Combined Effect of Environmental Factors on Spoilage Bacteria

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Effect of environmental factors such as temperature, pH and sodium chloride concentrations, individually and at various combinations, on the growth and survival of spoilage bacteria was studied in flesh broth and Zobells broth. Eight isolates, belonging to three different genera namely, *Pseudomonas*, *Vibrio* and *Acinetobacter*, harvested from spoiled *Penaeus indicus* were stored at 5,15,30,35 and 50°C, pH 2,4,6,8,10 and 12 and sodium chloride concentrations of 0,1,3,6,10 and 15%. Optimum growth for all the isolates was found to vary from 15 to 45°C and pH from 5-10. The growth pattern of all the isolates, in response to various sodium chloride concentrations was not similar in flesh broth and Zobells broth. Optimum sodium chloride concentration was found to vary from 1 to 6%. Influence of more than one environmental factor on the growth of spoilage bacteria was tested at various levels in flesh media by maintaining one of the factors constant and altering the other two. Results suggested that at extreme conditions each individual factor had an independent influence on the growth of bacteria.

Environmental factors influence the growth, survival and death of microorganisms. The bacterial flora of fish is a function of the environment from where they are caught and subsequently stored (Disney, 1976). In fish spoilage factors such as temperature, pH and osmotic pressure determine the rate of perishability of the commodity. In fish preservation, while designing the nature of preservation, attention is paid to the environmental factors to control the propagation of bacteria. In order to control the growth and propagation of bacteria, a knowledge of their response to various environmental factors is warranted. In the present communication the effect of environmental factors on the growth and survival of spoilage bacteria isolated from *Penaeus indicus* is presented.

Materials and Methods

Bacteria used in the present study were isolated from *Penaeus indicus* stored at 5,15,30,45 and 60°C. The cultures were identified to various genera based on their morphological and biochemical characteristics (Shewan *et al.*, 1960; Anon, 1974; Cowan, 1974). Eight isolates from the culture collection were selected for the present study based on their predominance during spoilage and also their properties like proteolysis, lipolysis, production of trimethylamine (TMA) from trimethylamine oxide (TMAO), production of off odours in flesh broth and formation of clear zone around the colony on flesh agar (Chandrasekaran *et al.*, 1985). The selected isolates belonged to three genera, namely. *Pseudomonas* (PR8, PL97, PF 152), *Vibrio* (PR42, PL146, PF10) and *Acinetobacter* (PL14, PF88).

ZoBell's 2216e broth and prawn flesh broth were used to find out the effect of temperature, pH and NaCl concentration on growth and survival of the cultures. Prawn flesh broth was prepared according

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Table 4. *Effect of pH on the survival of spoilage bacteria (percentage of growth index)*

Organism	Time, h	pH 2.6	4.2	6.0	8.6	9.6	10.6
Pseudomonas PR8	6	2.6	3.6	85.5	100.0	75.3	3.1
	12	1.7	2.1	77.4	93.2	72.9	1.7
	18	0.9	1.2	74.2	90.2	58.2	0.5
	24	0.0	0.6	70.4	88.5	51.4	0.3
	30	0.0	0.0	69.2	82.6	48.6	0.0
	36	0.0	0.0	68.8	80.1	48.0	0.0
*Vibrio*PR42	6	8.2	14.8	100.0	79.5	67.9	3.6
	12	4.1	9.4	91.9	74.9	61.5	2.3
	18	0.6	1.7	85.6	73.8	60.5	0.6
	24	0.0	0.4	68.9	66.7	57.3	0.5
	30	0.0	0.0	65.2	55.4	41.2	0.0
	36	0.0	0.0	63.6	50.2	25.5	0.0
Pseudomonas PL97	6	1.5	3.8	100.0	78.2	72.2	13.9
	12	0.5	2.5	83.2	74.2	58.4	4.0
	18	0.3	1.7	81.7	70.4	55.7	2.4
	24	0.0	0.1	77.2	59.9	51.5	0.6
	30	0.0	0.0	73.5	47.8	40.4	0.0
	36	0.0	0.0	72.4	41.4	38.6	0.0
Acinetobacter PL114	6	5.1	8.9	100.0	98.5	85.5	14.5
	12	2.2	2.5	88.8	82.7	84.6	5.8
	18	0.2	0.8	76.4	71.4	72.3	3.8
	24	0.0	0.0	73.8	68.5	66.4	0.9
	30	0.0	0.0	71.4	66.4	56.3	0.0
	36	0.0	0.0	68.3	59.2	24.5	0.0
Vibrio PF 146	6	14.9	22.5	94.8	100.0	68.2	45.3
	12	0.0	4.7	93.6	93.7	58.4	19.8
	18	0.0	0.6	84.6	89.5	41.6	5.6
	24	0.0	0.4	74.8	87.9	36.7	0.2
	30	0.0	0.0	64.3	74.4	36.2	0.0
	36	0.0	0.0	42.9	64.4	30.4	0.0
Vibrio PF 10	6	18.9	30.2	100.0	94.6	86.4	38.0
	12	14.6	23.9	99.8	87.4	83.1	20.5
	18	0.0	11.2	98.4	83.7	72.5	8.7
	24	0.0	0.8	90.3	78.9	68.3	0.4
	30	0.0	0.0	84.6	75.3	57.6	0.0
	36	0.0	0.0	76.7	68.9	44.6	0.0
Acinetobacter PF88	6	15.7	47.2	100.0	95.7	88.9	47.2
	12	9.6	19.1	96.8	85.3	83.4	8.9
	18	0.0	7.6	95.8	81.3	80.6	0.2
	24	0.0	0.0	94.2	77.7	64.9	0.0
	30	0.0	0.0	64.9	61.9	49.5	0.0
	36	0.0	0.0	57.6	58.2	41.6	0.0
Pseudomonas PF152	6	13.3	23.9	91.4	100.0	95.2	17.1
	12	2.2	4.8	80.4	91.2	82.1	8.7
	18	0.0	1.3	78.2	85.8	71.8	5.7
	24	0.0	0.0	67.6	82.4	65.7	0.2
	30	0.0	0.0	54.9	64.9	57.7	0.0
	36	0.0	0.0	52.4	50.9	27.2	0.0

Table 5. *Effect of sodium chloride concentrations (%) on the growth (percentage of growth index) of spoilage flora grown in two different media*

Organism	Flesh broth						ZoBell's broth					
	0	1	3	6	10	15	0	1	3	6	10	15
Pseudomonas PR8	53.7	85.3	100.0	91.2	36.0	-	35.9	49.6	100.0	42.7	15.4	-
Vibrio PR42	10.1	82.2	81.1	100.0	19.5	-	45.8	45.6	46.1	100.0	30.6	-
Pseudomonas PL97	49.6	28.7	100.0	41.1	20.9	-	48.4	100.0	43.4	41.8	8.2	-
Acinetobacter PL114	8.7	100.0	38.4	19.6	14.7	-	100.0	83.7	44.9	75.5	9.3	-
*Vibrio*PL146	16.5	81.7	74.2	100.0	1.1	-	53.9	75.0	100.0	34.6	5.8	-
*Vibrio*PF10	26.9	89.3	89.3	100.0	8.6	-	34.1	80.3	100.0	63.7	-	-
Acinetobacter PF88	6.8	100.0	6.9	5.7	4.6	-	49.8	100.0	88.2	9.2	-	-
Pseudomonas PF152	100.0	81.4	97.1	94.3	65.7	-	74.2	100.0	76.3	65.0	44.3	-

growth was observed between pH 6 and 10 in both the media and no growth was observed at pH 2 and 12. In ZoBell's broth four cultures showed maximum growth at pH 6.0, three at pH 8.0 and one at pH 10.0, whereas in flesh broth all cultures except two Pseudomonas (PL97 and PF152) showed maximum growth at alkaline pH (8.0 and 10.0). This confirms the earlier finding (Vanderzant & Nickelson, 1972) that bacteria prefer alkaline pH for growth in flesh. Better growth in flesh broth at alkaline pH suggests that the nutrients in prawn flesh may favour proliferation of the bacteria.

All the cultures survived well at pH 6.0 to 9.6 and were highly sensitive to acidic (≤ 4.2) or alkaline (≥10.6) pH (Table 4). However, they survived a few hours (12 to 18 h) at pH 4.2 and 10.6. Vanderzant & Nickelson (1972) reported that *V.parahaemolyticus* in shrimp homogenate was very sensitive to pH below 6.0 and no survivor was detected at pH 1 to 4. Generally the pH of spoiled flesh exceeds 7.0 (Iyengar *et al.*, 1960; Pillai *et al.*, 1961) and may be favourable for these bacteria.

From the results in Table 5 it is clear that the optimum requirement of sodium chloride depends on the growth media and varied from strain to strain. The *Pseudomonas* strain PR8 showed maximum growth at 3% NaCl in both the media whereas PL97 showed maximum growth at 3% NaCl in flesh broth and 1% NaCl in Zobell's broth. All the three *Vibrio* strains showed maximum growth at 6% NaCl in flesh broth whereas in ZoBell's broth maximum growth was observed at 6% NaCl for PR 42 and at 3% NaCl for PL 146 and PF10. *Acinetobacter* strains PL 114 and PF88 showed maximum growth at 1% NaCl in flesh broth and at 0% and 1% respectively in ZoBell's broth.

Results presented in Table 6 indicate that all the strains survived well at 0 to 6% NaCl whereas above 6% growth was affected in most cases. At 15% NaCl all the strains were destroyed within 18 h. This suggests that the spoilage flora are not halophilic.

The combined effect of different factors on the growth of the cultures is presented in Fig.1. Strains of *Acinetobacter* and *Pseudomonas* showed feeble growth at pH 10.0 at all temperatures and NaCl levels. 30°C favoured the growth of all the isolates at 3% NaCl and at pH 6.0 and 8.0. The requirement of NaCl for growth increased when temperatures were above ambient,

Table 6. *Effect of sodium chloride concentration on the survival of spoilage bacteria (percentage of growth index)*

Organism	Time, h	NaCl (%) 0	1	3	6	10	15
Pseudomonas PR8	6	20.3	100.0	38.3	38.2	26.1	21.8
	12	17.9	85.6	35.4	32.6	17.1	5.2
	18	14.8	79.6	31.5	27.6	10.5	0.0
	24	7.2	69.7	28.2	21.4	4.7	0.0
	30	3.9	64.2	27.2	8.2	0.8	0.0
	36	3.1	58.2	26.1	4.8	0.0	0.0
Vibrio PR 42	6	100.0	99.6	85.7	83.6	81.8	78.9
	12	98.9	94.6	84.5	77.8	47.5	14.6
	18	90.4	87.5	80.9	70.8	17.2	0.0
	24	86.1	86.6	79.6	58.2	0.0	0.0
	30	77.8	75.8	71.7	12.4	0.0	0.0
	36	70.8	70.1	60.3	0.0	0.0	0.0
Pseudomonas PL 97	6	83.9	90.8	100.0	88.2	58.5	48.9
	12	75.4	88.2	94.2	84.3	41.5	11.5
	18	68.8	85.7	92.2	80.8	12.7	0.0
	24	61.6	75.4	88.6	70.3	8.4	0.0
	30	59.5	60.8	80.8	68.6	2.2	0.0
	36	51.4	55.6	78.6	58.6	0.0	0.0
Acinetobacter PL 114	6	61.8	100.0	92.4	66.2	56.2	34.7
	12	51.4	92.4	70.2	63.9	50.7	23.9
	18	43.2	70.2	66.1	59.3	39.6	0.0
	24	38.9	62.1	59.5	50.0	6.2	0.0
	30	30.6	50.0	38.9	47.9	0.0	0.0
Vibrio PF 146	6	82.6	100.0	20.5	20.1	2.4	2.2
	12	78.7	98.6	19.4	18.6	1.2	1.1
	18	77.5	89.3	19.1	16.9	0.8	0.7
	24	76.5	87.2	17.0	13.6	0.0	0.0
	30	60.2	86.8	16.5	9.6	0.0	0.0
	36	58.9	84.5	13.8	1.9	0.0	0.0
Vibrio PF 10	6	100.0	74.8	69.6	54.1	48.9	43.9
	12	95.3	65.9	52.6	50.9	45.7	37.2
	18	76.0	52.6	44.8	18.1	31.1	0.0
	24	60.8	47.7	35.5	6.2	12.7	0.0
	30	54.4	44.4	25.3	5.4	0.0	0.0
	36	49.2	35.4	18.6	2.1	0.0	0.0
Acinetobacter PF 88	6	78.2	100.0	19.1	9.6	9.6	9.6
	12	45.8	93.4	17.2	5.8	5.6	0.0
	18	40.0	87.6	16.1	0.0	0.0	0.0
	24	38.1	78.1	8.6	0.0	0.0	0.0
	30	37.2	71.4	2.0	0.0	0.0	0.0
	36	36.1	70.4	0.9	0.0	0.0	0.0
Pseudomonas PF 152	6	100.0	95.0	85.8	74.8	68.8	32.0
	12	97.2	75.8	72.3	67.8	55.0	25.1
	18	85.4	69.9	64.4	64.0	31.2	11.5
	24	66.0	67.9	63.2	51.4	2.0	0.0
	30	50.5	66.0	59.8	4.8	0.0	0.0
	36	48.5	41.8	35.6	3.1	0.0	0.0

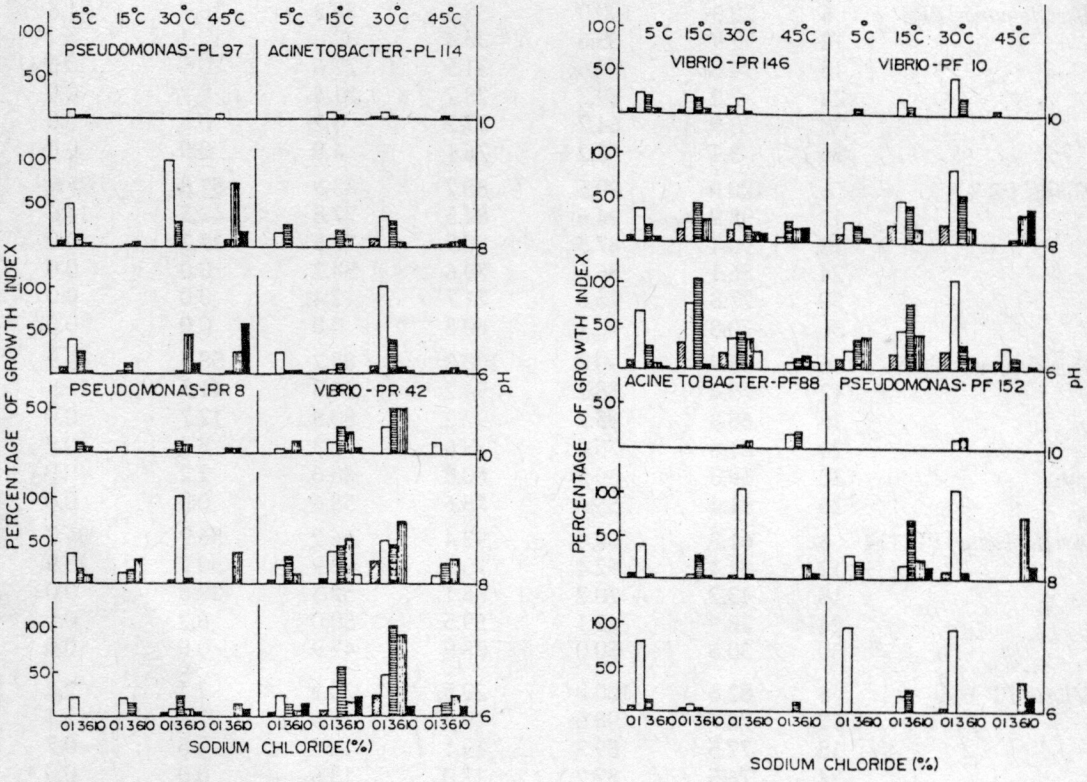

Fig. 1. Combined effect of temperature, pH and sodium chloride concentration on the growth of spoilage bacteria

whereas at 5°C very low concentration of NaCl (1%) was sufficient for enhancing growth. Covert and Woodburn (1972) showed that NaCl ranging from 0 to 12% in Trypticase soy broth afforded protection against lethality at 48° in *V.parahaemolyticus*. Similarly Beuchat (1975) reported that cells grown in Tryptic soy broth containing 3% NaCl displayed more resistance to heat than those grown in 0.5% NaCl. The positive relationship observed between NaCl. The positive relationship observed between NaCl concentration and incubation temperature on the growth of spoilage bacteria might be a property induced in them by the combined effect of temperature and NaCl concentration independent of pH within the optimum range.

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