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Genetic Analysis of Bacteriophage ϕ29 of *Bacillus subtilis*: Integration and Mapping of Reference Mutants of Two Collections

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Reference mutants of *Bacillus subtilis* phage ϕ29 of the Madrid and Minneapolis collections were employed to construct a genetic map. Suppressor-sensitive and temperature-sensitive mutants were assigned to 17 cistrons by quantitative complementation. Three-factor crosses were used to assign an unambiguous order for the 17 cistrons. Recombination frequencies determined by two-factor crosses were used to construct a linear genetic map of 24.4 recombination units. The genes were numbered sequentially from left to right (1 to 17) according to their relative map position.

Bacillus subtilis phage ϕ29 has a unique complex morphology (2), and seven viral structural proteins resolved by electrophoresis have been identified as components of the head, neck, and tail (12).

The genome of ϕ29 is a DNA duplex with a molecular weight of about 11×10^6 (2, 3). When isolated from the virion, the molecule is in a closed form with the ends attached by a specific protein. This observation of Ortin et al. (14) is consistent with the fact that transfection by ϕ29 DNA is sensitive to treatment with proteolytic enzymes (10).

At least 23 viral-induced proteins have been identified after ϕ29 infection of UV-irradiated *B. subtilis*, and these proteins account for more than 90% of the information content of the ϕ29 DNA (7, 9, 11, 15). Most of the viral-induced proteins that appear early during infection can be synthesized by an *Escherichia coli* cell-free system directed by ϕ29 DNA (6).

Temperature-sensitive (*ts*) and suppressor-sensitive (*sus*) mutants have been isolated and mapped (4, 8, 11, 13, 17, 18, 20, 21). Moreno et al. (13), using both *ts* and *sus* mutants, have defined 17 cistrons by complementation and constructed a linear genetic map.

We have combined the mutants of two collections into a single set of representative mutants to derive an estimate of the number of cistrons, to provide a more definitive and useful genetic map, and to facilitate the adoption of a common nomenclature. The mutants tested (8, 13, 17, 18, 20, 21) fall into 17 complementation groups and have been placed on a linear genetic map by two- and three-factor crosses. *sus* mutants are available in 16 of 17 cistrons, and both *ts* and *sus* mutants are available in 11 cistrons.

MATERIALS AND METHODS

In this study each laboratory employed its standard materials and methods whenever appropriate and, in general, the details have been published.

Bacteria and phage. (i) **Madrid.** The nonpermissive host *B. subtilis* 110 NA and the permissive bacterium *B. subtilis* 168 MO-99 were employed (13).

(ii) **Minneapolis.** The properties of the permissive host *B. subtilis* L15 and the nonpermissive host *B. subtilis* SpoA12 have been described (18).

Both *ts* and *sus* mutants of each collection were employed in this study (8, 13, 18, 20).

Lysate production and bacteriophage assay. Our methods, media, and solutions for lysate preparation and viral storage have been described (3, 12, 13, 16), and standard phage techniques were used (1).

Genetic methods. With the following exceptions, qualitative and quantitative complementation, two-factor and three-factor crosses, recombinant virus construction, and antiserum preparation have been described (4, 13, 16, 17, 18, 20, 21).

(i) **Madrid.** The qualitative complementation test was modified. Representative mutants of each cistron were used to infect the nonpermissive host, *B. subtilis* 110 NA, at a multiplicity of infection of 0.1 to 0.2, and 10^8 of these infected cells were used per complementation test plate. Mutant phage were transferred by toothpick from the permissive host to the test set and to both the permissive and nonpermissive host strains.

An incubation temperature of 42°C was used to study quantitative complementation with *ts* mutants.

(ii) **Minneapolis.** Cells (10^8) of the nonpermissive host *B. subtilis* SpoA12 were suspended in antibiotic medium 3 (Difco; PB) containing 0.002 M NaCN to

measure quantitative complementation with mixed *ts* × *sus* infection. *ts* mutants at a multiplicity of infection of 5 and *sus* mutants at a multiplicity of infection of 10 were adsorbed for 15 min at ambient temperature. The infected cells were treated with antiserum (K value, 2,000) for 5 min at 43°C and diluted by a factor of 10^3 into unsupplemented PB at 43°C, the infected culture was lysed by the addition of 10 μg of lysozyme (Calbiochem) per ml.

RESULTS

We employed quantitative complementation and three-factor crosses to construct a genetic map that would permit us to utilize a nomenclature similar to that of Studier with phage T7 (19). The ϕ29 genes were also numbered sequentially from left to right (1 to 17), according to their relative map position. We will begin to use the new nomenclature when discussing the genetic results. An example of the transition would be the designation of *sus* mutants A628 (Minneapolis) and F513 (Madrid) as *sus*2(628) and *sus*2(513), respectively.

Complementation. A list of the reference mutants used is presented in Table 1, and the results of quantitative complementation are given in Table 2. The reference *sus* and *ts* mutants of the Minneapolis collection that failed to complement are listed in Table 2. The mutant *ts*5(219) complemented reference *sus* mutants of each cistron (data not shown). In both Madrid and Minneapolis, all *ts* and *sus* mutants were tested upon arrival by qualitative complementation, and the results (data not shown) were consistent with published data. Then quantitative complementation was employed by each laboratory to establish the identity of cistrons defined by mutants of both collections. The results in Table 2 from Madrid and Minneapolis are in good agreement and indicate that at least 17 cistrons can be identified by complementation. The mutant *sus*14(1241) has the phenotype of delayed lysis, and progeny phage released by lysozyme can be titered on the permissive host (13). The assignment of mutants *sus*17(741) and *sus*17(112) to gene 17 is based on mapping and phenotype analysis (see Discussion) as well as complementation.

TABLE 1. *Reference mutants used*

Gene	Minneapolis		Madrid	
	sus	*ts*	*sus*	*ts*
1	B629			
2	A628	A35	F513	F98
3	C713		K91	K132
4	D369		O56	
5		P219		C17
6	E626	E204		Q1360
7	G614	G224		J116
8	F769	F205		G93
9	H756	H136	A422	A18
10	I302		E136	E54
11	O683		H542	H119
12	J305	J225	B47	B108
13	K330		L55	I810
14			M1241	
15	N724		N212	
16	L300	L218	D241	D46
17	M741		P112	

Genetic mapping. The results of a series of three-factor crosses were used to establish the relative order of cistrons and to suggest additional two-factor crosses that would supplement quantitative data reported by Moreno et al. (13).

The data from series A of Table 3 suggest the cistron order 1-2-3-4-6-7. However, the results of the *sus*4(369) × *sus*3(713)-*sus*6(626) cross are not convincing.

Infection by a mutant with the clear plaque (c) phenotype permits the uncleaved appendage precursor protein P(J) to accumulate with a parallel reduction in burst size (22). The recombinants *sus*3(713)-c, *sus*4(369)-c, and *sus*6(626)-c were constructed and used in the three-factor crosses of series B of Table 3. The cistron order 3-4-6 seems reasonable.

The data in series C of Table 3 orient the mutant *ts*5(219) and suggest the cistron order 4-5-6. The additional data are included because we have not previously employed *sus-ts* recombinants in these crosses, and because they support our conclusion that the cistron order is 4-5-6.

The first cross of series D of Table 3 defines the limits of the segment of the genome under investigation and suggests the cistron order 13-16-17. The three-factor crosses of Moreno et al. (13) indicate the cistron order 13-14-15. Although the results of the second cross of series D of Table 3 are ambiguous, the next three sets of series D data imply the cistron order 14-15-16, 15-16-17, and 13-15-16.

The above information was used in the planning of the two-factor crosses reported in Table 4. When these data are used to supplement those of Moreno et al. (13), a genetic map of ϕ29 consisting of 24.4 recombination units is obtained (Fig. 1).

DISCUSSION

We have defined 17 cistrons of the ϕ29 genome by quantitative complementation. Although the mutant *sus*17(112) gave a relatively

TABLE 2. *Integration of ϕ29 mutant collections by quantitative complementation*[a]

Gene	Minneapolis complementation			Minneapolis complementation			Madrid complementation		
2	A628 × A35			A628 × F513			A628 × F513		
	(0.38)	(1.7)	1.8	(0.36)	(0.36)	0.48	(0.15)	(0.1)	0.43
3				C713 × K91			C713 × K91		
				(0.44)	(0.95)	0.14	(0.2)	(0.08)	0.45
4				D369 × O56			D369 × O56		
				(0.47)	(0.82)	0.73	(0.2)	(0.001)	0.1
5							P219 × C17		
							(1.8)	(0.002)	2.2
6	E626 × E204			E626 × Q1360			E626 × Q1360		
	(0.52)	(0.70)	0.85	(0.61)	(1.9)	1.7	(0.4)	(0.05)	0.47
7	G614 × G224			G614 × J116			G614 × J116		
	(0.15)	(0.75)	0.70	(0.29)	(0.60)	0.53	(0.04)	(0.01)	0.02
8	F769 × F205			F769 × G93			F769 × G93		
	(0.64)	(1.2)	0.3	(0.21)	(0.31)	0.57	(0.08)	(0.02)	0.06
9	H756 × H136			H756 × A422			H756 × A18		
	(0.64)	(1.8)	1.9	(0.8)	(0.21)	0.40	(0.001)	(0.015)	0.07
10				I302 × E136			I302 × E54		
				(0.7)	(0.16)	0.28	(0.15)	(0.015)	0.02
11				O683 × H542			O683 × H119		
				(0.53)	(0.17)	0.23	(0.11)	(0.02)	0.07
12	J305 × J225			J305 × B47			J305 × B108		
	(0.31)	(1.7)	1.2	(0.6)	(0.21)	0.38	(0.08)	(0.05)	0.02
13				K330 × L55			K330 × L55		
				(0.31)	(0.44)	0.42	(0.19)	(0.18)	0.19
15							N724 × N212		
							(0.2)	(0.1)	0.35
16	L300 × L218			L300 × D241			L300 × D241		
	(0.16)	(1.8)	1.7	(0.29)	(0.12)	0.24	(0.002)	(0.002)	0.004
17							M741 × P112		
							(0.8)	(12)	14

[a] The values in parentheses under each mutant designation indicate the complementation index after single infection. The values to the right indicate the complementation index after mixed infection. The complementation index reflects the ratio of the burst size of mutant-infected bacteria to the wild-type burst size in the nonpermissive host multiplied by 100.

TABLE 3. *Results of three-factor crosses*

Series	Reference mutants			Tests (no.)	Recombination frequency[a]		
	1	2	3		1 × 23	2 × 13	3 × 12
A	*sus*1(629)	*sus*2(628)	*sus*3(713)	4	0.63	0.07	2.1
	*sus*2(628)	*sus*3(713)	*sus*4(369)	3	2.5	0.22	0.77
	*sus*3(713)	*sus*4(369)	*sus*6(626)	4	0.89	0.52[b]	0.80
	*sus*4(369)	*sus*6(626)	*sus*7(614)	5	2.3	0.40	1.8
B	*sus*3(713)[c]	*sus*4(369)[c]	*sus*6(626)[c]	4	1.1	0.32	2.3
C	*sus*4(369)	*ts*5(219)	*sus*6(626)	4	1.2	0.24	1.5
	*sus*6(626)	*ts*5(219)	*sus*7(614)	4	0.24	0.77	1.7
	*sus*7(614)	*ts*5(219)	*sus*8(769)	4	0.17	3.6	1.3
D	*sus*13(330)	*sus*16(300)	*sus*17(112)	3	2.7	0.61	5.8
	*sus*13(55)	*sus*14(1241)	*sus*15(212)	6	1.2	0.20	0.25
	*sus*14(1241)	*sus*15(212)	*sus*16(300)	4	0.72	0.21	5.0
	*sus*15(212)	*sus*16(300)	*sus*17(112)	4	1.9	0.48	5.8
	*sus*13(330)	*sus*15(212)	*sus*16(300)	5	1.2	0.31	2.0

[a] The central mutant is listed in column 2; thus, 1-2-3 order is the left to right cistron order.
[b] Eight experiments were carried out to obtain the average value.
[c] In this series the single parent also carried the clear (c) mutation (4).

TABLE 4. *Recombination percentages among mutants of Madrid and Minneapolis collections*[a]

Cistron and (mutant)	Recombination (%)														
	1[b] (629)[c]	2 (628)	3 (91)	3 (713)	4 (56)	4 (369)	5 (219)	6 (626)	7 (116)	7 (614)	8 (769)	8 (93)	9 (422)	9 (756)	10 (302)
2 (513)	0.7^{2}	0.5	2.0				1.8^{2}	6.6							
3 (91)	1.3^{2}			0.1^{2}	1.1	1.4	0.6^{2}	3.6			5.1				
3 (713)							0.5^{2}								
4 (56)							0.05^{2}			2.2^{2}	3.1^{2}				
5 (17)							0.05^{2}								
6 (626)							1.6		2.1^{2}	1.4^{3}	3.0^{3}	3.4	13.8		
7 (116)										0.15^{2}	1.0^{3}	2.1^{2}	5.1		
7 (614)											1.0^{3}	2.2^{2}	3.7		
8 (769)												0.8^{2}	3.7^{2}	4.6[7]	
8 (93)													2.6		
9 (18)														0.16	
9 (422)														0.32	1.5^{2}
9 (756)															1.2
10 (136)															0.06^{2}

Cistron and (mutant)	Recombination (%)										
	11[b] (683)[c]	11 (525)	12 (305)	12 (47)	12 (662)	13 (44)	13 (55)	13 (330)	15 (212)	16 (241)	17 (112)
11 (542)	<0.01										
11 (683)		0.8	3.6^{7}		5.6^{7}						
12 (108)			1.0^{2}	1.8^{3}		2.9^{3}					
12 (305)				1.1^{2}	1.0^{6}	2.7^{2}					
12 (47)						1.9					
12 (662)								2.7			
13 (44)							1.2	1.0			
13 (53)							0.9	0.8			
13 (55)								<0.01			
15 (212)										0.9	
15 (724)									0.09	1.0	
16 (300)										0.07	
17 (741)											0.06

[a] The experiments were carried out as described in Materials and Methods. The superscripts indicate the number of experiments carried out to obtain the average values.
[b] Cistron.
[c] Mutant.

high burst size (Table 2), it does not complement mutant *sus*17(741). These mutants recombine at a very low frequency (Table 4). Moreover, the same proteins are missing from the electrophoretic profile after infection of the nonpermissive indicator by mutants *sus*17(741) and *sus*17(112), and revertants of both mutants can synthesize the proteins (unpublished observations). This evidence supports the conclusion that cistron 17 exists and that the mutants *sus*17(741) and *sus*17(112) are properly named.

Moreno et al. (13) reported that mutant *sus*I44 gave low complementation values with *sus*L53 and *sus*L55. However, because protein XII (7) was not synthesized after *sus*I44 infection of the nonpermissive host and it was synthesized after infection with mutants *sus*L53 and *sus*L55, the mutant was assigned to a separate cistron. Mutant *sus*K330 gave low complementation values (less than 2%) with all three mutants (Table 2; data not shown). Protein XII is not synthesized during infections with revertants of mutant *sus*I44, but this protein is synthesized after infection with mutant *sus*I342 (13) (unpublished observations). This information suggests that the mutants of cistrons I, L, and K be provisionally placed in a single cistron, cistron 13.

Moreno et al. (13) were unable to map the mutant *ts*5(17), previously designated C17. Mutant *ts*5(219) has been mapped by three-factor crosses (Table 3).

The results of the three-factor crosses reported in this paper are consistent with the map of Moreno et al. (13) and complement published three-factor crosses (17, 18). We believe

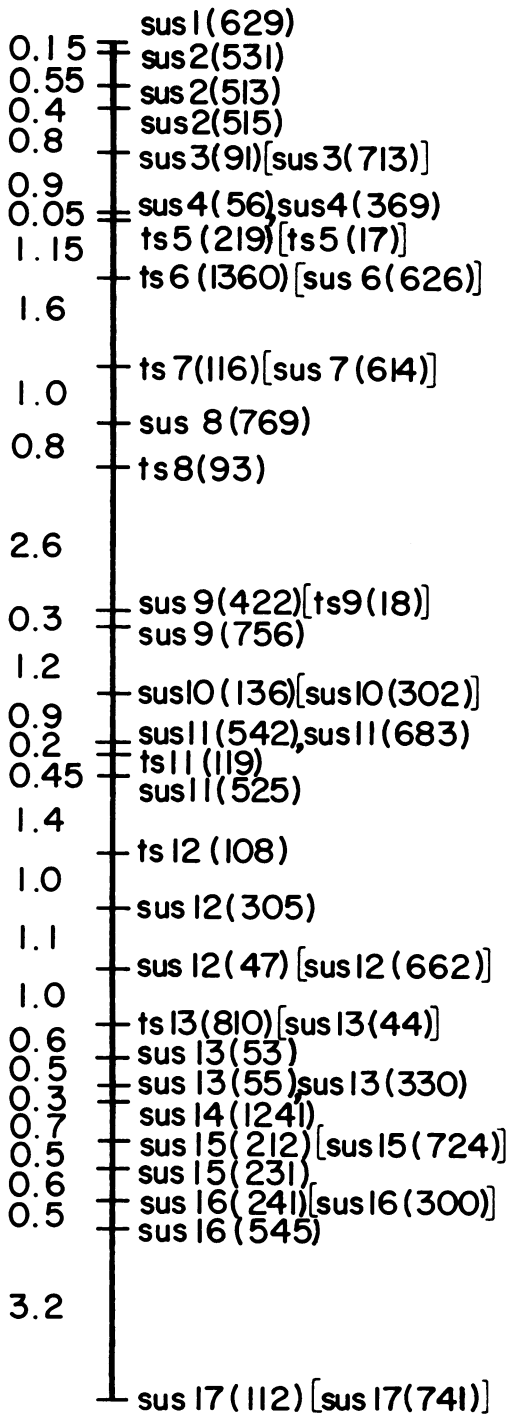

FIG. 1. *Genetic map of bacteriophage φ29. The position of the mutants in the map is based on the recombination units obtained by Moreno et al. (13) and the values given in Table 4. Mutants separated by a comma give <0.01% recombination with respect to the reference mutant. Mutants in brackets are close to the reference mutant (≤0.2% recombination), but their relative order has not been established.*

these data establish an unambiguous order for the 17 cistrons identified by our laboratories (Fig. 1).

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