

Sporulation of *Clostridium perfringens* Type A in Vacuum-Sealed Meats

F. M. DEWORK, JR.

Departments of Food Science and Microbiology, Clemson University, Clemson, South Carolina 29631

Received for publication 19 July 1972

Type A strains of *Clostridium perfringens* inoculated into vacuum-sealed packages of hamburger, roast beef, ground beef plus cream sauce, and turkey roll produced high levels of spores.

Clostridium perfringens and staphylococci are the leading causative agents of food illness in the United States (7). It is believed that *C. perfringens* sporulate only to a limited extent in meat products (1, 6). Vacuum-sealing of meats and meat products has become a common method of preservation, and a study of sporulation by *C. perfringens* in vacuum-sealed meats was undertaken (presented in part at the 70th Annual Meeting of the American Society for Microbiology, 26 April to 1 May 1970, Boston, Mass.).

Type A strains of *C. perfringens* (BP6K, 2617, and Hobbs type 1) were obtained from V. R. Dowell, Center for Disease Control, Atlanta. Stock cultures were grown at 37 C for 24 hr in Robertson cooked meat medium (Difco) and stored at 5 C.

The inoculum was prepared by growing the desired strain in thioglycolate broth (Difco) for 5 hr at 46 C. This medium supported only vegetative growth. The cells were harvested by centrifugation, washed with 0.067 M phosphate-peptone (0.1%) buffer, and suspended in the same buffer to give an optical density at 600 nm of 0.6. This suspension was diluted 100-fold and yielded approximately 3×10^5 cells/ml.

The meats (roast beef, hamburger, turkey roll, and hamburger plus cream sauce in 94- to 113-g portions) had been previously cooked (2) and held at 0 C in vacuum-sealed packages formed from polyethylene laminate film (R-525 and R-540, W. R. Grace Co.) which has low permeability to oxygen. The pouches were aseptically opened, and each meat sample was injected with a total of 1.0 ml of inoculum. Approximately 0.16-ml portions of the inoculum were injected at six evenly spaced locations. The meats were resealed under vacuum and incubated at the appropriate temperature for various times.

Using aseptic conditions, spore counts were made as follows. The contents of a vacuum-sealed package were mixed in a Waring blender at low speed for 1 min with 200 ml of phosphate-peptone buffer. A 10-ml portion of the slurry was held at 80 C for 20 min, diluted decimally with buffer, and plated in duplicate on sulfite polymixing sulfadiazine agar (Difco) containing 0.1% starch. Plates were incubated in Brewer Anaerobic Jars in an atmosphere of 80% N₂-10% CO₂-10% H₂ at 37 C for 24 hr, and black colonies were counted. Representative black colonies were picked and confirmed to be *C. perfringens* if the organisms were gram-positive, nonmotile rods which demonstrated lecithinase production on McClung-Toabe egg yolk agar; produced nitrite from nitrate; and showed β -hemolysis on blood agar (4, 9).

Temperature is a critical factor for sporulation of *Clostridium* and *Bacillus* species (8). Vacuum packages of hamburger meat inoculated with strain BP6K or 2617 were incubated at 25, 37, and 46 C. At various times, duplicate packages were examined for viable spore counts, and the results are shown in Table 1. Highest spore crops were obtained at 37 C, and this incubation temperature was used thereafter.

Vacuum-sealed packages of turkey roll, roast beef, hamburger, and hamburger plus cream sauce were inoculated with strains BP6K, 2617, or Hobbs type 1, and incubated at 37 C. At various times, the meats were examined for spore production. The results are shown in Table 2. For all strains and meats tested, spore production was essentially complete after 12 to 24 hr of incubation. Spore production was highest in hamburger for all three strains of *C. perfringens* tested. For all strains, spore production was at least 100-fold less in turkey than in hamburger. Spore production by

Hobbs 1 and BP6K strains was similar, whereas strain 2617 generally showed lower sporulation in all meats. Vegetative culture counts of *C. perfringens* (no heat treatment) were from 3×10^7 to 8×10^7 cells/g after 24 hr of incubation. The vegetative and spore counts in uninoculated, cooked, control samples never exceeded 5/g at any time during the incubation period. This low count of *C. perfringens* was probably due to the prior cooking treat-

ment. Uninoculated, raw hamburger meat obtained from two local supermarkets had vegetative counts of 300 to 2,000 cells/g.

This study has shown that high levels of *C. perfringens* spores can occur in meat products. The type of meat is important, and precaution must be taken in the holding time and temperature of beef products, as these meats are frequently involved in *C. perfringens* food illness (5). Stews, casseroles, and chili are often stored and reheated. If sporulation has occurred, reheating could heat shock the spores and allow germination and growth of *C. perfringens* to possible infective levels (3). This could be a problem in the food service industry. The fact that spores were produced in high numbers in beef and turkey products establishes the need for reexamination of spore production by *C. perfringens* in other food products.

This investigation was supported by Public Health Service traineeship ITIES60-03 from the Department of Health, Education, and Welfare.

I thank M. J. B. Paynter and M. G. Johnson for their critical and constructive review of the manuscript.

LITERATURE CITED

1. Barnes, E. M., J. E. Despaul, and M. Ingram. 1963. The behaviour of a food poisoning strain of *Clostridium welchii*. *J. Appl. Bacteriol.* **26**:415-427.
2. Bjorkman, A., and K. A. Delphin. 1966. Sweden's Nacha Hospital food system centralizes preparation and distribution. *Cornell H.R.A. Quart.* **31**:84-87.
3. Dishe, F. E., and S. D. Elek. 1957. Experimental food poisoning by *C. welchii*. *Lancet* **2**:71-74.
4. Dowell, W. R., and T. M. Hawkins. 1968. Laboratory

TABLE 1. Effect of incubation temperature on sporulation of *Clostridium perfringens* strains BP6K and 2617 in vacuum-sealed packages of cooked hamburger incubated at 25, 37, and 46 C

Strain	Time after inoculation (hr)	Spores/g ^a		
		25 C	37 C	46 C
BP6K	5		1.1×10	0
	9		4.3×10	2
	15	1.3×10	1.2×10^3	12
	24		5.0×10^3	32
	48	8.4×10^3	7.1×10^3	35
	96	2.3×10^3		
	120	2.5×10^3		
2617	5		2.0×10	0
	10	3.4×10	4.0×10	0
	19		7.0×10^3	7
	21	7.0×10^2		21
	27		2.3×10^4	23
	45	2.1×10^3		25
	72	1.7×10^3		

^a Mean of duplicates.

TABLE 2. Spore production by *C. perfringens* at 37 C in vacuum-sealed meat products^a

Strain		Time (hr)					
		8	10	12	18	24	45
BP6K	Hamburger		1.3×10^4	4.1×10^4	4.1×10^5	8.7×10^5	1.4×10^6
	Hamburger plus cream sauce		6.1×10^3	7.4×10^3	1.3×10^4	1.4×10^5	1.5×10^5
	Roast beef		4.2×10^3	2.1×10^4	2.4×10^5	2.5×10^5	2.5×10^5
	Turkey		2.5×10^3	3.7×10^3	1.1×10^4	1.2×10^4	1.3×10^4
2617	Hamburger	7.8×10	5.8×10^2	4.1×10^3	2.8×10^4	4.7×10^4	
	Hamburger plus cream sauce	5.3×10	1.2×10^2	4.0×10^2	3.8×10^3	7.1×10^3	
	Turkey	4.6×10	8.2×10	3.6×10^2	4.2×10^2	4.4×10^2	
Hobbs type L	Hamburger	3.2×10^2	1.1×10^3	8.2×10^4	6.3×10^5	8.1×10^5	
	Hamburger plus cream sauce	1.1×10^2	4.6×10^3	7.7×10^4	9.5×10^4	8.9×10^4	
	Roast beef	5.1×10	1.3×10^2	10^3	9.0×10^3	8.9×10^3	
	Turkey	3.5×10	8.8×10	6.1×10^2	6.6×10^3	6.6×10^3	

^a Spores/g, mean of duplicate counts on two packages.

- methods in anaerobic bacteriology. U.S. Dept. H.E.W. Public Health Service Publ. 1803.
5. Hall, H. E., and R. Angelotti. 1965. *Clostridium perfringens* in meat and meat products. Appl. Microbiol. 13:352-357.
 6. Kim, C. H., R. Cheney, and M. Woodburn. 1967. Sporulation of *C. perfringens* in a modified medium and selected foods. Appl. Microbiol. 15:871-876.
 7. Center for Disease Control. 1971. Foodborne outbreaks annual summary. Center for Disease Control, Atlanta.
 8. Ordal, Z. J. The effect of nutritional and environmental conditions on sporulation, p. 18-26. 1957. In H. O. Halvorsen, (ed.), Spores Publ. no. 5. American Institute of Biological Sciences, Washington, D.C.
 9. Thatcher, F. S., and D. S. Clark. 1968. Microorganisms in food. Univ. of Toronto Press., Toronto, Canada.