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Differential Plating Medium for Quantitative Detection of Histamine-Producing Bacteria

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A histidine-containing agar medium has been devised for quantitative detection of histamine-producing bacteria that are alleged to be associated with scombroid fish poisoning outbreaks. The responsible bacteria produce a marked pH change in the agar, with attendant color change of a pH indicator adjacent to the colonies, thus facilitating their recognition. *Proteus morganii* and *Klebsiella pneumoniae* were the two most common histidine-decarboxylating species isolated from scombroid fish and mahi mahi.

Scombroid poisoning (histamine toxicity) occasionally results from consuming scombroid fish (saury, tuna, bonito, butterfly kingfish, mackerel) as well as certain other species such as mahi mahi (*Coryphaena hippurus*). These fish are characterized by having high levels of free histidine in their tissues (3).

Onset of the poisoning symptoms ranges from a few minutes to a few hours after eating. Victims generally experience a flushing of the face and neck, a throbbing headache, dizziness, faintness, and an itching, burning sensation in the mouth and throat. The fish, as consumed, may have a sharp, peppery flavor and invariably contain high levels of free histamine, usually in excess of 100 mg/100 g (1). Histamine accumulates in the tissue as a result of bacterial decarboxylation of the free histidine in the fish muscle tissue (2). Other than high histamine content as detected chemically, no other evidence of spoilage or decomposition may be evident in the affected fish.

The responsible histidine-decarboxylating bacteria may be difficult to detect because they generally comprise a minority of the bacterial flora in freshly caught fish. Also, they appear to be killed easily by subsequent freezing of spoiled fish, and therefore ultimately may constitute a rather small proportion of the flora after thawing.

To facilitate quantitative detection of histidine-decarboxylating bacteria, a differential plating medium was devised that permits their recognition. The medium was comprised of 0.5% tryptone (Difco), 0.5% yeast extract (Difco), 2.7% L-histidine·2HCl, 0.5% NaCl, 0.1% $CaCO_3$, 2.0% agar, and 0.006% bromocresol purple (pH 5.3). The medium was autoclaved for 10 min or less to avoid excessive hydrolysis of the agar at the low pH. Decimal dilutions were prepared using the conventional pour-plate technique, and the solidified agar was subsequently overlaid with about 5 ml of the same medium to suppress spreading colonies.

Duplicate plates were incubated, one set at 25°C and the other at 35°C, for 36 to 72 h, then examined for purple colonies with a purple halo on the yellow background. The pH surrounding the decarboxylating colonies increased at least 1.5 units as the result of accumulation of the more alkaline histamine. The principle is similar to the conventional Moeller broth used in the identification of the *Enterobacteriaceae* species, but modified by the addition of agar.

Histidine-decarboxylating colonies generally appeared larger than the surrounding background flora on the differential agar, thereby aiding in their recognition. One such colony among several hundred was readily apparent. Their more vigorous growth may be the result of the pH increase, thus creating a more favorable microenvironment. We have no evidence that histidine is metabolized as an energy source.

Of the two incubation temperatures, 35°C appeared to be distinctly superior. Only on rare occasions were decarboxylating colonies detected only on the 25°C plates.

Several hundred colonies showing the characteristic purple halo on the agar plates were isolated, and the pure cultures were tested for their histidine decarboxylase activity. The cultures were inoculated into a broth having the same composition as the plating medium, but without the agar. In addition, numerous pure cultures were inoculated into a tuna homogenate prepared by macerating in a Waring blender 400 g of canned water-packed tuna in 1 liter of water containing 10 g of peptone and 25 g of histidine·2HCl (pH 5.5). The macerate was distributed into tubes and autoclaved before inoculation. Histamine was determined in the cultures after 48 h at 35°C by a modified fluorometric method

(4). Without exception, all cultures representing positive colonies on the plating medium produced high amounts of histamine in the broth cultures, some in excess of 2%. All cultures tested that were negative on the plates failed to produce histamine in broth cultures.

Among the histamine-positive cultures that had been isolated from raw tuna, mahi mahi, and mackerel, an attempt was made to identify 28 by using the API system. *Proteus morganii* proved to be the most common species (15 cultures), followed by *Klebsiella pneumoniae* (4), *Escherichia coli* (2), *Enterobacter aerogenes* (2), *Enterobacter cloacae* (1), *Proteus* sp. (1), *Edwardsiella* sp. (1), and *Vibrio* spp. (2). *P. morganii* previously has been reported to be associated with numerous scombroid poisoning outbreaks (2); *K. pneumoniae* (5) and some other members of the *Enterobacteriaceae* have also been found.

As a matter of interest, all *P. morganii* strains in our collection readily decarboxylated histidine; five of the six *K. pneumoniae* isolates also produced histamine, but only one of seven *E. cloacae* strains and two of six *E. coli*.

It is possible that the low pH of the plating medium might exclude growth of some histamine-positive bacteria. Indeed, the observed counts on this medium were generally lower by approximately one order of magnitude than counts obtained on plate count agar (Difco). Nevertheless, despite repeated attempts, we were not successful in isolating from plate count agar histamine-positive bacteria that could not grow in the differential plating medium. Histidine decarboxylase is not an uncommon enzyme among the various bacterial species, and it might be concluded that the plating medium would detect only those bacteria that vigorously produce histamine in large amounts. Such bacteria, however, are the very ones that would be expected to be associated with scombroid poisoning outbreaks.

With *P. morganii* as a test culture, histamine was produced rather vigorously in the tuna homogenate medium at temperatures ranging from 20 to 40°C; optimum temperature was about 35°C. There was no sharp pH optimum for growth and subsequent histamine production; activity was high over a pH range of 5.3 to 6.0. Histamine was produced equally well by the growing culture either under aerobic or anaerobic incubation.

It is likely that a plating medium and technique similar to those described here might be employed to detect other amino acid decarboxylases among the various bacterial species. Adjustments in basal medium composition, pH, and indicator system might be required to fit the requirements of the specific taxa being tested.

Through use of the differential plating medium, our studies have confirmed that the microorganisms responsible for histamine accumulation in susceptible fish appear to be exclusively mesophiles, nearly all of which belong to the family *Enterobacteriaceae*. These microorganisms do not appear to be part of the normal flora on or in the fish as they are caught; rather, the fish become contaminated from contact with unclean surfaces. If then held at favorable growth temperatures for several hours, the microorganisms grow very rapidly, with attendant increase in histamine in the tissues. It is not surprising, then, that scombroid poisoning is more commonly associated with consumption of scombroid and related fish from tropical climates, where refrigeration facilities are not available for adequate chilling or freezing of the fish shortly after they are caught. None of the psychrotrophic bacteria that we tested that normally comprise the majority of the indigenous microflora of fish produced histamine in detectable quantities in the differential medium.

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The histamine assays that were necessary in this study were conducted by R. W. Vetro.

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