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Research Note

Isolation and Identification of Histamine-forming Enterobacteria in Freshly Landed Tuna (*Euthynnus affinis*) Using a Dichotomous Scheme

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Histamine poisoning has been attributed mainly to the accumulation of toxic levels of histamine in a spoiling fish. Histamine is reportedly produced by a wide range of microorganisms, majority of which are gram-negative rods of *Enterobacteriaceae* family (Frank *et al.*, 1985; Taylor and Sumner, 1986; Klausen and Huss, 1987; Okuzumi *et al.*, 1994).

For the detection and enumeration of histamine forming bacteria (HFB), Niven's differential agar medium (Niven *et.al.*, 1981) has been widely used (Baranowski, 1985; Chen *et al.*, 1989; Roig-Sagues, 1997). Modification to Niven's medium has been reported for the detection of HFB in general with respect to changes in pH and histidine content (Yoshinaga and Frank, 1982; Chen *et al.*, 1989; Mavromatis and Quantick, 2002). The positive isolates from Niven's medium are reported to be further characterized by using rapid identification kits such as API 20E test strips or PASCO Gram Negative Identification System (Frank *et al.*, 1985; Lopez-Sabater *et al.*, 1996). Several investigators have adopted conventional biochemical tests for characterization of these isolates (Subburaj *et al.*, 1984; Gopakumar *et al.*, 1988; Lakshmanan *et al.*, 2002) . Polymerase chain reaction techniques and DNA probes have also been reported for the detection of HFB (Alves *et al.*, 2002; Kim *et al.*, 2003). The aim of this work was to formulate a dichotomous scheme with minimal number of conventional biochemical tests for the identification of HFB belonging to *Enterobacteriaceae* family detected on a differential agar medium.

The study was undertaken using fresh tuna (*Euthynnus affinis*) procured from Cochin fishing harbour. The aerobic plate count (APC) was estimated as per the standard method (Maturin and Peeler, 1995) using tryptone glucose agar. Violet red bile glucose agar (VRBGA) (HiMedia, Mumbai) was used for the enumeration and isolation of *Enterobacteriaceae* according to ICMSF (1978).

About 28 well-isolated purple colonies surrounded by a purple halo on a VRBGA plates giving 33 colonies were purified and then subjected to gram-staining, oxidase and nitrate reduction tests for provisional identification as *Enterobacteriaceae*. Motility and other conventional biochemical tests such as O/F test, indole production, MR-VP, citrate utilization, malonate utilization, phenylalanine deaminase, hydrogen sulfide on TSI, ONPG, urease and carbohydrate utilization tests were performed according to Edwards and Ewing (1972) and MacFaddin (1980).

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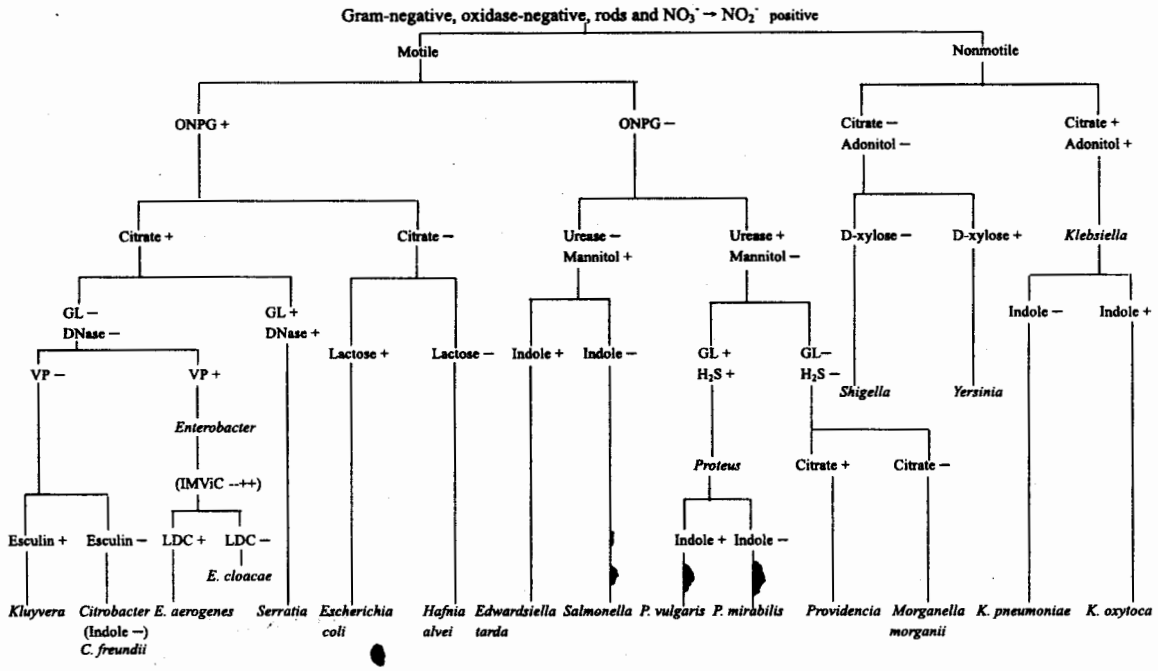

Abbreviations: ONPG, o-nitrophenyl-β-D-galactopyranoside; DNase, Deoxyribonuclease; GL, Gelatin Liquefaction; VP, Voges-Proskauer; IMViC, Indole-Methyl Red-Voges-Proskauer-Citrate and LDC, Lysine Decarboxylase

Fig. 1. A dichotomous key for identification of enterobacterial genera with selected species of histamine-forming bacteria

Histamine formation by the isolates was confirmed by using the medium of Yamani and Untermann (1985). The histamine-positive isolates were identified up to species level using the dichotomous key of Prescott *et al.* (1996) after modifications (Figure 1) with the help of conventional biochemical tests given by Kreig and Holt (1984).

Table 1. Histamine-forming enterobacteria isolated from fresh tuna.

Organism	No. of isolates	HDC[a] positive isolates
Citrobacter spp.	2	1
Enterobacter aerogenes	2	2
Enterobacter spp.	2	1
Escherichia coli	3	3
Klebsiella pneumoniae	1	1
Klebsiella oxytoca	2	2
Morganella morganii	1	1
Proteus mirabilis	3	3
Proteus vulgaris	1	1
Proteus spp.	1	–
Serratia spp.	3	2
Yersinia spp.	2	–
Untypable	5	–
Total	28	17

[a] Histidine decarboxylase

The APC and enterobacterial count of the fresh tuna were observed to be 1.5×10^5 cfu/g and 2.17×10^2 cfu/g respectively. The incidence of enterobacterial HFB observed in fresh tuna is detailed in Table 1. It is seen that the majority of the enterobacterial genera encountered have the ability to decarboxylate histidine to histamine. However, they have been reported to vary in the rate of histamine production (Arnold and Brown, 1978). *H. alvei*, another potent histamine-forming organism, could not be detected in the isolates.

The present study revealed that the proportion of enterobacterial HFB has accounted for less than 0.1 % of the APC that was closely related to the low enterobacterial count in fresh tuna.

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