Effect of Gamma-Irradiation on the Microflora of Rice

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ABSTRACT

Unpolished and polished rice harvested in Japan, and polished rice imported from Spain, were investigated. Chromogenic *Pseudomonas* and fluorescent *Pseudomonas* were the main microflora of unirradiated, unpolished or polished Japanese rice. Of microorganisms found on Spanish rice, 20 to 30% are molds; the rest are chromogenic *Pseudomonas* and fluorescent *Pseudomonas*. The principal microorganisms of rice which are chiefly responsible for rice damage, including molds such as *Penicillium* and *Aspergillus,* can be sterilized with 0.2 to 0.3 Mrad. When rice was irradiated with 0.2 to 1.2 Mrad, red *Pseudomonas* was the main survivor. Radiation-resistant yeasts have been isolated from unpolished rice irradiated with 1 Mrad or more. No increase in the number of microorganisms was observed on irradiated rice packed in a polyethylene pouch after storage for 30 days at 10° and 30°C.

The damage in cereal grains caused by insects or microorganisms is severe in Japan because summer humidity is high enough for mold or bacterial growth on improperly stored rice. Damage is even more severe in imported grains. In our laboratory we have carried out since 1948 a series of studies (1–8) on microflora of normal and damaged rice from Thailand, Burma, China, and Japan. On the basis of the results obtained from such studies, an attempt has been made to prevent microbiological damage in rice by use of gamma-irradiation.

There seems to be no prior study of the microbiological effects of ionizing radiation on rice, although some investigation (9) was made of the disinfestation of rice by such radiation. The present study reports the effects of gamma-irradiation on the microflora and keeping quality of rice.

MATERIALS AND METHODS

Materials

A sample of unpolished Japanese rice of Nakashin 120 species produced in Yamagata Prefecture in 1964, a commercial sample of Japanese polished rice of unknown origin, and a commercial sample of Spanish polished rice were used for the tests. The latter two samples were obtained in January 1965. The work was carried out in 1965.

Procedure for Gamma-Irradiation

The gamma-ray source used was a 1,000-curie cobalt-60 irradiator in the Komagome Branch of the Japan Atomic Energy Research Institute. The dose rate at the irradiation position was 6.8×10^4 rad/hr. as determined by a Fricke dosimeter.

The irradiation method used was as follows: Two rice samples of 200 grains and 1 g. were each sealed in a polyethylene pouch. Fourteen pairs were irradiated with the following doses: 0.01, 0.04, 0.10, 0.17, 0.20, 0.24, 0.30, 0.40, 0.51, 0.61, 0.81, 1.02, and 1.36 Mrad. One pair was kept unirradiated as a control.

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Determination of Sterilization Effect

The sterilization effect was estimated by counting the surviving microorganisms by two methods. A third method was used to identify the different species of microorganisms that survived the irradiation treatment. The three methods are given below.

Method 1. Add 50 ml. of sterile water to 200 grains of irradiated rice. Wash by shaking sufficiently and then dilute the washings in several steps. Take a 0.2-ml. aliquot of each diluted sample and spread it on a nutrient agar plate, and another 0.2 ml. on a potato agar plate. Incubate at 30°C. for 3 days and count the colonies formed. The nutrient agar will count bacteria and the potato agar the yeasts and molds on the surfaces of the rice grains.

Method 2. Crush 1 g. of irradiated rice in a mortar. Add 50 ml. of sterile water and dilute the resultant suspension in several steps. Take a 0.2-ml. aliquot of each diluted sample and spread as in method 1 on nutrient agar and potato agar. Incubate at 30°C. for 3 days and count colonies formed. This method determines the total microorganisms of the rice.

Method 3. Place 5 grains of irradiated rice on each of nutrient agar and potato agar plates. Incubate at 30°C. for 3 days. Observe the change in microflora before and after irradiation.

Effect of Storage Time on Irradiated Rice

About 10 g. of rice containing 14–16% moisture was sealed in a polyethylene pouch, irradiated with the various doses described above, and stored at 10° and 30°C. for 30 days. The residual microorganisms were counted by methods 1 and 2 and compared with the count obtained initially shortly after irradiation.

RESULTS

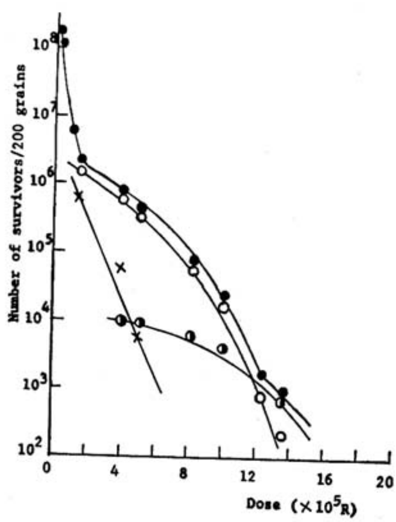
Unpolished Japanese Rice

Iizuka (1,2) has reported that the characteristic microflora of normal, new unpolished rice consists mostly of chromogenic *Pseudomonas* and fluorescent *Pseudomonas;* the rest consists of *Aerobacter, Micrococcus, Brevibacterium,* and molds such as *Helminthosporium* and *Alternaria* which are parasites of the plant.

In this work similar results have been observed in the unirradiated samples of unpolished Japanese rice. We usually found that most of the microflora of unirradiated rice was chromogenic *Pseudomonas* and fluorescent *Pseudomonas,* and the rest included *Aerobacter, Micrococcus, Brevibacterium,* and *Bacillus*. Figure 1 shows the results obtained with method 1 and with nutrient agar plates. The total number of microorganisms on unirradiated rice was about 5×10^5 per grain. In the low-dosage region of 0.01–0.2 Mrad, the total number of survivors decreases rapidly with increasing dosage. Above 0.2 Mrad, the higher the dose, the smaller is the rate of decrease. After a decrease in total number of survivors, the residual microflora consisted of species different from those in the initial unirradiated microflora. The main microorganisms of normal rice, such as chromogenic *Pseudomonas* and fluorescent *pseudomonas,* were inactivated with a dose of 0.2 Mrad or more. At doses of more than 0.2 Mrad, the main microflora consisted of red colony-marked bacteria which are rare in unirradiated rice. In addition to this bacterium some yeasts, *Brevibacterium*, and *Flavobacterium* were also isolated from the irradiated unpolished rice. The red colony-marked bacteria were also isolated from irradiated samples of polished Japanese rice and Spanich rice, as will be seen later. The morphological and physiological nature of this bacterium is like that of the genus *Pseudomonas* (see Chart, p. 510). However, this bacterium is somewhat different from the ordinary species of the genus *Pseudomonas;* it produces pink to reddish water-insoluble pigments and oil drops in its cells, and is radiation-resistant. Hence, in this paper we will call this bacterium "red *Pseudomonas*".

In Fig. 1 are shown the survival curves (plots of numbers of residual microorganisms *vs*. dose) for red *Pseudomonas, Brevibacterium,* and *Flavobacterium,* yeast, and total survivors (the sum of the preceding four species). These microorganisms were taken from the surface of the rice grains. The residual bacteria consist mainly of red *Pseudomonas* for the dose range from 0.2 to 1.1 Mrad (Fig. 1).

When rice was irradiated with a dose of more than 0.6 Mrad, radiation-resistant asporogenous yeasts were also isolated in considerable amount. Above a dose of 1.2 Mrad, yeasts predominated. From unpolished rice irradiated with 0.1 to 0.5 Mrad, *Brevibacterium, Flavobacterium,* and *Rhodotorula* also were isolated. Further, a small number of *Bacillus* was isolated from rice irradiated with less than 0.5 Mrad. The survival curves in Fig. 1 are for 200 grains of irradiated rice counted on nutrient agar. From Fig. 1 the number of microorganisms per one grain of rice before irradiation is estimated as follows:



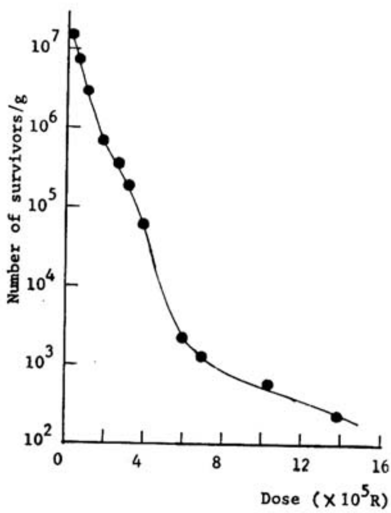


Fig. 1 (left). Survival curves of microflora on grains of unpolished Japanese rice (sample not crushed before incubation). ●, total survivors; ○, red *Pseudomonas;* ×, *Brevibacterium* and *Flavobacterium;* white-and-black symbols indicate yeast.

Fig. 2 (right). Survival curve of microorganisms on and in grains of unpolished Japanese rice (sample crushed before incubation).

Chromogenic *Pseudomonas* + fluorescent *Pseudomonas*	about 5×10^5
Red *Pseudomonas*	about 1×10^4 to 1×10^5
Brevibacterium + *Flavobacterium*	about 1×10^4
Yeast	about 5×10^1

The survival curve of total microorganisms on and in the grains of unpolished Japanese rice from the 1-g. sample is shown in Fig. 2; the changes in the microflora showed behavior similar to that shown in Fig. 1. With method 3, the main surviving microorganisms on the unpolished rice irradiated at more than 0.4 Mrad were red *Pseudomonas* and yeasts. Colonies of these two have been observed on all the grains. When less than 0.1 Mrad was used, many grains were also covered with mold colonies. With the plate method of counting, no molds could be isolated from unirradiated or irradiated rice. The number of bacterial colonies produced on a potato agar plate were usually less than were produced on a nutrient agar plate.

Polished Japanese Rice

The microflora on unirradiated polished Japanese rice consists mainly of chromogenic *Pseudomonas* and fluorescent *Pseudomonas* (the situation is similar to that of unpolished rice). The total number of various microorganisms found was about 2×10^3 per grain. When polished rice was irradiated with more than 0.1 Mrad, one of the main survivors was red *Pseudomonas*. About 16 per grain of red *Pseudomonas* were found on the rice sample irradiated with 0.1 Mrad. A secondary contaminant of rice (6) was also isolated, such as *Bacillus*, about 10 per grain of unirradiated rice. Even when unpolished rice was irradiated with a high dose, radiation-resistant yeast could not be isolated. Mold was not isolated from polished rice even when it had not been irradiated. The data in Fig. 3 were obtained by method 1. Very few microorganisms survived in the high-dose region; e. g., only one per grain at 0.7 Mrad. The color of polished rice was unchanged by irradiation with 0.2 Mrad or less, but was charged markedly from white to pale brown by irradiation with more than 0.5 Mrad.

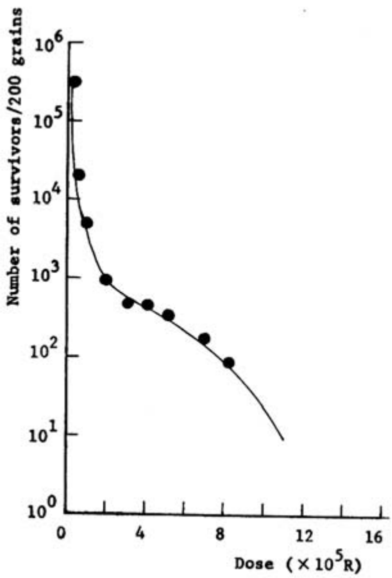
Polished Spanish Rice

The total number of microorganisms found on polished unirradiated Spanish rice is about 1.5×10^2 per grain. From 20 to 30% of the microorganisms on the rice were molds, mainly of genus *Penicillium*. The remainder of the microflora consisted of chromogenic *Pseudomonas*, fluorescent *Pseudomonas*, red *Pseudomonas*, *Bacillus*, etc. Figure 4 shows survival curves obtained with method 1. The total number of microorganisms decreases rapidly with doses up to 0.2 Mrad. Above this, the main survivors were red *Pseudomonas*. All molds were sterilized with less than 0.2 Mrad. No color change was observed with this rice even in the samples irradiated with high doses.

Effect of Storage on Microorganisms on Irradiated Rice

To observe the storage effect, microorganisms were counted by method 1 on rice sealed in a polyethylene pouch, irradiated with gamma-rays, and stored for 30 days at 10° or 30°C.

Survival curves for unpolished Japanese rice, obtained after 30 days'



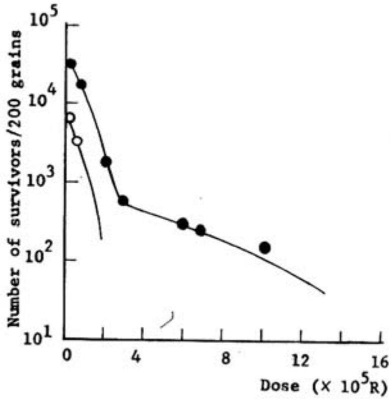


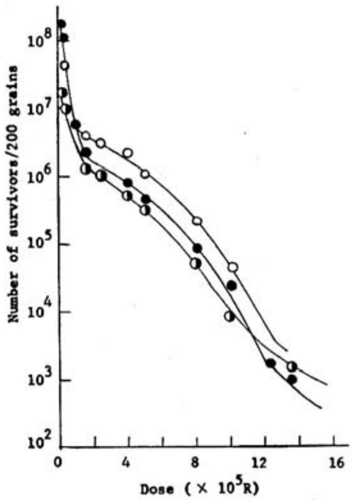
Fig. 3 (left). Survival curve of microorganisms on grains of polished Japanese rice (sample not crushed before incubation).

Fig. 4 (right). Survival curves of microorganisms on grains of polished Spanish rice (sample not crushed before incubation). ●, total survivors; ○, mold.

storage, are shown in Fig. 5. In the sample irradiated with a low dose (0–0.1) and stored at 30°C., chromogenic *Pseudomonas* and fluorescent *Pseudomonas* were much less than observed before storage; red *Pseudomonas* which was able to survive high-dose radiation was just slightly less. The total number of microorganisms is, in spite of the dose difference, less than what is observed before storage (Fig. 5). In contrast, at 10°C. storage, the chromogenic *Pseudomonas* and fluorescent *Pseudomonas* were not changed in number by the storage. The increase in total number of microorganisms in the high-dose region, more than 0.2 Mrad, seems to be due to the increase of red *Pseudomonas*. No change was found in the number of rice-spoilage microorganisms such as molds and *Bacillus*.

As shown in Fig. 6, the total number of microorganisms on polished Japanese rice was independent of the dose when the rice was stored at 10°C. At 30°C. storage, an increase in the number of microorganisms is seen in the high-dose region, in *Bacillus,* etc., but not in red *Pseudomonas*. In the low-dose region, on the other hand, the decrease is mainly in the number of chromogenic *Pseudomonas* and fluorescent *Pseudomonas,* which is different from that for unpolished rice.

With Spanish rice the total number of microorganisms was decreased by storage at 30°C. and the decrease occurred at every dose (Fig. 7). The decrease was mainly in chromogenic *Pseudomonas* and fluorescent *Pseudomonas*. The number of surviving molds which are indigenous to Spanish



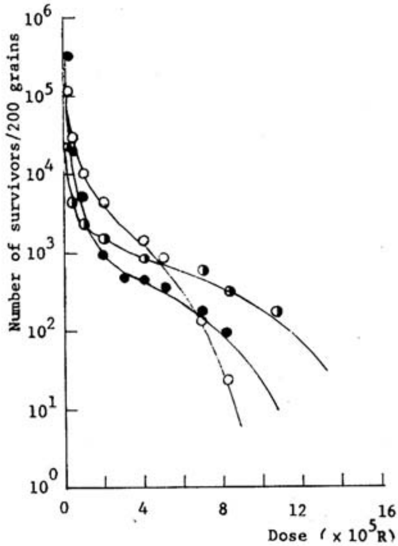


Fig. 5 (left). Survival curves of microorganisms on grains of unpolished Japanese rice, observed after 30-day storage (sample not crushed before incubation). Sample was sealed in a polyethylene pouch, irradiated, and stored. ●, before storage; ○, stored at 10°C.; white-and-black symbols: stored at 30°C.

Fig. 6 (right). Survival curves of microorganisms on grains of polished Japanese rice, observed after 30-day storage (not crushed before incubation). Sample was sealed in a polyethylene pouch, irradiated, and stored. ●, before storage; ○, stored at 10°C.; white-and-black symbols: stored at 30°C.

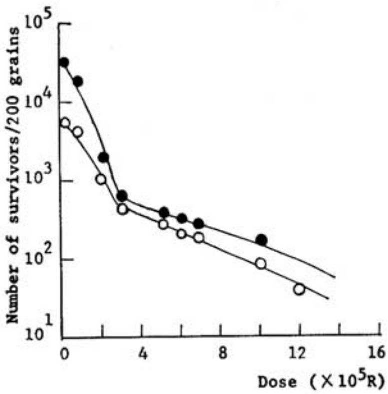


Fig. 7. Survival curves of microorganisms on grains of polished Spanish rice, observed after 30-day storage (sample not crushed before incubation). Sample was sealed in a polyethylene pouch, irradiated, and stored. ●, before storage; ○, stored at 30°C.

rice was little changed by storage. No insect infestation was found in any irradiated sample in the storage experiments.

DISCUSSION

In the unirradiated rice samples, the main microorganisms were chromogenic *Pseudomonas* and fluorescent *Pseudomonas,* both of which have been studied by Iizuka and Komagata (3,4,5). These two groups of species were found to be sensitive to radiation and are sterilized by 0.2 Mrad or less. This dose is for the species "on rice," and is much higher than for the same species of *Pseudomonas fluorescens* "in phosphate buffer solution," the latter dose being about 0.06 Mrad. This means that this species in the dried state "on rice" is much more resistant to radiation. The main microflora surviving after irradiation with more than 0.2 Mrad were red *Pseudomonas* in all rice samples. The vegetative cell of this bacterium was found as radiation-resistant as the endospores, e.g. of *Bacillus subtilis,* but not as heat-resistant as those endospores. This bacterium was sterilized when kept at 80°C. for 10 min. The gamma-ray dose necessary for sterilization of red *Pseudomonas* suspended in 0.066*M* phosphate buffer solution is 0.8 Mrad for the RP-C strain and 3.5 Mrad for the O-1 strain. The percent survival of these strains decreased exponentially against doses.

Since red *Pseudomonas* is supposed to survive for a long period on rice grains as compared with other *Pseudomonas,* the microflora on the grains of old unhulled Japanese rice which had been stored for 4 years was examined after aseptic hulling of the rice. The total number of microorganisms counted is about 3.7×10^2 per one grain: about 50% red *Pseudomonas,* about 25% *Flavobacterium,* and the rest, *Brevibacterium, Bacillus,* and molds. Radiation-resistant asporogenous yeast has been isolated only from unpolished Japanese rice irradiated with more than 0.4 Mrad; the number of survivors for this yeast is only about 50 per one grain, independent of the dose. On the basis of preliminary observations in the taxonomical and radiation-biological studies on red *Pseudomonas* and radiation-resistant yeast, it is evident that these two species are not likely to cause rice spoilage during storage or transportation. *Penicillium,* which is able to damage rice, has been found on Spanish rice. This species is sensitive to radiation, the sterilization dose being 0.2 to 0.3 Mrad, the same as for *Penicillium* and *Aspergillus* suspended in phosphate buffer solution. If the loss of rice grain caused by molds such as *Penicillium* and *Aspergillus* is a serious problem, then radiation pasteurization, e.g. of imported rice, might be promising.

High-density polyethylene film (0.05 mm. thick) was used for packaging the rice samples used in this work. Tripp (10) reported that the radiation dose used for sterilizing food, 4.8 Mrad, produces slight changes in the physical properties of the polyolefin plastics, but 1 Mrad or less shows a negligible effect. Packages made of single films thicker than 0.05 mm. are resistant to penetration of microorganisms even after damage by irradiation. In this investigation, no radiation damage of the film was observed. No rapid increase was observed in the number of microorganisms on the rice packed in polyethylene films and stored at 30°C.

Although both nutrient agar and potato agar were used as media for plating to get reliable counts, all numbers of survivors reported in this paper are from nutrient agar alone, because the number of colonies produced on

CHART OF TAXONOMIC CHARACTERISTICS OF SO-CALLED RED *Pseudomonas;* DESCRIPTION OF ISOLATED STRAINS RP-C AND O-1

Rods: 0.5 to 0.8 by 2.0 to 3.0 μ. Motile with single polar flagella. Gram negative. Contain oil drop in cell.

Nutrient agar colonies: Circular, smooth, convex, entire, glistening, pink to red, translucent.

Nutrient agar slant: Moderate growth, filiform, pink to red, medium unchanged.

Glutamate agar slant: Moderate growth, filiform, red, medium unchanged.

Potato agar slant: Abundant growth, filiform, pink, medium unchanged.

Nutrient broth: Membranous (strain RP-C), sediment (strain O-1), moderately turbidic.

Nutrient gelatin stab: No liquefaction.

Potato plug: Moderate growth, dull reddish brown.

Milk: Unchanged.

Litmus milk: Alkaline, not peptonized, litmus not reduced.

Nitrate reduced to nitrite in succinate-nitrate.

Indole not produced.

Hydrogen sulfide produced.

Starch slightly hydrolyzed.

Acetyl methyl carbinol not produced.

Urease-positive.

Acid but no gas from xylose, glucose, and glycerol; no acid and no gas from sucrose, lactose, and starch in synthetic media.

Glucose, gluconate, 2-keto-gluconate, citrate, succinate, ethanol, methanol, benzoate, salicylate, and *p*-hydroxy benzoate assimilated.

Phenol not assimilated.

Cytochrome oxidase: Positive.

Catalase: Positive.

Optimum temperature: 20° to 32°C.

Aerobe.

Source: Japanese unpolished rice (strain RP-C), Japanese old rice (strain O-1).

potato agar was less and because of the larger dispersion of data from potato agar.

Acknowledgment

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