

PRESERVATIVE SENSITIVITY OF THERMALLY TREATED SPORES OF *BACILLUS CEREUS*

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A knowledge of the resistance of bacterial spores to chemical and physical agents singly, in combination or sequentially, is important in both the food and pharmaceutical industries.

The germination, outgrowth and viability of *Bacillus cereus* T spores after exposure to elevated temperatures and/or chemical preservatives have been investigated. Spores in sterile glass-distilled water were held for 30 min at temperatures ranging from 60 to 80°C. Spore germination and outgrowth were determined by measuring the optical density at 500nm of heated and unheated spores incubated in the nutrient broth acting as germination and outgrowth medium. Samples were also removed for viability determinations. In some experiments, subinhibitory concentrations of important food or pharmaceutical preservatives (chlorocresol, chlorhexidine diacetate, sodium benzoate and potassium sorbate) were incorporated into the medium. The pH of the medium was 7.2 except when sodium benzoate and potassium sorbate (both pH 5.5) were used. Results are summarized in Table 1.

High temperatures produced no loss of spore viability, but the rate of germination of *B.cereus* T spores was slower after exposure to 80°C and considerably reduced when these heated spores were treated with sub-inhibitory concentrations of chlorocresol in the germination medium. Chlorhexidine diacetate, in contrast had little effect on the germination of spores, even when these had previously been heated, but sub-inhibitory concentrations reduced the rate and extent of outgrowth. The rate of germination of *B.cereus* T in the presence of sorbate or benzoate was reduced both by prior heating at 80°C and by increasing the preservative concentration. Control (30°C) cells germinated and outgrew normally in broth at pH 5.5 or 7.2 but cells treated at 60 or 80°C germinated more slowly in broth at the acidic pH and outgrowth was extremely slow.

Table 1. Effect of sublethal heat treatment and/or preservatives on germination and outgrowth of *B.cereus* T

Treatment	Conc ⁿ . (% w/v)	Inhibition of germination (G) or outgrowth (Og)
None	-	G and Og normal
Chlorocresol	0.02	G inhibited
Chlorhexidine	0.001	Og inhibited, little effect on G
Sodium benzoate	0.2	G inhibited
Potassium sorbate	0.15	G inhibited
Moist heat (80°C)	-	G rate reduced
Heat + chlorocresol*	0.01	G rate reduced (> heat alone)
Heat + chlorhexidine*	0.0005	Og inhibited, little effect on G
Heat + benzoate*	0.1	G rate reduced (> heat alone)
Heat + sorbate	0.075	G rate reduced (> heat alone)

* Chemical agents incorporated at subinhibitory concn. into nutrient broth.