Genetic Analysis of Spore Germination Mutants of *Bacillus subtilis* 168: the Correlation of Phenotype with Map Location

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(Received 21 September 1978)

The isolation and characterization of 29 new germination (Ger) mutants of *Bacillus subtilis* 168 is described. These were classified, along with previously described mutants, into seven groups according to map location. The mutations in 26 GerA mutants mapped between *cysB* and *thr*; detailed mapping of two of these has located them very close to *citG*. These mutants were deficient in germination in alanine, but responded to the germinative combination of asparagine, glucose, fructose and KCl. One GerB mutant mapped on the origin-proximal side of *hisA*; it was normal in germination in alanine, but deficient in germination in a mixture of asparagine, glucose, fructose and KCl. Two GerC mutants were linked to *lys*, but were separable from a temperature-sensitive growth deficiency mapping between *lys* and *trp*. The GerC mutants had a similar germination phenotype to the GerA mutants. Three GerD mutants did not germinate in either of the above germinants or in Penassay Broth. They were located on the side of *ery* distal to *cysA*. The GerE mutant, which did not germinate in any of the three germinants, was located very close to *citF* and possessed an altered spore coat. The two GerF mutants were defective in germination in all three germinants and mapped on the origin proximal-side of *hisA*, but much closer to *his* than did the GerB mutant. A phosphoglycerate kinase-negative mutant altered in germination mapped between *cysB* and *hisA* (GerG). These mutants have established a minimum of seven locations important to germination, and will be useful in the development and appraisal of theories of spore germination.

INTRODUCTION

The differentiation process in a spore-forming bacterium is complex. It involves the formation of a dormant and resistant spore, its release from the dormant state (germination of the spore) and its subsequent outgrowth into a vegetative cell. Mutants defective in sporulation (Piggot & Coote, 1976), germination (Wax *et al.*, 1967; Trowsdale & Smith, 1975; Vary, 1975; Foerster, 1971) and outgrowth (Nukushina & Ikeda, 1969; Galizzi *et al.*, 1973, 1975) are being used to analyse this process and its control.

Germination of the bacterial spore is accompanied by changes in the structure and metabolic capacity of the spore (Gould, 1969) and can proceed in the presence of inhibitors of transcription and translation (Keynan & Halvorson, 1965). It can be induced by chemical or physical stimuli. Amino acids, nucleosides and sugars are common chemical germinants; *Bacillus subtilis* 168, for example, is stimulated to germinate by a number of amino acids, alanine being particularly effective (Woese *et al.*, 1958). Germination mutants, in which spore germination does not proceed at the normal rate in response to germinants, have been obtained in a number of *Bacillus* species, but only in a few cases has a corresponding (and perhaps causative) biochemical defect been identified (Foerster & Foerster,

1973; Prasad *et al.*, 1972). The study of such mutants can provide information on requirements for germination and relationships between different germinants. The isolation of germination mutants in *B. subtilis* 168 has permitted some genetic analysis and they have been shown to result from mutations at one of several locations on the chromosome (Trowsdale *et al.*, 1974; Trowsdale & Smith, 1975; Lafferty & Moir, 1977; Smith *et al.*, 1977; Smith *et al.*, 1978). This paper describes the isolation, phenotypic characterization, mapping and attempted classification of a spectrum of germination mutants of *B. subtilis* 168.

METHODS

Bacterial strains. All strains were derivatives of *Bacillus subtilis* 168 (Table 1). Germination mutants described in this paper (Table 2) include six of those isolated by Trowsdale & Smith (1975) which have been studied further. All mutants were derived by mutagenesis of strain 1604, except for 1484, 1498, 1503, 1487, 1488, 1558 and 4548 which were derived from strain 1408.

Media. Difco Penassay Broth (PAB) was used as a liquid growth medium. Solid media were potato glucose yeast extract (PGYE agar; Dring & Gould, 1971) and Tryptose Blood Agar Base (TBAB; Difco) solidified with 1.5% (w/v) Oxoid no. 1 agar. TBAB top layer contained 1% agar. Minimal agar (MA; Anagnostopoulos & Spizizen, 1961) contained glucose (5 mg ml⁻¹) and was supplemented as necessary with amino acids (50 µg ml⁻¹) except for arginine (200 µg ml⁻¹). Sodium DL-lactate (5 mg ml⁻¹) and L-alanine (2 mg ml⁻¹) were used as alternative carbon sources. For growth of strain 61111, L-malic acid, adjusted to pH 7.0 with NaOH, was added to PAB and TBAB at 5 mM and to MA at 10 mM. G agar was MA supplemented with CaCl₂ (60 µM), MnCl₂ (100 µM), L-asparagine (10 mM) and D-fructose (1 mg ml⁻¹). NA-bromocresol purple agar was the purification medium of Carls & Hanson (1971).

Preparation of spores. This was as previously described (Lafferty & Moir, 1977). PGYE agar was the medium usually employed for preparation of spores, but occasionally the resuspension method of Sterlini & Mandelstam (1969) was used.

Germination studies. (i) The germination of spore suspensions was followed by measuring the fall in absorbance at 580 nm. Readings taken at 15 min intervals over a 60 min period after addition of germinant were normalized to the initial value and represented graphically. This produced a curve describing the proportional fall in absorbance with time. Since a spore population is asynchronous with respect to the time of exposure to germinant before germination occurs (microlag) and since the actual duration of the germination-associated changes (microgermination) is short, the curve reflects the distribution of microlag time in the population. The term germination 'rate' is used in this paper to signify the maximum gradient of the curve; a steeper curve (narrower distribution of microlag) was correlated with a shorter mean microlag.

Germinants used included: L-alanine (10 mM), ALA; L-asparagine (10 mM), ASN; D-glucose (1 mg ml⁻¹), GLC; D-fructose (1 mg ml⁻¹), FRU; KCl (10 mg ml⁻¹); PAB; SPAB, i.e. PAB+ALA+ASN+GLC+FRU+KCl. Germination was in 0.1 M-Tris/HCl, pH 7.4, except when PAB or SPAB was used.

(ii) Most germination mutants (Ger⁻) could be distinguished from the wild type (Ger⁺) by the tetrazolium overlay technique, based on that of Lafferty & Moir (1977). Spore-containing colonies on PGYE agar were exposed to chloroform vapour, then overlaid with agar containing 2,3,5-triphenyltetrazolium chloride. After incubation, the Ger⁺ colonies appeared red as a result of reduction of the tetrazolium (Tzm-Red) while Ger⁻ colonies remained unstained (Tzm-White). The response to tetrazolium was dependent on the efficiency of sporulation in the colony, and this was affected by some auxotrophic markers. *CysB* strains, for example, sporulated better and gave a clearer response in the test if PGYE agar was supplemented with cysteine.

(iii) In some cases, Ger⁺ and Ger⁻ phenotypes could be distinguished by replica plating after chloroform treatment to G agar and incubating at 25 °C for 16 h. The replicas of Ger⁻ clones showed much fainter growth than those of Ger⁺ clones, the distinction diminishing after further incubation at 25 °C.

Isolation of germination mutants. Exponential phase cultures of strain 1604 in PAB were either treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (75 µg ml⁻¹) for 1 h at 37 °C, or resuspended in 100 mM-MgSO₄ and irradiated with ultraviolet light (0.1% survival). Samples (0.2 ml) of undiluted and 10-fold diluted mutagenized cultures were used to prepare spores as described above. The procedures for enrichment for germination mutants were described by Lafferty & Moir (1977). Several mutants were retained from the same mutagenized culture only if their germination phenotypes were clearly different.

Transduction. Phage PBS1 lysates were prepared and used in transduction as described by Jamet & Anagnostopoulos (1969). Transductants were selected and purified on the same selective medium and a single colony was used for determining unselected markers. Cit⁺ and phosphoglycerate kinase⁺ (PGK⁺) recombinants were selected for their ability to grow on MA plus lactate and MA, respectively, and were

Table 1. *Strains of Bacillus subtilis* 168

Strain designation	Genotype	Origin
1408	<i>trpC2</i>	Trowsdale & Smith (1975)
BD111	<i>cysB3 thr-5 trpC2</i>	D. Dubnau
SB137	<i>aroB2 hisH2</i>	Nester <i>et al.</i> (1963)
1696	<i>trp hisH2</i>	J. Trowsdale. Aro ⁺ and Trp ⁻ transduced into SB137
1604	<i>trpC2</i>	Cys ⁺ and Thr ⁺ from 1696 transduced into BD111
BD92	<i>cysB3 hisA1 trpC2</i>	C. Anagnostopoulos
1621	<i>nov-3 trpC2</i>	Thy ⁺ revertant of VUB33 (Trowsdale & Anagnostopoulos, 1976)
4650	<i>cysB3 nov-3 trpC2</i>	Nov ^R and Thr ⁺ from 1621 transduced into BD111
60229	<i>ald trpC2</i>	E. Freese
61111	PGK ⁻ <i>met trp</i>	Freese <i>et al.</i> (1972)
BD163	<i>hisA1 argC4</i>	D. Dubnau
1420	<i>hisA1</i>	From BD163
BD112	<i>cysA14</i>	D. Dubnau
4248	<i>cysA14 ery-1</i>	Goldthwaite <i>et al.</i> (1970)
4620	<i>cysA14 ery-1 trpC2</i>	Cys ⁻ and Ery ^R from 4248 transduced into 1604
Mu8u5u16u24	<i>leuA8 metB5 purA16 sacA24</i>	N. Sueoka
1612	<i>metB5 aroI906 sacA321 dal</i>	J. Lepesant
GSY254	<i>lys-1 trpC2</i>	C. Anagnostopoulos
1506	<i>lys-1</i>	Trp ⁺ revertant of GSY254
BD40	<i>phe-12 argA3</i>	D. Dubnau
GSY374	<i>leuA8</i>	C. Anagnostopoulos
WB2281b	<i>aroA</i>	E. W. Nester
JH402	<i>citF2 trpC2</i>	Ohné <i>et al.</i> (1973)
JH326	<i>citF78 leu</i>	Ohné <i>et al.</i> (1973)
JH404	<i>citG4 trpC2</i>	Rutberg & Hoch (1970)

washed in minimal salts before plating. Antibiotic resistance was scored on TBAB agar plus erythromycin (1 µg ml⁻¹) or novobiocin (2 µg ml⁻¹).

Determination of the germination phenotype of transductants. For large numbers of transductants the tetrazolium overlay reaction was used, except where otherwise stated. To confirm the validity of this method for the donor and recipient strains concerned, the germination characteristics of spore suspensions of at least six Ger⁺ and Ger⁻ recombinants were determined for each mutant mapped. The variation in both the germination phenotype and tetrazolium response of spores of different *B. subtilis* 168 strains can be as great as that between the wild type and germination mutants. Wherever possible, recipient strains whose spores germinated and gave the same tetrazolium reaction as the Ger⁺ strain 1604 were used.

RESULTS

Choice of 'wild-type' strain

Neither Ger⁺ nor Ger⁻ phenotypes could be selected; reliability of the tetrazolium overlay reaction was essential for recognizing and scoring germination defects (Trowsdale & Smith, 1975; Lafferty & Moir, 1977). It was therefore necessary to use a Ger⁺ starting strain which grew, sporulated and germinated normally and which reduced 2,3,5-triphenyl-tetrazolium chloride efficiently, becoming dark red on incubation after overlay. Trowsdale & Smith (1975) were aware that their *B. subtilis* 168 wild-type strain 1408 did not meet all these criteria. However, they showed that some *ger* mutations were linked to *cysB* and that the Ger⁺ and Ger⁻ phenotypes of Cys⁺ transductants of BD111 (Table 1) could be clearly distinguished both by the germination ability of washed spore suspensions and by the tetrazolium reaction. Thus Cys⁺ transductants of BD111 satisfied our conditions, and moreover, BD111 would be an appropriate strain into which to transfer *cysB*-linked germination mutations. It was therefore used as the major genetic background of the Ger⁺ starting 'wild-type'. The cysteine requirement of BD111 was removed by transduction to Cys⁺Thr⁺ using a lysate grown on strain 1696 (Table 1). The germination characteristics of spores of this strain (1604) are shown in Fig. 1. Spores responded well to all germinants

Fig. 1. Germination of spore suspensions of strain 1604. Spores produced on PGYE agar at 42 °C were germinated at 25 °C (●) and 42 °C (○) in: (a) 0.1 M-Tris/HCl, pH 7.4, containing L-alanine (10 mM); (b) 0.1 M-Tris/HCl, pH 7.4, containing L-asparagine (10 mM), D-glucose (1 mg ml⁻¹), D-fructose (1 mg ml⁻¹) and KCl (10 mg ml⁻¹); (c) Penassay Broth.

tested, except that spores prepared at 25 °C germinated poorly in ASN + GLC + FRU + KCl at 25 °C. Heat activation stimulated the rate of, but was not essential for, germination of strain 1604. Most of the spore germination mutants were derived from this strain.

Germinants

Three germinants were chosen. (i) L-Alanine – this is the amino acid to which spores of *B. subtilis* 168 respond most sensitively (the rate of germination is further enhanced if KCl is added). (ii) A mixture of L-asparagine, D-glucose, D-fructose and KCl – germination is induced only if all four components are present and these may satisfy the same metabolic requirements for germination as alanine (Wax & Freese, 1968); thus germination mutants altered in response to alanine might remain normal for the mixture, and vice versa. (iii) Penassay Broth – this is a complex medium in which a number of compounds whose nature has not been determined may be involved in germination.

Mutant isolation

After mutagenesis of vegetative cells and subsequent enrichment of their spores for germination mutants in ALA, PAB or SPAB, colonies from surviving spores were picked and purified on TBAB. Their spore germination phenotype was then tested by the tetrazolium reaction and by replica plating. Spores were produced from those isolates (1 to 10% of the total) distinguishable from the wild type in this preliminary screening, and their germination response to different germinants was measured.

All the mutants isolated, except 4592 (*ger-18*), were Tzm-White; spores of all germinated poorly (< 10% fall in absorbance in 60 min) in at least one of the germinants. Their phenotypes are described in Table 2. All the mutants grew normally on MA plus tryptophan, except for 4586 (Thr⁻), 4600 (His⁻) and 4521 (cold sensitive for growth). The germination mutations could be separated by PBS1 transduction from the mutations responsible for these phenotypes.

The type of germination mutant obtained was influenced by the nature of the germinants used. Enrichment in ALA resulted in isolation of mutants which germinated in ASN + GLC + FRU + KCl but not in ALA. Enrichment in PAB resulted not only in mutants of this Ger⁻ phenotype, but also one which germinated in ALA but not in ASN + GLC + FRU + KCl and another which was defective in germination in all three germinants; five

Table 2. *Origin and properties of germination mutants*

Strain designation	Mutation	Mutagen used	Enrichment	Germination* in:		
				PAB	ALA+KCl	ASN+GLC+FRU+KCl
4522	<i>ger-1</i>	UV	ALA	+	—	+
4507	<i>ger-2</i>	NG	ALA	+	hs	+
4582	<i>ger-3</i>	UV	PAB	±	—	+
4514	<i>ger-4</i>	NG	PAB	±	—	+
4502	<i>ger-5</i>	NG	PAB	±	—	+
4521	<i>ger-6</i>	NG	PAB	±	—	+
4583	<i>ger-7</i>	UV	PAB	±	—	+
4584	<i>ger-8</i>	UV	PAB	±	—	+
4506	<i>ger-9</i>	NG	ALA	±	—	+
4505	<i>ger-10</i>	NG	PAB	±	—	+
4585	<i>ger-11</i>	UV	SPAB	—	—	+
4586	<i>ger-12</i>	UV	SPAB	hs	hs	+
4587	<i>ger-13</i>	UV	SPAB	—	—	+
4588	<i>ger-14</i>	UV	SPAB	—	—	+
4589	<i>ger-15</i>	NG	PAB	—	—	+
4590	<i>ger-16</i>	NG	PAB	hs	hs	+
4592	<i>ger-18</i>	UV	PAB	+	+	—
4593	<i>ger-19</i>	UV	SPAB	—	—	—
4594	<i>ger-20</i>	UV	SPAB	—	—	—
4595	<i>ger-21</i>	UV	PAB	—	—	—
4518	<i>ger-26</i>	NG	PAB	±	—	+
4504	<i>ger-28</i>	NG	PAB	±	—	+
4500	<i>ger-29</i>	NG	ALA	±	—	+
4512	<i>ger-31</i>	NG	PAB	±	—	+
4510	<i>ger-32</i>	UV	ALA	±	—	+
4548	<i>ger-35</i>	NG	ALA	+	—	+
4600	<i>ger-36</i>	UV	SPAB	—	—	—
4601	<i>ger-37</i>	UV	SPAB	—	—	—
4728	<i>ger-45</i>	UV	SPAB†	—	—	—
1484	<i>ger-84</i>	NG	ALA	+	—	+
1498	<i>ger-98</i>	NG	PAB	±	—	+
1503	<i>ger-89</i>	NG	PAB	±	—	+
1487	<i>ger-87</i>	NG	PAB	±	—	+
1488	<i>ger-88</i>	NG	PAB	+	hs	+
1558	<i>ger-58</i>	NG	PAB	+	hs	+

UV, Ultraviolet light; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; ALA, Tris/HCl containing L-alanine; PAB, Penassay Broth; SPAB, PAB containing L-alanine, L-asparagine, D-glucose, D-fructose and KCl; ASN+GLC+FRU+KCl, Tris/HCl containing L-asparagine, D-glucose, D-fructose and KCl.

* Germination rates relative to wild-type spores: + = > 50 %, ± = 15 to 50 %, — = < 15 %. hs, Germination rate slower at 42 °C than at 25 °C.

† Spores produced by the resuspension method.

out of 15 of the mutants from this enrichment germinated well in PAB. Enrichment in SPAB resulted in approximately equal proportions of mutants responsive to neither ALA nor PAB and mutants not germinating in any of the three test germinants. Although the enrichment procedures did not yield only the anticipated types of mutant, a higher proportion of the mutants were unresponsive to a wide range of germinants when several germinants were present during the enrichment.

Phenotypes and mapping of Ger mutants

The genetic and physiological analysis enabled the assignment of mutants to groups GerA to GerG according to phenotype and map location (Lafferty & Moir, 1977; Smith *et al.*, 1977; Smith *et al.*, 1978). These phenotypic designations, which also reflect approximate map location, are not equivalent to genotypes and are regarded as a temporary convenience.

Fig. 2. Germination of spore suspensions of strain 4586 (*ger-12*). Details as in Fig. 1 legend.

Mutants in which germination is conditional on the nature of the germinant

GerA mutants. Strains carrying the mutations *ger-1* to -16 inclusive and *ger-26*, -28, -29, -31, -32 and -35, along with *ger-84*, -86, -87, -89, -97 and -98 of Trowsdale & Smith (1975) form a distinct phenotypic group. All failed to germinate in ALA+KCl, but responded well to ASN+GLC+FRU+KCl. Their response to PAB varied from that of wild type (*ger-1*) through intermediate levels (*ger-4*) to less than 10% of wild type (*ger-11*). As indicated in Table 2, the germination of several mutants was heat-sensitive; they germinated less well at 42 °C than at 25 °C. Strain 4586 (*ger-12*), for example, was heat-sensitive in ALA and to some extent in PAB (Fig. 2). Mutants which germinated to an intermediate extent in PAB responded less efficiently at 26 °C than at 42 °C; since the *Ger*⁺ strain 1604 also responded more quickly at 42 °C than at 26 °C (Fig. 1), it was not possible to define the degree of cold sensitivity attributable to the *ger* mutation. Where tested (*ger-1*, -2, -5, -6, -9, -10, -26 and -28) the spores of *GerA* mutants did not germinate in L-valine (10 mM) or α -aminobutyrate (10 mM).

The addition to ALA+KCl of GLC+FRU stimulated germination of all of these mutants. The addition of GLC alone sometimes resulted in partial germination, especially if the spores were produced at 42 °C (Fig. 3). Because of this dependence on sporulation temperature it is difficult to classify mutants according to whether they require FRU in addition to GLC for germination in ALA+KCl, although this distinction has previously been employed (Trowsdale & Smith, 1975; Lafferty & Moir, 1977).

Six of the nine germination mutants described by Trowsdale & Smith (1975) share phenotype and map location with the other *GerA* mutants. Of these (Table 2), strain 1484 is included because *ger-84* has now been mapped (Table 3) and 1487, 1498 and 1503 are included because further work has revealed that they germinate as well as the wild type in ASN+GLC+FRU+KCl and to a significant extent in PAB. Like the other mutants, the inability to germinate in ALA+KCl was overcome by the addition of GLC or GLC+FRU.

In order to map the *ger* mutations, phage lysates prepared on *ger trpC2* mutants were used to transduce auxotrophic markers in *Ger*⁺ reference strains to prototrophy, and the germination phenotype of a random sample of transductants was tested by the tetrazolium overlay method. Co-transduction frequencies of *ger* mutations in strains of the *GerA* phenotype with *cysB* and *hisA* were determined (Table 3). In addition, BD111 was transduced to Thr⁺; the linkages to *thr* of *ger-1*, -3, -7, -8, -15, -16 and -84 were 40, 39, 42, 49, 43, 41 and 36% respectively. Mutations *ger-26*, -28, -29, -32 and -35 were 33, 20, 12, 25 and 25% co-transduced with *ald* in crosses with strain 60229 as recipient. Co-transduction

Fig. 3. Effect of temperature of spore formation on the germination of strain 4583 (*ger-7*). Spores produced on PGYE agar at 25 °C (a) and 42 °C (b) were germinated in 0.1 M-Tris/HCl, pH 7.4, containing L-alanine (10 mM) and KCl (10 mg ml⁻¹): ●, without further additions; □, with D-glucose (1 mg ml⁻¹); ■, with D-glucose (1 mg ml⁻¹) and D-fructose (1 mg ml⁻¹).

Table 3. Genetic analysis of group A germination mutants

Co-transduction frequencies with *cysB* were obtained using strain BD111 as recipient and those with *hisA* using BD92 (for *ger-1*, -3, -7, -9, -10 and -16) or BD163 (all other *ger* mutations). Three factor crosses, selecting Cys⁺, were between BD111 as recipient and *ger trpC2* as donor except for the cross locating *ger-12*(*), in which BD92 was the recipient and *ger-12 thr trpC2* was the donor.

Mutation in donor	Co-transduction (%) with		Three factor cross No. of transductants per recombinant class			
	<i>cysB</i>	<i>hisA</i>	Thr ⁻ Ger ⁺	Thr ⁻ Ger ⁻	Thr ⁺ Ger ⁺	Thr ⁺ Ger ⁻
<i>ger-1</i>	80	6	17	43	2	33
<i>ger-2</i>	78	7	18	25	0	38
<i>ger-3</i>	74	9	24	40	1	31
<i>ger-4</i>	70	2	20	32	1	18
<i>ger-5</i>	83	4	16	19	0	60
<i>ger-6</i>	77	8	18	30	0	32
<i>ger-7</i>	74	8	24	41	1	30
<i>ger-8</i>	81	ND	18	32	0	45
<i>ger-9</i>	75	13	17	20	0	31
<i>ger-10</i>	82	3	12	28	1	32
<i>ger-11</i>	76	6	23	41	1	35
<i>ger-12</i>	78	7	2*	21*	32*	41*
<i>ger-13</i>	77	6	23	54	0	24
<i>ger-14</i>	76	9	25	56	0	24
<i>ger-15</i>	80	ND	18	33	0	41
<i>ger-16</i>	69	12	29	51	1	15
<i>ger-26</i>	80	5	11	14	1	34
<i>ger-28</i>	86	9	12	20	0	52
<i>ger-29</i>	72	ND	22	25	1	33
<i>ger-31</i>	59	ND	31	20	0	25
<i>ger-32</i>	77	ND	19	33	1	30
<i>ger-35</i>	69	ND	24	38	1	17
<i>ger-84</i>	69	ND	23	25	1	29

ND, Not done.

Table 4. *Three and four factor transduction crosses ordering ger, nov and cysB in group A germination mutants*

Thr⁺ transductants were selected in crosses between *ger thr-5 trpC2* as donors and strains 1621 (*nov-3 trpC2*) and 4650 (*cysB3 nov-3 trpC2*) as recipients.

Recipient strain	Mutation in donor	No. of transductants per recombinant class			
		Nov ^R Ger ⁺	Nov ^R Ger ⁻	Nov ^S Ger ⁺	Nov ^S Ger ⁻
1621	<i>ger-1</i>	71	19	2	110
	<i>ger-3</i>	66	16	0	115
	<i>ger-11</i>	89	26	1	120
	<i>ger-13</i>	74	18	1	143
	<i>ger-14</i>	55	17	0	87
4650		Cys ⁻ Cys ⁺	Cys ⁻ Cys ⁺	Cys ⁻ Cys ⁺	Cys ⁻ Cys ⁺
	<i>ger-7</i>	29 18	0 14	0 0	0 96
	<i>ger-14</i>	5 5	0 6	0 0	0 54
	<i>ger-15</i>	5 7	0 3	0 0	0 20
	<i>ger-16</i>	27 3	0 8	0 0	0 50

frequencies were calculated from a screening of 90 to 100 transductants in most crosses, but of only 60 to 90 transductants in 12 of the 23 crosses establishing linkage with *cysB*, in two of the 15 with *hisA* but in all five with *ald*. All GerA mutants contained *ger* mutations linked to *cysB* and to *hisA*, *thr* and *ald* where tested. The mean co-transduction frequencies were: *cysB* 76%, *hisA* 7%, *thr* 41% and *ald* 23%.

The presence in BD111 of two linked auxotrophic markers, *cysB3* and *thr-5*, to which *ger* mutations were linked permitted the analysis of recombinant classes in a three factor cross (Table 3). In every cross, the members of one of the four possible combinations of unselected phenotypes were in a clear minority. The order of markers which would be responsible for the observed distribution was *thr-ger-cysB* for each of the 23 GerA mutants tested. This conclusion was entirely consistent with the co-transduction frequencies obtained from two factor crosses and was supported for *ger-1*, -3, -6, -7, -8, -9, -10, -15 and -84 by the analysis of further three factor crosses (A. Moir, unpublished results). The order of genetic markers is therefore *thr-ger-cysB*; the higher co-transduction frequency with *cysB* would indicate that the *ger* mutations are located closer to *cysB* than to *thr*.

Ger thr-5 trpC2 strains, constructed by transduction of Cys⁺ and Ger⁻ from *ger trpC2* strains into BD111, were used as recipients in transduction crosses using strains 1621 or 4650, carrying *nov-3*, as donors (Table 4). The distribution of recombinants amongst the possible phenotypic classes in three factor crosses clearly indicate that the sequence for *ger-1*, -3, -11, -13 and -14 is *thr-nov-ger*. The results of four factor crosses involving *ger-7*, -14, -15 and -16 were consistent with this deduction, although the number of recombinants from the cross involving *ger-15* was low. Therefore, for all of the GerA mutants tested, the map location was *thr-nov-ger-cysB* and no distinction could be made from these crosses between the map location of different GerA mutants.

Crosses were performed to compare the location of *ger* mutations with that of *citG*, which also maps between *thr* and *cysB* (Rutberg & Hoch, 1970). Strains of genotype *ger-3 cysB3 trpC2* and *ger-7 cysB3 trpC2* were constructed by transduction of BD111 to Thr⁺ by lysates produced on *ger trpC2* hosts. These strains were used as donors in three factor transduction crosses with JH404 as recipient (Table 5, crosses 1 and 2). *CitG* was co-transduced with *ger-3* and *ger-7* at a very high frequency (98 and 97%, respectively). Because of this, there were too few Cit⁺Ger⁺ recombinants to permit allocation of an unequivocal order to the *ger*, *citG* and *cysB* genes. If the order were *citG-ger-cysB*, then Cys⁻Ger⁺ recombinants should be less common than Cys⁺Ger⁺ recombinants, because of the requirement for an increased number of crossover events in their formation. However,

if the order were *ger-citG-cysB*, then there should be no rare class, and assuming an absence of interference effects, the proportions of Ger⁺ and Ger⁻ phenotypes amongst the Cys⁻ transductants should be similar to their proportions amongst the Cys⁺ transductants. The distribution of phenotypes (Table 5) is most consistent with the order *citG-ger-cysB*, the evidence being stronger for *ger-7* than for *ger-3*.

The GerB mutant. *Ger-18* was the only mutation producing the phenotype of normal germination in ALA + KCl and PAB but failure to germinate in ASN + GLC + FRU + KCl (Table 2). The concentration of ALA required to induce germination of *ger-18* spores at half the maximal rate (Smith *et al.*, 1978) was 80 μ M, similar to that of the Ger⁺ strain. The extent of germination in ASN + GLC + FRU + KCl (a 10% fall in absorbance in 60 min) was not increased by raising the concentration of GLC or FRU by 10- or 100-fold or of ASN by 10-fold.

Spores of a mutant lacking fructose-1-phosphate kinase were unable to germinate in ASN + GLC + FRU + KCl but did so if mannose was substituted for fructose (Prasad *et al.*, 1972). Strains carrying *ger-18* did not respond to this substitution although the wild type germinated equally well if either mannose or fructose was present. Thus the effect of *ger-18* on the germination phenotype is different from that of a fructose-1-phosphate kinase deficiency.

Strains carrying *ger-18* were not distinguishable from the wild type by the tetrazolium reaction. However, a two factor cross using BD163 as recipient and *ger-18 trpC2* as donor yielded His⁺ transductants of two types which were distinguishable as slow- and fast-growing in the germination replica plating test. Spores were produced from three transductants of each of the putative Ger⁺ and Ger⁻ classes; measurement of the germination response of spore suspensions in ASN + GLC + FRU + KCl and ALA + KCl confirmed the validity of the scoring technique and thus the 34% (39/116) co-transduction of *ger-18* with *hisA*.

Differences in the auxotrophic markers present in transductants interfered with the scoring of *ger-18* by replica plating. Scoring by microscopic observation (Lafferty & Moir, 1977) proved unreliable, and measurement of the fall in absorbance of fully washed spore suspensions was the only satisfactory method of scoring the germination phenotype of transductants in crosses between BD92 (*cysB3 hisA1 trpC2*) and *ger-18 trpC2*. Strain BD92 was transduced to His⁺ and Cys⁺ in separate experiments using phage produced on *ger-18 trpC2*. *HisA* and *cysB* were 15 to 20% co-transduced (A. Moir, unpublished results). The germination phenotypes of approximately equal numbers of transductants carrying wild-type and mutant alleles of the unselected auxotrophic marker were tested. From the selection for Cys⁺, 8 His⁺ and 10 His⁻ transductants were tested; all were Ger⁺. From the selection for His⁺, 12 Cys⁺ and 14 Cys⁻ were tested; all 12 Cys⁺ were Ger⁺, 8 Cys⁻ were Ger⁺ and 6 Cys⁻ were Ger⁻. No co-transduction of *ger-18* with *cysB* was detected in the Cys⁺ recombinants tested, and three factor analysis of these crosses was therefore impossible. The co-transduction frequency with *hisB* and low co-transduction with *cysB* are consistent with the order *cysB-hisA-ger-18*.

GerC mutants. Two strains, 1488 (*ger-88*) and 1558 (*ger-58*), which did not germinate in ALA at 42 °C but did germinate in ASN + GLC + FRU + KCl were also altered in vegetative growth. They grew slowly on PGYE agar, particularly at high temperature, but normally on MA. The 'weird' growth phenotype (Wrd⁻) mapped near *lys* and was not separated from the defective germination phenotype in any of the 16 transductants tested by Trowsdale & Smith (1975). These authors also found that mapping in this region was complicated by the presence in the wild-type strain 1408 (from which 1488 and 1558 were derived) of a mutation causing colonies to remain white after the tetrazolium overlay (Tzm-White) unlike the red colour developed by colonies of 1604 or 1506.

In order to improve the analysis of the GerC mutants, transduction of strain 1506 by

Table 5. *Three factor transduction crosses establishing the location of ger mutations in groups A, D, E, F and G germination mutants*

Cross no.	Recipient	Donor	Selection	Recombinant class	No. in class
1	<i>citG4 trpC2</i>	<i>ger-3 cysB3 trpC2</i>	Cit ⁺	Cys ⁺ Ger ⁺	3
				Cys ⁺ Ger ⁻	54
				Cys ⁻ Ger ⁺	1
				Cys ⁻ Ger ⁻	158
2	<i>citG4 trpC2</i>	<i>ger-7 cysB3 trpC2</i>	Cit ⁺	Cys ⁺ Ger ⁺	7
				Cys ⁺ Ger ⁻	82
				Cys ⁻ Ger ⁺	0
				Cys ⁻ Ger ⁻	182
3	PGK ⁻ <i>met trp</i>	<i>cysB3 hisA1 trpC2</i>	PGK ⁺	Cys ⁺ His ⁺	35
				Cys ⁺ His ⁻	24
				Cys ⁻ His ⁺	56
				Cys ⁻ His ⁻	32
4	<i>cysB3 hisA1 trpC2</i>	PGK ⁻ <i>met trp</i>	His ⁺	Cys ⁺ PGK ⁺	2
				Cys ⁺ PGK ⁻	17
				Cys ⁻ PGK ⁺	62
				Cys ⁻ PGK ⁻	14
5	<i>thr-5 cysB3 trpC2</i>	PGK ⁻ <i>met trp</i>	Thr ⁺	Cys ⁺ PGK ⁺	26
				Cys ⁺ PGK ⁻	14
				Cys ⁻ PGK ⁺	109
				Cys ⁻ PGK ⁻	0
6	<i>cysA14 ery-1</i>	<i>ger-19 trpC2</i>	Cys ⁺	Ery ^S Ger ⁺	23
				Ery ^S Ger ⁻	170
				Ery ^R Ger ⁺	29
				Ery ^R Ger ⁻	2
7	<i>cysA14 ery-1</i>	<i>ger-20 trpC2</i>	Cys ⁺	Ery ^S Ger ⁺	21
				Ery ^S Ger ⁻	111
				Ery ^R Ger ⁺	7
				Ery ^R Ger ⁻	0
8	<i>cysA14 ery-1</i>	<i>ger-37 trpC2</i>	Cys ⁺	Ery ^S Ger ⁺	8
				Ery ^S Ger ⁻	176
				Ery ^R Ger ⁺	16
				Ery ^R Ger ⁻	1
9	<i>ger-36 pheA12</i>	<i>leuA8</i>	Phe ⁺	Leu ⁺ Ger ⁺	1
				Leu ⁺ Ger ⁻	153
				Leu ⁻ Ger ⁺	185
				Leu ⁻ Ger ⁻	41
10	<i>ger-36 argA3</i>	<i>leuA8</i>	Arg ⁺	Leu ⁺ Ger ⁺	31
				Leu ⁺ Ger ⁻	221
				Leu ⁻ Ger ⁺	124
				Leu ⁻ Ger ⁻	0
11	<i>citF2 trpC2</i>	<i>ger-36 argA3</i>	Cit ⁺	Arg ⁺ Ger ⁺	7
				Arg ⁺ Ger ⁻	57
				Arg ⁻ Ger ⁺	0
				Arg ⁻ Ger ⁻	43
12	<i>hisA1</i>	<i>ger-21 cysB3 trpC2</i>	His ⁺	Cys ⁺ Ger ⁺	19
				Cys ⁺ Ger ⁻	190
				Cys ⁻ Ger ⁺	14
				Cys ⁻ Ger ⁻	64
13	<i>hisA1 cysB3 trpC2</i>	<i>ger-45 trpC2</i>	His ⁺	Cys ⁺ Ger ⁺	7
				Cys ⁺ Ger ⁻	47
				Cys ⁻ Ger ⁺	12
				Cys ⁻ Ger ⁻	83

Table 6. Mapping of mutations in the *lys-trp* region

Lys⁺ transductants were selected in a cross between strain 1506 (Tzm-Red *lys-I*) and 1558 (Tzm-White Wrd⁻ *trpC2*).

Recombinant class	No. in class
Trp ⁻ Tzm-White Wrd ⁻	163
Trp ⁻ Tzm-White Wrd ⁺	3
Trp ⁻ Tzm-Red Wrd ⁻	1
Trp ⁻ Tzm-Red Wrd ⁺	1
Trp ⁺ Tzm-White Wrd ⁻	19
Trp ⁺ Tzm-White Wrd ⁺	0
Trp ⁺ Tzm-Red Wrd ⁻	66
Trp ⁺ Tzm-Red Wrd ⁺	28

lysates produced on 1558 was repeated. The Trp, Tzm and Wrd phenotypes of Lys⁺ recombinants of strain 1506 were scored (Table 6). Their distribution between phenotypic classes was consistent with the order *lys-wrd-tzm-trp*. Since Wrd⁻ strains did not grow on PGYE agar at 37 or 42 °C, spores were prepared at 26 °C from transductants of each common phenotype, and germination in ALA+KCl was measured at 42 °C. Wrd⁺Tzm-Red Trp⁺ transductants (seven tested) germinated at the same rate as 1604, the Ger⁺ Tzm-Red wild type; a Wrd⁻Tzm-Red Trp⁺ transductant (one tested) germinated equally fast. Wrd⁻Tzm-White Trp⁺ recombinants (five tested) germinated as fast as the Ger⁺Tzm-White strain 1408 from which the mutants were derived; the maximum rate of germination of strain 1408 was 50% that of 1604. Wrd⁻Tzm-White Trp⁻ recombinants (two tested) germinated only very slightly (5 to 7% fall in absorbance in 90 min). Therefore the Wrd⁻ mutation is not responsible for the defective germination, although it appears that a mutation (or mutations) responsible for the germination phenotype may be located in the same region. The characteristics of this *ger* mutation must be examined in recombinants in which it has been separated from the Wrd and Tzm mutations of 1558.

The GerG mutant. Of a number of mutants lacking glycolytic enzymes (Prasad *et al.*, 1972) only one, a 3-phosphoglycerate kinase (PGK⁻) mutant also produced spores that had lost the ability to germinate in ALA+KCl without the addition of GLC and FRU. The PGK mutation has been mapped by three factor crosses (Table 5, crosses 3, 4 and 5) using the requirement of PGK⁻ strain 61111 for both glucose and malate as carbon sources (Freese *et al.*, 1972) to select PGK⁺ transductants or to score PGK⁺ and PGK⁻. The data are consistent with the location of the PGK⁻ mutation between *cysB* and *hisA*. Co-transduction frequencies of PGK with *thr* (9%), with *cysB* (58%) and with *hisA* (38%) are also consistent with this map position.

The germination phenotype of the PGK mutant (Prasad *et al.*, 1972) is somewhat similar to that of the GerA mutants (Trowsdale & Smith, 1975; Lafferty & Moir, 1977). The *ger* mutations in GerA strains, however, are located quite separately from that responsible for the PGK deficiency, and GerA strains do not lack a glycolytic enzyme since their growth on glucose is normal.

Mutants altered in response to all three germinants

GerD mutants. The mutations *ger-19*, *ger-20* and *ger-37* are responsible for defective germination in PAB, in ALA+KCl and in ASN+GLC+FRU+KCl (Table 2). They were co-transduced, in two factor crosses with Mu8u5u16u24, 1612 and BD112, with *purA* (4 to 10%), with *aroI* (10 to 18%) and with *cysA* (77 to 88%), respectively. Cys⁺ recombinants were selected in the presence of methionine (Piggot, 1973) in three factor crosses with 4248 (*cysA ery-I*) as recipient (Table 5, crosses 6, 7 and 8). The order of markers which can be deduced is *cysA-ery-ger* for all three mutants.

Confirmation of the germination phenotype of transductants (performed for *ger-20* and *ger-37*) was difficult. Erythromycin-sensitive (Ery^s) strains sporulate normally at 42 °C, and spores of the Ery^s transductants, both Tzm-White and Tzm-Red, produced at 42 °C possessed the original mutant and wild type germination properties, respectively. However, erythromycin-resistant (Ery^r) strains sporulate very poorly at 42 °C (Tipper *et al.*, 1977). To overcome this, spores were prepared at 26 °C from transductants of all four recombinant classes where available. None of these spores germinated in ALA or in ASN + GLC + FRU + KCl, but spores from Tzm-Red Ery^s and Ery^r transductants germinated in PGYE broth if heat-activated at 80 °C for 10 min, whereas spores of Tzm-White transductants did not. Thus the Ger⁻ phenotype scored by the tetrazolium response was still associated with a decrease in ability to germinate. The map location of the *ger* mutants was therefore validated, although the recipient strain 4248 possessed a sporulation temperature-dependent deficiency in germination. Since this deficiency was retained by all transductants tested, it did not map close to *cysA*.

The GerE mutant. Strain 4600 (*ger-36*) did not germinate in any of the three test germinants. The *ger* mutation was not co-transduced with either *cysB* or *cysA*. It was co-transduced with *aroA* (23%), *argA* (35%), *leuA* (92%) and *pheA* (59%) in two factor crosses using recipients WB2281b, BD40, GSY374 and BD40, respectively. The order *argA-ger-36-leuA-pheA* was established in three point crosses (Table 5, crosses 9 and 10). The recipients in these crosses were constructed by transduction of BD40 to Ger⁻Arg⁺ and Ger⁻Phe⁺, respectively, by a lysate grown on strain 4600.

The frequency of co-transduction of *ger-36* with *citF2* was 93% (Table 5, cross 11). Since the number of Ger⁺ transductants was very low, the assignment of a gene order to *ger*, *cit* and *arg* must remain tentative. The most probable order, however, is *arg-ger-36-citF2*. *Ger-36* was co-transduced with *leu* and *citF78* [not with *citF78* alone, as reported by Smith *et al.* (1978)] at a frequency of over 99%; the *ger* mutation may be close to *citF* or between *citF78* and *leu*. Strain 4600 and recombinants carrying *ger-36* in the genetic background of BD40 appeared normal in vegetative growth both in rich and minimal media and, unlike *citF* strains, grew at the same rate as the wild type on MA plus lactate and formed purple colonies on NA-bromocresol purple agar (Carls & Hanson, 1971).

Ger-36 was the only *ger* mutation observed to affect spore formation. Spores of the mutant strain and of Ger⁻ transductants were formed on PGYE agar, but preparations contained up to 50% of cells which had not formed phase-bright prespores. The spores appeared normally phase-bright but were smaller than those from Ger⁺ strains and were frequently not released from the sporangium. The progress of sporulation of *ger-36* strains in resuspension sporulation medium (Sterlini & Mandelstam, 1969) was monitored (A. Moir, unpublished results). Populations of Ger⁺ and Ger⁻ cells initiated sporulation in synchrony, but at 42 °C *ger-36* strains produced partially bright forespores which did not develop normal resistance to heat or to organic solvents and did not accumulate dipicolinic acid. At 26 °C sporulation of *ger-36* strains proceeded to completion. Spores, whether produced at 26 °C in resuspension sporulation medium or at 26 or 42 °C on PGYE agar, were defective in germination in all three germinants. Electron microscopy of thin sections of *ger-36* spores (A. Moir & G. Dring, unpublished results) showed that the coat layers were incomplete in the mutant. The lysozyme-sensitivity and altered germination behaviour of *ger-36* spores was reminiscent of the properties of coat-defective mutants of *Bacillus cereus* (Aronson & Fitz-James, 1975; Cheng *et al.*, 1978).

GerF mutants. Spores of strains 4595 and 4728 (carrying *ger-21* and *ger-45*, respectively) germinated poorly in all three germinants tested (Table 2). Spores of strains carrying *ger-21* germinated to some extent in PAB and ALA + KCl (25 and 15% fall in absorbance in 60 min, respectively) if they were produced at 26 °C, although the fall in absorbance was

only 10% in each case if the spores were produced at 42 °C. Spores of strains carrying *ger-45* did not germinate in any of the three germinants (< 5% fall in absorbance in 60 min), irrespective of the temperature of sporulation.

In crosses with BD111 and BD92 as recipients, *ger-21* and *ger-45* were not co-transduced with *thr*, but were 16 and 31% co-transduced with *cysB*, and 93 and 89% co-transduced with *hisA*, respectively. Three factor crosses between strain 1420 and *ger-21 cysB3 trpC2* (constructed by transduction of BD92 to His⁺Ger⁻ by a phage lysate grown on 4595) and between BD92 and 4728 were carried out (Table 5, crosses 12 and 13). In cross 12, 17% of His⁺ transductants were Cys⁻; in order to determine the proportions of Ger⁺ and Ger⁻ phenotypes amongst both Cys⁺ and Cys⁻ transductants with reasonable accuracy, additional Cys⁻ transductants were screened for germination phenotype and are included in the data in Table 5. This was not necessary for cross 13, where the co-transduction of *cysB* and *hisA* was more frequent. In both crosses, no rare class of recombinant phenotypes was observed; the unequal distribution in the classes was consistent with the high frequency of co-transduction of *ger* with *hisA*. The sequence *cysB-hisA-(ger-21, -45)* can thus be deduced from the three factor crosses. This result is consistent with the two factor analysis and with further three factor crosses between BD92 and 4595 (A. Moir, unpublished results).

DISCUSSION

The mutants described were isolated as defective in response to at least one of three test germinants. However, a random sample of germination mutants has not been studied. All mutants were required to grow and sporulate on PGYE agar, only a limited variety of germinants were used for enrichment, and only strains which could be distinguished by the tetrazolium overlay method or by replica plating were examined. In addition those mutants whose spores responded to an intermediate extent to germinants at the concentrations used were not chosen for detailed analysis. How likely is it that the complex Ger⁻ phenotypes of the mutants result from single mutations? Genetic analysis has involved the transfer of *ger* mutations by transduction into an unmutagenized background. While this does not prove that the Ger⁻ phenotype observed in transductants is the result of a single mutation, it does imply that if multiple mutations are responsible, they are located close together on the chromosome, since they were not separated in any of the recombinants whose phenotype was tested.

Accepting these limitations, the new mutants, along with those of Trowsdale & Smith (1975), can still be conveniently subdivided according to the pattern of their response to the germinants, namely mutants germinating in ASN+GLC+FRU+KCl but not ALA, those germinating in ALA but not in ASN+GLC+FRU+KCl and those not germinating in either ALA or ASN+GLC+FRU+KCl (Table 2). *Ger* mutations have been located in six different regions of the *B. subtilis* 168 chromosome; if the PGK⁻ mutant (Prasad *et al.*, 1972) is included the number is seven. *Ger* mutations mapping in the same region have similar effects on the germination phenotype, although mutations in several different regions can be responsible for similar germination phenotypes. The distribution of mutants between groups and locations is extremely uneven. Of the 28 mutants whose spores were altered specifically in their germination response only to ALA, 26 map close together near *citG* between *thr* and *cysB* (the GerA group). They may represent a gene or group of genes particularly important in the germinative response to ALA. The other two, GerC mutants, are located in the *lys-trp* region. Representatives of other locations are also few. The one GerB mutant is altered in response to ASN+GLU+FRU+KCl and maps on the origin-proximal side of *hisA* and the PGK⁻ mutant (GerG) maps between *cysB* and *hisA*. Among six mutants deficient in response to ALA, ASN+GLC+FRU+KCl and PAB, the GerD mutants map near *cysA* and the GerE and GerF mutants close to *citF* and *hisA*, respectively. Since mutants with this germination phenotype are so few and already three map

locations have been recognized this could well mean that more than three genes are involved in the response of spores to these germinants.

Thus, interpretation of the available data in terms of the number and properties of genes involved in germination remains speculative. In addition, no fine structure mapping has yet been performed for mutations, like the GerA group, which are closely linked. The inability to select for either the Ger⁺ or Ger⁻ phenotype means that comparison of different *ger* mutations by the recombination index method (Lacks & Hotchkiss, 1960) will be time consuming. Even after such analysis, it would not be possible to assign individual gene designations with confidence unless complementation analysis between *ger* mutants is performed. Merodiploidy is at present limited to certain regions of the *B. subtilis* chromosome (Karmazyn *et al.*, 1973; Trowsdale & Anagnostopoulos, 1976) but the cloning of genes into plasmids which are stably maintained in *B. subtilis* provides a promising alternative (Keggins *et al.*, 1978). GerA and GerE mutants are located very close to *cit* genes (Table 5); thus the possibility of relationships between *ger* genes and these closely linked genes can be explored. Other aspects of the genetic analysis, such as the identification of nonsense mutations, the analysis of revertants and the identification of suppressor loci are now being attempted.

Some consideration of the nature of the deficiencies of Ger mutants in relation to possible mechanisms of spore germination is justified. The germination of spores of *ger* mutants is either conditional on the germinant used (GerA, B, C and G) or is defective in responses to the three germinants tested (GerD, E and F). GerA mutants do not respond to ALA, although response to the germinative combination of ASN+GLC+FRU+KCl remains normal. Thus the response to ALA involves a component, altered in GerA mutants, which is not required for the response to ASN+GLC+FRU+KCl. The germination response of the GerB mutant suggests that the converse is also true. The efficiency of an interaction between germinant and a germinant-specific receptor or of the germinant-specific transduction of the initial stimulus may be altered in these GerA and B mutants (Smith *et al.*, 1978). This deficiency is unlikely to be in the general permeability of the spore to small molecules or in any critical enzyme effecting hydrolytic changes observed in the spore during germination unless these were germinant-specific. GerA and B germination properties could be the result of mutational blocks in the metabolism of germinants in the spore. However, any deficiency in the metabolism of glucose or alanine by the mutants is not sufficient to affect their utilization as carbon sources by vegetative cells (A. Moir, E. Lafferty & R. Sammons, unpublished). The defects in GerD, E and F mutants appear to be more general in nature (although testing a wider spectrum of germinants may reveal that some of these, too, are conditional). The mutations could affect a molecule whose role is common to a wide variety of germinants such as a common receptor or transducer of the germination stimulus, or a regulatory molecule controlling the synthesis of proteins important in germination. Alternatively, they could affect spore structure and have an indirect effect on germination, perhaps by decreasing the permeability of the outer layers of the spore to germinants, or by decreasing the accessibility of receptor sites. Mutant spores could fail to provide the structure in which macromolecules important in germination may be located, or fail to produce germination-associated proteins because of alterations in the pattern of gene expression during sporulation. Only in the GerE mutant has any actual structural abnormality been identified.

The mapping of *ger* mutations provides information of considerable importance to comparisons between Ger⁻ mutant phenotypes, and relevant to the development and testing of theories of germination. The knowledge that different gene products are altered in mutants of similar phenotype may help to qualify any theories based on the properties of such mutants. Likewise, similarities and differences in phenotypes of mutants may assume increased significance if they are known to map very closely together. Identification of the nature and role of the altered gene products will require a much more detailed

analysis of the physiology and biochemistry of the mutants than has so far been carried out. However, the work described should provide a basis for an approach to the analysis of germination complementary to biochemical studies of normal germination.

The work was supported by an SRC research grant to D.A.S. and CASE studentship to E.L. We are grateful to Hazel Howell and Sue Thomas for technical assistance and to Jane Calvert and Teresa Bovingdon for the isolation of *ger-45* during an undergraduate project. We thank all those who sent us bacterial strains, and Mr G. Dring, Professor G. W. Gould, Drs J. Trowsdale and P. Piggot for valuable discussion.

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