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# Initiation of Germination of Bacterial Spores by Hydrostatic Pressure

## By G. W. GOULD AND A. J. H. SALE

Unilever Research Colworth/Welwyn Laboratory, Colworth House, Sharnbrook, Bedford

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#### SUMMARY

Hydrostatic pressure initiated germination of bacterial spores in nutrientfree media. Those spores which were most dormant towards chemical germinants at I atmosphere pressure were also the most resistant to germination by pressure treatment. Germination by high pressure treatment was characterized by temperature and pH optima, like germination at atmospheric pressure. Germination initiated by pressure was inhibited by metabolic poisons and was potentiated by low concentrations of various nutrients including some of those which are normally germinative (at higher concentrations) at atmospheric pressure. In particular, L-alanine and closely related  $\alpha$ -amino acids, but not their breakdown products, potentiated germination initiated by pressure. Study of potentiation by D-alanine (which strongly inhibits germination initiated by L-alanine at I atmosphere pressure) revealed that high pressures caused an increase in the rate of racemization of alanine by spores. Germination by pressure probably resulted from acceleration of some germination reaction which is normally negligibly slow at a pressure of I atmosphere, and also from an increase in permeability of some barrier within the spore to L-alanine and related  $\alpha$ -amino acids.

### INTRODUCTION

Inactivation of bacterial spores by hydrostatic pressures, unlike the inactivation of vegetative bacteria, occurred in two stages (Clouston & Wills, 1969; Sale, Gould & Hamilton, 1970). First, pressure caused germination of the spores, and then it inactivated the germinated forms. However, pressures of only a few hundred atmospheres could still cause germination, but were too low to inactivate the resulting germinated forms. The overall effect of such pressures was therefore solely to cause germination, i.e. heat-sensitization of the spores, optical, structural and chemical changes which normally occur during germination. Study of environmental factors showed that pressure inactivation of spores was strongly influenced by temperature, and less strongly by pH value, water activity and ionic strength.

Pressure germination is of interest as a potential step in preservation procedures aimed at eliminating spores from perishable materials. It is also worth studying as a novel initiator of germination, because the way in which spore germination is initiated by any germinant system is not yet understood.

Germination and inactivation of spores of *Bacillus pumilis* by pressures up to 1700 atmospheres (atm.) was originally described by Clouston & Wills (1969). The present paper is concerned with the germinant action of hydrostatic pressure on spores of

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