Journal of Food Protection, Vol. 53, No. 4, Pages 296-299 (April 1990)

Thermal Inactivation of *Borrelia burgdorferi,* the Cause of Lyme Disease

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(Received for publication August 4, 1989)

ABSTRACT

Borrelia burgdorferi strain EBNI was cultivated in BSK-II medium at 34°C, then cultures at different physiological states were heat-treated at temperatures in the range of 50 to 70°C. Numbers of survivors were estimated by the Most Probable Number technique. Log MPN was plotted against treatment time, and resulting survivor curves were linear. Estimated D-values for cultures incubated at 34°C for 7 d before heat-treatment were 5.5, 4.3, 2.7, .47, and .14 min at 50, 55, 60, 65, and 70°C, respectively. Spirochetes in the lag phase had greater resistance to heat than those in the stationary phase, with the latter being more resistant to heat than spirochetes in the same phase of growth but refrigerated at 4°C for 3 d. D-values for *B. burgdorferi* are generally less at 50°C, and greater at 70°C than those reported for other nonsporeforming pathogens. When $\log_{10}$ MPN was plotted against treatment temperature, two linear segments for each thermal death curve were obtained. Our data show the spirochete had higher z-values than most nonsporeforming pathogens. The pH of the medium, in the range of 5.0 to 7.6, did not affect resistance of *B. burgdorferi* to heat.

Borreliae were first discovered in 1868 when Otto Obermeier detected spirochetes in the blood of patients during an epidemic of relapsing fever in Berlin *(4)*. Borreliae also caused skin lesions that were known in Europe as Erythema Chronicum Migrans *(6)*. In 1975, cases of the disorder (attributed to *Borrelia burgdorferi* by Burgdorfer and co-workers in 1982) were first discovered in the USA in Lyme, Connecticut, and thus the name Lyme disease resulted *(19)*. It is now known that *B. burgdorferi* causes an infectious disease (Lyme disease) and that the bacterium is transmitted primarily by the ticks *Ixodes dammini* and *I. pasificus*. In humans, symptoms of the disease include skin rash, arthritis, and cardiac and neurologic disorders. The major natural reservoirs of *B. burgdorferi* are the white-tailed deer, *Odocoileus virginianus,* and the white-footed mouse, *Peromyscus leucopus (8)*.

Burgess et al. *(9)* examined specimens of cerebrospinal fluid, urine, saliva, blood, milk, lungs, kidney, spleen, heart, brain, and liver from a cow infected with *B. burgdorferi*. The researchers detected high antibody titers against the pathogen in serum, milk, and synovial fluids, but the spirochete was isolated only from the lungs and liver. In another study, some samples of blood serum (282/430), colostrum (2/3), and synovial fluids (5/10) obtained from cows suspected of having clinical disease caused by *B. burgdorferi* were positive for antibody to the pathogen. No positive milk samples (0/44) were noted. When samples were cultured, 7/156 blood, 1/14 synovial fluids, 1/3 colostrum, 0/44 milk, and 2/10 urine samples yielded *B. burgdorferi (7)*. The study also revealed that infected animals do not always show clinical symptoms of the disease.

Several decades ago, Sergent *(18)* detected borreliae in milk, and some guinea pigs that consumed the contaminated milk became infected. In a recent study with mice (*P. leucopus* and *P. maniculatus*) susceptible to *B. burgdorferi*, Burgess et al. *(10)* showed that these species can transmit the disease by direct contact. Mice *(P. maniculatus)* inoculated orally with *B. burgdorferi* developed antibodies to the bacterium, which was isolated from the blood of 1 of 10 infected mice *(8)*.

The findings just discussed indicate that *B. burgdorferi* occurs in some organs and blood, and thus it is likely that the pathogen is present in the meat of infected animals. Although one study showed that colostrum but not normal milk of infected cows contained the pathogen, further studies are needed to establish if *B. burgdorferi* occurs in milk either from environmental contamination or through shedding by infected cows. It also appears that food may function as vehicle for transmission of *B. burgdorferi*. Hence, this study was done to investigate the sensitivity of *B. burgdorferi* to heat-treatments similar to those used during processing of foods.

MATERIALS AND METHODS

Microorganism

B. burgdorferi strain EBNI was obtained from the laboratory of Dr. E. C. Burgess, School of Veterinary Medicine, University of Wisconsin-Madison. The strain is an isolate from *I. dammini*. This culture was transferred weekly in BSK-II medium (pH 7.6), and between transfers was stored at 4°C.

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Medium

B. burgdorferi was grown in the BSK-II medium *(3)*. The same medium was used as the menstruum for heat-treatment of the bacterium.

Culture

B. burgdorferi was transferred into tubes of BSK-II medium (inoculum level was 1%). Culture tubes were incubated at 34°C for 7 d and then the contents were examined with a darkfield microscope to ascertain culture purity. The last culture was then transferred (1% inoculum) into BSK-II medium and treated as follows: incubated at 34°C for 12 h (C1), incubated at 34°C for 7 d (C2), or incubated at 34°C for 7 d and then refrigerated at 4°C for 3 d (C3). Portions (1 ml each) of C1 culture were dispensed into 15 x 45 mm borosilicate glass vials with screw-caps, and then heat-treated. C2 and C3 cultures were first diluted 1:10 in fresh BSK-II medium, before dispensing and heating.

Heat-treatment

Vials containing cultures of *B. burgdorferi* were completely immersed in a shaking water bath adjusted to the appropriate temperature to be studied. Temperature of BSK-II medium in a vial was monitored using a thermometer. After the desired heating temperature appeared on the thermometer, timing for the treatment began. Vials were removed from the water bath at fixed intervals and immediately cooled in an ice-water mixture. Cultures were heated at 50, 55, 60, 65, or 70°C. Preliminary experiments were done for each heating temperature to determine the range of heating times needed to obtain results appropriate for constructing survivor curves.

Heat-treatment at different pH values

The pH of BSK-II medium was adjusted to 5.0, 6.5, and 7.6 using 0.1 N solution of NaOH or HCl. *B. burgdorferi* propagated as in the C2 culture was inoculated into the tubes of medium at the various pH values and heat-treated at 65°C, as described earlier.

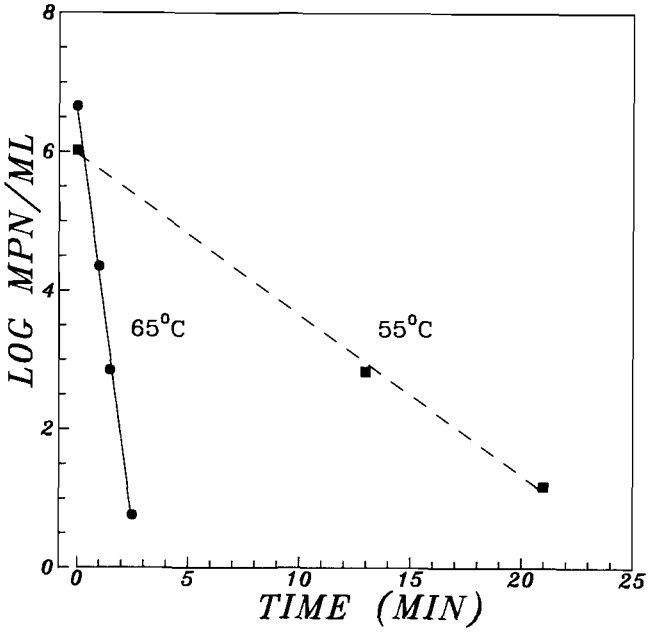

Figure 1. *Survivor plot for* B. burgdorferi *obtained from a culture incubated at 34°C for 7 d and then heated at 55 or 65°C in BSK-II medium at pH 7.6. Results represent data from two replicates.*

Enumeration of B. burgdorferi

Untreated and heat-treated cultures were diluted serially in BSK-II medium, three test-tubes of each dilution were made. All dilutions were incubated at 34°C for 7 d. Tubes were considered as positive when the color of the medium changed to yellow, as a result of growth by *B. burgdorferi*. Microscopic examination was done to verify that the change in color of the medium was caused by growth of *B. burgdorferi*. The Most Probable Number (MPN) of surviving cells in cultures at each heating interval was determined using the three-tube MPN table *(21)*.

Data analyses

Survivor curves were constructed by plotting $\log_{10}$ MPN/ml vs. heating time. Data of survivor plots were analyzed by regression to estimate the line of best fit. D-values (the negative reciprocal of the slope) were calculated from the resulting regression equations. z-Values were estimated by regressing $\log_{10}$ D-values vs. heating temperatures. Experiments were done in duplicate. D- or z-values for *B. burgdorferi* obtained from cultures treated in three different ways were compared by analysis of variance and least significant difference (LSD), using SAS Statistical Software.

RESULTS AND DISCUSSION

BSK medium solidified with agarose permits growth of *B. burgdorferi* as a lawn rather than discrete colonies *(3)*. Although Kurtti et al. *(17)* reported formation of colonies when the pathogen was grown on a solidified medium, such colonies were generally small, some were diffuse, and required an incubation period of 3 to 4 wk for accurate enumeration. Because of these reported difficulties in obtaining countable colonies of *B. burgdorferi*, we used the MPN technique to estimate numbers of viable cells before and after heat treatments.

In the first part of the study, thermal destruction of cells of *B. burgdorferi* of different physiological states was studied. Our results show that numbers of *B. burgdorferi* decreased linearly during the course of heating. Examples of survivor curves are in Fig. 1. Calculated D-values for *B. burgdorferi* obtained from the three different cultures (Fig. 2) were analyzed statistically. Results of the analysis indicate that cultural conditions significantly ($p<0.01$) affected resistance of *B. burgdorferi* to heat. Cells of *B. burgdorferi* incubated at 34°C for 12 h before heating (C1 culture) had the greatest heat resistance (Fig. 2). Cells of this cultures were in the lag phase (data are not shown). White *(20)* found that resistance of *Streptococcus faecalis* to heat was variable during the phases of growth and it increased appreciably during the lag phase. *B. burgdorferi* incubated at 34°C for 7 d (C2 culture) before heat-treatment had significantly (LSD at 5% level) lower D-values than the C1 culture. The *B. burgdorferi* culture that was incubated at 34°C for 7 d and then refrigerated at 4°C for 3 d (C3 culture) had significantly (LSD at 5% level) lower D-values than did the C2 culture. Refrigerated storage may have stressed cells in the C3 culture so they became more susceptible to heat than those of the other cultures.

Results of a comparison of D-values of *B. burgdorferi* with those previously reported for other foodborne patho-

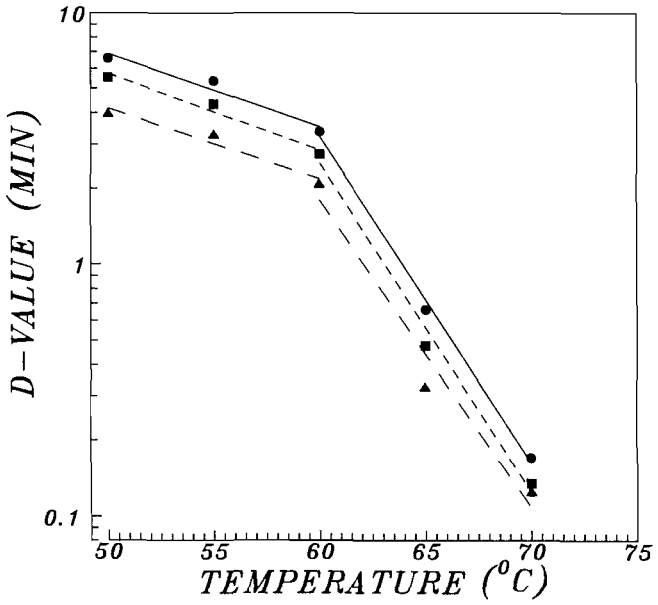

Figure 2. *Thermal death curves for* B. burgdorferi *at three differnt cultural conditions.*
●---● Culture incubated at 34°C for 12 h.
■---■ Culture incubated at 34°C for 7 d.
▲--▲ Culture was incubated at 34°C for 7 d and then refrigerated at 4°C for 3 d.

gens should be cautiously interpreted because, *(a)* D-values for the foodborne pathogens were determined in food or media different than that we used with *B. burgdorferi*, and *(b)* D-values for the other pathogens at certain temperatures were not reported, so had to be estimated by extrapolating reported data. The $D_{50°C}$ for *B. burgdorferi* were 4.0-6.95 min, depending on condition of the culture. These values are less than those of *Listeria monocytogenes (5,13), Salmonella* spp., and *Staphylococcus aureus (1)*, but are similar to those of *Campylobacter jejuni (16)*. The $D_{70°C}$ of *B. burgdorferi* is greater than that of *L. monocytogenes (5,13), C. jejuni (16), Salmonella manhattan,* and *S. aureus (1)*, but less than that of a heat-resistant strain of *Salmonella senftenberg (1)*.

When the $\log_{10}$ D-value was plotted against heating temperature, we obtained a series of points that were not linear (Fig. 2). It is evident that there were two temperature ranges for heat-inactivation of *B. burgdorferi;* 50-60°C (R1) and 60-70°C (R2). The pathogen was more sensitive to changes in temperature within the second than the first range. This discontinuity in the thermal destruction curve suggests that the mechanism of inactivating *B. burgdorferi* by heat in the two ranges of heat-treatment is different. A similar observation was reported for *L. monocytogenes* at temperatures ranging from 4 to 75°C *(22)*. Because of this discontinuity in the thermal destruction curve, two series of z-values (corresponding to the two ranges of heat-treatments) were calculated (Table 1). Results of statistical analysis indicate that z-values for cells obtained from the three cultural conditions were not significantly different ($p>0.1$) within each of the two ranges (R1 or R2) of temperature. z-Values of *B. burgdorferi* obtained in this study (Table 1) are larger than those reported for many nonsporeforming bacteria (4 to 6°C) *(14)*. These high z-values for *B. burgdorferi* indicate that the pathogen is less sensitive to changes in temperatures than many nonsporeforming bacteria.

The Code of Federal Regulations indicates that cooked beef and poultry products should be heated to an internal temperature of 62.8 and 71.1°C, respectively *(12)*. Results of several studies show that these cooking temperatures may be insufficient to inactivate nonsporeforming pathogens (of similar or less heat resistance than *B. burgdorferi*) in meat and poultry products. For example, cooking chicken breasts at 71.1°C decreased the population of *L. monocytogenes* by only 1.8-1.9 orders of magnitude *(11,15)*. *L. monocytogenes* in processed meat products was more resistant to heat-processing than when present in meat extract broth or other liquid media. To inactivate large populations of *L. monocytogenes*, processed meat products needed such high heat-treatments that the products were organoleptically unacceptable *(2)*. Assuming that *B. burgdorferi* in meat behaves like other nonsporeforming pathogens, the spirochete, if present in large populations, may survive heat-treatments normally used during cooking of meat and meat products. The estimated $D_{71.7°C}$ for *B. burgdorferi* is 4.0 to 5.87 s, depending on the physiological condition of the cells. Therefore, a heat-treatment similar to that used to pasteurize milk by the High-Temperature Short-Time method (HTST, at 71.7°C for 15 s) is only a 2.6- to 3.8-D process for *B. burgdorferi*. These results, however, should be considered cautiously since *B. burgdorferi* may behave differently during heating of foods rather than in a culture medium.

Foods that may harbor *B. burgdorferi* (e.g. meat) usually have pH values lower than that of BSK-II medium (7.6) that we used in this study. Therefore, we also determined the combined effect of heat and different pH values. The heating menstruum was adjusted to different pH values, and inoculated with *B. burgdorferi*. The pathogen in

TABLE 1. *z-values of* Borrelia burgdorferi *given three different treatments before heating.*

Culture	Temperature range	Replicate 1	Replicate 2	Average
C1[a]	50-60	35.5	33.1	34.3
	60-70	7.7	7.71	7.71
C2[b]	50-60	28.7	37.6	33.2
	60-70	7.69	7.48	7.58
C3[c]	50-60	33.2	37.8	35.5
	60-70	8.30	7.87	8.09

[a]Culture incubated at 34°C for 12 h.
[b]Culture incubated at 34°C for 7 d.
[c]Culture incubated at 34°C for 7 d and then refrigerated at 4°C for 3 d.

this pH-adjusted medium was heat-treated at 65°C. Averages for $D_{65°C}$ were 0.42, 0.39, and 0.35 min at pH 7.6, 6.5, and 5.0, respectively. $D_{65°C}$ values at the different pH-values were not significantly different ($p>0.05$), indicating that sensitivity of *B. burgdorferi* to heat did not change markedly when the pH was varied in the range of 5.0 to 7.6.

In conclusion, resistance of *B. burgdorferi* to heat was generally less at 50°C, and greater at 70°C than that of other nonsporeforming pathogens. Sensitivity of the pathogen to heat was affected by the physiological condition of the cells, but not by pH in the range of 5.0 to 7.6. Heat treatments similar to that of HTST pasteurization of milk are expected to decrease the population of *B. burgdorferi* by only 2.6-3.8 orders to magnitude. If *B. burgdorferi* behaves like other nonsporeforming pathogens when present in meats in large numbers, it is likely that the spirochete will survive heat-treatments sometimes used to process these products.

ACKNOWLEDGMENTS

Research supported by the College of Agricultural and Life Sciences, the Doosan Research Laboratory of Korea through support of Si K. Lee and the Center for Dairy Research of the University of Wisconsin-Madison. We thank Dr. E. C. Burgess, School of Veterinary Medicine, University-Madison, for providing the spirochete isolate.

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