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Original Research Article

Isolation and characterization of bacterial population of Khairi Bhandan River, Mayurbhanj, Odisha (India)

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A B S T R A C T

Soil samples were collected from three sites of river Khairi Bhandan and tested for physico-chemical properties and microbial analysis. The result of the study indicates high bacterial populations from the study site due to decomposition of organic matter which is normally found in river bank and sediment soil. The pH of the soil were found to be acidic in nature thus resulting into high bacteria count since most of the bacteria grow in slightly acidic to natural pH. The bacterial populations are found to be pH, moisture and organic carbon dependent but no significant co-relation was observed for potash and phosphorus which suggest that the bacterial population is independent on this element for their nutrition. The soil samples were not found to be coli form positive which indicate that the soil samples are not polluted by pathogenic bacteria.

Keywords

Soil samples, Khairi Bhandan River, Physico-chemical properties, Microbial analysis

Introduction

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem. It is a critical resource not only for agricultural production and food security but also towards maintenance of most life processes. The functions of soil biota are central to decomposition processes and nutrient cycling. Soil is considered a store house of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. Therefore, major microbial activity is confined to the 'hot-spot', i.e. aggregates with accumulated organic matter, rhizosphere. Microbial ecologist has, in particular, studied microbial community composition since it exerts important control over soil processes. Diversity and community structure in the rhizosphere is however influenced by both, plant and soil type. The soil is highly favorable to life, which proliferates abundantly in its environment. In soil, life mostly occurs in the form of numerous minute organisms embedded in the soil matrix and invisible to the naked eye. At the microbial scale, the soil matrix is highly heterogeneous, a condition favoring the emergence of biodiversity. Among biological diversity microbial diversity is considered one of the

richest in the earth. The microbial diversity includes all types of microscopic organisms present in our environment. The microbial world specially bacteria are highly ubiquitous and can be found in everywhere. Soil bacteria are extraordinary diverse group of bacterial population and distributed in all possible biotopes of the world. The organisms are excellent materials for investigation by ecologist, physiologist, biochemist and biotechnologist. They possess a number of unique biological characteristic and they are considered to be one of the potential organisms which can be useful to mankind in various ways.

Studies on microbial population in soil sample by Acostal- Martinez *et al*. (2008), Alabouvette *et al*. (1979), Allen *et al.* (1995), Bent *et al.* (2006), Brady *et al.* (1999), Buckley *et al.* (2003), Davis *et al.* (2005), Gray *et al.* (2003), Hackl *et al.* (2004), Jonson *et al.* (2003), Kuske *et al.* (1997), Liesack *et al.* (1997), Norris *et al.* (2002), Stackebrandt *et al.* (1993), Torsvik *et al.* (1990), have shown that the proportions and diversity of microbial population in different soil samples vary widely from one region to another in accordance to the physico-chemical properties of soil samples. Soil bacteria play an important role in soil processes that determine plant productivity. For successful functioning of introduced microbial bioinoculants and their influence on soil health, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats.

Study area

The study was conducted in Mayurbhanj district of Odisha state situated between 21º 16'-23º 34' North latitude and 85º 40'-87º 91' East longitude. The district is primarily dominated by tribal population and the total rice cultivable area in the district is about 4,47,214 hectare. Rice cultivation is mainly possible due to the presence of some major rivers in the district like Budhabalanga, Gangahar, Suna and Deo. The river side or coastal water is very fertile and good cultivation is done in this area. This is due to the presence of different kinds of microorganisms in the sediment soil, so in this present study three sites of river Khairi Bhandan were selected for sample collection to know the microbial population of sample.

Materials and Methods

Soil samples were collected from three sites of river Khairi Bhandan.

Site 1: Ramatirtha (RT) (21°57'53.66"N, 86° 3'51.40"E)

Site 2: Khairi (K) (21°57'46.15"N, 86° 4'17.06"E)

Site 3: Bhandan (BH) (21°57'34.59"N, 86° 4'47.02"E)

Soil samples were collected during the month of February, 2010. The samples were collected with a sickle from 10-15 cm depth. The sample put in a sterile polybags and immediately brought into the laboratory. The samples were assigned with numbers and collection date for record in the field note book before they are processed. The physico-chemical properties of soil like pH, salinity, conductivity, moisture, NPK were determined. The p H of the soil was determined by making a soil solution and measured by digital meter. Conductivity is the ability of a substance to conduct the electric current and the presence of various ionic species. The conductivity of the soil samples were determined by conductivity meter and conductivity method. The organic

carbon was determined by colorimetric method. Phosphorus occurs in natural water bodies as phosphates in solution and also in soil sample. Available soil phosphorous was determined in the laboratory. Nitrogen and potash present in the dissolved state have been often considered to the most important nutrients in the primary production of fresh water body that influence the growth and distribution of phytoplanktons. Moisture is a major factor which varies with temperature. The moisture content of the soil was determined after sample collection. One gram of the each soil sample was taken in Petri plates and dry under hot air oven at 70ºC for 6 h until constant weight. The soil moisture content was determined as:

$$\frac{Initial\ weight - Fianl\ weight}{initial\ weight} \times 100$$

Microbial Analysis

Microbial works are done very carefully and seriously. To isolate the bacteria from soil sample several processes are done in sequential process. The main processes are

1. Preparation of culture media
2. Serial dilution
3. Colony observation
4. Preparation of slant
5. Description of colony morphology
6. Identification and characterization of bacteria using staining technique.

The survival and continued growth of microorganism depend on as adequate supply of nutrients of nutrients and a favorable growth environment. The primary purpose of a growth medium in to enable or encourage one or more organisms to grow and divide. So it is very important to select a culture media which favors growth of microorganisms. For bacterial growth nutrient Agar media is most suitable and was selected for the present study. For determination of coliforms differential medium i.e. Maconkey agar was used.

Preparation of Nutrient Agar and nutrient broth medium

1. 150ml of distil water was taken 2gm Nutrient broth and 2.25gm nutrient Agar were measured in the physical balance. Than they were mixed with the distil water to make a solution.
2. After the solution was prepared, than it is subjected to autoclaving.
3. Before autoclaving the Petri plates beaker, conical flask, micropipette tips all the apparatus used in the working procedure all ware wrapped with crab paper and the solution containing conical flask was caped with non-absorbing cotton, which wrapped with the paper. After this all ware put in the pressure cooker for autoclaving. When the pressure cooker whistled, the gas was shimmed, kept for 25 minutes and put off.
4. All the apparatus were taken into Laminar-Air-flow chamber and media poured aseptically into the Petri plates. The plates were kept until the media solidified.

Serial dilution

For isolation of a particular organism from its natural habitat required a pure culture. To isolate the individual colonies of the microorganisms serial dilution technique was used.

1. First 8 test tubes were taken. Then 9ml of distil water was added to each test tube. All test tubes were plugging and kept them in the pressure cooker for autoclaving.

2. Then 10gm of soil sample was taken and

dissolved it in 100ml of distil water taken in a conical flask. It was shaken well and when the suspension was prepared allows it to settle for sometimes.

3. Serial 10 fold dilution was prepared by taking 1ml of the soil suspension and adding into the 1st test tube. From this serial dilution was made by adding 1ml of the suspension of the first test tube into 2nd test tubes and so on. Dilution was made up to10^{-6} dilution factor.
4. From each dilution 1ml of the suspension of each sample were incubated at 37ºC for 25 hours.

Bacterial colonies growing on the plates were counted and expressed as Colony Forming Unit (CFU). The isolated colonies were picked into nutrient agar slant and keep in refrigerator for further used. The viable colony was calculated as:

$$\frac{\textit{dilution factor} \times \textit{number of colony}}{\textit{amount of inoculum}}$$

Characterization of the bacterial isolates

The bacterial isolates were characterized by morphological character by observing under microscope using gram-staining techniques.

To bring out chemical differences existing within the cell or on its surface spherical differential staining techniques have been introduced. Differential staining requires the use of at least three chemical reagents that are applied sequentially to a heat-fixed smear. The gram stain, usually employed for the classification of bacteria, is a differential stain having primary stain, decolorizing agent and counter stain. Gram positive bacteria possess a component, apparently in the cell wall, which complexes with crystal violet and iodine, the later Bering relatively insoluble in alcohol Gram negative bacteria either do not possess this component or the complex is more easily removed with alcohol thus reflecting a difference in cell wall permeability. Before staining, all bacteria are colorless. After gram staining, all positive bacteria are violet and gram negative bacteria are strained red.

This differentiate bacteria into two groups the Gram-positive (those retaining the blue color) and the Gram negative (those which can be decolorized and counter-stained red).

Preparation of a smear of bacteria on the slide:

One or two drop of tap water was placed on the slide. Then a wire loop was touched lightly to the bacterial colony. The bacteria were transferred to the slide using the loop the smear was allowed to become perfectly dry in air.

Results and Discussion

The physico-chemical properties of the sediment river soil like pH, conductivity, percentage of organic carbon, phosphorus and potash varies from different sites and seasons. At different locations the pH of the soil varies from 6.4 to 6.6. Percentage of carbon varies from 0.47 to 0.62 and salinity 0.5 respectively. Similarly phosphorus and potash varies from 18.8 kg/h to 22 kg/h and 286 kg/h to407 kg/h respectively. The physico-chemical parameter like pH, salinity, organic carbon, available phosphorus, and available potash varies with reference to the study sites.

Table 1 shows that isolated bacterial colony with their morphological characteristics. Three sites contain various colors likes yellow, pale yellow, white, pale white. And also contain various shapes like irregular complete, irregular incomplete, small circular colony around with irregular

complete colony wavy, whitish creamy circular colony, uneven growth, whitish with pale yellow irregular complete.

Table 2 shows site-1 and site-2 have p H value 6.6 and site3 have 6.4, it is acidic in nature. The soil samples of three sites have maintaining constant salinity and percentage of organic carbon is very slightly. Availability of phosphorus content is high in site -1 i.e.; 22kg/H and low in site- 2 i.e.; 18.8 kg/H. It shows that p H, moisture, salinity and organic carbon are co-related with the bacterial population and amount of phosphorus and potash are do not vary widely.

Table 3 reveals that using gram staining method the total bacterial population can be divided into gram negative. In site-1, 10 gram positive bacteria are found and no gram negative bacteria are present. Like this in site-2, 10 gram positive bacteria are present and there are no gram negative bacteria. In site-3, 10 number of gram positive and no gram negative.

Table 4 reveals that total Isolate number of bacteria, gram positive or negative and their microscope observation. All are gram positive in nature. Most bacteria are short, circular and cocci. Among them a very few are short rod shaped in chain, ladder like and bacillus.

Table 5 reveals the number of bacterial population in Site-1. It is 82 when the dilution factor is 10^2. Gradually the bacterial population decreases with increasing dilution. In 10^4 dilutions factor the colony forming unit is 59. Like this in Site-2 the bacterial population is 76 and in Site-3 it is 59.

Table 6 reveals Site-1, Site-2 and Site-3 have correlation curve between pH and bacterial population of sites. In site 1, R^2=0.8671, the graph shows correlation with increasing pH and bacterial population. In site-2, R^2=0.989 the graph shows linear correlation and in site-3, R^2=1, here the graph shows high correlation. From this result the bacterial population is positively correlated with pH of the soil sample.

Table 7 reveals three sites and three correlation curve between phosphorus and bacterial population. In site-1 the R^2=0.3714, here the correlation is very low.

In site 2 R^2=0.5949 and in site-3, R^2=0.8768. The above three graph are rarely co-related. So the bacterial population is independent of availability of phosphorous present in soil sample.

Table 8 reveals 3 sites and 3 co-relation curve between organic carbon (%) and bacterial population. In site-1 R^2=0.9858, in site-2 R^2 =0.9267, in site-3 R^2=0.9169,in all the three sites the graph shows positive co-relation and bacterial population is depend upon the percentage of organic carbon.

Table 9 reveals 3 sites and 3 co-relation curve between potash kg/h and bacterial population. In site-1 the R^2 =0.2262, in site-2 R^2=0.6494 and site-3 R^2=0.7429.Here also the graph show very low co-relation. Site-1 is negatively co-related. So the bacterial population also independent of availability of potash in soil sample.

Table 10 reveals that 3 sites having 3 co-relation curve between moisture and bacterial population. In site-1, R^2 =0.9627 and the graph show positive co-relation between moisture and bacterial population. In site-2 R^2=0.9797 the graph show high positive co-relation. In site-3 R^2=0.9888. Bacteria population is depend upon moisture content of soil sample.

From the above result, it is clear that the

bacterial population is depending upon the physico-chemical property like Ph, moisture, and organic carbon and independent of phosphorus and potash. Using MacConkey agar medium in 3 sites has no coli form.

An ecological niche is composed of many microhabitats; each microhabitat is composed of a microscopic diversity which includes bacteria, protozoa, fungi, nematodes, and a macroscopic diversity that includes plants and insects.

Soil is a complex medium in which one can encounter many kinds of microbial communities. Application of nucleic acid-based methods to analyze soil microbial communities has revealed high prokaryotic diversity. The microbial diversity communities present in soil depend on the composition of the soil and many physical chemical properties that the medium possesses. Also the flora and decomposing organic matter on the surface on the soil will influence vastly with the microbial diversity present. For example the fallen trees, barks and flowers provide nutrients broth to the microbes and plants present, through microbial degradation of carbohydrates, lipids and proteins to 4sugar, fatty acids, glycerol and amino acids and respectively to mineralization. Besides providing these nutrients, plant secondary metabolites that are generally toxic to micro organisms will need to be degraded or detoxified by certain microbes.

These degrade microbes are selectively pressured and ultimately evolve to produce novel secondary metabolites possibly to counteract the toxic plant secondary metabolites. There are different types of soils, and their composition depends on the amount of minerals, plants and the type of soil chemistry that is present in an area. Research has demonstrated that soil with a high content of organic material and high humidity has a much higher microbial diversity. When soil is colonized by a high number of plants, it will tend to be richer in nutrients and microbial diversity since all the organic material that the plant disperses to the environment will accumulate on the surface of the soil. This is important in plant and microbe interactions since the populations of microbial decomposers will increase. Bacteria are important group of prokaryotic microbes which are found in diverse habitats and soil type. Bacterial diversity is mostly studied from rhizosphere and forest soil. However, bacteria form other habitats mostly from sediment soil is less explored or understudies. There is need to study bacteria associated with sediment soil because these soils also provide unique ecological niche to explore new and interesting bacteria. In the present study an attempt has been to study the bacterial population of sediment soil of Khairi Bhandan River. Environmental variables like p H, moisture and organic carbon (%) mostly govern microbial populations and vary from different places to places. Here also these variables were found to be slightly acidic in nature ranging from pH 6.4 to 6.6.

The acidic nature of soil may be due to more litter decomposition in soil of that region. The soil moisture and organic carbon also vary among the sites. Presence of moisture is an important factor for rich microbial population. Many bacteria cannot survive in dry soil, due to desiccation of cell and eventually decreases its population. The presence of high organic carbon content indicates available of nutrient in the soil sample.

Table.1 Colony morphology of bacterial population

Sites	**Description of bacteria colony**	**Sites**	**Description of bacteria colony**	**Sites**	**Description of bacteria colony**
RT-1	Pale yellow, irregular, complete, small circular colony around it.	K-1	White, wavy, complete, small circular colony around it.	BH-1	Pale white, irregular, complete, small circular colony around it.
RT-2	Yellow, irregular, complete, small circular colony around it.	K-2	White, irregular, complete, small circular colony around it.	BH-2	Yellow and creamy color colonies are found.
RT-3	Pale white, irregular, complete, small circular colony around it.	K-3	Pale white, wavy, in-between yellowish circular colonies are present.	BH-3	Pale white, wavy, complete.
RT-4	White, wavy, complete.	K-4	White colony, irregular, small circular colonies are present around it.	BH-4	White, wavy, complete.
RT-5	White with one yellow colony, irregular growth, small circular colony around it.	K-5	White, wavy, complete.	BH-5	Pale white, irregular, complete.
RT-6	Pale yellow, irregular, complete, small circular colony around it.	K-6	Pale white, wavy, complete.	BH-6	White, wavy, complete.
RT-7	White, wavy, complete.	K-7	Pale white, wavy, complete, small circular colony around it.	BH-7	White, wavy, complete.
RT-8	White, circular, complete.	K-8	Pale white, wavy, complete, small circular colony around it.	BH-8	Creamy, wavy, complete.
RT-9	White, circular, whitish creamy circular colony, uneven growth.	K-9	Pale white, yellowish, circular colony around it.	BH-9	White, wavy, complete.
RT-10	White with pale yellow, irregular, complete.	K-10	Pale white, wavy, complete.	BH-10	Pale white, irregular, complete, small circular colony around it.

Table.2 Properties of soil in different sites of study area

LOCATIONS	RT-1	K-2	BH-3
pH	6.6	6.6	6.4
SALINITY	0.5	0.5	0.5
ORGANIC CARBON (%)	0.47	0.58	0.62
PHOSPHORUS(Kg/h)	22	18.8	21.6
POTASH(Kg/h)	407	426	286
MOISTURE (%)	20	30	30

Table.3 Using Gram staining method Gram positive and gram negative bacterial population were determine

LOCATIONS	RT-1	K-1	BH-1
Total isolate no.	10	10	10
Gram positive	10	10	10
Gram negative	0	0	0

Table.4 Microscopic observation of bacterial isolation from soil sample using Gram staining

ISOLATED NUMBER	MICROSCOPIC OBSERVATION OF BACTERIAL ISOLATION FROM SOIL SAMPLE USING GRAM STAINING
RT(01)D1	Gram positive, round shape, cocci species.
R(02)D1	Gram positive, round shape, cocci species.
R(03)D1	Gram positive, round shape, cocci species
R(04)D1	Gram positive, round shape, cocci species
R(05)D1	Gram positive, round shape, cocci species
R(06)D1	Gram positive, round shape, cocci species
R(07)D1	Small rod shaped with short chain, gram positive, bacillus species
R(08)D1	Round shape, gram positive, cocci species
R(09)D1	Round shape, gram positive, cocci species
R(10)D1	Round shape, gram positive, cocci species
K(01)D2	Round shape, gram positive, cocci species
K(02)D2	Round shape, gram positive, cocci species
K(03)D2	Rod shape, gram positive, bacillus species
K(04)D2	Round shape, gram positive, cocci species
K(05)D2	Round shape, gram positive, cocci species
K(06)D2	Round shape, gram positive, cocci species
K(07)D2	Round shape, gram positive, cocci species
K(08)D2	Round shape, gram positive, cocci species
K(09)D2	Rod shape, gram positive, bacillus species
K(10)D2	Round shape, gram positive, cocci species
BH(01)D3	Short rod shaped, gram positive, bacillus species
BH(02)D3	Rod shape, gram positive, bacillus species
BH(03)D3	Rod shape, gram positive, bacillus species
BH(04)D3	Rod shape, gram positive, bacillus species
BH(05)D3	Rod shape, gram positive, bacillus species
BH(06)D3	Rod shape, gram positive, bacillus species
BH(07)D3	Round shape, gram positive, cocci species
BH(08)D3	Rod shape, gram positive, bacillus species
BH(09)D3	Round shape, gram positive, cocci species
BH(10)D3	Round shape, gram positive, cocci species

D1- Site1, D2- Site2, D3- Site3

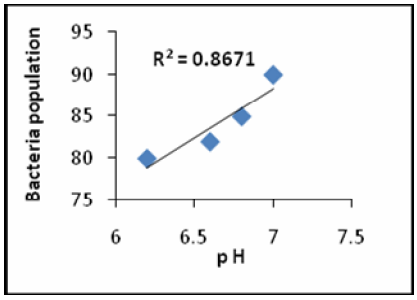
Table.5 Colony forming unit of bacterial isolation from three sites

SITES	103	102	104	105	106
RT	-	82	-	-	-
K	-	>300	76	-	-
BH	>300	>300	59	-	-

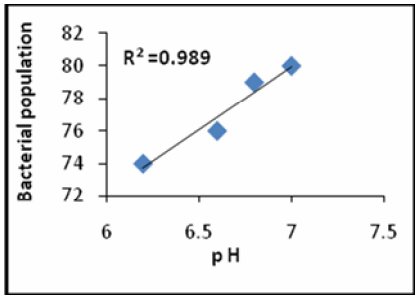
Table.6 Characterization of bacteria from soil samples (Ph)

Sl. no.	RT		K		BH	
1	6.6	82	6.6	76	6.6	59
2	6.2	80	6.2	74	6.2	55
3	6.8	85	6.8	79	6.8	60
4	7	90	7	80	7	62

Graph.1



Graph.2



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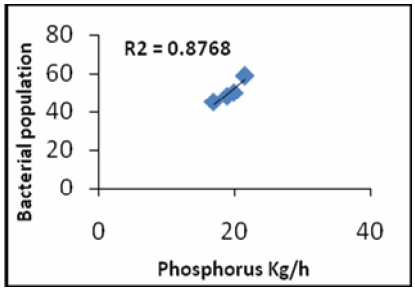
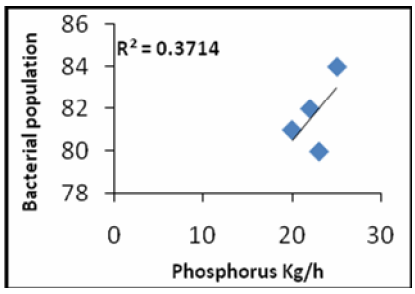


Table.7 Characterizations of bacteria from soil samples (Phosphorus Kg/H)

Sl. no.	RT		K		BH	
1	22	82	18.8	73	21.6	59
2	20	81	16	70	20	50
3	23	80	15	72	19	48
4	25	84	10	79	17	45

Graph.1



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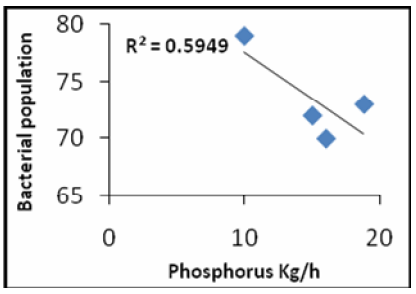

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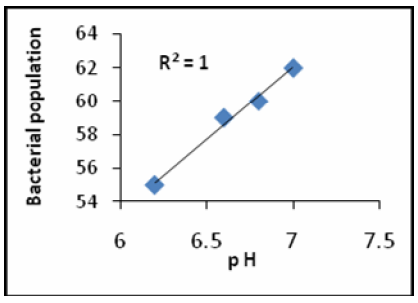
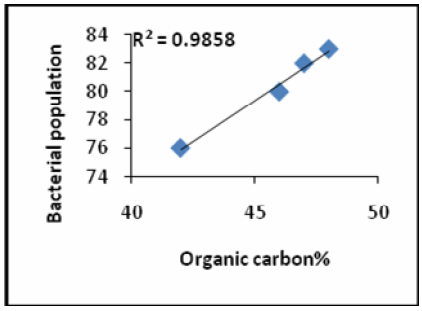


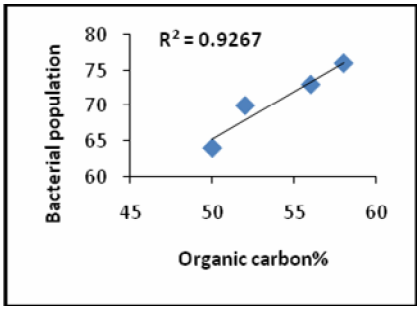
Table.8 Characterization of bacteria from soil samples (organic carbon %)

Sl. no.	RT			K		BH	
1	47	82		58	76	62	59
2	46	80		56	73	60	48
3	48	83		52	70	57	42
4	42	76	50	64	59	46	

Graph.1



Graph.2



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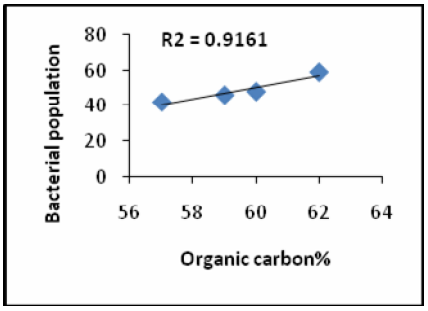
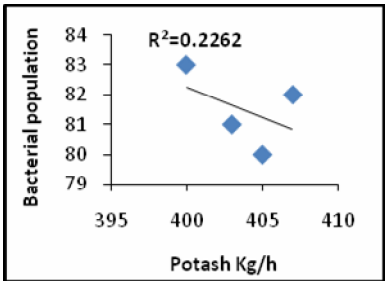


Table.9 Characterization of bacteria from soil samples (potash Kg/H)

Sl. no.	RT		K		BH	
1	407	82	426	73	286	59
2	405	80	420	70	270	53
3	403	81	423	71	275	50
4	400	83	421	69	274	51

Graph.1



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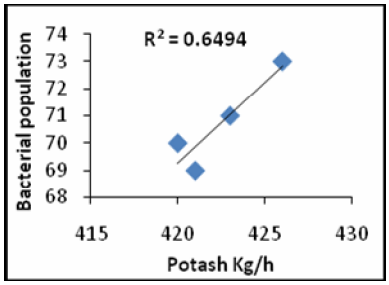

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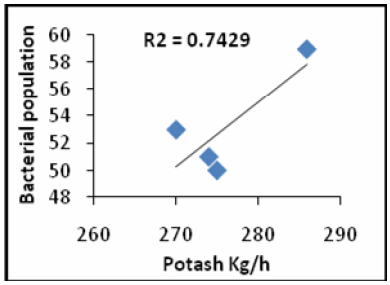
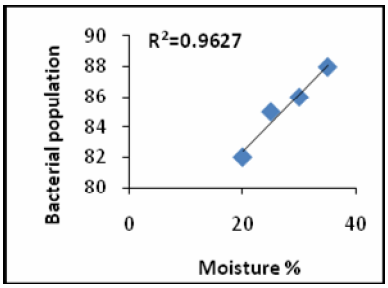


Table.10 Characterization of bacteria from soil samples (moisture %)

Sl. no.	RT		K		BH	
1	20	82	20	76	20	59
2	25	85	25	77	25	62
3	30	86	30	79	30	64
4	35	88	35	81	35	66

Graph.1



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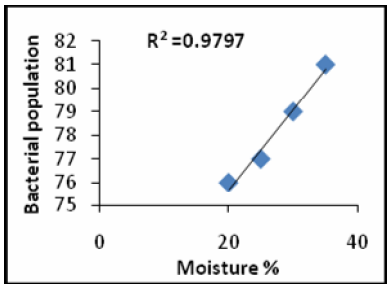
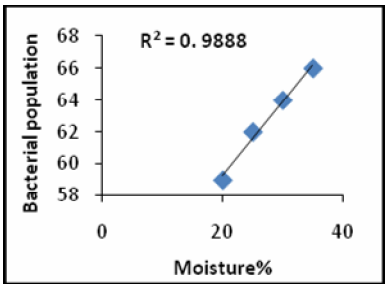


Graph.3



Further the high bacterial population in the soil samples may also be attributed to the suitable environmental condition governed by environmental variable like pH, soil moisture and organic carbon content. All these variables are found to have positive co-relation to bacterial population with R values greater than 9. However, very low co-relation was observed for potash and phosphorus content to the bacterial population indicating that these variables may not have a role in bacterial population. Microbial activity in soil is controlled by several environmental factors, such as availability of carbon, mineral nutrients and growth factors, availability of water, favorable temperature and pH, composition of soil micro flora and ecological interactions between micro-organisms.

Micro-organism plays an important role in soil fertility because they oxidize organic matter and promote the biogeochemical cycles of carbon, nitrogen, phosphorus and sulphur. Soil enzymes activities are involved in soil nutrient cycling dynamics and can catalyze the conversion of nutrient from unavailable to form readily assailable by plants and micro-organisms. In the present study no coli forms were detected in the soil samples which indicate that the soil are free from contamination by pathogenic bacteria and indicates that bacteria isolated were mostly saprophytes in nature.

In conclusion, the result of the study indicates that high bacterial populations were encountered from the study site. The high bacteria count may be attributed to the available nutrition of the soil sample. This may be due to decomposition of organic matter which is normally found in river bank and sediment soil. The pH of the soil were found to be acidic in nature which might have resulted into high bacteria count since most of the bacteria grow in slightly acidic to natural pH. The bacterial populations are found to be pH,

moisture and organic carbon dependent as found out in the co-relations graph when compared with this variables. However, no significant co-relation was observed for potash and phosphorus which suggest that the bacterial population may not depend on this element for their nutrition. The soil samples were not found to be coli form positive which indicate that the soil samples are not polluted by pathogenic bacteria. Many bacteria are found to produce secondary metabolites for biotechnological applications. In the present study also attempt could be made to screen this bacterial isolates for production of secondary metabolites although the present work is preliminary in nature, it elucidate the rich bacterial population of this ecosystem.

References

Acosta-Martinez, V., Dowd, S., Sun, Y., Allen, V. 2008. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type affected by management and land use. *Soil Biol biochem,* 40: 2762–2770.

Alabouvette, C., Rouxel, F., Louvet, J. 1979. Characteristics of fusarium wilt suppressive soils and prospects for their utilization in biological control. *In soil-borne plant pathogens*, ed. B Schippers, W Gams, pp. 165–82. Academic press, London.

Allen, E. B., Allen, M. F., Helm, D. J., Trappe, J. M., Molina, R., Rincon, E. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. *Plant and soil,* 170:47–62.

Bent, B., Yin, B., Figueroa, A., Ye, J.,Fu, Q. 2006. Development of a 9,600-clone procedure for oligonucliotide fingerprinting of rRNA genes that correlate in abundance with the development of avocado root rot. *J. Microbiol. Methods*, 67: 171–80.

Brady, N.C., Weil, R.R. 1999. Elements of the nature and property of soils, 1st ed. Prentice Hall, Upper Saddle River, NJ

Buckley, D.H., Schmidt, T.M. 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. *Environ. Microbiol*., 5: 441–452.

Davis, K.E.R., Joseph, S.J., Janssen, P.H. 2005. Effects of growth medium, inoculum size,incubation time on culturabiliy and isolation of soil bacteria. *Appl. Environ. Mcrobiol.,* 71: 826–834.

Gray, N. D., Hastings, R. C., Sheppard, S. K., Loughnane, P., Lloyd, D. 2003. Effects of soil improvement treatments on bacterial community structure and soil processes in an upland grassland soil. *FEMS Microbiol. Ecol.*, 46:11–22.

Hackl, E., Boltenstern, S., Bodrossy, L., Sessitsch, A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. *Appl. Environ Microbiol.,* 7:5057–5065.

Jonson, D., Booth, R.E., Whiteley, A.S., Bailey, M.J., Read, D.J., Grime, J.P. 2003. Plant community composition affects the biomass, activity and diversity of microorganisms in limestone grassland soil. *Eur. J. Soil Sci.,* 54: 671–677.

Kuske, C. R., Barns, S. M.,Busch, J. D. 1997. Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. *Appl. Environ. Microbial.,* 63: 3614–3621.

Liesack, W., Janssen, P. H., Rainey, F. A., Stackebrandt, E. 1997. Microbial diversity in soil: the need for a

combined approach using molecular and cultivation techniques, Pp. 375–439.

Norris, T. B., Wraith, J. M., Castenholz, R. W., McDermott, T.R. 2002. Soil microbial community structure across a thermal gradient following a geothermal heating event. *Appl. Environ. Microbial.,* 68: 6300–6309.

Stackebrandt, E., Liesack, W., Goebel, B. M. 1993. Bacterial diversity in a soil sample from a subtropical Australian environment as determined by 16S rDNA analysis. *Faseb. J.,* 7: 232–236.

Torsvik, V., Salte, K., Sorheim, R., Goksoyr, J. 1990. Comparison of phenotypic diversity and DNA heterogeneity in a population of soil bacteria. *Appl. Environ. Microbiol.,* 56: 776–781.