Salt Tolerance of Bacteria Isolated from Tropical Marine Fish and Prawn*

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Salt tolerance of selected cultures of *Pseudomonas, Moraxella, Vibrio, Micrococcus, Acinetobacter,* and *Flavobacteria/Cytophaga* were determined. More than 80% of the cultures belonging to each of the above genera, were capable of growth in presence of 1.5 to 3.5% salt (NaCl) and at least 25 to 30% of the cultures in each group required 1.5 to 3.5% salt for growth. Forty per cent each of *Pseudomonas* and *Vibrio* strains and 30% each of *Moraxella, Micrococcus* and *Flavobacteria/Cytophaga* strains tolerated 10% salt. Majority of the cultures belonging to the genera *Pseudomonas, Vibrio, Moraxella, Micrococcus, Acinetobacter* and *Flavobacteria/Cytophaga* were slightly halophilic (2 to 5% salt tolerant), about 25% especially of *Micrococcus* spp; moderately halophilic (5 to 20% salt tolerant) and none from *Pseudomonas, Vibrio, Moraxella, Acinetobacter* and *Flavobacteria/Cytophaga* spp. extremely halophilic (20 to 32% salt tolerant).

Bacteria growing in presence of high salt (NaCl) concentrations had long been known. Certain marine bacteria not only tolerated high salt concentrations, but have a specific requirement for salt. All bacteria requiring salt from 2 to 32% are grouped as halophilic bacteria (Shewan, 1942).

These halophilic bacteria are further subdivided into slightly halophilic (requiring 2–5% salt), moderately halophilic (requiring 5–20% salt) and extremely halophilic (requiring 20–32% salt) bacteria. The extreme halophiles are usually characterised by the production of an intense red or pink pigment and hence known as the "red or "pink" halophiles. They comprise at least two groups of organisms, one consisting of Gram-negative asporogenous polar flagellated rods and generally classified as *Halobacterium* and the other belonging to *Micrococcus* or *Sarcina* group. Most marine bacteria are either halotolerant or slightly to moderately halophilic and comprise species belonging to the genera *Pseudomonas, Moraxella-Acinetobacter* and *Vibrio*. They grow best in sea water media or in isotonic mineral solution.

Simudu & Aiso (1962) isolated 475 bacterial strains from Kamogawa Bay, Japan and found that 82% of them were halophilic types, showing optimal growth at about 3% of sodium chloride. Their halophilic group mainly consisted of *Pseudomonas, Achromobacter, Vibrio* and *Photobacterium.* Karthiyani & Iyer (1967) and Surendran (1980) found that the distilled water based media containing 0.5% NaCl could recover only a significantly small portion of the bacteria from marine fish and prawn, caught off Cochin.

This paper reports the attempts of the authors to determine the salt tolerance behaviour of the bacteria isolated from tropical fish and prawn, with a view to get a true picture regarding the salt requirement in their recovery media.

Materials and Methods

Bacterial cultures were isolated from marine oil sardine *(Sardinella longiceps),* mackerel *(Rastrelliger kanagurta)* and prawn *(Penaeus indicus),* caught off Cochin. Cultures were isolated, morphologically and biochemically characterised and classified as described by Surendran (1980) and Surendran & Gopakumar (1981). Typical strains belonging to the major bacterial genera were selected for their salt tolerance studies.

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Salt tolerance of the cultures were determined in distilled water based medium, consisting of 1% (w/v) peptone (Difco) and 0.3% (w/v) beef extract (Difco) in distilled water pH being 7.2.

To the basal medium, analar (BDH) sodium chloride was added to get NaCl concentrations of 0, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.1, 2.2, 2.3, 2.5, 3, 3.5, 4, 5, 7.5, 10, 15 and 20 per cent (w/v). Ten ml of the medium was distributed in test tubes and sterilized at 1.05 kg/cm^2. Cultures grown in sea water peptone (SWP) medium for 48 h were harvested by centrifugation and the cells were washed twice with sterile distilled water (DW) and then suspended in sterile DW. The suspension was diluted with sterile DW, so as to get a fixed turbdity. One drop from this suspension was used for immediate inoculation to the above levels of NaCl in the basal medium in test tubes. Each tube was prepared in duplicate, one tube was incubated at 28±2°C (room temperature, RT) and the other at 36±1°C for one week.

In another set, cultures grown at 8±1°C in SWP were used for inoculation, after centrifuging, washing with sterile DW twice and re-suspending in sterile DW. This set was incubated at 8±1°C for 10 days. Growth was assessed turbidimetrically, by measuring the absorption at 660 nm in a photoelectric colorimeter (Evelyn photo-electric colorimeter, Rubican Instrument, U. S. A.).

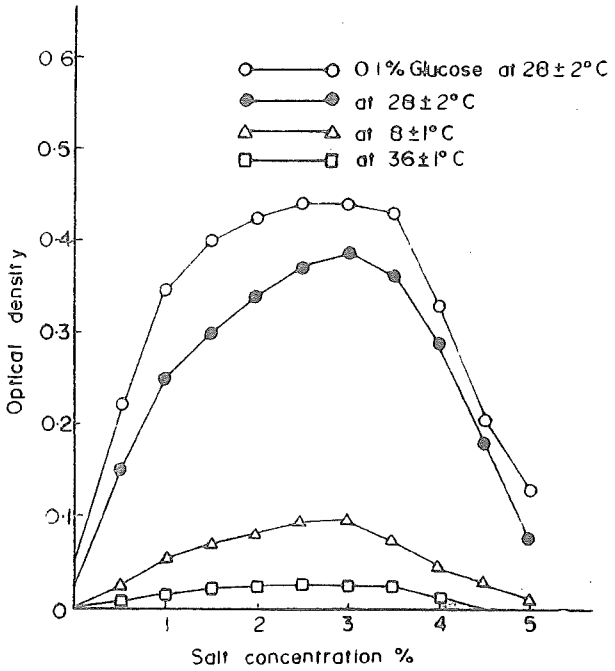

Fig. 2. Salt tolerance of a *Vibrio* strain at various temperatures and in presence of glucose

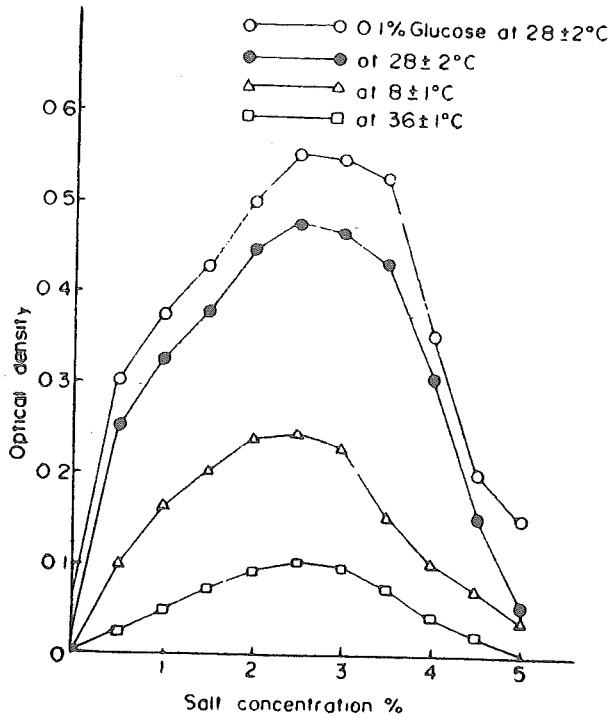

Fig. 1. Salt tolerance of a *Pseudomonas* strain at various temperatures and in presence of glucose.

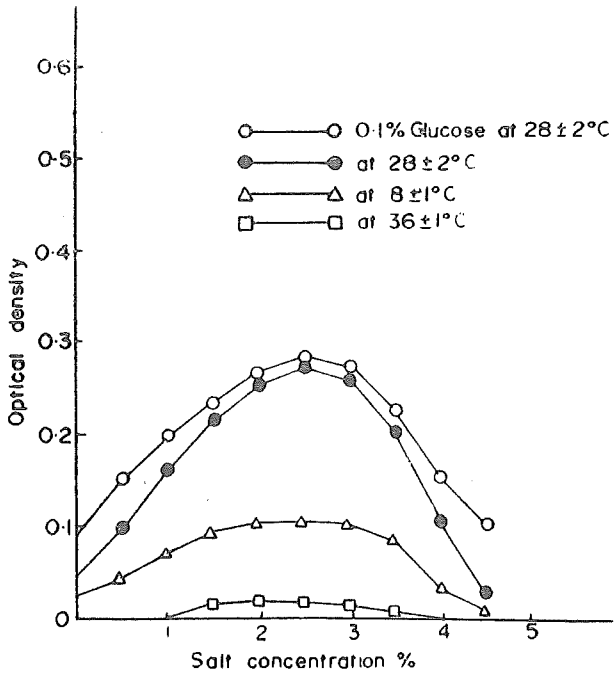

Fig. 3. Salt tolerance of a *Acinetobactor* strain at various temperatures and in glucose

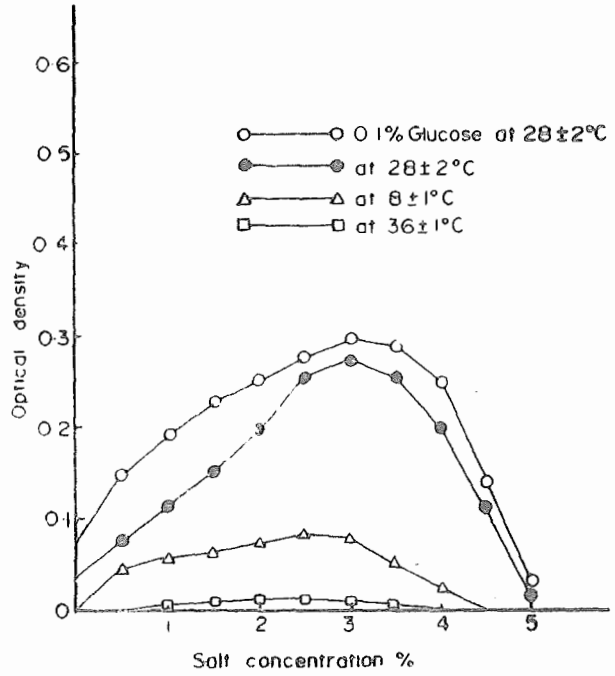

Fig. 4. Salt tolerance of a *Moraxella* strain at various temperatures and in presence of glucose

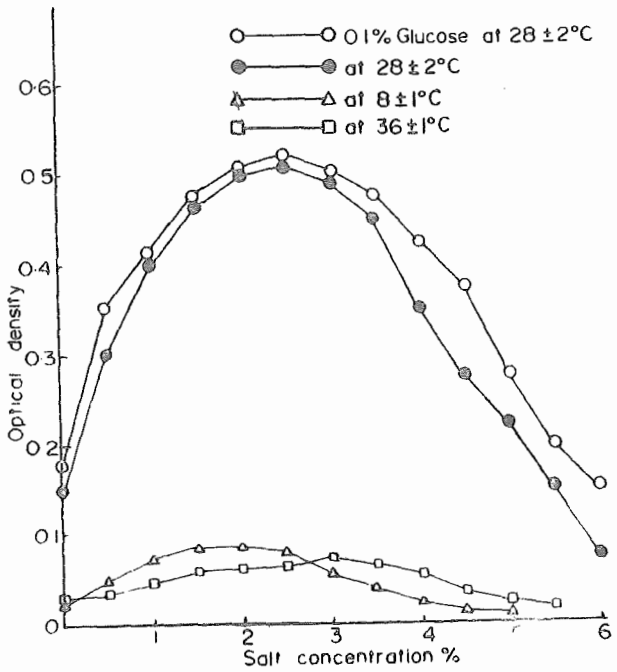

Fig. 6. Salt tolerence of a *Micrococcus* strain at various temperatures and in presence of glucose

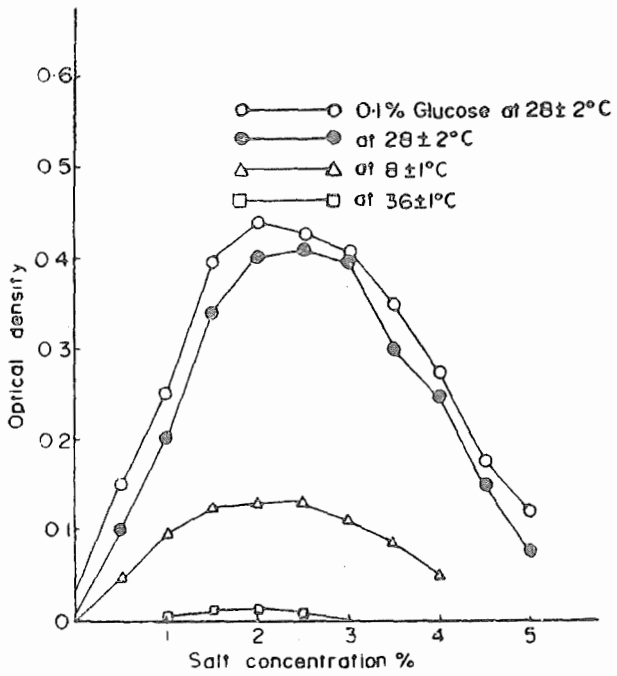

Fig. 5. Salt tolerance of a *Flavobacterium/Cytophaga* strain at various temperatures and in presence of glucose

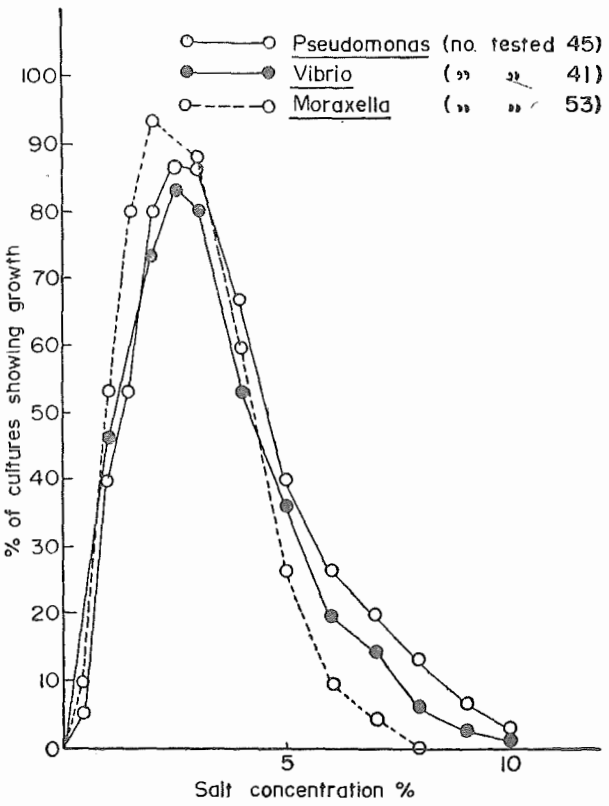

Fig. 7. Salt tolerance of various *genera* of bacteria at ± 2° C.

Table 1. *Salt tolerance of selected bacterial cultures at* 28 ± 2°C

Bacterial genus and culture number	Growth expressed as optical density at salt concentrations (%)															
	0	0.1	0.25	0.5	0.75	1.0	1.5	2.0	2.2	2.5	3.0	3.5	4.0	7.5	10	20
Pseudomonas																
SS1	0.03	0.12	0.16	0.20	0.22	0.28	0.35	0.38	0.41	0.44	0.52	0.49	0.40	0.16	0.01	0.0
SG7	0.0	0.04	0.05	0.09	0.12	0.15	0.19	0.22	0.23	0.26	0.34	0.37	0.33	0.21	0.06	0.0
SI15	0.0	0.08	0.20	0.30	0.34	0.38	0.42	0.50	0.52	0.55	0.53	0.51	0.35	0.09	0.0	0.0
PK7	0.02	0.06	0.11	0.24	0.27	0.33	0.39	0.45	0.45	0.46	0.45	0.40	0.32	0.12	0.0	0.0
Moraxella																
SS12	0.05	0.08	0.12	0.18	0.23	0.29	0.35	0.39	0.42	0.45	0.47	0.44	0.36	0.08	0.0	0.0
MS1	0.12	0.16	0.19	0.22	0.26	0.30	0.34	0.37	0.39	0.41	0.40	0.36	0.24	0.11	0.02	0.0
Acinetobacter																
SG1	0.06	0.09	0.11	0.12	0.14	0.17	0.21	0.26	0.27	0.29	0.29	0.20	0.10	0.0	0.0	0.0
MI15	0.02	0.10	0.14	0.17	0.20	0.23	0.27	0.31	0.33	0.36	0.34	0.27	0.14	0.04	0.0	0.0
Vibrio																
SI73	0.025	0.09	0.12	0.16	0.19	0.25	0.28	0.36	0.38	0.40	0.44	0.41	0.33	0.16	0.07	0.0
MS80	0.00	0.05	0.10	0.14	0.17	0.19	0.22	0.26	0.28	0.31	0.31	0.30	0.27	0.09	0.0	0.0
PN7	0.04	0.08	0.12	0.14	0.18	0.21	0.23	0.29	0.32	0.34	0.37	0.33	0.24	0.10	0.04	0.0
Micrococcus																
SS8	0.15	0.19	0.23	0.26	0.28	0.30	0.39	0.48	0.50	0.52	0.49	0.45	0.35	0.08	0.03	0.03
MI30	0.07	0.12	0.16	0.19	0.21	0.24	0.27	0.31	0.33	0.40	0.43	0.45	0.40	0.15	0.07	0.05
Flavobacteria/ Cytophaga																
SG8	0.00	0.03	0.08	0.12	0.16	0.20	0.24	0.27	0.27	0.33	0.31	0.27	0.20	0.0	0.0	0.0
SI42	0.00	0.07	0.14	0.18	0.21	0.27	0.30	0.36	0.39	0.37	0.32	0.24	0.17	0.0	0.0	0.0

Optical density was plotted against salt concentration for each culture and from the salt tolerance curve, the optimum, minimum and maximum salt tolerance of each culture were determined.

Also, in another series of experiment, glucose was added to the level of 0.1% in the medium in order to ascertain whether glucose could influence the salt tolerance of the cultures.

Results and Discussion

Table 1 gives the growth (expressed as optical density) of selected number of bacterial cultures belonging to the common genera present in fish, in presence of various salt concentrations, ranging from 0 to 20%. Table 2 presents the minimum, optimum and maximum amount of sodium chloride tolerated by these cultures.

It can be noted that certain strains of *Pseudomonas, Vibrio* and *Flavobacteria/Cytophaga* spp. could exhibit growth in the complete absence of salt, although the growth density was very little (only 6% of the growth at optimal salt concentration in the case of the *Pseudomonas* strain). But almost all cultures required a certain amount of salt for optimal growth. This amount was in the range of 2.5 to 3.5% for *Pseudomonas* spp. 2.5 to 3% for *Moraxella, Acinetobacter* and *Vibrio* spp; 2.5 to 3.5% for *Micrococcus* and 2.2 to 2.5% for *Flavobacteria/Cytophaga* spp. The maximum amount of salt tolerated was in the range of 10 to 20% for *Pseudomonas, Vibrio* and *Micrococcus* spp. For *Acinetobacter* and *Moraxella* spp. the range was 7.5 to 10% and for *Flavobacteria/Cytophaga,* less than 7.5%.

Figs. 1 to 6 represent the effect of temperature and glucose on salt tolerance of typical

Table 2. *Mininum, optimum and maximum salt tolerances of selected bacterial cultures at* 28±2 °C

Bacterial genus and culture number	Percentage of NaCl tolerated		
	Minimum	Optimum	Maximum
Pseuodomonas			
SS1	0.0	3.0	10
SG7	0.1	3.5	above 10
SI15	0.1	2.5	above 10
PK7	0.0	2.5	above 10
Moraxella			
SS12	0.0	3.0	above 7.5
MS1	0.0	2.5	10
Acinetobacter			
SG1	0.0	2.5–3.0	above 4.0
MI51	0.0	2.5	above 7.5
Vibrio			
SI73	0.0	3.0	above 10.0
MS80	0.1	2.5–3.0	above 7.5
PN7	0.0	3.0	above 10.0
Micrococcus			
SS8	0.0	2.5	above 20
MI30	0.0	3.5	above 20
Flavobacteria/Cytophaga			
SG8	0.1	2.5	above 4.0
SI42	0.1	2.2	above 4.0

cultures belonging to the six genera of bacteria. Effect of temperature and glucose appeared to be more or less similar on all the six genera, but for slight difference in quantitative aspects. The temperature did not alter the salt tolerance range for the cultures, but affected the growth rate. The presence of glucose improved the growth quantitatively, but the range of salt tolerance was unaffected.

Figs 7 and 8 show the percentage of cultures belonging to each genus, growing in various salt concentrations. It is interesting to note that more than 80% of the cultures belonging to *Pseudomonas, Moraxella, Vibrio, Micrococcus, Acinetobacter* and *Flavobacteria/Cytophaga* spp. were not only capable of growth in presence of 1.5 to 3.5% salt, but at least 25 to 30% of the cultures in each group required 1.5 to 3.5% salt for growth. Nearly 40% of the *Pseudomonas* and *Vibrio* strains and 30% of *Moraxella, Micrococcus* and *Flavobacteria/Cytophaga* strains could tolerate 5% salt, but only 15% of *Acinetobacter* strains could tolerate 5% salt. Maximum salt tolerated seemed to be 8% in the case of *Moraxella* and *Acinetobacter* and 10% in *Flavobacteria/Cytophaga*. A small percentage of *Pseudomonas* and *Vibrio* spp. appeared to grow beyond 10% salt, while nearly 3% of the *Micrococcus* grew in 15% salt.

Simudu & Aiso (1962) found that 82% of the cultures isolated from marine environment was halophilic, showing optimal growth at about 3% of sodium chloride. Most of these cultures belonged to *Pseudomonas, Achromobacter* (present *Acinetobacter- Moraxella* group), *Vibrio* and *Photobacterium*. According to Scholes & Shewan (1964), most marine bacteria are either halotolerant or slightly to moderately halophilic (2 to 5% and 5 to 20% salt tolerating). It is evident from the present study, that majority of the cultures belonging to the genera *Pseudomonas, Moraxella, Vibrio, Micrococcus, Acinetobacter and Flavobacteria/Cytophaga* (Figs. 7 & 8) were slightly halophilic (2 to 5% salt tolerating) according to Shewan's classification of halophilic bacteria (Shewan, 1942). About 25% of the cultures belonging to *Micrococcus* are moderately halophilic (5 to 20% salt tolerating). None from *Pseudomonas, Moraxella, Acinetobacter, Vibrio*

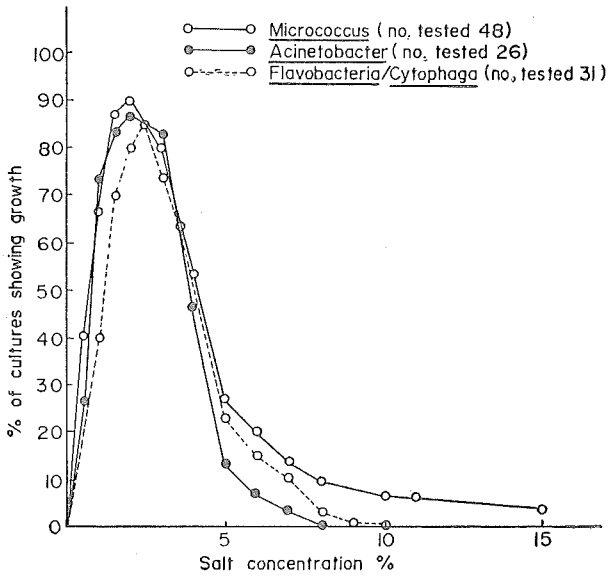

Fig. 8. Salt tolerance of various genera of bacteria at 28±2°C.

and *Flavobacteria / Cytophaga* spp. appears to be extremely halophilic (20 to 32% salt tolerating). This behaviour of the bacterial cultures explain the higher bacterial counts for marine fish and prawn, obtained on sea water agar media, compared with those on distilled water agar media, which contains only 0.5% or less salt.

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