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Resistance of *Clostridium perfringens* Type A Spores to γ-Radiation

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ABSTRACT

MIDURA, T. F. (The University of Michigan, Ann Arbor), L. L. KEMPE, J. T. GRAIKOSKI, AND N. A. MILONE. Resistance of *Clostridium perfringens* type A spores to γ-radiation. Appl. Microbiol. **13**:244–247. 1965.—The radiation resistance of the spores of a classical strain and of an atypical, heat-resistant strain of *Clostridium perfringens* was determined. Spores were produced in Ellner's and in a Trypticase broth medium. Approximately 10^6 viable spores per milliliter were suspended in 0.06 M phosphate buffer and irradiated with γ rays from cobalt-60; the survivors were counted in Tryptone-yeast extract-agar by the Prickett-tube technique. Radiation *D* values for spores of the atypical strain in phosphate buffer and in cooked-meat broth were 0.23 and 0.30 Mrad, respectively, and the *D* value of the classical strain was 0.25 Mrad in phosphate buffer. Spores of the classical and atypical strains of *C. perfringens* type A are characterized by differences in heat resistance; yet, all strains tested demonstrated similar radiation resistance. Also, the spores were more resistant to ionizing radiation in cooked-meat broth than in phosphate buffer.

Foodborne enterotoxemia and dysentery in animals have been associated with four *Clostridium perfringens* serotypes, namely, B, C, D, and E. Type F has been established as the causative agent of necrotic enteritis in humans (Zeissler and Rassfelt-Sternberg, 1949), but type A is perhaps the most widely known of the group, even though it was not associated with foodborne disease in humans until 1945 (McClung, 1945). A few years later, atypical type A strains were identified with food poisoning in humans (Hobbs et al., 1953); these strains were considered atypical because of the high heat resistance of their spores. Classical strains have also been found to cause food poisoning (Collee, Knowlden, and Hobbs, 1961; Hall et al., 1963). Since foods can be processed with ionizing radiations, the resistance of *C. perfringens* to such radiations must be known. Midura et al. (1963) reported on the resistance of *C. perfringens* type A, Hobbs serotype 2, to γ rays with the spores suspended in phosphate buffer. Recently Matsuyama, Thornley, and Ingram (1964) evaluated the resistance of *C. perfringens* type A, Hobbs serotype 1, to these radiations in a cooked-meat medium. Radiation resistances of classical and atypical strains of *C. perfringens* are compared in the present paper.

MATERIALS AND METHODS

Two *C. perfringens* type A strains were selected. The classical type A strain G47, isolated, from a wound infection, was obtained from T. F. Wetzler, The University of Michigan, Ann Arbor; the Hobbs "heat resistant" strain, E2, isolated from a food-poisoning event, was obtained from H. Hall, of the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

Stock cultures were maintained in modified Robertson's chopped-heart medium (Wetzler, Marshall, and Cardella, 1956). Hydrogen was used for all work done under anaerobic gaseous environments. Suspensions of spores were prepared in Ellner's (1956) medium and in a Trypticase broth medium. Spore yields in Ellner's medium varied with different subcultures of the same strain in the same batch of medium; for example, yields of approximately 10^5 per milliliter were obtained with strain G47, in marked contrast to strain E2 which either did not sporulate or produced spores in very limited numbers.

The following procedure consistently encouraged the sporulation of strain E2. Stock cultures were grown in tubes of modified Robertson's chopped-heart medium at 37 C. These tubes were then refrigerated at 4 C. For use, one tube was placed in a water bath at 80 C for 15 min; then 0.5 ml of the culture was inoculated into a freshly steamed and cooled tube of modified Robertson's chopped-heart medium which was incubated for 24 hr at 37 C. Next, approximately 45 ml of freshly prepared Trypticase Soy Broth in 25 by 200 mm test tubes (Kimble Glass Division, Owens-Illinois Glass Co., Toledo, Ohio) were inoculated with 1 to 2 ml of the chopped-heart culture. The concentration of Trypticase (BBL) in this broth was increased to 4%, and the medium was designated

as TSB-4. The inoculated tubes were incubated for 48 hr at 37 C.

Spore crops were harvested by pooling the contents of 5 tubes in one bottle, centrifuging at 3,000 rev/min for 30 min at 0 C, and washing three times with distilled water. After removal of the last supernant fraction, sterile distilled water was added in the least amount sufficient to facilitate pipetting the spore suspension into screw-cap test tubes. Viable spores were counted after heating at 80 C for 15 min. The spore suspension was stored at 4 C.

Five media were tested to select the most suitable medium for determining survival of *C. perfringens* spores subjected to γ-radiation and heating at 80 or 100 C. These media were: pork-pea infusion agar (Andersen, 1951), Tryptone-yeast extract-agar (Mossel et al., 1956), reinforced clostridial agar (Oxoid), iron-sulfite-agar (Oxoid), and sulfite-polymyxin-sulfadiazine-agar (Angelotti et al., 1962).

The number of viable spores was finally determined by dilution counts with the use of Miller-Prickett tubes (Miller, Garrett, and Prickett, 1939) and the basal medium of Mossel et al. (1956). The latter contains 1.5% Tryptone (Difco), 1% yeast extract (Difco), and 1.5% agar (Difco).

For the irradiation studies, approximately 10^6 spores per milliliter were suspended in 0.06 M phosphate buffer at *p*H 7.0, or in the supernatant liquid of cooked-meat medium (Difco) at *p*H 6.8. Samples (4-ml) of these suspensions were dispensed into 5-ml ampoules (Kimble Glass Division). The ampoules were heat-sealed and immersed in ice water, and the spore suspensions were irradiated with γ rays at a rate of 72,000 or 74,000 rad per hour. The cobalt-60 source at the Phoenix Radiation Facility of The University of Michigan was employed.

Viable spores were enumerated, as previously described, to construct radiation dose-survivor curves. Each curve was based upon duplicate runs; a single straight line was determined by the method of least squares. Radiation *D* values were obtained from these curves.

The heat resistance of spore suspensions to be used in the radiation experiments was tested by the following procedure. Samples (4-ml) of diluted spore suspensions were transferred to 5-ml ampoules which were then sealed in an oxygen flame. All of the ampoules were held in ice water prior to heat treatment. The ampoules were submerged in a thermostatically controlled bath containing water and ethylene glycol at 100 (±0.5) C. At the end of the prescribed heating time, the ampoules were rapidly transferred to ice water and then to a refrigerator (4 C). The time required for spore suspensions in the vials to reach the bath temperature was 2 min. This was determined by thermocouple measurements. The heating times were corrected for this 2-min heating period by use of the assumptions and the techniques described by Kempe (1955). Viable-spore counts were then determined as previously described.

RESULTS AND DISCUSSION

Critical morphological, cultural, and physiological characteristics of two selected strains of *C. perfringens* were examined before and after irradiation to establish their identity as *C. perfringens* type A cultures. Physiological characteristics of the cultures are given in Table 1, and carbohydrate fermentations are given in Table 2. No differences were evident between irradiated and nonirradiated spores on the basis of these tests.

TABLE 1. *Physiological characteristics of Clostridium perfringens type A**

Differential test	Strain G47	Strain E2
BCP iron milk	ACG	ACG
Gelatin liquefaction	+	+
Acrolein production	+	+
Hydrogen sulfide	+	+
Nitrate reduction	+	+
Motility	−	−
Indole formation	−	−
Gram reaction	+	+
Lecithinase activity	+	+
Anaerobiosis	+	+
Spores	O, S	O, S

* ACG = acid, coagulation, gas; BCP = bromocresol purple; O = oval; S = subterminal; + = positive; − = negative.

TABLE 2. *Carbohydrate fermentations of Clostridium perfringens type A**

Carbohydrates	Strain G47	Strain E2
Adonitol	−	−
Arabinose	+	+
Dextrose	+	+
Dextrin	+	+
Dulcitol	−	−
Galactose	+	+
Glycogen	+	−
Glycerol	+	+
Inositol	+	−
Inulin	−	−
Lactose	+	+
Levulose	+	+
Maltose	+	+
Mannitol	−	−
Mannose	+	+
Raffinose	+	+
Rhamnose	−	−
Salicin	+	−
Starch	+	+
Sucrose	+	+
Trehalose	+	−

* Symbols: + = acid and gas; − = no reaction.

The recovery medium was found to influence the number of organisms counted as surviving exposure to heat or irradiation. Pork-pea infusion agar, reinforced clostridial agar, and Tryptone-yeast-agar gave higher counts than did sulfite-polymyxin-sulfadiazine-agar or iron-sulfite-agar, irrespective of strain of organism or severity of treatment. Tryptone-yeast extract-agar was selected because fragmentation by the gas was minimal.

The data show that the log per cent of the spores surviving is linear with increasing amounts of γ-radiation applied. Figure 1 depicts the effect of γ-radiation on the survival of strain E2 spores suspended in phosphate buffer, or in cooked-meat broth. The radiation D values for E2 spores in phosphate buffer and in cooked-meat broth were 0.23 and 0.30 Mrad, respectively. Experience with other spores, such as *C. botulinum*, has indicated greater radiation resistance in foods than in phosphate buffer (Kempe et al., 1956). The greater radiation resistance of *C. perfringens* spores in cooked-meat broth suggests that a protective effect may be exerted by foods. A similar protective action of cooked-meat broth on the heat resistance of *C. perfringens* organisms has been previously discussed in connection with *C. perfringens* food poisoning (Collee et al., 1961).

Figure 2 shows the effects of γ-radiation on the survival of the spores of both the classical and the atypical strains of *C. perfringens*. These spores were produced in Ellner's medium, suspended in phosphate buffer, and irradiated. D values for the G47 and E2 spores were 0.25 and 0.28 Mrads, respectively. The similarity of D values for *C. perfringens* type A spores (0.23 to 0.28) in phosphate buffer indicates that both the classical and atypical spores have similar resistances to radiation. The results agree with the observations of Matsuyama et al. (1964) with a related organism. They found the D value for spores of a heat-resistant strain, Hobbs serotype 1, in Robertson's cooked-meat medium to lie in the range of 0.21 to 0.48 Mrad.

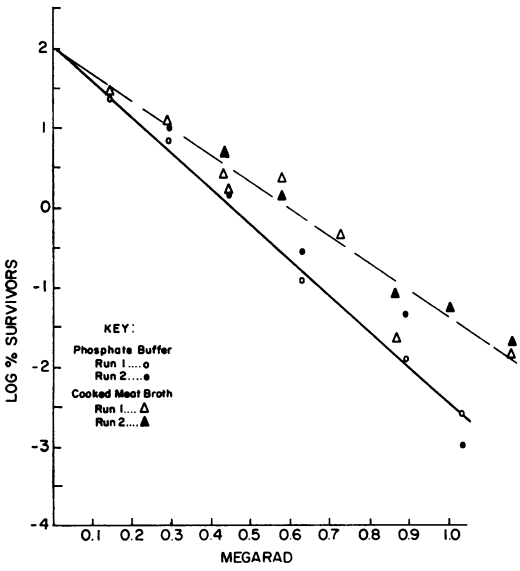

FIG. 1. *Effect of γ-radiation on the survival of Clostridium perfringens type A strain E2 spores.*

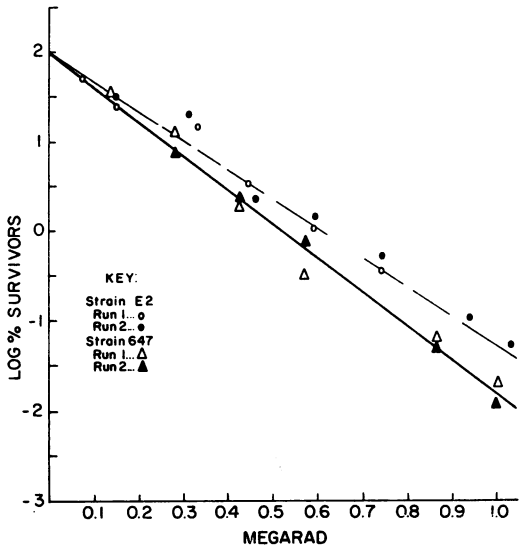

FIG. 2. *Effect of γ-radiation on the survival of Clostridium perfringens type A spores suspended in 0.06 M phosphate buffer (pH 7.0).*

C. perfringens type A spores were found to be more radiation-resistant than *C. botulinum* type E spores, which are reported to have a D value of approximately 0.14 Mrad (Schmidt, Nank, and Lechowich, 1962).

Spores of *C. perfringens* atypical type A strains are reported to have greater heat resistances than spores of classical strains (Hobbs et al., 1953). Samples of the spore suspensions which were prepared for the irradiation studies were heated at 100 C for varying times. In phosphate buffer, spores of the classical type A strain did not survive exposure to 100 C for 15 min, whereas strain E2 survived exposure to 100 C for 2 hr in cooked-meat broth. These survival times at 100 C were in agreement with the work of Headlee (1931) and Hobbs et al. (1953). Although spores of the atypical strain were characteristically more heat-resistant than spores of the classical strain, the data from the present investigation indicate that the radiation resistances of the spores are similar.

In summary, the radiation resistances of the spores of an atypical, food-poisoning strain and of a classical strain of *C. perfringens* type A appear to be similar in phosphate buffer. *C. perfringens* spores exhibit varying resistance to γ-radiation, depending upon the substrate in which they are irradiated; resistance is greater in cooked-meat broth than in phosphate buffer. Although the spores of a food-poisoning strain were found to have characteristically greater heat resistance than spores of the classical strain, they did not have greater radiation resistance.

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