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Isolation, Identification and Comparative Study of Fungal and Bacterial Strains Found in Organic and Inorganic Soils of Different Agricultural Fields

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Abstract

Soil microbial communities are arguably the most diverse communities on earth and soil represents a favorable habitat for microorganisms. Microorganisms are found in prominent amount with great species diversity in the soil of the earth. In the present investigation the extent of the diversity of microorganisms in organic soil was seen enormous comparatively in Inorganic soil. This wide range of microorganisms is also involved in important soil functions. The development and application of methods to explore microbial diversity in organic and Inorganic soil has revealed that a remarkable diversity of microorganisms were found in organic soil as compared to Inorganic soil. The reason for this was that the organic soil nourished with organic matter (manures) provided an important habitat for microorganisms. The results so far showed that the abundance of different microbial groups and total microbial biomass was generally increased by organic matters in comparison to inorganic fertilizers. It was found that with organic manures was rich in bacterial diversity (859 colonies), fungal diversity (130 colonies) and other number of microorganisms. However species richness was higher in fungi as eighteen genera comprising of 39 species and 7 genera comprising of 8 species of bacteria and has found that the organic soil was highly diverse than inorganic soil. Thus organic fertilizers have changed soil microbial community structure and we propose the fact that the soil treated with organic fertilizers is the key factor determining that soil microbial diversity is related to the complexity of the microbial interactions in soil, including interactions between microorganisms and soil, and microorganisms and plants. From the present study we thus conclude that microbial diversity of organic soil is greater and higher as compared to inorganic soil.

Key Words: Organic, Soil microbiology, Bacteria, Fungi and Agronomic fields

Introduction

Soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms. Microorganisms are found in large numbers in soil usually between one to ten million microorganisms are present per gram of soil with a dominant number of bacteria and fungi. Soil organisms contributes to important soil functions such as supporting the growth of plants both in natural plant communities and those grown for food, fibre, or energy and absorbing, neutralizing and transforming compounds that might otherwise become pollutants in the environment. Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contribution of these microbes. Soil microbial diversity is influenced by both organic and inorganic matter. Soil organic matter is generally used to represent the organic constituents in the soil, excluding undecayed plants and animal tissues, their partial decomposition products and the soil biomass [1]. The soil organic matter provides a favorable habitat for the microorganisms to grow as compared to inorganic soil. The bacterial diversity present in the soil is greatly influenced by organic matter. It has been consistently reported that soil organic matter favors the growth of bacteria present in soil. The studies have revealed that bacterial diversity is approximately one hundred times greater than the other microbial diversity [2].

Bacteria are one of the most important components of the soil micro biota and don't occur freely in the soil solutions but are closely embedded in organic matter even often adding as the dispersing agents [3]. Moreover they play a major role in organic matter decomposition, biotransformation, biogas production, nitrogen fixation. In particular they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like potassium, phosphorous and nitrogen which are required for plant growth [4].

In most of the aerated or cultivated soils fungi share a major part of the total microbial biomass. Many important plant

pathogens and plant growth promoting microorganisms are fungi. Fungi are also critical decomposers in soil habitat like bacteria; fungi derived nutrients for their growth from organic matter [5]. The rest being actinomycetes, protozoa, algae and many other also constitute the microbial diversity of soil. Microbial biomass in the soil display a positive linear relationship with annual net primary productivity, demonstrating that the growth of microorganisms and of crops can be controlled and influenced by using organic matter [6].

Study site

For the comparison of microbial diversity in the organic and inorganic soil fields, the soil samples were taken from the "Biodiversity Conservation Farm" of NAVDANYA RESEARCH FOUNDATION run by an eminent environmentalist Dr. Vandna Shiva of India. This farm is located at Ramgarh about 16 km away from Dehradun. The Navdanya Research Foundation is working under the thought of organic farming and the methods for agriculture and post cropping activiyies are met with the global sustainable use of soil, organic fertilizers etc. Futhermore, the "*movement of protection of seeds"* is successfully running from the foundation, especially the grain seeds and pulses used in the hilly areas of Uttarakhand since time immemorial.

Materials and method

Isolation of bacteria

Isolation of microbes from soil took many steps which includes field trips, lab work etc. Soil samples were collected by sterile methods from organic and inorganic sample plots/fields visited during the time of fructification of crop and brought to the lab in the air tight polybags. The vertical samples were taken from 5 and 10 cm depth. The samples were processed using soil dilution plate method. One gram of soil sample was serially diluted with sterilized distilled water upto 10^{-1}, 10^{-2}, $10^{-3,}$ 10^{-4}, 10^{-5} 10^{-6}, 10^{-7} and 100 ml of each dilution was added to 20ml of nutrient agar medium in 90mm diameter sterile Petri dishes and then enumerated. Single separate colonies on the agar plates were selected at random according to standard medium and streaked on the nutrient agar slants and incubated for 24 hrs at ± 30^0 C. Code names were given to each of the isolated plates and stored at ± 4^0 C for characterization and identification by standard methods. Once colonies rose in the media, the sub culturing was continued until a pure isolate was obtained. Identification of microbes was done with the help of standard literature. For isolation of bacteria different media like Nutrient agar medium, Nutrient broth medium etc. (Hi media) were prepared and to differentiate between gram +ive and gram –ive bacteria Gram's staining was done. Thereafter for identification different biochemical tests were performed for both organic and inorganic bacterial colonies [7].

Isolation of fungi

The soil borne fungi was isolated and their total population was enumerated by following the method as given below:

First soil samples were collected from both the organic and inorganic fields, then 3 flasks (250 ml) were taken and 90 ml distilled water was transferred into each flask. Each flask was plugged properly, labeled 1-3 and autoclaved at 15lb/inch^2 for 30 minutes. 1 gm of soil sample was weighed and transferred into the flask 1containing 90 ml. It gives the dilution 1:10 i.e. 10^{-1}.Then it was shacked for five minutes gently with a stirrer to get homogenous soil suspension. 1.0 ml soil suspension was transferred from 10^{-1} dilution into flask 2 containing 90 ml distilled water to get dilution 10^{-2} and then mixed it gently. Similarly 1ml of soil suspension was serially transferred from 10^{-2} dilution into flask 3 containing 90 ml water to get the final dilution of 10^{-3} and mixed it gently. 1ml of soil suspension was aseptically poured from 10^{-3} dilution in different media plates. The plates were gently rotated so as to spread the suspension on medium. The plates were incubated at ± 25^0 C for 4-5 days. Different media like Czapek Dox Agar medium, Potato Agar medium, Martin's Rose Bengal medium, etc. were prepared for isolation of fungi. For identification of fungi lacto phenol and cotton blue stain was used also called as mounting fluid. The slides were observed under microscope and fungi were identified by following the mycological literature [8].

Table 1: Occurrence of fungal colonies in serial dilution method in both organic and inorganic fields

S.No.	Field	Dilution	Organic field No. of colonies in serial Dilution method	Organic field No. of organisms per gram of soil	Inorganic field No. of colonies in serial Dilution method	Inorganic field No. of organisms per gram of soil
		10^{-4}	15		7	
		10^{-5}	5		3	
1	A*	10^{-6}	2	22×10^{-7}	1	11×10^{-7}
		10^{-7}	-		-	
		10^{-4}	12		7	
		10^{-5}	8		4	
2	B*	10^{-6}	6	26×10^{-7}	1	12×10^{-7}
		10^{-7}	-		-	
		10^{-4}	12		5	
		10^{-5}	8		3	
3	C**	10^{-6}	3	23×10^{-7}	1	8×10^{-7}
		10^{-7}	-		-	
		10^{-4}	14		4	
		10^{-5}	6		1	
4	D**	10^{-6}	2	22×10^{-7}	-	5×10^{-7}
		10^{-7}	-		-	
	Overall Total colonies		93		37	

*Wheat field; **Pea field in both organic and inorganic field

Table 2: Percentage of occurrence of various fungus species colonies in examined field areas

S. No.	Fungi from organic field	Fungi from organic field	Fungi from organic field	Fungi from Inorganic field	Fungi from Inorganic field	Fungi from Inorganic field
	Species	No. of colonies	Occurrence %	Species	No. of colonies	Occurrence %
1	*Absidia ramosa*	2	2.10	Not found	-	-
2	*Acaulospora elegans*	2	2.10	Not found	-	-
3	*Acremonium stomaticum*	2	2.10	Not found	-	-
4	*Alternatia brassicicola*	3	3.15		2	5.4
5	*Aspergillus niger*	5	5.26		4	10.8
6	*A. nidulens*	4	4.21		3	8.1
7	*A.terreus*	3	3.15	Not found	-	-
8	*A.versicolor*	2	2.10		1	2.7
9	*A.candidus*	1	1.05		1	2.7
10	*A.flavus*	1	1.05		1	2.7
11	*A.fumiculosus*	2	2.10	Not found	-	-
12	*A.ustus*	1	1.05	Not found	-	-
13	*Chrysosporium sp.*	3	3.15		1	2.7
14	*Cladosporium cladosporoides*	4	4.21		1	2.7
15	*C.lunata*	2	2.10	Not found	-	-
16	*C.elatum*	1	1.05	Not found	1	2.7
17	*Fusarium oxysporium*	2	2.10		2	5.4
18	*F.moniliformae*	4	4.21		2	5.4
19	*Gigaspora gigentium*	2	2.10	Not found	-	-
20	*G. remisporophora*	1	1.05	Not found	-	-
21	*Glomus mosseae*	3	3.15		1	2.7
22	*G. fasciculatum*	1	1.05	Not found	-	-
23	*G.ambisporum*	1	1.05	Not found	-	-
24	*G.formosanum*	2	2.10	Not found	-	-
25	*Humicola grisea*	3	3.15		2	5.4
26	*H. fusco-atra*	2	2.10	Not found	-	-
27	*Mucor mucido*	4	4.21		2	5.4
28	*M. hiemallis*	1	1.05		1	2.7
29	*Penicillum rubrum*	5	5.26		4	10.8
30	*P. puberrulum*	3	3.15		1	2.7
31	*P. purprogenum*	3	3.15	Not Found	-	-
32	*Rhizopus arrhizus*	4	4.21		2	5.4
33	*R. oryzae*	2	2.10		2	5.4
34	*Sclerocystis rustiformis*	1	1.05	Not found	-	-
35	*Scutellospora pellucid*	2	2.10	Not found	-	-
36	*S. gregarea*	1	1.05	Not found	-	-
37	*Trichoderma lignorum*	4	4.21		2	5.4
38	*T. viride*	3	3.15		1	2.7
39	*Verticillum candelabrum*	1	1.05	Not found	-	-
Total		93			37	

Table 3: Occurrence of Bacterial and actinomycetes colonies in serial dilution method in both organic and inorganic fields

S.No.	Field	Dilution	Organic field: No. of colonies in serial Dilution method	Organic field: No. of organisms per gram of soil	Inorganic field: No. of colonies in serial Dilution method	Inorganic field: No. of organisms per gram of soil
		10^{-4}	84		45	
		10^{-5}	60		43	
1	A*	10^{-6}	17	168×10^{-7}	9	98×10^{-7}
		10^{-7}	7		1	
		10^{-4}	77		39	
		10^{-5}	49		32	
2	B*	10^{-6}	17	149×10^{-7}	6	77×10^{-7}
		10^{-7}	6		-	
		10^{-4}	62		43	
		10^{-5}	54		32	
3	C**	10^{-6}	16	135×10^{-7}	2	77×10^{-7}
		10^{-7}	3		-	
		10^{-4}	55		39	
		10^{-5}	26		11	
4	D**	10^{-6}	17	102×10^{-7}	3	53×10^{-7}
		10^{-7}	4		-	
	Overall Total colonies		554		305	

*Wheat field; **Pea field in both organic and inorganic field

Table 4: Showing the description of unknown samples isolated from organic field

S. No.	Experimental Procedure	Observations and Results					
		C1	C2	C3	C4	C5	C6
1	Gram stain	+	+	-	-	-	-
2	Shape and arrangement	Rod shaped	Rod shaped	Thick Rod shaped	Thin Rod shaped	Thick Rod Shaped	Thick Rod shaped
3	Cultural Characteristics	Round creamy white	Transparent white	Round creamy white	Irregular creamy white	Round creamy white	Round creamy white
4	Carbohydrate fermentation						
	a)Glucose	+	+	+	+	+	+
	b)Sucrose	+	+	+	+	+	+
	c)Fructose	+	+	+	+	+	+
5	Starch hydrolysis	+	+	-	+	-	-
6	Catalase activity	-	+	-	+	-	+
7	Indole activity	+	-	-	+	-	-
8	Methyl Red test	+	+	+	-	+	+
9	Voger Proskauer test	-	+	-	+	+	-
10	Oxidase test	+	-	+	+	-	+
11	Citrate utilisation	-	+	-	-	+	+

+ive = Present
-ive = Absent

Table 5: Showing the description of unknown samples isolated from Inorganic field

S. No.	Experimental Procedure	Observations and Results				
		C1	C2	C3	C4	C5
1	Gram stain	+	+	+	-	+
2	Shape and arrangement	Rod shaped in chains	Thin Rod chains	Thick Rod shaped	Rod shaped	Rod Shaped in chains
3	Cultural Characteristics	Round creamy white	Round creamy white	Irregular transparent	Light yellowish colony	Round creamy white
4	Carbohydrate fermentation					
	a) Glucose	+	+	+	+	+
	b) Sucrose	+	+	+	-	+
	C) Fructose	+	-	+	+	+
5	Starch hydrolysis	+	-	-	+	+
6	Catalase activity	-	+	+	+	-
7	Indole activity	+	+	-	+	-
8	Methyl Red test	-	-	+	+	+
9	Voger Proskauer test	+	+	+	-	-
10	Oxidase test	-	+	-	+	+
11	Citrate utilization	+	+	+	-	+

+ive = Present
-ive = Absent

Table 6: Percentage of occurrence of various bacterial species in examined field area

S.No.	Bacteria from Organic field			Bacteria from Inorganic field		
	Species	No. of colonies	Occurrence %	Species	No. of colonies	Occurrence %
1	Pseudomonas florescens	115	20.75		94	30.8
2	Bacillus megaterium	99	17.87		75	24.5
3	Bacillus cocii	87	15.70		62	20.3
4	Clostridium perfringens	43	7.76	Not found	-	-
5	Azotobacter vinelandii	78	14.07		59	19.3
6	Escherichia coli	37	6.67	Not found	-	-
7	Flavobacterium	74	13.35	Not found	-	-
8	Streptomyces	21	3.79		15	4.9
Total		554			305	

Results and Discussions

An acceptable number of fungi in 1g of fertile soil are around 400000 [9]. However in the present study a total of 989 isolates of microbes comprising of bacteria, fungi, actinomycetes were obtained from the analysis of 8 soil samples taken 4 from organic and 4 from inorganic sample fields in September 2008. The serial dilution method was followed to determine the microbial diversity of soil. The identification of these isolates resulted in 47 species of microbes including bacteria (7 species), fungi (39 species) and actinomycetes (1 species). The genera with the greater number of species in fungi were *Aspergillus* (8 species), *Glomus* (4 species), *Penicillium* (3 species). *Cladosporium* (3 species) in the serial dilution plate method. The most widely distributed and abundant colony forming taxa were *Penicillium* (16 colonies), *Aspergillus* (29 colonies), *Rhizopus* (10 colonies) *Trichoderma* (10 colonies), *Fusarium* (10 colonies), *Glomus* (8 colonies), *Cladosporium* (8 colonies) and *Humicola* (7 colonies) in both soil sample fields. The richest genera in terms of the number of species were *Aspergillus* and *Penicillium* and the most common ones in both sample fields were *Aspergillus niger*, *A. versicolor*, *A. flavus*, *A. candidus*, *Penicillium rubrum*, *P. puberlum*, *Cladosporium cladosporiodes*, *Trichoderma lignorum* and *Glomus mosseae*. Thirty nine fungal species representing 18 genera were isolated and identified from organic and inorganic fields. Three species belonged to genus *Penicillium* and *Cladosporium*, four to *Glomus a*nd eight to *Aspergillus*. Two species belonged to each genus of *Rhizopus, Mucor, Humicola, Fusarium, Gigaspora*, *Trichoderma* and *Scutellospora.* While the rest of the genera *Acremonium, Verticillium, Acaulospora*, *Albidia, Alternaria*, *Chrysosporium* and *Sclerocystis* were represented by a single species.

In bacteria the results showed that the number of colonies was found higher in organic field (554 colonies) in comparison to inorganic field (305 colonies). The study also showed that bacterial colonies grown on potato dextrose and malt extract medium were higher than fungal colonies which proved the earlier research that bacteria produce different kinds of enzymes which inhibit the other fungal species in soil whether that are useful or pathogenic to the crops. Pseudomonas and bacillus genera were found both in organic and inorganic fields and were proved higher in species richness among other bacterial species. This data proved that both the bacterial genera were able to tolerate adverse microclimate in soil and decompose organic material and can synthesize inorganic minerals. Moreover they can sporulate properly and could help in making soil much nutritive with the help of different types of enzymes produced by them [10]. On the other hand *Clostridium Perfringens, Streptomyces, Flavobacterium sp., Azotobacter vinelandii, Azospirillum* sp. and *Escherchi coli* were not isolated from inorganic soil samples may be due to their non occurrence in these types of soils or could not rose in used culture medium.

The isolation of various fungal, bacterial species showed that the soil of organic field is quite rich in microbial flora. A total of 93 colonies of fungi and 554 colonies of bacteria were isolated from organic field, while a total of 37 colonies of fungi and 305 colonies of bacteria were isolated from inorganic field.

The results showed that soil was rich in bacterial diversity (859 colonies) comparatively to fungal diversity (130 colonies), however species richness were high in fungi as eighteen genera comprising of 39 species were found in both types of fields, while only 7 genera comprising of 8 bacterial species has been found. Since the soil samples were taken during fruiting time of crop, at the fruiting time exchange of mineral production of different micronutrient material by bacteria was so high, thus many colonies were found on triplicate agar plates. The organic field nourished with cow dung, ashes, mulches, vermicompost etc. produced high number of bacteria and fungi comparatively to inorganic fields where only 3 species of bacteria and 21 species of fungi were recorded which is nourished with chemical fertilizers. Among 39 species of fungi, 20 species were isolated from both organic and inorganic fields while 19 species could not found in inorganic field. The occurrence of plenty of species in genus *Aspergillus* (8) and *Penicillium* (3) are probably due to their capability of producing a diverse range of antibiotics and mycotoxins which protect them from other soil organisms and may also hinder the growth of other fungal species. The percent occurrence of these fungal species is given in the table 2. *Aspergillus niger* and *Penicillium rubrum* both showed occurrence frequency of 5.3% in organic field and 10.8% in inorganic field. *A. nidulens, cladosporium cladosporoides, Fusarium oxysporium, Mucor mucido, Rhizopus arrhizus, Trichoderma lignorum*, showed 4.3% occurrence frequency in organic field, while in inorganic field the percent occurrence is 8.1%, 2.7%, 2.7%, 5.4%, 5.4%, 5.4% and5.4% respectively. Whereas the lowest occurrence frequency was shown by *A. flavus, A. candidus, A. ustus, Cladosporium, C. elatum, Gigaspora remisporophora, Glomus fasciculatum, Mucor heimalis, Sclerocystis gregarea and Verticillum candelabrum* as there were isolated not more than 0.76% of total colonies of fungi in both fields. Some other species i.e, *Absidia ramosa, Acaulospora elegans, Acremonium stomaticum, Aspergillus versicolor, A. terreus, A. fumiculosus, Alternaria brassicola, Cladosporium lunata, Gigaspora gigantea, Glomus mosseae, G. formosanum, Humicola grisea, H.fusco-atra, Penicillium puberrulum, P. purprogenum and Trichoderma virde* have showed moderate percentage of occurrence. The same results were found with the bacteria where the percentage occurrence was higher in organic field comparatively to inorganic fields.

These results showed that a positive correlation exists between percentage of occurrence and the number of soil samples from which fungal species were collected. The significance of this correlation is that within a specific area we can determine the distribution of a particular fungal species from its occurrence frequency [11]. The study extends to relate occurrence frequency with sporulation and growth rate on culture. 21 species were isolated from both types of fields with much variation in microclimatic conditions. These include *Aspergillus flavus Aspergillus niger, Aspergillus versicolor, Cladosporium cladosporioides,Mucor hiemalis, Penicillium rubrum, P. purprogenum and Trichoderma lignorum.* Their isolation from both types of localities and especially those one with highest occurrence frequency showed that these are physiologically more versatile, not only in coping with the harshness of the microclimate but also have an extensive

enzyme system to degrade organic matter of various nature [12]. This study also proved that organic fertilizers have great capacity to give a good atmosphere for microbial growth comparatively than in inorganic fertilizers because synthetic fertilizers depends on the chemical reactions while due to organic fertilizers, natural physiological activities occur among various microbes.

The consequence of the present study that the organic farm soils have a great capacity to give space to the microbial survival which renders a fruitful outcome in the form of good crop production having a great tolerance to atmospheric pathogens and diseases. At the same time inorganic soil has less microbial diversity which proved that some bacteria or fungal species may be found in inorganic fields but not able to use properly the microclimate or micronutrients as can be used by organic microbes. The mycorrhizal species i.e. *Gigaspora, Glomus, Sclerocystis, and Scutellospora* have been found in ample amount in organic fields while only a single colony has been found in inorganic field. Furthermore the results indicate that common practices of using synthetic fertilizers harms the soil quality in time and consequently low fertility of soil can be observed.

Thus from the results we concluded that organic soil was rich in microbial diversity in comparison to Inorganic soil whether the microbes were bacteria, fungi, etc. It was because the soil with organic matter provides a favorable habitat for microbial diversity whereas inorganic soil with chemical fertilizers has harmful effect on the growth of microbial diversity and cannot survive.

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