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

least ten single cell cultures of each isolate were examined. The formulae of the three agar solidified sporulation media that were investigated have been described (Merritt & Hurley, 1971). Slopes were made of 5 ml volumes of media in 20 ml screw capped glass bottles. Inoculated slopes were incubated at 25°. Samples were taken from each slope at regular intervals, heat fixed to a glass slide and stained with 0·5% safranin. A count was made of at least 500 structures to find the percentage of cultures in which at least 1% of the structures were asci. The ascospores of *K. fragilis* are released from the ascus as they mature, so the number of ascospores could be determined, but not the number of asci. The results for *K. fragilis* are for the percentage cultures in which at least 1% of the structures are ascospores.

Of the three media studied, the sodium acetate medium allowed optimum sporulation of all four species. The 6% *S. cerevisiae* cultures which were apparently nonsporing, all contained some asci, but not to 1% of the structures. The asci production on this medium was predictable and large variations in asci yields between replicates was unusual. The Gorodkowa medium was of little value for three of the species studied, although some of the cultures contained a few asci. The V8 medium was useful and occasionally gave relatively higher yields of asci than the sodium acetate medium. However, the medium was unreliable because a few cultures would fail to contain any asci when replicates were sporing freely.

For the yeasts of medical importance that I have investigated the sodium acetate medium is very suitable for inducing sporulation. The small amount of nutrient included in the formulation allows some cell division and compensates for some variation in inoculum levels, although this may delay the onset of sporulation by 1–2 days. I can recommend this unbuffered sodium acetate sporulation medium for routine isolates of yeast-like fungi which may be *Candida* species.

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The effect of cetyltrimethylammonium bromide on the cytochrome system of *Escherichia coli*

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The effect of a number of agents on cytochrome difference spectra of cells of *Escherichia coli* NCTC 1093 have been studied. Cells were grown as described by Rye & Wiseman (1966). harvested from the exponential phase of growth by membrane filtration and suspended in glucose-free medium to give a cell concentration of between 15 and 20 mg/ml. Up to 0·2 ml volumes of water or of solutions of substrates or reagents were added to 4 ml aliquots of these suspensions and difference spectra between pairs of them measured using a Unicam SP 700 recording spectrophotometer.

The spectra obtained between blanks of washed aerated cells and similar test suspensions to which had been added sodium succinate showed absorbancy peaks in the visible light region at 533, 560, 593 and 630 nm corresponding to those reported for *E. coli* by Smith (1954). The addition of potassium cyanide to the succinate respiring cells in such pairs of suspensions resulted in the elimination of the 630 nm cytochrome a_2 peak whilst the other peaks remained unaffected. This indicates that the terminal cytochrome had become oxidised, being no longer reducible by the remainder of the electron transport chain when complexed with cyanide.

In the spectra obtained between blanks of washed aerated cells and test suspensions of succinate respiring cells treated with some concentrations of CTAB the 630 nm cytochrome a_2 peak was again eliminated, with the other peaks unaffected. This indicated that CTAB is capable of specifically uncoupling the terminal cytochrome from the remainder of the electron transport chain. Similar elimination of the 630 nm peak also followed treatment of respiring cells with chlorhexidine diacetate. The final concentrations of CTAB and of chlorhexidine having these effects were 300 to 400 and 100 to 200 μg/ml respectively; lower concentrations had no effect on the cytochrome spectra whilst higher concentrations caused