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IMPACT OF CARBON-STARVED *PSEUDOMONAS AERUGINOSA* STRAIN IE-6S$^+$ ON THE DIVERSITY OF CULTURABLE FUNGI IN THE RHIZOSPHERE AND WITHIN ROOTS OF MUNGBEAN

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Abstract

Mungbean was grown repeatedly in sandy loam soil which was either left untreated (control) or was treated with *Pseudomonas aeruginosa* strain IE-6S$^+$ or its carbon-starved derivatives. Bacterial inoculants were applied to the soil at the start of each 52-day-long mungbean growth cycle and their effect on the diversity of the rhizosphere populations of cultureable fungi was assessed at the end of the first and fourth cycles. A total of 23 fungal species belonging to 18 genera were isolated from the rhizosphere of mungbean. There was a marked difference among the growth cycles with respect to fungal community composition; only slight differences occurred across the bacterial treatments. At fourth growth cycle, general diversity and equitability were lower in soils treated with wild type IE-6S$^+$ while higher in those treated with IE-6S$^+$PBK1 or IE-6S$^+$KUC2. Following IE-6S$^+$KUC2 treatment, fungal abundance pattern was described by geometric series while those following treatment with IE-6S$^+$ or IE-6S$^+$PBK1 were found to conform to MacArthur's broken-stick model at first growth cycle. Nine fungal species comprising 7 genera were found to colonize mungbean root tissues. Roots grown in untreated soils gave high colony forming units of the parasitic fungi while those treated with bacterial treatments harboured mostly saprotrophic fungi. When compared to the controls, general diversity, equitability and species richness of the culturable endophytic fungi were slightly higher in mungbean roots treated with the bacterial inoculants. Abundance patterns of root-fungi in the controls and following bacterial treatments could be described by both geometric and broken-stick models.

Introduction

Application of microorganisms into soil or the rhizosphere has been proposed for biological control of soil-borne crop diseases (Cook, 1993). However, the release of large populations of biocontrol agents into the environment raises important biosafety issues related to the possible ecological consequences of such introductions on resident populations and ecosystem functioning (Défago *et al.,* 1997). This case is of primary concern in case of the biocontrol agent adapted to carbon limited environments and in many countries the existing regulatory framework makes their deliberate field release strictly dependent on detail assessment of their potential environmental impact and associated risk (Nuti *et al.,* 1994).

Soil-borne fungal pathogens cause considerable damage to crop plants and they have often been targeted in biocontrol. However, the majority of soil fungi are non-pathogenic, and a large number of them may even be beneficial to plants and/or contribute positively to ecosystem functioning. The measurement of fungal diversity has

received increased attention over the last decade, in part, due to the fact that fungi have great potential for industrial and biotechnological applications (Hawksworth *et al.*, 1995). Adaptation to nutrient-limited conditions by repeated culture on soil agar media was found to induce resistance to osmotic, oxidation, thermal and pH stress as well as carbon-limited culture conditions in *P. aeruginosa* strain IE-6S$^+$ (Siddiqui & Shaukat, 2003). Induction of carbon starvation in IE-6S$^+$ resulted in enhanced production of secondary metabolites including salicylic acid and hydrogen cyanide and improved biocontrol activity against root-knot nematode in tomato. It is anticipated that such carbon-starved bacteria with enhanced tolerance to certain stress factors may enable bacterial cells to survive for a longer duration under harsh environmental conditions that prevail in arid regions and subsequently also provide long term biological control of soil-borne plant pathogens. However, before application of carbon-starved bacteria under natural conditions their effects on non-target rhizosphere fungi need to be assessed. The objective of the present investigation was to assess whether introduction of carbon-starved *P. aeruginosa* in the soil could have an impact on the composition and structure, in particular species diversity of rhizosphere and root microfungal assemblages of mungbean.

Materials and Methods

Pseudomonas aeruginosa strain IE-6S$^+$ and its carbon-starved derivatives, IE-6S$^+$PBK1 and IE-6S$^+$KUC2 (Siddiqui & Shaukat, 2003) were used throughout the study.

Sandy loam soil (pH 8.0) was collected from the surface horizon of a fallow field in Gharo, Southern Sindh, that was moderately saline (electrical conductivity = 4.2 dSm^{-1}). The soil was air-dried and passed through a 5-mm-mesh sieve to discard grass rhizomes, pebbles and stones. The four treatments applied were as follows: (i) inoculation with IE-6S$^+$; (ii) inoculation with IE-6S$^+$PBK1; (iii) inoculation with IE-6S$^+$KUC2 and (iv) no treatment (control). At the start of the experiment, the soil was drenched with either a cell suspension of bacterial inoculants or sterile distilled water (100 ml kg^{-1} of soil). Inoculation resulted in 3 x 10^7 cfu of the introduced *P. aeruginosa* per g of dry soil. The soil was mixed thoroughly to ensure an even distribution of inoculants and filled in 8-cm-diam plastic pots @ 400 g pot^{-1}. Subsequent to bacterial application, the upper 1 cm soil surface was removed from each pot and eight mungbean seeds sown. The removed soil was returned and sprayed with 40 ml sterile distilled water. The number of seedlings was adjusted to three per pot at 10 days after sowing. Each treatment was replicated 5 times and randomized on a glasshouse bench. In each pot, 0.3 g urea was applied at every alternate week. The pots were emptied at 45 days of growth. The root systems were shaken-off to remove the adhering soil, which was mixed with fresh untreated soil and put back into the pots. On the same day, each treatment was applied a second time on the same pots as mentioned above. Mungbean seeds were sown and a second 52-day-long cycle of plant growth was carried out. The same procedure was repeated twice for a total of four cycles of mungbean growth.

The effects of bacterial inoculants on the culturable rhizosphere microfungi were investigated in the first and fourth cycles of mungbean growth. At the end of each of the two cycles, one plant was randomly chosen from each pot to examine fungi. The roots were excised and weighed after the excess soil had been removed. The roots were then shaken vigorously in a test tube containing sterile distilled water, blotted dry, and reweighed. A serial dilution of the soil suspensions was prepared and divided into 2 equal halves. One half of the sample was stored in a refrigerator (for two hours) for the

estimation of bacterial populations while the other was tested for the enumeration of fungi. A 0.5 ml aliquot from 10^3 dilutions was plated on Malt extract agar (MEA) or Czapek Dox agar (CDA) medium, supplemented with penicillin (100,000 units l^{-1}) and streptomycin sulphate (0.2 g l^{-1}) to avoid bacterial contamination. After incubation at 28 °C, the plates were examined for total fungal counts. Most isolates were obtained after a few days of incubation, which were excised subsequent to observations, but the plates were re-examined over several weeks to allow isolation of slow-growing fungi. Developing fungal colonies were sub-cultured as pure isolates and identified as described previously (Shaukat & Siddiqui, 2003).

The roots of all plants (including the ones which were examined for the rhizosphere fungi) were cut into small (5 mm) segments and after surface sterilization in 1% $Ca(Ocl)_2$ for 3 min., 5 such segments were plated on potato dextrose agar (PDA) plates supplemented with penicillin (100,000 units l^{-1}) and streptomycin sulphate (0.2 g l^{-1}). The plates were incubated at 28°C for one week and fungi emerging from each root segment were identified. The colonization percentage was determined using the following formula:

$$\% \text{ Colonization} = \frac{\text{No. of root pieces colonized by a fungus}}{\text{Total no. of root pieces}} \times 100$$

The soil sample that had previously been stored in the refrigerator was tested for the re-isolation of viable bacteria. A serial dilution was made and a 100 µl aliquot from a dilution was plated onto KB medium was amended with an appropriate amount of antibiotic as mentioned above for the isolation of bacteria. The plates were incubated at 28°C for 48 h and numbers of cfu recorded.

Data sets were subjected to factorial analysis of variance (FANOVA) using STATISTICA ver. 5.0 (StatSoft, Inc. 1995, Tulsa, OK, USA). The post hoc tests included Fisher's least significant difference (LSD) and Duncan's multiple range tests. Bacterial population counts were transformed to $\log_{10}$ (x+1) prior to analysis to render the data closer to normal distribution and to achieve homoscedasticity. Hierarchical agglomerative cluster analysis was performed using unweighted pair group method of averaging (UPGMA) with Euclidean distance as the resemblance function.

Diversity was analysed using diversity indices as well as by fitting species abundance distributions (Magurran, 1988). Diversity indices represent a useful means for quantifying community diversity and have been instrumental in revealing the impact of biocontrol inoculants on resident populations (Natsch *et al.*, 1997). The general species diversity of the fungal communities was measured by popular Shannon-Wiener information theory function:

$$H' = -\sum_{i=1}^{s} p_i \log p_i$$

where H' is the general species diversity and p_i the proportion of total number of cfu for fungi or counts for nematodes, N belonging to the *i*th species (Shannon & Weaver, 1963). The variance of general diversity var (H') was calculated in accordance with Magurran (1988), as follows:

$$\text{Var } (H') = \sum pi \, (\log p_i)^2 - (\sum p_i \log p_i)^2/N + (S-1)/2N^2 \quad i = 1,\ldots s$$

Dominance concentration (complement of diversity) was measured by using Simpson's index (Southwood & Henderson, 2000) as: $D = \sum \{[n_i(n_i-1)]/[N(N-1)]\}$ in which n_i = number of cfu for fungi (i=1,…s). The general diversity incorporates two components of diversity: species richness which expresses the number of species (S) as a function (ratio) of the total number of individuals (N), and equitability that measures the evenness of allotment of individuals among the species (Magurran, 1988). The equitability component of diversity and its variance were measured in accordance with Pielou (1975): $J' = H'/H'_{max.}$ The equitability index J' is the ratio between observed (H') and maximal diversity (H'_{max}). Variance of equitability was estimated as: Var (J') = Var (H')/(log S)^2. Non-parametric estimates of species richness were obtained in two different ways:

(i) The jackknife estimate was obtained in accordance with Burnham and Overton (1978) and Heltshe & Forrester (1983). This estimate relies on the number of species found (S_{obs}), the number occurring in only one sample (U), and n, the number of samples collected, as follows: $\hat{S}_{jack}=S_{obs}+U(n-1/n)$.

(ii) The bootstrap estimator derived by Smith & van Belle (1984) was calculated as described by Shaukat & Siddiqui (2003).

Abundance patterns were investigated by applying three common abundance distribution models including geometric, log-normal and MacArthur's broken-stick distribution. A program package for diversity models and indices was developed by one of us (S.S.S.) in Microsoft FORTRAN 77 and is available on request.

Results

Effects of bacteria on populations and diversity of microfungi in mungbean rhizosphere: Regardless of the growth cycles and different treatments, a total of 18 genera and 23 species of fungi were isolated from the rhizosphere of mungbean (Table 1). *Fusarium*, *Aspergillus* and *Penicillium* were among the dominating genera with greater number of species and greater number of colony forming units. The most frequently isolated fungi were predominant in all the treatments and controls. However, a few fungal species were exclusively suppressed following a specific treatment. *Cephaliophora tropica* and *Trichoderma viride* were specifically isolated from the bacteria-treated soils. On the other hand, soil application with wild type strain IE-6S$^+$ of *P. aeruginosa* completely suppressed *Aspergillus flavus* while carbon-starved strain IE-6S$^+$KUC2 inhibited *Fusarium oxysporum*. Regardless of the treatments, in general, greater number of fungal species with high populations was isolated from the rhizosphere of mungbean at 4th growth cycle compared to the first growth cycle.

The dendrogram resulting from UPGMA cluster analysis, based on rhizosphere fungal communities, showed two major groups comprising of samples from each of the two growth cycles (Fig. 1). The microfungal composition of the control was similar to that of IE-6S$^+$ treated soil at both the growth cycles. On the other hand, the samples from IE-6S$^+$PBK1 and IE-6S$^+$KUC2 treated soils clustered together at both the cycles.

Table 1. Effect of soil treatment with *Pseudomonas aeruginosa* and its carbon-starved derivatives (IE-6S$^+$PBK1 and IE-6S$^+$KUC2) on culturable microfungal community structure in the rhizosphere expressed as $\log_{10}$ (x+1) at two cycles of mungbean growth.

Fungal species	[(log cfu g^{-1} fresh root wt.)+1]							
	Control		IE-6S$^+$		IE-6S$^+$PBK1		IE-6S$^+$KUC2	
	Cycle for the mungbean growth							
	1st	4th	1st	4th	1st	4th	1st	4th
Alternaria alternata	0.34	0.56	0.34	0.34	0.44	0.56	0	0.44
Aspergillus flavus	0	0.34	0	0	0	0.34	0.44	0.56
Aspergillus fumigatus	0.34	0.84	0.49	0.56	0.34	0.44	0	0.34
Aspergillus niger	1.43	2.15	1.38	1.06	0.84	1.26	0.56	1.41
Botryotrichum piluliferum	0.44	1.26	0.99	1.38	0.34	0.99	0.34	0.84
Cephaliophora tropica	0	0	0.44	0	0.34	0.56	0	0.34
Chaetomium sp.	0.34	0.56	0	0.84	0.44	0.99	0.34	0.34
Cladosporium sphaerospermum	1.26	0.99	0.84	2.15	0.49	1.06	0.56	1.38
Cochliobolus spicifer	0.49	0.34	0.34	0.56	0	0.44	0.34	0.84
Drechslera hawaiiensis	0.34	0	0	0.34	0.84	0.56	0.34	0.44
Fusarium semitectum	0.34	1.59	0.44	1.06	0	0.99	0.34	0.84
Fusarium oxysporum	0.44	0.84	0	0.56	0.34	0.34	0	0
Fusarium solani	0.34	0.44	0.99	1.26	0.56	2.15	0.44	1.38
Fusarium sp.	0.44	0.34	0.49	0.56	0.34	0.34	0	0.44
Macrophomina phaseolina	1.06	1.26	0.84	0.49	0.84	0.99	0.56	0.99
Mycelia sterilia	0	0.34	0.84	0.44	0	0.56	0.34	0
Paecilomyces lilacinus	0	0.34	0.56	0.49	0.34	0.44	0.34	0.56
Penicillium brefeldianum	1.41	2.15	1.06	2.69	0.84	1.74	0.56	2.15
Penicillium sp.	0.34	0	0.44	0.34	0	0.34	0.56	0.49
Phoma herbarum	0.34	0.34	0	0.34	0.44	0	0	0.34
Rhizoctonia solani	0.56	0.84	0.34	0	0	0.49	0.34	0.44
Trichoderma viride	0	0	0.34	0	0.56	0.44	0.34	0.49
Ulocladium consortiale	0.44	0.56	0.34	0.49	1.26	0.34	0	0.44
Total species	18	19	18	19	17	22	16	21
Total genera	13	14	14	14	14	16	13	16

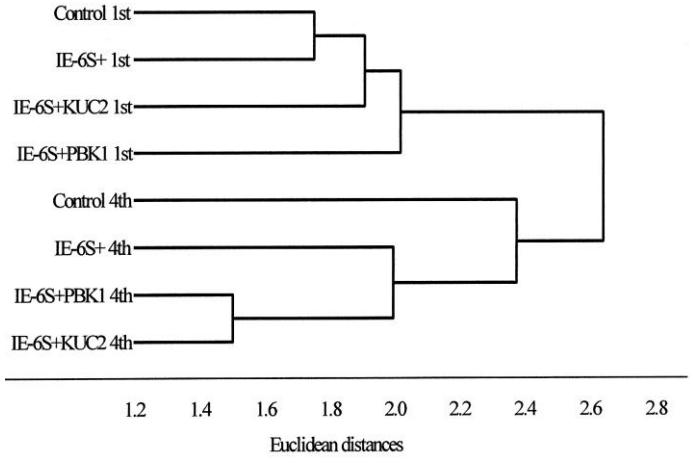

Fig. 1. Dendrogram derived from unweighted paired group method of averaging (UPGMA) of the fungal density data pertaining to bacterial treatments and controls. 1st = 1st growth cycle; 4th = 4th growth cycle.

Table 2. General diversity (H′), equitability (J′) and species richness (d), dominance (D), non-parametric estimators (S_{jack} and S_{boot}) of the rhizosphere fungal communities affected by *Pseudomonas aeruginosa* strain IE-6S$^+$ and its carbon-starved derivatives (IE-6S$^+$PBK1 and IE-6S$^+$KUC2) at two cycles of mungbean growth.

Diversity indices	[(log cfu g^{-1} fresh root wt.)+1]							
	Control		IE-6S$^+$		IE-6S$^+$PBK1		IE-6S$^+$KUC2	
	Cycle for the mungbean growth							
	1st	4th	1st	4th	1st	4th	1st	4th
H′	2.33	1.84	2.57	1.22	2.57	2.0	2.74	1.96
Variance (*H′*)	0.0094	0.0038	0.0069	0.0027	0.0087	0.0067	0.0053	0.0074
J′	0.80	0.62	0.89	0.41	0.91	0.65	0.99	0.64
Variance (*J′*)	0.0011	0.0004	0.0008	0.0003	0.001	0.0007	0.0007	0.0008
D	1.66	0.93	1.76	0.69	1.93	1.26	2.43	1.25
D	0.13	0.24	0.09	0.47	0.08	0.25	0.04	0.27
S_{Jack}	27.6	24.6	26.0	25.0	24.2	29.2	24.8	28.2
S_{boot}	22.4	21.8	21.7	20.7	20.5	25.6	20.0	24.5

Table 3. Percent colonization of the fungi isolated from the roots of mungbean growing in soils treated with *Pseudomonas aeruginosa* strain IE-6S$^+$ and its carbon-starved derivatives (IE-6S$^+$PBK1 and IE-6S$^+$KUC2) at two growth cycles of mungbean.

Fungal species	[(log cfu g^{-1} fresh root wt.)+1]							
	Control		IE-6S$^+$		IE-6S$^+$PBK1		IE-6S$^+$KUC2	
	Cycle for the mungbean growth							
	1st	4th	1st	4th	1st	4th	1st	4th
Alternaria alternata	0	2	0	0	0	2	0	0
Aspergillus flavus	0	0	2	0	7	10	5	3
Aspergillus niger	2	5	2	13	3	0	0	5
Chaetomoim sp.	2	0	8	0	0	7	7	0
Fusarium solani	15	38	25	17	12	13	7	27
Fusarium oxysporum	7	23	0	12	3	8	5	2
Macrophomina phaseoilina	32	57	23	38	5	17	22	10
Rhizoctonia solani	12	17	7	10	5	10	0	17
Trichoderma viride	0	0	17	8	3	0	8	5
Total species	6	6	7	6	7	7	6	7
Total genera	6	5	6	5	5	6	5	5

In general, the species diversity (*H'*) and equitability (*J'*) of fungal communities were higher at 4th growth cycle of mungbean compared to first growth cycle (Table 2). An opposite trend was depicted by Simpson's index of dominance concentration. The non-parametric estimates of species richness (S_{jack} and S_{boot}) were slightly higher at first cycle compared to fourth for the controls and IE-6S$^+$ but the reverse was true for IE-6S$^+$PBK1. Both general diversity and equitability at first growth cycle were consistently higher in the bacterized soils over the controls, whilst at fourth growth cycle general diversity and equitability were lower in soils treated with IE-6S$^+$ and higher in those treated either with IE-6S$^+$PBK1 or IE-6S$^+$KUC2. Species richness estimates were slightly lowered following IE-6S$^+$ inoculation but elevated following treatment with starved bacteria particularly at the fourth growth cycle.

Distribution pattern of fungal species in the mungbean rhizosphere of the controls did not exhibit close fit to any of the abundance distributions but those following treatment with IE-6S$^+$KUC2 at first growth cycle gave a close fit to geometric distribution while those following treatment with IE-6S$^+$ and IE-6S$^+$PBK1 at first cycle were found to conform to MacArthur's broken-stick model.

Table 4. General diversity (*H'*), equitability (*J'*) and species richness (d), dominance (D), and non-parameter estimates of richness (S_{jack} and S_{boot}) of the endophytic communities of fungi affected by *Pseudomonas aeruginosa* strain IE-6S$^+$ and its carbon-starved derivatives (IE-6S$^+$PBK1 and IE-6S$^+$KUC2) at two cycles of mungbean growth

Diversity indices	[(log cfu g^{-1} fresh root wt.)+1]							
	Control		IE-6S$^+$		IE-6S$^+$PBK1		IE-6S$^+$KUC2	
	Cycle for the mungbean growth							
	1st	4th	1st	4th	1st	4th	1st	4th
H'	1.42	1.44	1.64	1.63	1.81	1.82	1.61	1.61
Variance (*H'*)	0.0083	0.0031	0.0052	0.0036	0.009	0.0032	0.007	0.008
J'	0.79	0.80	0.84	0.91	0.93	0.94	0.90	0.83
Variance (*J'*)	0.0025	0.001	0.001	0.001	0.0024	0.0008	0.002	0.0023
D	0.71	0.50	0.76	0.61	1.13	0.85	0.81	0.84
D	0.28	0.27	0.21	0.22	0.16	0.16	0.22	0.23
S_{Jack}	7.6	6.8	8.6	6.8	7.8	9.9	9.2	9.3
S_{boot}	6.7	6.4	7.8	6.5	9.0	8.2	7.3	8.1

Effects of bacterial antagonists on root colonization and diversity of fungi: A total of 9 fungal species comprising 7 genera were isolated from the surface-sterilized mungbean roots (Table 3). In general, regardless of the treatments, fungal colonization was greater at fourth growth cycle compared to the first growth cycle. Application of bacterial inoculants did not markedly alter fungal populations in mungbean roots. Root colonization by *F. solani*, *F. oxysporum* and *M. phaseolina* was markedly lower following soil inoculation with *P. aeruginosa* strain IE-6S$^+$ or its carbon-starved derivatives. *Aspergillus flavus* and *Trichoderma viride* were isolated only from bacteria-treated mungbean roots while *Alternaria alternata* failed to colonize mungbean roots in soils treated with either strain IE-6S$^+$ or its carbon-starved derivative IE-6S$^+$KUC2.

Both general diversity and equitability of the culturable fungi did not differ markedly at first and fourth growth cycles (Table 4). Compared to controls, general diversity and equitability of the fungi were slightly higher in the rhizosphere treated with the bacterial inoculants. Likewise, species richness estimates were also usually elevated by treatments over the controls, particularly at fourth growth cycle. The abundance patterns of root-fungi in the controls and following bacterial treatments could, in general, be described both by geometric and broken-stick models.

Bacterial rhizosphere colonization: Initially, at the start of first cycle, the populations of introduced pseudomonads were 3.5-4.6 × 10^7 CFU per g of dry soil. Wild type strain IE-6S$^+$ was present at 5.5±0.26 and 4.1±0.21 log CFU g^{-1} of root, IE-6S$^+$PBK1 at 5.9±0.33 and 4.7±0.30 log CFU g^{-1} of root while IE-6S$^+$KUC2 at 6.4±0.29 and 4.2±0.27 log CFU g^{-1} of root at the end of the first and fourth cycles of mungbean growth, respectively.

Discussion

Carbon-starved derivatives of *P. aeruginosa* strain IE-6S$^+$ had a relatively minor and transient effect on the number of fungi in the rhizosphere that could grow on malt extract agar or Czapek's dox agar medium. This suggests that strains pre-adapted to carbon limitation could have a negative impact on some soil-borne culturable fungi. Studies of non-target effects of microbial inoculants examine only culturable fungi, though most agriculture soils also harbour fungi, including group of glomales that are difficult or nearly impossible to cultivate on agar medium. Therefore, the fungi isolated in the

present study do not reflect the entire spectrum of the rhizosphere fungi and are limited only to those fungi that can grow only on malt extract and/or Czapek dox agar medium at certain temperature.

A total of 18 genera and 23 fungal species were recorded from mungbean rhizosphere. The microfungi isolated from the rhizosphere exhibited a fungal spectrum that exists in most agricultural soils, rhizosphere and roots of crop plants. However, the number of fungi recovered in the present study was lower compared to those isolated by Girlanda *et al.,* (2001). The fungal spectrum in the current investigation overlaps the one observed by Mandeel (2002) who found 28 microfungal species comprising 20 genera from the rhizosphere soil of the halophytic plant *Zygophyllum qatarense* inhabiting saline and non-saline habitats of the arid desert environment of Bahrain. Likewise, Shaukat & Siddiqui (2003) studying the effects of *P. fluorescens* strain CHA0 on fungal communities isolated 20 genera and 29 species from the rhizosphere of mungbean. Of the fungi isolated in the present study, *Cladosporium, Aspergillus, Penicillium* and *Fusarium* were the dominants in the rhizosphere of mungbean, a finding consistent with that documented for the data from similar arid Sahara ecosystem (Mandeel, 2002).

In the present study, the rhizosphere fungal assemblages in most cases did not exhibit a close fit to any of the models tested. However, IE-6S^{+}KUC2 treatment at first growth cycle fitted the geometric series while IE-6S^{+} and IE-6S^{+}PBK1 gave a close fit to broken-stick distribution. In contrast, Girlanda *et al.,* (2001) while investigating the effect of *P. fluorescens* bicontrol strain CHA0 and its genetically modified derivatives on rhizosphere fungal diversity found that fungal assemblages were best described by truncated lognormal distribution in most cases, while Zak (1992) found a logarithmic series to conform to root surface fungal assemblages. The close fit to geometric or broken stick distributions, in our study, seems to be due to comparatively lower species richnesss and also because the fungal species mostly belonged to a homogeneous taxonomic group. Geometric model generally gives a good fit to assemblages from harsh (stressed) environments where one or a few species are dominant and preempt a significant portion of the limiting resources. Thus, the observed geometric distribution could be due to relatively greater production of secondary metabolites in mungbean rhizosphere which created stressed conditions for the survival and proliferation of certain rhizosphere fungi. Though the population levels of the biocontrol psedomonads at the end of each growth cycle remain at a level sufficient for disease suppression, it is possible that the impact on culturable rhizosphere fungi is greater at first compared to subsequent growth cycles and that mostly reselience of fungal populations occurred in the later cycles (Natsch *et al.,* 1997).

Populations of certain root infecting fungi including, *F. solani*, *F. oxysporum, M. phaseolina* and *R. solani* were suppressed following bacterial treatments while the populations of certain saprotrophic fungi such as *Aspergillus, Chaetomium* and *Trichoderma* were favoured which resulted in an increased diversity. In the present study, a total of 8 genera and 9 fungal species were isolated from the root tissues of mungbean. The observed numbers of fungal species from mungbean roots are consistent from the previous studies where Shaukat & Siddiqui (2003) recovered 8 fungal species from surface-sterilized mungbean roots. Endophytic fungal communities were best described by both geometric and broken-stick models. MacArther's model suggests that some insight into resource allocation may be gained from a study of trophically related species. MacArther (1957), assumed that component species do not overlap on any resource gradient in short supply, suggesting strong species interactions that give rise to an arrangement of non-overlapping but contiguous niches, the size of each niche determining the abundance of each species.

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