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Alkalotolerant and Alkalophilic Bacteria in Seawater

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ABSTRACT: Numerous alkalotolerant and alkalophilic bacteria, with concentrations ranging from 10 to 10^3 cells 100 ml^{-1} water sample, were isolated from seawater in the Pacific and Indian Oceans using a buffered medium of pH 10.6. The pH effects on growth rates were used to define the occurrence of three types of alkalotolerant and alkalophilic bacterial groups: (1) bacteria exhibiting high growth rates throughout the pH range of 7.3 to 10.6; (2) bacteria growing well at pH 9 and below, but to a lesser extent also at pH 10.6; (3) bacteria which grow only at pH 10 to 10.6. Isolates of Type (1) are more abundant than those of the other two groups. Types (1) and (2) are tentatively named alkalotolerant; Type (3), alkalophilic. In the alkaline medium, pH fluctuation during growth was not observed, probably due to the buffer action of Na_2CO_3 added to the medium. The alkalotolerant bacteria isolated from the Pacific Ocean consisted of *Pseudomonas* spp.; those isolated from Sagami Bay were *Vibrio* and *Pseudomonas* spp.; these species are common in the water area investigated.

INTRODUCTION

It has generally been assumed that most bacterial species grow best in media with relatively restricted pH ranges, i. e. close to neutrality. This would be in line with the situation in natural ecosystems which usually feature neutral pH conditions, except in extreme environments. However, even in normally neutral environments, wide fluctuations of pH are often observed. Rapid growth of phytoplankton, for example, can cause the environmental pH to increase temporarily to more than 10, even in seawater. Under such circumstances, certain bacterial populations may become subject to rapid pH fluctuations. Hitherto, the question has remained unanswered whether or not these bacteria represent particular strains or are common members of the local ecosystem.

While there are several reports concerning the occurrence of alkaline bacteria in terrestrial soil and fresh water, this paper is the first assessment of the distribution of alkaline bacteria in the marine environment. The generic composition of the bacteria isolated and their growth versus pH fluctuations have been determined. The bacteria concerned are *Pseudomonas* spp. and *Vibrio* spp., isolated from the Pacific Ocean and from Sagami Bay, Japan.

MATERIALS AND METHODS

Water Samples

Seawater samples were collected using a sterilized ORIT sampler (Taga, 1968) at the stations shown in Figure 1. Seawater temperatures at different water depths were measured using protected reversing thermometers attached to the Nansen Bottles.

Media

The composition of the alkaline medium for the growth of alkaline bacteria (alkalotolerant and alkalophilic) was as follows (g) : NaCl, 18.0; KCl, 0.6; $MgCl_2 \cdot 6\ H_2O$, 8.1; $CaCl_2$, 0.8; Bacto-peptone, 1.5; Bacto-soytone, 1.5; Bacto-yeast extract, 1; ferric citrate, 0.1; agar, 15; Na_2CO_3, 10; 1000 ml distilled water. Na_2CO_3 was sterilized separately and added to the other components after cooling to 50 °C. This gave the medium a pH of 10.6 and a Na^{+1} concentration of about 0.4 M. For media of pH 7.3, 8.0, 9.0 and 10.0, the amount of Na_2CO_3 added to the basic medium was 0.07, 0.15, 0.42 and 2.35 g l^{-1}, respectively.

The composition of the medium for heterotrophic

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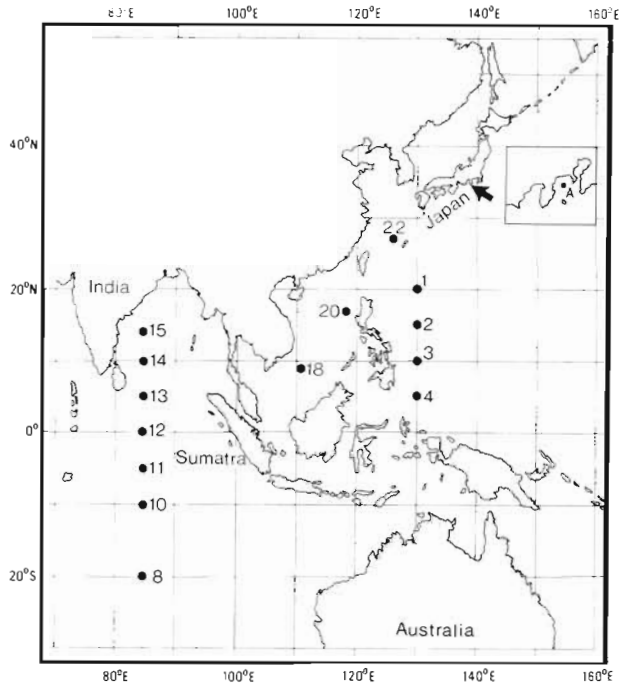

Fig. 1. Stations for seawater sampling

bacteria was as follows (g): Bacto-peptone, 2; Proteose-peptone No. 3, 1; Bacto-soytone, 1; Bacto-yeast extract, 1; ferric citrate, 0.1; agar, 15, 1000 ml seawater. pH was adjusted to 7.4.

Estimation of Bacterial Number

A suitable volume of seawater was filtered through a sterilized Nuclepore filter (0.2 μm pore size; 47 mm diameter) and the filters were placed on plates of alkaline medium or the medium for heterotrophic bacteria mentioned above. All plates were incubated at 20 °C for 2 weeks before the bacterial colonies were counted. Bacteria were isolated for further study by picking all colonies from a suitable plate and by transferring them to fresh medium for purification and storage.

Bacterial numbers in the samples are defined as the number of colony forming units (c. f. u.) per 100 ml of seawater.

Identification of Bacteria

The generic composition of the isolates, especially the alkalotolerant bacteria, was determined according to the scheme of Shewan et al. (1960). The vibriostatic compound 0/129 (2,4-diamino-6,7-diiso-prophyl-pteridine) was not used as means to distinguish *Vibrio* from *Aeromonas*. These were identified on the basis of differences in gas production using glucose as the substrate. The mode of glucose metabolism of the isolates was determined using Hugh-Leifson's medium (1953) based on artificial seawater.

Alkalophilic bacteria which grew only in the medium of pH 10 and above were not identified because of the difficulty in working under high pH conditions during the identification procedure.

Determination of Bacterial Growth

Alkalotolerant and alkalophilic bacteria were preincubated in the pH 8 medium for 2 d and in the pH 10.6 medium for 7 d respectively; 0.5 ml of the preincubated culture was then used to inoculate 50 ml of liquid medium. After a suitable incubation period – 2 d for the alkalotolerants and 7 d for the alkalophilic bacteria – the bacterial cells were centrifuged and washed three times with 3 % (w/v) NaCl. The amount of bacteria in the centrifuge tube was measured in terms of protein concentration determined by the method of Lowry et al. (1951).

RESULTS

A large number of the bacterial cells present in the seawater samples were able to grow in the alkaline medium (pH 10.6). The numbers of these microbes

Table 1. Generic composition of alkaline (alkalotolerant and alkalophilic) bacteria from the Pacific Ocean and Sagami Bay and their growth as a function of pH

Strain No.	Genus	pH 7.3	8.0	9.0	10.0	10.6
P 1	*Pseudomonas*	2	2	2	2	2
P 2	*Pseudomonas*	2	2	2	2	2
P 3	*Pseudomonas*	2	2	2	2	2
P 5	not identified	0	0	0	1	1
P 6	*Pseudomonas*	2	2	2	2	1
P 7	*Pseudomonas*	2	2	2	1	1
P 8	*Pseudomonas*	2	2	2	2	2
P 9	*Pseudomonas*	2	2	2	2	2
P 16	*Pseudomonas*	2	2	2	2	1
P 25	not identified	0	0	0	2	2
S 1	*Vibrio*	2	2	2	1	1
S 3	*Pseudomonas*	1	1	1	1	1
S 4	*Pseudomonas*	2	2	2	2	2
S 5	*Pseudomonas*	2	2	2	2	2
S 6	*Vibrio*	2	2	2	2	2
S 7	*Vibrio*	2	2	2	2	2
S 15	not identified	0	0	0	1	1

P: collected in the Pacific Ocean.
S: collected in Sagami Bay.
1. colony diameter below 5 mm.
2: colony diameter above 5 mm.

ranged from 10 to 10^3 100 ml^{-1} in the upper layers to less than 10^2 100 ml^{-1} in the lower layers of the profile (Fig. 2). The results also show that, at most depths, alkaline bacteria formed a relatively constant proportion of the heterotrophic bacterial population.

The growth of bacterial strains, P 2, P 3, P 5, P 6, P 7 and P 25, versus various pH values are shown in Table 1. The following tentative grades of growth were adopted: 1 indicates colonies with diameters below 5 mm; and 2, colonies above 5 mm diameter, after 2 weeks incubation on the 5 agar media of different pH. Three types of growth patterns can be distinguished: (1) bacteria producing high yields throughout the pH range of 7.3 to 10.6, even if their optimum pH was near 7; (2) bacteria growing well at pH 9 and below, but which can also grow at pH 10 and 10.6 though to a lesser degree; (3) bacteria which only grow at pH 10 to 10.6. These three growth patterns were also recogniz-

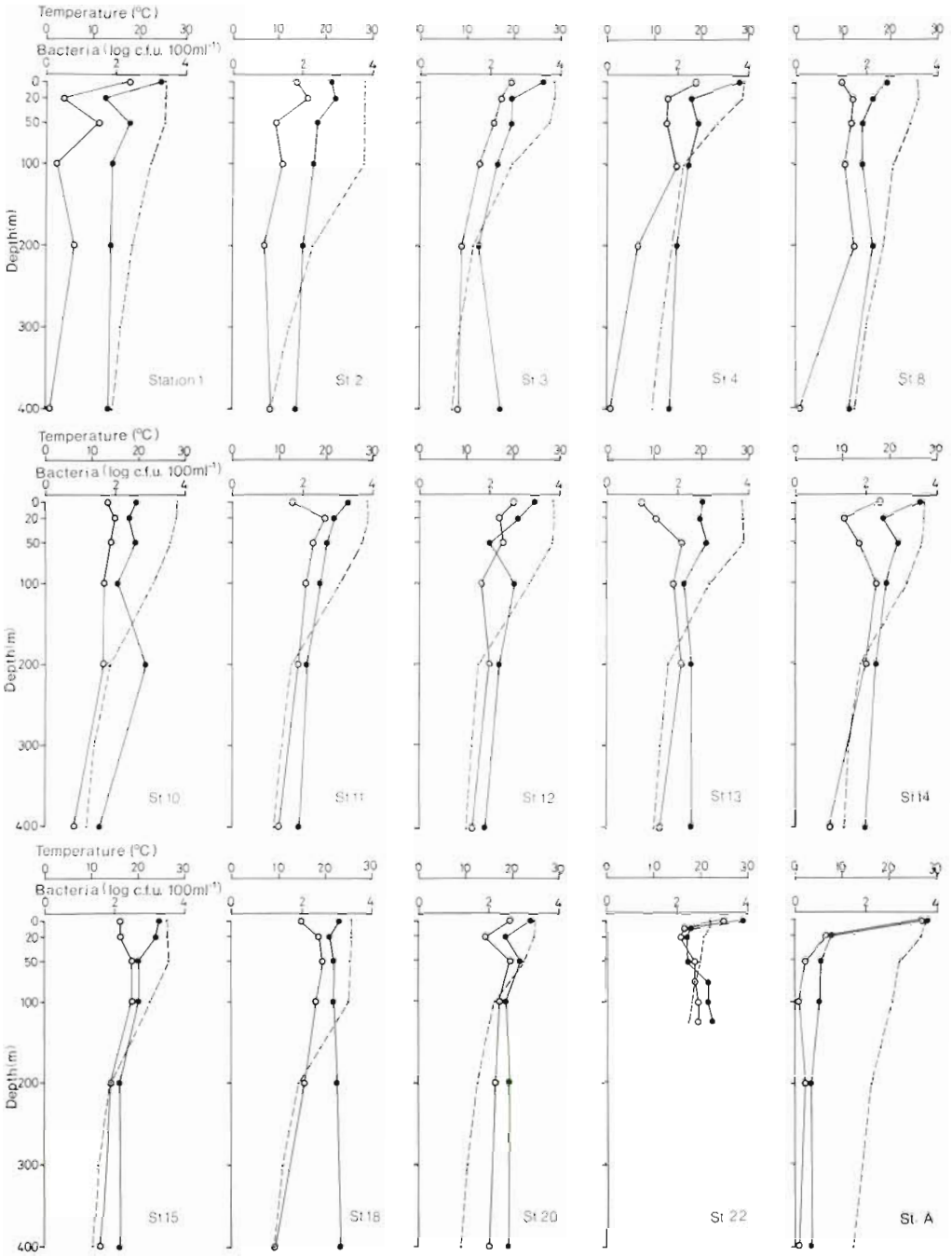

Fig. 2. Profiles of heterotrophic and alkaline (alkalotolerant and alkalophilic) bacteria in seawater. (○) alkaline bacteria; (●) heterotrophic bacteria; broken line: water temperature

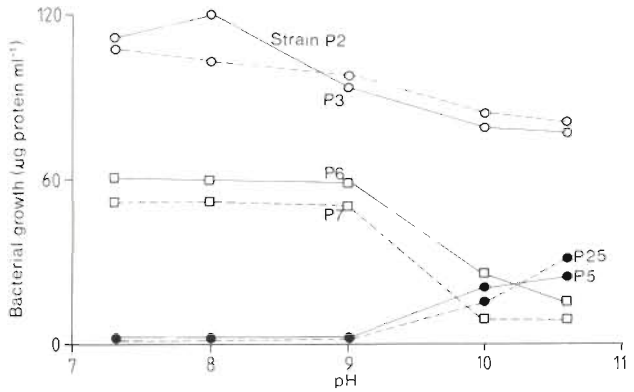

Fig. 3. Bacterial growth as a function of pH

able when the strains were cultivated in the liquid medium with various pH values (Fig. 3). We call Types (1) and (2) 'alkalotolerant', and Type (3) 'alkalophilic' bacteria. The isolates belonging to Type (1) were more abundant as compared to the other two groups. In the culture liquids, pH fluctuations were not observed during growth, probably because of the strong buffer action of Na_2CO_3.

Table 1 shows the generic composition of alkalotolerant bacteria in the samples. The isolates from the Pacific Ocean (Stations 1 and 4) were identified as *Pseudomonas* spp., those from Sagami Bay as *Pseudomonas* spp. and as *Vibrio* spp., respectively.

DISCUSSION

We found an unexpectedly large number of bacteria, isolated from the seawater, that were able to grow in alkaline medium. On reculturing, all isolates grew again in the new alkaline medium. This suggests that these microbes on the agar plate used for isolation do not maintain their metabolism by mutual effects, but are able to maintain their cell metabolism independently in a high alkaline environment.

The results obtained regarding pH effects on microbial growth show the occurrence of three types of alkalotolerant and alkalophilic bacterial groups as mentioned under 'Results'. Among these groups, alkalophilic bacteria had never been found using the ordinary medium of near neutral pH.

As shown in Table 1, the generic composition of the alkalotolerant microbes consisted of *Pseudomonas* spp. in the Pacific Ocean and *Pseudomonas* spp. and *Vibrio* spp. in Sagami Bay; all these are very common genera in seawater (Sieburth, 1971; Simidu et al., 1977). The bacteria belonging to Type (1), i. e. those which grow well from pH 7.3 to pH 10.6, are the most abundant of the three groups. These results suggest that the species commonly present in seawater are capable of adapting to rapid environmental change including fluctuations in pH. In contrast, in the terrestrial environment specific forms, such as *Bacillus*, have been isolated in an alkaline medium (Horikoshi, 1971).

The reason why a substantial number of bacteria in seawater possesses alkalotolerant or alkalophilic characteristics requires further investigation (the pH values of all seawater samples collected were less than 8.2). A possible explanation may be the connection between alkaline bacteria and the microenvironment of suspended aggregates, where NH_3^{+1} concentration should be high as the result of bacterial degradation of organic matters. The position of marine bacteria in the microbial evolution processes should also be taken into account. In addition, many of the alkaline bacteria isolated, especially the alkalotolerants, concentrate and accumulate calcium ions from the medium around their cells; this might represent one of the processes of metal-lump formation in the sea.

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