

Heat Inactivation of In Vivo- and In Vitro-Grown Mycobacteria in Meat Products

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Heat inactivation of mycobacteria from lesions and from culture was compared in meat products. In vivo-grown organisms were more easily heat inactivated than were in vitro-grown organisms.

Heat inactivation of in vitro-grown mycobacteria in meat products has been reported (2, 3). However, concern has been expressed that the in vivo-grown organisms within tubercular lesions might be more heat resistant than are in vitro-grown organisms.

To conduct a comparison of in vivo-grown organisms with in vitro-grown organisms, we obtained the liver and the spleen from a naturally infected pig detected at slaughter. Massive tubercles were present in the spleen, and smaller tubercles were present in the liver. We used these tubercles to prepare wieners with in vivo-grown organisms. The organisms in these tissues later were found to be *Mycobacterium avium* serovar 2. Liver and spleen from a nontuberculous pig were used for the emulsion prepared with in vitro-grown organisms isolated from the infected pig. The in vitro-grown organisms were cultured and prepared for incorporation into the emulsion in the same manner as that previously reported (2).

The emulsions were prepared and stuffed in wiener casings as previously described (2). Each emulsion consisted of 270 g of pork fat, 990 g of lean beef, 240 g of tuberculous or normal spleen, 540 g of tuberculous or normal liver, 600 g of ice, 15 g of spice mixture, 56 g of dextrose, and 72 g of NaCl. The wieners were heated in a water bath at central internal temperatures of 55 to 65°C in 2.5°C intervals for periods of 0, 1, 5, 10, 20, 40, and 60 min. The wieners were processed and cultured as previously described (2).

After the heating cycle and cooling, the wieners were placed in Stomacher blender bags and weighed. Four volumes of 0.3% benzalkonium chloride, used as a decontaminating solution, was added, and the contents were blended for 1 min. The top of the bag was folded tightly to act as a coarse filter, and a portion of the filtrate was strained into a screw-capped tube. The filtrate was allowed to stand for 1 h at room

temperature. Then the tube was shaken to assure an even distribution of fat droplets, and 0.9 ml of the filtrate was added to 0.9 ml of saline (0.85% NaCl) to provide a 10^{-1} dilution of the wiener. Subsequent serial 10-fold dilutions were prepared. The inocula consisted of 0.25 ml of the dilutions pipetted onto the surface of egg yolk medium in 25-cm² tissue culture flasks. The flasks were rotated to assure even distribution of the inocula on the surface. The cultures were incubated for 30 days at 38°C before colonies were counted and recorded. Because of the dilution factor, counts of less than 40 viable units per g of meat would not have been detected.

Survival curves of the in vivo- and in vitro-grown mycobacteria are illustrated (Fig. 1). The viability of in vitro-grown organisms was reduced only slightly after 60 min at 55 or 57.5°C. In this menstruum, the decimal reduction value (D-value) of in vitro-grown organisms of this strain was 28 min at 60°C. The D-value of the in vivo-grown organisms at 60°C was 1 min. At internal temperatures above 60°C, the in vivo-grown organisms were no longer detected at 0 time. Far more of the in vivo-grown organisms than in vitro-grown organisms were inactivated during the period in which the wieners were attaining water-bath temperatures.

The use of lesions from a tuberculous pig infected with the more heat-resistant (1) serovar 4, 8, or 10 organisms may have been desirable, but because most swine tuberculosis in this area (central United States) is caused by *M. avium* serovar 2 organisms, a set of extensive lesions selected at random would not likely have contained organisms of any of these serovars. From a human pathogenicity standpoint, lesions due to *Mycobacterium bovis* may have been desirable to use, but in addition to the relative scarcity of such lesions, the number of organisms per gram of tissue usually is much smaller in lesions due to *M. bovis* than in lesions due to *M. avium*.

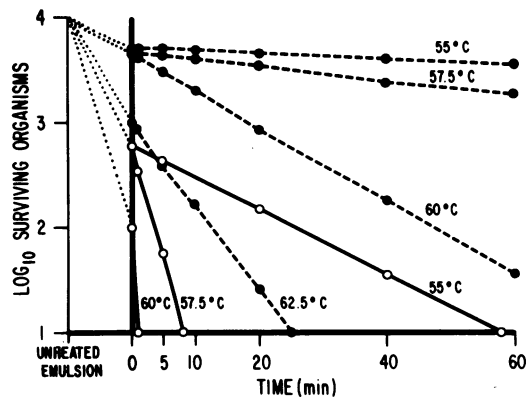


FIG. 1. Survival of in vivo-grown (—) and in vitro-grown (---) (same strain) *M. avium* serovar 2 in wieners submerged in a water bath at various temperatures. The losses of viability during the heating-up period are shown (···). At the highest temperatures, none of the organisms survived the heating-up period.

These data show that the in vivo-grown *M. avium* organisms were more easily heat inactivated than were in vitro-grown organisms of the same strain. Because we have no reason to believe that other mycobacteria would behave differently, we conclude that heat-inactivation data gathered by using in vitro-grown organisms provide time-temperature relationships that are more than adequate when applied to naturally infected tissues.

LITERATURE CITED

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