

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	1.7×10^{-4}	
Buffer†		1.2×10^{-3}
Yeast glucose broth	1.2×10^{-4}	
Anaerobic		
Buffer plus thioglycollate‡		1.0×10^{-3}
R C M‡	1.3×10^{-4}	

* Pressurization was for 30 min. at 70°. Ungerminated spores were estimated by viable counts.

† Buffer was 0.1 M-sodium phosphate (pH 8.0); sodium thioglycollate was used at 0.1% (w/v).

‡ Reinforced Clostridium medium (Gibbs & Hirsch, 1956).

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

Organism	Addition‡	Germination (%)* after pressurization† at						
		0°	20°	30°	40°	50°	60°	70°
<i>Bacillus coagulans</i>	None	0	0	1	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
<i>B. subtilis</i> var. <i>niger</i> (syn. <i>globigii</i>)	None	1	1	2	3	10	10	8
	L-Alanine (250 μM)	1	1	50	50	75	30	25
	L-Alanine (1 mM)	1	1	68	>90	70	36	13
<i>B. cereus</i> T	None	0	0	21	40	64	5	—
	L-Alanine (250 μM)	12	>95	>99	>99	>99	>99	95

* 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.

‡ Suspending medium was 0.1 M-sodium phosphate (pH 8.0).

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-α-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.

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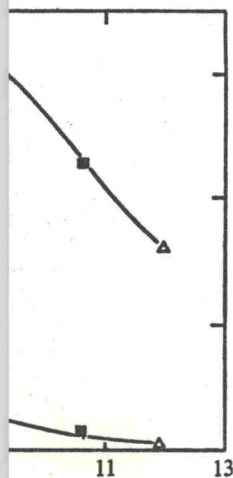
B. subtilis var. *niger* and *B. cereus* T in

heat activation

completely by pressure than were activated at higher pressures, i.e. heat activation than germination

germination

initially near neutral pH, but the spores initiated by nutrients at



Bacillus cereus T were activated (10 mM) and subjected to pressures of 1000 atm. sodium citrate/phosphate (O); sodium bicarbonate (□); germination was estimated by measuring

of aerobic spores

pressure as effectively in media (Table 2). Germination of spores of *B. cereus* T was unaffected by anaerobiosis.

and other compounds

(about 1000 atm.) was markedly reduced, for example, the effect of inosine with rising pressure and became more pronounced. Observations that germination