

Heat Inactivation of *Mycobacterium avium*-*Mycobacterium intracellulare* Complex Organisms in Aqueous Suspension

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Isolants from swine and from humans representing serotypes 1, 2, 4, 8, and 10 of the *Mycobacterium avium*-*Mycobacterium intracellulare* complex were compared for heat tolerance in aqueous suspension. The most heat-resistant isolant found was a serovar 10 isolated from a human. This isolant was examined further to determine the rate of kill at various temperatures and pH's, the effect of meat protein and fat, and the effect of nitrite. Kill rates were not significant at 60°C or below. Decimal reduction values were 4 min or less at 65°C and 1.5 min or less at 70°C. Kill rates were slightly higher at pH values of 6.5 and 7.0 than at 5.5 or 6.0. The water-soluble fraction of wiener emulsion did not alter kill rates, but the saline-soluble fraction protected the organism somewhat. Fat did not affect the survival of the organisms except to eliminate the protective effect of saline extract when the suspension contained 50% fat. The addition of sodium nitrite to the suspension did not alter the heat sensitivity of the organisms.

Most mycobacteriosis lesions in swine are due to members of the *Mycobacterium avium*-*Mycobacterium intracellulare* complex (3). These organisms may be present in the muscle tissue as well as in the lesion site (1, 3, 6) and have been isolated from lymph nodes of swine with no visible lesions (2, 7).

This study was designed to determine the heat sensitivity in aqueous suspension of mycobacteria that may be present in pork. Conditions pertinent to commercial processing of pork products were considered. A subsequent report will deal with the organisms in meat products (4).

MATERIALS AND METHODS

All records of viable organisms were based on colony counts from decimal dilutions of hand-agitated tubes. Ultrasonic dispersion was not used at any stage.

Medium selection. In preliminary tests to determine the best culture medium for these determinations, 7H10 agar containing fraction V albumin, serum agar, and egg yolk agar (5) were compared. After heat shock, the greatest numbers of colonies consistently developed on egg yolk agar. The presence or absence of 2 µg of ferric mycobactin per ml in the egg yolk agar did not alter the numbers of colonies formed after heating. Egg yolk agar neutralizes the benzalkonium chlorides that would later be used as a decontaminant in isolating the organisms. Therefore, egg yolk agar without mycobactin was used throughout these tests.

Selection of the most heat-resistant strain. Two swine isolants and two human isolants of serotypes 1, 2, 4, 8, and 10 were used. The swine isolants were obtained from C. O. Thoen, National Veterinary Services Laboratories, United States Department of

Agriculture, Ames, Iowa, and the human isolants were obtained from K. L. McClatchey, National Jewish Hospital, Denver, Colo. The donors had serotyped the isolants. The isolants were designated by the serovar plus either A or B if from swine or C or D if from humans.

A large quantity of each isolant was grown on egg yolk agar. The organisms were collected, washed three times in saline, suspended to an optical density of 0.15 when diluted 1:100 (10^{10} to 10^{11} organisms per ml), and stored in 2-ml portions at -70°C.

To find the most heat-resistant isolant, a 2-ml portion of each was thawed and 10 10-fold dilutions of 20 ml each were made in 0.15 M phosphate buffer at pH 6.0. For each time and temperature to be tested, 1-ml portions of these primary dilutions were then added to tubes (13 by 100 mm). A water bath was used to heat the dilutions to temperatures of 60 and 65°C. The surface of the water in the bath was always at least 1 cm above the level in the tubes, which were heated without agitation. At the end of each time interval (1, 2, 5, 10, 30, and 60 min), a row of tubes containing dilutions from 10^{-1} to 10^{-10} were removed from the heat to an ice-water bath. Tissue culture flasks (25 cm²) containing egg yolk agar were inoculated with 0.25 ml from each heated dilution and from the unheated control dilutions and stored in an incubator at 37°C. The colonies were counted after 1 month.

Effect of pH on heat sensitivity. The pH of freshly slaughtered pork muscle dropped over a 24-h period from about pH 7.0 to pH 5.5. Because the lowering of pH in meat is due primarily to lactic acid, the effect of pH was determined with both lactate and phosphate buffers. Sterile pH solutions were made with 0.15 M phosphate buffer of pH 5.5, 6.0, 6.5, and 7.0. Lactate buffers were prepared by adjusting 1%

lactic acid solutions with NaOH to pH 5.0, 6.0, and 7.0. The procedure used in the previous heat treatment was followed for diluting isolant 10C in each pH solution and for arranging the rows of dilutions for each time and temperature to be tested. Tubes with the varying pH solutions were held in the water bath at the designated temperatures and lengths of time, then cooled immediately in an ice bath and inoculated, incubated, and recorded as previously described. The times shown are total immersion times. As illustrated in Fig. 1, when tubes containing the prediluted suspensions of organisms were immersed in the water bath or ice bath, 1 to 2 min lapsed before the dilutions reached the new temperature. To reduce the effects of these lag periods at higher temperatures and shorter

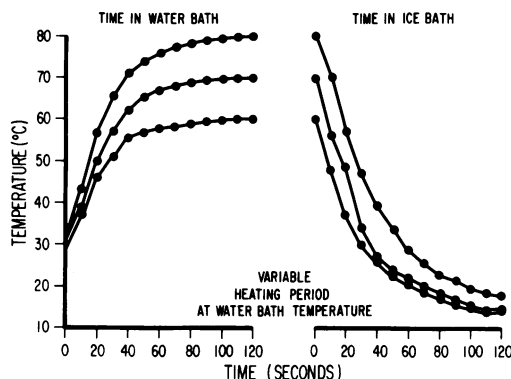


FIG. 1. Temperature rise within the aqueous suspensions when the tubes were immersed in a water bath at various temperatures. Temperature decrease within the suspensions when removed from the water bath to an ice bath.

times, the less accurate method was used of pipetting a heavy suspension of organisms into 9 volumes of preheated buffer solution. After brief heating periods, samples of the heated suspension were pipetted into 9 volumes of prechilled (4°C) buffer. Tenfold serial dilutions were made from the chilled suspensions.

Effect of rate of temperature increase. To determine whether the rate of temperature increase (come-up time) within time periods practical in the commercial processing of wieners might alter the final temperature necessary to destroy the organisms, isolant 10C was suspended, as in previous tests, in 0.15 M phosphate buffer at pH 5.5, 6.0, and 6.5. Rows of the dilutions were immersed in a water bath in which the temperature was raised gradually from 50 to 75°C at varying rates as illustrated in Fig. 2. The rows of dilutions were removed at 5°C intervals and were cooled immediately in an ice bath. The time required to reach each temperature was recorded. The suspensions were inoculated, incubated, and recorded as previously described.

Effect of nitrite on heat sensitivity. Test solutions were made with pH 6.0 phosphate-buffered saline. Nitrite (as NaNO_2) was added at levels of 0, 0.003, 0.006, and 0.012 g/100 ml. Isolant 10C organisms were added, diluted, heat treated, and inoculated as before. Duplicate series were heated to 60 and 65°C for periods of 2, 5, 10, 30, and 60 min.

Wiener emulsion extracts. Raw wiener emulsion from a commercial meat processor was combined with 3 volumes of distilled water and then thoroughly mixed. This mixture was centrifuged, and the supernatant was drawn off. The supernatant was filter sterilized (0.22 μm). The residue was further extracted with 1% saline which was centrifuged and filtered as before. The final residue was extracted with acetone, then with petroleum ether, and finally with diethyl

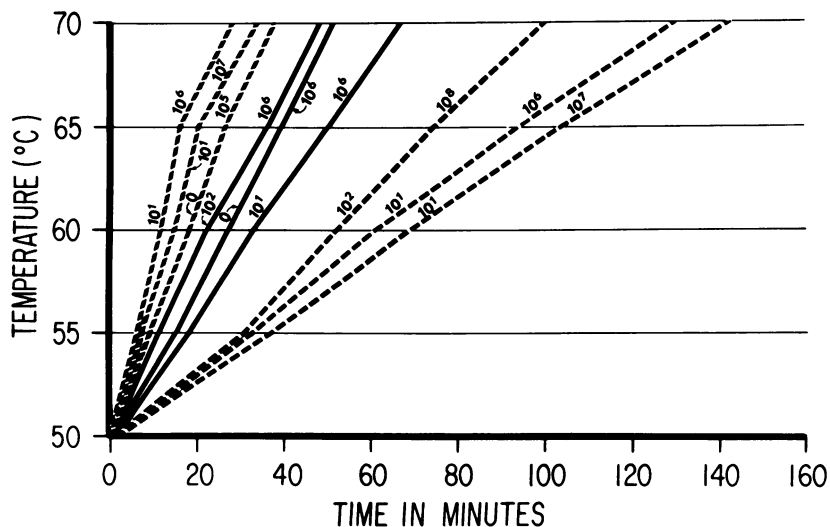


FIG. 2. Effect of rate of temperature increase on numbers of 10C killed in 0.15 M, pH 6.5, phosphate buffer during commercially feasible heating rates. The lines represent the temperature increases with time. The numbers on the lines represent the numbers of 10C killed when temperatures had reached 60 and 65°C. More than 10^{10} had been killed at all rates when the temperature had reached 70°C.

ether to obtain the fat. The water and saline extracts and fat were used singly and in combinations at varying concentrations as diluents for the 10C organisms. These dilutions were heated at 60 and 65°C for periods of 2, 5, 10, 30, and 60 min in a water bath. After cooling, the samples were inoculated onto egg yolk medium and incubated.

RESULTS

Strain resistance. For purposes of comparison, the kill time was designated as the time at a given temperature to reduce the number of viable units by 2 logs. Figure 3 illustrates the kill times of the *M. avium*-*M. intracellulare* complex organisms tested.

The *M. avium* group (serovars 1 and 2) tended to be more heat sensitive than the *M. intracellulare* group. In the *M. avium* group, the isolants from humans (C and D) were more heat sensitive than the isolants from swine (A and B), but in the *M. intracellulare* group the isolants from humans seemed to be slightly more heat tolerant. Isolant 10C was chosen for subsequent tests because serotype 10 organisms were overall more heat tolerant than the other groups.

Effect of pH. The heat inactivation of 10C in 0.15 M phosphate buffers is illustrated in Fig. 4. The heat resistance of the organisms increased as the pH decreased when either phosphate or lactate buffers were used. The D-value, or decimal reduction time, is the time at a given temperature required to reduce the population 90%, or a 1-log reduction. In these studies, the D-values increased with decreasing pH. No loss of viability was detected at any pH in 2 h at 50 or 55°C, and organisms were killed very slowly by heating at 60°C. At 60°C, the D-value rose from 17.8 at pH 6.5 to 120 at pH 5.5. The D-values were from 2 to 4 min at 65°C and from 0.3 to 1.5 min at 70°C. Short-time heating trials at 75 and 80°C yielded D-values of about 0.2 and 0.1 min,

respectively; destruction was too rapid to assess the effect of pH.

Influence of temperature on time of thermal death. Figure 5 illustrates the effect of temperature at pH 5.5 in 0.15 M phosphate buffer. At all pH levels, death time decreased sharply when the temperature was increased from 60 to 65°C. In the short-time heat trials at 75 and 80°C, the reduction in viable units exceeded 10⁶ during the first 2 min at all pH levels.

Death rate curves (log₁₀ survivors versus time) usually were not straight lines, as would have been expected if thermal death was based on first-order kinetics; the curves were bent slightly downward, suggesting a slight cumulative thermal effect or a consistent experimental error.

Effect of rate of temperature increase.

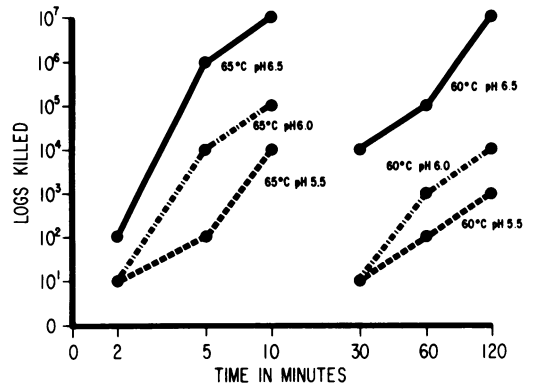


FIG. 4. Effect of pH at 60 and 65°C in phosphate buffers on loss of viability of isolant 10C.

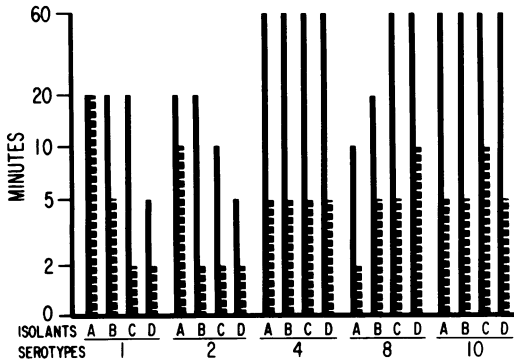


FIG. 3. Kill time—time in the water bath at 60°C (solid lines) or 65°C (broken lines) to reduce the number of viable units of mycobacterial isolants by 2 logs.

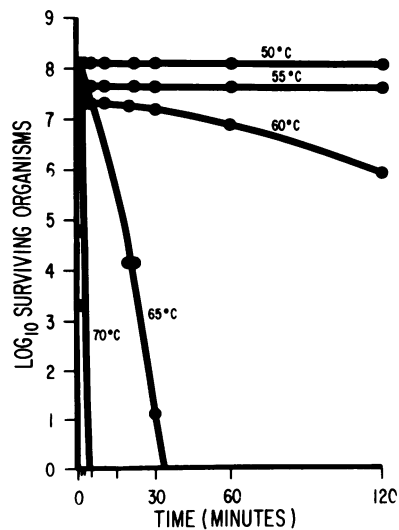


FIG. 5. Reduction in viable units of isolant 10C at pH 5.5 in phosphate-buffered saline at various temperatures.

Figure 2 illustrates the come-up time in temperature test at pH 6.5, and it is typical of the tests done at all pH intervals. Regardless of heating rate, from 0 to 2 logs of 10C organisms had been killed by the time the temperature reached 60°C. When the temperature had reached 65°C, 5 to 8 logs had been killed. Within the time frames studied, increase in the number of organisms killed as the time to reach 65°C was increased was not consistent. By the time the tubes had reached 70°C, all organisms (10+ logs) were dead. Within this range of practical come-up times or heating lags, no significant differences in the numbers of organisms killed were detected at any pH.

Effect of nitrite. The presence or absence of nitrite in the suspensions of isolant 10C organisms had no effect on the heat sensitivity of the organisms.

Effect of meat extracts. The distilled water extract did not produce a measurable effect on the thermal resistance of 10C organisms. However, high concentrations of the saline extract reduced the numbers of organisms killed. Figure 6 illustrates that after 5 min of heating at 60°C, organism survival was greatest in the more concentrated dilutions of saline extract. At dilutions of 1/64 or greater, the saline extract no longer protected the organisms. Fat did not alter the thermal resistance when suspended in saline alone or when incorporated at 15 or 30% in the saline extract suspensions, but when fat constituted 50% in the saline extract suspensions, it eliminated the protective effect of concentrated saline extract.

DISCUSSION

The predominant mycobacteria detected in slaughtered swine are the *M. avium* serovars 1 and 2. *M. intracellulare* isolants represent a

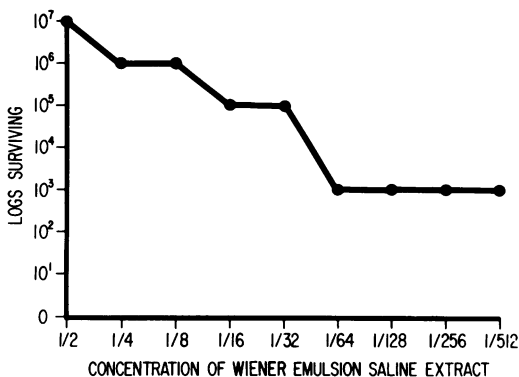


FIG. 6. Survival of 10C after 5 min in saline extract of wiener emulsion held 5 min in a 60°C water bath.

small proportion of the serovars detected but in the present study proved to be slightly more heat resistant than *M. avium*.

These tests were designed to study the thermal destruction of the *M. avium*-*M. intracellulare* complex. A representative of serotype 10 was among the most heat-tolerant isolants we tested and was chosen to evaluate the effects of some variables that may occur in meat products.

We have found no loss of viability in 2 h at 50 or 55°C and extremely long D-value times at 60°C. D-values of 4 min or less at 65°C and 1.5 min or less at 70°C indicate that safe products can be produced by processing at 65 or 70°C for appropriate time periods. In addition, 5 or more logs of organisms would ordinarily be destroyed during the times commonly required in wiener processing to achieve temperatures of 65°C or higher. However, during the very short heating times that occur in deep-fat or radiant heat cooking of precooked sausages, substantially fewer organisms would be killed as maximum temperature is reached.

Most meat products are at pH values between 5 and 6. Contrary to the situation with many organisms, isolant 10C was slightly more heat tolerant at these pH values than at neutrality. In addition, the saline-soluble protein component of meat adds a further degree of heat tolerance, except when the fat levels are very high. The presence of sodium nitrite did not alter thermal resistance. Many other variables obviously exist in the actual meat products which can best be evaluated in those products. The destruction of these mycobacteria in meat products is the subject of another report (4).

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