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SHORT COMMUNICATION

The effect of yeast cells in the heating medium on the heat resistance of *Bacillus stearothermophilus* spores

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THE heat resistance of bacterial spores is greatly influenced by the nature of the medium in which the spores are heated. Organic matter present during heating can afford protection to spores and many workers have shown that carbohydrates, proteins and fats can provide this. Dead bacterial cells have protected living cells of the same species subjected to heat (Lange, 1922). No protective effect was found with *Clostridium botulinum* spores when vegetative cells or heat killed spores of the same species were added (Sugiyama, 1951), and similarly for *Bacillus coagulans* spores when vegetative cells were added (Frank & Campbell, 1957). However, the spores of *Clostridium sporogenes* were protected from heat by living cells or spores of micro-organisms of different species (Amaha & Sakaguchi, 1954); killed suspensions had no effect. The experiments reported are part of a series designed to investigate the nature and extent of this protective effect with reference to an acknowledged heat resistant spore. Yeast cells were used as a source of organic matter as they are of uniform size.

EXPERIMENTAL

Spores of *Bacillus stearothermophilus* NCIB 8919 were obtained using the method of Cook & Brown (1964), except that the sporulation conditions were seven days at 60°. Vegetative cells were separated from the spores by washing ten times using a refrigerated centrifuge. The heating and counting techniques used were those described by Cook & Brown (1964) but the recovery medium, antibiotic assay medium with 0·1% starch, was of pH 7·3.

A special moist yeast marketed as "Yeast for B.S.I. C/10 tests"* was used to prepare a 10% dry weight suspension of yeast in distilled water. The suspension was prepared, sterilised by heating at 115° for 20 min and assayed for moisture content using the methods given in B.S. 808: 1938.

RESULTS AND DISCUSSION

Typical time survivor curves for *B. stearothermophilus* spores heated in water and in a killed yeast suspension are given (Fig. 1). Both curves show a characteristic period of heat activation followed by an exponential death rate. The exponential part of the curve for spores heated in the presence of yeast is less steep than that of the control curve, and when both regression lines are extrapolated back to zero time they show nearly the

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same extrapolation number (Alper, Gillies & Elkind, 1960). This would indicate that the yeast cells afford some protection from heat to the *B. stearothermophilus* spores. If the difference between the slopes was due to initial differences in rate of heat transfer, then the exponential parts of each curve would be parallel. Thermocouple readings indicated that 115° was reached in less than 1 min for spores in water and in less than 2 min for spores heated in the yeast suspension.

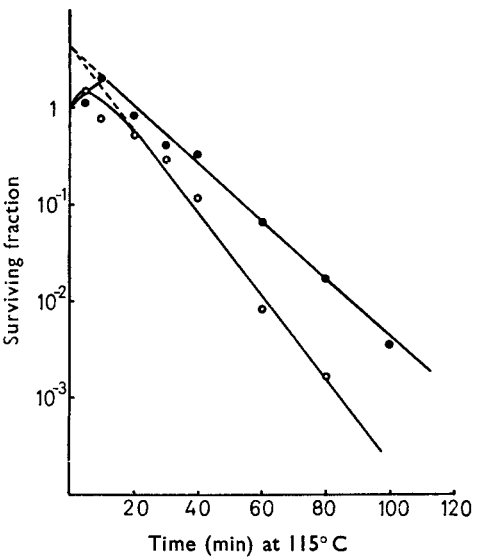

FIG. 1. Effect of heating at 115°C in different substrates upon the colony count of *B. stearothermophilus* spores. ○ Water ● 10% (dry weight) yeast suspension.

It was thought unlikely that yeast carried over from the heated suspension accounted for the increased recovery since the medium used already contained 0·3% yeast extract. This was confirmed by heating spores in water and subculturing into antibiotic assay medium containing additional heated yeast suspension; no significant increase in colony counts was observed. Experiments are continuing to examine this protective effect quantitatively and qualitatively.

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