African Journal of Microbiology Research Vol. 4 (4), pp. 255-266, 18 February, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Estimation of bacterial diversity in soil and vermi compost using sole source carbon utilization (SSCU) profile

Nipun Silawat[1*], Shweta Chouhan[1], Pramod Sairkar[1], R. K. Garg[1], Neetu Vijay[2] and N. N. Mehrotra[3]

[1]Centre of Excellence in Biotechnology, M.P. Council of Science and Technology (MPCST) Vigyan Bhawan, Nehru Nagar, Bhopal-462003 (M.P.), India.
[2]Indian Council of Medical Research, New Delhi, India.
[3]Central Drug Research Institute, Chattar Manzil Palace, Lucknow - 226 001, Uttar Pardesh, India.

Accepted 17 December, 2009

In this research study, three samples were collected from the T.S. Murthi Udhyan, Obedullahganj out of which two samples were from agriculture field soil and the third sample was from compost. Pure cultures of bacteria were isolated by using serial dilution and spread plate methods. The isolated bacterial cultures were separated according to the morphotypes and then bio-chemically tested. They were further identified with the help of bacteria identification program (PIBWin 2007). The identified bacterial cultures are *Achromobacter group F, Acinetobacter calcoacet, Pseudomonas aeruginosa, P. malli, P. putida, P. cepacia, P. shutzeri, P. pseudomalli, P. malli* and *P. pickettii.* The value of Simpson's index (*D*) is 0.09, which represented high diversity. Isolated bacteria were inoculated in micro titer plates and Niche overlap index (NOI) was calculated after two days of inoculation. The results of study indicate that direct incubation of samples in micro titer plates produces patterns of metabolic response useful in the classification and characterization of microbial communities. Principal component analysis of colour responses quantified from digitized images of plates revealed distinctive patterns among microbial habitats and spatial gradients within soil and compost sites. Correlation of the original carbon source variables to the principal components gives a functional basis to distinctions among communities. Intensive spatial and temporal analysis of microbial communities with this technique can produce ecologically relevant classifications of heterotrophic microbial communities.

Key words: Microdiversity, compost, sole source carbon utilization, testing.

INTRODUCTION

Over three decades of molecular-phylogenetic studies, researchers have showed that microbial diversity is distributed among three primary relatedness groups or domains: Archaea, Bacteria, and Eucarya. The general properties of representatives of the three domains indicate that the earliest life was based on inorganic nutrition and that photosynthesis and use of organic compounds for carbon and energy metabolism came comparatively later. The application of molecular-phylogenetic methods to study natural microbial ecosystems without the traditional requirement for cultivation has resulted in the discovery of many unexpected evolutionary lineages; members of some of these lineages are only distantly related to known organisms but are sufficiently abundant that they are likely to have impact on the chemistry of the biosphere (Pace, 1997).

Carbon is a key factor governing microbial growth in

*Corresponding author. E-mail: silawat.nipun@gmail.com, shweta.geminibest31@gmail.com

soil, and functional aspects related to substrate utilisation can provide important information beyond that afforded by taxonomic level investigations or structural investigations based on rRNA analysis (Insam and Goberna, 2004). The functional diversity of microorganisms, particularly as defined by the substrates used for energy metabolism. Indeed, it has been argued that it is diversity at the functional level rather than at the taxonomic level that is crucial for the long-term stability of an ecosystem. Community level carbon source utilization profile has recently been introduced as a means of classifying microbial community on the basis of heterotrophic metabolism. Such a classification system might allow microbial ecologist to compare the metabolic roles of microbial communities from different environments without involving tedious isolation and identification of community members.

Community (CLPP) involves inoculating mixed microbial communities into micro plates that contain 31 single carbon sources in addition to a tetrazolium dye. The utilization of any carbon source by the microbial community results in the respiration-dependent reduction of the dye and purple colour formation that can be quantified and monitored over time.Community level physiological profiling (CLPP) owes its beginnings to the development of the BIOLOG system in the late 1980s. The system was developed to identify bacteria of clinical importance by assessing each bacterium's usage of any of the 95 different carbon sources in one micro titer plate.

The method was originally developed for medical strain identification. The principal users of the BIOLOG system were pharmaceutical, biotech, cosmetics, and medical devices companies, as well as labs testing for diseases of humans, animals and plants, labs performing environmental monitoring, and companies or organizations involved in production or testing of food and drinks and beverages. In the 1990s, environmental researchers realized that useful physiological data concerning "whole" communities could be generated by inoculating mixed microbial assemblages in to the BIOLOG plates and noting the response of the mixed community to the carbon sources.

Since prokaryotic communities can be considered functional units characterized by the sum of the metabolic properties of individual bacteria, CLPP represents a sensitive and rapid method for assessing the potential metabolic diversity of microbial communities. Furthermore, the ecological relevance of certain contaminants such as hydrocarbons, pesticides and metals to soil bacterial communities can also be assessed.

This study was carried out in order to known the bacterial community persisting in the ecological niche of vermi compost and their behaviour in accordance to their carbon utilization pattern. Utilization of same carbon source when supplied to all isolates are been taken up by some and avoided by some. This brings up the diversity based on physiological properties basically carbon utilization pattern.

MATERIALS AND METHODS

Three soil samples were collected, 2 from field area of T. S. Murty Udyan, Obedullaganj, and 1 from compost added soil. The isolation of bacterial colonies was done on Nutrient Agar Medium. CFU were calculated and then the isolated colonies were pure cultured through four way streak technique followed by Gram staining. The pure isolated colonies were then further subjected to series of procedures for identification.

Growth at different temperatures

Cultures were inoculated in Nutrient broth and flasks were incubated at different temperatures and growth was observed.

Growth on selective and differential media

EMB, Mannitol agar and Mac Conkey agar media plates were prepared and isolates were grown just to check the growth on selective and differential media (Deshmukh, 1997).

Staining

All the pure isolated cultures were subjected to Gram staining to identify the shape and gram positivity and gram negativity of the isolated culture.

Biochemical testing

All the pure isolated cultures were subjected to a series of biochemical testing for identification like - IMVic test, fermentation of carbohydrates, hydrogen sulphide production, urease, casein hydrolysis, catalase, amylase production, motility, triple sugar iron agar test etc (McFaddin, 1980).

Software analysis

The results of gram staining and biochemical testing were entered into PIBWIN software version 1.9.2. (Bryant, 2004) (www.som.soton.ac.uk/staff/tnb/pib.htm) and runned. This gives species specific of the isolated cultures. PIBWIN is probabilistic identification of bacteria window a version of DOS program.

Simpson's diversity indices

Simpson's index (Lambshead et al, 1983 and Simpson, 1949) measures the probability that any two individuals drawn at random from an infinitely large community will belong to same species. The value of *D* ranges between 0 and 1, 0 represents infinite diversity and 1represents no diversity. Bigger the value of *D*, the lower the diversity. It is calculated by the formula:

$$D = \frac{? \, n\,(n-1)}{N\,(N-1)}$$

Where, n is the total number of individuals of each species and N represents the total number of organism of all species.

Shannon index

Shannon Index (Lambshead et al, 1983 and Shannon and Weaver 1949) This is a widely used index which is calculated by following formula:

$$H_s = ? \sum_{i=1}^{S} p_i \ln p_i$$

Where, p_i is the proportion of individual found in the i species and ln denotes natural logarithm. The value obtained from a sample is in itself no significance. The index is off more value while comparing two or more sites.

Sole source carbon utilization (SSCU) test

SSCU is basically a substrates utilization test which provides important information regarding the variant physiological activities in spite of same genetic makeup of closely related species (Chouhan et al 2009). The functional diversity of microorganisms, particularly as defined by the substrates used for energy metabolism, is integral used for energy metabolism, is integral to our understanding of biogeochemistry. All the isolated strains were subjected to SSCU test.

Carbon source

A total of 31 carbon sources were selected which were most relevant to compost community for isolates utilization. These included carbohydrates, amino acids and organic acids.

D – Sorbitol, D – Mannitol, Dextrose Monohydrate, Lactose Monohydrate, Sucrose, D (+) Maltose Monohydrate, β – Cyclodextrin, D – Fructose, D (+) Galactose, L – (+) Rhmnose Monohydrate, D (+) Mannose, D (+) Arabitol, L (+) Arabinose, D (+) Cellobiose, D (+) Trehalso Dihydrate, DL Malic Acid, Citric Acid Anhydrous, Malonic Acid, Succinic Acid, DL – Methonine, L – Histidine Hydrochloride, L – Alanine, L – Leucin, L – Asparagine Monohydrate, DL – Asparatic Acid, DL – Alanine, L – Valine, L – Threonine, DL – Serine, L – Arginine Hydrochloride, L – Histidine.

Preparation of Bacterial Suspension: Pure single colony of bacterial cultures was inoculated in 50 ml of King's B broth. Flasks were incubated at 28 ± 2℃ for two days until log phase growth. Centrifugation was done to obtain pellet at 10,000 rpm for 10 min. Supernatant was discarded and remaining pellet was washed with 0.85% NaCl. Centrifugation at 10,000 rpm for 10 min. Above step was repeated 2 times. O.D. was taken at 456 nm (Jaspers and Overmam, 2004).

Preparation of Micro titre Plates: For growth test, each micro titre well received 50 µl of bacterial suspension, 50 µl of Triphenyl Tetrazolium Chloride dye (TTC) (0.5%), 50 µl of C-source (10%) and 50 µl of M-9 medium devoid of glucose. The plates were incubated for 5 days at 30 ± 2℃. The activity in the plates was measured using a microplate reader set for absorbance at 590 nm.

(I) Niche Overlap Index (NOI): NOI is calculated by total number of carbon sources utilized by two strains A and B divided by the total number of substrates utilized by either of the two (Wilson and Lindow, 1994).

$$NOI = \frac{N\ A \cap B}{N\ total}$$

If value, = 1 cannot coexist, < 1 stable coexist.

Binary matrix

For the dendrogram, Binary matrix was prepared from the results obtained from SSCU tests. 0 was written for negative result and 1 for positive results. Binary matrix was so prepared on note pad.

Distance matrix and Dendrogram (Jaccard)

Distance matrix was generated by PAST (Paleontological Statistics) Software (Hammer et al., 2001) using Jaccard's coefficient and UPGMA. Dendrogram was prepared employing the un-weighted pair group method with arithmetic average.

RESULTS

Physicochemical properties of collected samples

Some physicochemical properties of agriculture field soil and compost were shown in Table 1. Temperature of the sample ranges from 28 - 38℃. Sample of vermi compost was taken at the initial stage of composting and it was well irrigated while sample of soil was collected in summer 2008 so its temperature was 10℃ higher than that of vermi compost. All the samples were alkaline in nature. The concentration of sodium ion in both SD 45 and SD15 soil sample was 0.121 and 0.093 mg/gm respectively and in vermi compost its concentration was 0.312 mg/gm. The concentration of potassium ion in both SD 45 and SD15 soil sample was 0.460 and 0.420 mg/gm respectively and in compost its concentration was 0.288 mg/gm.

Colony count

By using serial dilution plating method of both vermi compost and soil samples the morphological count and CFU (Colony forming unit) has been calculated and the results are listed in Table 2, 3 and 4 respectively.

Growth at different temperatures and differential media

Temperature is one of the most important physical factor affecting microorganisms. Growth performance of bacteria at different temperature conditions is listed in Table 5. The selective media was used to permit the growth of some of specific group of organisms while preventing or retarding the growth of other, thus facilitating bacterial isolation. A differential medium is that which will cause certain colonies to develop differential

Table 1. Physiochemical qualities of soil and Vermi compost.

Sample	Temperature	pH	Na (mg/gm)	K (mg/gm)
SD45	38℃	8.22	0.121	0.460
SD15	38℃	8.82	0.093	0.420
V.C.1	28℃	8.21	0.321	0.288

Table 2. Bacterial colony morphology and count of Vermi compost sample, V.C. 1.

Dilution	Colony Morphology				Bacteria Id	Total No. of colonies	CFU (No. of cells/ml)
	Colour	Margin	Form	Elevation			
10^{-5}	Cream	Entire	Circular	Convex	003	5	41 x 10^{5}
	Translucent	Entire	Circular	Convex	004	13	
	Cream	Lobate	Circular	Convex	005	6	
	Cream	Undulate	Circular	Flat	006	6	
	Translucent	Entire	Circular	Convex	007	1	
	Cream	Lobate	Irregular	Flat	008	10	

Table 3. Bacterial colony morphology and CFU of soil sample, SD 45.

Dilution	Colony Morphology				Bacteria Id	Total No. colonies	CFU (No. of cells/ml)
	Colour	Margin	Form	Elevation			
10^{-8}	Cream	Lobate	Irregular	Raised	009	5	5x10^{8}
10^{-5}	Cream	Entire	Circular	Convex	010	13	13x10^{5}

Table 4. Bacterial colony morphology and CFU of soil sample, SD 15.

Dilution	Colony morphology				Bacteria Id	Total No. of colonies	CFU (No. of cells/ml)
	Colour	Margin	Form	Elevation			
10^{-8}	Cream	Entire	Circular	Convex	011	4	10x10^{8}
	Translucent	Entire	Circular	Convex	012	1	
	Cream	Entire	Circular	Convex	014	2	
	Translucent	Undulate	Irregular	Raised	015	2	
	Yellow	Entire	Circular	Convex	017	1	
10^{-5}	Cream	Lobate	Irregular	Flat	013	5	12x10^{5}
	Translucent	Entire	Circular	Flat	016	7	

from other certain colonies to be develop differentially from other organisms present by producing a characteristic change in the bacterial growth. Media used are Mannitol agar, Mac Conkey and Eosine methylene blue (EMB). The growths of different pure culture on those media are listed in Table 6.

Identification of Bacteria

The results of gram's staining of the pure culture isolated from vermi compost and soil sample is listed below in the Table 6. For identification of isolated microbes various biochemical tests have been done. The results of those biochemical tests listed in Table 7 On the basis of results of the gram's staining, growth on selective and differential media and biochemical analysis, the isolated bacteria were identified using the software PIBWin-2007. The results of analysis are listed in Table 8.

Diversity and Correlation between Samples

Simpson’s index (D) was calculated. The value of D is

Table 5. Growth of bacterial isolates at different temperatures.

Bacteria Id	Temperature					
	42ºC	48ºC	55ºC	60ºC	65ºC	70ºC
003	+	+	_			_
004	+	+	+	+	+	_
005	+	+	_	_	_	_
006	+		_	_	_	_
007	+	+	_	_	_	_
008	+	+	_	_	_	_
009	+	+	+	+	+	_
010	+	_	_	_	_	_
011	+	+	_	_	_	_
012	+		_	_	_	_
013	+	+	_	_	_	_
014	+		_	_	_	_
015	+	+	_	_	_	_
016	+	_	_	_	_	_
017	+	+	_	_	_	_

+ = Growth, - = No growth.

Table 6. Growth of bacterial isolates on selective and differential media, Gram's staining and arrangement of cells.

Bacteria Id	Mannitol agar	MacConkey	EMB	Gram (+/-), arrangement of cell
003	+	-	-	G (-), Streptococcus
004	-	-	-	G (-), Streptobacillus
005	-	-	-	G (-), Streptococcus
006	-	+	-	G (-), Streptobacillus
007	-	+	-	G (-), Streptococcus
008	+	-	-	G (-), Streptobacillus
009	+	-	-	G (-), Streptococcus
010	+	-	-	G (-), Streptococcus
011	+	-	+	G (-), Streptococcus
012	-	-	-	G (-), Streptobacillus
013	+	+	-	G (-), Streptobacillus
014	-	-	+	G (-), Streptococcus
015	+	-	-	G (-), Streptococcus
016	_	-	-	G (-), Streptococcus
017	+	-	-	G (-), Staphylococcus

+ = Positive result, - = Negative result.

0.09, Where 0 represents infinite diversity and 1, no diversity. That is the bigger the value of D, the lower the diversity. Here, the value of D is 0.09 that is it has high diversity. The result of Simpson's Index (D) is listed in Table 9. The graph of the Shannon index was plotted by using Biodiversity Pro software. This graph shows the H_s diversity. Here, the value of D is 0.09 that is it has high diversity. The result of Simpson's Index (D) is listed in Table 9. The graph of the Shannon index was plotted by using Biodiversity Pro software. This graph shows the H_s value of V.C.1 is highest, and the H_s value of SD 45 is the lowest. The graph of the Shannon index is depicted in Figure 1. Descriptive statistics of the three samples are shown in Table 10 and Rank Correlation (Sokal and Rohlf, 1969) of samples is shown in Table 11.

Results of sole source carbon utilization (SSCU)

The BLOLOG redox technology based on tetrazolium

Table 7. Biochemical test performance of the bacterial isolates.

BAC Id	Fermentation Test			IMVic				Motility	Urease	Starch	H_2S Prod.	Casein	Catalase	TSIA			
	Glu	Suc	Lac	Indole	MR	VP	Citrate							Acid	Gas	Pb	H_2S
003	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-
004	+	+	+	-	-	+	-	-	+	-	-	-	+	-	-	-	-
005	+	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
006	+	+	+		-	+	_	+	+	-	-	-	+	-	-	-	-
007	+	+	+	-	-	+	+	+	-	-	-	-	+		-	-	-
008	+	+	+	-	-	+	+	-	+	-	-	+	+	-	-	-	-
009	+	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-
010	+	+	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-
011	+	+	+	-	+	-	+	+	+	-	-	+	+	-	-	-	-
012	+	-	+	-	-	+	-	-	+	-	-	-	+	+	-	-	-
013	-	+	+	-	+	-	+	-	+	+	-	+	+	+	-	-	-
014	-	+	-	-	-	+	-	+	+	-	-	-	+	+	-	-	-
015	+	+	+	-	-	+	+	-	+	+	+	+	+	+	-	-	-
016	+	+	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
017	-	-	+	-	-	+	-	+	+	-	-	+	+	+	-	-	-

+ = Positive result, - = Negative result.

Table 8. Identification of isolated cultures from sample.

S. No.	Bacteria Id	Identified bacteria
1	Bac 003	*Pseudomonas aeruginosa*
2	Bac 004	*P. malli*
3	Bac 005	*Achromobacter group F*
4	Bac 006	*P. putida*
5	Bac 007	*P. shutzeri.*
6	Bac 008	*Acinetobacter calcoacet*
7	Bac 009	*P. pseudomalli*
8	Bac 010	*Pseudomonas*
9	Bac 011	*P. pseudomalli*
10	Bac 012	*P. malli*
11	Bac 013	*P. pickettii*
12	Bac014	*Achromobacter group F*
13	Bac 015	*P. cepacia*
14	Bac 016	*Achromobacter group F*
15	Bac 017	*Achromobacter group F*

dye reduction as an indicator of sole-carbon-source utilization was evaluated as a rapid, community-level method to characterize and classify heterotrophic microbial communities (Bochner and Savagrave, 1977, Bochner 1989 (a) 1989 (b)). The result of SSCU of 14 isolated bacteria is listed in Table 12 and 13 and the colour change is shown in Figures 2 and 3.

Survey of some features of bacterial population

Bacteria from three different microbial habitats (two soils and one vermi compost) had distinctive pattern of sole source carbon utilization on the basis of transformed colour response data. Bacteria present in SD 15 sample utilized citric acid and malic acid which is not utilized by any bacteria of other two samples. 80.64, 80.64 and 90.32% carbon source were utilized by VC 1, SD 45 and SD 15 respectively. Survey of some features of bacterial population is listed in Table 14.

Niche overlap index (NOI)

Calculation of NOI was based upon *in vitro* use as the

Table 9. Simpson's Index of isolated cultures from samples.

Bacterial ID	Identified bacteria	Number (N)	n (n-1)
003	*Pseudomonas aeruginosa*	5	20
004	*P. malli*	13	156
005	*Achromobacter group F*	6	30
006	*P. putida*	6	30
007	*P. shutzeri.*	1	0
008	*Acinnetobacter calcoacet*	10	90
009	*P. pseudomalli*	5	20
010	*Pseudomonas*	1	0
011	*P. pseudomalli*	4	12
012	*P. malli*	1	0
013	*P. pickettii*	5	20
014	*Achromobacter group F*	2	2
015	*P. cepacia*	2	2
016	*Achromobacter group F*	7	42
017	*Achromobacter group F*	1	0
	Total	69	424

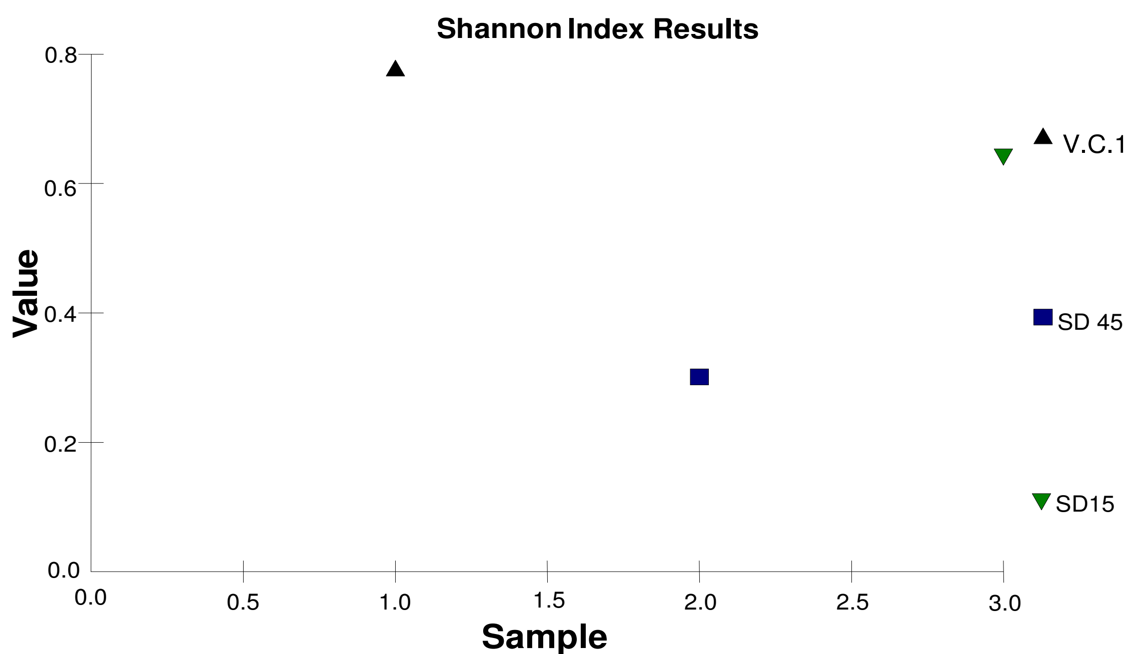

Figure 1. Biodiversity graph of bacteria from different samples.

Table 10. Descriptive statistics of the three samples.

Sample	Mean individual	Variation	Standard deviation	Standard error	Total individual	Total species	Maximum	Mean confidence interval
V.C. 1	0.6	0.267	0.516	0.163	6	6	1	0.166
SD 45	0.2	0.178	0.422	0.133	2	2	1	0.11
SD 15	0.7	0.9	0.949	0.3	7	5	3	0.558

Table 11. Rank Correlation of samples.

	V.C. 1	SD 45	SD 15
V.C.1	1	*	*
SD45	0.0303	1	*
SD 15	0.0512	0.3182	1

Table 12. The result of SSCU of 14 isolated bacteria.

Bacteria	Bacteria Id	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14	SU-15	SU-16
Pseudomonas aeruginosa	Bac 003	_	_	_	+	+	+	+	+	+	+	+	+	+	+	_
P. malli	Bac 004	+	+	+	_	_	_	_	+	+	+	+	+	+	+	+
Achromobacter group F	Bac 005	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. shutzeri	Bac 007	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinnetobacter calcoacet	Bac 008	+	+	+	+	+	+	_	+	+	+	+	+	+	+	+
P. pseudomalli	Bac 009	+	+	+	_	_	+	_	+	+	+	+	+	+	+	+
Pseudomonas	Bac 010	+	+	+	+	_	+	+	+	+	_	_	+	+	+	+
P. pseudomalli	Bac 011	+	+	+	+	+	+	_	+	+	+	+	+	+	+	+
P. malli	Bac 012	+	+	+	+	_	_	_	+	+	+	+	+	_	+	+
P. pickettii	Bac 013	+	_	+	_	+	_	_	+	_	+	+	+	+	+	_
Achromobacter group F	Bac 016	+	+	+	+	+	+	+	+	+	_	+	+	+	+	+
P. cepacia	Bac 015	+	+	+	_	+	+	+	+	+	+	+	+	_	+	+
Achromobacter group F	Bac 017	+	+	+	+	_	_	+	+	+	+	+	+	+	+	+

+ = coloured well, - = colourless well

sole carbon source of the 31 carbon compounds (Wilson and Lindow, 1994). Results of NOI are listed in Table 15.

Diversity between isolated micro organisms

For the study of diversity between isolated microorganisms from soil and vermicompost samples, on the basis of SSCU a dendrogram was prepared by employing the un-weight pair group method with arithmetic average (UPGMA) (Figure 3).

DISCUSSION

Three samples were collected from the T.S. Murthi Udhyan, Obedullahganj. Two samples were of soil, the third sample was of vermicompost. Pure cultures of bacteria were isolated by using serial dilution, spread plate and streak plate methods. The isolated bacterial cultures were separated according to the morphotypes and then bio chemically tested. On putting these data in bacteria identifier program, PIBWin, 2007 all isolated bacterial cultures were identified. These identified bacterial cultures are *Pseudomonas aeruginosa, P. malli, Achromobacter group F, P. putida, P. shutzeri. Acinnetobacter calcoacet,*

Table 13. The result of SSCU of 14 isolated bacteria.

Bacteria	Bacteria Id	SU-17	SU-18	SU-19	SU-20	SU-21	SU-22	SU-23	SU-24	SU-25	SU-26	SU-27	SU-28	SU-29	SU-30	SU-31	SU-32
Pseudomonas aeruginosa	Bac 003	_	_	_	+	+	+	+	+	_	_	+	+	+	_	_	+
P. malli	Bac 004	_	_	_	_	_	+	+	+	+	_	+	+	+	+	+	+
Achromobacter group F	Bac 005	_	_	_	_	_	+	+	+	+	_	+	+	+	+	+	+
P. shutzeri	Bac 007	_	_	_	_	+	+	+	+	+	_	+	+	_	+	+	+
Acinnetobacter calcoacet	Bac 008	_	_	_	_	_	+	+	+	+	_	+	+	+	+	+	+
P. pseudomalli	Bac 009	_	_	_	_	+	+	+	+	+	_	+	+	+	_	+	+
Pseudomonas	Bac 010	_	_	_	_	_	_	_	_	+	_	+	+	+	+	_	_
P. pseudomalli	Bac 011	_	_	_	_	_	+	+	+	+	_	+	+	+	+	+	+
P. malli	Bac 012	_	_	_	_	_	_	+	+	+	_	_	_	_	_	_	_
P. pickettii	Bac 013	_	_	_	_	_	+	_	+	+	_	_	_	_	_	_	_
Achromobacter group F	Bac 016	_	_	_	_	_	+	+	+	+	_	+	+	+	+	+	+
P. cepacia	Bac 015	_	_	_	_	_	+	+	+	+	+	+	_	+	_	_	+
Achromobacter group F	Bac 017	_	+	+	_	+	+	+	+	+	_	+	+	+	+	+	+

+ = Coloured Well, - = Colourless Well.

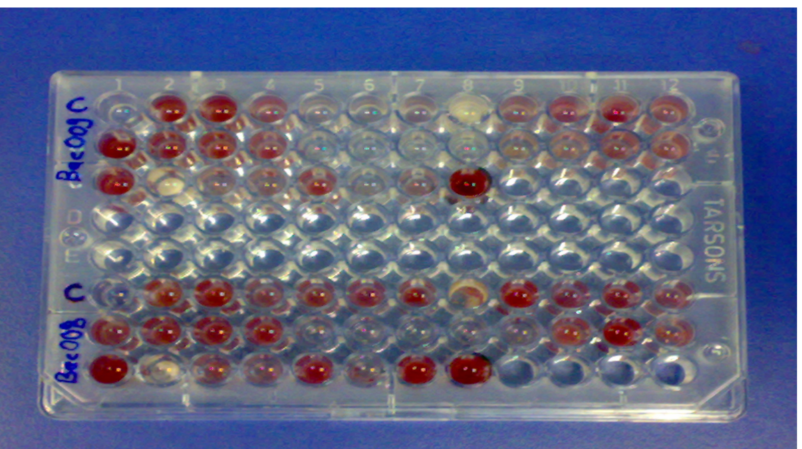

Figure 2. Micro titre plate showing result of SSCU.

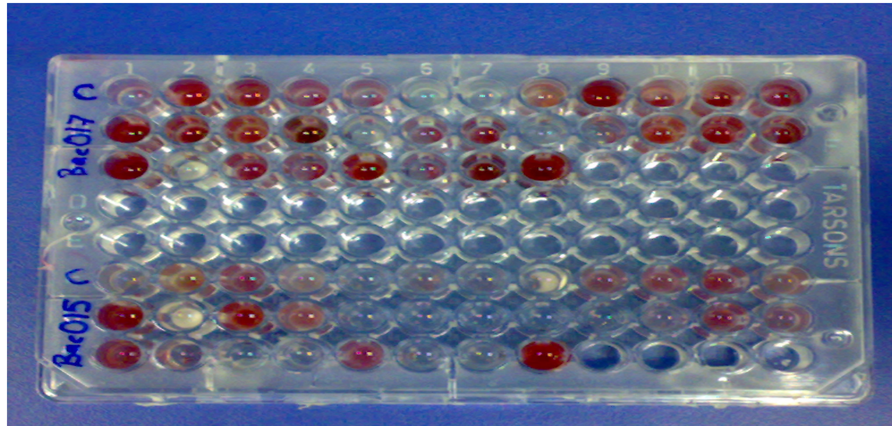

Figure 3. Micro titre plate showing result of SSCU.

Table 14. Survey of some features of bacterial population.

Carbon source	% of occurrence of individual features		
	V.C. 1	S.D. 45	S.D. 15
D – Sorbitol	100	100	100
D – Mannitol	100	100	83
Dextrose	100	100	100
Lactose	60	50	80
Sucrose	60	50	50
D (+) Maltose	80	50	80
β – Cyclodextrin	60	50	33.3
D – Fructose	80	100	100
D (+) Galactose	100	100	83
L – (+) Rhmnose	80	50	83
D (+) Mannose	100	50	100
D (+) Arabitol	100	100	100
L (+) Arabinose	100	100	100
D (+) Cellobiose	100	100	100
D (+) Trehalose	100	100	100
DL Malic Acid	0	0	0
Citric Acid	0	0	16
Malonic Acid	0	0	16
Succinic Acid	0	0	0
DL – Methonine	0	50	16
L – Histidine	100	50	100
L – Alanine	100	50	100
L – Leucin	100	50	100
L – Asparagine	80	100	100
DL – Asparatic Acid	0	0	0
DL – Alanine	80	100	80
L – Valine	100	100	80
L – Threonine	80	100	83
DL – Serine	80	50	80
L – Arginine	100	100	90
L - Histidine	100	50	80

Table 15. Results of Niche Overlap Index of the bacteria.

Bacteria	Niche overlap index (NOI)												
	P. aeruginosa	*P. malli*	*Achromobacter group F*	*P. shutzeri*	*A. calcoacet*	*P. pseudomalli*	*Pseudomonas*	*P. pseudomalli*	*P. malli*	*P. pickettii*	*Achromobacter group F*	*P. cepacia*	*Achromobacter group F*
P. aeruginosa	2	2	2.20	2.20	2.15	2.05	1.85	2.15	1.70	1.60	1.80	1.65	2.25
P. malli	2	2	2	2	2.15	2.05	1.85	2.15	1.70	1.60	1.80	1.65	2.25
Achromobacter group F	1.83	1.83	2	2	1.96	1.87	1.70	1.95	1.58	1.50	1.66	1.54	2.04
P. shutzeri	1.83	1.83	2	2	1.96	1.87	1.70	1.95	1.58	1.50	1.66	1.54	2.04
Acinnetobacter calcoacet	1.87	1.87	2.04	2.04	2	1.91	1.73	2	1.60	1.52	1.69	1.56	2.08
P. pseudomalli	1.95	1.95	2.14	2.14	2.09	2	1.80	2.09	1.66	1.57	1.76	1.6	2.19
Pseudomonas	2.18	2.18	2.41	2.41	2.35	2.23	2	2.35	1.82	1.70	1.94	1.76	2.47
P. pseudomalli	1.87	1.87	2.04	2.04	2	1.91	1.73	2	1.60	1.52	1.69	1.56	2.08
P. malli	2.43	2.43	2.71	2.71	2.64	2.5	2.21	2.64	2	1.85	2.14	1.92	2.78
P. pickettii	2.66	2.66	3	3	2.91	2.75	2.41	2.91	2.16	2	2.33	2.06	3.08
Achromobacter group F	2.25	2.25	2.50	2.50	2.43	2.31	3.87	2.43	1.87	1.75	2	1.81	2.56
P. cepacia	2.53	2.53	2.84	2.84	2.77	2.61	2.30	2.76	2.07	1.92	2.20	2	2.92
Achromobacter group F	1.80	1.80	1.96	1.96	1.92	1.84	2.88	1.92	1.56	1.48	1.64	1.52	2

P. pseudomalli, Pseudomonas, P. pseudomalli, P. malli, P. pickettii, Achromobacter group F, P. cepacia, Achromobacter group F, Achromobacter group F.

The value of Simpson' index (*D*) is 0.09, Where 0 represents infinite diversity and 1, no diversity. That is the bigger the value of *D*, thelower the diversity. Here, the value of *D* is 0.09 that is it has high diversity.

Thirteen samples were inoculated in micro titer plates and data was recorded after two days inoculation. Niche overlap index (NOI) was calculated by number of carbon source utilized by both strains divided by total number of carbon source utilized by either of two. Wilson and Lindow (1994) calculated niche overlap index for Ice (Sup+) *P .syringae* strain with respect to Ice (Sup-) *P. syringae* TLP2 del. It was uniformly high indicated that they were ecologically similar but had low level of coexistence. They found that in the phyllosphere resource partitioning among different bacterial species with NOI values of 0.25 to 0.59 allowed stable coexistence, whereas catabolically identical strains (NOI 1.0), even if they belong to different species cannot coexist. Measure of niche overlap index (NOI) does not provide information about the types of substrates that are utilized by the bacterial strains. Four strains showed identical NOI but still catabolized different substrates. Beside NOI, a consistent relationship among strains was made by cluster analysis, based on the presence or absence of utilized. Jaspers and Overmann (2004) used a combination of cultivation based methods with 9 molecular biological approaches to investigate whether planktonic bacteria with identical 16S rRNA gene sequences can represent distinct eco and genotypes.

Community level carbon source utilization profile has recently been introduced as a means of classifying microbial community on the basis of heterotrophic metabolism. Such a classification system might allow microbial ecologist to compare the metabolic roles of microbial communities from different environments without involving tedious isolation and identification of community members. The results of this research indicate that direct incubation of samples in micro titer plates

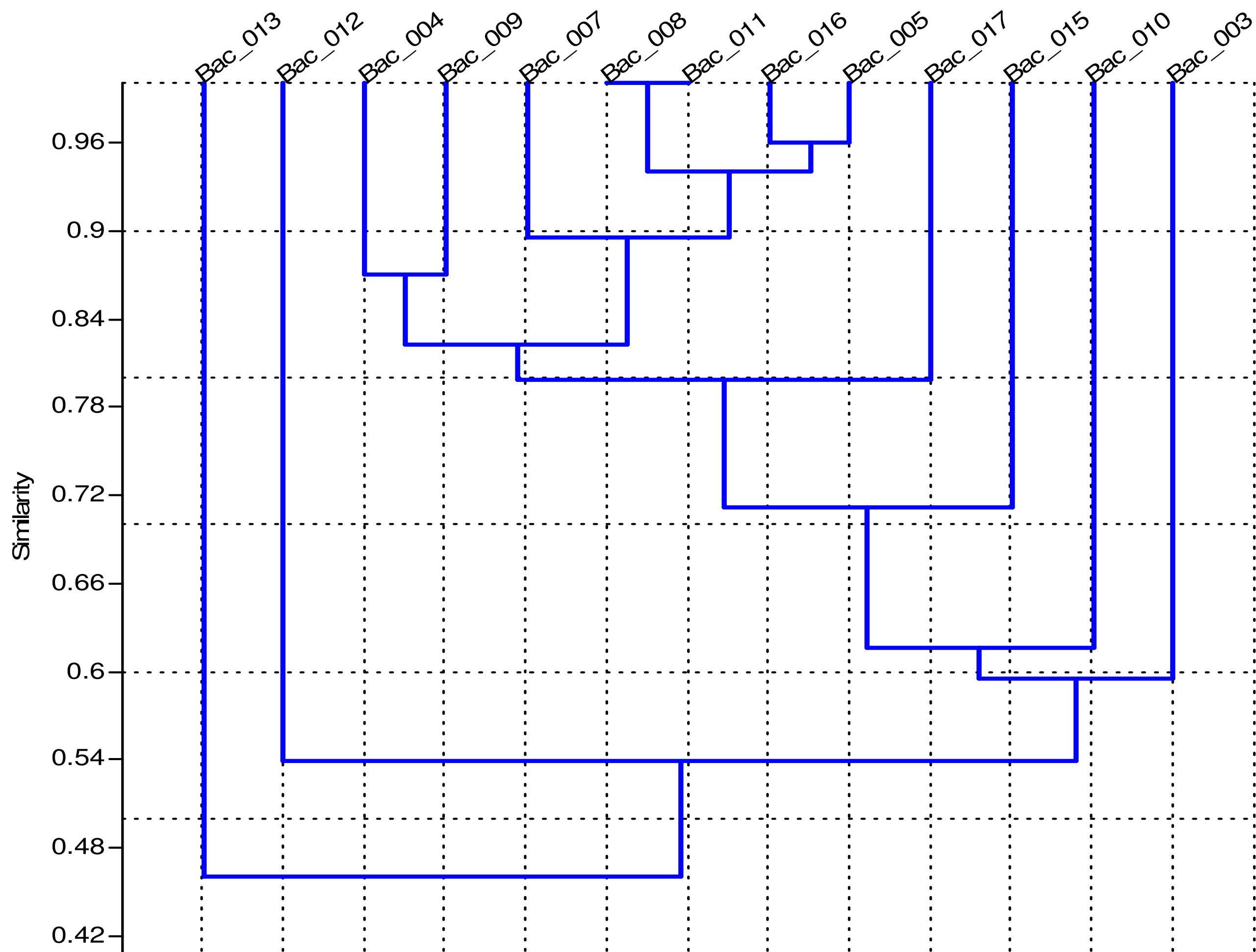

Figure 4. Dendrogram was prepared with the Cluster Vis by employing the un-weight pair group method with arithmetic average (UPGMA).

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