Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2011, 2 (4):208-216

ISSN: 0976-8610
CODEN (USA): AASRFC

Characterization of Rhizobacteria Diversity Isolated from *Oryza sativa* Cultivated at Different Altitude in North Himalaya

Parvin Joshi[1] *, Vidit Tyagi[2], Ajay Ballabh Bhatt[1]

[1]*Department of Botany and Microbiology, H.N.B. Garhwal University Srinagar Garhwal, Uttarakhand, India*
[2]*Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun, Uttarakhand, India*

ABSTRACT

The aim of this study was to understand of diversity and activity of dominant bacterial populations in the rhizosphere of rice. Two sites at different altitudes were selected in this study. A total of 335 isolates were recovered from irrigated and rainfed rice plants rhizosphere from both altitude. In which Bacillus sp. (27%) was found to be dominant followed by Pseudomonas sp. (26%), Azotobacter sp. (5%), Flavobacterium sp. (7%), Serratia sp. (4%) and Klebsiella sp. (6%). Enterobacter sp., Micrococcus sp. and Staphylococcus sp., were also observed in the isolates though in low frequency. Genera identified in rhizosphere isolates were also found in the rhizoplane. The spread plate technique was used to isolate and purify all the isolates. The characteristics of the bacterial strains were determined using the colony morphology, gram staining as well as biochemical properties. All isolates were screened for plant growth promoting activities such as siderophore production, indole-3-acetic acid production and phosphate solubilizaton. The lowest Shannon-Wiener index was 1.663 from Jangal rainfed rice and highest was 2.164 from Suddhowala irrigated rice field of Dehradun.

Key words: Rhizosphere, diversity, rice, Himalaya,

INTRODUCTION

Rice (*Oryza sativa*) is a cereal foodstuff which forms an important part of the diet of many people worldwide. Rice, which is being cultivated for several years in the Indian sub continent, is just not a grain, it is life line and second most important to maize (*Zea mays*) [1]. Rice rhizosphere contains a high diversity of plant growth promoting bacteria. Microbial diversity in soil is considered important for maintaining the sustainability of agriculture production systems.

However, the links between microbial diversity and ecosystem processes is not well understood [2]. The rhizosphere is a region of intense microbial activity where root exudates allow the development of many rhizosphere communities [3].

Assessing both structural and funcional diversity are fundamental in order to fully understand the dynamics of the rhizosphere microbial communities. Functional diversity refer to the number of functional groups in a community [4]. Specially, the distinct processes of functions that can potantially be performed by the microbial communities discribe the functional diversity, whereas, functional redundancy is the measure of the number of different species within a functional goup [5].

Several microorganisms are able to promote the plant growth. Several microbial products directly promote growth or indirectly protect from disease [6].

MATERIALS AND METHODS

Research Sites:
Two sites at different altitudes were selected. One of the site was Uttarkashi (30^o44'N 78^o27'E and 30^o.73'N 78^o.45E at an elevation of 1550 meter amsl) and second was Dehradun (30° 19' 48"N, 78° 3' 36'E) at 600 meter amsl. Each sites had four localities in which two were rainfed and two were irrigated.

Isolation and enumeration of rhizobacteria from rice plant root
Bacteria isolates were isolated from the rhizosphere soil and rhizoplane of rice plants grown in fields of district Uttarkashi and Dehradun during the 2008-2009. To estimate the number of soil microflora, using the pour plate methods and triplicate samples of 1 gm soil, and an appropriate dilutions.

Plant Growth Promoting Mechanisms
Siderophore detection
Siderophore was detected by the formation of orange halos surrounding bacterial colonies on CAS agar plates after 48 hour at 28°C [7].

Phosphate solubilization
Phosphate solubilization detected by formation of transparent halos surroundings bacterial colonies on the Pikovskaya agar after 72 hour incubation at 28°C [8].

Indole-3- acetic acid production
Bacteria cultures were incubated in Luria Bertani broth at 28°C. The bacterial cells were removed from the culture medium by centrifugation at 8000 × gm for 10 min. 1ml of supernatant was mixed vigorously with 2ml of Salkowaski's reagent (4.5 gm of $FeCl_3$ per liter in 10.8 M H_2SO_4) and incubated at room temperature in the dark for 30 min and take absorbance at 530 nm [9].

Soil Analysis

For the soil analysis, the composite samples were mixed well individually before use. The sample dried at 20^0C to 25^0C [10]. Soil organic carbon was determined by Walkely and Black's rapid titration method [11]. Total nitrogen (%) was measured using Kjeldahl's procedure [15]. Exchangeable phosphorus (P) and available potassium (K) was determined by Jackson [12].

Statistical Analysis: Analysis of variance (ANOVA) was used to contrast the results. A correlation was established between organic carbon and colony forming units, altitude and colony forming units. All statistical analysis was performed using PAST Software.

RESULTS

Distinct bacterial morphotypes were selected on the basis of their colony characteristics (margin, elevation, surface). Isolated morphotypes were identified on the basis of colony morphology, biochemical characteristics. A total of 93 morphotypes were recovered from Uttarkashi irrigated rice fields. The rhizobacteria were identified as *Bacillus* sp. (26%) documented the most dominant genera followed by *Peseudomonas* sp. (23%), *Azotobacter* sp. (10%), *Flavobacterium* sp. (7%) were dominantly colonize the rice rhizosphere. From rainfed rice 73 morphotypes were isolated in which *Bacillus* sp. (32%) constitute the most dominant genera followed by *Peseudomonad* (30%), and *Flavobacterium* sp. (6%) were present.

A total 97 morphotypes were isolated from Dehardun irrigated rice fields in which *Bacillus* sp. (26%), *Pseudomonas* sp. (25%), *Azotobacter* sp. (9%) and *Klebsiella* sp. (7%) were dominantly present in rhizosphere. In Rainfed rice fields 72 morphotypes were recovered in which *Pseudomonas* sp. (28%) *Bacillus* (26%), *Klebsiella* sp. (7%) and *Enterobacter* sp. (7%) dominantly present.

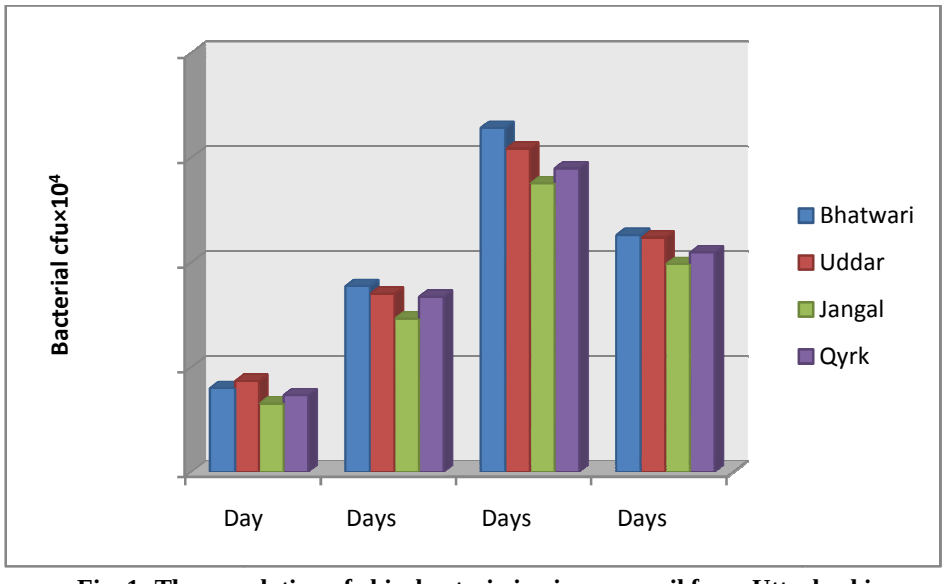

Fig. 1: The population of rhizobacteria in rice crop soil from Uttarkashi.

The significant differences in population count of rhizobacteria were observed between zero to 90 days in both altitude. A graduals increase in population count was observed in zero day to 60

days. The colony forming units were 4.5×10^4 to 1.9×10^7 in Uttarkashi (fig. 1) and 6.8×10^4 to 2.0×10^7 in Dehradun rice (fig. 2). The results were statistically significant ($p<0.01$). The results showed that in rice crop cfu count was maximum in August *i.e.* 60 days old crop. Bacterial cfu count progressively increased in the first three month then declined.

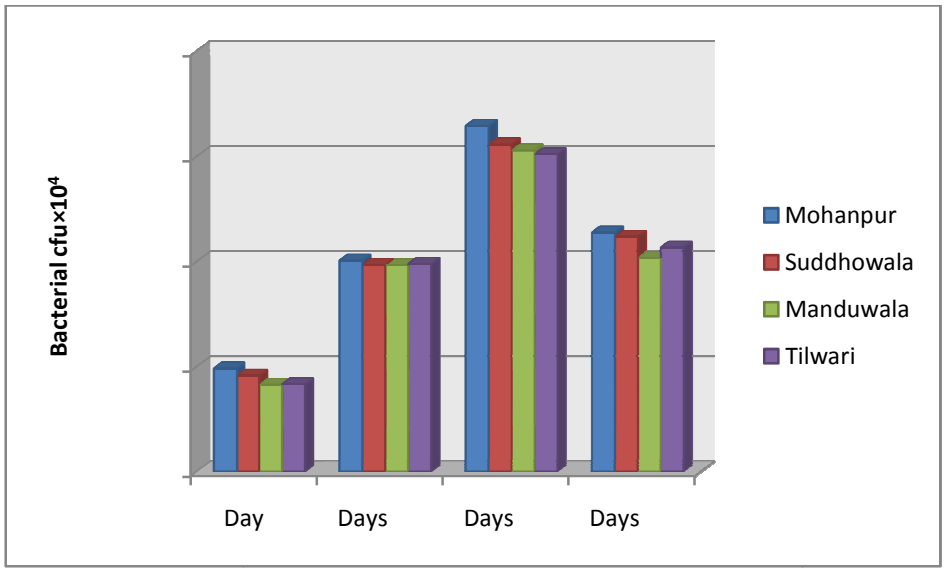

Fig. 2: The population of rhizobacteria in rice crop soil from Uttarkashi.

Table 2: Assessment of the localities wise diversity as calculated by Shannon Wiener diversity index (H') in rhizosphere

Indices	Rice							
	Uttarkashi				Dehradun			
	Renfed		Irrigated		Rainfed		Irrigated	
	Qyrak	Jangal	Bhatwari	Uddar	Manduwala	Tilwari	Mohanpur	Suddhowala
Dominance	0.1995	0.2383	0.1616	0.1489	0.2022	0.1626	0.1552	0.1571
Shannon index	1.932	1.663	2.059	2.119	1.862	2.032	2.139	2.164
Simpson index	0.8005	0.7617	0.8384	0.8511	0.7978	0.8374	0.8448	0.8429
Equitability	0.8059	0.7999	0.8587	0.8838	0.8085	0.8826	0.8609	0.8438

The diversity indices were calculated and compared the bacterial diversity between irrigated and rainfed fields, and also different altitude *i.e.* Dehradun and Uttarkashi (table-2). The lowest Shannon-Wiener index was 1.663 from Jangal rainfed rice and highest was 2.164 from Suddhowala irrigated rice field of Dehradun.

Functional Diversity: Each isolates were screened for plant growth promoting traits such as siderophore production, phosphate solubulization and indole acetic acid production.

Phosphate solubilization: Qualitatively phosphate solubilization was detected on pikovaskya agar plate, evident by halo around the inoculated spot**.** A total 51 isolates showed positive results. Isolates which were positive in qualitative screening were further investigated for quantitatively.

The phosphate solubilization from rice rhizosphere isolates from Uttarkashi ranged from 6.75-91.48 µg/ml. The results were statistically significant ($p<1.0$). Isolate R_0UjanRp1 solubilized phosphate maximum (91.48µg/ml). The phosphate solubilization worked out from rice rhizosphere isolates from Dehradun ranged from 3.20-91.85 µg/ml ($P<1.0$).

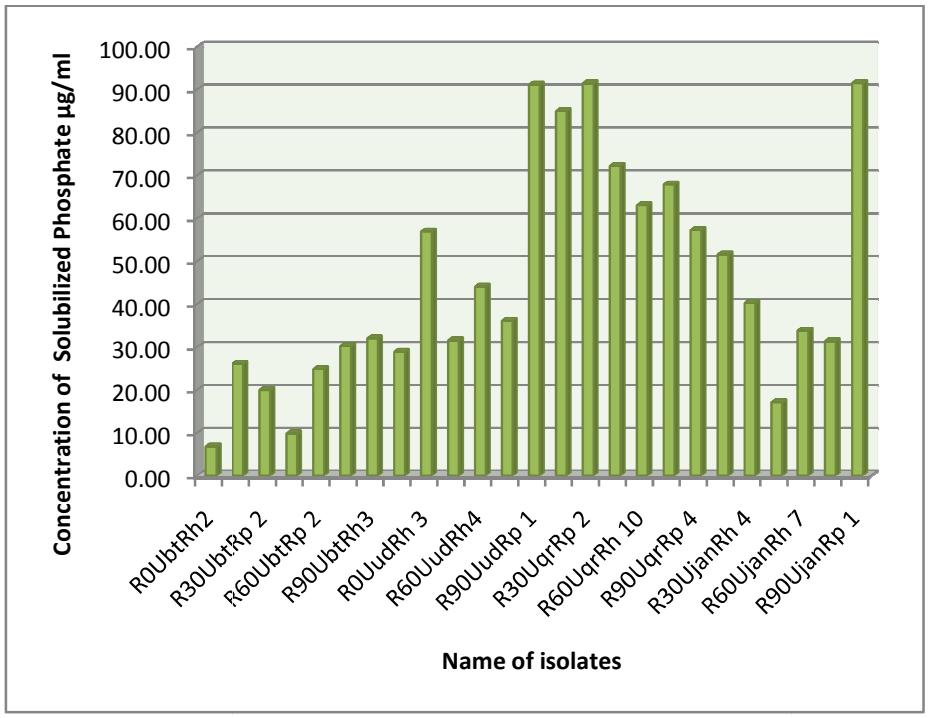

Fig.3: Quantity of Phosphate solubilization by bacterial isolates obtained from rice rhizosphere of Uttarakashi localities

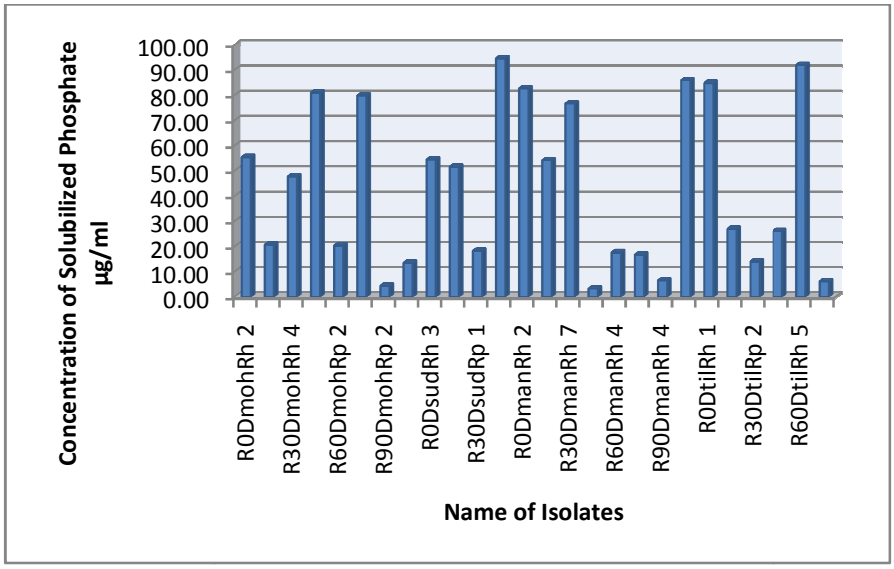

Fig. 4: Quantity of Phosphate solubilization by bacterial isolates obtained from rice rhizosphere of Dehradun localities.

Indole-3-acetic acid: A total 9 isolates showed IAA production range from 0.82 to 6.56 µg/ml isolated from Uttarkashi fields and total 11 isolates produced IAA range from 1.05 to 9.68µg/ml recovered from Dehradun localities.

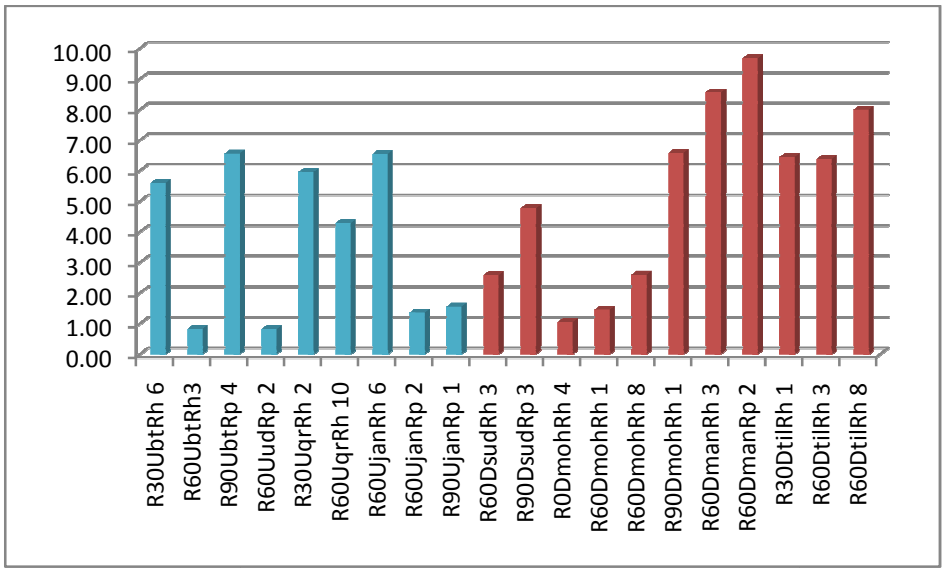

Fig. 5: Indole-3-acetic acid production

Siderophore production: A total 35 and 37 isolates showed positive results of siderophore production isolated form Uttarkashi and Dehradun rice fields respectively.

Physical properties of soil *viz* soil temperature and moisture, measured every month during sampling. Moisture content and temperature of Dehradun rice field soil ranged from 17.5 to 43.7% and 18^0C-29^0C respectively. Moisture content and temperature of Uttarkashi rice field soil ranged from 14.7 to 37.8% and 18^0C-32^0C respectively.

Table 2: Chemical properties of soil

S. No.	Detail of Samples	pH	Total Nitrogen (%)	Available Phosphorus (ppm)	Exchangeable Potassium (ppm)	Organic Carbon (%)
1	**Bhattwari**	5.79±0.03	0.106±0.003	25.23±0.12	374.33±0.58	1.156±0.002
2	**Uddar**	5.87±0.02	0.226±.002	8.26±0.006	137.67±0.0.58	2.145±0.0006
3	**Qyrk**	5.50±0.02	0.296±0.002	47.47±0.11	715±1.0	2.44±0.58
4	**Jangal**	5.98±0.58	0.119±0.05	6.46±0.42	55±1.0	0.955±0.23
5	**Mohanpur**	6.07±0.02	0.318±0.003	51.90±0.1	578±0.58	2.965±0.02
6	**Sudhowala**	6.03±0.03	0.193±0.004	20.00±0.58	97±1.0	2.466±0.11
7	**Manduwal**	6.64±0.02	0.110±0.002	20.48±0.05	76±1.15	2.060±0.05
8	**Tilwari**	6.57±0.06	0.208±0.003	48.93±0.04	156±1.15	2.558±0.03

Soil at both altitudes was acidic in nature. The pH of the soil of Uttarkashi district ranged from 5.50 to 5.98 and that of Dehradun district ranged from 6.03 to 6.64. The organic carbon (0.955 to 2.44%) and nitrogen (0.106 to 0.296 ppm) were found higher to be in Dehradun soil than that of Uttarakashi soil. A positive correlation was found between nitrogen content and total organic

matter (r = 0.80). Soil contents were significantly correlated ($p<0.01$). Chemical properties of soil in different samples at both the locations were represented in the Table -2.

DISCUSSION

Microbial diversity studies are important in order to understand the microbial ecology in soil and other ecosystems [13]. Micro-organisms are environment specific; therefore, for understanding the microbial diversity and their applications, investigation on occurrence of various groups of microorganisms from different environment is essential [14].

In this study it was observed that eight gram negative genera were present in rhizosphere. Atlas and Bartha, [15] reported the rhizosphere is colonized by a predominantly gram-negative microbial community.

In our study *Bacillus* (27%) was dominant group. *Bacillus* species are also a major component of the microbial flora, which live in close association with various types of agricultural crops. However, previous study shows *Bacillus* as the dominant genera in the rhizosphere of *Elaeagnus angusti folia* L. [16].

In this study, predominance of *Bacilllus* is due to its ability to efficiently use the nutrients provided by the plant through exudates. In additions, *Bacillus* has the ability to inhibit the growth of other strains. Many strains of *Bacillus* have been reported to produce substances that act as growth inhibitors for other microorganisms [17].

The diversity of rhizobacteria was calculated by Shannon diversity index. There was significant difference between altitudes, irrigated and rainfed system in associated rhizobacteria of rice rhizosphere. The Shannon diversity index was grater in irrigated rice field of Dehradun (2.164) and lowest was rainfed rice field of Uttarkashi (1.663). The value of Shannon-diversity index was clearly showed that low altitude and irrigated rice had more diversity.

There was significant difference in bacterial population in high altitude and low altitude. A highly negative correlation (r = -0.81) observed between low and high altitude bacterial counts. It is according to Pandey *et al.,* [18], microbial population of these three groups, bacteria, actinomycetes and fungi decreased with increasing altitude.

The rhizosphere effect increased progressively with increase in plant age until the maturity and then declined. The number of rhizobacteria bacteria in the rhizosphere soil and rhizoplane followed the same pattern of progressive increase from first month to maturity and then declined. It is of common occurrence for numbers of microorganisms in the rhizosphere and rhizoplane to increase with plant age [19, 20] which will be due to the progressive interaction between the roots and the microorganisms accompanied by continuous availability of nutrients for the growth of the microorganisms. Our study supported above statements, bacterial cfu count progressively increased in the first three month then declined. The colony forming units were 4.5×10^4 to 1.9×10^7 in Uttarkashi (fig. 1) and 6.8×10^4 to 2.0×10^7 in Dehradun rice (fig. 2).

The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on plant. To achieve the maximum growth promoting interaction between PGPR and nursery seedlings it is important to discover how the rhizobacteria exerting their effects on plant and whether the effects are altered by various environmental factors, including the presence of other microorganisms [21]. The phosphate solubilization from rice rhizosphere isolates from Uttarkashi ranged from 6.75-91.48 µg/ml. Isolate R_0UjanRp1 solubilized phosphate maximum (91.48µg/ml). The phosphate solubilization worked out from rice rhizosphere isolates from Dehradun ranged from 3.20-91.85 µg/ml. A total 5.97% isolates produced IAA and 21.49% isolates produced siderophores. Siderophores provide a competitive advantage to producer organism over fungal pathogens for the absorption of available iron. The role of siderophores in the control of diseases has been well documented by Baker *et al*., [22]. The quantity and activity of microorganisms are determining factor of the productivity of any kind of soil [23].

CONCLUSION

Genera *Bacillus* is dominant in rhizospheric soil of rice. The actual composition of the microbial community in the root zone is dependent on root type, plant species, plant age soil type. Bacterial population decreased with increasing altitude. The findings of the present investigation highlighted that plant growth promoting rhizobacteria from local soil could be easily isolated and may be exploited after strain improvement for local use.

REFERENCES

[1] Kapoor, N., Arya, A., Siddique, A.M., Kumar, H. and Amir, A. *Am. J. Plant Physiol.*, **2011**, 6(1): 28-35
[2] Stark, C., Condron, L.M., Stewart, A., Di, H.J. and O'Callaghan M., *Applied Soil Ecology*, **2007**, 35: 79-93.
[3] Curl, E.A., and Truelove, B. The rhizosphere. (Springer Verlag, Berlin, **1986**), 288.
[4] Smith, T. and Huston, M., *Vegetatio,* **1989**, 83: 49-69.
[5] Gaston, K.J; Biodiversity: Biology of Number and Difference. Oxford, UK, **1996.**
[6] Nelson, L.M. Online. *Crop Management*, 2004. doi: 10.1094/CM-2004-0301-05-RV.
[7] Schwyn, B. and Neilands, J.B. *Analytical Biochemistry,* **1987,** 160: 47-56.
[8] Pikovskaya, R.E. *Microbiologia,* **1948,** 17: 362-370.
[9] Fischer, S.E., Fischer, S.I., Soledad, M. and Mori, G.B. *World J. Microbiol. Biotecnol.,* **2007,** 23: 895-903.
[10] Jackson, M.L. Soil Chemical Analysis. (Prentice-Hall Inc., USA. **1958**), 498.
[11] Walkely, A.E. and Black, J.A. *Soil Science*, **1934,** 37: 29.
[12] Bremmer, J.M. Black, C.A. (eds.), (Madison. WI: American Society of Agronomy. **1965**), 1149-1178.
[13] Garland, J.L. and Mills, A.L. *Appl. Environ. Microbiol.* **1991,** 57(8): 2351.
[14] Pandey, A., Das, N., Kumar, B., Rinu, K., and Trivedi, P., *World J. Microbiol. Biotechnol.*, **2008,** 24: 97-102.
[15] Atlas, R.M. and Batrha, R. Microbial Ecology: Fundamentals and Applications. (Benjamin/Cummings. **1993**), 563.
[16] Romos, B., Pozuelo, J.M., Acero, N. and Gutierrez Manero, F.J. *Orsis*, **1998**. 13: 7-16.

[17] Lilinares, F., Muñoz-Mingarro, D., Pozuelo, J.M., Ramos, B. and Bermúdez de Castro, F. *Geomicrobioogy Journal***, 1994**, 11: 149-156.
[18] Pandey, A., Trivedi, P., Kumar, B., Chaurasia, B., and Palni, L.M.N. NBA Scientific Bulletin No. 5, (National Biodiversity Authority, Chenni, Tamil Nadu, India**, 2006)**, 64.
[19] Rovira, A.D. *Ann. Rev. Microbiol.*, **1965**, 19: 241-266.
[20] Rawat, S., Izhari, A. and Khan, A. *Advances in Applied Science Research*, **2011**, 2(2):351-356.
[21] Bent, E., Tuzun, S., Chanway, C.P. and Enebak, S. *Can. J. Microbiol.*, **2001,** 47: 793-800
[22] Baker R., Flad, Y. and Sneh, B. In: Swineburne, T.R., (Ed), Iron, Siderophores and Plant Diseases, (Plenum press, New York, **1986**), 77-84.
[23] Madhanraj, P., Manorajan, S., Nadimuthu, N. and Panneerselvan, A. *Advances in Applied Science Research*, **2010,** 1(3): 161-167.