Competitive Growth of Chicken Skin Microflora and Clostridium botulinum Type E after an Irradiation Dose of 0.3 Mrad

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ABSTRACT

Chicken skins with chicken exudate were used as a model system to determine if low dose irradiation might cause a health hazard by eliminating the natural flora and allowing Clostridium botulinum type E spores, if present, to produce toxin in the absence of typical spoilage. Irradiation (0.3 Mrad, 5°C) reduced the natural flora from 10^4 to 10^5 cells/cm^2, whereas C. botulinum type E (Beluga) spores were reduced only by one log10. At 10°C, the irradiation survivors of the natural flora were able to multiply and produce spoilage odors within 8 d, whereas the C. botulinum survivors could not produce toxin within 14 d. At an abuse temperature of 30°C, the natural survivors grew faster than C. botulinum spores and produced an off-odor before the sample was toxic.

Fresh broiler carcasses in retail outlets normally have an initial contamination level of 10^4 to 10^5 microorganisms per cm^2 (3,20) and normally can only be stored for 1 to 2 d at 5°C and still maintain their freshness (1). Storage for 4 to 6 d at 4.4°C results in spoilage (20). The major organisms causing spoilage of poultry stored at refrigeration temperature are Pseudomonas spp. (2,3,20). These organisms cause an off-odor when they are present in high numbers (10^7 cells/cm^2).

Methods for prolonging the freshness of poultry have constantly been sought. Different chemicals such as chlorine (11,15), sorbates (13,14) and succinic acid (5) have been tested. The use of low dose irradiation (0.2 to 0.7 Mrad) was proposed for prolonging the shelf-life of refrigerated poultry. The Netherlands has approved for sale broilers irradiated with a maximum dose of 0.3 Mrad. In 1978, South Africa approved the sale of irradiated chicken (0.2 to 0.7 Mrad). Both Canada and the USSR approved experimental batches of irradiated chickens (6). The use of low-dose irradiation will not only increase shelf-life but also will reduce Salmonella, which is recognized as one of the main pathogens associated with poultry (19). Irradiation of carcasses with 0.25 Mrad was found to be highly effective in destroying Salmonella in chilled broiler carcasses (12). However, a concern often raised regarding low-dose irradiation of fish, and certainly will be brought forth in the case of irradiated poultry, is that of a potential Clostridium botulinum type E health hazard.

The ability of C. botulinum type E spores to germinate, multiply and produce toxin at refrigeration temperatures has been well-documented (4,9,16,18). In fish products and other marine species inoculated with C. botulinum type E, the use of low dose irradiation resulted in toxic samples before the product was rejected (1,8,17).

In a previous article (7) we showed that C. botulinum type E could grow and produce toxin on chicken skins at 10°C. However, after irradiation (0.3 Mrad), the surviving spores were unable to develop and produce toxin at 10°C. This investigation was undertaken to obtain a better understanding of the effects of irradiation on the normal flora and the ability of the survivors to compete with C. botulinum type E at 10 and 30°C.

MATERIALS AND METHODS

Preparation of chicken skins

Skins from breast of broilers were prepared as described previously (7). A round piece of skin was embedded in wax so that an area of 7 cm^2 of skin was exposed. When sterility was desired, the skins were irradiated with 4.1 Mrad (-30°C) in a 60Co gamma source (dose rate of 14,000 rad/min).

Bacterial counts

After each experiment the skin sample was removed from the wax with sterile forceps and placed in a small blender jar with 100 ml of sterile 0.1% peptone and blended for 1 min. After appropriate dilution, 1-ml portions were inoculated in duplicate. The natural flora of chicken skins was enumerated in PBP medium (Plate count broth; Difco + 2% agar; BBL). The plates were stratified with the same medium and incubated at 20°C for 48 h aerobically. Clostridium botulinum type E was enumerated in tubes containing TPG (typticase, peptone, glucose) media as described previously (7).

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Growth experiments

Filter-sterilized chicken exudate (0.3 ml) was added to non-sterilized chicken skins. The skins were stored at 10°C under both aerobic and anaerobic conditions. The anaerobic samples were packed in flexible pouches and vacuum-sealed. The aerobic samples were stored in a desiccator containing water. The growth of C. botulinum type E (Beluga) was studied by adding approx. $10^5$, $10^7$ spores/cm$^2$ to irradiation sterilized skins containing 0.3 ml of filter-sterilized chicken exudate.

Irradiation survival curves

Preliminary results showed that there was no difference in irradiation survival curves between natural flora grown on chicken skins or natural flora that grew in plate count broth and thereafter transferred to the skins. However, the latter gave more uniform results. Skins were inoculated with $10^9$ to $10^9$ cells/cm$^2$ of the aerobic mixture or approx. $5 \times 10^7$ cells/7 cm$^2$ of the anaerobic mixture. The samples were vacuum-sealed in flexible pouches (61 cm Hg for the aerobic and 4 cm Hg for the anaerobic cultures), and irradiated at 5°C in a 60Co gamma source. The resistance of C. botulinum type E spores ($5 \times 10^8$ spores/7 cm$^2$) was determined in the same manner as for the anaerobic flora.

Isolation of radiation resistant anaerobic flora

Four 7 cm$^2$ pieces of irradiated (0.3 Mrad) chicken skins were suspended in PCB and incubated at 10°C for 5 d in an anaerobic jar. This culture (6 ml) was irradiated with 0.3 Mrad at 5°C in vacuum-sealed Pyrex tubes (16 x 150 mm). The irradiated culture was added to fresh PCB and incubated at 10°C for 5 d. After alternatingly growing and irradiating for four cycles, the resulting culture's radiation resistance on chicken skins was determined as described above.

Growth of normal flora surviving irradiation

Uninoculated, non-sterilized chicken skins with 0.3 ml added non-sterilized chicken exudate were sealed in flexible pouches, irradiated with 0.3 or 0.5 Mrad and aerobically or anaerobically incubated at 10 or 30°C as described above.

Toxin production of C. botulinum type E cells grown in competition with surviving normal flora on irradiated skins

Non-sterilized chicken skins with 0.3 ml of non-sterilized chicken exudate were inoculated with C. botulinum type E spores (approx. $5 \times 10^7$ spores/7 cm$^2$), vacuum-sealed in a flexible pouch, irradiated with 0.3 Mrad at 5°C and incubated at 30°C. At periodic intervals, samples were checked for aerobic plate counts, toxin and odor. Preliminary experiments indicated that C. botulinum cells did not form colonies on PCA. Furthermore, the medium used for the recovery of C. botulinum cells supported the growth of the normal flora. Therefore, the counts of C. botulinum in Fig. 4 are from sterilized skins inoculated with C. botulinum and treated as above.

Toxin was formed in competition with the surviving normal flora and was determined as described previously (7).

RESULTS AND DISCUSSION

Skins from the breast of broilers obtained from local supermarkets had counts of $10^5$ to $10^7$ spores/cm$^2$, which is similar to counts reported for other studies (3,20). The chicken exudate, which was the liquid accumulated in trays or absorbed in packing paper which supported chickens, was usually more heavily contaminated, having counts of $10^7$ to $10^9$/ml. Exudate was found to be a good medium for the growth of bacteria (13).

Growth of natural flora of chicken skins on skins having added (0.3 ml), filter-sterilized chicken exudate and held at 10°C aerobically and anaerobically is shown in Fig. 1. The natural flora multiplied faster and gave a higher yield when incubated aerobically. C. botulinum type E grew slower than the natural flora under anaerobic conditions. When the number of cells per 7 cm$^2$ reached $10^7$ to $10^8$, an off-odor could be detected. The off-odor became stronger with prolonged incubation. The off-odor was noted 3 d and 6 d when the skins were incubated aerobically and anaerobically, respectively. For the radiation-sterilized skins inoculated with C. botulinum spores and incubated anaerobically, the first toxic samples were detected after 3 d (data not shown). These results suggest that under anaerobic conditions the skins may become toxic before an off-odor was observed. However, this was observed in the absence of competitive microflora.

The irradiation survival curves of the natural flora grown either aerobically or anaerobically in plate count broth and inoculated on the chicken skins could be described by two straight lines; a sharp decline resulting from a dose of 0.05 Mrad, and a more moderate decline with higher doses (Fig. 2). The initial sharp decline was due to inactivation of irradiation sensitive strains, whereas the more moderate decline was due to more resistant strains. To show that the break in the survival curve was due to different populations, the resistant flora was selected by alternately growing and irradiating the normal flora for four cycles. After enrichment, the survival curve could be described by a straight line passing through the origin, with a slope similar to the resistant portion of the anaerobic population (Fig. 2). The relative numbers of the radiation resistant strains in the original population could be obtained by extrapolating the line obtained for the higher doses (0.05-0.30 Mrad) to

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Figure 2. Radiation resistance of the natural flora of chicken skin, before and after four cycles of growth-irradiation, and C. botulinum type E spores on chicken skin at 5°C. Symbols: ●, natural flora grown aerobically before irradiation; ■, natural flora grown anaerobically before irradiation; ◇, anaerobic resistant flora after four cycles of growth-irradiation; ▲, C. botulinum type E spores.

the ordinate. For example, as shown in Fig. 2, for the aerobic mixture the interception with the ordinate is at about log-2. This indicates that about 99% of the total population is very sensitive to irradiation, whereas 1% is more resistant. The main difference between the three natural populations was in the relative number of sensitive strains. The D-values (the dose required to destroy 90% of the bacteria) obtained for the dose range of 0.05-0.30 Mrad were 0.05, 0.06, and 0.07 Mrad for aerobic, anaerobic, and resistant flora, respectively. The survival curve of C. botulinum type E spores on chicken skins had a shoulder of 0.1 Mrad, and the D-value obtained for the dose range 0.1-0.30 Mrad was 0.12 Mrad.

Barnes (2) stated that, when the chief spoilage organisms are inhibited, microorganisms producing less offensive odors may be present in much higher numbers without affecting acceptability of the product. Therefore, experiments were designed to determine if irradiation with 0.3 Mrad might allow C. botulinum type E to produce toxin, in the absence of spoilage.

In the case of non-sterilized chicken skins with 0.3 ml of non-sterilized chicken exudate, a major portion of the natural flora (10² to 10⁶/7 cm²) was eliminated by an irradiation dose of 0.3 Mrad at 5°C. However, one could always recover at least 10 cells/7 cm². The growth of these survivors on chicken skins at 10°C after irradiation, under both anaerobic and aerobic conditions, is shown in Fig. 3. The growth rate under both conditions was similar. It was shown previously (7) that irradiated (0.3 Mrad) C. botulinum type E spores were unable to develop on chicken skins when incubated anaerobically at 10°C for 18 d. It is evident from Fig. 3 that the normal flora surviving a dose of 0.3 Mrad was able to multiply at 10°C aerobically or anaerobically and produce spoilage odors by 9 d. Therefore, it could be concluded that there should not be any botulism hazard with irradiated chicken if the product is refrigerated.

Spores of C. botulinum type E surviving 0.3 Mrad could grow and produce toxin on chicken skins at 30°C (7). Therefore, non-sterilized skins were inoculated with approx. 5 × 10⁴ spores/7 cm², vacuum-sealed, irradiated with 0.3 Mrad and incubated at 30°C to determine if spoilage off-odors would be produced before the product became toxic (Fig. 4). The natural flora grew faster than C. botulinum at 30°C. When C. botulinum grew together with the natural flora, no toxin was detected after 1 d but a slight off-odor could be observed. The first toxic sample was detected after 2 d. At the same time, a distinct off-odor was produced by the natural flora. Although our results do not directly show a competitive advantage of irradiation survivors over C. botulinum type E, there is indirect evidence that the normal flora would have a competitive advantage under either aerobic or anaerobic conditions (Fig. 3,4). In addition, at 30°C, a definite spoilage odor was present by the time toxic samples were observed. The
competitive advantage would be greater under aerobic than anaerobic conditions since C. botulinum would take longer to produce toxin (7).

When non-sterilized, noninoculated chicken skins were irradiated with 0.5 Mrad (5°C), less than 10 cells were present per 7 cm² (data not shown). At least 8 d were needed at 10°C to get >20 colonies in the first dilution. Some skins contained less than 10 cells/7 cm², even after 21 d at 10°C. At 30°C, it took 4 d for survivors to reach a count of 10 cells/7 cm², and 6 d for skins to develop an off-odor. Therefore, irradiating chicken with a dose higher than 0.3 Mrad may create a potential C. botulinum type E hazard only if the product is severely abused by holding at 30°C.

Results obtained in this study indicate that it is highly unlikely that irradiating chicken carcasses with a dose of 0.3 Mrad to reduce Salmonella and extend shelf-life would result in a C. botulinum type E hazard.

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REFERENCES


GROWTH OF RADIATION SURVIVORS ON CHICKEN SKIN

Figure 4. Aerobic plate counts of normal flora, odor and toxin production on chicken skins inoculated with C. botulinum type E spores, irradiated (0.3 Mrad, 5°C) and incubated at 30°C. (A) Bacterial counts, symbols: O natural flora on skins inoculated with C. botulinum spores; □ C. botulinum type E cells inoculated on radiation-sterilized chicken skins. (B) Odor (●) of skins in which natural flora and C. botulinum grew together and the percent of samples that became toxic (■).