Proceedings of the Seventeenth Sessions of the Sri Lanka Association for Fisheries and Aquatic Resources, 19th and 20th May, 2011. Auditorium of National Aquatic Resources Research and Development Agency, Colombo 15.

Detection of histamine forming Enterobacteriaceae bacteria in fish using Polymerase Chain Reaction

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Fish is a nutritious article rich in protein, fat, vitamins and minerals. As it is a perishable food item, it spoils easily when decarboxylase enzymes form by bacterial growth causing generation of bioactive amine including histamine. In most amine containing foods, the majority of amines are generated by decarboxylation of corresponding amino acids through substrate specific enzymes designed from micro organisms present in the foods. The formation of biogenic amines is important for not only from the stand point of their toxicity, but also because they can be used as the freshness or spoilage indicators for food. Histamine is formed by free histidine, an amino acid, which is present in large amounts in the muscle tissues of fish belonging to Scombridae family such as tuna and mackerel, decarboxylating with decarboxylase enzyme of bacterial histidine. There are various bacteria species facilitating decarboxylation of histidine, mostly the group of gram negative enteric bacteria including *Morganella morganii*, *Klebsiella* spp, *Enterobacter* spp.

Presence of histamine decarboxylasing gene (*hdc*) in bacteria can give a clear idea about those which can form histamine. In this study, mackerel (*Rastrelliger kanagurta*) were collected in local markets and samples were prepared in Maximum Recovery Diluent (MRD) and the colonies were grown on Violet Red Bile Glucose Agar (VRBG). Further, the colonies were streaked on Nutrient Agar and used for the PCR. Positve result for the *hdc* gene gave a band size of 709 bp, gene which is responsible for the formation of histamine. Out of 216 isolates, 46 gave positive results for *hdc* gene. Pink colour cells were obtained in Gram staining. Colonies were biochemically identified with the test of urease test, oxidase test, indole test, citrate test, TSI reaction and VP test and they were identified as *Enterobacter* spp, *Klebsiella* spp, *Proteus* spp. and *Morganella morganii*. The use of this method can detect histamine forming gram negative bacteria early and rapidly and also this helps evaluating the potential of histamine formation in fish.