Role of alanine racemase in pressure germination

The anomalous situation, that D-alanine (which inhibits germination at 1 atm.) potentiated pressure germination, suggested that pressure might cause racemization to the L-form. This was so (Fig. 3). Consequently, under pressure there was a rapid formation of L-alanine (which stimulated germination) from the inhibitory D-enantiomorph. Furthermore, rate studies showed that pressure germination was in fact initially

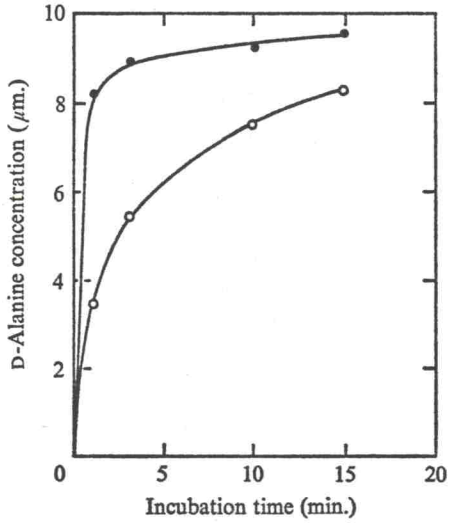

Fig. 3

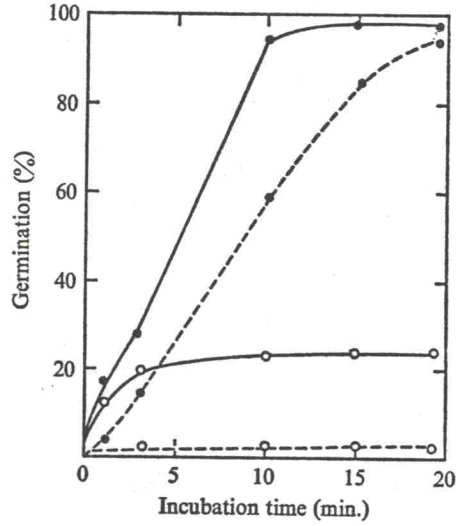

Fig. 4

Fig. 3. Racemization of alanine under pressure. Spores of *Bacillus cereus* T were incubated (25°) with L-alanine (25 μM) in sodium phosphate (0·1 M, pH 8·0) at 1 atm. (○) or 250 atm. (●) pressure. Samples were assayed for racemization by measuring formation of D-alanine as described in Methods.

Fig. 4. Germination of spores in L- and D-alanine. Spores of *B. cereus* T were incubated (25°) in 0·1 M-sodium phosphate (pH 8·0) containing 250 μM of L-alanine (continuous lines) or D-alanine (dashed lines) at 1 atm. (○) or 250 atm. (●) pressure. Samples were assayed for germination by determining the percentage of spores which had become phase-dark.

potentiated more rapidly by L- than by D-alanine, although the difference was small after the first few minutes of pressurization (Fig. 4). The hypothesis that D-alanine potentiated pressure germination *via* formation of L-alanine was further substantiated by using an inhibitor of alanine racemase, *O*-carbamyl-D-serine (OCDS). This substance strongly *antagonized* the potentiating action of D-alanine (presumably by arresting racemization to L-alanine), whilst *increasing* the potentiating action of L-alanine (presumably by arresting racemization to D-alanine) (Table 5). Interestingly, with the exception of phenylalanine, OCDS had little effect on the potentiation of pressure germination by other amino acids, suggesting that alanine was not involved as an intermediate in these instances (Table 5).

Stimulation of pressure germination by ribosides and related compounds

Ribosides, which are strong potentiators of the germination of spores of certain species initiated by amino acids at 1 atm., were much less effective than amino acids