OCCURRENCE OF *VIBRIO* DURING FISH SPOILAGE

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ALTHOUGH the species of *Vibrio*, as commensal bacteria of freshly harvested fin fish and shell fish, have been extensively reported,[1-6] the incidence of this bacterium as a component of spoilage flora during storage period of sea foods is rather limited.[6,7] The role of this bacterium in the spoilage process has been completely neglected. This paper reports the occurrence of *Vibrio* sp. as commensal and spoilage bacteria in tropical white prawn *Penaeus indicus* stored at higher and lower temperatures (28, 4 and −18°C), not reported earlier from Indian waters.

P. indicus collected live from Cochin backwater were killed by shock treatment and thoroughly washed with sterile saline. They were stored in raw unprocessed conditions of 'whole', 'headless', 'peeled and undeveined' (PUD) and 'peeled and deveined' (PD), at the three different temperatures. Samples were periodically drawn at different intervals, and analysed for spoilage.[8]

Bacteriological analysis: Total aerobic heterotrophic bacteria (THB) was estimated using ZoBell's 2216e agar, employing pour plate method. The plates were incubated for 5–7 days at room temperature (28 ± 2°C). Bacterial cultures were isolated from all samples by the random technique, checked for their purity and maintained on ZoBell's 2216e agar slants throughout the period of study. The genera were identified by their morphological and biochemical characters.[9,10]

The bacterial flora of fresh prawns, *P. indicus*, water and sediment samples, collected from Cochin backwater during April 1981 is presented in table 1. The data show that *Vibrio* sp was maximum (30.8%) followed by *Pseudomonas* (25.6%) and *Acinetobacter* (8%) in prawns. Similar dominance of *Vibrio* sp was also recorded in water and sediment. Gram-negative bacteria were high in prawn, water and sediment, and constituted 77.2%, 67.7% and 62.5% respectively. The presence of various genera of THB in prawn suggests the possibile influence of the environment.[4] The

Table 1 *Generic distribution of heterotrophic bacteria of fresh prawn Penaeus indicus, water and sediment*

	Sample (%)		
Genera	*P. indicus*	Water	Sediment
Vibrio	30.8	27.7	18.8
Aeromonas	–	6.7	6.3
Pseudomonas	25.6	13.3	18.8
Alcaligenes	–	6.7	–
Moraxella	7.7	6.7	–
Acinetobacter	8.0	6.7	12.5
Enterobacteriaceae	5.1	–	6.3
Micrococci	7.7	12.3	18.8
Bacillus	7.4	13.3	12.5
Corynebacterium	7.7	6.7	6.3
Gram positive	22.8	32.3	37.5
Gram negative	77.2	67.7	62.5

predominance of *Vibrio* sp in all the samples agrees with earlier reports from fresh gulf shrimps and shrimps harvested from ponds of Mexico.[11-14] Similarly high counts of *Vibrio* were reported in *Metapenaeus dopsoni*,[6] in Cochin backwater.

Species of *Vibrio* dominated in all the samples throughout the storage period at 28°C, except for a few instances where it showed predominance. In sample, whole, the *Vibrio* were 57.9%, 97.1%, 69.9% and 94.7% at 4, 8, 12 and 24 hr respectively. They were predominant in headless samples at 4 hr (40%) and subsequently the dominance of *Vibrio* was recorded at 8, 12 and 24 hr (72.4%, 100% and 98.4% respectively). *Vibrio* were at high level in PUD (94.9%, 100%, 93.3% and 62% at 4, 8, 12 and 24 hr respectively). In PD *Vibrio* (100%) alone were incurred except at 24 hr (95.5%). This is the first attempt of its kind to study the microbial flora of the stored white prawns at room temperature and to record the dominance of *Vibrio* sp during spoilage.

At 4°C this genera formed a predominant flora at several instances constituting a component of late spoilers on a few occasions. They were recorded on the 10th day in all samples *viz* 20.3% in whole, 62.5% in headless and 38.6% in PD. Their number decreased on the 15th day, 2.2% in whole, 14.6% in headless and 22% in PD. On the 30th day of storage, they were recorded in headless (36.5%), PUD (2.0%) and PD (22.0%). Prawns stored at −18°C showed disappearance of *Vibrio* sp during storage, but the reocurrence at a few instances was recorded on whole on the 21st day (26.4%) and 100th day (20.2%), on 34th day (40%) and on 100th day (12.5%). The observations made with

storage of prawns at 4 and −18°C agree with that of ice stored shrimps[6,11,13,14]. Dominance and predominance of *Vibrio* sp at a few instances at reduced temperature were similar to the observations made with ice-stored paraben-treated sardines (*Sardinella longiceps*) where *Vibrio* sp dominated among the late spoilers.[7]

Spoilage of fish and prawn commences immediately after rigormorties, progresses rapidly at higher temperatures and perishes within a short period before the harvested commodity goes to the fish processors. The dominant flora at the time of catch have ample chance to invade the flesh, progress rapidly and form a part of dominant flora or fully command spoilage. Reports on ice-stored and frozen prawn and fish, confirm *Pseudomonas* and *Achromobacter* as kings of spoilers. But the present study strongly suggests the possible association of *Vibrio* as spoilage flora of fresh tropical white prawns.

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SYNTHESIS AND PHYSIOLOGICAL ACTIVITY OF SOME NEW PYRANO-BENZOXAZOLES

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PYRANOBENZOXAZOLES have been reported to possess antibacterial activity[1,2]. It was therefore considered interesting to synthesise some new pyranobenzoxazoles from hydroxyaminocoumarins and hydroxyaminochromones.

The starting materials were 5-amino-6-hydroxy-, 6-amino-7-hydroxy-4-methyl-, 8-amino-7-hydroxy-4-methyl- coumarins and 6-amino-7-hydroxy-8-bromo-2-methyl-, and 8-amino-7-hydroxy-2-methyl-chromones. These were prepared by hydrogenation of the known nitro compounds[3–6] in the presence of palladium/charcoal catalyst.

Refluxing the amino hydroxy compounds with acetic anhydride for 1 hr and then decomposing over crushed ice, directly afforded the pyrano-2-methylbenzoxazole derivatives as crystalline solids in 80–85% yield.

Similarly, when equimolecular quantities of the amino-hydroxy compounds and aromatic or heterocyclic acids were heated with PPA at 150–60° for 1.5 hr and later at 200–205° for a further period of 3 hr the corresponding 2-substituted oxazoles were isolated as crystalline solids in 60–70% yields.

The pyranobenzoxazoles are listed in table 1. A typical pyranobenzoxazole (IIIa) showed λ_{max}^{MeOH} (log ε): 225 (4.57) and 275 (4.11) nm. Its IR (nujol) spectrum showed bands at 1710 (> C=O), 1570, 1350, 1050 (characteristic of oxazole ring system) cm^{-1}. Its NMR spectrum (TFA) showed δ 2.76 (3H, s, $-CH_3$); 6.8 (1H, s, C_7-H); 7.8–8.7 (7H, m, C_4H, C_5H and $C_{2'-6'}H$). The benzoxazole (V–d) showed λ_{max}^{MeOH} (log ε): 224 (4.62) and 273 (4.13) nm. Its IR (nujol) spectrum showed bands at 1655 cm^{-1} (> C=O), 1570 cm^{-1}, 1350 cm^{-1}, 1050 (characteristic of oxazole ring system) cm^{-1}. Its NMR spectrum ($CDCl_3$) showed δ 2.5 (3H, s, $-CH_3$); 2.75 (3H, s, $-CH_3$); 6.15 (1H, s, C_2H); 7.1–8.03 (6H, m, ArH). All the above compounds were