## In vitro Susceptibility of Strains of *Penicillium viridicatum* and *Aspergillus flavus* to β-Irradiation

Penicillium and aspergillus fungi grow well on foods, especially fruits, vegetables, grains and ground nuts, and cause spoilage of economic consideration. The growth of fungi may also be a health hazard, due to the production of highly dangerous toxins such as the aflatoxins of *A. flavus*. Food irradiation shows promise to control microbial growth either by inhibiting or by killing the organisms.

Earlier investigations have disclosed[1-4] the comparative susceptibility of some fungi to ionizing radiations. In this paper we wish to report effects of β-radiation on growth and development of penicillium and aspergillus fungi which have not been previously reported.

*Materials and methods.* Seven strains of *P. viridicatum* (Nos. 64, 127, 128, 129, 142, 174, 185) and 6 strains of *A. flavus* (Nos. 365, 370, 372, 373, 376, 400) were obtained from Dr. H. FRANK of Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe. These strains of fungi were grown on Potato Dextrose Agar at pH 6 in petri dishes. A water suspension containing Tween 80 (0.1%) was made from conidia of each culture and 0.02 ml of the suspension was used to inoculate each petri dish at 5 different locations. The experiments were conducted in 15 replications. The prepared petri dishes were irradiated at several doses up to 0.5 Mrad with a 1-Mev van de Graaff accelerator at 60 Mrad/h by employing the beam power of 5 Watts. The doses and dose rate were calculated according to the method of GRÜNEWALD[5].

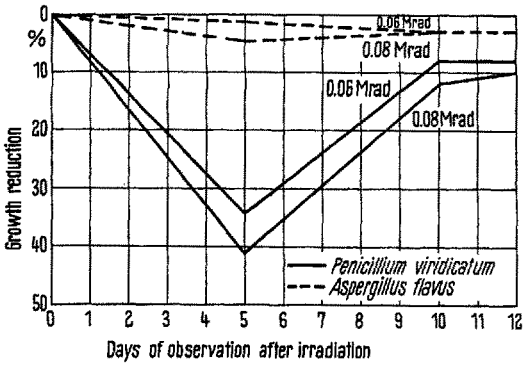


Fig. 1. Growth reduction and recovery of 3-week-old cultures of *P. viridicatum* and *A. flavus* after β-irradiation.

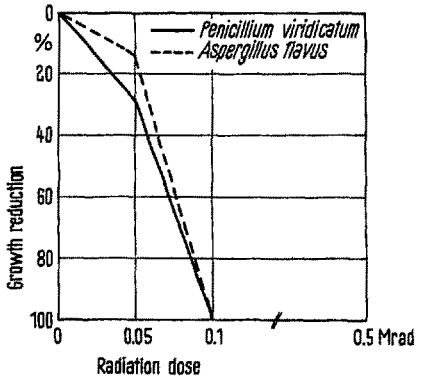


Fig. 2. Growth reduction of 6-month-old cultures of *P. viridicatum* and *A. flavus* strains 5 days after β-irradiation.

Subsequent to irradiation, control as well as irradiated petri dishes were incubated at 25 °C and 80% relative humidity. The representative samples were periodically taken out for stereo-microscopic observations and measurements of colony diameter. Growth reduction of irradiated cultures was calculated by comparison with non-irradiated controls.

*Results.* For the sake of simplification, the data obtained on different strains were pooled for each species. It is apparent from Figures 1, 2, 3 (a and b) that with the increase in the radiation dose, the mycelial growth and the development of conidia was retarded, regardless of the species and strains studied. 3-week-old cultures of

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[5] TH. GRÜNEWALD, *Über die Dosisverteilung bei der Bestrahlung mit van-de-Graaff-Elektronenbeschleunigern* (Dissertation, Technische Hochschule, Karlsruhe, Germany 1965).

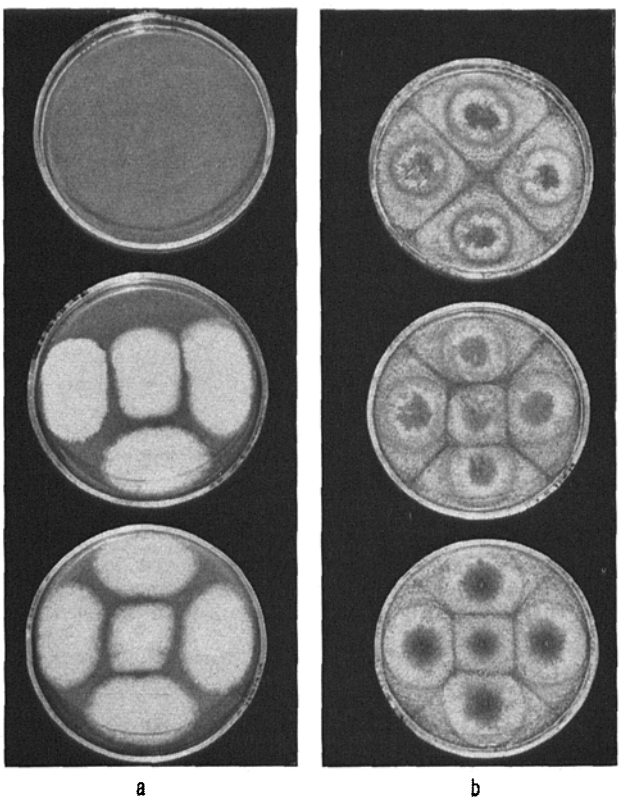


Fig. 3. Comparative radiation damage of (a) *P. viridicatum* strain No. 64 and (b) *A. flavus* strain No. 400 with increasing doses of radiation, i.e. 0, 0.06, and 0.08 Mrad. (a) Petri dishes bottom to top. Bottom: non-irradiated, profuse conidial growth rings spreading from centre outwards. Middle: at 0.06 Mrad, fewer and thinly spread conidial growth rings. Top: at 0.08 Mrad, no growth. (b) Petri dishes bottom to top. Bottom: non-irradiated, profuse conidial growth at the centre and less profuse in the outer ring. Middle: at 0.06 Mrad, less profuse conidial growth at the centre and narrower outer ring with much less conidial growth. Top: at 0.08 Mrad less profuse conidial growth at the centre with outer narrower ring of white mycelia without conidial development.

*P. viridicatum* strains showed a radiation damage of 34% at 0.06 and 41% at 0.08 Mrad (Figure 1) during the first 5 days; recovery set in after this period. On the tenth day *P. viridicatum* strains at both doses showed an average recovery of about 28%. No conspicuous further recovery was observed on or after the twelfth day. In contrast, however, *A. flavus* strains had a negligible damage by the same radiation doses. Age of the fungi strains plays an effective role in the susceptibility to β-radiation, i.e. conidia of the strains of 6-month-old *A. flavus* and *P. viridicatum* cultures were more susceptible to irradiation than 3-week-old (Figures 1 and 2).

A difference in the susceptibility among strains of *P. viridicatum* as well as of *A. flavus* was noted. One strain (No. 128) of the former showed no growth at all at 0.08 Mrad and the other (No. 129) showed no conidial growth both at 0.06 and 0.08 Mrad. Of *A. flavus* strains the most susceptible was No. 376. Regardless of the age of fungal species and strains studied, *P. viridicatum* and *A. flavus* growth was inhibited when irradiated with 0.2 Mrad and above. Of 195 inoculation points in petri dishes irradiated at 0.2 Mrad, and another 195 irradiated at 0.5 Mrad, not a single one showed any growth. In general *P. viridicatum* strains were more sensitive to β-radiation than those of *A. flavus*. Figure 3, a and b clearly show the inhibiting effect of irradiation on mycelial and conidial growth. Studies are now in progress to correlate differential radiation sensitivity with differences in mycelial and conidial cell wall structure[6].

*Zusammenfassung.* Die β-Strahlen-Empfindlichkeit von 7 *P.-viridicatum*-Stämmen und 6 *A.-flavus*-Stämmen wurde an Kulturen verschiedenen Alters untersucht. Eine Dosis von 0,2 Mrad verhinderte das Wachstum 3 Wochen alter Kulturen beider Gattungen vollständig, während bei 6 Monate alten Kulturen eine Dosis von 0,1 Mrad genügte.

D. S. Malla, J. F. Diehl and D. K. Salunkhe[7]

*Institut für Strahlentechnologie der Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe (Germany), 15th December 1966.*

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[7] Alexander von Humboldt fellow and Guest Professor from Utah State University, Logan (Utah, USA).

## 4-Methylcatechol, a Metabolite of Homoprotocatechuic Acid

The metabolism of homoprotocatechuic acid (3,4-dihydroxyphenylacetic acid) in rats and rabbits has been studied by Booth et al.[1,2] and Scheline, Williams and Wit[3]. The latter authors, using (carboxy-$^{14}$C) homoprotocatechuic acid given to rabbits orally at a dose level of 100 mg/kg, found that all of the administered radioactivity could be recovered in the urine after 8–9 days. The radioactivity in the 44 h urines amounted to about 85% of the dose and was found in the following metabolites: homoprotocatechuic acid (63%), homovanillic acid (5.6%), *m*-hydroxyphenylacetic acid (14%) and *p*-hydroxyphenylacetic acid (1.4%) of the dose. Similar experiments using 3 rats (Scheline, Williams and Wit, unpublished results) showed that the 44 h urines contained about 80% of the radioactivity which was found in the above metabolites to the extent of 55, 18.5, 6.5 and 1.4% of the dose, respectively. The mean recovery of radioactivity in the urine and faeces was 93% after 13 days.

The dehydroxylation of catechol acids has been suggested to be a reaction which is carried out by the microflora of the intestinal tract (Shaw et al.[4]). Booth and Williams[5,6] reported that several catechol acids, including homoprotocatechuic acid, are converted to *m*-hydroxy derivatives by rat faecal and caecal contents, and Perez-Silva et al.[7] have recently isolated a strain of Psuedomonas *sp.* from rat faeces which could dehydroxylate caffeic acid (3,4-dihydroxycinnamic acid). As part of a study to investigate the metabolic capabilities of the gastrointestinal flora towards foreign organic compounds, the dehydroxylation reaction was studied according to the method described previously[8,9]. When homoprotocatechuic acid was incubated anaerobically with rat faecal extracts, *m*-hydroxyphenylacetic acid was observed chromatographically in 7 of 9 experiments. In addition, a prominent spot was observed on all the chromatograms which was not seen when homoprotocatechuic acid was incubated with the medium alone or with faecal contents and oxytetracycline (8 μg/ml). This substance was also formed when rat caecal contents were used. A 0.01 *N* HCl solution of this substance obtained from a thin-layer chromatogram showed an absorption maximum at 279 nm (Beckman DB) and a fluorescence maximum at 323 nm (Aminco-Bowman Spectrophotofluorometer, uncorrected value). These values suggested that the substance was a catechol and its Rf value in benzene-glacial acetic acid-water (6:7:3) (BAW) suggested that it was a catechol slightly less polar than pyrocatechol.

It has been found that a number of *p*-hydroxybenzoic acid derivatives, including protocatechuic acid, are decarboxylated to the corresponding phenols by rat faecal and caecal contents[8]. A similar decarboxylation of the phenylacetic acid derivatives would lead to the 4-methylphenols and, in the case of homoprotocatechuic acid, to

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