Nitrogen Fixing Bacteria, *Azotobacter* sp. in Aquatic Sediment

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Non-symbiotic, free living, nitrogen fixing bacteria, *Azotobacter* sp. was estimated in sediments of estuarine, marine, backwater and mangrove environments of Portonovo. Number of colony forming units (CFU) of *Azotobacter* sp. was less (5 to 27 cells/g of dry sediment). CFU of total heterotrophic bacteria (THB), actinomycetes and fungi were between 4.1 x 10^6 and 4.5 x 10^7, 0.8 x 10^5 and 4.9 x 10^5, 1.1 x 10^5 and 3.8 x 10^5/g respectively. Mangrove sediments contained more CFU of the above microbial groups.

Nitrogen is an essential biogeneic component and the productivity of the aquatic environment depends on its amount present and the character of its component (Thomas, 1970; Skelef *et al.*, 1971). Biological nitrogen fixation which transforms molecular nitrogen to ammonia or organic nitrogen, namely the process in which the atmospheric nitrogen enters into biosphere is one of the most important process in nitrogen cycle in aquatic environments. Microorganisms are active in the process of fixing atmospheric nitrogen and the possible participation of various groups of bacteria in the transformation process of the nitrogenous compounds in the aquatic environments is reported (Kawai & Sugahara, 1972). The role of *Azotobacter* in the nitrogen fixing process was reported from various seawater regions and sediments (Herbert *et al.*, 1977). Information on the occurrence, distribution and ecological significance of the non-symbiotic nitrogen fixing bacteria *Azotobacter* in soils are available and in India attempts have been made to enumerate *Azotobacter* population from paddy soils (Sadasivam, 1965). However the above information from aquatic environment is lacking. The present communication reports the occurrence of *Azotobacter* in sediments of Portonovo coastal zone.

Materials and Methods

Sediment samples were collected from marine, estuarine, backwater and mangrove environments of Portonovo (Fig. 1) using the Peterson's grab and about 50 g was transferred to a sterile polyethylene bag, kept in an insulated ice box and transported to the laboratory. The number of CFU of *Azotobacter*, THB, actinomycetes and fungi were enumerated following pour plate method using mannitol agar, Zobell 2216 e agar, Kuster's agar and Martin Rose Bengal agar. All determinations were carried out in triplicate and the results are expressed per g dry weight of the sediment. All the typical colonies on mannitol agar were transplanted to mannitol agar slants, restreaked repeatedly to ensure purity and were identified (Gibbs & Shapton, 1960).

Physico-chemical variables such as temperature and pH were measured using a mercury bulb thermometer and a pH meter

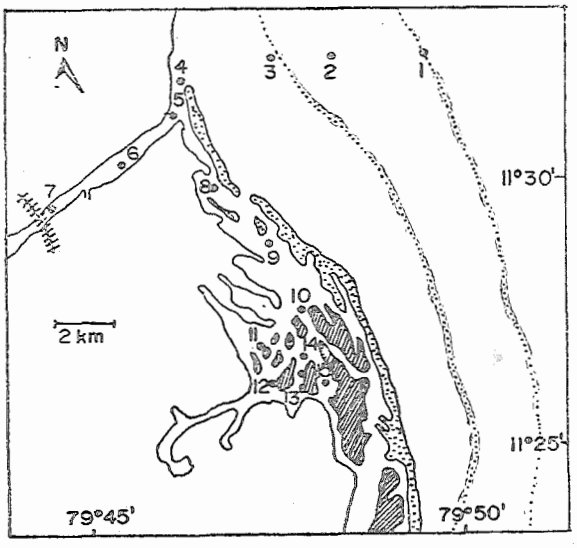

Fig. 1. Sampling stations

(ELICO model L1-10) respectively. A portion of the air dried sediment was powdered and passed through 85 μ mesh sieve. Total organic carbon (Wakeel & Riley, 1956), total nitrogen (Barnes, 1959) and total phosphorus (Murphy & Riley, 1962) were also estimated.

Results and Discussion

The environmental parameters presented in Table 1 showed no remarkable difference in temperature and pH between the stations. The values of C/N ratio suggest that the organic matter in bottom sediments is very rich in mangrove environment and gradually decomposed by heterotrophic bacteria.

Table 1. *Physico-chemical parameters measured in the sediments*

Station	Temp. °C	pH	C/N ratio	Total phosphorus (%)
1	30.5	8.05	5.0	0.13
2	30.5	8.10	5.9	0.16
3	30.5	8.10	6.3	0.08
4	30.5	8.00	8.0	0.06
5	30.5	8.10	7.8	0.10
6	30.5	7.90	6.3	0.13
7	30.0	8.00	4.0	0.06
8	30.5	8.10	3.1	0.02
9	30.0	7.90	3.4	0.05
10	30.5	8.00	2.3	0.06
11	29.0	8.00	4.7	0.03
12	30.5	8.20	7.6	0.04
13	30.0	8.10	10.2	0.04
14	29.0	8.05	10.2	0.05
15	29.5	8.20	9.8	0.06

The number of THB, actinomycetes, fungi and *Azotobacter* in the sediment is given in Fig. 2. THB in sediment was very high (10^6–10^7 /g) and that of *Azotobacter* was extremely low (5 to 27 cells/g). Actinomycetes recorded between 10^4 and 10^5/g while fungi was at 10^5/g level. *Azotobacter* in marine sediment was between 0.8 to 1.7 x 10 cells/g while THB were fairly abundant (10^6/g). Similar trend was also observed in estuarine and backwater sediments where *Azotobacter* was 0.5 to 1.9 x 10 cells/g while THB varied between 10^6 and 10^7 cells/g. In mangrove sediment *Azotobacter* fluctuated between 2.4 and 2.7 x 10 cell/g and THB was 10^7 cells/g.

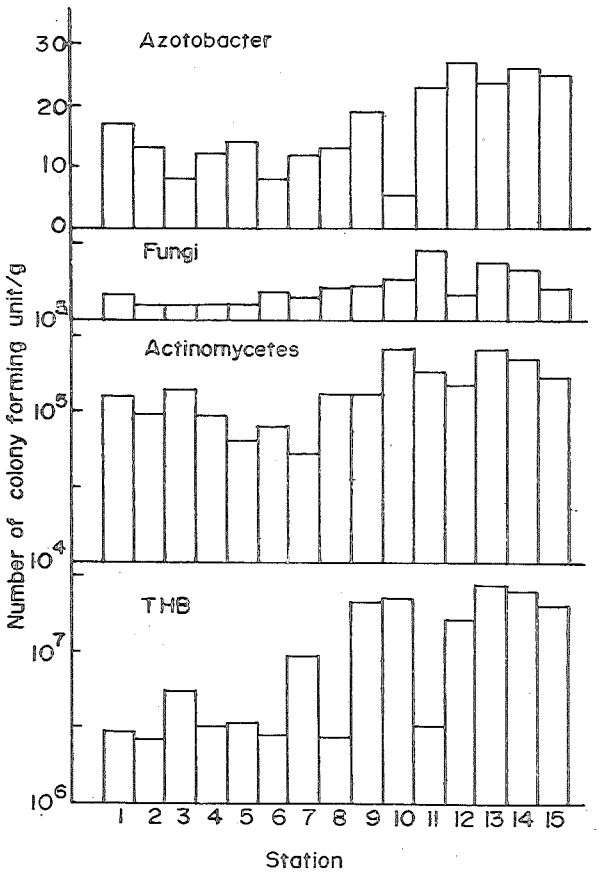

Fig. 2. Distribution of microorganisms in sediment.

The presence of *Azotobacter* in the bottom sediments was extremely small. Similar findings were also reported by Kawai and Sugahara (1971a) from East China Sea (0.1–10 cells/g) and 100 cells/g from Maizuri Bay and Kumihama Bay. It is obvious that mangrove sediments contained slightly larger quantities of *Azotobacter* and this could be attributed to the muddy sediments which contain higher quantities of organic matter and to the rich fauna and flora inhabiting the area. Normally the bottom deposits are conditioned by a varying content of the mineral compounds and organic substances which are the source of carbon and energy for these organisms. Also more strongly developed vegetation, the small depth and immense mass of particles of organic matter probably of planktonic origin

and leaves, litter and animals, being only part mineralized, may be conducive environment to this group of bacteria.

The ratio of the number of THB to that of *Azotobacter* was found to be high (10^6/g) when compared to previous reports. Kawai *et al.* (1971) reported 10^2–10^4/g in culture ponds and Kawai & Sugahara (1971a, b) recorded 10^3–10^4 in Surugu Bay, Sagami Bay, East China Sea, 10^2 in Kumihama Bay and 10^2–10^3 in Maizuru Bay. It indicates that the standing crop *Azotobacter,* in general, was extremely small in the bottom sediments of these environments, eventhough a slightly higher level could be found in mangrove sediments.

Kawai & Sugahara (1971a) reported that the number of THB was usually at a level of 10^5 cells/g while the number of nitrogen fixing bacteria gradually decreased from coastal to offshore but in the present study the distribution of *Azotobacter* from coastal to offshore was found uneven. No correlation was observed between *Azotobacter* and various environmental parameters. Similarly Kawai & Sugahara (1971c) also could not find any relationship between the number of nitrogen fixing bacteria and other environmental factors in Lake Biwa and Lake Yunoko. However the seasonal fluctuations of the number of nitrogen fixing bacteria in bottom deposits of Ilawa Lake was correlated with the reproductive periods of bottom invertebrates (Zukowska, 1960) and also with dying planktonic and benthic organisms which enrich the sediment and nourish bacteria.

The present investigation thus confirms the existence of free living aerobic nitrogen fixing bacteria, *Azotobacter* sp. in different aquatic biotopes of the coastal zone. Some of the strains were found to be potent atmospheric nitrogen fixers (Lakshmanaperumalsamy *et al.*, 1975). This group of bacteria along with phosphobacteria could be used as biofertilizer to enrich the prawn farm.

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References

Barnes, H. (1959) *Apparatus and Methods of Oceanography.* Part I Chemical, Allen & Unwin, London

Gibbs, B. M. & Shapton, D. A. (1960) *Identification Methods for Microbiologists.* Academic Press, London

Herbert, R. A., Brown, C. M. & Stanely, S. D. (1977) in *Aquatic Microbiology.* (Skinner, F. A. & Shewan, J.M., Eds.) Academic Press, London

Kawai, A. & Sugahara, I. (1971a) *Bull. Jap. Soc. Sci. Fish.* 37, 981

Kawai, A. & Sugahara, I. (1971b) *Bull. Jap. Soc. Sci. Fish.* 37, 747

Kawai, A. & Sugahara, I. (1971c) *Bull. Jap. Soc. Sci. Fish.* 37, 740

Kawai, A. & Sugahara, I. (1972) *Bull. Jap. Soc. Sci. Fish.* 38, 292

Kawai, A., Sugiyama, M & Sugahara, I. (1971) *Bull. Jap. Soc. Sci. Fish.*37, 985

Lakshmanaperumalsamy, P., Chandramohan D & Natarajan, R. (1975) *Bull. Dept. Mar. Sci. Univ. Cochin,* 8, 103

Murphy, J. & Riley, J. P. (1962) *Anal. Chim. Acta.* 27, 31

Sadasivam, K. V. (1965) *Studies on Azotobacter Population in Soil.* M.Sc. Thesis, Annamalai University, Annamalainagar, India

Skelef, G. Oswald, W. J. & Goluske, G. C. (1971) *Proc. 5th Int. Cong. Water Pollution Res.* 111, 25

Thomas, W. H. (1970) *Limnol. Oceanogr.* 15, 386

Wakeel, S. K. & Riley, J. P. (1956) *J. Cons. int. Explor. Mar.* 22, 180

Zukowska, I. (1960) *Observations on Bottom Fauna of Jeziorak Lake.* B. S. Thesis, Univ. M. Kopernik, Torun