Changes in Bacterial and Actinomycetes Diversity of Groundnut Phyllosphere with reference to Plant age, Kind of leaves and Season Adopting Culture Dependent Methods

S Namasivayam, K Sahayaraj

Citation

S Namasivayam, K Sahayaraj. *Changes in Bacterial and Actinomycetes Diversity of Groundnut Phyllosphere with reference to Plant age, Kind of leaves and Season Adopting Culture Dependent Methods*. The Internet Journal of Microbiology. 2008 Volume 6 Number 1.

Abstract

Objective; To study the changes in bacterial and actinomycetes diversity of groundnut phyllosphere of different age of the plant, kind of leaves and season adopting culture dependent methods.

Methods Leaf washing method was used to isolate bacteria and actinomycetes from groundnut leaves of 20, 40, 60 and 80 days old with different kinds viz healthy, aphids lepidopteron infested and diseased sampled during summer and khariff season.

Results. The bacterial and actinomycetes diversity showed variation with respect to the age, kind of leaves and season… Bacterial count was found to be maximum in summer. Irrespective of season aphids infested leaves recorded highest bacterial count and diseased leaves recorded least population of both.367 bacterial isolates belonging to 9 bacterial species and 127 actinomycetes isolates belonging to 3 genera were recovered from different kind of leaves, ages and season. But their composition showed variation with respect to age, season and kind of leaves.

INTRODUCTION

Phyllosphere or plant leaf surface is a habitat for many microorganisms [1] (Raimen, 1961). The leaf surface microbes are important in several ways. Some of them are known to fix atmospheric nitrogen [2,3] (Murty, 1983; Favilli and Messini, 1990), produce plant growth regulators [4] (Buckley and Pugh, 1971) and can control plant parasites either by stimulating plants to synthesize phytoalexins [5] (Last and Warren, 1972) or by producing antibacterial (Mc Cormack et al., 1994) [6] and antifungal compounds (Starmer et al., 1987). [7] The epiphytic microflora are also known to produce sugars, aminoacids, peptides, enzymes, vitamins, organic acids and nucleotides which exert influence on the plant growth (Chandramohan and Mahadevan, 1968). [8] The microbial communities on leaves vary drastically from one leaf to another and undergo constant change in size and composition (Hirane and Upper, 2000). [9] The microflora are challenged with the tharsh condition of the leaf environment (Beattie and Lindow, 1995) including highly fluctuating water availability, exposure to UV radiation from sunlight and limited access to nutrients. [10] The present study was undertaken to evaluate the bacterial including actinomycetes diversity of groundnut phyllosphere of different ages, seasons and kind of levees adopting culture dependent method.

MATERIALS & METHODS

SAMPLE COLLECTION

12 different Groundnut fields in an around Shencottai Taluk (77 25 [1] E LONG, 8 97 [1] N ALT), Tirunelvli district, Tamil Nadu, India were selected in this study. Sampling was done at the age of 20, 40, 60 and 80 DASE (Days After Seedlings Emergence) in both summer (February to May) and khariff (June to August). The leaves of healthy, pest infested aphids and lepidopteron) and diseased leaves were collected in sterile polythene bags kept in icebox, brought to the laboratory and identification of the leaves was done immediately. Pests' infestation and diseased leaves (Leaf spot) were identified by the criteria suggested by Wightman

and Rao (1993) [11] and Sokhi (1983) respectively. [12]

MICROBIOLOGICAL ANALYSIS

Five gram of (each kind) leaves were suspended separately in 100 ml of sterile distilled water with 0.01% Tween 80 and shaken in rotatory shaker at 100 rpm for 30 minutes. The suspension was serially diluted and 0.1 ml of aliquote was spread plated on standard plate count agar (Hi media,India). and starch casein agar(Hi media,India) The seeded plates were incubated at 37 C for 48 hours and 37 C for 14 days (actinomycetes) Developed colonies were counted and then randomly chosen and purified for identification. The purified colonies were stored on respective agar slants at 4 C in refrigerator. All the bacterial including actionmycetes by Bergey's manual of systemic bacteriology (Buchanon and Gibbons, 1979; [13] Kirieg and Holt 1984) [14]

STATISTICAL ANALYSIS

The microbial populations were correlated with the season and kind of leaves using STATISTICA statistical package. Similar statistical package has also been used for the comparison of health leaves to infested and infected leaves. The significant was expressed at 5% level.

RESULTS

ENUMERATION OF TOTAL HETEROTROPHIC BACTERIAL AND ACTINOMYCETES POPULATION

The total heterotrophic bacterial (THBP) and actinomycetes population varied according to the season and the kind of leaves investigated. A strong positive correlation (r = 1.0) was observed between the population and the season, as well as kind of leaves. During summer season, bacterial population was found to be maximum in all kind of leaves, except diseased leaves. In the case of healthy leaves the bacterial population increased from 20 days old plant (17.6 x 10^5 CFU/g) to 80 days old plant (13.2 x 10^7 CFU/g) (r = 0.9812). But during khariff season, the bacterial population recorded at the age of 20, 40, 60 and 80 days were 9.3 x 10^3 , 17.7 x 10^3 , 23.1 x 10^3 and 27.6 x 10^3 CFU/g respectively (r = -0.5134) (Table 1). As observed for healthy leaves THBP was same or more or less similar at all ages as well as both seasons in lepidopteron-infested leaves. Irrespective of season, the aphids infested leaves recorded highest bacterial population and this was statistically significant ($P < 0.05$). In aphid-infested leaves, the bacterial population increased from 23.7 x 10^7 CFU/g to 71.7 x 10^8 CFU/g as the plant advanced from 20 to 80 days of age respectively in summer season. Similar trend was also observed during khariff season. But the diseased leaves consist of least bacterial population in both seasons as well as in all ages than the other kind of leaves recorded (Table 1).

Actinomycetes population fluctuated according to the season. The actinomycetes population at the age of 20, 40, 60 and 80 days recorded in healthy leaves during summer season were 6.4 x 10^2 , 27.4 x 10^3 , 16.4 x 10^3 and 29.0 x 10^2 CFU/g respectively but during khariff season actinomycetes population was not recorded at 20 and 40 DASE. Aphids infested leaves did not showed any effect on actinomycetes population as in bacteria. The diseased leaves of 40 DASE consist of 17.5 x 10^1 CFU/g in summer and the actinomycetes count was absent during khariff.

Table 1. shows bacterial and actinomycetes population from groundnut phyllosphere

Figure 1

Table 1: Enumeration of total heterotrophic bacterial, actinomycetes, fungal and yeast population (CFU/g) in phyllosphere of groundnut with different kinds and season

Crop age (days)	Microorganisms	Healthy		Aphids infested		Lepidopteron infested		Diseased leaf	
		Summer	Khariff	Summer	Khariff	Summer	Khariff	Summer	Khariff
20	Bacteria	17.6 x 10^5	9.3 x 10^3	23.7 x 10^7	12.4 x 10^7	02.4 x 10^5	12.4 x 10^5	-	-
	Actinomycetes	06.4 x 10^2	-	06.3 x 10^2	-	01.3 x 10^2	-	-	-
40	Bacteria	39.1 x 10^5	17.7 x 10^3	47.1 x 10^8	37.6 x 10^7	17.6 x 10^7	17.1 x 10^7	21.1 x 10^2	11.3 x 10^2
	Actinomycetes	27.4 x 10^3	-	06.2 x 10^2	-	-	-	17.5 x 10^1	-
60	Bacteria	57.2 x 10^7	23.1 x 10^3	69.7 x 10^8	73.2 x 10^7	49.1 x 10^7	41.2 x 10^5	07.1 x 10^2	07.1 x 10^2
	Actinomycetes	16.4 x 10^3	7.6 x 10^2	07.2 x 10^3	17.2 x 10^3	11.2 x 10^3	11.2 x 10^3	-	-
80	Bacteria	13.2 x 10^7	27.6 x 10^3	71.7 x 10^8	89.2 x 10^7	99.7 x 10^7	27.2 x 10^3	09.3 x 10^3	07.6 x 10^2
	Actinomycetes	29.0 x 10^2	11.2 x 10^2	08.1 x 10^3	10.1 x 10^3	16.2 x 10^3	21.2 x 10^3	-	-

BACTERIAL COMPOSITION

367 bacterial isolates belonging to different species were isolated from the phyllosphere of groundnut with different kinds of leaves. The bacterial composition has showed variation with respect to age, kind of leaves and season. Pseudomonas fluorescens, Chromobacterium violaceum and Bacillus cereus were reported at 20 days age of healthy leaves and B. polymyxa reported with the above three bacterial species at 40 and 60 days age. But P. fluorescens and B. cereus persisted on leaves of 80 days age. The same bacterial species with small differences occur in lepidopteron-infested leaves as in healthy leaves. Among the various bacterial species, P. fluorescens was found to be dominant at all ages followed by Chromobacterium violaceum at 20 days, B. cereus at 40, 60 and 80 days age respectively. But specific differences in colorization and persistence pattern could be observed in khariff season. Enterobacter sp. and Alcaligenes sp. were reported in all kind of leaves except diseased leaves. P. fluorescens, Enterobacter sp. and Alcaligenes sp. were reported in all

ages in healthy and lepidopteron infested leaves. During this season, Enterobacter sp. was found to be dominant in all ages except 80 days followed by P. fluorescens.

The aphids infested leaves had shown more bacterial species than the other leaves during both seasons. During summer, P. fluorescens, Chromobacterium violaceum, Enterobacter sp., Alcaligenes sp., P. stutzeri, Klebsiella sp. and Erwinia herbicola were isolated from aphids infested leaves of 20 DASE. There was no marked difference in bacterial composition ($P > 0.05$), except the presence of B. polymyxa at 40 DASE and the absence of Chromobacterium violaceum at 60 and 80 DASE and Pseudomonas stutzeri at 80 DASE. As observed in healthy and lepidopteron infested leaves, Pseudomonas fluorescens was dominant in all the ages of groundnut followed by Klebsiella sp. at 20 DASE, B. polymyxa at 40 DASE and B. cereus at 80 DASE. As recorded in summer, P. fluorescens was found to be dominant in all ages of groundnut during khariff followed by P. stutzeri, B. polymyxa, Alcaligenes sp. and B. cereus at 20, 40, 60 and 80 DASE respectively. Irrespective of the season and ages, B. cereus was found to be dominant followed by B. polymyxa in the diseased leaves. The remaining bacterial species was not observed in the diseased leaves.(Table 2)

Table 2 shows generic composition of bacterial species iso.lated from groundnut phyllosphere

Figure 2

Table 2: Bacterial composition (%) of groundnut phyllosphere of different kinds, ages and season

Plant Age (Days)	Species	Healthy		Aphids infested		Lepidopteron infested		Diseased	
		Summer	Khariff	Summer	Khariff	Summer	Khariff	Summer	Khariff
20	*Pseudomonas fluorescens*	43.6	29.8	36.4	22.1	50.1	43.6	-	-
	Chromobacterium violaceum	36.4	-	19.2	-	40.9	-	-	-
	Bacillus cereus	20.0	-	-	14.5	-	-	-	-
	Enterobacter sp.	-	41.3	-	12.5	-	30.0	-	-
	Alcaligenes sp.	-	28.9	-	13.4	-	26.4	-	-
	Pseudomonas stutzeri	-	-	12.3	16.4	-	-	-	-
	Klebsiella sp.	-	-	20.0	15.3	-	-	-	-
	Erwinia herbicola	-	-	12.1	5.8	-	-	-	-
40	*Pseudomonas flourescens*	39.2	32.0	24.6	21.3	35.4	48.0	-	-
	Bacillus polymyxa	14.7	-	23.4	17.7	21.3	-	59.4	64.7
	Bacillus cereus	24.3	-	-	11.1	24.1	-	40.6	35.3
	Chromobacterium violaceum	21.8	-	11.3	-	19.2	-	-	-
	Alcaligenes sp.	-	25.4	-	14.3	-	30.8	-	-
	Enterobacter sp.	-	42.6	-	13.2	-	29.2	-	-
	Klebsiella sp.	-	-	14.5	10.0	-	-	-	-
	Pseudomonas stutzeri	-	-	10.8	12.4	-	-	-	-
	Erwinia herbicola	-	-	15.4	-	-	-	-	-
60	*Pseudomonas flourescens*	41.6	35.0	23.6	19.4	57.4	46.2	-	-
	Bacillus cereus	31.4	-	17.2	16.3	42.6	-	39.4	43.3
	Chromobacterium violaceum	-	-	-	-	-	-	-	-
	Klebsiella sp.	-	-	14.5	14.2	-	-	-	-
	Enterobacter sp.			-	13.2	-	29.4	-	-
	Alcaligenes sp.	-	22.0	-	19.4	-	24.6	-	-
	Pseudomonas stutzeri	-	-	12.4	10.0	-	-	-	-
	Bacillus polymyxa	27.0	-	16.4	7.5	-	-	70.6	56.7
	Erwinia herbicola	-	-	10.7	-	-	-	-	-
80	*Pseudomonas fluorescens*	40.0	43.4	34.3	21.4	48.3	50.0	-	-
	Bacillus cereus	35.0	-	21.7	16.2	30.7	-	60.9	62.7
	Erwinia herbicola	-	-	19.4	-	-	-	-	-
	Klebsiella sp.	-	-	14.3	14.5	-	-	-	-
	Alcaligenes sp.	-	21.0	-	13.6	-	20.0	-	-
	Pseudomonas stutzeri	-	-	-	16.0	-	-	-	-
	Bacillus polymyxa	25.0	-	20.3	18.5	21.0	-	39.1	37.3
	Enterobacter sp.	-	35.6	-	-	-	30.0	-	-

- Absent

ACTINOMYCETES COMPOSITION

127 actinomycetes were isolated which belonging to three genera such as Micromonospora, Streptomyces and Actinomyces also showed variations with respect to age, season and kind of leaves. Micromonospora reported in all kind of leaves during 20, 40 and 60 DASE except in diseased leaves. Except in diseased leaves, Actinomyces and Streptomyces recorded on 40 and 60 DASE in all kind of leaves. But during 80 DASE, Streptomyces sp. was only observed in healthy, aphids infested and lepidopteron infested leaves. The diseased leaves greatly influenced the actinomycetes composition. For instance, actinomycetes belonging to Micromonospora spp reported diseased leaves of 40 DASE. Other genera were not recorded at 60 and 80 DASE of both the season. Among the actinomycetes, Micromonospors spp. was found to be dominant in all ages except 80 days.(Table 3)

Table 3 shows generic composition of actinomycetes

isolated from groundnut phyllosphere

Figure 3

Table 3: Actinomycetes composition (%) of groundnut phyllosphere of different kinds, ages and season

Plant age (Days)	Species	Healthy		Aphids infested		Lepidopteron infested		Diseased	
		summer	Khariff	Summer	Khariff	summer	Khariff	Summer	Khariff
20	Microsmonospora sp.	100.0	-	100.0	-	100.0	-	-	-
	Actinomyces sp.	-	-	-	-	-	-	-	-
	Streptomyces sp.	-	-	-	-	-	-	-	-
40	Microsmonospora sp.	61.6	-	60.0	-	50.0	-	100.0	-
	Actinomyces sp.	38.4	-	40.0	-	50.0	-	-	-
	Streptomyces sp.	-	-	-	-	-	-	-	-
60	Microsmonospora sp.	49.3	-	45.0	-	46.0	-	-	-
	Actinomyces sp	23.4	-	20.0	-	24.0	-	-	-
	Streptomyces sp.	27.3	-	35.0	-	30.0	-	-	-
80	Microsmonospora sp.	-	-	-	-	-	-	-	-
	Actinomyces sp	-	-	-	-	100.0	-	-	-
	Streptomyces sp.	100.0	-	100.0	-	-	-	-	-

- Absent

DISCUSSION

The microbial communities of leaves are diverse and include many different genera of bacteria, actinomycetes, filamentous fungi, yeast and less frequently protozoa and nematodes (Brighna et al, 1997). [15] The total heterotrophic microbial population varied according to the age of the crop, season and kind of leaves. Andrews et al. (1981), [16] Blakeman and Parbery (1977) [17], Kinkel (1997) [18] exclusively studied the factors influencing the microbial communities in leaves. They found that host plants, leaf age, leaf position, physical environmental condition and availability of immigrant inoculum were involved in determining the microbes in the phyllosphere. Distinct difference could not be observed in bacterial and actinomycetes population and composition according to the age. But seasonal impact in all ages could be observed. Summer season recorded maximum bacterial and actinomycetes population than khariff season. Irrespective of season and age's aphids infested leaves supported maximum growth of bacteria. Stadler and Muller (1996) [19] reported the effect of aphids infested leaves on the phyllosphere microflora of Picea abies (Linn.). They observed the presence of honeydew significantly increased the growth of bacteria, yeast and filamentous fungi. They further reported that honeydew have acted as a potential source of energy which might have promoted the growth of the microorganisms. Aphids present in Spraces, Beetch and Oak produced honeydew which was consumed by microorganism. As a result, bacteria, yeast and filamentous fungal population were increased three folds. Moreover leaf-feeding moth caterpillars also positively affected the growth of the microorganisms on leaves of Beetch and Oak (Stadler and Muller, 2000). [20] But in the present study, the lepidopteron infested leaves didn't show the distinct bacterial population population as observed by Stadler and Muller, 2000.) Similarly the diseased leaves recorded very least bacterial and actinomycetes population. The pathogens obviously change the metabolism of the host plant following the infection of the pathogen. The obvious change is a rise in the host respiratory rate which is especially due to the increase in the pentose phosphate pathway. This produces 4 and 5 carbon sugars. In addition to these sugars, pathogens were also responsible for the synthesis of phenols, flavonoids, isoperenoids and teopenoids which have antifungal and antibacterial activities. [21] These compounds might prevent the colonization of microflora..

In the present study, 367 bacterial isolates belonging to 9 bacterial species were isolated from different kinds of groundnut leaves during both summer and khariff. P. fluorescens was reported in all the ages of groundnut during both the season and in all kind of leaves except diseased leaves. Presence of Pseudomonas sp. in phylloplane of trees was reported by Jenny et al. (1989). [22] Nitrogen fixing Bacillus polymyxa and B. macerans in cotton phyllosphere was reported by Oliveira et al. (1993). [23] The production of indole-3 acetic acid by B. polymyxa was reported by Holl et al. (1988). [24] The diseased leaves during both the seasons at all ages consist of only B. cereus and B. polymyxa. Remaining bacterial species were such as in health, Aphids and Lepidopteron pests infected leaves were not detected. This might be due to the antimicrobial compounds produced by the host plant after the entry of pathogens and may prevent the colonization of microflora. Barelle et al. (1992) [25] reported that B. cereus is antagonist of leaf spot of peanut which can be used as a biological control agent with chitin as a amendment. Even though B. cereus was reported in diseased leaves, significant reduction of infection could not be observed.

Raujimakers et al (1995) [26] reported that effective colonization and high population size of the introduced bacterial biological control agents on plant surfaces have been considered to be important factor in the successful control of plant diseases. Ji and Wilson (2003) [27] also studied the enhancement of population size of a biological control

agent and their bio-efficacy in the control of bacterial speck of tomato through salicylate and ammonium surface amendments. In future, trials will be carried out to control leaf spot diseases using B. cereus and B. polymyxa with suitable amendments. Aphids infested leaves consisted of more bacterial strains. For instance Pseudomonas stutzeri and Erwinia herbicola were recorded only in this kind of leaves in both the season and in all ages except P. stutzeri at the age of 80 DASE. As mentioned earlier by Stadler and Muller (1996), [19] the aphids honeydew a carbon rich waste product might have promoted the growth of microorganisms. Bacterial composition also showed variation with respect to the season. Occurrence of Alcaligenes sp. and Enterobacter sp. were only reported in all kind of leaves except the diseased leaves. This may be due to the humid environment that favoured the colonization of these bacteria. Ercolani (1991) [28] found a distinct pattern of microbial colonization at different times of the year. The actinomycetes compositions were found to be greatly changed according to the season as well as age of the groundnut. Actinomycetes were not observed in most of the ages and their presence on leaves of certain ages may be recorded occasionally in the physllosphere.

The present study simply explained the bacterial and actinomycetes composition of the phyllosphere of groundnut by culture dependent methods. In contrast, Ching Hung Yang et al. (2001) [29] demonstrated that culture-independent methods revealed higher community complexity in the phyllosphere than conventional culture based methods. Their study revealed that majority of the 16s rRNA sequences recovered from the leaf washings of various bacterial species were not previously described as being found in the phyllosphere. Hence, for the better understanding of the groundnut phyllosphere both culture independent as well as dependent methods are essential in future.

References

1. Raimen, J. 1961.The phyllosphere- An ecological neglected mileus.Plant and Soil, 15 '81-106
2. Murty, M.G., 1983.Nitrogen fixation (acetylene reduction) in the phyllosphere of some economically important plants. Plant and Soil, 73; 151-153
3. Favill, Fi and Messini,A., 1990. Nitrogen fixation at the phyllosphere level in coniferous in Italy. Plant and Soil, 128; 90-95
4. Buckley, N.G and Pugh, G.J.F. 1971. Auxin production by phyllosphere fungi. Nature (London); 231; 332-335
5. Last, F.T and Warren, R.C 1972.Aspects et role the microbes non- parsitites colonistant surles fexiles ventes. Endessuare, 31; 143-150
6. Mc Cormack, P.J, Wildman, H.G and Jeffbius, P 1994.Production of antibacterial compounds by phyllosphere inhabiting yeast and yeast like fungi. Applied and Environment Microbiology, 60; 927-931
7. Starmer, W.T, Gunter, P.F, Aberdeen, V, Lachanter, M.A and Phaff, H.J. 1987.The ecological role of killer yeasts in natural communalities of yeasts. Canadian Journal of Microbiology, 33; 783-796
8. Chandramohan, D and Mahadevan, A. 1968. Epiphytic microorganisms and IAA synthesis. Planta, 81; 201-205.
9. Hiran, S.S and UpperC.D., 2000..Bacteria in the leaf ecosystem with emphasis of Pseudomonas syringae a pathogen ice nucleus and epiphyte. Microbiology and Molecular Biology Review, 64; 624-65
10. Beattie, G.A and Lindow, S.E, 1995.The secret life of foliar bacterial pathogen on leaves. Annual Review of Phytopathology, 33; 145-172
11. Wightman, J.A, Rao, G, V.R. A groundnut insect identification handbook for India, Information Bulletin No.39.ICRISAT, Putanchery, Andhra Pradesh, India; 1993.
12. Sokhi, .S.S.1983. Detached leaf culture of rusts of pearl millet and groundnut. Indian Phytopathology, 36; 100-102
13. Buchanon, R.E and Gibbons,N.E. 1979. Bergey's manual of determinative bacteriology. 5th edition, Williams and Wilkins, Baltimore, Maryland 234-243
14. Kirieg, N.R and Holt, J.R. (eds.) 1984.Bergey's manual of systemic bacteriology. Williams and Wilkiins, Baltimore, Maryland, Vol1; 476
15. Brighna, L.M, Ravenelli, A, Monelli, A and Ercoli, R.1997.. The use of epiphyte as bioindicator of air pollution in costa rica. Science and Environment, 198; 175-180
16. Andrews, J.H, Kenerley, C.M and Nordhim, E.V.1981. Positional variation in phylloplane microbial population within an apple tree campy. Microbial Ecology, 6; 71-84
17. Blakeman and Parbery,,D.G. 1977. Stimulation of appresorium formation in Collelotrichum acutatum by phylloplane bacteria. Physiological plant pathology, 1; 313-325
18. Kinkel, L.L.Wilson, W and Lindow, S.E 2000. Plant species and plant incubation conditions influence variability in epiphytic bacterial population size. Microbial ecology, 39; 1-11
19. Stadler,B and Muller,T. 1996..Aphid honeydew and its effect on the phyllosphere microflora of Picae albies (L) Karst. Oecologia, 106; 771-776
20. Stadler,B and Muller,T. 2000.. Effect of aphid and moth caterpillars on epiphytic microorganisms in canopies of forest trees. Canadian journal of forest research, 38(4); 631-638
21. Campbell. R. 1985. Plant microbiology. Edward Amole (publishers) Ltd, London p 101.
22. Jenny, B, Isch,C and Arango,M 1988. Nitrogen fixation by new strains of Pseudomonas pseudoflava and related bacteria. Journal of general microbiology, 135; 461-467

Author Information

S. Karthick Raja Namasivayam
Department of advanced Zoology and Biotechnology, Crop Protection Research Centre, St.Xavier's College (Autonomous)

K. Sahayaraj
Department of advanced Zoology and Biotechnology, Crop Protection Research Centre, St.Xavier's College (Autonomous)