## ALE

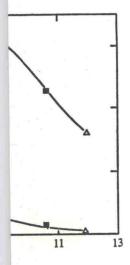
ulans and B. subtilis var. niger rmant spores of B. cereus T in

### eat activation

letely by pressure than were iated at higher pressures, i.e. activation than germination

#### rmination

lly near neutral pH, but the pores initiated by nutrients at



3acillus cereus T were activated M) and subjected to pressures sodium citrate/phosphate(); IC1(); sodium bicarbonate/n was estimated by measuring

# 1 of aerobic spores

ressure as effectively in media 2). Germination of spores of ly unaffected by anaerobiosis.

# 's and other compounds

out 1000 atm.) was markedly e, for example, the effect of th rising pressure and became bservations that germination at such high pressures was in general relatively unaffected by changes of environment or pretreatment of the spores, the experimental pressure range was restricted to 1000 atm. maximum.

Of a variety of substances tested, amino acids were clearly the most effective potentiators of pressure germination. For example, inspection of the results summarized in

Table 2. Pressure germination of Bacillus coagulans spores in aerobic and anaerobic media

Medium	Ungerminated spores (%) following pressurization* at				
	2000 atm.	3000 atm.			
Aerobic Water Buffer† Yeast glucose broth	$1.7 \times 10^{-4}$ $1.2 \times 10^{-4}$	I·2×IO-3			
Anaerobic Buffer plus thioglycollate† R C M‡	1·3×10 <sup>-4</sup>	I.0 × IO-3			

<sup>\*</sup> Pressurization was for 30 min. at 70°. Ungerminated spores were estimated by viable counts.

Table 3. Initiation of spore germination by pressure at different temperatures

	Addition‡	Germination (%)* after pressurization† at						
Organism		o°	20°	30°	40°	50°	60°	70°
Bacillus coagulans	None	0	0	I	0	50	60	0
	L-Alanine (250 µM)	0	0	5	>95	>95	>95	50
	L-Alanine (1 mm)	0	0	12	>95	>95	>95	50
B. subtilis var. niger (syn.	None	1	1	2	3	10	10	8
globigii)	L-Alanine (250 μм)	I	I	50	50	75	30	25
	L-Alanine (1 mm)	I	1	68	>90	70	36	13
	None	0	0	21	40	64	5	_
	L-Alanine (250 $\mu$ M)	12	>95	>99	>99	>99	>99	95

<sup>\*</sup> Spores were activated (70°, 30 min.) before use; germination was measured by recording the percentage of phase-dark spores.

† Pressure was 250 atm. maintained for 30 min.

Table 4 indicated that spores of *Bacillus cereus* could be caused to germinate at 1 atm. by a variety of amino acids, particularly at the higher concentration used (10 mM) and in the presence of inosine. Germination of *B. cereus* spores caused by pressure was similarly potentiated by a variety of amino acids. In contrast, spores of *B. coagulans* were much less responsive than those of *B. cereus* to amino acids at 1 atm., germinating rapidly only in L-alanine, and much less rapidly in L- $\alpha$ -aminobutyric acid and L-valine; similarly, the range of amino acids potentiating germination at 200 atm. pressure was much more restricted than the range potentiating germination of spores of *B. cereus*. Amino acids were effective germinants at 200 atm. at concentrations well below those effective at 1 atm.

<sup>†</sup> Buffer was o I M-sodium phosphate (pH 8·o); sodium thioglycollate was used at o I % (w/v). ‡ Reinforced Clostridium medium (Gibbs & Hirsch, 1956).

<sup>‡</sup> Suspending medium was o·I M-sodium phosphate (pH 8·o).