ISSN : 0974 - 7451 Volume 7 Issue 5

Trade Science Inc.

Environmental Science
An Indian Journal

Current Research Paper

ESAIJ, 7(5), 2012 [192-197]

Microbial diversity in flooded rice soil

A.Sridevi[1], G.Narasimha[2]*, B.Ramankrishnan[3]

[1]Department of Biotechnology, Sri Padmavati Mahila University, Tirupati.-517502, AP, (INDIA)

[2]Applied Microbiology Laboratory, Department of Virology, Sri Venkateswara University, Tirupati. 517502, AP, (INDIA)

[3]Central Rice Research Institute, Cuttack, Orissa, (INDIA)

Received: 23rd February, 2012 ; Accepted: 23rd March, 2012

ABSTRACT

Soil samples of both alluvial and acid sulfate saline were analyzed for their physico-chemical properties, microbial biomass and populations in the present study. Higher organic matter content with 1.88mg/ml was noticed in saline (pokkali) than alluvial soil. The soil pH was slight variation from 5.47 to 5.15. The contents of total nitrogen and sulfate are 0.09% and 0.0086% in alluvial where as 0.25% and 0.0072% in pokkali respectively. The microbial populations like heteotrophic, oligotrophic, aerobic and anerobic were enumerated on various media. The alluvial soil had considerable population of aerobic heterotrophic bacteria and the diluted nutrient agar supported least number of the total aerobic heterotrophic bacteria whereas the soil extract agar had the highest populations. The population of diluted nutrient broth bacteria in alluvial soils was considerable whereas the population of nutrient broth bacteria in flooded alluvial soils was more than that of the DNB bacteria under aerobic conditions. On the contrarily, DNB were dominated under anaerobic conditions. In this study, flooded conditions are favorable for the build of oligotrophic bacteria especially those capable of growing under anaerobic conditions. The populations of anaerobic oligotrophic bacteria are also more than the population of aerobic oligotrophic bacteria. Hence the study explains the role of microorganism on the dynamic and mechanisms of nutrient mineralization and sustainability of this flooded agro ecosystems greater significance now the even before.

KEYWORDS

Flooded rice soil;
Physical and chemical properties;
Microbial diversity.

INTRODUCTION

Flooded rice soils are the best source for the study of diversity of microbial community in an important agro-ecosystem. Microbial biomass of soil represents the total mass of microorganisms that have values of less than 5000 Um^{-3} and constitutes up to 50 case dry weight / hectare with diverse populations of bacteria fungi and micro fauna[1]. These microorganisms' acts as biocatalyst in nutrient cycle and ecosystem functioning. About 200-2000Ug microbial biomass carbon g-1 soil is often found in agricultural soils. This microbial community is made up by thousands of different species of which only 1% can only be cultivated and thus characterized[2]. The diversity of microorganisms is immense and is required for the functioning of the destruction process of

dead organic matter[3]. The relation between soil function and soil microbial diversity has been explained in several experimental studies[4,5], but so far, a clear picture has not been obtained, as the complex interrelation between the different soil organisms which may create unique niches for each other[6]. Another problem is that only a small portion of soil micro biota is actively governing the energy flux of the soil system while most of the soil microorganisms are presumably dormant[7,8]. This microbial cell biomass is involved in fixation of 100 – 600 kg nitrogen and 50-300 kg p/h in the upper 30 cms of soil[9]. These levels of nutrients exceed the annual application through fertilizers.

Microflora in rice soils alters in particular sequence that aerobic bacteria (in few days after the soil is flooded) to facultative anaerobes to anaerobes[10,11]. The highest agricultural priority in Asia is rice production and about 90% total world rice area is in Asian countries, with 75 million hectors harvested annually[12]. These high inputs provide staple food grains for one billion people productivity. Rice is preferable grown under flooded conditions since rice yields better under these conditions. But the mineralization of carbon in anaerobic flooded rice soils has lead to the production of methane which leads changes in global climate. Rice soils are considered as an important sources of methane contributing about 60 TG Y -1 with a range of 25 – 100 TG -1[13]. There are microbes in flooded rice soils which harbored oligotrophic or diluted nutrient broth (DNB) bacteria which are culturable only in 100 folds diluted nutrient broth medium. The studies on the occurrence of oligotrophic bacteria in anaerobic flooded rice soils are understand their roles in carbon mineralization. The microbial biomass is known to determine the rates of mineralization[14]. The estimated of soils biomass is considered to be a good indicator of microbial status and of soils health. Therefore experienments were conducted in the present study to compare different culture media for enumeration of bacteria and to isolate and enumerate DNB or oligotrophic bacterial population in soils and in roots of rice.

MATERIALS AND METHODS

Collection of soil samples

An alluvial soil from the experimental farm of Central Rice Research Institute, Cuttack and an acid sulfate (Pokkali) soil collected from Ernakulum, Kerala were used in various experiments of this study. The collected samples were air dried under shade and big soil clods were pounded with a wooden mallet. They were passed through a 2 mm sieve and stored at room temperature. The physico-chemical properties of the soil are measured by the standard procedures of Jackson (1967)[15].

Microbial analyses in soil samples

Enumeration of microbial population

One gram of soil sample was diluted by tenfold using sterile water (10 mL). After a thorough mixing, diluted sample (1 mL) was transferred to another sterile water blank (9 mL), which conformed to 10^2 dilutions. Similar procedure was followed upto 10^8 dilution.

Nutrient agar medium which consisted of (g/L): 3.0 beef extract, 5.0 tryptone and 20.0g agar and pH 7.0), a 100 X diluted nutrient medium, plate count- and soil extract agar for bacteria, and Rose Bengal and Czapek-Dox agar medias for fungi were autoclaved at 15 lb pressure and 121^0C for 20 minutes and used for enumeration of microbial populations including bacteria and fungi. For this an aliquot (0.5mL) of the diluted soil samplers (10^{-5}, 10^{-6} and 10^{-7}) was transferred to each sterile plate. After pouring 15 mL of the medium onto sterilized Petri-plate and swirling gently to mix the contents and the agar was allowed to solidify. Then, the plates were inverted and placed in a BOD incubator for aerobic conditions while an anaerobic jar was used for anaerobic conditions. Enumeration for bacteria of fungi was done after 4 to 7 days and after 30 days, for anaerobic oligotrophic bacterial population.

Extraction and estimation of microbial biomass

All the samples in the conical flask were shaken for 30 minutes in a shaker. The samples were allowed to settle down and the supernatant was filtered. A 5 mL of portion of filtrate of each sample was transferred to a 500 mL conical flask. To each of the flask 10 mL of 1N potassium dichromate and 10 mL of concentrated sulphuric acid were added. Instead of the filtrate 5 mL potassium sulphate solution was taken

in the blank samples. Two blank samples were taken. All the samples in 500 mL flasks were digested on a hot plate for 30-40 minutes. To all the samples, after cooling, 200 mL of distilled water was added. Diphenylamine indicator was added to each flask and samples were titrated against 0.25N ferrous sulphate solution. The biomass C by means of an extraction with 0.5 m K_2SO_4 was calculated according to Vance *et at.* (1987)[16].

Enumeration of aerobic bacteria and fungi

Populations of total aerobic bacteria (heterotrophic) and fungi in the air-dried alluvial soil sample were estimated by the standard dilution plate technique using different media. The culture plates were incubated at $28 \pm 2\ ^0C$ for aerobic growth in a BOD incubator.

Enumeration of diluted nutrient bacteria (DNB)

For the isolation and enumeration of oligotrophs, a 100X diluted nutrient broth[17] was used. Flooded soil samples after 10 days flooding were diluted using this medium. Plate technique was followed to enumerate the oligotrophic bacteria from soil. The culture plates were incubated at $28 \pm 2\ ^0C$ for aerobic growth in a BOD incubator.

Enumeration of total aerobic bacteria and diluted nutrient (DNB) or oligotrophic bacteria from rice roots

For sampling of roots, rice plants were uprooted gently from the experimental pots. After thoroughly washing under running tap water, roots were cut into 1 cm length. They were surface sterilized by immersing them in 0.1% $HgCl_2$ solution for 30 min and then, washed free of mercuric chloride using sterile water. Extracts from these sterile root-pieces were used for serial dilution. Plate technique was followed to enumerate the oligotrophic bacteria from roots of rice. The culture plates were incubated at $28 \pm 2\ ^0C$ for aerobic growth in a BOD incubator.

Populations of total anaerobic, oligotrophic bacteria from soil and rice roots in the air-dried alluvial soil sample were estimated by the standard dilution plate technique. For enumeration of all these anaerobic bacteria, the culture plates were immediately transferred to a 'GasPak' anaerobic jar following the standard procedure of BBL Microbiology Systems (Becton Dickinson and Co, Cockeysville, Maryland, USA). After 30 days of incubation, the colonies were counted for oligotrophic bacteria.

Estimation of microbial biomass content

For estimation of microbial biomass in flooded alluvial or acid sulfate soil, 50gm of air-dried soil sample was placed in containers of 120 mL capacity and flooded with sterile distilled water (1:1 soil and water ratio). Air bubbles developed during the initial periods of incubation were carefully removed. The flooded soil samples, after closed with aluminum foil, were incubated for 10 days at $28 \pm 2\ ^0C$. Microbial biomass C content was analyzed by the chloroform fumigation extraction method[18].

RESULTS AND DISCUSSION

Soil samples of both alluvial and acid sulfate saline (Pokkali) were analyzed for their physico-chemical properties and results were represented in the TABLE 1. There was slight variation in pH of soil from 5.47 in alluvial to 5.15 in acid-sulfate soil. Higher organic matter content of 1.88mg/ml was observed in pokkali than alluvial soil. The contents of total nitrogen and sulfate are 0.09% and 0.0086% in alluvial where as 0.25% and 0.0072% in pokkali respectively.

TABLE 1 : Physical characteristics of the flooded rice soil.

Properties	Alluvial	Acid sulfate saline (Pokkali)
pH[a]	5.47	5.15
Organic carbon	1.33	1.88
Sulfate ($SO_4 - S$)[c] (%)	0.0086	0.0072
Total nitrogen (%)	0.09	0.25

[a]Measured by taking 1:1.25 soil-water slurry; [b]Estimated by Walkely-Black method; [c]Estimated by Massoumi-cornfields method; [d]Estimated by Kjeldahl method.

Microorganisms of diverse nature and functions inhabit soil and they are responsible for nutrient cycling and ecosystem functioning. Since the populations of the microorganisms are heterogeneous and diverse in their physiological activities, qualitatative or quantitative methods to enumerate or identify all of them are inadequate. The alluvial soil had considerable population of aerobic heterotrophic bacteria (TABLE 2). A 100 X diluted nutrient agar supported least number of the total aerobic heterotrophic bacteria whereas the soil extract agar

had the highest. The soil extract agar had nutrients whose concentrations are similar to those found in normal, soils thus this nature of medium composition was good for the development of cultural bacteria from the soil in this study. Flooding of soil creates aerobic anaerobic and diluted nutrient environment with diverse nature for bacteria. There are reports of occurrence of bacteria which are obligate to the oligotrophic nature of flooded soils[19]. Hence the bacterial population in diluted nutrient agar would be different from that of full strength nutrient agar (TABLE 2). Similarly, the estimates of total fungi differed with the use of two media (TABLE 3). These results indicate the difference in the abilities of diverse physiological groups of microorganisms to appear in the standard media. Soils can be very different in the diversity of organisms present; Types, numbers, and biomass of organisms vary not only from soil to soil, but also within the same soil type. Generally fungi dominate the soil biomass with 10^3 to 10^6 colony-forming units' g-1 soil, while bacteria are most abundant in numbers. The diversity in microbial communities in flooded rice soils within a few days of flooding was also observed in other studies[10,11]. In the studies of Yoshida (1975)[10], generally bacteria were predominate in rice soils where as fungi and actinomycetes were more in upland soils. In some other studies also, the microbial biomass is influenced by many factors including soil type, temperature, moisture and other biological factors[20-22]. Flooded rice soils are predominantly with anaerobic environment with many micro sites of nutrient poor or rich conditions depending on the dilution by flood water and the diffusion of dissolved oxygen. The population of DNB bacteria in alluvial soils was considerable (TABLE 2) whereas the population of NB bacteria in flooded alluvial soils was more than that of the DNB bacteria under aerobic conditions (TABLE 4). On the contrarily, DNB were dominated under anaerobic conditions. Flooded conditions are favorable for the build of oligotrophic bacteria especially those capable of growing under anaerobic conditions. The populations of anaerobic oligotrophic bacteria are also more than the population of aerobic oligotrophic bacteria in the roots of rice (TABLE 5). However, the numbers of NB bacteria both aerobic and anaerobic were almost identical. Endorhyzosphere of rice roots is known to harbor many nitrogen fixing bacteria[23].

TABLE 2 : Bacterial population (total aerobic heterotrophic) in alluvial soil enumerated on various nutrient media

Medium	(cfu's) g^{-1} soil (X 10^7)
Nutrient	4.45 ± 3.5
Diluted nutrient agar	3.65 ± 4.9
Plate count agar	3.80 ± 0.0
Soil extract agar	8.40 ± 1.2

***values represented in the table are mean of duplicates**
Microbial populations measured in terms of colony forming units/g (CFU) of soil

TABLE 3 : Fungal populations in alluvial soil on two different medium

Medium	Fungal populations (cfu's) g^{-1} soil (X 10^4)
Rose-Bengal agar	5.20 ± 5.6
Czapek-Dox agar	3.20 ± 1.4

***values represented in the table are mean of duplicates**
Microbial populations measured in terms of colony forming units/g (CFU) of soil

TABLE 4 : Bacterial populations in nutrient-broth (NB) and diluted nutrient-broth bacteria (DNB) in an alluvial soil under aerobic and anaerobic conditions.

Group	Bacterial populations (C FU X 10^6 g^{-1} soil)	
	Aerobic	Anaerobic
NB bacteria	15.60	5.17
DNB bacteria	5.94	6.72

TABLE 5 : Bacterial population in rice plant roots on nutrient and diluted nutrient-broth

Group	Population (colony forming units X 10^4 g^{-1} soil)	
	Aerobic	Anaerobic
NB bacteria	27.00	27.30
DNB bacteria	5.05	6.75

***values represented in the table are mean of duplicates**
Microbial populations measured in terms of colony forming units/g (CFU) of soil

The biomass of micro flora is known to determine the rate of mineralization[14]. The microbial biomass is influenced by soil temperature, moisture and other biological factors[24,25]. Accumulation of CO_2 is also indicator of microbial activity especially soil respiration[26]. In this experiment the effects of different levels of soil microbial biomass content on the mineration rate were examined used full strength nutrient medium. Net accumulation of CO_2 was highest after 10 days of incubation amended with soil microbial biomass at 2-10 ng

ml^{-1} soil (TABLE 6). With the decrease in the levels of soil microbial biomass content, there was reduction in the net accumulation of CO_2. Anaerobic microorganisms are known to have slower metabolic rates compared to aerobic microorganisms[27]. However, net methane accumulation was comparable to that of aerobic conditions. Under both aerobic and anaerobic conditions, the rates of mineralization were lowered with decreasing levels of soil microbial biomass content (TABLE 7). Similarly, the elevated CH_4 production in flooded soils during the reproductive growth stages of lowland rice (*Oryza sativa* L.) was believed to result from decomposition of root exudates and autolysis root tissue in the studies of triggering of methane production in rice soils by root exudates[28]. In the studies of Sahrawat, application of or in-situ availability of terminal electron acceptors (oxidants) such as ferric iron or sulfate allows iron or sulfate reducers to successfully compete for substrates, hydrogen or acetate, with methanogens. This stops methane production. Electron acceptors also oxidize methane and reduce its emission.

TABLE 6 : Net accumulated carbon dioxide (mg mL-1) and methane (μg mL-1) in full-strength nutrient liquid medium amended with different levels of soil microbial biomass under aerobic conditions.

Soil microbial Biomass	Days of incubation			
(mg mL-1)	10		20	
	CO_2	CH_4	CO_2	CH_4
No addition	7.92	3.41	6.60	3.68
210.00	35.20 (4.4)	29.09 (8.5)	13.20 (2.0)	21.33 (5.8)
21.00	14.98 (1.9)	20.10 (5.9)	11.00 (1.7)	9.28 (2.5)
2.10	11.44 (1.4)	5.35 (1.6)	8.80 (1.3)	5.27 (1.4)
0.02	10.56 (1.3)	6.43 (1.9)	9.24 (1.4)	9.22 (2.5)

Values in the parentheses represent the number of folds of increase over the unamended control.

TABLE 7 : Net accumulation of carbon dioxide (mg mL^{-1}) and methane (μg mL^{-1}) in full-strength nutrient liquid medium amended with different levels of soil microbial biomass under anaerobic conditions (10 after incubation)

Soil microbial biomass (Mg mL^{-1})	Net CO_2 accumulation	Net CH_4 accumulation
No addition	7.92	2.90
210.00	13.64 (4.4)	22.04 (7.6)
21.00	7.04 (1.9)	3.22 (1.1)
2.10	4.40 (1.4)	2.29 (0.8)
0.02	1.76 (1.3)	1.90 (0.7)

Values in the parentheses represent the number of folds of increase over the unamended control.

REFERENCES

[1] M.Clarholm; Possible Roles of Roots, Bacteria, Protozoa and Fungi in Supplying Nitrogen to Plants, In A.H.Fitter, D.Atkinson, D.J.Read, M.B.Usher, (Eds); Ecological Interactions in Soil. Special Publication 4, British Ecological Society. Blackwell Scientific Publications Ltd., Oxford, 355-365 **(1985)**.

[2] V.Torsvik, R.Sørheim, J.Gorksøyr; J.Ind.Microbiol., **17**, 170-178 **(1996)**.

[3] R.Conrad, P.Frenzel; Flooded Soils. In Encyclopedia of Environmental Microbiology. G.Britton, (Ed); New York: John Wiley & Sons. 1316-1333 **(2002)**.

[4] B.S.Griffiths, K.Ritz, R.Wheatley, H.L.Kuan, B.Boag, S.Christensen, F.Ekelund, S.J.Sorensen, S.Muller, J.Bloem; Soil Biol.Biochem., **33**, 1713-1722 **(2001)**.

[5] B.P.Degens, L.A.Schipper, G.P.Sparling, L.C.Duncan; Soil Biology and Biochemistry, **33**, 1143-1153 **(2001)**.

[6] J.S.Waid; Applied Soil Ecology, **13(2)**, 151-158 **(1999)**.

[7] D.S.Jenkinson, J.N.Ladd; Microbial Biomass in Soil: Measurement and Turnover. In: Soil Biochemistry, E.A.Paul, J.N.Ladd, (Eds); Dekker, New York, 415-471 **(1981)**.

[8] W.Dejonghe, N.Boon, D.Seghers, E.M.Top, W.Verstraete; Environ.Microbiol., **3**, 649-657 **(2001)**.

[9] R.Martens; Biol.Fert.Soils, **19**, 87-99 **(1995)**.

[10] T.Yoshida; Microbial Metabolism of Flooded Soils. In: E.A.Paul, A.D.McLaren, (Eds); Soil Biochemistry, Dekker, New York, **3**, 83-119 **(1975)**.

[11] N.Sethunathan, V.R.Rao, T.K.Adhya, K.Raghu; CRC Crit.Rev.Microbiol., **10**, 125-172 **(1983)**.

[12] E.Mathews, I.Fung, J.Lerner; Global Biogeochem. Cycles, **5**, 3-24 **(1991)**.

[13] R.A.Houghton; Ambio., **25**, 267-272 **(1996)**.

[14] S.Simkins, M.Alexander; Applied and Environmental Microbiology, **47**, 1299-1306 **(1984)**.

[15] M.L.Jackson; Soil Chemical Analysis Prentice, Hall of India Private Limited, New Delhi, **(1967)**.

[16] E.D.Vance, P.C.Brookes, D.S.Jenkinson; Soil Biol.Bio-Chem., **19**, 703-707 **(1987)**.

[17] T.Hattori; Rep.Inst.Agr.Tohoku Univ., **27**, 23-30 **(1976)**.

[18] M.Buchanan, L.D.King; Biol.Fert.Soils, **13**, 211-217 **(1992)**.
[19] M.Drazkiewicz, T.Hattori; Pol.J.Soil Sci., **11**, 133-141 **(1978)**.
[20] O.Priha, A.Smolander; Biol.Fertil.Soils, **24**, 45-51 **(1997)**.
[21] T.McM.Adams, R.J.Laughlin; Journal of Agricultural Science, Cambridge, **97**, 319-327 **(1981)**.
[22] G.Voos, P.M.Groffman; Biology and Fertility of Soils, **24**, 106-110 **(1997)**.
[23] D.N.Nayak, V.R.Rao; Archives of Microbiology, **115**, 359-360 **(1977)**.
[24] M.B.Alvarez, S.Gagne, H.Antoun; Appl.Environ. Microbiol., **61**, 194-199 **(1995)**.
[25] P.K.Donnelly, J.A.Entry, D.L.Craw Ford Jr., K.Cromack; Microbial Ecology, **20**, 289-295 **(1990)**.
[26] G.Stotzky, M.W.Broder, J.D.Doyle, R.A.Jones; Advances in Applied Microbiology, **38**, 1-98 **(1993)**.
[27] M.Alexander; Introduction to Soil Microbiology. 2nd Edition, Krieger Publ.Co., Malabar, FL, 467 **(1991)**.
[28] S.Mitra, M.S.Aulakh, R.Wassmann, D.C.Olk; Soil Sci.Soc.Am.J., **69**, 563-570 **(2005)**.