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Review

Clostridium sporogenes PA 3679 and Its Uses in the Derivation of Thermal Processing Schedules for Low-Acid Shelf-Stable Foods and as a Research Model for Proteolytic *Clostridium botulinum*

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ABSTRACT

The putrefactive anaerobe *Clostridium sporogenes* PA 3679 has been widely used as a nontoxigenic surrogate for proteolytic *Clostridium botulinum* in the validation of thermal processes for low-acid shelf-stable foods, as a target organism in the derivation of thermal processes that reduce the risk of spoilage of such foods to an acceptable level, and as a research model for proteolytic strains of *C. botulinum*. Despite the importance of this organism, our knowledge of it has remained fragmented. In this article we draw together the literature associated with PA 3679 and discuss the identity of this organism, the phylogenetic relationships that exist between PA 3679 and various strains of *C. sporogenes* and proteolytic *C. botulinum,* the heat resistance characteristics of PA 3679, the advantages and limitations associated with its use in the derivation of thermal processing schedules, and the knowledge gaps and opportunities that exist with regard to its use as a research model for proteolytic *C. botulinum*. Phylogenetic analysis reviewed here suggests that PA 3679 is more closely related to various strains of proteolytic *C. botulinum* than to selected strains, including the type strain, of *C. sporogenes*. Even though PA 3679 is demonstrably nontoxigenic, the genetic basis of this nontoxigenic status remains to be elucidated, and the genetic sequence of this microorganism appears to be the key knowledge gap remaining to be filled. Our comprehensive review of comparative heat resistance data gathered for PA 3679 and proteolytic strains of *C. botulinum* over the past 100 years supports the practice of using PA 3679 as a (typically fail-safe) thermal processing surrogate for proteolytic *C. botulinum*.

For the purpose of thermal processing to eliminate spore-forming bacteria, foods may be grossly divided into two categories on the basis of their pH. While the specific details of thermal processing requirements and the exact definitions of ''low acid'' versus ''acid'' and ''acidified'' foods vary from country to country, foods with a pH of 4.6 or less generally receive relatively mild thermal processes. This is because the concentration of hydrogen ions is sufficient to prevent the germination and outgrowth of most spores, particularly after thermal processing. By comparison, foods with a pH greater than 4.6 must be given more severe heat treatments that reduce to an acceptable level the risk posed by *Clostridium botulinum,* the most heat-resistant pathogenic (toxigenic) bacterial sporeformer, assuming that growth of this pathogen is not otherwise controlled in the food, for example by water activity *(66, 101)*.

In the derivation of adequate thermal processing schedules for foods supporting the growth of *C. botulinum,* knowledge of the thermal death characteristics of this organism is fundamental. Data describing the time necessary to destroy spores of *C. botulinum* at various temperatures began to enter the literature in 1919 *(12, 23),* and in 1922 Esty and Meyer *(25),* having conducted a comparative study of a large number of strains of *C. botulinum* from diverse habitats, published a destruction curve describing the thermal resistance of spores of the most resistant strains of *C. botulinum*. However, independent research groups encountered difficulties in producing such heat-resistant spore crops *(5, 90, 99)*. This, together with the fact that it was unsafe to bring *C. botulinum* into commercial processing plants for the inoculated pack studies that were recommended as a means of validating calculated processes, acted to drive the search for a nontoxigenic surrogate for proteolytic *C. botulinum*. Thus, when a nontoxigenic putrefactive anaerobe, PA 3679, whose spores had a heat resistance in excess of the maximum recorded for spores of *C. botulinum* was isolated in the research laboratories of the National Canners Association (NCA), it was rapidly adopted as a surrogate for proteolytic *C. botulinum* in inoculated pack studies *(56, 99)*.

While heat treatments that assure the destruction of proteolytic *C. botulinum* spores are adequate to protect human health, more substantial processes designed to achieve commercial sterility are required to prevent the spoilage of ambient-stable, low-acid foods. In instances in which thermophilic spoilage has not been a concern, PA 3679 has often been used as the target organism *(94, 101)*

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and, more recently, as one of a number of target organisms *(104)*. Processes based on resistance values observed for spores of PA 3679 typically provide a considerable, albeit ill-defined, safety margin with respect to the destruction of spores of proteolytic *C. botulinum*.

In addition to its use as a surrogate for proteolytic *C. botulinum* and as a target organism for the production of commercially sterile low-acid foods, PA 3679 is sometimes used as a research model for proteolytic *C. botulinum* by laboratories interested in fundamental aspects of the heat resistance and thermal inactivation of spores of proteolytic *C. botulinum (51, 52)*. This is due to the health hazard associated with working with *C. botulinum* and to requirements pertaining to biosecurity *(4, 17)* that, in some countries, prevent most laboratories from working with *C. botulinum*.

Here we review for the first time the literature associated with PA 3679, discussing the origin and identity of this important organism and its relationship to various strains of *Clostridium sporogenes* and proteolytic *C. botulinum*. The heat resistance of spores of PA 3679 is compared with that of proteolytic strains of *C. botulinum,* and the advantages and limitations associated with the use of PA 3679 in the derivation of thermal processing schedules for low-acid shelf-stable foods are considered. Finally, we highlight the knowledge gaps and opportunities that exist with regard to the use of PA 3679 as a research model for proteolytic *C. botulinum*.

THE ORIGIN AND IDENTITY OF PA 3679

Putrefactive anaerobe no. 3679 was isolated by E. J. Cameron in 1927 from spoiled canned corn in the research laboratories of the NCA (Cameron as cited by *(99)*). PA 3679, as it became known, was described as being morphologically more similar to *C. botulinum* than any other organism *(5)*. However, its identity was not quickly established. While Cameron is cited as stating that PA 3679 resembled *C. sporogenes* but was probably not identical to it *(34)* and Gross et al. *(31)* indicated that PA 3679 was culturally and serologically distinct from *C. sporogenes* (Spray), Williams *(108)* and Sognefest and Benjamin *(87)* referred to PA 3679 as a strain of *C. sporogenes*. Subsequently, Michener *(58)* stated that PA 3679 was an unnamed strain that resembles *C. botulinum,* and Brown et al. *(10)* that ''while the taxonomic position of PA 3679 is not entirely clear, it is closely related to *C. sporogenes*.'' Echoing these sentiments, Fujioka and Frank *(27)* stated that PA 3679 is probably closely related to *C. sporogenes* but that its taxonomic position has never been clearly established.

The identity of PA 3679 was undoubtedly further confounded by researchers who isolated putrefactive anaerobes from other sources and, finding them to be culturally similar to PA 3679, began to refer to them as PA 3679 or as strains of PA 3679. For example, Gross et al. *(31)* isolated a putrefactive anaerobe, originally designated S_2, from spoiled canned meat. They reported that S_2 and PA 3679 were serologically and culturally identical, despite the fact that the two organisms exhibited different responses in the fermentation of sucrose. Subsequently S_2 was referred to as S_2 (PA 3679) by Gross et al. *(32),* as PA 3679 by Steinkraus and Ayres *(92),* and as PA 3679/S2 and PA 3679 by Roberts et al. *(74)*. Moreover, C. R. Stumbo, who was an author on the original paper describing S_2, subsequently published data pertaining to the heat resistance of PA 3679 in various foodstuffs, without identifying the original source of the organism *(95)*. In a similar manner to Gross et al. *(31),* Steinkraus and Ayres *(92)* isolated a putrefactive anaerobe from pork trimmings with biochemical reactions like those of PA 3679 and designated it *Cl.* species PA 3679. By 1982, a number of ''strains of PA 3679'' were in use, including 174, 194, 1075, 1077, PA 3679 SF, PA 3679 W, PA 3679 S_2, and PA 3679 h *(75)*.

Although no publication has been found specifically illustrating that PA 3679 is a strain of *C. sporogenes,* PA 3679 is now widely regarded as a strain of that species. This situation probably stems from the fact that many laboratories have obtained their cultures of PA 3679 from the American Type Culture Collection (ATCC), which holds it as a strain of *C. sporogenes,* or from one of the other culture collections that have obtained it from the ATCC. The ATCC has indicated that PA 3679 was deposited by the NCA in 1941, or earlier, as *C. sporogenes (111)*. 16S rRNA sequencing of PA 3679 obtained from the ATCC (ATCC 7955) was recently conducted in our laboratories, and Basic Local Alignment Search Tool analysis indicated that the sequence obtained exhibited 99% identity with that of a number of *C. sporogenes* strains as well as that of *C. botulinum* A str. Hall and *C. botulinum* A str. ATCC 19397 (data not shown). On the basis of that finding, and the fact that PA 3679 is nontoxigenic by mouse bioassay *(31),* we can state that according to the definition of the Judicial Commission of the International Committee on Systematic Bacteriology *(40)* PA 3679 is a strain of *C. sporogenes*.

RELATIONSHIPS BETWEEN PA 3679, OTHER STRAINS OF *C. SPOROGENES,* AND PROTEOLYTIC STRAINS OF *C. BOTULINUM*

As species, *C. sporogenes* and *C. botulinum* are heterogeneous, and consequently the degree of genetic relatedness between individual members of the two species is variable. While *C. botulinum* (nonproteolytic) was first recognized and isolated in 1896 by Emile van Ermengem *(102)* as the causative agent in an outbreak of botulism, *C. sporogenes* was described by Metchnikoff *(57)* as an inhabitant of the intestinal contents of healthy individuals and was thus considered, initially, to be nontoxigenic. During the years that followed, numerous morphologically and culturally similar anaerobic organisms were isolated from diverse habitats, and the toxigenic capabilities of the two species quickly became a source of confusion. Some researchers reported the occurrence of toxigenic strains of *C. sporogenes (68, 105, 107),* others the occurrence of nontoxigenic strains of *C. botulinum (34, 43, 45, 56, 71, 89, 98)*. Still others reported the occurrence of strains of *C. botulinum* or *C. sporogenes* that were toxigenic when

TABLE 1. $D_{121°C}$- *and* z*-values for PA 3679 suspended in phosphate buffer and selected foodstuffs*

Substrate	$D_{121°C}$-value (min)	*z*-value (°C)	pH	Reference
Phosphate buffer	0.81[a]		7.00	*88*
	2.033	10.50	7.00	*24*[b]
	1.984	9.89	7.00	*24*[c]
	3.50	10.67	7.00	*24*[d]
	0.62	12.00	7.00	*48*
	0.75		7.00	*49*
	0.70[a]	9.44	7.00	*99*[e]
	1.45[a]	11.89	7.00	*72*[f]
	0.92		7.00	*56*
		10.3		*59*
	1.3	11.67		*103*
	2.01[a]	12.78		*2*
	2.6	14.0	7.00	*15*
	1.1	11.0	5.00	*15*
	2.61	9.5	7.00	*77*[g]
	0.189[h]	9.11	7.00	*28*
	1.2	10.5	7.00	*28*
	1.51[h]	15.1	7.00	*30*[i]
	1.65	12.6	7.00	*76*
	1.43[j]	11.67		*73*
	1.37[a]	11.11	7.00	*97*
Artichoke (puree)	0.36	8.3	5.20	*76*
Asparagus	0.515[a]	11.36	5.11	*99*[e]
	0.88[k]	8.89[k]		*73*[l]
	0.50[k]			*73*[m]
	0.47[k]			*73*[n]
	1.50[j]	11.67[j]		*73*[l]
	1.10[j]	11.11[j]		*73*[m]
	1.14[j]	11.56[j]		*73*[n]
		7.44[k]		*41*
		10.50[j]		*41*
Asparagus (puree)	0.70[a]	8.89	6.70	*88*
	1.48	9.28	5.88	*78*
Asparagus (frozen)	0.357[h]	12.44	6.15	*24*[b]
	0.445[h]	11.11	6.15	*24*[c]
	0.53[a]	13.67	6.15	*24*[d]
Asparagus (canned)	0.364[h]	12.17	5.65	*24*[b]
	0.403[h]	11.61	5.65	*24*[c]
	0.51[a]	13.39	5.65	*24*[d]
Beans, green	0.70[a]	9.39	6.60	*72*[f]
Beans, green (fresh)	0.872[h]	10.22	5.85	*24*[b]
	0.387[h]	12.94	5.85	*24*[c]
	0.80[a]	12.78	5.85	*24*[d]
	0.932	9.00	6.00	*24*[b]
	0.997	9.50	6.00	*24*[c]
	1.50[a]	10.89	6.00	*24*[d]
Beans, green (puree)	0.54[a]	10.00	6.10	*88*
	2.00[a]	14.78	5.20	*72*[f]
Beans, green (frozen)	0.398[h]	12.67	6.03	*24*[b]
	0.459[h]	12.28	6.03	*24*[c]
	0.75[a]	12.33	6.03	*24*[d]
Beans, green (canned)	0.402[h]	12.22	5.82	*24*[b]
	0.436[h]	12.00	5.82	*24*[c]
	0.71[a]	11.06	5.82	*24*[d]
	0.751	12.33	5.70	*24*[b]
	0.769	9.89	5.70	*24*[c]
	1.08[a]	10.89	5.70	*24*[d]
	0.288	9.44		*84*
Beans, lima	2.63[k]	8.06[k]		*73*[l]
	1.74[k]	7.50[k]		*73*[m]

TABLE 1. *Continued*

Substrate	$D_{121°C}$-value (min)	*z*-value (°C)	pH	Reference
	1.49[k]	6.67[k]		*73*[n]
		6.56[k]		*41*
	2.56[j]	10.72[j]		*41*
Beans, snap	0.63[k]	11.39[k]		*73*[l]
	0.26[k]			*73*[m]
	0.26[k]	7.78[k]		*73*[n]
	1.68[j]	12.78[j]		*73*[l]
	1.07[j]	10.28[j]		*73*[m]
	1.05[j]	10.28[j]		*73*[n]
		7.89[k]		*41*
		10.56[j]		*41*
Beans, snap brine	0.79[a]	12.78	5.20	*97*
Beef	2.23[j]	11.78[j]		*41*
Beets	0.83[k]	11.94[k]		*73*[l]
	0.48[k]	10.83[k]		*73*[m]
	0.53[k]	10.56[k]		*73*[n]
	1.70[j]	11.11[j]		*73*[l]
	1.18[j]	10.56[j]		*73*[m]
	1.28[j]	10.56[j]		*73*[n]
		10.50[k]		*41*
		10.61[j]		*41*
Beets (puree)	0.69[a]	10.14	6.20	*88*
Beets (canned)	0.337	10.39		*84*
Carrots	0.47[k]	8.89[k]		*73*[l]
	0.34[k]	9.72[k]		*73*[m]
	0.37[k]	10.28[k]		*73*[n]
	1.50[j]	11.11[j]		*73*[l]
	0.79[j]	8.61[j]		*73*[m]
	0.87[j]			*73*[n]
		10.33[k]		*41*
		10.28[j]		*41*
Carrots (fresh)	1.115	10.22	6.18	*24*[b]
	1.000	10.11	6.18	*24*[c]
	1.33[a]	10.89	6.18	*24*[d]
Carrots (canned)	0.777	10.89	5.48	*24*[b]
	0.794	10.44	5.48	*24*[c]
	1.18[a]	11.06	5.48	*24*[d]
Corn	2.50[k]	10.28[k]		*73*[l]
	2.11[k]			*73*[m]
	2.14[k]	10.83[k]		*73*[n]
		11.11[k]		*41*
	2.00[j]	11.00[j]		*41*
Corn (whole kernel)	0.7[a]	10.78		*99*[e]
	1.18	9.22		*95*
Corn, white (puree)	0.91[a]	9.44	7.10	*88*
Corn, yellow (puree)	1.72[a]	11.67	6.80	*88*
Corn (frozen)	0.414[h]	12.11	7.20	*24*[b]
	0.485[h]	11.50	7.20	*24*[c]
	0.65[a]	12.22	7.20	*24*[d]
	1.099	9.89	6.64	*24*[b]
	1.033	10.56	6.64	*24*[c]
	1.63[a]	10.50	6.64	*24*[d]
Corn (frozen puree)	0.60[a]	8.56	7.98	*72*[f]
Corn (canned)	0.478[h]	11.39	6.48	*24*[b]
	0.410[h]	11.83	6.48	*24*[c]
	0.58[a]	13.17	6.48	*24*[d]
Corn, brine	1.35[a]	5.56	6.10	*97*
Cream	1.09[a]	12.83	6.30	*99*[e]
Cream (20% unsterilized)	1.52	10.44		*95*
Custard pudding, strained	0.328	9.56		*84*

TABLE 1. *Continued*

Substrate	$D_{121°C}$-value (min)	*z*-value (°C)	pH	Reference
Eggs (scrambled)	0.76		7.00	*48*
Milk, skim (17.69% solids)	0.89[a]	11.33	6.45	*99*[e]
Milk, skim (19.43% solids)	0.53[a]	10.78	6.26	*99*[e]
Milk, whole (25.61% solids)	1.20[a]	14.11	6.36	*99*[e]
Milk, whole (26.78% solids)	0.59[a]	10.83	6.24	*99*[e]
Milk (evaporated, presterilized)	1.29	10.89		*95*
Milk (evaporated, unsterilized)	1.425[h]	11.39		*95*
Mushrooms	1.45[k]	8.06[k]		*73*[l]
	1.22[k]	9.17[k]		*73*[n]
	1.45[j]	7.5[j]		*73*[l]
	0.61[j]			*73*[m]
	1.16[j]	7.22[j]		*73*[n]
	1.74[k]	12.22[k]		*41*
	1.98[j]	9.28[j]		*41*
Mushroom (extract)	1.50	9.59	6.65	*77*
Okra	0.53[k]	8.06[k]		*73*[l]
	0.34[k]	7.78[k]		*73*[n]
	1.18[j]	11.94[j]		*73*[l]
	0.69[j]			*73*[m]
	0.65[j]			*73*[n]
		7.50[k]		*41*
		9.33[j]		*41*
Peas	0.77[a]	9.39	5.98	*99*[e]
	1.5[k]	8.06[k]		*73*[l]
	1.20[k]	8.06[k]		*73*[m]
	1.12[i]	8.61[k]		*73*[n]
		8.83[k]		*41*
	2.23[j]	9.89[j]		*41*
Peas (fresh)	2.55[a]	13.33		*72*[f]
Peas (puree)	1.10[a]	8.89	6.60	*88*
	3.1	12.2	7.00	*15*
	1.0	9.2	5.00	*15*
	1.52	9.61		*95*
	1.67			*44*
Peas (frozen)	2.08[a]	10.44	7.10	*72*[f]
	0.955[h]	10.44	6.95	*24*[b]
	0.949[h]	10.56	6.95	*24*[c]
	1.05[a]	12.22	6.95	*24*[d]
	0.629[h]	11.67	6.75	*24*[b]
	0.748[h]	11.06	6.75	*24*[c]
	1.13[a]	11.50	6.75	*24*[d]
Peas (frozen puree)	2.35[a]	11.78	6.98	*72*[f]
Peas (canned)	0.430	10.00		*84*
	0.335[h]	12.78	6.27	*24*[b]
	0.407[h]	11.33	6.27	*24*[c]
	0.79[a]	12.56	6.27	*24*[d]
Peas (canned puree)	1.681	9.44		*93*
Pea, brine	1.53[a]	11.67	5.70	*97*
Pork	2.14[j]	10.17[j]		*41*
Pork (fresh uncooked)	1.39	10.00		*95*
Pork (cooked)	1.21	9.72		*95*
Pork (canned)	0.508	9.72		*84*
Potatoes (mashed)	0.61	10.00	6.30	*48*
Pumpkin	0.55[k]	10.56[k]		*73*[l]
	0.40[k]	9.44[k]		*73*[n]
	1.50[j]	11.11[j]		*73*[l]
		10.00[j]		*73*[m]
	1.09[j]	10.83[j]		*73*[n]
		9.44[k]		*41*
		10.67[j]		*41*

TABLE 1. *Continued*

Substrate	$D_{121^\circ C}$-value (min)	*z*-value (°C)	pH	Reference
Shrimp	1.68[a]	11.89	7.63	*72*[f]
Spinach	0.67[a]	10.11	5.39	*99*[e]
	1.23[a]	10.17	6.80	*72*[f]
	1.25[k]	10.00[k]		*73*[l]
	1.00[k]	10.00[k]		*73*[m]
	1.09[k]	10.00[k]		*73*[n]
	2.40[j]	11.39[j]		*73*[l]
	1.73[j]	9.72[j]		*73*[m]
	1.69[j]	10.28[j]		*73*[n]
		10.61[k]		*41*
		10.11[j]		*41*
Spinach (puree)	2.33[a]	12.72	6.18	*72*[f]
Spinach (frozen)	0.908[h]	11.11	6.69	*24*[b]
	0.883[h]	11.44	6.69	*24*[c]
	1.75[a]	10.44	6.69	*24*[d]
	0.997	9.72	6.56	*24*[b]
	1.100	10.00	6.56	*24*[c]
	1.40[a]	11.33	6.56	*24*[d]
Spinach (canned)	0.788[h]	10.28	5.55	*24*[b]
	0.731[h]	10.94	5.55	*24*[c]
	1.05[a]	11.83	5.55	*24*[d]
Soup, chicken (canned)	0.274	9.56		*84*
Squash		11.50[k]		*41*
		12.28[j]		*41*
	0.95[k]	11.11[k]		*73*[l]
Squash, Hubbard (fresh)	0.208[h]	14.72	5.73	*24*[b]
	0.270[h]	12.78	5.73	*24*[c]
	0.35[a]	15.39	5.73	*24*[d]
Squash, summer	0.60[k]			*73*[m]
	0.66[k]	11.11[k]		*73*[n]
	2.05[j]	12.22[j]		*73*[l]
	1.32[j]	10.28[j]		*73*[m]
	1.42[j]			*73*[n]
Squash (strained)	0.85	10.11		*95*
Sweet potato	1.45[k]	15.28[k]		*73*[l]
	0.85[k]	13.33[k]		*73*[m]
	0.84[k]	11.39[k]		*73*[n]
	1.90[j]	11.11[j]		*73*[l]
	1.22[j]	11.39[j]		*73*[m]
	1.00[j]	8.33[j]		*73*[n]
		10.61[k]		*41*
		8.61[j]		*41*
Sweet potato (puree)	0.7[a]	8.89	5.60	*88*
Sweet potato (7.5% syrup)	1.10[a]	10.39	7.00	*72*[f]
Sweet potato (50% syrup)	1.15[a]	9.94	6.67	*72*[f]
Vegetables and beef (canned)	0.37	9.89		*84*
Vegetables and bacon (canned)	0.365	9.89		*84*
Water, deionized	0.6			*1*
Water, distilled	0.80	9.78		*95*
	0.938	10.44		*24*[b]
	0.938[a]	10.56		*24*[c]
	1.20	10.56		*24*[d]
White sauce	1.23	9.22		*95*

[a] $D_{121^\circ C}$-value was calculated by us from an *F* value supplied by the original study.
[b] The original authors calculated their $D_{121^\circ C}$-values according to the methods described by Stumbo et al. *(95).*
[c] The original authors calculated their $D_{121^\circ C}$-values according to the methods described by Schmidt *(81).*
[d] The original authors obtained *F* and *z*-values by the extrapolation of survival and destruction end point curves.
[e] Data presented are based on the composite *F* and *z*-values. In instances where one or more composite values were determined, the average of the composite values is presented.
[f] Data presented are based on the *F* and *z*-values calculated by the authors using the method of least squares for 99.99% destruction.

isolated but that lost their toxigenicity or maintained a level of attenuated toxigenicity *(106)*. While some of these accounts were undoubtedly attributable to the contamination of stocks of *C. botulinum* with *C. sporogenes,* vice versa, or the contamination of stocks of *C. sporogenes* with other pathogens *(26, 36, 42, 70, 71, 91),* some argued that the so-called contaminants may in fact have been toxigenic, or nontoxigenic, variants of the same species *(7, 34, 35)*. The question of the existence of toxigenic and nontoxigenic variants of the two species was never fully resolved. Instead, toxin production was adopted as the trait used to differentiate the two species *(36, 40)*.

Today, *C. botulinum* is the taxonomic designation for a complex of anaerobic, spore-forming, rod-shaped bacteria that share the ability to produce one or more of the botulinal neurotoxins *(21)*. It comprises four phylogenetically and physiologically distinct groups of bacteria, known as *C. botulinum* groups I to IV, with the distinction between the groups considered strong enough to merit four different species *(65)*. Likewise, *C. sporogenes* is recognized as being a heterogeneous species comprising at least two variants *(60, 69)*. Phylogenetic analyses illustrate that PA 3679 and other strains of *C. sporogenes* are most closely related to group I (proteolytic) members of the *C. botulinum* complex *(21, 22, 37–39, 43)*. The relatedness of individual strains varies, however, with some strains of *C. sporogenes* being more closely related to certain strains of proteolytic *C. botulinum* than to other *C. sporogenes* strains and vice versa *(16, 37, 43, 46, 60, 109)*. With regard to PA 3679, Lee and Riemann *(46)* demonstrated that the DNA of PA 3679 is homologous with that of *C. botulinum* A62 and, thus, that the two strains are closely related. Moreover, it has been demonstrated that PA 3679 is more closely related to various strains of proteolytic *C. botulinum* than to selected strains of *C. sporogenes,* including 213, J-53, and NCIMB 10696, the type strain of *C. sporogenes (60, 83, 109)*.

A COMPARATIVE ANALYSIS OF THE HEAT RESISTANCE OF SPORES OF PA 3679 AND PROTEOLYTIC *C. BOTULINUM*

A considerable number of datasets describing the heat resistance of spores of PA 3679, and of various proteolytic strains of *C. botulinum,* have been published. However, these data have rarely, if ever, been drawn together. We have compiled these data and used the composite datasets (Tables 1 and 2) for the purposes of making a comparative analysis of the heat resistance of PA 3679 and proteolytic *C. botulinum*. While it is difficult to make direct comparisons between individual datasets, because of variations arising from the use of different strains, spore crops, heating and recovery methodologies, and methods of calculation, some general comparisons can be made. Figures 1 and 2 illustrate the maximum and mean of reported $D_{121^\circ C}$-values for PA 3679 and proteolytic *C. botulinum* derived from the composite dataset. Likewise, Figures 3 and 4 illustrate the maximum and mean of reported *z*-values.

In neutral phosphate buffer, the published $D_{121.1^\circ C}$-values for proteolytic *C. botulinum* range from 0.055 to 1.43 min, with a mean of 0.21 $\pm$ 0.27 min; and the *z*-values range from 8.1 to 14.06°C, with a mean of 9.89 $\pm$ 1.30°C. These values are in good agreement with the classical values quoted for proteolytic *C. botulinum* ($D_{121.1^\circ C}$ = 0.2 min, z = 10°C) *(6, 67, 96)*. The published $D_{121.1^\circ C}$-values for PA 3679 in neutral phosphate buffer are substantially higher than those of proteolytic *C. botulinum* (P = 3.29E−9), ranging from 0.189 to 3.5 min, with a mean of 1.51 $\pm$ 0.81 min. The published *z*-values for PA 3679 in neutral phosphate buffer range from 9.11 to 15.1°C, with a mean of 11.34 $\pm$ 1.64°C, and are thus around 1 to 1.5°C higher than those of proteolytic *C. botulinum* (P = 2.85E−3). This latter observation is in contrast with that of Townsend et al. *(99),* who reported that ''the *z*-value for No. 3679 is slightly lower than that for *Cl. botulinum* in phosphate . . . ,'' and indicates that the use of a *z*-value of 10°C for calculating process times that are designed to sterilize low-acid foods with respect to PA 3679 (as reported by Reed et al. *(72)* and Kaplan et al. *(41)*) may overestimate the influence of increasing temperature on lethal rates.

When the $D_{121.1^\circ C}$-values of PA 3679 in neutral phosphate buffer were compared with the $D_{121.1^\circ C}$-values obtained in other substrates, it was found that the maximum reported $D_{121.1^\circ C}$-value was higher in neutral phosphate buffer than in any of the other substrates examined (Fig. 1). The same was found to be true for proteolytic *C. botulinum* (Fig. 1). Similarly, the highest mean $D_{121.1^\circ C}$-value for PA 3679 drawn from the composite dataset was found to be that describing data obtained in neutral phosphate buffer (Fig. 2). By contrast, the highest mean $D_{121.1^\circ C}$-value for proteolytic *C. botulinum* was found to be that describing data obtained in green beans, although the difference between that and the mean of data obtained in neutral phosphate buffer was not significant ($P < 0.05$) (Fig. 2). These observations suggest that the majority of substrates examined either reduce the heat resistance of spores of

←

[g] Data presented are those obtained with a micropurge.
[h] $D_{121^\circ C}$-value was calculated from a *D*-value obtained at another temperature and the *z*-value.
[i] Data presented is that pertaining to the most heat-resistant spore crop.
[j] Specific reference is made to the fact that data were collected following the addition of medium to the thermal death time cans after processing and prior to incubation.
[k] Specific reference is made to the fact that data were collected without the addition of medium to the thermal death time cans after processing and prior to incubation.
[l] The authors calculated $D_{121^\circ C}$-values from thermal death time curves constructed from survival and destruction points.
[m] The authors calculated $D_{121^\circ C}$-values according to the methods described by Stumbo *(93)*.
[n] The authors calculated $D_{121^\circ C}$-values according to the methods described by Schmidt *(79)*.

TABLE 2. $D_{121°C}$- *and* z*-values for proteolytic strains of* C. botulinum *suspended in phosphate buffer and selected foodstuffs*

Substrate	$D_{121°C}$-value (min)	z-value (°C)	pH	Strain	Reference
Phosphate buffer	0.23	10	7–7.12	19, 23, 97	*25*
	0.158[a]	10.46	7.00	62A	*99*[b]
	0.175[a]	9.45	7.00	213B	*99*[b]
	0.364[c]	9.9	7.00	A16037	*62*
	1.43[a]		7.00	213B	*88*[d]
	0.055[a]	8.33	7.00	213B	*41*[e]
	0.070[a]	8.33	7.00	213B	*41*[f]
	0.133[g]	9.06	7.00	62A	*95*
	0.31[a]	11.61	7.00	62A	*72*
	0.16[a]	9.94	7.00	213B	*72*
	0.44[c]	8.22	7.00	213B	*28*
	0.106[c]	11.04	7.00	213B	*29*
	0.07		7.00	Various [B]	*63*
		10.7	7.00	RH 19 [B]	*82*
		10.7	7.00	73-211[B]	*82*
	0.14	10.06	7.00	PC [F]	*50*
	0.14	10.61	7.00	Langeland [F]	*50*
	0.23	14.06	7.00	4 YRC [F]	*50*
	0.080[c]	8.1	7.00	62A	*53*
	0.106[c]	9.2	7.00	A190	*53*
	0.173[c]	10.6	7.00	BIG4 [A]	*53*
	0.178[c]	10.0	7.00	62A (ATCC 7948)	*53*
	0.186[c]	9.0	7.00	62A (TEFRL)	*53*
	0.101[c]	10.1	7.00	BLamana	*53*
	0.098[c]	9.0	7.00	169B	*53*
	0.081[c]	8.5	7.00	213B	*53*
	0.13	10.4	7.00	62A	*54*
Asparagus	0.05[a]	8.36	5.23	62A	*99*[b]
	0.065[a]	8.81	5.23	213B	*99*[b]
	0.14	13.7	5.5	62A	*54*
Beans, green	0.22[a]	11.28	7.1	62A	*72*
	0.22[a]	12.06	7.1	213B	*72*
Beans, snap	0.074[a]	9.72		213B	*41*[e]
	0.075[a]	9.72		213B	*41*[f]
Beets	0.147[a]	10.83		213B	*41*[e]
	0.180[a]	11.11		213B	*41*[f]
Carrots	0.077[a]	9.44		213B	*41*[e]
	0.067[a]	9.44		213B	*41*[f]
Corn	0.189[a]	10.00		213B	*41*[e]
	0.173[a]	8.89		213B	*41*[f]
	0.27[a]	10.56	6.9	213B	*88*[d]
Corn (raw)	0.14[a]	10.28		62A	*99*[b]
	0.12[a]	8.22		213B	*99*[b]
Corn (frozen)	0.22[a]	9.83	7.13	62A	*72*
Corn (canned)	0.23[a]	11.56		62A	*99*[b]
	0.19[a]	9.56		213B	*99*[b]
Crabmeat	0.175	12.78		4 YRC [F]	*50*
Crabmeat, Zuwai	0.23	11.6	7.6	62A	*54*
Macaroni creole	0.128[c]	8.89	7.0	62A	*110*
Mackerel in water	0.15	10.6	6.3	62A	*54*
Mackerel in oil	0.41	12.7	6.3	62A	*54*
Milk, whole	0.07[a]	8.94	6.34	62A	*99*[b]
	0.065[a]	7.95	6.25	213B	*99*[b]
Mushroom (puree)	0.05		6.4	Various [B]	*63*
Mushroom	0.06	8.9	6.0	62A	*54*
Oysters in water	0.17	9.9	6.1	62A	*54*
Peas	0.04[a]	7.53	5.71	62A	*99*[b]
	0.155[a]	8.76	5.97	213B	*99*[b]
	0.10[a]	8.33	7.2	213B	*88*[d]
Peas (puree)	0.089	8.33		62A	*95*

TABLE 2. *Continued*

Substrate	$D_{121°C}$-value (min)	*z*-value (°C)	pH	Strain	Reference
	0.127			62A	*44*[b]
Rice (puree)	0.118[c]	8.61	7.0	62A	*110*
Spaghetti (puree)	0.108[c]	8.33	7.0	62A	*110*
Spinach	0.095[a]	9.20	5.38	62A	*99*[b]
	0.08[a]	8.61	5.39	213B	*99*[b]
	0.24[a]	10.78	6.68	62A	*72*
	0.158[a]	10.28		213B	*41*[f]
Squash (strained)	0.060[g]	8.17		62A	*95*
Tomato juice	0.10	9.4	4.2	62A	*62*
Tuna, in water	0.11	12.9	6.0	62A	*54*
Tuna, in oil	0.21	12.2	6.0	62A	*54*
Water, distilled	0.051[g]	8.5		62A	*95*

[a] $D_{121°C}$-value was calculated from an *F* value.
[b] In instances where one or more values were determined, the average is presented.
[c] $D_{121°C}$-value was calculated from a *D*-value obtained at another temperature and the *z*-value.
[d] In instances where one or more values were determined at the same pH, the average is presented. In all other instances, the data presented is that obtained at the natural pH of the product before heating.
[e] The authors calculated their $D_{121°C}$-values according to the methods described by Stumbo *(93).*
[f] The authors calculated their $D_{121°C}$-values according to the methods described by Schmidt *(80).*
[g] Values are incorrectly quoted by the International Commission on Microbiological Specifications for Foods (ICMSF; *Microorganisms in Foods 5: Characteristics of Microbial Pathogens,* 1996).

PA 3679 and proteolytic *C. botulinum* and/or inhibit the recovery and/or germination or outgrowth of heat-injured spores. Xezones and Hutchings *(110)* acidified several foods and reported that the $D_{121°C}$-values of proteolytic *C. botulinum* increased with increases in pH. That higher thermal death times were observed in neutral phosphate buffer than in the other substrates examined (Figs. 1 and 2) may thus be explained, at least in part, by the comparatively low pH of the latter (Tables 1 and 2). Interestingly, Xezones and Hutchings *(110)* observed that the value of *z,* unlike that of $D_{121°C}$, was not significantly influenced by pH. Comparing the heat resistance characteristics of spores recovered in substrates supplemented with a growth medium with those recovered in unsupplemented substrates, Reed et al. *(72)* observed that semiacid products exhibit an inhibitory effect upon the growth of the most heat-resistant spores, Reynolds et al. *(73)* that the majority of the vegetables they examined exhibited an inhibitory effect on the germination of spores of PA 3679, and Kaplan et al. *(41)* that higher thermal death times were observed when spores were recovered in food substrates modified with added peptone as compared with unmodified substrates. It is probable, then, that the observation of lower $D_{121.1°C}$-values in food substrates is attributable to both an increased sensitivity to the applied heat treatment and to an inhibition of recovery and/or germination or outgrowth of heat-injured spores.

When the *z*-values of PA 3679 in neutral phosphate buffer were compared with the *z*-values obtained in other substrates, it was found that the maximum reported *z*-value was obtained in neutral phosphate buffer (Fig. 3). Again, the same was found to be true for proteolytic *C. botulinum* (Fig. 3). The highest mean *z*-value for PA 3679 drawn from the composite dataset was found to be that describing data obtained in neutral phosphate buffer (Fig. 4), while for proteolytic *C. botulinum* it was that describing data obtained in green beans (Fig. 4). The difference between the mean *z*-values describing the data obtained in green beans and in neutral phosphate buffer was, however, not significant ($P < 0.05$). Overall, for both PA 3679 and proteolytic *C. botulinum,* the variation in mean *z*-values across the substrates was found to be low. This observation concurs with that of Kaplan et al. *(41)* who, upon conducting a statistical analysis of thermal death time data for PA 3679 heated in 15 low-acid foods, concluded that the observed variation in individual *z*-values may be attributable to experimental error rather than to the effect of specific food substrates.

In comparing the heat resistance of PA 3679 and proteolytic *C. botulinum* across a range of substrates, we found the mean and maximum $D_{121.1°C}$-values for PA 3679 to be higher than those of proteolytic *C. botulinum* in all of the substrates examined (Figs. 1 and 2). Statistical analysis indicated that the $D_{121.1°C}$-values reported for PA 3679 across the range of foods examined exceeded those reported for proteolytic *C. botulinum* ($P = 3.23E{-}14$). Likewise, the *z*-values reported for PA 3679 in foods exceeded those reported for proteolytic *C. botulinum* ($P = 7.76E{-}3$). The mean *z*-values for PA 3679 were higher than those of proteolytic *C. botulinum* in all cases except that of beans and beets (although the differences were not significant at $P < 0.05$), and the maximum reported *z*-values for PA 3679 were higher than those of proteolytic *C. botulinum* in all cases except that of asparagus (Figs. 3 and 4). Our findings are thus consistent with the prevailing idea that the heat resistance of PA 3679 exceeds that of proteolytic *C. botulinum (3, 18, 94, 108).*

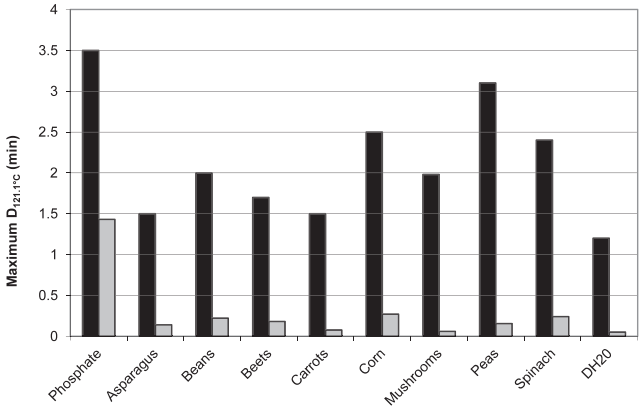

FIGURE 1. *Maximum reported* $D_{121°C}$ *(minutes) for PA 3679 (black bars) and proteolytic strains of* C. botulinum *(gray bars) in various substrates.*

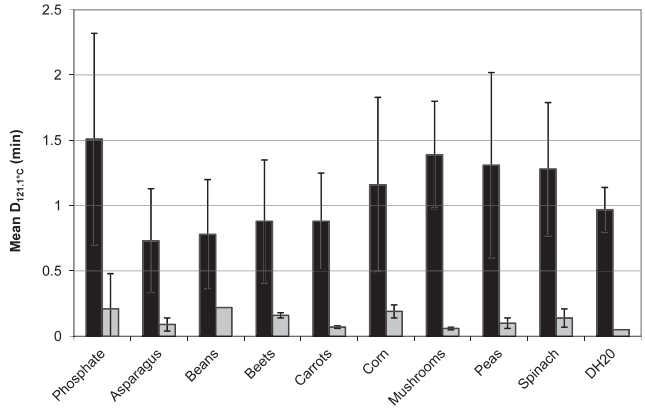

FIGURE 2. *Mean of reported* $D_{121°C}$ *(minutes) for PA 3679 (black bars) and proteolytic strains of* C. botulinum *(gray bars) in various substrates. Error bars indicate standard errors of the mean.*

PA 3679 AS A THERMAL PROCESSING SURROGATE FOR PROTEOLYTIC *C. BOTULINUM*

Shortly after Bigelow et al. *(8)* set out the first rational method for the derivation of thermal processing schedules, the concept of experimental packs inoculated with spores of known heat resistance was developed for the purpose of testing calculated processes *(14).* Since it was unsafe to bring *C. botulinum* into processing plants for such validation studies, and because it was difficult to produce crops of spores of proteolytic *C. botulinum* with a high degree of resistance, a surrogate was needed. PA 3679 was selected for this purpose because it was nontoxigenic, it was morphologically similar to *C. botulinum,* and the heat resistance of its spores was somewhat in excess of that of the maximum recorded for *C. botulinum* spores *(61, 100).* Some 70 years have passed since PA 3679 was adopted as a surrogate for proteolytic *C. botulinum,* and it seems pertinent as we reflect on this organism that we highlight those characteristics that make it a useful surrogate and the limitations associated with its use as such.

Among those characteristics that undoubtedly make PA 3679 a good thermal processing surrogate for proteolytic *C. botulinum* are its nontoxigenicity *(31),* the relative ease with which spore crops with a relatively high degree of heat resistance can be produced *(30, 113),* its ready detection through off-odor and gas formation *(33, 73, 88),* and its genetic similarity with proteolytic strains of *C. botulinum* ((*46)* and as indicated by the present study).

That the heat resistance of PA 3679 exceeds that of proteolytic *C. botulinum* is also typically considered one of the characteristics of PA 3679 that makes it a good thermal processing surrogate. However, as Townsend et al. *(99)* pointed out, the difference is so great that it must be kept in mind when using PA 3679 as a surrogate for proteolytic *C. botulinum* in inoculated pack studies. While some have attempted to obtain suspensions of PA 3679 having a heat resistance only slightly more than the maximum for *C. botulinum* by the use of less favorable growth media or by the fractionation of spore crops by centrifugation *(61),* a more standard approach to the problem has been to reduce the number of spores of PA 3679 inoculated to individual packs. The NCA Research Laboratories *(61)* suggested that a spore concentration of PA 3679 should be selected to give a thermal death time (*F* value) at 240°F in neutral phosphate buffer of between 12 and 16 min, since the maximum recorded thermal death time of 6×10^{10} *C. botulinum* spores under the same conditions was said to be 10 min. While such an approach takes account of the difference in the *D*-values of the two organisms at 240°F, it makes no allowance for the difference in *z*-values. As indicated in the preceding discussion, the *z*-values of PA 3679 were 1 to 1.5°C higher than those of proteolytic *C. botulinum* across a range of substrates. As the thermal profile of a process deviates from the temperature at which the *D*-values of the two organisms was obtained, then, the difference in the *z*-values of the two organisms will result in an underprediction of the safety of a product at temperatures above that at which the *D*-values were determined and an overprediction of safety at temperatures below the same. Whether the end result will be overprocessing or underprocessing will depend on whether the balance of the thermal process lies above, or below, the temperature at which the *D*-values were determined.

A further limitation of using PA 3679 as a surrogate for proteolytic *C. botulinum,* but one that is not unique to PA 3679, is the fact that the heat resistance characteristics of the spores of this organism vary from crop to crop as well as with the media and methods of spore production used. Thus the results of one inoculated pack study cannot necessarily be reproduced by independent processing facilities or at a later point in time.

In discussing the use of surrogate microorganisms, Busta et al. *(13)* stated that ''the ideal surrogate would be a nonvirulent strain of the test pathogen that retained all other characteristics except pathogenicity.'' In fact, a nontoxigenic mutant of *C. botulinum* 62A has recently been constructed by inserting a group II intron into the botulinum neurotoxin type A gene *(9).* The mutant was shown to retain the growth characteristics and sporulation properties of the parental strain, and, while the authors suggest that it may be used for food challenge tests in a laboratory situation, they do not advocate its use as a thermal processing surrogate for inoculated pack studies. In fact, such a strain could not be

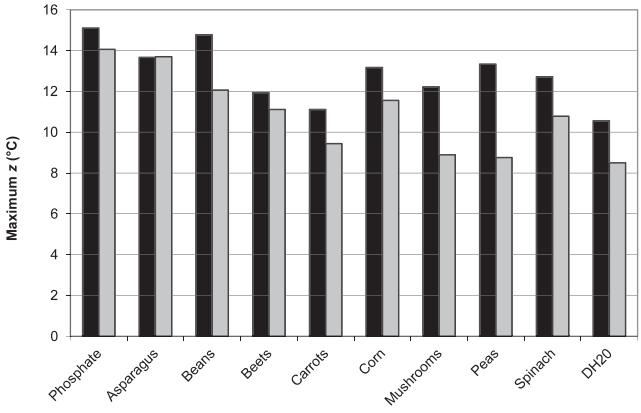

FIGURE 3. *Maximum reported* z*-values (degrees Celsius) for PA 3679 (black bars) and proteolytic strains of* C. botulinum *(gray bars) in various substrates.*

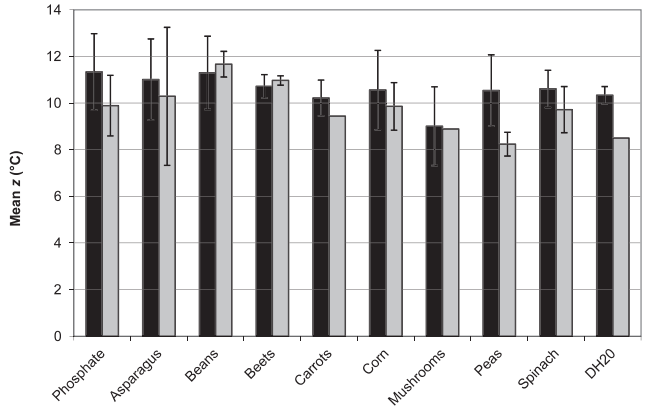

FIGURE 4. *Mean reported* z*-values (degrees Celsius) for PA 3679 (black bars) and proteolytic strains of* C. botulinum *(gray bars) in various substrates. Error bars indicate standard errors of the mean.*

considered for use in commercial environments because the possibility of reversion to the toxigenic state, albeit unlikely, remains.

Thus we concur with Townsend et al. *(99)* that, while the heat resistance characteristics of PA 3679 are unlike those of proteolytic *C. botulinum* in foods, this organism is the most suitable yet found to enlist as a surrogate.

PA 3679 AS A TARGET FOR SETTING PROCESSES DESIGNED TO ACHIEVE COMMERCIAL STERILITY

While the inactivation of proteolytic *C. botulinum* is the primary consideration with regard to the thermal processing of low-acid foods, consideration is also given to the risk of spoilage posed by nonpathogenic microorganisms *(108).* Since the heat resistance of many spoilage organisms exceeds that of proteolytic *C. botulinum,* the resistance values of one or more of these organisms often determine the process specifications for the production of commercially sterile foods. During the mid-1900s, the resistance values of PA 3679 were used widely for designing processes for low-acid foods that enabled the processor to achieve economically acceptable levels of spoilage. Rapid cooling of the products subsequent to processing, and storage at temperatures inhibitory to growth, were relied upon to prevent the growth of any of the more heat-resistant thermophiles that might have survived the thermal process *(73, 94).*

Today, it is common practice to use a number of target organisms in calculating a thermal process for the delivery of a commercially sterile product. The Campden and Chorleywood Food Research Association of the United Kingdom has defined eight groups of spoilage organisms based on their optimum growth temperatures and tolerance of oxygen. In establishing a thermal process, one must take into account the load of each of the groups of target organisms (based on raw ingredient loads), the target maximum load for each group based on good manufacturing practices, and the resistance values of each group, while at all times assuring that the probability of survival of proteolytic *C. botulinum* remains at an acceptably remote level (i.e., not exceeding 1 in 10^{12} for shelf-stable products). In such processing calculations the resistance values of PA 3679 are used for group VI spoilage organisms (as defined by the Campden and Chorleywood Food Research Association), the facultative mesophilic anaerobes. Whether or not this organism will eventually dictate the process depends on the load, the desired probability of survival, and the resistance values of all target organisms present, including proteolytic *C. botulinum (55).*

CONTEMPORARY USE OF PA 3679 AS A RESEARCH MODEL FOR PROTEOLYTIC *C. BOTULINUM*

While knowledge pertaining to the fundamental aspects of the heat resistance and thermal inactivation of spores of *Bacillus* species is being generated steadily *(11, 19, 20, 47, 64, 86, 112),* comparatively less parallel research is being conducted with *Clostridium* species. Given the importance of killing spores of proteolytic *C. botulinum* in foods, the knowledge gap that exists is significant *(85).* Current legislation in some countries has meant that *C. botulinum* can be used, or even held, only within specialized containment facilities where personnel are highly trained and approved to work with this organism *(4, 17).* Thus, the state of our knowledge is unlikely to change rapidly unless a nontoxigenic organism, such as PA 3679, is used as a research model. We believe that the use of PA 3679 as a model for studying fundamental aspects of the heat resistance of spores of proteolytic *C. botulinum* is readily justified because PA 3679 is closely related to proteolytic strains of *C. botulinum,* it forms highly heat-resistant spores, and it is an important organism in its own right given its use as a thermal processing surrogate for proteolytic *C. botulinum* and its occurrence as a spoilage organism. If PA 3679 is to be adopted as a research model for proteolytic *C. botulinum,* however, it will be essential that we add to our knowledge of this organism, and its relationship with proteolytic strains of *C. botulinum,* through genome sequencing and bioinformatics analyses.

CONCLUSIONS

Despite the historical and continuing importance of *C. sporogenes* PA 3679 in the derivation of thermal processing schedules for low-acid shelf-stable foods, and as a research model for proteolytic *C. botulinum,* our knowledge of this organism has remained fragmented. In this review we have drawn together the earliest and subsequent literature discussing the isolation and identification of PA 3679. The taxonomic relationship of PA 3679 to various strains of *C. sporogenes* and proteolytic *C. botulinum* has at times been somewhat confused; despite its nontoxigenicity, more recent phylogenetic analysis suggests that PA 3679 is more closely related to various strains of proteolytic *C. botulinum* than to selected strains, including the type strain, of *C. sporogenes*. However, the comprehensive review of comparative heat resistance data for PA 3679 and proteolytic *C. botulinum* compiled here supports the practice of using PA 3679 as a (typically fail-safe) thermal processing surrogate for proteolytic *C. botulinum*. While a nontoxigenic mutant of a proteolytic strain of *C. botulinum* is now available, it is unlikely that such mutants will find ready acceptance as thermal processing surrogates for proteolytic *C. botulinum,* due to the concern about the possibility of reversion to the toxigenic state. Interestingly, without knowledge of the genetic sequence of PA 3679, and the genetic reasons for its nontoxigenicity, it is not possible to compare the likelihood of mutant reversion with the likelihood of toxigenicity arising in this microorganism. Finally, the contemporary use of PA 3679 as a research model for proteolytic *C. botulinum* must be supported by details of the genetic sequence of PA 3679 and by bioinformatics analyses that confirm the relationship of these organisms.

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