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Radiation Sensitivity of Some Food Decay Fungi

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몇몇 음식 부식 균류의 방사선 감수성에 대한 연구

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ABSTRACT

Five species of food decay fungi, *Aspergillus flavus*, *Asp. uiger*, *Penicillium sp.*, *Botrytis cinerea*, and *Rhizopus stolonifer*, were examined for their radio sensitivity in several suspension media.

Asp. flavus, *Asp. niger* and *Penicillium sp.* have almost the same sensitivity toward gamma rays, with D value in the range of 30 to 35 K rad, whereas *Botrytis cinerea* has a D value of approximately 55 K rad and *Rhizopus stolonifer*, the most resistant fungus studied, has a D vlaue of approximately 100 K rad. Dry spores of *Asp. flavus* showed a considerable increase in their radioresistance when compared with spores irradiated in water.

Asp. flavus and *Penicillium sp.* spores irradiated in citrate buffer at pH 3-7 showed almost no change in their radiosensitivity with pH, but *Botrytis cinerea* spores showed a distinct decrease in their radioresistance at pH 6 and 7.

Penicillium sp. spores irradiated in sucrose solutions showed no significant change in their radioresistance. *Botrytis cinerea* spores displayed a higher radioresistance when they were irradiated in sucrose solution than in water.

INTRODUCTION

The discovery of ionizing radiation by Roentgen and Bequerel Singleton *et al*, in 1895 initiated research on its bactericidal properties. Thereafter, radiation resistance of microorganisms has been investigated to a great extent in the field of radiation biology in connection with food storage. The severity of this problem is recognized from the conservative estimates that about two percent of annual worldgrain produce is damaged by microorganisms (Reimann, H., 1969). The growth of fungi also may be a health hazard due to the production of mycotoxins such as aflatoxin which is produced by *Aspergillus flavus* or *Aspergillus parasiticus*(Jarvice, B., 1972).

The ionizing radiation has been used for controlling microbial growth in various foods and food products (Food Irradiation, 1966). Attempts to sterilize with ionizing radiation date back to early researches on its bactericidal properties (Prescott, S.C, 1904) soon

after the discovery of X-rays, and the natural radioactivity by Roentgen and Bequerel respectively. Brasch and Huber (Brasch, A., *et al*, 1947) were the first to report on the possibility of food preservation by accelerated electrons. During the past twenty years considerable interest has been expressed in the lethal effect of gamma rays on microorganisms (Buckley, P.H. *et al*, 1969, Mirza,M. *et al*, 1972, Padw al-Desal S.R. *et al*, 1972, Padwal Uesai S.R. *et al*, 1972). Only few of the early publications deal with fungi.

The objective of this study has been to investigate the effect of a number of different factors likely to affect the survival of fungi exposed to ionizing radiations.

MATERIALS AND METHODS

Of the fungi studied here, *Aspergillus flavus*, *Botrytis cinerea*, *Aspergillus niger*. *Rhizopus stolonifer*, and *Penicillium sp.* were used. The organism was maintained on Potato Dextrose Agar (Difco) slants at 5°C. A spore suspension of the fungi was prepared by the slant culture incubated at 25°C.

The spores were harvested from the slants with sterile demineralized water containing 0.01%Tween 80 solution as a wetting agent. This conidial suspension was filtered through sterilized cheese cloth (16 folds) to remove mycelia debris. The filtered suspension was centrifuged and washed three times by centrifugation with sterile solution of 0.01% Tween 80. Finaily the sporepellet was dispersed and diluted to the desired concentration (1×10^6 conidia per m*l.*) in the Tween solution. Spore concentration was determined with the Howard mold counter and microscopic examination confirmed the absence of spore clumps and mycelia fragments.

The washed spore suspension was stirred with a magnetic stirrer having a sterile bar. One m*l.* portions of the suspension were transferred to sterile vials containing 3m*l.* of the liquid in which the irradiation was carried out. All the fungi cited earlier were irradiated with gamma rays in water demineralized by means of a Bantam demineralizer. To this water 0.01% Tween 80 was added as a wetting agent.

Citrate buffer was used to obtain suspending liquid of a definite pH. The final buffer concentration was 0.075M. Pure sucrose crystals were dissolved in demineralized water. The dry spores were harvested in the same way as pescribed in wet spore harvesting, and dried in a vacuum desiccator. One m*l.* portions in demineralized water were dried in vials under 27.5″ of vacuum at 25°C.

All fungi were irradiated with gamma rays (Cobalt-60 sources) at dose-levels of 0 to 300 K rads. The dose rate varied according to the source used and the distance of exposure.

The irradiated spores were held overnight at the temperature of 5°C. The vials containing the irradiated spore suspension were well mixed before diluting the plating with the aid of a Vortex mixer. The plated spores on PDA medium were incubated at 25°C for 10 to 72 hours. The colonies were counted both visually by a Quebec colony counter and under a stereoscopic microscope with the exception of *Rhizopus stolonifer*, where a binocular microscope was used. Each treatment was done in at least 5 replicates and average values were plotted.

RESULTS AND DISCUSSION

Fourteen day old spores of *Asp. flavus* and *Rhizopus stolonifer*, 19-day old cultures of *Botrytis cinerea* and *Penicillium sp.*, and 7-day old spores of *Asp. niger* were harvested and

the spores were irradiated in several suspension media.

The wet and dried spore suspensions of *Asp. flavus* were exposed to 0, 50, 100, 150, 200, 250, and 300 K rad of gamma rays. The results of this experiment are summarized in Figure 1. It is apparent that the dried spores of *Asp. flavus* are considerably more resistant than the spores suspended in water. Bhattacharjee (Bhattachrjee, S.B., 1961). obtained similar results when he irradiated *E. coli* with X-rays. An approximate D value of 35 to 37. 5 K rad can be calculated in wet spores.

Stapleton and Hollaender (Stapleton, G.E. *et al*, 1952) showed that the higher the water content of *A. terrecus* spores, the lower the lethal dose. Several other investigators also demonstrated this striking increase of micro-decreases to lethal dose of *Asp. niger*. (Bhaltachrjee, S.B., 1961, Lea, D.E., 1955, Moose, W.S. 1952, Wood, T.H., 1961). The difference in the result may be due to the suspension media during the irradiation and the size of the original population.

Aqueous *Botrytis cinerea* spore suspensions containing 10^5 to 10^6 spores per m*l*. were exposed to gamma rays. The survival curve obtained was not a straight line. The results indicated a D value of about 55 K rad at the straight part of the line. The irradiation destroys only a small number of spores at the low doses, resulting in a lag which extends to 100 to 150 K rad.

The survival curve of *Penicillium sp.* is based on four separate experiments. Colony counting, two days after plating, indicated a D value of about 35 K rad.

In *Rhizopus stolonifer*, the survivals were

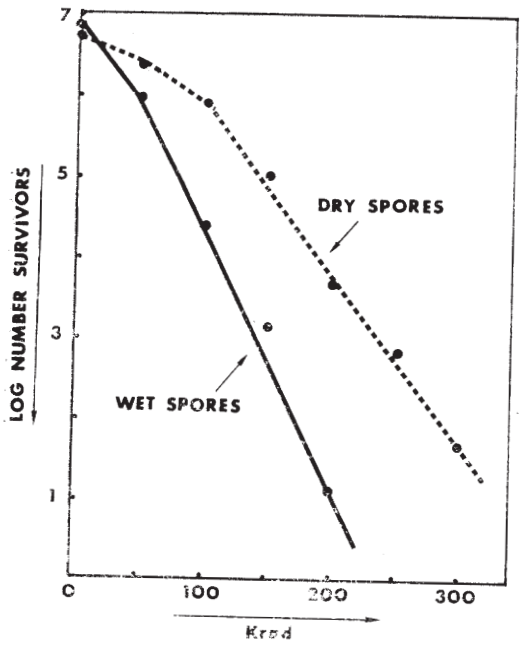

Fig. 1. Effects of gamma radiations on the viability of *Aspergillus flavus* conidia irradiated in water and dry.

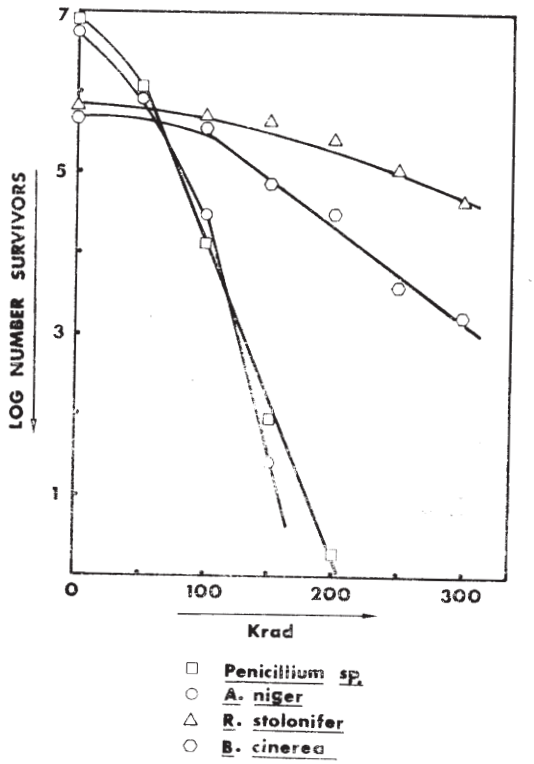

Fig. 2. Viability of gamma-irradiated food decay fungi spores in demineralized water containing 0.01% Tween 80.

counted after 20 to 24 hours of plating with the aid of a binocular because the colonies of this fungus older than 24 hours spread over adjacent colonies hindering the enumeration. *Rhizopus stolonifer* is the most resistant fungus checked. The results obtained are quite similar to those reported in the literature Beraha, L., 1961, Saravacos, G.D., 1962, Sommer, N.F. *et al.*, 1964) although a different procedure of harvesting, irradiating and determining the survival was used.

Asp. flavus, *Penicillium sp.*, and *Botrytis cinerea* spore suspensions at pH levels of 3 to 7 produced using citrate buffers were exposed to gamma rays at the doses 0 to 300 K rad. The results are summarized in Figures 3 and 4. These data indicate that pH has not an appreciable effect on the radiosensitivity of *Asp. flavus* and *Penicillium sp.*

Alper and Gillies[1] found some dependence of the pH on the survival of irradiated *E. coli*. The higher the acidity of the media, the higher was the number of survivors.

In *Botrytis cinerea*, the results indicated that the lower the pH, in the range studied, the higher the percent survivors at all doses. As the dose increased the number of survivors decreased more sharply at higher rather than at low pH levels.

Penicillium sp. and *Botrytis cinerea* spores were suspended in 0, 5, 10, and 15%(w/v) solutions of sucrose, and were exposed to 0, 100, and 200 K rad of gamma rays. The results are presented in Figure 5.

In *Penicillium sp.*, from these data it appears that sucrose, at the concentrations used, had little or no effect on the radioresistance of the fungus spores.

Botrytis cinerea appears that a great number

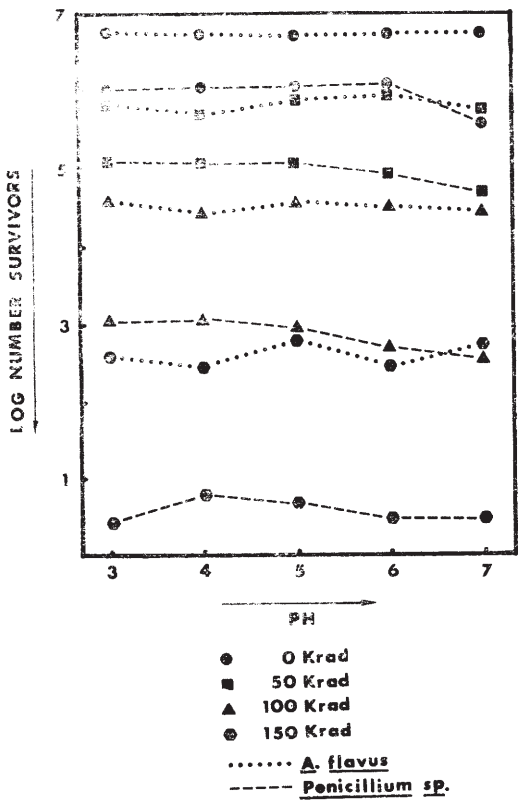

Fig. 3. Viability of gamma-irradiated *Aspergillus flavus* and *Penicillium sp.* spores in citrate buffer solution.

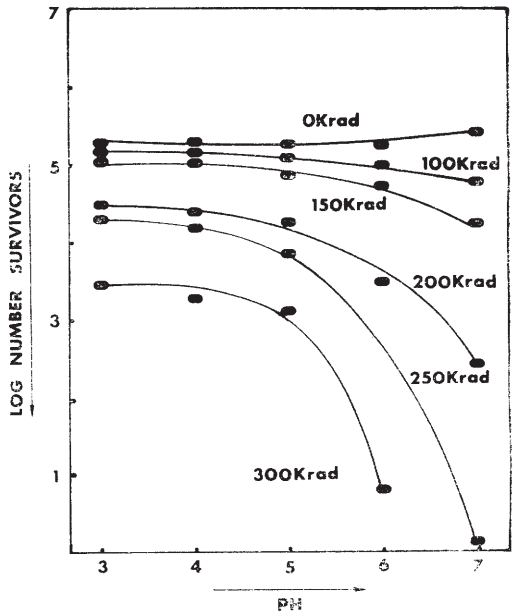

Fig. 4. Viability of gamma-irradiated *Botrytis cinerea* spores in citrate buffer solution.

of spores are likely to survive radiation destruction in sucrose solution than in water. However, no clear differences among the various sucrose concentrations tested can be ascertained in regard to radiation protection of the spores.

All the tested fungi produced small colonies after irradiation. The number of small colonies among survivors increased with increasing dose of irradiation, in an irregular manner. The small colonies did not reach the size of the regular colonies, even if they were held for longer incubation time. Laser (Laser, H., 1964) found that similar results were obtained when yeasts were exposed to X-rays.

A temporary inhibition of division and formation of visible colonies were observed among the spores that survived the irradiation. The duration of the delay increased with increasing dose. It is most apparent in the doses approaching the lethal dose. A similar retardation was reported by Lea (Lea, D.E.,1955) to be a general action of irradiation in a great variety of living cells.

It is well known that *Asp. flavus* produces aflatoxins. There are several reports that irradiation causes an increase or decrease in toxin production by the organism. In further studies, suspensions of conidia from a toxigenic strain of *Asp. flavus* will be subjected to low levels of gamma irradiation, and subsequently tested for aflatoxin production. It is possible that certain levels or irradiation modify the gene responsible for aflatoxin production and render the mold harmless.

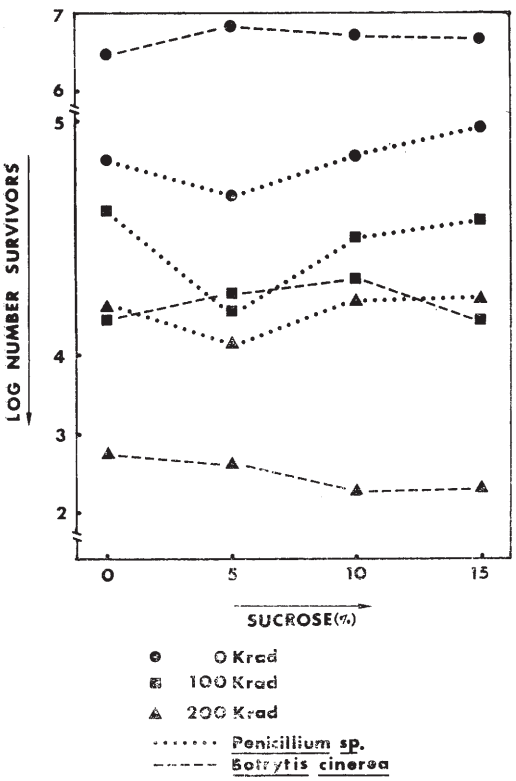

Fig. 5. Effect of gamma rays on the viability of *Penicillium sp.* and *Botrytis cinerea* spores suspended in sucrose solutions.

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