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## Physico-chemical properties and bacterial population in flooded rice soils

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### ABSTRACT

The physicochemical properties, aerobic and anaerobic bacterial population in alluvial and acid sulphate soils and roots of rice plants were studied in the present study. There was slight variation in pH of soil from 5.47 in alluvial soil to 5.15 in acid-sulfate soil. Higher organic matter content was observed in pokkali than alluvial soil. The total nitrogen and sulfate are higher in alluvial soil, than pokkali. Flooded rice soils are predominantly with anaerobic environment with micro sites of nutrient poor or rich conditions. The total population of DNB bacteria in alluvial soil was considerable whereas the population of NB bacteria in flooded alluvial soil was more than that of the DNB bacteria under aerobic conditions. On the contrary, DNB were dominated under anaerobic conditions. Flooded conditions are favorable for the growth of oligotrophic bacteria especially those capable of growing under anaerobic conditions. The anaerobic oligotrophic bacterial population was higher than the population of aerobic oligotrophic bacteria in the roots of rice plant.

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### KEYWORDS

Flooded rice soil;  
Alluvial and Acid sulfate soil;  
Bacterial community.

### INTRODUCTION

Rice is one of the most important food crops, as more than a billion people depend on its cultivation for their livelihoods and more than 3.5 billion people depend on rice for more than 20% of their daily calories<sup>[1]</sup> Microorganisms in rice field soil are important with respect to rice growing and greenhouse gas emission. They influence rice growth directly through symbiosis or indirectly through nutrient cycling and emit greenhouse gases during sequential reduction processes initiated by the flooding of rice field soil<sup>[2,3]</sup>. 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<sup>[4]</sup>. These microorganisms act as biocatalyst in nutrient is cycling and ecosystem functioning. This microbial community is made up by thousands of different species of which only 1% can only be cultivated and thus characterized<sup>[5]</sup>.

The diversity of microorganisms is immense and re-

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quired for the functioning of the destruction process of dead organic matter<sup>[6]</sup>. The relation between soil function and soil microbial diversity has been explained in several experimental studies<sup>[7,8]</sup>, 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<sup>[9]</sup>. 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<sup>[10,11]</sup>. 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<sup>[12,13]</sup>. The estimated of soils biomass is considered to be a good indicator of microbial status and of soils health. Therefore, experiments were conducted in the present study to compare different culture media for enumeration of bacteria and to isolate and enumerate DNB or oligotrophic bacteria 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<sup>[14]</sup>.

### Microbial analysis of the alluvial soil samples

#### Enumeration of aerobic bacteria

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  $28 \pm 2^\circ\text{C}$  for aerobic growth in a BOD incubator.

#### Enumeration of diluted nutrient bacteria (DNB)

For the isolation and enumeration of oligotrophs, a 100 X diluted nutrient broth 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  $28 \pm 2^\circ\text{C}$  for aerobic growth in a BOD incubator.

## RESULTS

### Collection of soil samples and their characteristics

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 alluvial and acid sulfate soils.**

Properties	Alluvial	Acid sulfate saline(Pokkali)
pH <sup>a</sup>	5.47	5.15
Organic carbon	1.33	1.88
Sulfate (SO <sub>4</sub> – S) <sup>c</sup> (%)	0.0086	0.0072
Total nitrogen (%)	0.09	0.25

<sup>a</sup> Measured by taking 1:1.25 soil-water slurry; <sup>b</sup> Estimated by Walkely-Black method; <sup>c</sup> Estimated by Massoumi-cornfields method; <sup>d</sup> Estimated by Kjeldahl method.

Microbial population in alluvial soil was enumerated on various media and shown in TABLE 2. Among four medias used in this study bacterial population were more in soil extract medium and nutrient medium is second and intermediate populations in remaining media. 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. Soils with Eh > 300mV are considered aerobic or upland. The bacterial populations in nutrient-broth and diluted nutrient-broth bacteria (DNB) in an alluvial soil under aerobic and anaerobic conditions were enumerated shown in table 3. Higher number of NB bacteria were observed than DNB (TABLE 3) The population of DNB bacteria in alluvial soils was considerable 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 4) However; the numbers of NB bacteria both aerobic and anaerobic were almost identical.

**TABLE 2 : Microbial populations in an alluvial soil enumerated on various media**

Medium	(cfu's) g <sup>-1</sup> soil (X 10 <sup>7</sup> )
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 mean of triplicates

**TABLE 3 : Bacterial populations in nutrient-broth and diluted nutrient-broth bacteria (DNB) in an alluvial soil under aerobic and anaerobic conditions**

Group	Bacterial populations(CFU X 10 <sup>6</sup> g <sup>-1</sup> soil)	
	Aerobic	Anaerobic
NB bacteria	15.60	5.17
DNB bacteria	5.94	6.72

**TABLE 4 : Population of nutrient-broth and diluted nutrient-broth bacteria in rice roots**

Group	Population (colony forming units X 10 <sup>4</sup> g <sup>-1</sup> soil)	
	Aerobic	Anaerobic
NB bacteria	27.00	27.30
DNB bacteria	5.05	6.75

Values represented in the tables are mean of triplicates

## DISCUSSION

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, qualitative or quantitative methods to enumerate or identify all of them are inadequate. The alluvial soil from the experimental form of this institute 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 microbes that are active in upland environments are inhabited in the anaerobic wetland soil environment. Which primarily due to absence of O<sub>2</sub> and change in soil pH (acid to neutral) under anaerobic conditions, Exactly microbial biomass decreases under saturated soil conditions. Hence the bacterial population in diluted nutrient agar would be different from that of full strength nutrient agar (TABLE 2). 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. Both aerobic and anaerobic bacterial populations were successfully enumerated from soils of alluvial and acid sulfate on various media and compared the populations both nutrient and diluted nutrient media, this is an indication of diversity of bacterial community in flooded rice soils.

## REFERENCES

- [1] Y.K.Jae-Hyung Ahn, Jaekyeong Song, Byung, Myung-Sook Kim, Jae-Ho Joa, Hang-Yeon Weon; Characterization of the Bacterial and Archaeal Communities in Rice Field Soils Subjected to Long-Term Fertilization Practices, *The Journal of Microbiology*, **50(5)**, 754–765 (2012).
- [2] W.Liesack, S.Schnell, N.P.Revsbech; Microbiology of flooded rice paddies. *FEMS Microbiol.Rev.*, **24**, 625–645 (2000).
- [3] I.Kögel-Knabner, W.Amelung, Z.Cao, S.Fiedler, P.Frenzel, R.Jahn, K.Kalbitz, Kölbl, M.Schlöter; Biogeochemistry of paddy soils. *Geoderma*, **157**, 1-14 (2010).
- [4] M.Schlöter; Biogeochemistry of paddy soils. *Geoderma*, Clarholm, M. 1985. Possible roles of roots, bacteria, protozoa and fungi in supplying nitrogen to plants, In A.H.Fitter, D.Atkinson,

## FULL PAPER

- D.J.Read, M.B.Usher, (Eds); *Cological interactions in soil*. Special Publication 4, British Ecological Society. Blackwell Scientific Publications Ltd., Oxford., **157**, 1-14, 355-365 (2010).
- [5] V.R.Torsvik J.Sørheim, J.Gorksoyr; Total bacterial diversity in soil and sediment communities-a review. *J.Ind.Microbiol.*, **17**, 170-178 (1996).
- [6] R.Conrad, P.Frenzel; Flooded soils. In *Encyclopedia of Environmental Microbiology*. G.Britton, (Ed); New York: John Wiley & Sons., 1316-1333 (2002).
- [7] B.S.Griffiths, K.Ritz., R.Wheatley, H.L.Kuan, B.Boag, S.Christensen, F.Ekelund, S.J.Sorensen, S.Muller, J.Bloem; An examination of the biodiversity ecosystem function relationship in arable soil microbial communities. *Soil Biol.Biochem*, **33**, 1713-1722 (2001).
- [8] B.P.Degens, L.A.Schipper, G.P.Sparling, L.C.Duncan; Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? *Soil Biology and Biochemistry*, **33**, 1143-1153 (2001).
- [9] J.S.Waid; Does soil biodiversity depend upon metabolic activity and influences? *Applied Soil Ecology*, **13(2)**, 151-158 (1999).
- [10] 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).
- [11] W.Dejonghe, N.Boon, D.Seghers, E.M.Top, W.Verstraete; Bioaugmentation of soils by increasing microbial richness: missing links. *Environ. Microbiol.*, **3**, 649-57 (2001).
- [12] T.Hattori; Plate count of bacteria in soil on a diluted nutrient broth as a culture medium. *Rep.Inst.Agr.Tohoku.Univ*, **27**, 23-30 (1976).
- [13] Tomoyoshi Hashimoto, Kyung-Sook Whang, Kazunari Nagaoka; A quantitative evaluation and phylogenetic characterization of oligotrophic denitrifying bacteria harbored in subsurface upland soil using improved culturability, *Biol Fertil Soils*, **42**, 179-185 (2006).
- [14] M.L.Jackson; *Soil Chemical Analysis* Prentice, Hall of India Private Limited, New Delhi, (1967).