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Radiation Sensitivity of Some Toxigenic Molds Isolated from Deteriorated Rice

by

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變質米에서 분리된 毒素生成곰팡이의 放射線 感受性

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Abstract

Seven toxigenic molds isolated from deteriorated rice in Korea, *Aspergillus clavatus*, *Asp. flavus* var. *columnaris*, *Asp. fumigatus*, *Asp. ochraceus*, *Penicillium citrinum*, *Pen. implicatum*, and *Pen. islandicum*, were examined for their sensitivity toward Co-60 gamma rays for survival.

Conidia of all tested species showed survival curves of sigmoidal type, from which decimal reduction doses were found to vary in the range of 14~33 krad, induction doses, in the range of 12~56 krad and inactivation factors at 200 krad, in the range of 4.6~12.8, all at a dose rate of 11.56 krad/min.

Dose rate effects on the radiosensitivity of *Asp. flavus* and *Pen. islandicum* conidia indicated that the higher dose rate (11.56 krad/min) caused decreases in decimal reduction doses as compared with the lower dose rate (2.67 krad/min).

Introduction

Radiation sensitivity of microorganisms has been investigated to a great extent in the field of radiation biology during the last three decades. On the other hand, ionizing radiation has been tried to use in extending the storage life of various food commodities. The use of low level doses of gamma irradiation seems to be feasible to reduce the microbial population and thereby extend the shelf life of certain foods[1-4].

Molds are a common and important cause of spoilage in grains and cereal products and many of them are capable of producing mycotoxins hazardous to a wide range of animals [5,6]. Iizuka *et al.* [7-10] have reported a series of papers about the effect of gamma irradiation on the microflora of rice and other grains with a hope to use irradiation for mold control in grain storage. While irradiation treatment will certainly reduce the fungal growth, mycotoxin-producing ability of molds might be altered and raise problems about the use of irradiation as a method of food preservation.

Radiation doses required for the destruction of aflatoxins were found to be very high[11-13]. However, exposure of aflatoxin-producing fungi, *Aspergillus flavus* and *Asp. parasiticus* to gamma irradiation doses of 200 krad and above tended to reduce the ability to produce aflatoxins whereas doses of 100 krad tended to stimulate aflatoxin production in subsequent cultures, according to Jemmali and Guilbot [14] and Bullerman *et al.* [15]. On the contrary, Applegate [16] reported that doses of 200 krad caused the increased production of aflatoxin by *Asp. flavus*. Frank *et al.*[17] found that repeated sublethal doses of beta irradiation (160～240 krad) for aflatoxin-producing and non-toxigenic strains of *Asp. flavus* lead to loss, recovery or gain of the ability to produce aflatoxins. This sort of discrepancy appears to be due to the differences in the radiosensitivity of microbial strains, environmental factors and irradiation conditions. Further studies are needed for better understanding of the effects of ionizing radiation on the ability of toxigenic fungi to survive and produce toxins.

This study was, therefore, initiated to investigate the radiosensitivity of some representative molds which were isolated from deteriorated rice in Korea and known to produce mycotoxins in literature. The results should be very useful in deducing ways of preventing the fungal spoilage of rice grains from food preservation and public health aspects.

Materials and Methods

1. Microorganisms

Seven species belonging to the *Aspergillus* and *Penicillium* genera isolated from deteriorated rice in Korea [18,19] and reported to produce mycotoxins were employed. The organisms were maintained on potato-dextrose-agar slants and allowed to grow 1～2 weeks at 30°C for complete conidia formation.

2. Irradiation

A spore suspension of the fungi was prepared by shaking the slant culture with ice-cooled M/10 phosphate buffer (pH 6.5) and passing through a 4-fold cheesecloth. The filtrate was adjusted with the buffer solution so as to contain 10^7～10^8 conidia per ml and a 2-ml aliquot of the spore suspension in a 1.2×13 cm test tube was irradiated with gamma-ray at a dose-rate of 2.67 krad/min or 11.56 krad/min by use of 9,000 Ci Co-60 panoramic irradiator or 15,000 Ci Co-60 BNL's shipboard irradiator, respectively, installed at this Institute.

3. Viable cell count

The irradiated spore suspension was properly diluted and poured on a Czapek agar plate in petri dishes (10 cm diameter). After 3～5 days incubation at 30°C depending on the growth rate of each species, isolated colonies (ideally 30～200 colonies per dish) were counted.

Decimal reduction doses (D_{10} values, the radiation doses required to kill 90% of the testing organism) and induction doses (shoulder doses or lag doses) were obtained from survival curves of individual organisms.

Results and Discussion

Some species in the *Aspergillus* and *Penicillium* genera known to produce various mycotoxins as listed.

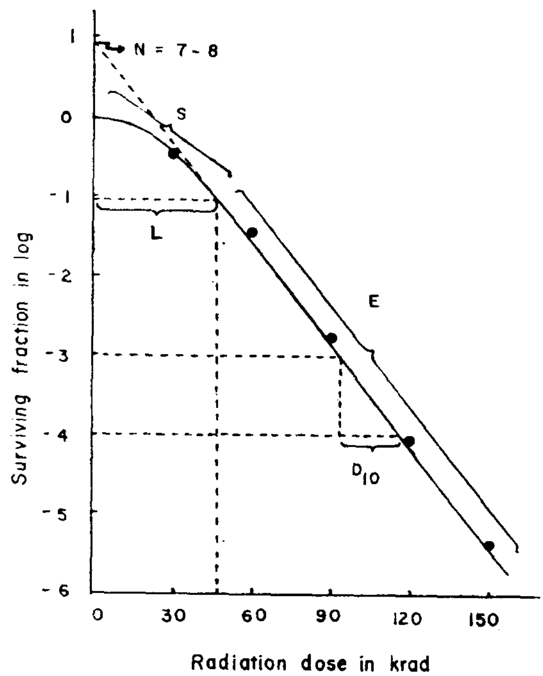

Fig. 1. The component parts of a sigmoidal radiation survival curve
N=extrapolation number or target number;
S=length of shoulder;
E=exponential decline portion;
L=induction dose

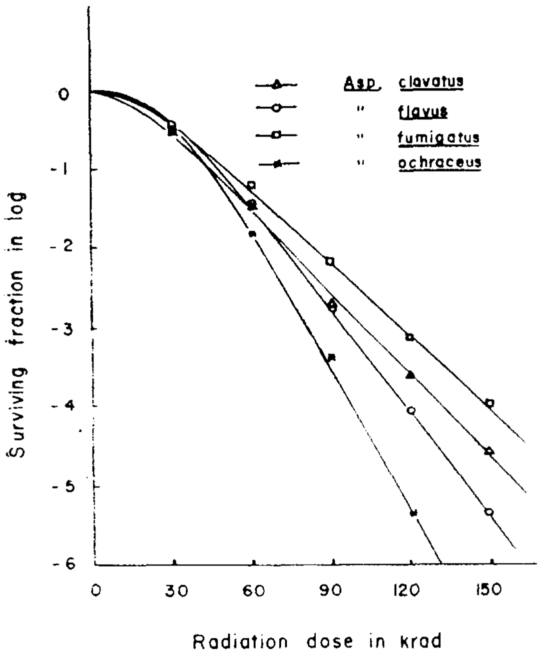

Fig. 2. Radiation survival curves for conidia of *Aspergillus* species at a dose rate of 11.96 krad/min

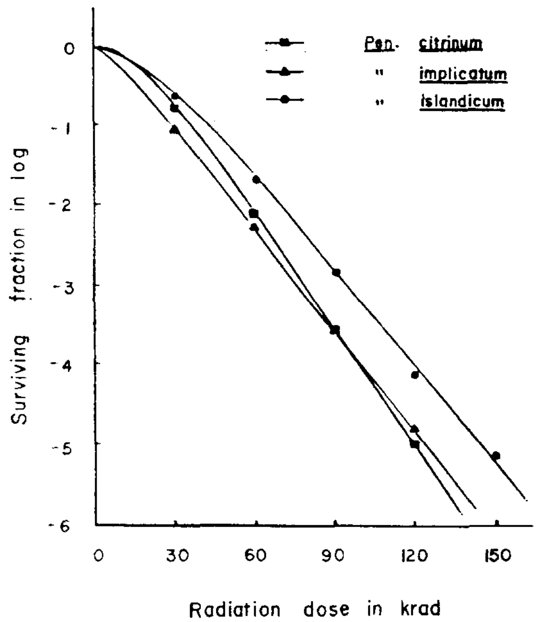

Fig. 3. Radiation survival curves for conidia of *Penicillium* species at a dose rate of 11.56 krad/min

Table 1. Radiation sensitivity of some toxigenic *Aspergillus* and *Penicillium* species isolated from deteriorated rice in Korea*

Mold	Mycotoxin reported[25,26]	D_{10} value (krad)	Induction dose (krad)	Inactivation factor at 200 krad
Asp. clavatus	patulin	29	24	6.1
Asp. flavus var. *columnaris*	aflatoxin, aspergillic acid, aspertoxin	24	48	6.3
Asp. fumigatus	fumagillin, gliotoxin, helvolic acid	33	48	4.6
Asp. ochraceus	ochratoxin	17	56	8.5
Pen. citrinum	citrinin	14	21	12.8
Pen. implicatum	citrinin	23	12	8.2
Pen. islandicum	islanditoxin, luteoskyrin	26	42	6.1

*Conidium suspensions of the organisms were irradiated at a dose rate of 11.56 krad/min.

in Table 1 were subjected to gamma irradiation in an aqueous suspension of their conidia. The results are shown as survival curves in Fig. 1-3, from which D_{10} values and induction doses were calculated as given in Table 1.

The susceptibility or resistance of a microorganism toward radiation may be best expressed as a survival curve since the shape of the curve varies with microbial species and other factors. Whether the shape of survival curve in a log surviving fraction vs. radiation dose plot is linear from the origin or sigmoidal type tells us the one-target theory or multi-target theory for the killing mechanism of microbial cells by ionizing radiation[20,21].

The radiosensitivity of a microorganism is often conveniently expressed in terms of decimal reduction dose or D_{10} value which is defined as the radiation dose in rads to reduce the microbial population by a factor of 10 (10% survival). Thus a D_{10} value can be obtained directly from the plot or according to the equation

(22): $\log N/N_0 = -D/D_{10}$ where N_0 is the initial population, N, the population after irradiation dose D. However, a correction may sometimes be made in a sigmoidal type of survival curve since an induction dose (L) corresponding to the initial dose just before the exponential declining portion should be added to the D_{10} value obtained from the straight portion of the plot. In consequence, a lethal dose (D) for a microorganism becomes $D = L + nD_{10}$ where n is called an inactivation factor to be decided from the microbial species and sterilization purposes.

All of the organisms tested in this study showed survival curves of sigmoidal type. D_{10} values were in the range of 14~33 krad and induction doses, in the range of 12~56 krad, of which *Asp. fumigatus* was the most resistant to the radiation. In general, the sterilization of fruits and other foods are expected to reduce the surviving fraction of existing organisms to 10^{-6}~10^{-7}. At a 200 krad level, inactivation factors of tested organisms were 4.6~12.8 as shown in Table 1; that is, irradiation of foods with 200 krad which is considered as the proper sterilizing dose for rice grains(7,23) will reduce the surviving fraction of microorganisms to $10^{-4.6}$~$10^{-12.8}$.

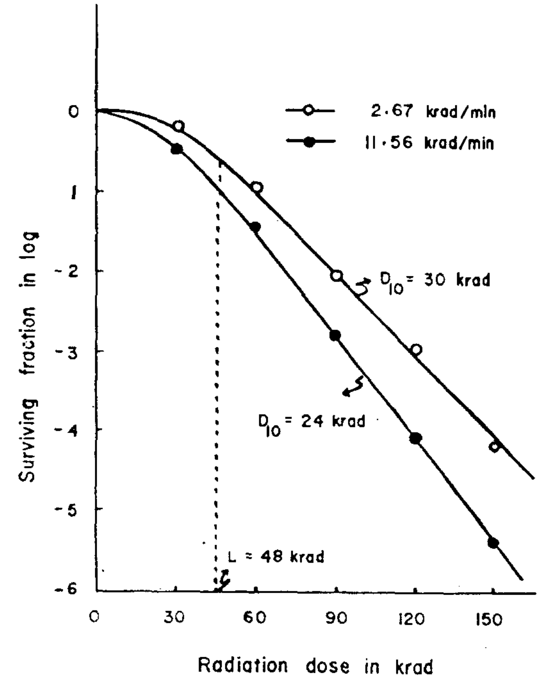

Fig. 5. Effect of radiation dose rate on the survival of *Pen. islandicum* conidia

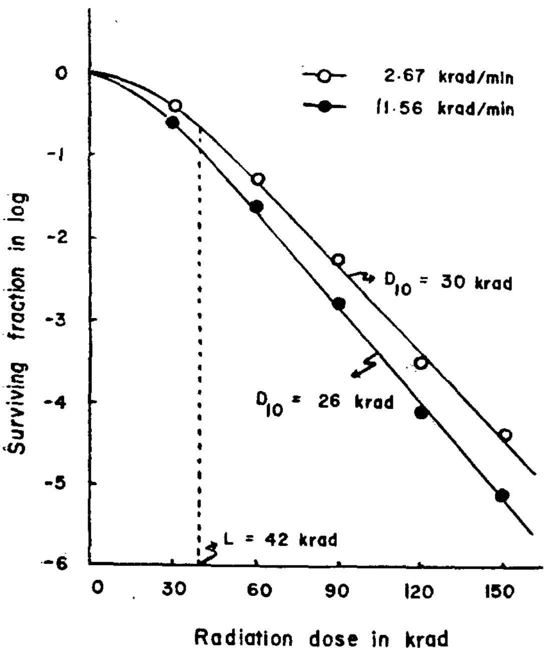

Fig. 4. Effect of radiation dose rate on the survival of *Asp. flavus* var. *columnaris* conidia

The effects of dose rate on the radiosensitivity of two representative molds, *Asp. flavus* and *Pen. islandicum* were investigated and the results are shown in Fig. 4 and 5. In both strains, the higher dose rate (11.56 krad/min) caused decreases in D_{10} values as compared with the lower dose rate (2.67 krad/min). For instance, irradiation at the higher dose rate needed 36 krad less than at the lower dose rate to bring about the 10^{-6} reduction of surviving fractions for *Asp. flavus* conidia. It was interesting to note that induction doses were not changed by different dose rates in both species. Whereas a higher dose rate would certainly shorten the necessary irradiation time, it should also bring about side effects on foodstuffs. It is, therefore, evident that a proper dose rate for sterilization of mycotoxin-producing fungi and maintenance of food quality should be chosen.

It is well known that *Asp. flavus* produces aflatoxins and *Pen. islandicum*, islanditoxin and luteoskyrin. There are several reports that irradiation causes an increase or decrease in toxin production by microorganisms(14-17,24). Thus, Bullerman *et al.*(15) and Jemmali and Guilbot[14] reported that aflatoxin production by *Asp. parasiticus* and *Asp. flavus* was increased by 100 krad and decreased by 200 krad irradiation whereas Applegate[16] found that its production by *Asp. flavus* was increased by 200 krad irradiation. It appears that the irradiation at such a sublethal dose may bring about mutation in the mycotoxin-producing ability of the fungi. Since aflatoxin itself in dry or crystalline form was known to be very resistant toward high radiation doses destruction of aflatoxin by irradiation does not seem feasible, though the possibility was not excluded. Rather the removal of mycotoxin-producing fungi from foods and animal feeds by irradiation and other treatments seems quite plausible and further research is needed.

요 약

국내의 變質米에서 분리, 동정된 7종의 毒素生成곰팡이 *Aspergillus clavatus*, *Asp. flavus* var. *columnaris*, *Asp. fumigatus*, *Asp. ochraceus*, *Penicillium citrinum*, *Pen. implicatum* 및 *Pen. islandicum*의 코발트-60 감마선에 대한 感受性을 조사하여 다음과 같은 결과를 얻었다.

供試한 모든곰팡이의 分生胞子에 대한 生存曲線은 S 자형을 나타냈으며 11.56 krad/min의 線量率에서 D_{10}값은 14～33 krad, 誘導線量은 12～56 krad, 200 krad 에서의 不活性化係數는 4.6～12.8이었다.

*Asp. flavus*와 *Pen. islandicum*分生胞子의 放射線 感受性에 미치는 線量率의 영향을 보면 高線量率(11.56 krad/min)은 低線量率(2.67 krad/min)보다 D_{10}값의 감소를 가져왔다.

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References

1) Niven, C. F.: *Ann. Rev. Microbiol.*, **12**, 507(1958).
2) Bellamy, W. D.: *Advan. Appl. Microbiol.*, **1**, 49 (1959).
3) Hannesson, G.: *Food Irradiation Information*, No. 1, 28 (1972).
4) Hartung, T. E., Bullerman, L. B., Arnold, R. G., and Heidelbaugh, N. D.: *J. Food Sci.*, **38**, 129 (1973).
5) Detroy, R. W., Lillehoj, E. B. and Ciegler, A.: *Microbial Toxins*, Vol. 6 *Fungal Toxins*, Academic Press, New York, p. 3 (1971).
6) Wilson, B. J.: *The Safety of Foods*, edited by Ayres, J. C. et al., AVI Pub. Co., Westport,Conn., Chapter 20 (1968).
7) Iizuka, H. and Ito, H.: *Cereal Chem.*, **45**, 503 (1968).
8) Ito, H., Shibabe, S. and Iizuka, H.: *Cereal Chem.*, **48**, 140 (1971).
9) Watanabe, H., Ito, H., Shibabe, S. and Iizuka, H.: *J. Agr. Chem. Soc. Japan*, **45**, 55 (1971).
10) Watanabe, H., Ito, H., Shibabe, S. and Iizuka, H.: *J. Agr. Chem. Soc. Japan*, **45**, 500 (1971).
11) Miyaki, K., Aibara, K. and Miura, T.: *Microbiological Problems in Food Preservation by Irradiation*, Proceedings of a Panel, IAEA, Vienna, p. 57 (1967).
12) Aibara, K. and Miyaki, K.: *Radiation Sensitivity of Toxins and Animal Poisons*, Proceedings of a Panel, IAEA, Vienna, p. 41 (1970).
13) Frank, H. K. and Gruenewald, T.: *Food Irradiat.* (*France*), **11**(1-2), 15 (1970) [*Nucl. Sci. Abstr.*, **25**, 4825 (1971)].
14) Jemmali, M. and Guilbot, A.: *Comp. Rend. Ser.* D, 269, 2271 (1969) [*Nucl. Sci. Abstr.*, 24, 1707 (1970)].
15) Bullerman, L. B., Barnhart, H. M. and Hartung, T. E.: *J. Food Sci.*, **38**, 1238 (1973).
16) Applegate, K. L.: *Ph.D. Thesis, Ohio State Univ.*, 135 p. (1972) [*Nucl. Sci. Abstr.*, **27**, 1893(1973)].
17) Frank, H. K.,Muenzner, R. and Diehl, J. F.: *Sabouraudia* (*W. Germany*), **9**, 21 (1971).
18) Cho, D. H., Chun, J. K. and Kim, Y. B.: *J. Korean Agr. Chem. Soc.*, **15**, 193 (1972).
19) Kim, Y. B. and Cho, D. H.: *J. Korean Agr. Chem. Soc.*, **17**, 54 (1974).
20) Matsuyama, A. and Kitayama, S.: *J. Japan. Biochem. Soc.*, **41**, 700 (1969).
21) Matsuyama, A.: *Radioisotopes*, **22**, 724 (1973).
22) FAO/IAEA: *Training Manual on Food Irradiation Technology and Techniques*, Technical Reports Series No. 114, IAEA, Vienna, p. 41 (1970).

23) Matsuyama, A.: *Radiation Preservation of Food*, Proceedings of a Symposium, IAEA, Vienna, p. 216 (1973).

24) Savagaon, K. A., Venugopal, V, Kamat, S. V., Kumta, U. S. and Sreenivasan, A.: *J. Food Sci.*, 37, 148 (1972).

25) Bamburg, J. R., Strong, F. M. and Smalley, E. B.: *J. Agr. Food Chem.*, 17, 443 (1969).

26) Morooka, N.: *J. Food Hyg. Soc. Japan*, 12, 459 (1971).