Bacterial Growth and Protein Degradation in Different Fish Flesh Extracts and Casein Media

Rosamma Philip and P. Lakshmanaperumalsamy*

School of Marine Sciences, Cochin University of Science and Technology, Kochi-682016

The four bacterial strains tested showed considerable variations in the growth pattern and extent of protein degradation in fish, prawn and clam extract media and casein medium. During the first 12h of incubation, growth rate of all the strains were higher in prawn flesh broth compared to that in other media except for *Pseudomonas* sp.Ca 173. On prolonged incubation these bacteria did grow equally well in other media also. Generally, in all the media, protein degradation commenced on the third day and progressed rapidly.

Bacterial proteolysis during fish spoilage has been reported by Dyer *et al.* (1950), Lerke *et al.* (1967), Nair & Lahiry (1968) and Venugopal & Lewis (1985). It is observed that spoilage may normally be initiated by the utilization of low molecular weight compounds such as amino acids and other non-protein compounds; the proteolysis thus becomes an event of advanced stages of spoilage (Jay, 1966; Lerke *et al* ., 1967 and Liston, 1973). During the progress of spoilage, considerable increase in the proteolytic bacterial population has been observed by Chai *et al.* (1968), Liston (1973), George (1979) Surendran & Gopakumar (1985). The present investigation is to study the time course of growth and protein degradation of selected strains of proteolytic bacteria in various flesh (prawn, clam and fish) and casein media.

Materials and Methods

Four strains of proteolytic bacteria, namely, *Pseudomonas* sp. CA 173 isolated from *Penaeus indicus, Pseududomonas* sp. Ca 386 from *Metapenaeus dobsoni, Vibrio* sp. Ca 377 from *Liza parsia* and *Vibrio* sp. Ca 761 from *Penaeus indicus* were used for the study.

Fish, prawn and clam flesh extract media and casein medium were employed for the study.

The tissue homogenates of fish, prawn and clam flesh were prepared separately as follows: 100 g of the flesh was taken in a waring blender, added 200 ml saline (0.5% NaCl) and homogenized for 5 min. This solution was made upto 1000 ml (1:10 w/v) before centrifuging at 5000 rpm for 30 min. The pH of the supernatant solution was adjusted to 7 and boiled for 30 min followed by centrifugation at 12000 rpm for the same duration. The supernatant was used as the flesh medium after autoclaving at 15 lbs pressure for 15 min.

Casein medium (pH 7) was prepared with casein (Hammerstein) as substrate (1%) in a mineral basal medium (K_2HPO_4, 1 g; $MgSO_4$. $7H_2O$, 0.5 g; NaCl, 5 g; $CaCl_2$, 0.1 g; Ferrous ammounium sulphate, 0.005 g; NH_4Cl, 1.5 g and Distilled water, 1 litre) and sterilized as suggested above.

Cell suspension was prepared by harvesting 18 to 20 h old nutrient agar slant culture in sterile physiological saline (0.80% NaCl). The optical density was adjusted to 1 and was used as the inoculum. One ml each of the cell suspension was inoculated to 100 ml of the various media prepared.

The bacterial growth was recorded by measuring the optical density of the broth

* Present address: Department of Environmental Sciences, Bharathiar University, Coimbatore-641046

culture at 600 nm in a Hitachi Model 200-20 UV Visible Spectrophotometer. Protein content of the media and the culture supernatant was estimated following the method of Lowry *et al.* (1951). The initial protein content of the casein medium and fish, prawn and clam flesh extract media were 645, 387, 387 and 167 mg/ml respectively.

Immediately after inoculation (O h) the cell density and the protein content of the media were estimated. The culture flasks were incubated at room temperature (28± 2°C) for 7 days and measured the growth and protein degradation at 24 h intervals. For estimating the protein content a portion of the culture broth was centrifuged at 7500 rpm for 15 min and the supernatant was taken for analysis. The percent degradation of protein was calculated from the initial protein content of the broth.

Results and Discussion

The growth pattern and the extent of protein degradation by the four bacterial strains in various media are shown in Figs. 1 to 4.

Variations in the growth pattern and the extent of protein degradation by the four strains of bacteria in the various media might well be accounted as one of the manifestations of differences in physiological traits of these bacteria and the variations in amount and kinds of different constituents in the media. Poor growth and protein degradation by all the strains in casein media except by *Pseudomonas* sp. Ca 386 shows their inability to attack directly the casein in the absence of easily assimilable organic carbon and nitrogen sources (Banwart, 1979). During the first 12 h of incubation, growth rate of all the strains was found to be higher in prawn flesh broth than in other media except for *Pseudomonas* sp.Ca 173. It is apparent that comparatively higher concentration of the free amino acids present in prawn meat have formed the first source of nitrogen for these bacteria to multiply faster (James, 1969 and Jay, 1978).

However, on prolonged incubation these bacteria did grow equally well in other media also. It is known that bacteria first

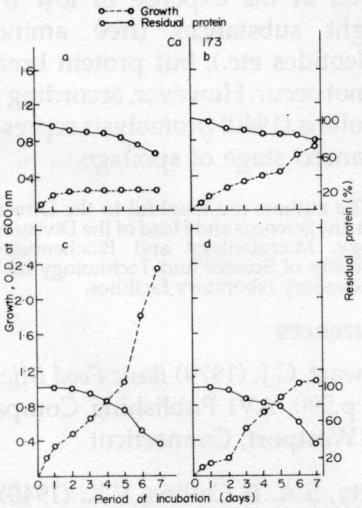

Fig. 1. Time course of growth and protein degradation by *Pseudomonas* sp. Ca 173, a: Casein b: Prawn flesh extract c: Clam flesh extract and d: Fish flesh extract media

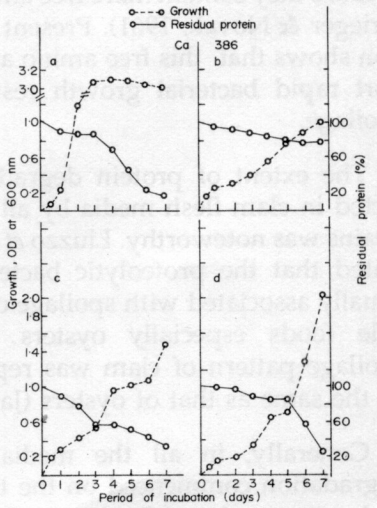

Fig. 2. Time course of growth and protein degradation by *Pseudomonas* Sp. Ca 386; a: Casein b: Prawn flesh extract c: Clam flesh extract and Fish flesh extract media

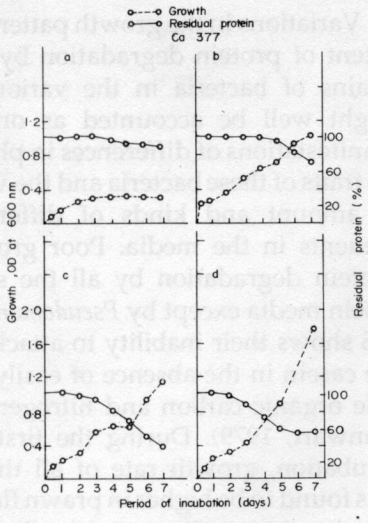

Fig. 3. Time course of growth and protein degradation by *Vibrio* Sp. Ca 377; a: Casein b: Prawn flesh extract c: Clam flesh extract and d: Fish flesh extract media

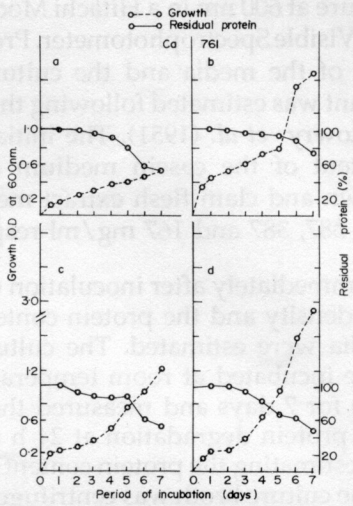

Fig. 4. Time course of growth and protein degradation by *Vibrio* Sp. Ca 761, a: Casein b: Prawn flesh extract c: Clam flesh extract and d: Fish flesh extract media

thrive on the free amino acids and other non-protein nitrogeneous substrates in the flesh and afterwards only attack the protein components (Liston, 1973). Prawns are reported to spoil more rapidly than finfish because they contain more free amino acids (Frieger & Novak, 1961). Present observation shows that this free amino acids support rapid bacterial growth resulting in spoilage.

The extent of protein degradation effected in clam flesh media by all the four strains was noteworthy. Liuzzo *et al.* (1968) stated that the proteolytic bacteria were usually associated with spoilage of perishable foods especially oysters and the spoilage pattern of clam was reported to be the same as that of oysters (Jay, 1978).

Generally, in all the media protein degradation commenced on the third day and progressed rapidly during the following days. This observation agrees well with the hypothesis that during incipient stage of spoilage, the utilization of the non protein nitrogeneous (NPN) compounds pool causes proteinase production and the subsequent proteolysis replenishing the NPN pool (Liston, 1973). Jay (1966) suggested that microbial spoilage of fresh meats occurred at the expense of low molecular weight substances (free amino acids, nucleotides etc.), but protein break down did not occur. However, according to Beatty & Collins (1940) proteolysis represented an advanced stage of spoilage.

The authors are thankful to the Director, School of Marine Sciences and Head of the Division of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology for providing the necessary laboratory facilities.

References

Banwart, G.J. (1979) *Basic Food Microbiology* p.590, AVI Publishing Company Inc., Westport, Connecticut

Beatty, S.A. & Collins, V.K. (1940) *J. Fish. Res. Bd Can.* **4**, 412

Chai, T., Chen, C., Rosen, A. & Levin, R.E. (1968)*Appl. Microbiol.* **16**,1738

Dyer, W.J., French, H.V. & Snow, J.M. (1950) *J.Fish. Res. Bd. Can.*. **7**,585

Freger, E.A. & Novak, A.F. (1961) in *Fish as Food* (Borgstrom, G.Ed.) p.561, Academic Press, New York

George, S. (1979) *Bacteriological and Biochemical Changes in Relation to Freshness of Shrimp*. M.F.Sc. thesis, p. 93, College of Fisheries, Mangalore

James, M.A. (1969) *Sci. Cult.* **35** 590

Jay, J.M. (1966) in *The Physiology and Biochemistry of Muscle as a Food* (Briskey, E.J., Cassens, R.G. & Trautman, J.C,. Eds.) p.387, Univ. of Wisconsia Press, Madison

Jay , J.M. (1978) *Modern Food Microbiology* p.479, D Van Nostrand Co., New York

Lerke, P., Farber, K. & Adams, R. (1967) *Appl. Microbiol.* **15**, 779

Liston, J. (1973) *GIAM IV S. PAULO* 23-28 p.645

Liuzzo, J.A., Farag, M.K. & Novak, A.F. (1968) *J.Food Sci.* **32**,678

Lowry, O.H., Rosenbrough, N.J., Farr, A.F., & Randall, R.J. (1951) *J. Biol. Chem.* **193**,265

Nair, R.B. & Lahiry, N.L. (1968) *J. Food Sci. Technol.* **5**, 109

Surendran, P.K. & Gopakumar, K. (1985) in *Harvest and Postharvest Technology of Fish*, p. 514. Society of Fisheries Technologists (India), Cochin

Venugopal, V. & Lewis, N.F. (1985) in *Harvest and Postharvest Technology of Fish*, p.424. Society of Fisheries Technologists (India), Cochin