PHYSICAL AND CHEMICAL RESISTANCE OF ION-EXCHANGE AND COAT DEFECTIVE SPORES OF BACILLUS SUBTILIS

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The susceptibility of ion-exchange forms and untreated spores of Bacillus subtilis (NCTC 10073) to heat and to commonly used sporicide formulations was determined with the aim of identifying common factors contributing to the high physical and chemical resistance of bacterial spores. Spores with coats damaged or removed were also prepared and susceptibility estimated. Spores of B. subtilis were prepared according to the method of Beeby & Whitehouse (1965), and subsequently converted to the calcium and hydrogen ion-exchange forms by the method of Kovacs-Proszt & Farkas (1976). Spore coats were partially or totally solubilized by treatment with either 10% v/v 2-mercaptoethanol, 8M urea (UME) at 60°C for 60 min., or 0.05M dithiothreitol, 1% w/v sodium lauryl sulphate, 8M urea in 0.1M saline (UDS), pH 10.5 at 37°C for 180 min. Thin-section electron-microscopy was used to determine the extent of removal of spore coat. Thermal death rates were determined in phosphate buffer (pH 7.0) at 90°C and chemical resistance by exposure to the following: 2000 ppm hypochlorite and 50% methanol at 20°C, iodophor containing 1% available iodine at 37°C, and 2% alkaline glutaraldehyde at 20°C. Calcium content was determined by atomic absorption spectrophotometry and dipicolinic acid by the method of Scott & Ellar (1978). The results for ion-exchange forms are shown in Table 1 and for coat defective spores in Table 2.

Table 1. The effect of heat and sporicidal agents on ion-exchange spores of B. subtilis

Sporicidal Agent	Time (min) to kill 90% of spore population		
	Untreated	H-form	Ca-form
90°C	10	5	17
hypochlorite/methanol	20	11	20
iodophor	23	20	48
alkaline glutaraldehyde	85	95	30

Table 2. The effect of heat and sporicidal agents on coat defective spores of B. subtilis.

Sporicidal Agent	D-value ratio of untreated to coat defective spores	
	UME-treated	UDS-treated
90°C	2.1	2.3
hypochlorite/methanol	4.0	>8.0
iodophor	2.5	>12.5
alkaline glutaraldehyde	2.1	>8.5

The results suggest that more than one factor is involved in the thermal resistance of B. subtilis spores. From the chemical resistance results the importance of the protective role of the spore coat is evident.

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