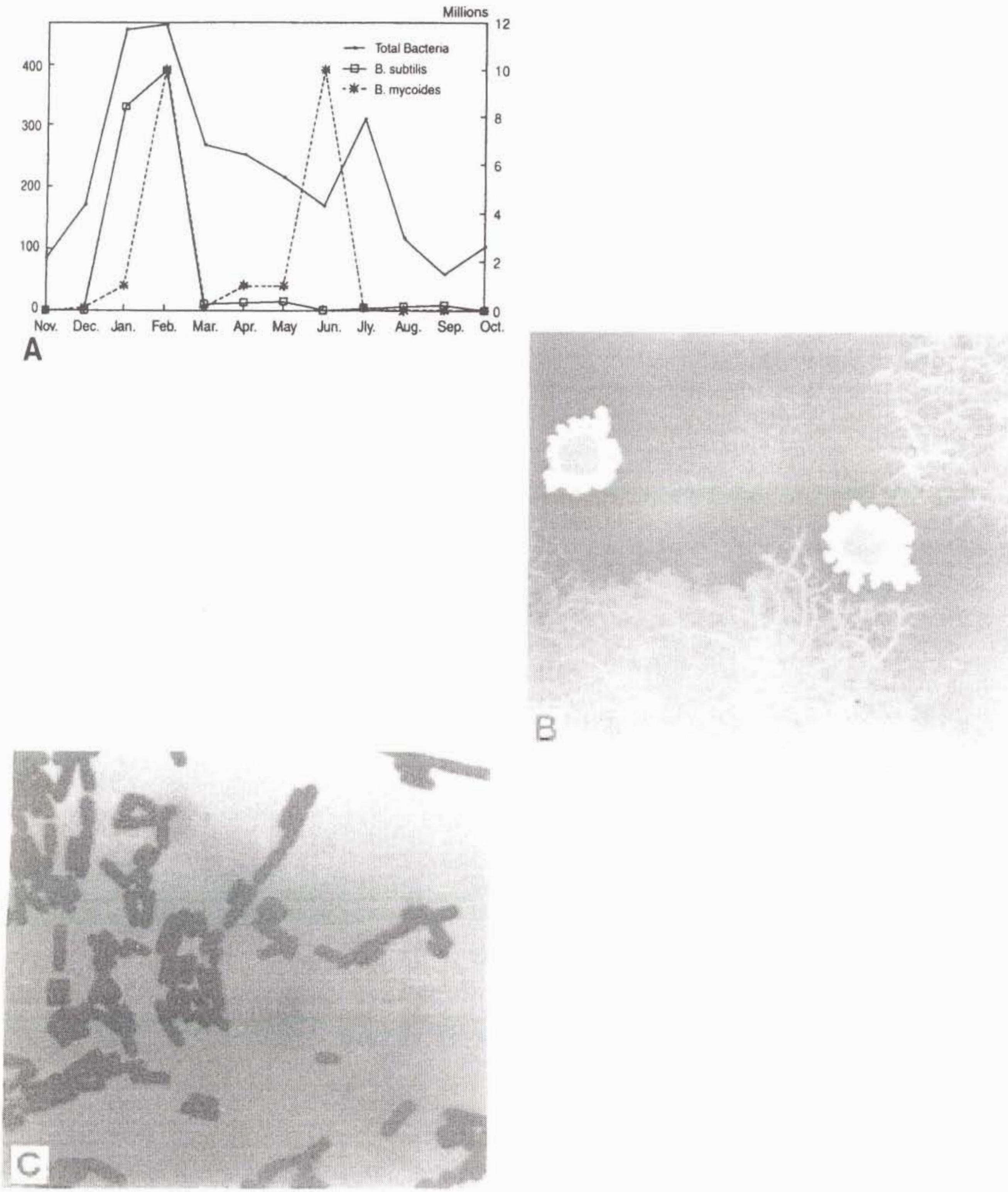

Fig. 1: Dominance of *Bacillus* spp. in established tea rhizosphere: (A) month-wise population of total *Bacillus* spp., *B. subtilis* and *B. mycoides*; (B) dominance of *B. subtilis* and *B. mycoides* on a soil dilution plate during extreme winters; (C) microscopic illustration of *B. mycoides* (x 1000).

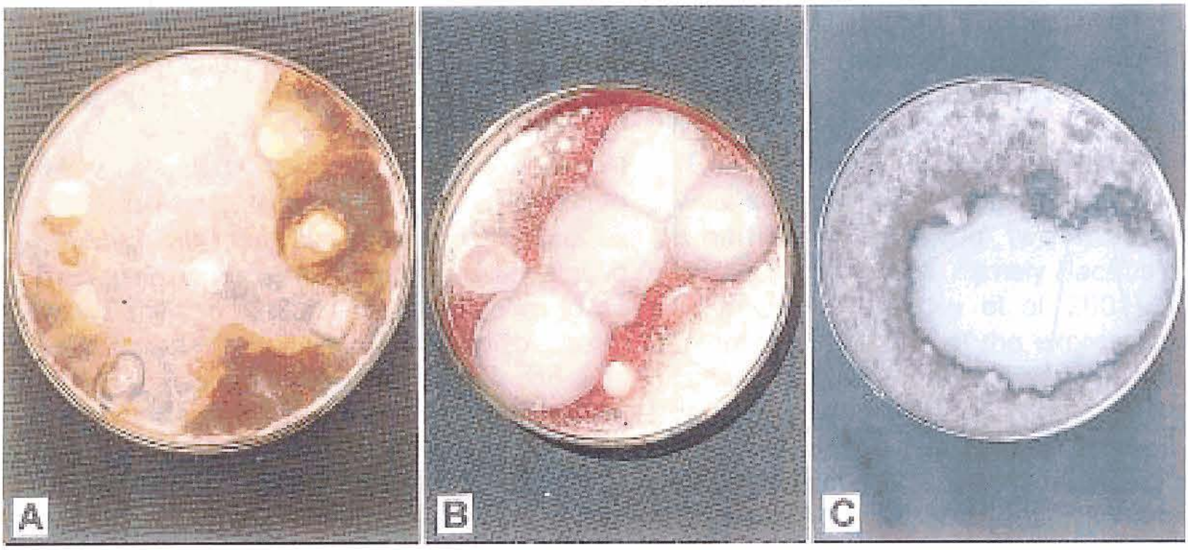

Fig. 2: Screening and selection of antagonistic bacteria from established tea rhizosphere: (a) fungal lawn inoculated with eight bacterial isolates; (b) bacterial isolates showing antifungal activity; (c) antifungal activity of *Bacillus subtilis* against a known pathogen of tea (*Botryodiplodia theobromae*).

Fig. 3: Growth promotion in seed raised tea due to bacterial inoculation (left) control (right) seedlings inoculated with *Bacillus subtilis*.

3. Applications

Hiltner (1904) had identified two major effects of rhizosphere on plants, along with areas to be intensely researched during the next decades. These were: (1) the relationship between the rhizosphere and plant nutrition, growth and development, and (2) the influence of rhizosphere phenomena on pathogens and pathogenesis (cited in Curl and Truelove 1986). During further developments in the field of rhizosphere microbiology efforts have been made towards the isolation and selection of rhizosphere microorganisms for plant growth promotion and biocontrol (Fravel 1988, Weller 1988, Kloepper et al. 1989, Pandey and Kumar 1989, 1990, Glick 1995, Glick and Bashan 1997, Pandey and Palni 1998). Natural rhizospheres are considered ideal sites for the isolation of beneficial microorganisms.

(3.1) Biocontrol

Tea is attacked by a number of fungal and bacterial pathogens. The major diseases in South East Asian Countries including India, Sri Lanka, Indonesia and Malaysia are blister blight and root rot. Brown blight and anthracnose are the main leaf diseases in China and Japan. The major diseases related to tea in African countries fall under the general category of 'root rot'. A number of articles on tea diseases and their management have been published (Chaudhuri 1937, Chen and Chen 1989, Arulpragasam 1992, Muraleedharan 1992, Rattan 1992, Chakraborty and Chakraborty 1997, Muraleedharan and Chen 1997, Agnihothrudu 1999). Important diseases of tea are listed in Table 6. Apple (1977) proposed 'Integrated plant disease management' programme including the use of cultural, chemical and biological control measures for disease control. The proposed measures have been reviewed by Muraleedharan and Chen (1997). In this chapter the discussion has been focused on the possible role of 'biological control' with emphasis on 'rhizosphere microorganisms' for the management of tea diseases. Though there are relatively few examples of successful biological control of tea pathogens, the potential of 'soil microbes' for this purpose should not be overlooked. A detailed account of 'potential of using biocontrol agents in tea' has been given by Agnihothrudu (1999). Various microorganisms, like species of *Bacillus*, *Pseudomonas*, *Gliocladium* and *Trichoderma*, have been recognized for their biocontrol potential against selected pathogens of tea. For example, black rot of tea can be controlled by *Bacillus subtilis* (cited in Agnihothrudu 1999). Chandra Mouli (1993) described the importance of *Trichoderma* in controlling tea pathogens at a field scale. He explained that the control of root diseases by injecting fumigants could be due to the development of a highly antagonistic microflora in the region where the soil is partly sterilized by the fumigants, and by preventing the re-entry of pathogen(s). Role of *Trichoderma* spp. as a biocontrol agent has been emphasized in recent years (Papavizas 1985, Lynch 1987). In addition to *Trichoderma* spp., various other soil fungi, e.g., *Penicillium*, *Fusarium*, *Mucor* and *Rhizopus* have also been reported as potential antagonists against red rot disease of tea roots caused by *Ganoderma pseudoferreum* (Martosupono and Arifin 1991). A siderophore producing strain of *Pseudomonas* as well as an *Actinomyces* strain, isolated from the tea rhizosphere, showed *in vitro* antibiosis against a number of plant pathogenic fungi (Dileep Kumar and Bezbaruah, 1996, 1999). Two strains of *Pseudomonas* spp. isolated from tea fields have been reported to have the ability to degrade organophosphorus insecticide (Tokuda et al. 1991). Goto et al. (1988) isolated ice-nucleation-active bacteria (INAB) (*Pseudomonas syringae* pv *syringae*) from the surface of tea leaves; the presence of these INAB induces ice formation and can be useful in the prevention of frost damage.

In a study based on long term work, the established rhizosphere of tea has been proposed as an excellent site for the isolation of biocontrol agents. While roots of established-old tea plants exert a negative rhizosphere effect on the microbial communities, clearly reflected in terms of R:S ratios, the tea rhizosphere was also found to harbour a large population of

Table 6:
Important diseases of tea and corresponding pathogens

Disease	Pathogen
Root Disease(s)	
Black rot disease	*Rosellinia arcuata* Petch
Red root disease	*Poria hypolateritia* Berk
Brown rot disease	*Fomes noxius* Corner
Root splitting disease	*Armillaria mellea* Vahl.
Violet root rot	*Sphaerostilbe repens* B. and Br.
Diplodia root rot	*Botryodiplodia theobromae* Pat.
Charcoal stump	*Ustulina zonata* Lev.
Tarry root rot	*Hypoxylon asarcodes* (Theiss) Mill.
Red root rot	Ganoderma pseudoferreum *(Wak.)*
Stem disease(s)	
Collar canker	*Phomopsis thea* Petch
Stem canker and die back	*Leptothyrium thea* Petch
Wood rot	*Hypoxylon serpens* Pers. Ex Fr. And *H. nummularium* Bull. Ex. Fr.
Die back	*Leptothyrium theae* Petch and *Nectria cinnabarina* Fr.
Branch canker	*Macrophoma theicola* Petch and *Poria hypobrunnea* Patch
Pink disease	*Corticium salmonicolor* B. and Br. And *Pellicularia salmonicolor* (B. & Br.) Rogers
Thorny stem blight	*Tunstallia aculeate* (Petch) Agnihoth
Leaf disease(s)	
Blister blight	*Exobasidium vexans* Massee
Gray blight	*Pestalozzia theae* Sawada & *P. longiseta* Spegazzini
Brown blight	*Colletotrichum camelliae* Massee
Copper blight	*Guignardia camelliae* (Cke.) Butler
Brown spot	*Phoma theicola* Petch
Scabbing of Leaves	Combined effect of *Phoma*, *Pestalozzia* & *Colletotrichum*
Red rust	*Cephaleuros mycoides* Karst.
Anthracnose	*Colletotrichum theae-sinensis* (Miyake) Yamamata
Bark disease	*Hendersonia Theicola* Cke.

Source of information: Chaudhury et al. 1937; Arulpragasam 1992, Chandra Mouli 1993, Muraleedharan and Chen 1997 and ICOS 2001.

antagonistic microoganisms (Pandey and Palni 1996, Pandey et al. 1997, Pandey and Palni 1999). During the twelve month investigation (Banuri Tea Experimental Garden), the soil dilution plates gave an indication that antagonistic activities amongst existing microbial communities play an important role in regulating the microbial population in an established tea rhizosphere. It is interesting to note that the overall interactions amongst tea roots, microbes and environmental conditions prevailing in the tea rhizosphere seem to favour the growth of microbes which are known to produce strong antibiotics with potential as biocontrol agents. *Bacillus* spp. amongst bacteria, species of *Penicillium* and *Trichoderma* amongst fungi, and several species of *Streptomyces* amongst actinomycetes have been found to survive and dominate the established tea rhizosphere (Pandey and Palni 1997, 1999).

(3.2) Role of antagonistic bacteria in hardening of tissue culture raised tea plants

Various laboratories have been successful in raising tea plants through micropropagation (Bisht et al. 1998, Palni et al. 1999). Tissue culture raised tea plants experience a high degree of mortality during or following lab to land transfer. The procedures adopted for hardening and acclimatization of tissue culture raised plants have not been very satisfactory in providing quality transplants for field (Ziv 1995). Amongst various abiotic and biotic causes, one major probable cause of high mortality of such 'aseptically' raised plants is sudden exposure of these plants to microbial communities present in the soil. Plants raised using tissue culture techniques are maintained under aseptic conditions and probably do not possess sufficient resistance to defend against soil microbial communities. Consequently a large number of plants following transfer to soil are attacked by soil microorganisms (especially fungi) and killed.

In this laboratory, a large number of bacteria isolated from both young as well as established tea rhizospheres from different locations, were screened for the presence of antifungal activity (Fig. 2*). *Bacillus subtilis* (from the established tea rhizosphere) and *Pseudomonas corrugata* (from young tea rhizosphere) were found to be excellent antagonists in petridish assays. These bacteria were used as inoculants in liquid formulation during lab to land transfer of tissue culture raised tea plants. The bacterial inoculation provided first line of defence to micropropagated tea plants and resulted in near 100% survival against 45-50% survival in control plants. The rhizoplane and rhizosphere soil analyses (of control and inoculated plants) suggested that the major cause of mortality during lab to land transfer was fungal attack, mainly by *Fusarium oxysporum* (Bag et al. 2001, Pandey et al. 2000, 2002).

(3.3) Plant Growth Promoting Rhizobacteria

The utility of rhizosphere bacteria with biocontrol potential would increase several fold if these agents also possess plant growth promoting properties. *Bacillus subtilis* and *Pseudomonas corrugata* were found also to have growth promoting property both in tissue culture as well as seed raised plants of tea (Fig. 3*; Pandey et al. 2000). Dileep Kumar and Bezbaruah (1996) have earlier reported both antibiosis and growth promotion in tea, pigeon pea, chick pea and maize by a *Pseudomonas* strain, isolated from the tea rhizosphere.

* See Plate on page 18

4. Future Prospects

The presence of 'negative rhizosphere effect' in tea (established bushes), along with associated features like colonization by a larger population of antagonistic microorganisms, lowering of soil pH, etc., opens up newer avenues for investigation in the field of 'rhizosphere microbiology'. Dominance of a specific microbial population, belonging to the community of antagonists represents a fine example of natural selection. Based on the available knowledge of microbial activities in the tea rhizosphere, following problems may receive priority attention.

(4.1) Chemical analysis of root exudates

Quantitative and qualitative analysis of root exudates and corresponding microbial populations using an age series in tea plants may provide some answers to the phenomenon of 'negative rhizosphere effect' which develops with age. Since tea is a long-lived plant, several microbial successions are likely to proceed the development of a microbial community which is specific to (established) tea plants. Molecular genetics offers an attractive approach for identifying significant/new traits. Researches with a view to identify traits (factors) that play an important role in root colonization and pathogen suppression are critical.

(4.2) Microbial inoculants

The modern microbial technology is full of promise for improving the nutritional status of tea plantations and providing protection against pathogens, ultimately enhancing the overall health of tea gardens. Association of antagonistic microbial populations with rhizosphere of established tea bushes provides an ideal opportunity for developing microbial inoculants for the tea industry. Use of bacterial cultures or fungal spores offers a feasible method of establishing beneficial biological agents directly in the rhizosphere to create an improved environment for enhancing the overall plant growth and for pathogen suppression. Dominance of *Pseudomonas* in the early stages of tea growth, *Bacillus* at a later stage, and association of

Trichoderma makes tea rhizosphere an excellent site for isolation and selection of beneficial organisms.

(4.3) Disease diagnostics

In many cases, particularly diseases that affect below ground parts, by the time symptoms appear on the aerial portion of the plant, it is already too late for taking effective control measures, except to uproot the entire plantation and burn or fumigate the area. In this regard development of detection kits, using poly or monoclonal antibodies or through the use of molecular markers, can be utilized for early detection of diseases (Palni 2001). Use of ultrasonic sensors in relation to live-wood termite infestation in tea plantations in Sri Lanka (Sivepalan 1999) and the use of infra-red photography for early detection of pests have been proposed.

(4.4) Use of bacteriocin producing rhizosphere microorganisms

Tea rhizosphere has been found to be dominated by various species of *Bacillus*; a number of them exhibiting antagonism to each other. In general bacteriocin producing microorganisms do not get preference over other microorganisms having wide spectrum activities due to production of the compounds of a highly specific nature. Development of agrocin-84 for the control of *Agrobacterium tumefaciens* is a unique example in this regard. Identification of other bacteriocin producing microbes, with potential of biocontrol for certain pathogenic microbes with wide host range may give useful results.

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