DEVELOPMENT OF BIOCIDE RESISTANCE IN *BACILLUS SUBTILIS* 168 AND ITS SPORULATION (SPO$^-$) MUTANTS

*A.G.Knott, *A.D.Russell, **B.N.Dancer, *Welsh School of Pharmacy and **School of Pure and Applied Biology, University of Wales College of Cardiff, Cardiff CF1 3XF, Wales

Comparatively little information is available about the development of biocide resistance during sporulation. It is, however, known that the dialdehyde, glutaraldehyde, is highly effective at low concentrations until a very late stage of sporulation (Power et al. 1988) whereas resistance to the bisbiguanide, chlorhexidine, is found at a much earlier stage (Shaker et al. 1988). In this report, we describe the stages involved in the development of resistance of Bacillus subtilis 168 and specific sporulation (Spo$^-$) mutants to phenolics, an organomercurial, a monoaldehyde, chlorine-releasing agents, an anionic surfactant and a quaternary ammonium compound. Synchronous sporulation in B.subtilis 168 was achieved by a step-down procedure, and mutant strains were grown by procedures described previously (Power et al. 1988; Shaker et al. 1988). With strain 168, samples were removed at 30min intervals from t = 0 to t = 12 and at t = 30hr, and exposed to antibacterial agents. Mutant strains were allowed to develop for 20hr before exposure. Viable counts were made before and after treatment. Toluene, 10%, moist heat at 80°C for 10 min and lysozyme (250 µg/ml, 10 min, 37°C) were used as markers of early, intermediate and late development of resistance, respectively.

The order of development of resistance to antibacterial agents (exposure time 10 min at 20°C, unless otherwise stated) was as follows: toluene (1 min), closely followed by aqueous formaldehyde (0.4% w/v) then sodium dodecyl sulphate (2% w/v) and the phenolics (chlorocresol 0.1%, m-cresol 0.5% and phenol 0.5% w/v). Resistance to phenylmercuric nitrate, chlorhexidine diacetate (200 µg/ml) and cetylpyridinium chloride (10 µg/ml) occurred just before the onset of resistance to moist heat (80°C). Resistance to sodium hypochlorite and sodium dichloroisocyanurate (both 10 ppm available chloride) developed marginally later, with resistance to lysozyme a much later event. It was confirmed that resistance to glutaraldehyde (2%, pH 7.9) was an even later occurrence.

Table 1. Development of resistance to B.subtilis 168 during sporulation and of Spo$^-$ mutants

Development of resistance		
Early (Stages 3-4)	Intermediate (Stages 4-5)	Late (Stages 6-7)
Toluene	Phenylmercuric nitrate	Lysozyme
Formaldehyde	Chlorhexidine diacetate	Glutaraldehyde
Anionic surfactant	Cetylpyridinium chloride	
Phenolics	Moist heat	
	Chlorine-releasing agents	

Power, E.G.M. et al. (1988) FEMS. Microbiol. Lett. 50: 223 - 226.
Shaker, L.A. et al. (1988) FEMS. Microbiol. Lett. 51: 73 - 76.