The heat resistance of bacterial spores after different vacuum drying treatments

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The resistance of bacterial spores to high temperatures of around 120° has been used in this work as a means of assessing damage induced in the spores by different vacuum drying treatments. The vacuum apparatus used enabled simultaneous measurements to be made, of pressure, sample weight and sample temperature changes, and allowed for correlation between physical measurements and biological response.

The effect of different drying treatments was assessed by exposing samples to a constant elevated temperature for different times and estimating the viability. Under all conditions it was found that the log surviving fraction (N/N_0)/heating time (t) curves exhibited a shoulder at high survival levels, but were linear below a surviving fraction of 0·1. The linear portion is described by $N/N_0 = ae^{-kt}$ where "a" is the intercept of the curve with the "y" axis. Two parameters have been used to characterize the response; firstly, the slope of the curve "k", and secondly, a shoulder constant "s" which is the heating time required to reduce the surviving fraction to 0·1 i.e. $s = t$, when $N/N_0 = 0{\cdot}1$.

The usefulness of the constants "k" and "s" in deducing possible lethal mechanisms relies on them changing with heating temperature in a meaningful way. Therefore the characteristics of the heat response of spores was investigated in aqueous suspension, and also after being subjected to sublimative low vacuum drying, and to high vacuum drying, where additional water is removed by isothermal desorption. Both "k" and "s" were found to be directly related to the heating temperature, and were shown to vary systematically and independently with the drying treatment.

When "k" values were treated according to the Arrhenius relationship $k = Ae^{-Ea/RT}$ it was found that the activation energy for the lethal mechanism (Ea) did not change with different drying treatments, being 155 kJ mol^{-1} (34 k cal mol^{-1}) in all cases. The susceptibility of the spore to these mechanisms, as indicated by the frequency factor (A), was, however, dependent upon the drying treatment, the value being 1000 times smaller after sublimative drying than in aqueous suspension, and 20 times greater after high vacuum than after sublimative drying.

A treatment of "s" in a similar way showed that the size of the shoulder decreased with increasing temperature, but that removal of water by sublimative drying caused an increase in "s" by a factor of 1000, the value after isothermal drying being 100 times lower than this.

A study of the variation of "k" and "s" under different conditions would lead us to doubt that they are representative of one mechanism. Rather we would postulate that "s", the shoulder constant, represents a lag time during which structural changes occur within the spore that cause it to become susceptible to the lethal mechanism represented by "k". The effects of different drying treatments have been analysed on this basis.

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Recognizing sporogenous yeast genera

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It is often important to the clinician that yeasts isolated from vaginal swabs are accurately identified. Conformation with Kochs postulates for pathogenicity has been established for several species of the imperfect genus *Candida* (Hurley, 1967), but not for any perfect yeast species. Some perfect species are distinguishable from *Candida* species only in their ability to form ascospores. I believe that the perfect yeasts are commensals. If an isolated yeast is established to be a commensal organism it will not mask the true cause of disease.

Isolates of four perfect yeast species, with postulated imperfect *Candida* stages, were used for this investigation. They were *Saccharomyces cerevisiae* (3 isolates), *Hansenula anomala* (2 isolates), *Pichia membranefaciens* (3 isolates) and *Kluyeromyces fragilis* (5 isolates). At