JOURNAL OF BACTERIOLOGY, Mar. 1971, p. 739-746

Vol. 105, No. 3
Printed in U.S.A.

Germination Requirements of *Bacillus macerans* Spores

L. E. SACKS AND P. A. THOMPSON

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Albany, California 94710

Received for publication 23 November 1970

2-Phenylacetamide is an effective germinant for spores of five strains of *Bacillus macerans*, particularly in the presence of fructose. Benzyl penicillin, the phenyl acetamide derivative of penicillin, and phenylacetic acid are also good germinants. L-Asparagine is an excellent germinant for four strains. α-Amino-butyric acid is moderately effective. Pyridoxine, pyridoxal, adenine, and 2,6-diaminopurine are potent germinants for NCA strain 7X1 only. D-Glucose is a powerful germinant for strain B-70 only. D-Fructose and D-ribose strongly potentiate germination induced by other germinants (except L-asparagine) but have only weak activity by themselves. Niacinamide and nicotinamide-adenine dinucleotide, inactive by themselves, are active in the presence of fructose or ribose. Effects of *p*H, ion concentration, and temperature are described.

Extensive studies on spore germination in the past 20 years have generally been limited to three species of *Bacillus*; *B. cereus*, *B. megaterium*, and *B. subtilis*, probably owing to the ease with which clean spores of these species are obtained and the rapidity with which they germinate. The possibility that an atypical picture of spore germination resulted was considered (4).

Preliminary studies showed that *B. macerans* NCA strain 7X1 has unusual germination requirements (16). These investigations have now been expanded to include five strains of *B. macerans*, in an effort to provide a more balanced view of the germination requirements of *Bacillus* spores. The results show that *B. macerans* spores have germination requirements quite distinct from those of *B. cereus*, *B. megaterium*, and *B. subtilis* spores, although there are many similarities. 2-Phenylacetamide, not previously known to be a spore germinant, is a potent germinant for *B. macerans* spores.

MATERIALS AND METHODS

Bacterial strains. *B. macerans* NCA strain 7X1 was obtained from the National Canners Association. *B. macerans* B-70, B-171, B-388, and B-430 were obtained from the Northern Regional Research Laboratory.

Spores. Spores were formed on a potato-agar $CaCO_3$ medium (17). Vegetative cells were removed by partitioning in a two-phase aqueous polymer system containing polyethylene glycol 1000 and potassium phosphate (17). A few early lots of spores of NCA strain 7X1 were formed on a liquid medium (16).

Germination. Germination rate and extent were determined turbidimetrically in 10-mm test tubes at 625 nm in a Bausch & Lomb Spectronic 20 colorimeter. Samples contained a buffer [usually *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) or potassium phosphate, *p*H 7], germinant, spores, and water. All experiments were conducted at 40 C unless otherwise stated.

Activation. Spores of strains B-70, B-171, and B-430 were activated by heating in a water suspension for 30 min at 64 C. Spores of strain B-388 required 120 min at 64 C for best activation. NCA strain 7X1 required an acid heat shock (9) of 60 min at 70 C while suspended in 0.01 M potassium acetate (*p*H 4.0); such spores were customarily washed and suspended in water before use. Spores of strain 7X1 grown on liquid medium (16) germinated reasonably well after shocking in water suspension at 64 C for 30 min.

Chemicals. Adenine, 2,6-diaminopurine, pyridoxine, pyridoxal, pyridoxamine, penicillin G (benzyl penicillin), niacinamide, nicotinamide adenine dinucleotide (NAD), D-ribose, L-glutamine, L-α-aminobutyric acid, D-glucosamine, 2-deoxy-D-glucose, and penicillinase were obtained from Calbiochem. D-Fructose, 2-phenyl acetamide, valeramide, isobutyramide, isovaleramide, and phenyl acetic acid were obtained from Eastman Kodak. L-Asparagine, D-asparagine, and 8-aza-2,6-diaminopurine sulfate were obtained from Cyclo Chemical. Salts of penicillin derivatives were obtained from Bristol Laboratories. Dipicolinic acid was obtained from Aldrich Chemical Co., Inc.

RESULTS

Compounds capable of initiating germination of *B. macerans* spores are listed in Table 1. The extremely low concentration (10^{-6} M) at which some of the germinants (pyridoxine, glucose, 2,6-diaminopurine, phenylacetamide) induce germi-

TABLE 1. *Minimum effective concentrations of germinants initiating B. macerans*[a]

Germinant	NCA-7X1 (M)	B-70 (M)	B-430 (M)	B-171 (M)	B-388 (M)
2-Phenylacetamide	10^{-4}	10^{-6}	10^{-5}	10^{-4}	10^{-4}
Phenylacetic acid		10^{-2}	(10^{-2})	10^{-2}	10^{-2}
Benzyl penicillin	10^{-3}	10^{-3}	10^{-3}		10^{-3} [b]
Phenethicillin	i[b]	3×10^{-3}	3×10^{-3}		
n-Valeramide	10^{-2}		10^{-3}	(10^{-2})	(10^{-2})
iso-Valeramide	10^{-2}		10^{-3}		
iso-Butyramide	(10^{-2})		10^{-2}	(10^{-2})	(10^{-2})
Valeric acid		10^{-2}			
L-Asparagine	i	2×10^{-4}	2×10^{-4}	10^{-3}	10^{-3}
D-Asparagine			10^{-2}		
L-Glutamine		i	(10^{-2})		
L-α-Aminobutyric acid	i	2×10^{-2}	2×10^{-3}	(3×10^{-2})	2×10^{-2}
D-Glucose	i	10^{-6}	i	i	(5×10^{-2})
D-Mannose	i	10^{-5}			
2-Deoxy-D-glucose	i	10^{-5}			
D-Glucosamine	i	10^{-4}			
Niacinamide	Fr10^{-2}[c]		(Fr10^{-2})	Fr10^{-2}	Fr10^{-2}
Nicotinamide adenine dinucleotide	Fr10^{-2}		i	Fr10^{-2}	Fr10^{-2}
Pyridoxine	10^{-6}			i	i
Pyridoxal	10^{-6}	i	i	i	i
Pyridoxamine	10^{-4}				
Adenine	8×10^{-5}	i	i	i	i
2,6-Diaminopurine	2×10^{-6}	i	i	i	i
8-Aza-2,6-diaminopurine	10^{-4}				

[a] Concentrations required to reduce optical density to <70% of initial value in 60 min. Figures in parentheses = reduces optical density to 70 to 85% of initial value. (Fructose and ribose at 0.05 M potentiate germination of all classes except those of L-asparagine and D-glucose.)

[b] i, Inactive.

[c] Fr, inactive; however, synergistic action with fructose reduces optical density to <70% of initial value in 60 min.

nation is noteworthy. Fructose (0.05 M) or ribose (0.05 M), when present with the germinants of Table 1, increased both rate and extent of germination (16). Only L-asparagine, D-glucose, and their analogues failed to show potentiation by fructose or ribose. Of the Table 1 germinants, only phenyl acetamide was effective for all strains.

Temperature effects. Increasing temperature in the range 20 to 40 C increased the rate and extent of germination (Fig. 1) in typical fashion (11). Germination rates were calculated by the method of O'Connor and Halvorson (11), and Arrhenius plots were constructed to determine the temperature characteristic (μ) of germination induced by various germinants. The results are given in Table 2; temperature range indicates range in which satisfactory linearity was obtained. The temperature characteristic for L-asparagine was close to the value of 20,000 determined previously for *B. cereus* on L-alanine (11) and *B. megaterium* on glucose (10). The other values ranged from 26,000 to 30,000, still consistent with enzymatic reaction values (19), but distinctly higher than previously determined levels. These high values may be related to the higher optimal growth and germination temperature of *B. macerans*.

pH optima. Strains B-70, B-430, and 7X1 showed relatively broad *p*H optima (*p*H 6.0 to 9.0) for all germinants tested (Fig. 2). Strains B-388 and B-171 were not tested. NCA strain 7X1 showed a *p*H response for germination on pyridoxine identical with that for adenine. Difference in *p*H response for the various strains germinating on different substrates seemed minor.

Ion effects. The effect of salt concentration (KCl, NaCl) on germination of strains 7X1 and B-70 in the presence of various germinants is shown in Fig. 3. Several points are noteworthy. Potassium is the preferred cation (14, 21) and an optimal concentration of 0.05 M to 0.1 M is

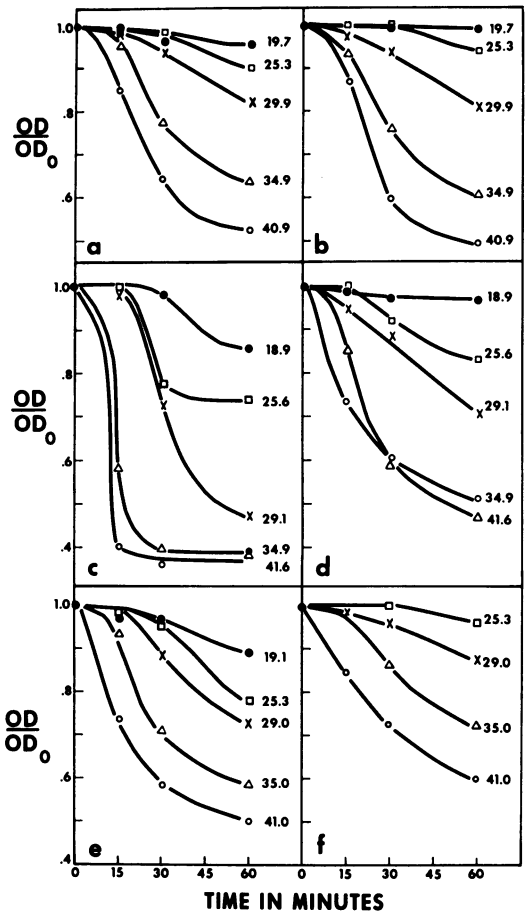

FIG. 1. *Influence of temperature on germination of B. macerans. (a) Strain 7X1 on adenine, 2 × 10^{-4} M. (b) Strain 7X1 on pyridoxal, 10^{-4} M. (c) Strain B-70 on glucose, 10^{-4} M. (d) Strain B-70 on phenylacetamide, 10^{-4} M. (e) Strain B-430 on L-asparagine, 10^{-3} M. (f) Strain B-430 on phenylacetamide, 10^{-4} M. All samples contain 0.05 M K-TES (pH 7.0). Temperature indicated on curves. OD/OD_0 is the ratio of the optical density at a given time to the initial optical density.*

common (3); higher concentrations of both salts are generally inhibitory. Adenine and pyridoxine germination, similar in many respects, differ considerably in sensitivity to high concentrations of Na^+ and K^+. Phenylacetamide germination is unique in being relatively insensitive to high salt concentration and in requiring higher salt concentrations for optimum germination.

Fructose germination shows a requirement for phosphate ion. The phosphate requirement is abolished when germination takes place in the presence of other germinants, except niacinamide. Fructose-niacinamide germination requires phosphate ion.

TABLE 2. *Temperature characteristic of germination of B. macerans spores on various germinants*

Germinant	Strain	Temp range	Temp characteristic (μ)
Pyridoxal	7X1	25.3–40.9	29,000
Adenine	7X1	19.7–40.9	30,600
Glucose	B-70	18.9–41.6	26,200
L-Asparagine	B-70	18.9–41.6	19,500
L-Asparagine	B-430	29.0–41.0	23,400
2-Phenylacetamide	B-70	18.9–34.9	29,000
2-Phenylacetamide	B-430[a]	29.9–41.6	26,200
2-Phenylacetamide	B-430	29.0–41.0	28,500

[a] Phosphate buffer.

Some characteristics of the germination induced by various germinants are given below.

2-Phenylacetamide. 2-Phenylacetamide is very effective for all strains in the presence of fructose; it is also effective alone, but to varying degrees (Table 1). Benzyl penicillin, a phenylacetamide derivative of penicillin, is also effective, but at higher concentration levels (Fig. 4; Table 1). Five other penicillin derivatives were tested. Phenethicillin, the R group of which is analogous to phenylacetamide, showed activity (Table 1), but ampicillin, methicillin, oxacillin and cloxacillin were inactive. Benzyl penicillin retained full activity after treatment with penicillinase, indicating that antibiotic activity is not associated with germinative activity.

Phenylacetic acid also showed good activity although at higher concentration levels. Benzamide was inactive. *n*-Valeramide, iso-valeramide, iso-butyramide, and *n*-valeric acid also showed activity at 10^{-2} to 10^{-3} M.

Phenylacetamide germination is favored by K^+ as compared to Na^+ and is not greatly retarded by high ionic strength.

L-Asparagine. L-Asparagine is an excellent germinant for all strains except 7X1 (Table 1). D-Asparagine and L-glutamine also showed activity, but at much higher concentration levels. L-Aspartic acid was inactive. Other amino acid amides (phenylalanine amide, leucine amide, alanine amide) were also inactive.

L-Asparagine germination was not potentiated by fructose or ribose.

Adenine. Adenine and 2,6-diaminopurine are effective germinants for NCA strain 7X1 only. 8-Aza-2,6-diaminopurine is also effective for 7X1, but at higher concentration levels. Many analogues were tested and found ineffective. (Inactive analogues of adenine are: hypoxanthine, guanine, xanthine, caffeine, allantoic acid, 6-methylpu-

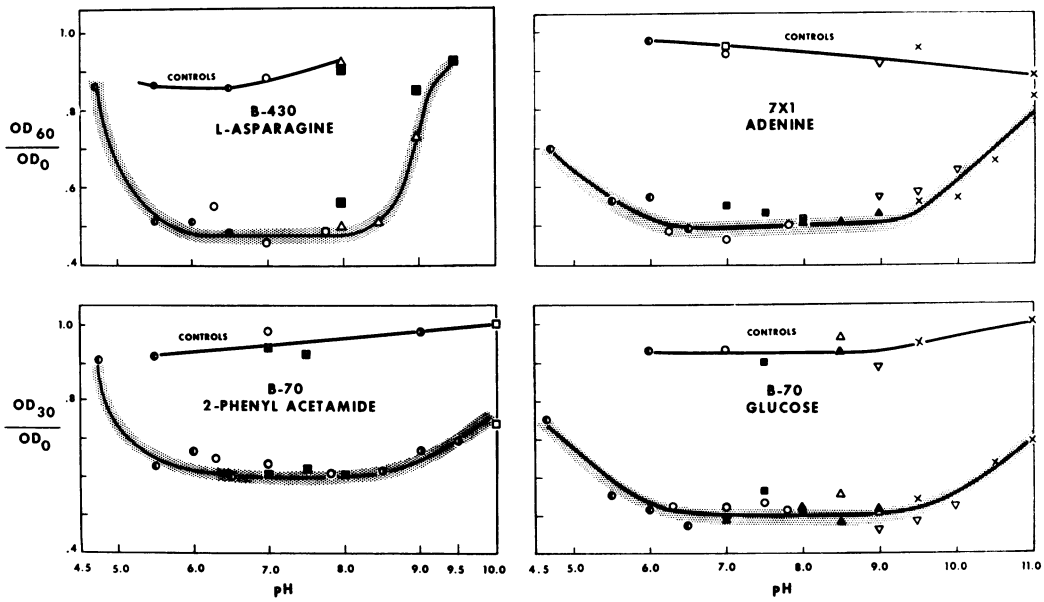

FIG. 2. *Influence of pH on germination of B. macerans spores. Germinant concentrations: Asparagine, 10^{-3} M; adenine, 2×10^{-4} M; phenylacetamide, 10^{-4} M; glucose, 10^{-4} M. All buffers are 0.05 M. Temperature 40 C. Abbreviations: CAPS, cyclohexylaminopropane sulfonic acid; TAPS, tris(hydroxy-methyl)methylamino propane sulfonic acid; TES, N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid; Tris, tris(hydroxymethyl)aminomethane. Symbols: ◐, citrate; ○, phosphate; ⊠, TES; ▲, TAPS; △, TRIS; ▽, glycine; ◑, NH_4OH; ×, CAPS; □, K_2CO_3. OD_{30}/OD_0 and OD_{60}/OD_0 are the ratios of the optical densities at 30 and 60 min, respectively, to the initial optical density.*

rine, 6-chloropurine, 6-methylaminopurine, 6-dimethylaminopurine, allopurinol, 8-azahypoxanthine, 8-azaguanine, 6,8-dihydroxypurine. Inactive ribosides are: adenosine, inosine, deoxyinosine, deoxyadenosine, guanosine, xanthosine, uridine, cytidine, deoxycytidine, 6-mercaptoguanosine, 6-mercaptopurine riboside, kinetin, psicofuranine. Inactive pyrimidines are: uracil, cytosine, 5-aminouracil, orotic acid, 5-methylcytosine, 6-methylcytosine, 4,6-dihydroxypyrimidine, isocytosine, 2-amino-4-methylpyrimidine, 4-hydroxypyrazolopyrimidine.)

Adenine germination shows the relatively broad *p*H optimum (Fig. 2) characteristic of *B. macerans* spores. Inorganic ions influence adenine germination strongly (Fig. 3), and sodium ions at relatively low concentration levels appear to be strongly inhibitory, much more so than potassium ions.

Adenine germination is potentiated by fructose; both rate and extent of germination are increased in its presence (16).

Pyridoxine and pyridoxal. Pyridoxine and pyridoxal are extremely effective germinants for strain 7X1; germination resembles adenine germination not only in strain specificity but in minimum effective concentration (Table 1), potentiation by fructose (Fig. 5), temperature response (Fig. 1b), and *p*H optimum (*not shown*). However, high salt concentrations are much less inhibitory to pyridoxine germination (Fig. 3b, d), and sodium ions do not appear to be inhibitory.

Pyridoxamine is also very effective, but at concentration levels appreciably higher than those required for activity of pyridoxine or pyridoxal.

Fructose and ribose. Although sugars are not good germinants for strains other than B-70, D-fructose and D-ribose, at relatively high concentration (0.05 M), strongly potentiate germination induced by adenine, pyridoxine, phenylacetamide, α-aminobutyric acid, niacinamide, and their active analogues.

Fructose is fully effective only at relatively high concentration levels (ca. 0.05 M); when used alone or together with niacinamide it requires phosphate ion. Spore germination induced in a fructose-phosphate medium is often characterized by a marked lag period (16).

Ribose may be substituted for fructose at about the same concentration level. For B-430 spores, ribose appears to be superior to fructose as a germinant.

Glucose. Glucose is an extremely effective germinant for strain B-70, inducing rapid and complete germination at 10^{-6} M (Table 1; Fig. 1c). D-Mannose, 2-deoxy-D-glucose, D-glucos-

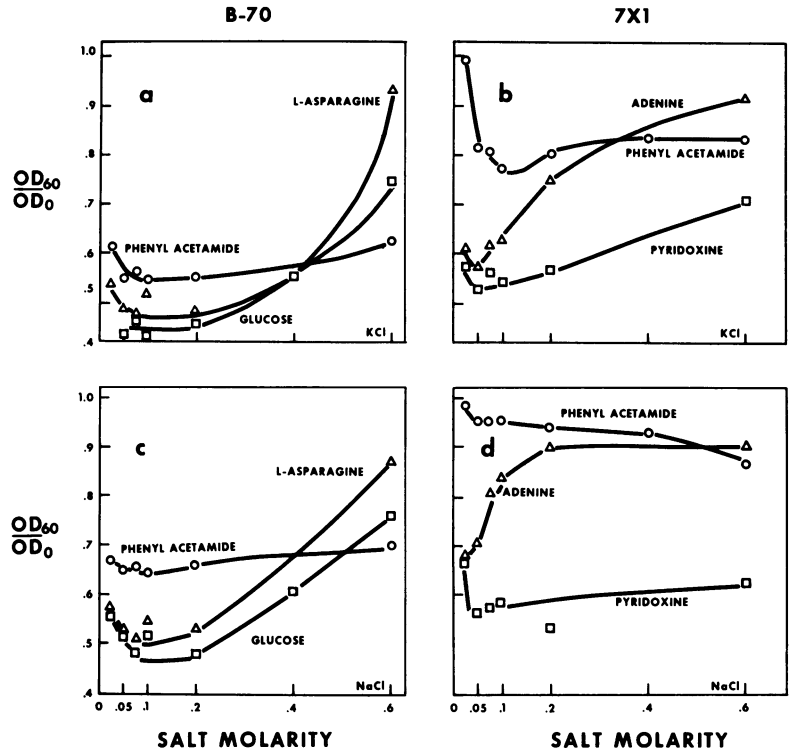

FIG. 3. *Influence of cation concentration on germination of B. macerans spores. (a,c) Strain B-70. (b,d) Strain 7X1. (a,b) Contain KCl at the molarity indicated. (c,d) Contain NaCl. Reagent concentrations: adenine, 2 × 10⁻⁴ M; pyridoxine, 10⁻⁴ M; phenylacetamide, 10⁻⁴ M; glucose, 10⁻⁴ M; L-asparagine, 10⁻³ M. K-TES (0.05 M, pH 7), was present in all tubes. Temperature, 40 C.*

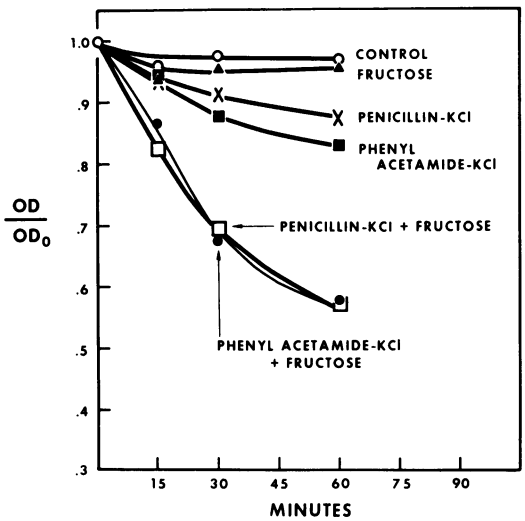

FIG. 4. *Synergistic action of fructose with phenyl acetamide or benzyl penicillin on the initiation of germination of B. macerans 7X1 spores. Reagent concentrations: fructose, 5 × 10⁻² M; 2-phenyl acetamide, 10⁻⁴ M; benzyl penicillin, 10⁻³ M. All tubes contain 0.05 K-TES (pH 7) and 0.2 M KCl. Temperature, 40 C.*

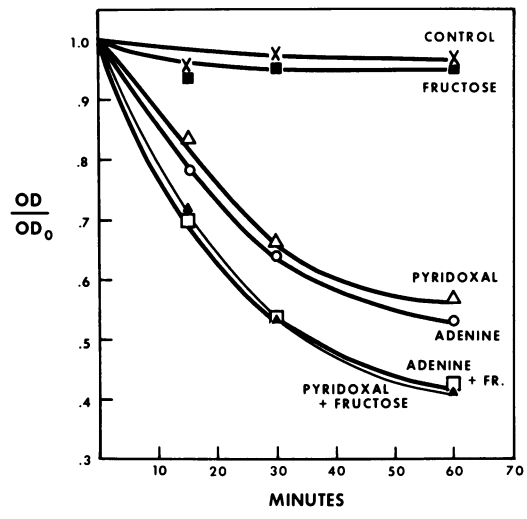

FIG. 5. *Initiation of germination of B. macerans 7X1 spores on adenine and pyridoxal in the presence and absence of fructose. Reagent concentrations: adenine; 2 × 10⁻⁴ M; pyridoxal, 10⁻⁴ M; fructose, 5 × 10⁻² M. All tubes contained 0.05 M K-TES (pH 7). Temperature, 40 C.*

amine, and D-fructose are also very effective although at increasing concentration levels (Table 1). L-Glucose is ineffective.

Strain B-388 germinates to an appreciable extent in the presence of a high concentration (0.05 M) of glucose or fructose.

α-Aminobutyric acid. α-Aminobutyric acid is a germinant for four strains (Table 1); germination is markedly potentiated by fructose or ribose. Other amino acids (including L-alanine) were inactive.

Niacinamide. Niacinamide is inactive by itself, but in combination with fructose it is quite effective (Table 3). However, it is inactive at concentration levels below 10^{-2} M. NAD is also active at these concentration levels. Nicotinic acid is inactive. Phosphate is required for fructose-niacinamide germination.

Combination of germinants. Fructose, a weak germinant for several strains, powerfully synergizes germination induced by nearly all germinants, except L-asparagine. When concentrations of adenine sufficient to induce maximum response of 7X1 spores are used, the presence of additional amounts of pyridoxine does not increase the rate or extent of germination. This fact, considered with the similarities in germination response to adenine and pyridoxine, may indicate that these germinants are acting at the same site or by a common pathway. Penicillin or phenylacetamide, however, can increase both the rate and extent of adenine-fructose germination (Fig. 6). This fact, considered with other differences between adenine and phenylacetamide germination, suggests that adenine and phenylacetamide act at different sites or by different mechanisms.

Hydrogen peroxide. Hydrogen peroxide, a nonphysiological "germinant," did induce "germination" of strain B-70 at concentrations of 0.025 to 0.1%. Other strains were not tested.

Inactive germinants. L-Alanine, adenosine, inosine, L-leucine, L-tyrosine, L-arginine, and L-lactic acid, compounds active in initiating germination of spores of other species, all failed to initiate germination of *B. macerans* spores. *n*-Dodecylamine and calcium dipicolinate (CaDPA), nonphysiological germinants effective for spores of many species, appear to be inactive for *B. macerans* spores. However, 0.01 M K_2DPA was active for B-70, B-430, and B-171, in the presence of fructose.

TABLE 3. *Synergistic action of fructose and niacinamide on germination of B. macerans spores*

Substance	OD_{60}[a]/OD_0	
	B-388	B-171
Potassium phosphate, 0.05 M, *p*H 7	0.959	0.960
Plus fructose, 0.05 M	0.816	0.886
Plus niacinamide, 0.01 M	0.935	0.891
Plus fructose + niacinamide	0.635	0.675

[a] Optical density.

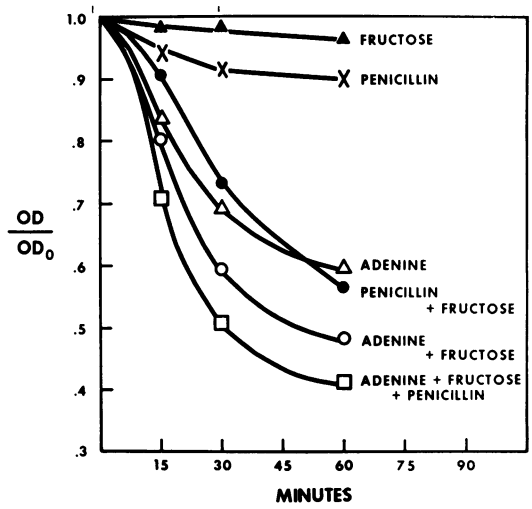

FIG. 6. *Influence of combinations of germinants on germination of B. macerans 7X1 spores. Reagent concentrations: fructose, 5 × 10^{-2} M; penicillin, 10^{-3} M; adenine; 2 × 10^{-4} M. All tubes contained 0.05 M K-TES (pH 7) and 0.01 M KCl. Temperature, 40 C.*

DISCUSSION

The germination requirement pattern exhibited by *B. macerans* spores is distinct from that of *B. cereus*, *B. megaterium*, and *B. subtilis* spores. However, there are many similarities. It may be instructive to consider the points of convergence and divergence. Adenosine and inosine are ineffective, but adenine and 2,6-diaminopurine are highly effective for NCA 7X1. L-Alanine is ineffective but α-aminobutyric acid, generally less effective than alanine (6, 22), is effective. Phenylalanine (5) is ineffective, but phenylacetamide is highly effective.

Other *B. macerans* germinants, D-glucose (5, 7, 15), L-asparagine (5, 21), pyrodoxine (13), and niacinamide (18), all have been shown to have germinative properties for other species. Potassium (21) and phosphate (3, 6) ion requirements have also been reported, as has the potentiation of germination by fructose (21).

Although the five *B. macerans* strains tested exhibit appreciable diversity in their germination requirements, the pattern seems to be one of variation on a limited number of themes (15, 20).

There is a considerable range of concentrations at which the various germinants are capable of acting. Adenine, 2,6-diaminopurine, pyridoxine,

pyridoxal, glucose, 2-deoxy-D-glucose, and 2-phenylacetamide are capable of initiating germination at 10^{-5} or 10^{-6} M. On the other hand, D-fructose and D-ribose have reduced activity below 0.05 M. This, of course, raises the possibility that traces of impurity in these latter compounds are the true germinants. Although this possibility cannot be entirely excluded, it seems unlikely since high purity samples from several manufacturers show the same activity.

Fructose has been shown to chelate iron and other cations (2) and the possibility that the germination effects of fructose were due to its chelation properties was considered. Neither ethylenediaminetetraacetic acid (EDTA), citrate, nor any of a number of common chelating agents were able to stimulate initiation or duplicate the fructose effect in any way. Spores treated with EDTA and washed did not show any differences in germination when compared with untreated spores.

B. macerans spores resemble spores of other species in their ionic requirements (3, 4), germinating best at an ionic strength of 0.05 M to 0.1 M with considerable retardation at higher ionic strength. However, the pyridoxine and phenylacetamide germination proceeded quite well at high ionic strength. The fact that the inhibitory action of high salt concentrations varies markedly with the germinant suggests that high salt concentration may influence an early step in the germination mechanism, if we assume that late steps are common to germination initiated by any physiological germinant.

Thus, it might be presumed that high concentrations of ions are more likely to influence binding of the trigger to the receptor protein than they are to influence the dissociation of the hypothetical spore CaDPA complex (3) or the activity of the "lytic enzyme" (4). The stimulatory effects of low concentrations of ions might reflect an influence on either or both of these events.

The broad *p*H optimum (*p*H 6.0 to 9.0) exhibited by *B. macerans* spores of all strains regardless of the nature of the germinant is noteworthy. Few studies have been made which employ a variety of germinants and buffers and cover a wide *p*H range. Church et al. (1) showed a similar broad optimum *p*H in the range *p*H 7 to 10 for the germination of *B. cereus* spores on alanine-adenosine. *B. megaterium* germinated quite well in the range *p*H 5 to 8 on seven germinants, although glucosamine and L-proline germination appeared to be favored by alkaline *p*H levels (7). Some *B. subtilis* spores show distinct and sharply different *p*H optima for glucose and L-alanine (20); a distinct *p*H optimum was demonstrated for *B. subtilis* germinating on L-alanine when k_m (maximal rate) was plotted against *p*H (21).

2-Phenyl acetamide was not previously reported to possess germinative properties, nor have phenyl acetic acid or benzyl penicillin.

Most physiological germinants are of low molecular weight and are almost universally present in biological materials. Their release from decomposing matter might constitute a signal indicating the presence of a potential food source (12). Phenyl acetamide has been found in some plant materials (8) and may represent a specialized signal which does not really differ significantly from the other members of this class.

B. macerans has some unique germination requirements and may prove to be a useful species to explore some aspects of germination.

LITERATURE CITED

1. Church, B. D., H. Halvorson, and H. O. Halvorson. 1954. Studies on spore germination: its independence from alanine racemase activity. J. Bacteriol. **68**:393-399.
2. Davis, P. S., and D. J. Deller. 1966. Prediction and demonstration of iron chelating ability of sugars. Nature (London) **212**:404-405.
3. Fleming, H. P., and Z. J. Ordal. 1964. Responses of *Bacillus subtilis* to ionic environments during sporulation and germination. J. Bacteriol **88**:1529-1537.
4. Gould, G. W. 1969. Germination, p. 397-444. *In* G. W. Gould and A. Hurst (ed.), The bacterial spore. Academic Press Inc., New York.
5. Hachisuka, Y., N. Asano, N. Kato, M. Okajima, M. Kitaori, and T. Kuno. 1958. Studies on spore germination. I. Effect of nitrogen sources on spore germination. J. Bacteriol. **69**:399-406.
6. Hermier, J., and M. Rousseau. 1967. La germination de la spore de *Bacillus subtilis*. IV. Role des acides amines dans la perte de réfringence de la spore. Ann. Inst. Pasteur **113**:327-340.
7. Hyatt, M. T., and H. S. Levinson. 1962. Conditions affecting *Bacillus megaterium* spore germination in glucose or various nitrogenous compounds. J. Bacteriol. **83**: 1231-1237.
8. Isogai, Y., T. Okamoto, and T. Koizumi. 1963. Isolation of 2-phenyl acetamide, indole-3 acetamide and indole-3-carboxaldehyde from etiolated seedlings of *Phaseolus*. Chem. Pharm. Bull. (Tokyo) **11**:1217-1218.
9. Keynan, A., and Z. Evenchik. 1969. Activation, p. 359-396. *In* G. W. Gould and A. Hurst (ed.), The Bacterial Spore. Academic Press Inc., New York.
10. Levinson, H. S., and M. T. Hyatt. 1970. Activation energy for glucose-induced germination of *Bacillus megaterium* spores. J. Bacteriol. **103**:270-271.
11. O'Connor, R. J., and H. O. Halvorson. 1961. L-Alanine dehydrogenase: a mechanism controlling the specificity of amino acid-induced germination of *Bacillus cereus* spores. J. Bacteriol. **82**:706-713.
12. Pulvertaft, R. J. V., and J. A. Haynes. 1951. Adenosine and spore germination; phase-contrast studies. J. Gen. Microbiol. **5**:657-663.
13. Rode, L. J., and J. W. Foster. 1961. Physiological and chemical germination of spores of *Bacillus megaterium*. Z. Allg. Mikrobiol. **1**:307-322.
14. Rode, L. J., and J. W. Foster. 1962. Ions and the germination of spores of *Bacillus cereus* T. Nature (London) **194**: 1300-1301.

15. Rode, L. J. 1968. Correlation between spore structure and spore properties in *Bacillus megaterium*. J. Bacteriol. **95:** 1979–1986.
16. Sacks, L. E. 1967. Adenine and 2,6-diaminopurine as germinants for *Bacillus macerans* spores. J. Bacteriol. **94:** 1789–1790.
17. Sacks, L. E. 1969. Modified two-phase system for partition of *Bacillus macerans* spores. Appl. Microbiol. **18:**416–419.
18. Shoesmith, J. G., and K. T. Holland. 1967. The germination requirements of spores of *Clostridium tetani*. Biochem. J. **107:**38.
19. Sizer, I. W. 1943. Effects of temperature on enzyme kinetics. Adv. Enzymol. **3:**35–62.
20. Thorley, C. M., and J. Wolf. 1961. Some germination factors of mesophilic spore formers, p. 1–13. *In* H. O. Halvorson (ed.), Spores II. Burgess Publishing Co., Minneapolis.
21. Wax, R., E. Freese, and M. Cashel. 1967. Separation of two functional roles of L-alanine in the initiation of *Bacillus subtilis* spore germination. J. Bacteriol. **94:**522–529.
22. Woese, C. R., H. J. Morowitz, and C. A. Hutchison III. 1958. Analysis of action of L-alanine analogues in spore germination. J. Bacteriol. **76:**578–588.