Increase of Radiation Resistance of a Soil Microflora Exposed to Long-Term Gamma Irradiation

W. H. ERIKSEN† and C. EMBORG‡
Accelerator Department, Research Establishment Risø, DK-4000 Roskilde, Denmark

Received for publication 10 August 1978

Soil microflora were exposed to long-term (18 months) gamma irradiation in an open-air facility at three different doses, 15, 150, and 1,500 krads/18 months. The radiation resistance increased at all doses when compared with the radiation resistance of the microflora from soil shielded from the irradiation with a lead wall.

Microorganisms with radiation-induced radiation resistance have often been demonstrated under laboratory conditions (3, 5–8, 10–13). The question of whether similar changes take place in the surroundings of reactors, irradiation plants, and other areas with a high level of irradiation has not yet been fully answered. Investigations in the field have been carried out, and radiation-resistant microorganisms were isolated (9, 14).

In the present investigation we took advantage of the 20-Ci, open-air 60Co irradiation facility at Risø, Denmark (2), to establish a controlled experiment with long-term irradiation at different doses.

Well-mixed field soil was placed in iron boxes and used as growth medium for radiation-resistant strawberry plants (Senga sengana). The boxes were placed near the gamma source in a way which facilitated sampling at various distances (0.3 to 3 m) from the source. A box was placed 7.5 m from the source and shielded by a 10-cm-thick lead wall. Samples from this box served as controls. At the beginning of the experiment the dose rates at the sampling sites were: 0.3 m, 180 rads/h; 1 m, 18 rads/h; and 3 m, 1.8 rads/h, based on the original calibration of the gamma source and a half-life for 60Co of 5.2 years. No attempt was made to measure the total amount of radiation absorbed by the soil at the sampling site.

After irradiation for 9 and 18 months, samples were taken after removing 1 to 2 cm of the upper soil. A special sampler which digs the soil to a depth of 10 cm was used. The samples were dried overnight in a laminar air flow bench, sieved, homogenized, and subdivided into lots of 1 g. Within the period of sampling, the initial number of microorganisms varied from $10^5$ to $10^9$/g, as judged by dilution and plating on TGY agar (Tryptone [Difco], 5 g; yeast extract [Difco], 3 g; d-glucose [Analar], 1 g; agar [Difco], 15 g; water up to 1 liter; pH 7.0) and incubation at 30°C for 1 week. At each sampling the counts from the various sites did not differ by more than one logarithmic step. All agar plates from the initial counts of the soil samples contained an abundance of various species of microorganisms. No soil sample was dominated by a few types of microorganisms.

One-gram lots were placed in sterile tubes with cotton stoppers and, after being sealed in Mylortene foil (a laminated foil of polyethylene and mylar), irradiated with 2, 3, 4, and 5 Mrads in a 10-MeV linear electron accelerator (2), 30 tubes at each dose for each soil sample. After irradiation, the tubes received 20 ml of sterile TGY broth. The tubes were incubated for 10 weeks at 30°C and inspected for visible growth every week. The results are presented in Table 1.

Table 1 shows that gradual increases in overall radiation resistance of the microflora occurred. Even samples with only 15 krads of long-term gamma irradiation gave a higher number of test tubes with growth than the controls. (At this site, the 3-m site, the strawberry plants yielded delicious strawberries.)

Figure 1 shows radiation inactivation curves for dried preparations (4) of two strains (K.3.24 and K.3.25) isolated from control samples irradiated at 3 Mrads (gram-positive, red-pigmented rods) and two strains isolated from the 0.3-m site samples irradiated with 5 Mrads. Strain 30.5.11 is a gram-positive, red-pigmented rod resembling Brevibacterium and Arthrobacter isolated in the field studies (9, 14).

Similar strains were isolated from the 3- and 1-m sites. Strain 30.5.16 is a gram-positive, red-
pigmented Micrococcus resembling M. radiodurans (1). Similar strains were also isolated from the 1-m site. The radiation inactivation curve of M. radiodurans strain R1, obtained by the same technique, is shown for comparison.

The data do not reveal the mechanism behind the shift in radiation resistance. Explanations may be selections and mutations in combination with oscillating environmental factors such as temperature and humidity. The data indicate that there is a significant possibility of special radiation-resistant microflora around radiation sterilization and decontamination plants.

We thank J. P. Skou, V. Hähr, and J. Berg for assistance with the setup at the open-air 60Co source and M. E. Andersen, I. Hansen, B. Nielsen, and L. H. Pedersen for technical assistance.

LITERATURE CITED


