Isolation of *Aeromonas* Sp. ATCC 29063, a Phenol-Producing Organism, from Fresh Haddock

T. C. CHEN* AND R. E. LEVIN*

Department of Food Science and Nutrition, University of Massachusetts, Amherst, Massachusetts 01002

Received for publication 20 November 1974

Attempts to isolate phenol-producing organisms from stale haddock fillets failed. Several such isolates, however, were readily obtained from fresh haddock and were designated *Aeromonas* sp. Phenol was produced from L-tyrosine by these isolates.

This laboratory (2) previously reported that the major high-boiling compound produced in haddock fillets during refrigerated storage is phenol. The present study documents the characteristics of an organism isolated from fresh haddock and allocated to the genus *Aeromonas* which converts L-tyrosine to phenol.

**MATERIALS AND METHODS**

Cultures. *Aeromonas hydrophila* NCMB 37 and *A. punctata* NCMB 76 were obtained from the Torry Research Station, Aberdeen, Scotland. *A. salmonicida* (isolated from brook trout), *A. liquefaciens* (isolated from brook trout), and *A. liquefaciens* (isolated from lamprey) were obtained from L. A. McDermott, University of Guelph. *Aeromonas* 3.35 (isolated from brook trout) was obtained from G. L. Bullock, Eastern Fish Disease Laboratory, Leetown, W.Va.

Biochemical and cytological tests. Throughout this study, nutrient broth (Difco) and nutrient agar (Difco) contained 0.5% NaCl. All incubation was at 20°C unless otherwise specified. Gas chromatography was performed on a 12-foot (ca. 3.7 m) Carbowax 20 M column at 140°C (2). Other tests and stains were performed as previously described (5).

Preparation of fish juice. Fish juice was prepared by blending fresh haddock tissue for 5 min with an equal weight of distilled water containing 0.5% NaCl. The resulting slurry was centrifuged at 6,000 rpm for 20 min, and the supernatant was sterilized and transferred to sterile tubes.

Preparation of resting cell suspensions. Cells were grown in 250-ml volumes of Trypticase soy broth without dextrose (BBL) at 20°C in 1-liter Erlenmeyer flasks with reciprocal agitation and were harvested, washed, and microscopically enumerated as previously described (2).

Assay for phenol produced by resting cells and in fish juice cultures. Phenol production by resting cells was assayed by first transferring 8.0 ml of the reaction mixture to an ice-chilled 250-ml flask; after 30 s the reaction mixture was transferred to a prechilled centrifuge tube and centrifuged at 10,000 rpm for 5 min at 2°C. An aliquot of the cell-free reaction mixture (3.0 ml) was then extracted with 3.0 ml of diethyl ether, and 1 μl of the extract was injected into the chromatographic column. Fish juice cultures were clarified by centrifugation as above, 5.0 ml was extracted with an equal volume of diethyl ether, and 1 ml of the extract was injected into the chromatographic column.

Aminoantipyrine assay for phenol in tissue. Fish tissue (25 g) was blended with 75 ml of distilled water for 2 min and centrifuged at 2°C for 10 min at 10,000 rpm. An aliquot of the clear supernatant (2.0 ml) was diluted to 100 ml and assayed for phenol by the modified aminoantipyrine method (1).

**RESULTS**

Effect of time and temperature on the production of phenol in haddock. Preliminary studies indicated the presence of phenol in stale samples of haddock, cod, and flounder. With a haddock fillet stored at 2°C, free phenol was not detected until after a storage period of 8 days (Table 1), whereas at a storage temperature of 20°C phenol production was detected on day 4 of storage.

Isolation of phenol-producing organisms. When tubes of fish juice were inoculated with 1 loopful of the mixed microbial flora directly from a stale haddock fillet and with fishery isolates of *Pseudomonas putrefaciens*, *P. fluorescens*, and achromobacters, only the mixed culture from the stale haddock produced phenol. Numerous attempts to isolate a phenol-producing organism directly from stale haddock using nutrient agar, Trypticase soy agar, and peptone iron agar failed.

Several methods of selective enrichment were attempted for isolation of the phenol-producing organisms from stale haddock, including incubation at various temperatures and the addition

---

1Paper no. 1035, Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst.
2Present address: Poultry Science Department, Mississippi State University, State College, Miss. 39762.
Table 1. Production of phenol in haddock fillets stored at 2 and 20°C

| Days of storage | Phenol (mmol/kg of tissue)* |
|-----------------|-----------------------------|
|                 | 2°C                        | 20°C                      |
| 0               | <0.001*                    | <0.001*                   |
| 2               | <0.001*                    | <0.001*                   |
| 4               | <0.001*                    | 0.028                     |
| 8               | 0.078                      | 0.660                     |
| 12              | 0.168                      |                           |
| 17              | 0.222                      |                           |

* Concentration of phenol was determined with the use of the modified aminomontpyrine method (1).

Aeromonas sp. ATCC 29063. The optimum growth temperature was found to be 30°C, the maximum 40°C, with no growth observed at 45°C; sustained growth occurred at 2°C (Table 2). From these results, it can be concluded that the strain has a slower rate of growth at 2°C than reported for most facultative psychrophiles on fish (4). This slower growth rate at 2°C most probably accounts for a low percentage of this organism at the time of spoilage and the failure to isolate the organism from stale fish.

Metabolic origin of phenol. The ability of Aeromonas sp. ATCC 29063 to grow and produce phenol in 0.5% vitamin-free casein hydrolysate suggested the phenol precursor to be an aromatic amino acid. Resting cells were found to produce phenol directly from L-tyrosine (Fig. 2) but not from L-phenylalanine. Tryptophane was not studied since it is absent from VFCH. Resting cell suspensions of A. hydrophila

![Graph](image_url)

**Fig. 1. Gas chromatogram of a 4-day-old culture of Aeromonas sp. incubated at 20°C in 0.5% vitamin-free casein hydrolysate. Uninoculated broth served as the blank. Injection volume was 3 μl. Attenuation ×20.**

Table 2. The generation time of Aeromonas sp. at different incubation temperatures

| Incubation temp (C) | Generation time (min) |
|---------------------|-----------------------|
| 2                   | 66                    |
| 20                  | 88                    |
| 25                  | 79                    |
| 30                  | 52                    |
| 35                  | 46                    |
| 40                  | 61                    |
| 45                  | 0                     |

* Cultures were grown in 250-ml Erlenmeyer flasks containing 50 ml of Trypticase soy broth without glucose and incubated with reciprocal agitation.
NCMB 37, A. punctata NCMB 76, A. salmonicida, A. liquefaciens, and Aeromonas 3.35 failed to produce chromatographically detectable phenol from L-tyrosine.

DISCUSSION

We have previously shown (3) that the concentration of phenol in fish tissue increases during refrigerated storage of haddock and is associated with an increase in the bacterial population. An earlier study dealing with the production of phenethyl alcohol by fishery achromobacters failed to detect phenol production by commonly encountered fishery isolates belonging to the genera Pseudomonas and Achromobacter (2). From this earlier study a priori reasoning led to the conclusion that phenol-producing organisms on haddock constituted a minority of the total bacterial population at the time of refrigerated spoilage. Our repeated inability to isolate phenol-producing organisms from stale haddock has confirmed this earlier conclusion. The growth rate of Aeromonas sp. strain ATCC 29063 at 2 C indicates that the organism grows notably slower than vigorous-spoilage isolates belonging to the genera Pseudomonas and Achromobacter (4) and hence cannot be expected to be readily isolated from stale fish.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Research grant FD00153-09 from the Food and Drug Administration and by Massachusetts Agricultural Experiment Station project no. 194.

LITERATURE CITED

1. American Public Health Association. 1960. Standard methods for the examination of water and waste water, 11th ed. American Public Health Association, Inc., New York.
2. Chen, T. C., and R. E. Levin. 1974. Taxonomic significance of phenethyl alcohol production by Achromobacter isolates from fishery sources. Appl. Microbiol. 28:681-687.
3. Chen, T. C., W. W. Nawar, and R. E. Levin. 1974. Identification of major high boiling volatile compounds produced during refrigerated storage of haddock fillets. Appl. Microbiol. 28:679-680.
4. Levin, R. E. 1967. The effectiveness of EDTA as a fish preservative. J. Milk Food Technol. 30:277-283.
5. Rosen, A., and R. E. Levin. 1970. Vibrios from fish pen slime which mimic Escherichia coli on violet red bile agar. Appl. Microbiol. 20:107-112.