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# Detection and Incidence of Specific Species of Spoilage Bacteria on Fish

## II. Relative Incidence of *Pseudomonas putrefaciens* and Fluorescent Pseudomonads on Haddock Fillets

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*Pseudomonas putrefaciens* has been found to constitute one of the major species of spoilage bacteria on haddock fillets. The initial population of this organism on fillets of high bacterial quality is uniformly below 4% and most frequently no greater than 1%. During refrigerated storage, the organism increases at a more rapid rate than the total psychrophilic population, comprising 50 to 90% of the total population when the total count exceeds $10^6$/g of tissue. Fluorescent pseudomonads were shown to constitute a second group of predominant pseudomonads constituting up to 19.3% of the total population after 8 days of refrigerated storage. Of a total of 45 fluorescent pseudomonads isolated from haddock fillets, 14 (31.1%) were found to be potent fish spoilers. The use of a soft-agar-gelatin plating technique showed a parallel increase of proteolytic organisms with total count indicating that proteolytic organisms other than *P. putrefaciens* and fluorescent pseudomonads increase at a slower rate than these two groups.

The major spoilage bacteria of fish have been shown by numerous workers to consist primarily of members of the genera *Pseudomonas* and *Achromobacter* (1, 2, 4, 6, 7). Studies to determine the predominant bacterial spoilage species on North American fish before and during refrigerated storage are notably lacking. Previous studies (3, 5, 8) have implicated *P. putrefaciens* as one of the predominant spoilage pseudomonads on North American white fish. The direct enumeration of *P. putrefaciens* from fishery products on the basis of pink or salmon pigmentation of colonies is not reliable because the intensity of pigmentation varies depending on whether colonies are above or below the agar surface. In addition, some isolates produce only a slightly detectable amount of pigmentation which varies with the salt content and type of agar medium, whereas those isolates of *P. putrefaciens* producing a deep pink coloration can easily be mistaken for members of the genus *Flavobacterium*. Previous studies (8) indicated the suitability of Peptone Iron Agar (Difco) as a direct differential plating medium for detection of *P. putrefaciens* owing to the formation of black colonies resulting from FeS formation and the notable absence of members of the genus *Proteus* on haddock. The specific enumeration of this organism and its numerical relationship to other members of the spoilage flora of haddock have not been reported previously.

### MATERIALS AND METHODS

*Differential enumeration of bacterial flora.* The bacterial population of haddock fillets was determined, as previously described (8), by the use of Peptone Iron Agar for detection of *P. putrefaciens* and soft-agar-gelatin for detection of proteolytic organisms. Detection of fluorescent pseudomonads was accomplished by spreading with a sterile glass rod 0.1 ml of broth dilutions of blended fish tissue onto the surface of Pseudomonas Agar F (Difco) plates which were incubated at 20 C for 3 days. Fluorescent colonies were detected with a Raymaster lamp using a type B black Raymaster ultraviolet tube (George M. Gates & Co., Long Island, N.Y.) with peak energy at 360 nm.

*Spoilage of fish tissue by pure cultures of fluorescent pseudomonads.* Nutrient Agar (Difco) plates containing 0.5% NaCl were inoculated by uniformly spreading 0.2 ml of individual broth cultures onto the agar surfaces followed by incubation at 20 C for 2 days. The skin from high quality haddock was completely removed without attempting to obtain sterile fish tissue. Approximately 50-g pieces were sliced off, smeared onto the surface of the pregrown plate cultures, and then wrapped in polyethylene bags. This technique is predicated on the consistent observations that hand filleting of haddock does not yield more than $5 \times 10^5$ bacteria/inch$^2$ of exposed surface tissue and that the heavy inoculum applied to the tissue will result in rapid spoilage by potent spoilage cultures, in

contrast to slower spoilage of tissue inoculated with nonspoilage cultures and uninoculated control tissue.

## RESULTS AND DISCUSSION

*Enumeration of proteolytic and $H_2S$-producing organisms on haddock.* Individual haddock fillets of high quality, procured the same day they were filleted, were used in detecting the relative increase in numbers of proteolytic and $H_2S$-producing organisms when the fillets were stored at 1 C. Results of one set of data are tabulated in Table 1 and are shown graphically in Fig. 1. With this fillet, the black colonies of *P. putrefaciens* were observed to increase at a more rapid rate than did the total count during the first 2 days of refrigerated storage, starting with 0.6% of the total count on the first day and reaching 13% of the total count on the eighth day. Results from a second fillet procured at a different time are tabulated in Table 2 and shown graphically in Fig. 2. The initial total count was $3.2 \times 10^5$/g, 27.4% of which were proteolytic organisms and 3.4% of which were $H_2S$-producing *P. putrefaciens*. After storage for 10 days at 1 C, the total count was $2.3 \times 10^8$, 40.5% of which were proteolytic organisms and 29.6% of which were $H_2S$-producing *P. putrefaciens*. The more rapid rate of increase of *P. putrefaciens* than that of the total count and this organism's eventual increase to 29.6% of the psychrophilic flora at the time of refrigerated spoilage indicate that this organism is one of the major and predominant spoilage species. The initially low percentage of *P. putrefaciens* confirms the originally high bacterial quality of the fillets.

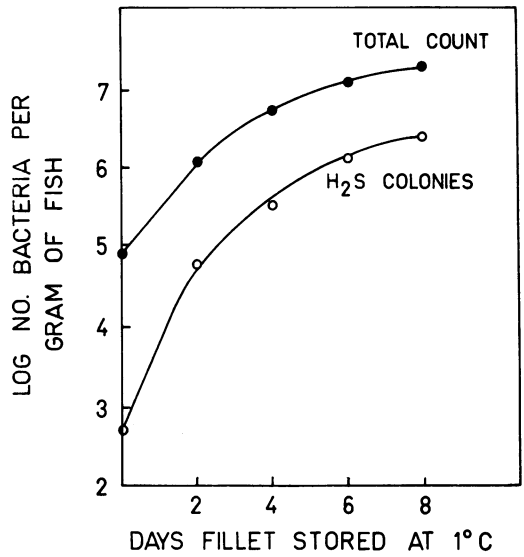


FIG. 1. *Increase in total bacterial population and $H_2S$-producing P. putrefaciens on a haddock fillet stored at 1 C. All plates were incubated at 20 C for 4 days.*

TABLE 1. *Relationship between increase in $H_2S$-producing colonies of P. putrefaciens and total count on a haddock fillet during storage at 1 C*[a]

| No. of days fillet stored at 1 C | Total count per gram of fish | $H_2S$ colonies per gram of fish | $H_2S$ colonies |
|---|---|---|---|
| | | | % |
| 0 | $8.1 \times 10^4$ | $5.0 \times 10^2$ | 0.6 |
| 2 | $1.2 \times 10^6$ | $6.5 \times 10^4$ | 5.0 |
| 4 | $5.5 \times 10^6$ | $3.5 \times 10^5$ | 6.4 |
| 6 | $1.3 \times 10^7$ | $1.3 \times 10^6$ | 10.0 |
| 8 | $2.0 \times 10^7$ | $2.6 \times 10^6$ | 13.0 |

[a] Data shown graphically in Fig. 1.

*Relationship of P. putrefaciens and fluorescent pseudomonads to total count.* This study was performed entirely on a single haddock fillet. The initial total count was $2.85 \times 10^5$/g, with

TABLE 2. *Relationship between increase in proteolytic, $H_2S$-producing colonies of P. putrefaciens and total count on a haddock fillet during storage at 1 C*[a]

| No. of days fillet stored at 1 C | Soft agar-gelatin overlay | | | Peptone iron agar | | |
|---|---|---|---|---|---|---|
| | Total count | Proteolytic count | Proteolytic colonies | Total count | $H_2S$ colonies | $H_2S$-producing |
| | | | % | | | % |
| 0 | $3.1 \times 10^5$ | $8.5 \times 10^4$ | 27.4 | $3.2 \times 10^5$ | $1.1 \times 10^4$ | 3.4 |
| 2 | $2.4 \times 10^6$ | $6.0 \times 10^5$ | 25.0 | $3.8 \times 10^6$ | $5.8 \times 10^5$ | 15.3 |
| 4 | $1.9 \times 10^7$ | $6.5 \times 10^6$ | 34.2 | $1.8 \times 10^7$ | $4.7 \times 10^6$ | 26.0 |
| 6 | $7.3 \times 10^7$ | $3.0 \times 10^7$ | 41.2 | $6.7 \times 10^7$ | $1.5 \times 10^7$ | 22.4 |
| 8 | $1.2 \times 10^8$ | $4.5 \times 10^7$ | 37.5 | $1.2 \times 10^8$ | $4.1 \times 10^7$ | 34.2 |
| 10 | $2.1 \times 10^8$ | $8.5 \times 10^7$ | 40.5 | $2.5 \times 10^8$ | $7.4 \times 10^7$ | 29.6 |

[a] One haddock fillet was obtained fresh with an initial low count, stored at 1 C, and counts were performed every 2 days for a 10-day period. All plates were incubated at 20 C for 3 days. Data presented graphically in Fig. 2.

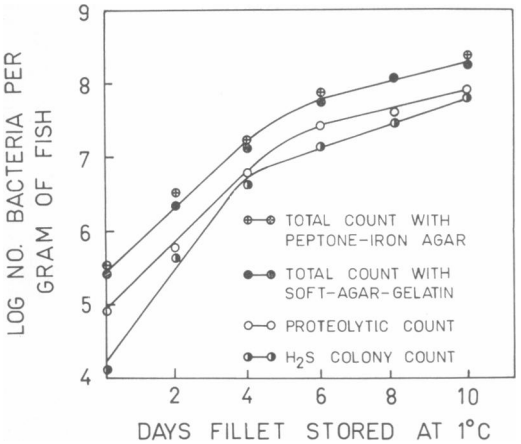


FIG. 2. *Increase in total count, proteolytic organisms, and $H_2S$-producing P. putrefaciens on a haddock fillet stored at 1 C. All plates were incubated at 20 C.*

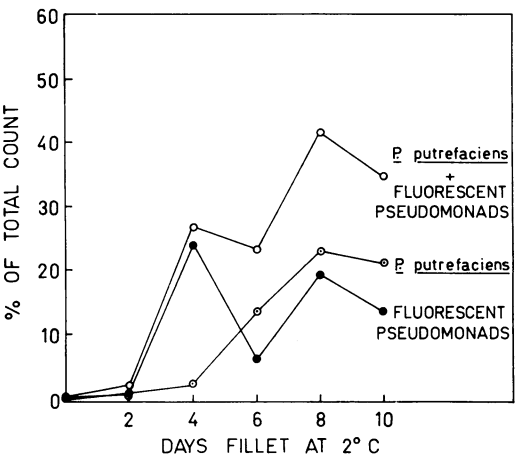


FIG. 3. *Relative proportion of P. putrefaciens and fluorescent pseudomonads to total count on a haddock fillet stored at 2 C.*

0.44% *P. putrefaciens* and 0.35% fluorescent pseudomonads. By the eighth day of refrigerated storage, the total count was $4.1 \times 10^8$/g, of which *P. putrefaciens* accounted for 22.9% and fluorescent pseudomonads, 19.3% (Fig. 3). The rapid and dramatic rise of both these psychrophilic groups in comparison to the total count is shown in Fig. 4.

*Spoilage of fish tissue by fluorescent pseudomonads.* Out of a total of 45 fluorescent cultures isolated from haddock, 14 (31.1%) produced spoilage odors of strong intensity on haddock at 2 C (Table 3). Castell et al. (3) reported the failure of green fluorescent pseudomonads to reduce trimethylamine oxide in contrast to its reduction by *P. putrefaciens*. Lerke et al. (4) used

TABLE 3. *Organoleptic spoilage of fish tissue by fluorescent pseudomonads isolated from haddock fillets*

| Number of cultures | Intensity of ammonia formed | Intensity of putrid odor |
|---|---|---|
| 17 | — | — |
| 5 | + | — |
| 5 | ++ | — |
| 2 | +++ | — |
| 1 | — | + |
| 2 | — | ++ |
| 5 | — | +++ |
| 1 | — | ++++ |
| 1 | + | + |
| 1 | ++ | +++ |
| 3 | +++ | ++ |
| 1 | ++++ | +++ |
| 1 | ++++ | ++++ |

[a] Based on odor of inoculated fish tissue in comparison to uninoculated tissue after storage for 6 days at 2 C. Symbols: (—), no reaction; (+), slight reaction; (++), moderate; (+++), strong; and (++++), very strong.

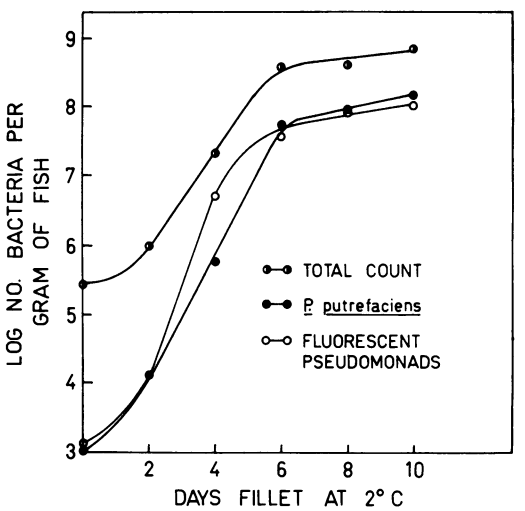


FIG. 4. *Relative increase of total count, P. putrefaciens, and fluorescent pseudomonads on a haddock fillet stored at 2 C.*

the production of volatile reducing substances and trimethylamine in sterile fish juice as an indication of the spoilage ability of 70 fluorescent cultures and were able to designate only two as spoilers. Shewan et al. (7) concluded from the relatively low percentage of detectable fluorescent pseudomonads on iced cod that the spoilage odors of white fish result from the action of non-pigmented pseudomonads. Castell (1), in a tabulation of 11 bacterial types, listed *P. fluo-*

TABLE 4. *Relationship between total count and percentage of P. putrefaciens on haddock fillets*[a]

| Total count | No. of fillets | *P. putrefaciens* (%) | | | |
|---|---|---|---|---|---|
| | | < 1 | 1 to 10 | 10 to 50 | 50 to 100 |
| $10^4$ to $10^5$ | 3 | 2 | 1 | 0 | 0 |
| $10^5$ to $10^6$ | 20 | 2 | 12 | 5 | 1 |
| $10^6$ to $10^7$ | 23 | 0 | 0 | 16 | 7 |
| $>10^7$ | 5 | 0 | 1 | 0 | 4 |

[a] All fillets were obtained randomly from retail sources and plated for bacterial counts on the same day they were procured.

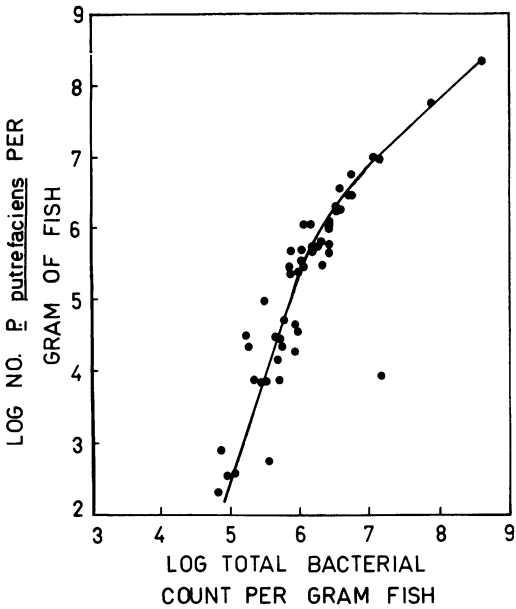


FIG. 5. *Relationship between log of number of P. putrefaciens and log of total bacterial population per gram of fish. All fillets were obtained randomly from retail sources and plated for bacterial counts on the same day they were procured.*

*rescens* as being capable of producing a putrid odor on cod fillets. With the exception of the last report, the spoilage ability of fluorescent pseudomonads appears to be somewhat discounted and is not consistent with our findings.

*Pseudomonas putrefaciens as an indicator of bacterial quality of haddock fillets.* With Peptone Iron Agar as an indicator medium for detecting $H_2S$-producing *P. putrefaciens* and total counts, 51 haddock fillets obtained randomly from 13 retail sources were assessed for bacterial content (Table 4). Of the 3 fillets with total counts below $10^5$/g, two had less than 1% *P. putrefaciens* and one had 1%. Of the 20 fillets with total counts between $10^5$ and $10^6$/g, 14 had less than 10% *P. putrefaciens*, five had between 10 and 50%, and one had 60%. It is particularly significant that out of the 28 fillets with total counts above $10^6$/g, all except one had over 10% *P. putrefaciens* (Table 4). The relationship between the log of the number of *P. putrefaciens* and the log of the total count is further illustrated in Fig. 5. Studies to date indicate that the quantity of *P. putrefaciens* on recently processed fillets is usually below 1% and that it has not as yet been found to exceed 4%. The percentage of *P. putrefaciens*, therefore, offers an additional index of bacterial quality and reflects the time fillets have been held in refrigerated storage.

ACKNOWLEDGMENTS

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