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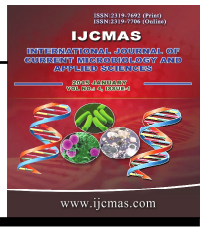

Original Research Article

Isolation and Identification of Soil Microflora of National Parks of Gujarat, India

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A B S T R A C T

In this paper, a database of topsoil soil microbial flora for four national parks of Gujarat is highlighted. The microbial community initially colonizes in a disturbed environment then will change the surroundings by decomposition of present organic material, thereby establishing a link between soil and environment. Then that primary community eventually gets replaced by a new community of microorganisms which keeps altering the ecosystem. Thus identification of microflora in forest soil types will surely help in suggesting strategies for sustainable forest management and also in order to describe the interrelationships and controlling mechanisms between out flux and influx of nutrients and energy in the soil, identification of soil microflora is obligatory. Identification of regional soil microflora was done which will help evaluating the role of microflora in soil in mitigation of greenhouse gas emissions through carbon sequestration. All four national parks showed the presence of all kinds of soil microflora including nitrifying and denitrifying bacterial species and some distinct fungal species.

Keywords

Soil Microbial Diversity, Soil Microflora, Top soil, climate change

Introduction

Soil is a complex environment offering a variety of microhabitats. The microbial community is one of the most important components of soil. This is one reason why microbial diversity in soils is much greater than that found in any other environment. The population compositions in the activity of microorganism are largely regulated by soil properties and by climate and vegetation (Jha *et al.*, 1992). Soil formation is the result of the combined action of weathering and colonization of geologic material by micro flora. It is also a favorable habitat for the proliferation of micro-organisms, and the number of micro-organism in soil habitats, typically 10^6 to 10^9/g is usually much higher than that in freshwater or marine habitats (Atlas, 1998). Microbial diversity depends on available nutrients and their varied concentrations (Willey *et al.*, 2008). Said to be evenly distributed throughout the soil column, higher numbers of microorganisms occur in the organically rich surface layers than in the underlying mineral soils (Atlas,

1998). A quantitative and qualitative estimation of soil microorganisms is, therefore, necessary to know the role of different microbes, operating in the soil which, in turn, helps in various ways (Aery 2010). The majority of microorganisms found in the rhizosphere are bacteria, but fungi also congregate in this region (Heritage *et al.,* 1999). These microorganisms decompose organic matter and play important role in various biological transformations taking place in the soil.

Soil microorganisms are important because they affect the soil's physical, chemical, and biological properties where several common groups of bacteria are especially important to ensure the health of the soil (Egger, 2010). Organic colloids and materials synthesized by soil fungi and bacteria are important in the formation of soil aggregates (Stallings, 1952). Bacteria are the largest group of soil microbes, both in total number and in diversity. Organic matter on and in forest soil helps improve and maintain soil structure (Lutz and Chandler, 1947). Soils used for agriculture, however, do not usually have enough organic matter in the surface layers to maintain the large populations of microorganisms found in forest soils. Indeed the presence of bacteria gives freshly dug soil its characteristic 'earthy' smell (Heritage *et al.,* 1999). Bacteria are the only life-forms capable of the biological fixation of nitrogen (Heritage *et al.,* 1999). Soil microorganisms significantly contribute to the maintenance of the matter and energy turnover in terrestrial environment. Soil represents one of the most significant places for biogeochemical processes, in which mineralization has a very important role (Luo and Zhou, 2006). Fungi plays significant role in soils and plant nutrition and also play important role in the degradation/decomposition of cellulose, hemi cellulose, starch, pectin, lignin in the organic matter added to the soil.

A global biogeochemical cycle is a conceptual model of pathways and flows of individual chemical elements and/or their compounds in the surface environment of the earth, which is made up by atmosphere, hydrosphere, lithosphere and biosphere as the major reservoirs (Lerman, 1988). Soil microbial biodiversity plays a key role by decomposition in the biogeochemical cycling of elements.

Just as marine primary production helps prevent and even more rapid increase in atmospheric CO_2, terrestrial forests are a tremendous CO_2 'sink.' As the soil microbes respiration rates increased, so does the volume of CO_2 released. If these findings can be extra polated to a world where CO_2 levels continue to increase, forest ecosystems may sequester less carbon than predicted (Willey *et al.,* 2008).

Materials and Methodology

Study area

Chose the study area for random sampling. Area considered was from the following weblink.
http://gujenvfor.gswan.gov.in/wildlife/wildlife-national-parks-sanctuaries.html

1) The Gir National Park (also known as Sasan- Gir) is a dry deciduous forest in the semi-arid western part of India covering area of 258.71 square kilometer in Gujarat, India established in 1965. It is the sole home of the Asiatic Lions and most important protected areas in Asia due to its supported species. Gir has a topography made up of succession of rugged ridges, isolated hills, plateaus and valleys.

2) Velavadar National Park: Established in 1976 in the in the Bhavnagar District of Gujarat state, India. It is spread over an area of 34.08 sq.km and typically Grassland, semi-arid bio-geographical zone about 50 kilometers west of the Gulf of Cambay.

3) **Marine National Park:** Situated on the southern shore of the Gulf of Kachchh in the Jamnagar District of Gujarat state, India. It is Mangrove forest and area 162.89 sq.km. There are 42 islands; best known ones are Pirotan, Narara, Sikka and kalubhar on the Jamnagar coast in the Marine National Park, most of them surrounded by reefs.

4) **Vansda National Park**: Representing moist deciduous forest covering area of 23.09sq.km having thick woodlands of the Dangs and southern Gujarat, in the mountains of Western Ghats or Sahyadris. is situated in the Navsari District of Gujarat state, India. Vansda, the town is an important trading place for the surrounding area where the majority of the population is represented by adivasis.

Thus, four National Parks of Gujarat state were chosen as the study area. Random sampling was done considering the different topography inside the national park falling under different eco-zones.

Soil Sample collection and Preservation

The Soil Samples were collected from four National parks mentioned above. The random soil sampling was done from the surface at the depth of 0-30 cm using GPS. Sampling Tool Hand auger used was also sterilized. Samples were put in sterile Plastic zip locked bags and immediately refrigerated in Laboratory. The numbers of samples were decided according to the area of the national park i.e., at least 25% of the area of the national park, published by FAO data.

Soil analysis (Physiochemical and Microbial)

A. Physicochemical analysis of soil
The physicochemical characteristics of soils were analyzed by standard methods. Soil pH, Electrical conductivity of soil, Bulk density of soil, Soil organic carbon percentage, Soil organic carbon density, these all characteristics were measured by standard methods like respectively.

1. The newer methods of measuring soil pH in a laboratory by taking 1:5 suspension of soil in 0.01 M $CaCl_2$ is being followed to measure pH of all four national park soil samples.
2. Electrical Conductivity was measured by standard Electrical Conductivity Meter.
3. The core method was used for the collected of samples for the bulk density determination. Soil samples were air dried ground and passed through a 2mm sieve before being used for analysis.
4. The organic c estimates in the soil samples were determined using the wet combustion method (Walkley and Black, 1934).
5. Carbon density was computed using the % C concentration, depth thickness and bulk density.

B. Microbial analysis of soil: Microbial analysis of soil was done by Media preparation, Autoclaving, Serial Dilution, Inoculation of the media for Isolation of organisms, Colony counting and identification of the same.

Media preparation: The media used were Nutrient Agar, Sabouraud Dextrose Agar, Actinomycete Isolation Agar, Asparagine Nitrate Medium and Inorganic Salt Medium. They were prepared as mentioned in Frankland *et al.* (1995) respectively to isolate different species of microflora. Appropriate amount of media powder was suspended in respective amount of distilled water. To dissolve the medium completely, it was heated till boiling. The media was autoclaved at 121°C and 15 lbs pressure.

Isolation and identification: An accurately weighed sample of 1g fresh soil was mixed with 10ml of D/W to make sample suspension. This sample was diluted up to 10^{-3} dilution by serial dilution method and the suspension was spread over media with the help of sterile glass spreader in aseptic condition (Robertson and Egger, 2010). The spread plates were permitted to absorb the inoculums at 37°C temperature for 24 hrs. They were inverted and incubated as desired. Morphological characteristics of all different colonies were observed which included size, shape, colour, elevation and transparency. Each and every different colony was identified with Gram stain Method (Gupta, 2006) then was followed by Colony Counting (Atlas Ronald, 1984). Plates with 30 to 300 colony forming units' i.e., CFUs/plate were used to calculate CFUs/ml. If fewer than 30, it runs into greater statistical in accuracy and if greater than 300, the colonies would be tedious to count and also would tend to run together (Jett *et al.*, 1997).

Result and Discussion

Keeping in mind, all the samples from all national parks were being tested and identified to confirm the identification up to the species level for each microbiological community.

Through microbiological analysis, following results were obtained shown in table 1

Table 1 shows National park wise results of Microbiological Analysis.

To give a glance of presence of particular species present in four national parks, site wise results of numbers of species obtained on nutrient agar plate is graphically shown in Figure 2.

Presence of fungi and presence of *Rhizobium species*

Critter *et al.* (2002) evaluated bacteria and fungus in soil samples quantitatively using agar plate counts and according to them both group of microorganisms when found in soil, play an essential role in nutrient transformations. Our results showed the presence of three different types of fungi on Sabouraud Dextrose Agar medium. They were found in all four National Parks which were identified as *Aspergillus niger* (GenBank Accesion no. KJ850946.1 and KJ850947.1), *Aspergillus tubingensis* (Genbank Accession No. KJ850945.1) and *Fusarium species (*GenBank: KJ850949.1). Presence of different types of fungi indicates freshness of soil.

Presence of Rhizobium species found in all four national parks.

Soil microbes act as important determinants of plant community diversity and productivity (Wardle *et al.*, 2004) and hence the input and output of quality and quantity of carbon input to soil (De Deyn *et al.*, 2008). Thus in Gir and Vansda, soil microflora species found must be closely in association with changes occurring in carbon as well as other biogeochemical cycles as they show presence of maximum species. Soil microbial biodiversity plays a key role in cycling of plants and animal

remains in ecosystem. It can help major effects on global fluxes of a variety of reactive gases, such as ammonia, hydrogen sulfide, and dimethyl sulfide. These reactive gases tend to be produced in more waterlogged environment (Willey *et al.,* 2008). Thus in marine national park species such as *Seratia spp*. and Actinomyces must be playing some role in change in global fluxes of these gases. Thomas *et al.* (2012) reported the dominance of archaebacteria in extreme environments of the Little and Great Rann of Kutch and identified haloarcheal linage which could sustain wide variation in the concentration of salts and extreme weather conditions. But we did not find any such archaebacterial species in sites of Marine national park as well as Velavadar with such similar sites. By uncertainty about how reactive different microbial groups and species are to temperature change and whether short-term increases in carbon mineralization—which are commonly observed in warming experiments in the field (Bardgett *et al.,* 2008)—will be sustained due to depletion of substrate availability and acclimation of soil microbial communities to higher temperature (Kirschbaum, 2006). Thus, the occurrence of Bacillus spp. in higher proportion in Gir and Vansda and also marking its presence in other two national parks shows that it might be temperature resilient species. Certain microorganisms carry out photosynthesis, rivaling plants in their role of capturing carbon dioxide and releasing oxygen into the atmosphere (Willey *et al.,* 2008) so as *Streptococcus spp., Bacillus spp* and *Nitrobacter spp*. shows their presence in all four national parks in abundance proving beneficial to adjoining plant community. The results are also in accordance with (Bhatt and Banmeru, 2014) where the maximum soil microbial biomass carbon obtained was in Gir and here maximum species seen was in Gir followed by Marine national park, followed by Vansda and least number of species marked in Velavadar National Park.

This study is a modest effort to understand Soil microbial diversity in major terrestrial ecosystems of Gujarat, with a focus on preliminary assessment and identification of soil microflora of forest soil types. As soil microbial biodiversity plays the key role in decomposition and acts as the major contributor to biogeochemical cycling. Change in this diversity surely changes the decomposition rates leading to disturbed biogeochemical cycling of elements like C, N and S. The potential to mitigate climate change by reducing greenhouse gas emissions through managing terrestrial microbial processes is a tantalizing prospect for the future (Singh *et al.,* 2010). Thus, such estimates may provide a database as inputs to models for aspects related to biogeochemical cycling and climate change in Gujarat context. However, the other aspects of identification of soil micro flora of forest including identification of organisms up to species level, future predictions of various human dimensions to climate change for policy generation at state level needs to be elucidated. In the light of the work done, this study could be forerunner to studies aiming at a detailed and improved understanding of soil micro flora in Gujarat, the various factors influencing it, and its coupled interactions with another major biogeochemical cycle of carbon. This has an important bearing on policy issues for controlling nitrogen pollution as well as implications for changes in Carbon cycle, which are very important in global context. This microbial process level uncertainty extends to unreliable model predictions of soil carbon feedbacks to climate change (Kirschbaum, 2006) and resolving this issue represents a major research challenge for the future.

Table.1 shows National park wise results of Microbiological Analysis.

Name of the National park	Bulk density	Soil Organic Carbon (%)	Soil organic carbon density	Electrical conductivity	pH
Gir National Park	1.1461	1.95± 0.59	20.69±0.97	0.16	7.639
Velavadar National Park	1.2311	1.24 ± 0.56	15.66±2.15	7.83	8.519
Vansda National Park	1.0867	1.08±0.44	11.33±1.40	0.14	7.368
Marine National Park	1.0767	0.47±0.056	5.07±0.61	6.77	8.609

Sr. no.	**Species**	**Vansda**	**Velavadar**	**MNP**	**Gir**
		These numbers are in percentage (%) form.			
1	*Nitrosomonas spp.*	-	-	30	16
2	*Streptococcus spp.*	29	19	13	16
3	*Staphylococcus spp.*	14	24	-	11
4	*Neisseria spp.*	-	5	-	9
5	*Micrococcus spp.*	-	-	-	4
6	*Bacillus spp.*	29	9	5	18
7	*Pseudomonas spp.* (GenBank: KJ850948.1)	-	-	10	9
8	*Nitrobacter spp.*	14	19	13	12
9	*Acinetobacter spp.*	-	24	12	5
10	*Enterococcus spp.*	14	-	-	-
11	*Actinomyces spp.*	-	-	12	-
12	*Seratia spp.* (GenBank: KJ850950.1)	-	-	5	-

Figure.1 Map of Gujarat showing four national parks marked as Site Description

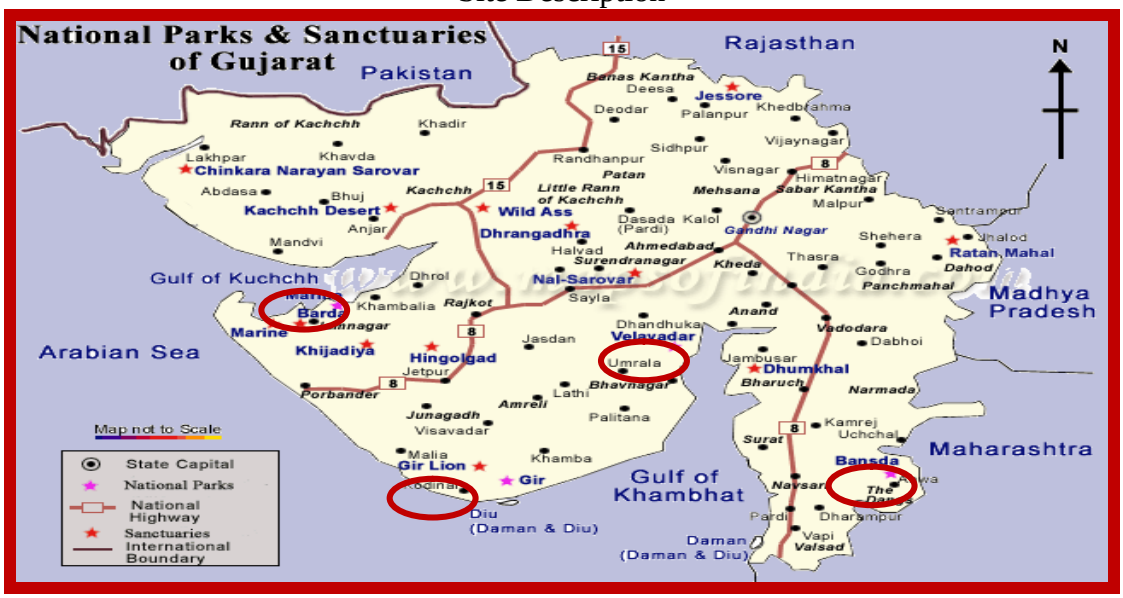


Figure.2 Site wise results of numbers of species obtained on Nutrient Agar plate.

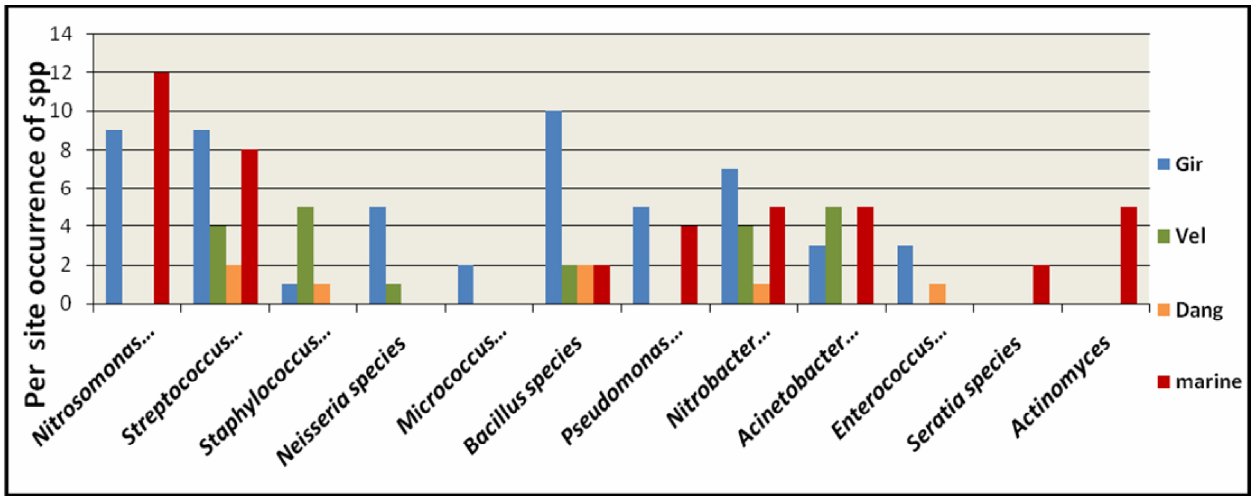


Fig.3 Gram Negative Cocci

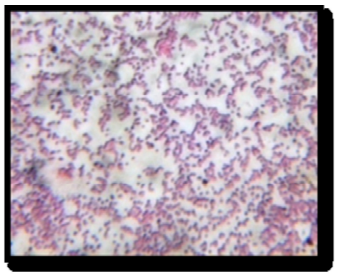


Fig.4 Gram Positive Bacilli

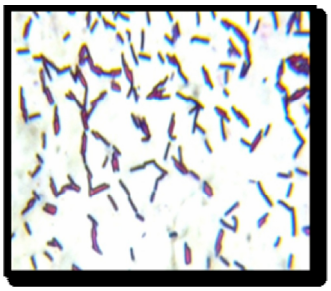
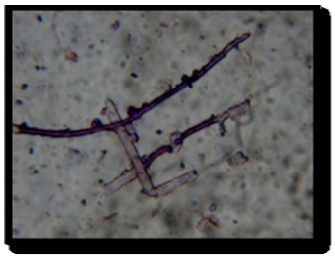
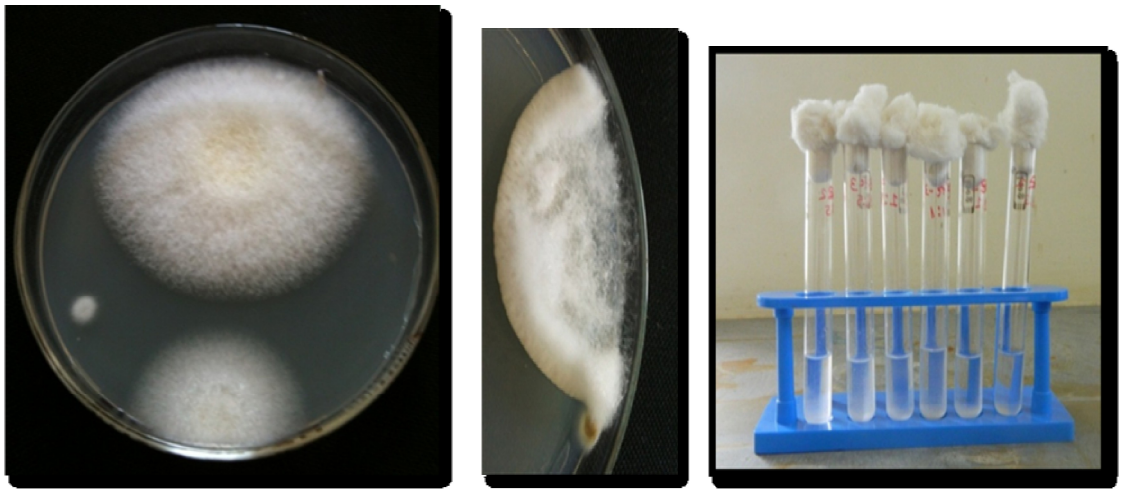


Fig.5 Microscopic views of hyphae



Luxuriant growth of Rhizobia seen in Inorganic Salt Medium



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