Spoilage Bacteria of *Penaeus indicus*

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Bacteria isolated from raw (untreated and unprocessed) prawn *(Penaeus indicus)* stored at 28 ± 2°C, 4°C and –18°C were tested for spoilage potential, namely, production of protease, lipase, amylase, reduction of trimethylamineoxide (TMAO) to trimethylamine (TMA), production of off odours from flesh broth and halo zone around the colony grown on flesh agar. About 63% of the total isolates tested were potential spoilers. Members of *Vibrio, Pseudomonas* and *Acinetobacter* were found to be dominant potential spoilers at all temperatures.

Fish spoilage is attributed to bacterial action on flesh substrates and several authors have reported various means to assess spoilage potential of bacteria isolated from fish or prawn. Bacteria isolated from spoiled fish that could hydrolyse fat, reduce trimethylamine oxide (TMAO) to trimethylamine (TMA), decompose proteins like gelatin and casein (Castell & Mapplebeck, 1952), grow at low temperatures and exhibit proteolytic property (Shewan *et al.*, 1960), produce off odours, volatile reducing substances (VRS), TMA, total volatile vases (TVB) (Lerke *et al.*, 1965; Shaw & Shewan, 1968), volatile sulphide compounds from flesh (Herbert *et al.*,1971) and produce clearing area around the colony grown on flesh agar (Chandrasekaran *et al.*, 1985) are considered to be potential spoilage bacteria. We report here the existence of potential flesh spoiling bacteria as a commensal flora in *Penaeus indicus* stored as raw, untreated and unprocessed samples at three different temperatures.

Materials and Methods

Penaeus indicus collected live from Cochin backwaters were killed by shock treatment, thoroughly washed with sterile saline and stored in raw unprocessed conditions as whole, headless, peeled and undeveined (PUD) and peeled and deveined (PD), at three different temperatures (28° ± 2°C, 4°C and -18°C). Samples were periodically drawn and analysed for spoilage characteristics.

Bacteriological analyses

Total heterotrophic bacteria (THB) was estimated using ZoBell's 2216e agar employing pour plate technique and incubated for 5–7 days at room temperature (28 ± 2°C). Bacterial cultures were isolated randomly, checked for their purity and maintained on ZoBell's agar slants. All the isolates were identified to various genera based on their morphological and biochemical characteristics (Bunchanan & Gibbons, 1974; Cowan, 1974).

Assessment of spoilage potential of bacteria

Bacteria selected for assessment of spoilage potential were checked for their proteolytic, lipolytic and amylolytic properties(Harrigan & McCance, 1972), reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA) (Wood & Baird, 1943; Laycock & Reiger, 1971) and for the production of off odours in flesh broth and clearing zone around the colony grown on flesh agar prepared according to Chandrasekaran *et al.* (1985). 100g of *Penaeus indicus* flesh was homogenized with an equal volume of 1% NaCl solution in a homogenizer. The homogenate was made upto 1000 ml and centrifuged at 1000 *g* for 30 min at room temperature (28° ± 2°C).

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Table 1. *Percentage composition of potential spoilers isolated from prawns with respect to storage at three different temperatures*

Storage temperature	28±2° C			4°C			−18°C			Total		
General	Total isolates	No. of spoilers	% of spoilers	Total isolates	No. of spoilers	% of spoilers	Total isolates	No. of spoilers	% of spoilers	No. of isolates	No. of spoilers	% of spoilers
Vibrio	21	16	72.0	9	8	89.0	10	10	100	40	34	85.00
Aeromonas	1	1	100.0	0	0	0	0	0	0	1	1	100.00
Pseudomonas	6	5	83.3	39	27	69.2	32	14	43.8	77	46	59.74
Alcaligenes	0	0	0	2	2	100.0	0	0	0	2	2	100.00
Acinetobacter	9	2	22.2	21	14	66.7	20	13	65	50	29	58.00
Enterobacteriaceae	2	1	50.0	9	3	60.0	2	1	50	9	5	55.56
Micrococcus	0	0	0	8	5	62.5	12	4	33.3	20	9	45.00
Bacillus	0	0	0	7	4	57.1	8	5	62.5	15	9	60.00
Corynebacterium	0	0	0	5	3	60.0	0	0	0	5	3	60.00
Total	39	25	64.1	96	66	68.8	84	47	56	219	138	63.00

The supernatant was boiled to remove coagulable protein and used as broth after autoclaving at 121°C for 15 min. For solidifed media 1.5% agar (Difco) was added to the broth. When cultures were tested, a loopful of 18 h old broth culture (10^3/ml), previously cultured in Zobell's broth, was inoculated to 10 ml of flesh broth in test tubes and spot inoculated in flesh agar medium.

Results and Discussion

Among 219 isolates tested, 138 representing species of *Vibrio, Aeromonas, Pseudomonas, Alcaligenes, Acinetobacter,* Enterobacteriaceae, *Micrococcus, Bacillus* and *Corynebacterium* were positive for production of protease, lipase and amylase enzymes and reduction of TMAO to TMA. These 219 strains were selected and tested for off odour production and halozone formation in flesh broth and flesh agar respectively. They were originally recorded as dominant groups among the bacterial flora existed on prawns during storage at the three different temperatures (Chandrasekaran *et al.* unpublished data). Results indicated that 63% of the 219 isolates examined were capable of producing off odours and halozone formation. All the isolates of *Aeromonas* and *Alcaligenes* tested were found as spoilers. Maximum number of *Vibrio* (85%) followed by *Bacillus* (60%), *Corynebacterium* (60%), *Pseudomonas* (59.7%), *Acinetobacter* (58%), Enterobacteriaceae (55.6%) and *Micrococcus* (45%) were spoilers. Members of *Vibrio, Aeromonas, Pseudomonas, Acinetobacter, Alcaligenes,* Enterobacteriaceae and *Micrococcus* were reported earlier to produce off odours from flesh juice (Lerke *et al.* 1965; Cox & Lovell, 1973; van Spreekens, 1977). In the present study all the above said groups and species of *Bacillus* and *Corynebacterium* were recorded to produce off odours in flesh broth and produce clearing area around their colony grown on flesh agar indicating utilisation of flesh substrates, mainly protein and lipid (Chandrasekaran *et al.*, 1985) of *Penaeus indicus.* Their ability to produce hydrolytic enzymes and halo formation on flesh agar confirms the existence of spoilage bacteria in *P. indicus* during storage and its spoilage.

From the results summarised in Table 1 it is clear that maximum percentage of spoilers were present in prawns stored at 4°C (68.8%) than 28±2° C (64.1%) and at –18°C (56%). At all the three storage temperatures, strains of *Vibrio, Pseudomonas, Acinetobacter* and Enterobacteriaceae showing spoilage activity were recorded. During storage at 28±2°C, *Vibrio* sp. formed the major dominant group in all the four types of samples. At 4°C *Pseudomonas* formed the major dominant group. However at –18°C both *Acinetobacter* and *Pseudomonas* formed the major group (Chandrasekaran, 1985). At 4°C and –18°C, when one of these groups was dominant, the rest of the groups formed second dominant groups. However, when they were assessed for their spoilage potential *Vibrio* sp. and *Acinetobacter* sp. represented increasing number of spoilers with decrease in storage temperature. However, *Pseudomonas* recorded a decrease in the percentage of spoilers along with decrease in storage temperature which was rather unusual. According to Adams *et al.* (1964), during storage, a wide variety of organisms may be present, of which only a small percentage cause spoilage. The rest of the groups probably exist as free riders or perhaps are involved in some synergism with weak spoiler. One should not say an organism isolated from spoiled fish or prawn as a non-spoiler just because in pure culture it is unable to spoil fish. The same organism may in mixed culture, play an important role (Adams *et al.*, 1964). Hence in the present study although only 63% of the isolates tested showed them to be active spoilers of prawn flesh, the rest of the groups also might play an unknown role in the spoilage of prawns during storage.

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