Pseudomonads and Achromobacters in the Spoilage of Irradiated Haddock of Different Preirradiation Quality

R. A. LAYCOCK and L. W. REGIER

Halifax Laboratory, Fisheries Research Board of Canada, Halifax, Nova Scotia

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The effect of initial quality of fish on postirradiation (100 krad) changes in the bacterial flora of haddock fillets during aerobic storage at 3°C has been investigated, with emphasis on the Pseudomonas and Achromobacter groups. The quality was related to the length of time the eviscerated fish had been stored in ice prior to filleting. Increased numbers of organisms, in particular Pseudomonas putrefaciens, were found initially on fillets cut from older fish. Pseudomonads were reduced by 2 to 3 log orders by irradiation, and achromobacters and gram-positive isolates predominated in the immediate postirradiation flora. Little difference could be detected in either types or relative proportions of organisms occurring during storage of unirradiated fish of different quality. Pseudomonads outgrew achromobacters and dominated the spoilage flora in all cases. After spoilage, however, the growth rate of pseudomonads declined markedly. In irradiated fish, achromobacters predominated throughout storage. In fish of better initial quality, bacterial numbers were 1 to 2 log orders higher at spoilage than in their unirradiated counterparts and in the poorer quality of irradiated samples. The increased number of organisms was accompanied by a radical change in the character of the predominant achromobacters. Pseudomonads were found to increase in numbers during storage of irradiated fish, in particular in poorer quality fish on which they were initially present in higher numbers. Detection of pseudomonads, even when present in high numbers, was found to be limited by the identification techniques normally used.

Low level gamma irradiation has been shown by many workers to extend successfully the refrigerated storage life of fresh fish (23). This extended storage life is a direct result of changes both quantitative and qualitative in the microbial flora of fish. A dose of 100 krad has usually been found to bring about a reduction in bacterial numbers of between 1 and 2 log orders, and pseudomonads, which constitute the major spoilage organisms of untreated fish (20), are generally found to be replaced as the principal component of the spoilage flora of irradiated fish by Achromobacter in aerobically stored fish (11, 16) and by lactobacilli in vacuum-packaged fish (11, 15).

The quality of the fillets used in the investigation may be another factor affecting composition of the postirradiation flora. It is at the time of filleting that the muscle tissue receives its initial load of microbial contaminants. The level and type of contamination and the condition of the muscle tissue itself, i.e., the nature and amounts of substrates available for microbial attack, may all be expected to affect the numbers and kinds of bacteria developing during storage.

In the present investigation, the effect of initial quality on the bacterial flora of haddock fillets given 100-krad irradiation has been studied with particular emphasis on the fate of the “normal” spoilage flora, pseudomonads and achromobacters, during aerobic storage at 3°C.

MATERIALS AND METHODS

The haddock (Melanogrammus aeglefinus) used in the experiments were caught by side trawler and filleted at local processing plants after 2, 5, and 9 days in ice. Henceforth, they will be referred to as 2-, 5-, and 9-day fish. They were chosen to represent good, medium, and poor quality fish arriving at the plant. Because days in ice cannot automatically be related to quality (4), they were also graded by Department of Fisheries inspectors (Table 1). The 9-day fish would not normally have appeared on the fresh fish market. However, it is by no means certain that such fish are always excluded, and they were included in the experiments for the sake of completion. After filleting on a regular processing line, the fillets were

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TABLE 1. Quality of eviscerated haddock after different periods of storage in ice

| Time held in ice prior to filleting (days) | Grade | Remarks |
|-------------------------------------------|-------|---------|
| 2                                         | 1     | Premium |
| 5                                         | 1     | Borderline grade 2 |
| 9                                         | 2     | Low grade 2 |

sealed singly in 1-mil polyethylene bags prior to transportation back to the laboratory, where half were given 100 krad of irradiation in a Gammaxcell 220. Samples were taken for bacteriological analysis immediately after irradiation. Further bacteriological (quantitative and qualitative) and sensory evaluations were made during storage at 3 C.

Quantitative bacteriological analysis. Five fillets were taken for each evaluation and blended with an equal volume of cold, sterile, 0.1% peptone solution in a sterile 1-gal blender. A 20-g subsample was serially diluted in 0.1% peptone, and three appropriate dilutions were plated on Trypticase Glucose Extract Agar (BBL) with added 0.5% peptone and 0.5% sodium chloride. Three replicates were plated on each dilution, and plates were counted after 5 days at 20 C.

Qualitative bacteriological analysis. Organisms to be identified were systematically isolated from countable dilution plates, as many organisms as possible being taken from each plate and a usual total of about 100 isolates being taken from each treatment. A multiple replication system similar to those described previously (18) was used in the transfer of isolates to various media. Isolates were grown in a liquid medium (similar to that used in primary isolation but without agar) in racks of 20 tubes. Transfers to solid media were made with a multiple Pasteur pipette inoculator, and transfers to liquid media were made by using a multi-needle inoculator. Isolates were characterized by the following tests: Gram stain; morphology; pigmentation, including fluorescent pigment production, on the medium of King, Ward, and Raney (8); oxidase activity (9); metabolism of glucose (7) and arginine (24); sensitivity to penicillin, 2.5 IU/ml, and oxytetracycline, 10 µg/ml; growth on MacConkey agar, on phenyl ethyl alcohol agar, and on Staphylococcus 110 medium; gelatin liquefaction and production of trimethylamine oxide, from trimethylamine oxide, by using a modification of the method of Wood and Baird (26). Gram stains were made on 24-hr cultures grown on the isolation agar. A modification of the procedure with n-propanol (2) was used. All reagents were in coplin jars, and racks of 25 slides, each containing eight heat-fixed smears (200 in all), were stained at one time, excess water being removed from the racks where necessary with absorbent paper. This procedure proved very satisfactory in practice. The oxidase test was also performed on the same plates. A reagent-soaked filter paper (7 cm) was pressed over the surface of the plate. Positive reactions occurred within 10 sec, the colonies being identified by their position on the plate.

Antibiotic sensitivities were determined by incorporating the antibiotic into isolation medium just prior to pouring the plates. Organisms were regarded as sensitive if they showed any reduction in growth. All tests were performed in duplicate, and doubtful ones were repeated. Motility was checked under phase-contrast microscopy, and flagella were stained by the method of Leifson (10) and, periodically, on selected isolates by electron microscopy. Pseudomonads were grouped by the method of Shewan et al. (21), with the exception of Pseudomonas putrefaciens, the taxonomic status of which is in doubt (1).

The achronobacter group is also in a state of taxonomic flux at present. For ease of comparison with other work and because the published reclassifications of the group are somewhat contradictory, we have continued to refer to these organisms as Achromobacter, by the description of Shewan et al. (21), but reference is made to their possible positions in other classifications.

Sensory evaluation. Sensory evaluations of the fillets at various periods during storage were carried out by taste panels composed of 10 people. For each taste panel assessment, two fillets of each treatment plus two frozen control fillets were baked in foil-covered pans at 260 C. Panels were asked to assess the coded samples for odor and flavor, and results were scored on a 10-point scale, on which 4 was taken as the limit of acceptability (based on Can. Dept. Agr. Publ. no. 1284, 1967.)

RESULTS AND DISCUSSION

Initial composition of the bacterial flora. The composition of the bacterial flora of 2- and 9-day fish immediately after filleting is shown in Tables 3 to 5, respectively. In each case, the flora can be seen to be dominated by the gram-negative, psychrophilic achronobacters and pseudomonads. Other organisms forming a small proportion of isolates on fillets from fish of all three ages were flavobacteria, micrococci, coryneforms, and vibrios. Bacillus subtilis was also isolated from 2- and 5-day fish, its presence being considered due to the conditions which exist in certain of the older filleting plants.

The total bacterial numbers initially found on the fillets are shown in Table 2, together with the calculated numbers of the predominant groups of organisms. In spite of the effect which must be exerted on the fillet microflora by the general contamination in the plant, it is possible (Table 2) to detect certain quantitative changes in the bacterial flora which may be attributed to the length of time the fish had spent in ice prior to filleting. Total bacterial numbers were found to be higher on fillets from older fish. Isolates showing the greatest numerical increases were P. putrefaciens and Pseudomonas II, the numbers of which were found to be over one log order higher on 9-day fish than on 2-day fish. P. putre-
faciens has been found to be a particularly potent spoilage organism on North American whitefish (5), and members of *Pseudomonas* group II have also been long recognized to be capable of producing the characteristic odors of spoiling fish (19). *Achromobacter* and the fluorescent *Pseudomonas* I were also found in higher numbers on 9-day fish than on 2-day fish. *Achromobacter* is commonly isolated from spoiling fish although its role in spoilage has not been clearly established. One way in which it may contribute to spoilage is by producing textural changes. Electron microscopic study of irradiated beef during low-temperature storage (25) has shown that bacteria producing mucopolysaccharide capsules can cause physical disruption of muscle tissue without concomitant proteolysis. Fluorescent pseudomonads have been found to produce strong spoilage odors on haddock (6).

**Effect of 100-krad irradiation on the bacterial flora.** The change in composition of the bacterial flora immediately after irradiation is shown in Tables 3 to 5. As in the unirradiated fish, *Achromobacter* predominated; pseudomonads, however, were considerably reduced; and gram-positive isolates, in particular *B. subtilis*, formed an increased proportion of the flora. These irradiation-induced changes are similar to those reported by other workers for irradiated sea food (23). Gram-positive isolates, in particular the sporeformers, are more radiation resistant than achromobacters, which in turn are more resistant than pseudomonads. Since only a limited number of isolates, usually about 100, can be identified at each stage in the analysis of a bacterial flora, the detection of such radiation-sensitive organisms as pseudomonads immediately after irradiation depends very much on their proportion relative to the more radiation-resistant organisms in the preirradiation-flora.

It can be seen (Tables 3–5) that the highest proportion of pseudomonads immediately after irradiation was found on the 9-day fish, on which pseudomonads formed 41% of the preirradiation flora and the incidence of gram-positive isolates, particularly *Bacillus*, was low.

### Table 2. Total bacterial counts and calculated numbers of predominant isolates on haddock fillets before and after 100 krad of irradiation

| Time in ice prior to filleting (days) | Irradiation dose (krads) | Log no. of bacteria | Pseudomonas | Achromobacter | Total count |
|--------------------------------------|--------------------------|---------------------|--------------|---------------|-------------|
|                                      |                          |                     | Group I fluorescent | Group II | Group III and IV | |                         |                         |                         |
|                                      |                          |                     | 52rfaciens | 52rfaciens | 52rfaciens |                         |                         |                         |
| 2                                    | 0                        | 3.83                | 3.88        | 3.57        | 3.18        | 4.53                    | 4.88                    |
| 5                                    | 0                        | 3.80                | 4.55        | 3.80        | 4.04        | 4.98                    | 5.20                    |
| 9                                    | 0                        | 4.60                | 5.04        | <3.40       | 4.74        | 5.39                    | 5.70                    |
| 2                                    | 100                      | <1.75               | <1.75       | <1.75       | <1.75       | 3.67                    | 3.95                    |
| 5                                    | 100                      | 2.35                | <2.06       | <2.06       | <2.06       | 4.03                    | 4.35                    |
| 9                                    | 100                      | 2.08                | <1.85       | <2.06       | <2.06       | 3.95                    | 4.08                    |

### Table 3. Percentage composition of the bacterial flora from irradiated (100 krad) and unirradiated haddock, filleted after 2 days in ice, and stored aerobically at 3°C

| Radiation (krads) | Time of storage (days) | No. of isolates identified | Pseudomonas I and II | Pseudomonas III and IV | Pseudomonas parafaciens | Achromobacter | Flavobacterium | Micrococci | Bacillus | Coryneforms | Vibrio |
|-------------------|------------------------|-----------------------------|----------------------|------------------------|------------------------|---------------|----------------|------------|----------|-------------|--------|
| 0                 | 0                      | 200                         | 19                    | 5                      | 2                      | 45            | 15             | 4          | 2        | 4           | 2      |
|                   | 4                      | 100                         | 43                    | 27                     | 19                     | 1             | 2              | 2          | 8        |             |        |
|                   | 8                      | 60                          | 45                    | 42                     | 12                     | 2             | 2              | 1          | 1        |             |        |
|                   | 13                     | 130                         | 50                    | 15                     | 29                     | 1             | 2              | 1          | 1        |             |        |
| 100               | 0                      | 159                         | 12                    | 52                     | 152                    | 15            | 4              | 45         | 3        |             |        |
|                   | 4                      | 37                          | 12                    | 78                     | 6                      | 2             | 2              |            |          |             |        |
|                   | 8                      | 60                          | 3                     | 95                     | 2                      | 2             |                |            |          |             |        |
|                   | 13                     | 144                         | 3                     | 97                     | 2                      | 2             |                |            |          |             |        |
|                   | 18                     | 100                         | 100                   |                        |                        |                |                |            |          |             |        |
Irradiation (100 krad) reduced the total bacterial count by approximately one log order (Table 2). The reduction observed was found to be to some extent dependent on the amount of contamination of the fillets by B. subtilis. Achromobacter was also reduced in numbers by about one log order by 100-krad irradiation. Pseudomonads, which were isolated immediately after irradiation from fillets of 5- and 9-day fish, seem to have been reduced by between 2 to 3 log orders. When no pseudomonads were identified, the maximum number of pseudomonads which could be present before one isolation might be expected was calculated by dividing the total numbers of bacteria by the total numbers of identified isolates (Table 2). From these figures it can be seen that, in those instances where pseudomonads were not isolated immediately after irradiation, reductions in numbers varying between 2.5 log orders for Pseudomonas II on 5-day fish and 1.5 log orders for P. putrefaciens on 2-day fish would have been sufficient to place these organisms below the level at which they could be expected to be detected. Other evidence, to be described, indicated that P. putrefaciens was, in fact, present in numbers of the order of 10^3 per g on 2-day fish and 10^2 per g on 5-day fish immediately after irradiation. These numbers are consistent also with a reduction in numbers of 2 to 3 log orders on irradiation.

**Changes in the bacterial flora of unirradiated haddock fillets during aerobic storage at 3 C.** The percentage composition of the bacterial flora of unirradiated fish during storage is shown in Tables 3 to 5. Pseudomonas I and II, P. putrefaciens, and to a lesser extent Achromobacter predominated throughout storage on all fillets. The storage life of the fillets was found by sensory evaluation to be 9 days for the 2-day fish, 7 days for the 5-day fish, and 5 days for the 9-day fish. The relative proportions of pseudomonads and Achromobacter in the flora can be seen to change as spoilage progresses (see tables). Up to the time of sensory rejection, pseudomonads formed

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**Table 4. Percentage comparison of the bacterial flora from irradiated (100 krad) and unirradiated haddock, filleted after 5 days in ice, and stored aerobically at 3 C**

| Radiation (krads) | Time of storage (days) | No. of isolates identified | Pseudomonas | Achromobacter | Flavobacterium | Micrococci | Bacillus | Coryne-forms | Vibrio |
|------------------|------------------------|---------------------------|-------------|---------------|---------------|------------|----------|-------------|--------|
|                  |                        |                           | I and II    | III and IV    | p.tre-    |            |          |            |        |
| 0                | 0                      | 200                       | 11          | 2             | 7             | 60         | 3        | 6           | 2      | 8       | 1      |
|                  | 4                      | 100                       | 31          | 37            | 28            | 1          |          | 2           |        |         |        |
|                  | 8                      | 58                        | 35          | 43            | 21            |            |          |              |        |         |        |
|                  | 13                     | 170                       | 28          | 35            | 34            | 1          |          |              |        |         |        |
| 100              | 0                      | 196                       | 1           |               | 53            | 1          | 12       | 33          |        |         |        |
|                  | 4                      | 40                        |             |               | 58            | 2          | 40       |              |        |         |        |
|                  | 8                      | 40                        |             |               | 98            |            |          |              |        |         |        |
|                  | 13                     | 69                        |             |               | 100           |            |          |              |        |         |        |
|                  | 18                     | 100                       |             |               | 1             | 99         |          |              |        |         |        |

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**Table 5. Percentage comparison of the bacterial flora from irradiated (100 krad) and unirradiated haddock, filleted after 9 days in ice, and stored aerobically at 3 C**

| Radiation (krads) | Time of storage (days) | No. of isolates identified | Pseudomonas | Achromobacter | Flavobacterium | Micrococci | Bacillus | Coryne-forms | Vibrio |
|------------------|------------------------|---------------------------|-------------|---------------|---------------|------------|----------|-------------|--------|
|                  |                        |                           | I and II    | III and IV    | p.tre-    |            |          |            |        |
| 0                | 0                      | 199                       | 30          | 11            | 49            | 2          | 1        | 5           | 1      |
|                  | 4                      | 100                       | 48          | 30            | 22            |            |          | 3           |        |         |        |
|                  | 8                      | 53                        | 53          | 21            | 23            | 3          |          |              |        |         |        |
|                  | 13                     | 60                        | 48          | 20            | 28            |            |          |              |        |         |        |
| 100              | 0                      | 154                       | 4           | 4             | 75            | 13         | 2        | 2           |        |         |        |
|                  | 8                      | 60                        | 0           | 85            |              | 13         |          |              |        |         |        |
|                  | 13                     | 100                       | 8           | 5             | 84            | 1          | 1        |              |        |         |        |
SPOILAGE OF IRRADIATED HADDOCK

an ever increasing proportion of the flora in all unirradiated samples, whereas the proportion of Achromobacter in each case declined steadily. Analyses of the flora several days after the time of sensory rejection showed that the proportion of Achromobacter had increased significantly but the proportion of pseudomonads had decreased. Other organisms forming a small percentage of isolates at various periods during storage were Flavobacterium, coryneforms, Bacillus, and micrococci. Little difference due to the age of the fish prior to filleting could be detected in either the types of organisms occurring or their relative proportions during storage.

The total bacterial counts found during storage of unirradiated fillets are shown in Fig. 1, and the calculated numbers of the predominant isolates are presented in Fig. 2 to 4. Apart from the initially higher numbers of organisms on fillets from older fish, no differences in bacterial numbers attributable to the differences in age of the fish from which the fillets were cut could be detected during storage. The bacterial counts in all cases levelled off at about $10^8$ per g after 8 days of storage. At the times of sensory rejection, however, numbers of bacteria on 2- and 5-day fish were about 1 log order higher ($10^8$ per g) than those on 9-day fish.

**Fig. 1.** Change in bacterial numbers during aerobic storage at $3^\circ$C of irradiated haddock fillets held 2, 5, and 9 days in ice prior to filleting.

**Fig. 2.** Change in numbers of Achromobacter during aerobic storage at $3^\circ$C of irradiated haddock fillets held 2, 5, and 9 days in ice prior to filleting.
During storage prior to sensory rejection, P. putrefaciens (Fig. 3) and Pseudomonas I and II (Fig. 4) grew very rapidly, whereas Achromobacter increased in numbers at a lower rate. The most rapid rates of increase observed for pseudomonads were on the fresh 2-day fish which initially had the lowest numbers of these organisms. After the time of sensory rejection, the rate of increase of pseudomonads fell off, very sharply in 2- and 5-day fish and less sharply in the 9-day fish. The increase in the relative proportion of Achromobacter in the flora during this period noted earlier can in fact be attributed to the decline in the growth rate of pseudomonads.

Changes in the bacterial flora of irradiated haddock fillets during aerobic storage at 3°C. The percentage composition of the bacterial flora of irradiated fish during storage is shown in Tables 3 to 5. Achromobacter predominated in all samples throughout storage. The mesophilic B. subtilis, which was present initially in high proportions on 2- and 5-day fish, formed only a small proportion of the isolates on continued storage, being unable to compete with the psychrophiles...
at the low temperature of storage. Micrococci which formed 40\% of isolates from 5-day fish after 4 days of storage were otherwise only occasionally isolated. Flavobacterium was found to form a small proportion of isolates from 2- and 5-day fish during the initial stages of storage but was not otherwise encountered. Pseudomonads were isolated sporadically from 2-, 5-, and 9-day fish during storage.

The irradiated samples were judged spoiled by sensory evaluation after about 18 days of storage for 2- and 5-day samples and after about 13 days of storage for 9-day samples. The storage lives of the irradiated fillets can only be given approximately as, unlike the unirradiated fillets, they did not deteriorate rapidly in quality prior to spoilage, thus giving a sharp end point. Irradiated fillets were instead found to decline slowly in quality throughout storage so that the exact onset of spoilage was difficult to determine.

At the time at which the irradiated samples were regarded as spoiled, 100\% of 2-day fish isolates, 99\% of 5-day fish isolates, and 84\% of 9-day fish isolates were *Achromobacter*. One per cent of the 5-day fish isolates and 13\% of the 9-day fish isolates at spoilage were pseudomonads.

The total bacterial count during storage of irradiated fish is shown in Fig. 1, and the calculated numbers of *Achromobacter*, *P. putrefaciens*, and *Pseudomonas* I and II are seen in Fig. 2 to 4, respectively. The total bacterial counts were found to increase logarithmically throughout storage in 2- and 5-day fish and by the time of sensory rejection had reached over $10^8$ per g. In 9-day fish, bacterial numbers had reached $10^8$ per g after 8 days of storage and were still of the same order at the time of sensory rejection—13 days. Bacterial numbers at spoilage were between one and two log orders higher on 2- and 5-day irradiated samples than on similar unirradiated samples. An increased number of bacteria at spoilage in irradiated seafood has been previously reported (23). Numerically, *Achromobacter* (Fig. 2) dominated the postirradiation flora. In 2- and 5-day samples, *Achromobacter* increased logarithmically with no apparent lag throughout the storage period and was responsible for the very high number of organisms encountered at the time of sensory rejection. In 9-day samples, *Achromobacter*, although still the predominant isolate, levelled off in numbers at $10^8$ per g several days before sensory rejection. This contrast between the spoilage of irradiated 9-day fish and that of irradiated 2- and 5-day fish was further emphasized when the characteristics of the *Achromobacter* isolates were examined.

The taxonomy of *Achromobacter* and related gram-negative genera is at present confused, and it is certain that this group of organisms will eventually be reclassified. In a recently proposed classification of the gram-negative cocci (4), presence or absence of oxidase activity, reflecting as it does differences in the cytochrome complement of the organisms, has been given considerable emphasis. On the basis of this classification, oxidase-negative and oxidase-positive achromobacters would be placed in separate genera. Oxidase-negative achromobacters would be placed in the genus *Acinetobacter*, and oxidase-positive achromobacters would possibly be in the genus *Moraxella*.

Table 6 shows the response of our *Achromobacter* isolates to test for oxidase activity and sensitivity to 10 $\mu$g of oxytetracycline per ml. During the first 13 days of storage, in irradiated and unirradiated samples alike, the overwhelm-

| Table 6. Changes in characteristics of achromobacters during storage at 3°C |
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| Time in ice prior to filleting (days) | Irradiation dose (krd) | Time of storage at 3°C (days) | Isolates giving responses indicated* (%) |
| --- | --- | --- | --- |
| 2 | 0 | 0 | 99 |
| 2 | 0 | 4 | 96 |
| 5 | 0 | 13 | 80 |
| 5 | 0 | 4 | 100 |
| 9 | 0 | 13 | 84 |
| 9 | 0 | 4 | 100 |
| 2 | 100 | 0 | 100 |
| 2 | 100 | 4 | 61 |
| 2 | 100 | 13 | 100 |
| 5 | 100 | 0 | 96 |
| 5 | 100 | 4 | 92 |
| 9 | 100 | 0 | 97 |
| 9 | 100 | 8 | 100 |
| 9 | 100 | 13 | 100 |

* Kovac's oxidase reaction, sensitivity to 10 $\mu$g of oxytetracycline per ml.
ing majority of achromobacters were oxidase-positive and oxytetracycline-sensitive, i.e., *Moraxella*-like (Table 6). Irradiated 2- and 5-day samples required a further 5 days of storage before becoming unacceptable to the taste panel. During this period, achromobacters increased in number from $10^3$ per g to well over $10^4$ per g. This increase in numbers was accompanied by a marked change in the character of the *Achromobacter* isolates. Whereas after 13 days of storage all of the *Achromobacter* isolates from irradiated 2- and 5-day fish were oxidase-positive and oxytetracycline-sensitive, after 18 days of storage more than three quarters of the *Achromobacter* isolates were oxidase-negative and oxytetracycline-insensitive and, thus, would be placed in the genus *Acinetobacter*. In addition, these oxidase-negative oxytetracycline-insensitive achromobacters were found to differ from all other *Achromobacter* isolates, both in their colonial morphology and in their consistent reactions to all of the tests used in their characterization, and it is likely that they represented a single species. Thus, the low-level irradiation of good quality fish has been found to permit, as a result of the increased storage life, very large numbers of a group of organisms not normally encountered.

Numbers of *P. putrefaciens* and *Pseudomonas* I and II found during storage of irradiated fish are shown in Fig. 3 and 4, respectively. The red-brown colored colonies of our *P. putrefaciens* isolates were found to be quite characteristic, and the organism could be readily picked out and identified from dilution plates containing thousands of bacteria, when these were available. In this way, *P. putrefaciens* was found to be present immediately after irradiation on 2- and 5-day fish and was also found to be present in numbers of about $10^7$ per g on 2-day fish at the time they were judged unacceptable by the taste panel. *P. putrefaciens* was also present on 5- and 9-day fish at about $10^7$ per g at the time of their sensory rejection (Fig. 3). Thus, *P. putrefaciens* was present in all irradiated fish at the time of sensory rejection. In other studies we have carried out on 100-krad irradiated cod fillets, *P. putrefaciens*, identified by use of an iron-containing medium (22), has been found to be present throughout storage and also to reach a level near $10^7$ per g at spoilage.

*Pseudomonas* I and II (Fig. 4) were not isolated from 2-day fish immediately after irradiation but were isolated after both 4 and 8 days of storage at levels of between $10^4$ and $10^5$ per g. No further isolations were made during continued storage. Apart from one *Pseudomonas* I isolate immediately after irradiation, no isolations of *Pseudomonas* I and II were made from 5-day fish during storage to the time of sensory rejection. However, in 9-day fish, *Pseudomonas* I and II were found to increase from less than $10^8$ per g immediately after irradiation to $10^8$ per g at the time the samples were judged spoiled. *Pseudomonas* I and II could certainly have been present in similar numbers on 2- and 5-day fish at spoilage without ever being isolated.

The fate of pseudomonads on irradiated seafoods is of importance because it has been considered that the elimination of these biochemically active organisms from the spoilage flora may potentiate the outgrowth of organisms of public health significance (particularly those which have a selective advantage on irradiation) through reduced microbial competition combined with a long extended storage life.

It has been stated or implied by many workers that pseudomonads are eliminated from the spoilage flora of irradiated fish by doses as low as 100 krad. Our experiments have indicated that pseudomonads can be present in considerable numbers during the spoilage of 100-krad irradiated fish and yet remain at or below the limit of their detectability by the usual methods.

In contrast to unirradiated haddock, pseudomonads have not been found to outgrow *Achromobacter* in irradiated haddock during storage. Factors which may influence this are the effects of radiation on the bacterial cells and increased competition from the relatively greater numbers of *Achromobacter* after irradiation.

An examination of data obtained in studies on 100-krad irradiated petrale sole (15, 16) also showed that, in aerobically stored samples, *Pseudomonas* failed to outgrow *Achromobacter* and formed only a small proportion of the isolates (2%) after 2 weeks of storage. However, in irradiated vacuum-packaged petrale sole, in which *Achromobacter* was at a selective disadvantage, *Pseudomonas* formed a significant proportion (30%) of the flora after a similar storage period. It is also interesting to note that the 2% of *Pseudomonas* on the aerobically stored fish actually represented a larger number of organisms than the 30% of *Pseudomonas* on the vacuum-packaged fish.

Studies of spoilage parameters such as odor and trimethylamine production provide further evidence of the kind of spoilage taking place in irradiated fish. The results of several studies have indicated that there are differences in the spoilage characteristics of fish given different doses of low level irradiation. Fish and shellfish irradiated at levels of 200 krad and above have usually been found to show very long, extended storage lives. These fish have low trimethylamine values throughout storage and indeterminate end points.
which are characterized by a variety of such odors as sweet, sour, and burnt (13, 17), but not putrid, ammoniacal. It has also been shown (11) that inoculation of such samples with *Pseudomonas* after irradiation results in development of high trimethylamine values and putrid ammoniacal odors. In contrast to fish irradiated at higher doses, spoilage in 100-krad irradiated haddock, like that in unirradiated haddock, was found to be accompanied by the logarithmic increases in trimethylamine values and ammoniacal and putrid odors typical of pseudomonad spoilage. Similar observations have been made for petrale sole (13), crab (14), and haddock (17). Thus, it would appear that, whereas the contribution of pseudomonads to the spoilage of irradiated fish is determined largely by the dose to which they are subjected, the detection of pseudomonads in the postirradiation flora, by conventional techniques, is dependent upon their initial inoculum level, the relative proportion of more radiation-resistant organisms in the flora, and the conditions of storage.

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