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Cotransduction and Cotransformation of Genetic Markers in *Bacillus subtilis* and *Bacillus licheniformis*

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Bacteriophage SP-15, a large generalized transducing phage of *Bacillus*, was compared with phages PBS-1 and SP-10 for the ability to cotransduce pairs of genetic markers exhibiting different degrees of linkage. When auxotrophs of *B. subtilis* W-23 were used as recipients, SP-15 and PBS-1 effected a much higher frequency of cotransduction than did SP-10 with markers that were not closely linked. With more closely linked loci, the differences were not as great. SP-15 cotransduced linked markers at a higher mean frequency than PBS-1, suggesting that SP-15 is able to transfer a larger fragment of the *Bacillus* genome than any phage heretofore described. The frequency of the joint transfer of genetic markers in *B. licheniformis* was lower via transforming deoxyribonucleic acid than by transduction with phage SP-10. The availability of three procedures for genetic exchange—transduction by SP-15 and SP-10 as well as transformation—each of which reveals a different degree of linkage, makes *B. licheniformis* 9945A especially amenable to genetic analysis.

Recently, Tyeryar et al. (28) demonstrated in *Bacillus licheniformis* that for a given pair of genetic markers the frequency of cotransduction by phage SP-15 was higher than the frequency of cotransformation. This result could be interpreted in at least three ways. (i) Phage SP-15 can transfer longer pieces of the *B. licheniformis* genome than those transferred by transforming deoxyribonucleic acid (DNA). (ii) *B. licheniformis* transforming fragments are unusually small, and the DNA segments transferred by SP-15 appear large only by comparison. (iii) SP-15 transfers very long pieces of the *B. licheniformis* genome, as does phage PBS-1 for *B. subtilis* (4, 21); in addition, the transforming DNA fragments of *B. licheniformis* are unusually small.

We attempted to choose from among these hypotheses by comparing the frequencies of joint transfer of linked markers by SP-15, SP-10, and transforming DNA in *B. licheniformis*. We also directly compared SP-15 and PBS-1, a phage that transfers large pieces of the *B. subtilis* chromosome, for the ability to cotransduce multiple auxotrophs of *B. subtilis* W-23 containing markers that exhibited different degrees of linkage when transduced with phage SP-10.

MATERIALS AND METHODS

Bacterial strains. The strains of *B. licheniformis* and *B. subtilis* used in this study are given in Table 1. Multiply marked strains of *B. licheniformis* were isolated after ultraviolet irradiation employing a modification of the method of Iyer (12) or were constructed by bacterial transformation by the method of Thorne and Stull (27). Multiply marked strains of *B. subtilis* W-23 were usually produced in two steps. The first marker was introduced by ultraviolet treatment (12); the subsequent markers were obtained after exposure to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) by employing modifications of the method of Altenbern (2). An 18-hr Penassay broth (Difco antibiotic medium no. 3) culture was harvested, and the cells were suspended in the same volume of 0.85% saline containing 200 μg of NTG per ml, *p*H 5.5. After 20 min of incubation at 37 C, the cells were harvested, washed once with minimal 1 broth and resuspended in the same broth supplemented with 25 μg of the first requirement per ml and 25 μg of the desired second requirement, per ml. After 4 to 6 hr of additional incubation, appropriate dilutions were plated on nutrient broth plus yeast extract (NBY; 26) agar. Characterization of mutants was accomplished by picking to appropriately supplemented minimal media. Strain TD-1 (*phe⁻arg⁻*) was constructed by transduction with PBS-1 by using a *phe⁻* donor and M1-M143 (*leu⁻arg⁻*) as recipient. An *arg⁻phe⁻* transductant was then selected from among the *leu⁺* transductants by picking to appropriately supplemented media.

Media and cultural conditions. Minimal 1 agar was prepared as described by Thorne and Stull (27). Phage assay (PA) broth and hard and soft PA agars were prepared as described by Taylor and Thorne (23, 24). Peptone diluent contained 10 g of Difco

TABLE 1. *Description of Bacillus cultures*

Designation	Tentative genotype	Origin or reference
Bacillus licheniformis		
9945A	Wild type	Thorne & Stull (27)
FD01	*str-1*[a]	Tyeryar et al. (28)
FD28	*pep-1 pur-1 leu-2 arg-3*	Tyeryar et al. (28)
FD52	*pep-1 pur-1 trp-1 tyr-1*	Tyeryar et al. (28)
FD55	*pep-1 pur-1 met-1 trp-1*	Tyeryar et al. (28)
FD59	*pep-1 pur-1 leu-2 lys-3 tyr-1*	Tyeryar et al. (28)
FD60	*pep-1 pur-1 leu-2 his-3 gly-2*	Ft. Detrick
FD084	*pep-1 gly-3 his-9*	Ft. Detrick
B. subtilis		
W-23	Wild type	Ft. Detrick
W-23 M1-M143	*leu-1 arg-1*	UV, NTG[b]
W-23 M2-M235	*leu-2 phe-2*	NTG, NTG
W-23 M5-M200	*met-1 ile-3*	UV, NTG
W-23 M10-M62	*lys-1 tyr-1 ile-2*	UV, NTG
W-23 TD-1	*arg-1 phe-2*	Transduction[c]
W-23-S^r	*str*	Taylor & Thorne (24)
W-23-S^r M9-13	*lys-3 thi*	UV, UV
168-M4	*ind lys-2*	Ft. Detrick
168	*ind*	Ft. Detrick

[a] *pep*, Inability to synthesize glutamyl polypeptide. Abbreviations used for nutritional requirements: *arg*, arginine; *gly*, glycine; *his*, histidine; *ile*, isoleucine; *ind*, indole; *leu*, leucine; *lys*, lysine; *met*, methionine; *phe*, phenylalanine; *pur*, purine; *ser*, glycine or serine; *thi*, thiamine; *trp*, tryptophan; *tyr*, tyrosine; for resistance marker: *str*, resistance to streptomycin.

[b] UV, ultraviolet irradiation; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

[c] See Materials and Methods.

peptone per liter. Triple-distilled water was used in the preparation of all media.

Spores were produced on potato extract agar, which consisted of potato extract medium (26) plus 2.5% Difco agar, or in PA broth.

Viable counts were determined by plating on NBY agar (26).

DNA. DNA for transformation was prepared from *B. licheniformis* FD01 by the benzoate-phenol procedure of Kelly and Pritchard (15). The DNA concentrations were determined by the method of Burton (6).

Phage DNA used to determine buoyant density and melting point was isolated by phenol extraction (25). Sedimentation coefficients were determined by using phage DNA isolated by the perchlorate procedure of Freifelder (10).

Physical methods. The buoyant densities and sedimentation coefficients of SP-15 DNA and PBS-1 DNA were determined in collaboration with R. C. Moyer and A. A. Fuscaldo of these laboratories by use of an ultracentrifuge (Spinco model E) equipped with electronic speed control and ultraviolet optics. The method of Shildkraut et al. (19) was used to determine the buoyant density in CsCl, except that the centrifugation was at 44,000 rev/min (An-F-rotor). The equation of Shildkraut et al. (19) relating buoyant density to distance from the center of rotation was altered to $\rho = \rho_0 + 0.0089\ (r^2 - r^2_0)$. *Micrococcus lysodeikticus* DNA was used as a reference of 1.731 g/cm^3.

Boundary velocity sedimentation in 1 M NaCl was performed in an An-D rotor at a speed of 22,000 rev/min. Experiments were done at several DNA concentrations from 0.19 to 0.60 OD_{260} (optical density at 260 nm) and the plot of S_{20} versus OD_{260} was linear. A factor of 1.136 was used to convert S_{20} 1 M NaCl to $S_{20,w}$. The cells were filled from the window end while partially assembled, thus avoiding the shear associated with the conventional filling method. The films were traced by means of a Beckman Model RB Analytrol with a film densitometer attachment. Mean sedimentation coefficients were calculated from the motion of the mid-point of the concentration curves.

The melting point of SP-15 DNA was determined in collaboration with D. D. Gwinn of our laboratory by the method of Marmur and Doty (16).

Transformation. *B. licheniformis* mutants were transformed by the procedures described by Thorne and Stull (27) and Tyeryar et al. (28).

Propagation of bacteriophages. Transducing lysates of SP-10 and SP-15 were prepared from infected spores of *B. subtilis* W-23-S^r and *B. licheniformis* as described by Taylor and Thorne (23, 24) with one exception. For propagation of SP-15 from spores of *B. subtilis*, PA broth was supplemented with 1 μg of $ZnSO_4 \cdot 7H_2O$ and 40 μg of $FeCl_3 \cdot 6H_2O$ per ml. SP-15 lysates prepared in this medium retained good transducing ability for several months when stored without additions at 4 C. The high-titer SP-10 used for antiserum production was propagated in TY broth (18) by the method of Goldberg and Bryan

(11). Phage PBS-1 was provided by J. Spizizen and W. R. Romig. The lysates of PBS-1 used in transduction experiments usually were prepared in PA agar (bottom layer 1.5% agar; top layer 0.5% agar) seeded with *B. subtilis* W-23-S^r spores and phage. Incubation was for 18 hr at 37 C. Occasionally, lysates with poor transducing activity were produced. Although we do not fully understand the reason for this variation in transducing activity, we were always able to regain activity by propagation from a single plaque. Some of our highest efficiency lysates originated from plaques with a clearer center than those produced by the majority of the parent PBS-1 particles.

Assay of bacteriophages. SP-10 and SP-15 were assayed by the procedures of Taylor and Thorne (23) on PA agar, *p*H 5.9. PBS-1 was assayed by the soft agar overlay procedure (1) on PA agar with the soft layer containing 0.5% agar. Seed consisted of 10^8 *B. subtilis* W-23-S^r spores.

Transduction. Transductions by SP-10 and SP-15 were usually done by the procedures of Taylor and Thorne (23, 24). For transduction of strain TD-1 by SP-15, recipient cells were not the usual 6-hr subculture but were cells of a clone grown for 18 to 24 hr after spreading on NBY agar and then evenly suspended in minimal 1-yeast extract-glycerol broth (24) to a concentration of 10^9 to 2×10^9 cells/ml. Whenever transduction mixtures were diluted for plating, we used 1% peptone diluent containing 7.5% glycerol (v/v).

During the early stages of the problem, recipient cells for PBS-1-mediated transduction were grown for 6 hr at 37 C in Penassay broth. Cells and phage were incubated at 37 C for 45 min. Our linkage studies, however, were done with recipient cells that had been grown for 6 hr at 37 C in minimal 1-yeast extract-glycerol broth (24). For mutant W-23 M1-143, the concentration of yeast extract was doubled. Mixtures consisting of 0.1 ml of phage, 0.05 ml of deoxyribonuclease (1 mg/ml in 0.1 M $MgSO_4 \cdot 7H_2O$) and 0.1 ml of peptone diluent with 15% glycerol (v/v) were preincubated for 15 min to avoid possible complication of the results by transformation. Cells (0.9 ml) were added and the incubation was continued for 10 min. Control mixtures of the same composition but lacking phage were run at the same time. Efficiencies of 10^{-6} to 5×10^{-6} transductants per plaque-forming unit were routinely obtained when PBS-1 lysates propagated on W-23-S^r were used to transduce auxotrophs of W-23.

Scoring of transformants and transductants. Suitable dilutions of transduction or transformation mixtures were spread on minimal 1 agar supplemented with 25 or 50 μg of the appropriate nutritional requirements per ml. The transductants obtained with SP-10 and PBS-1 were plated in the presence of homologous phage antiserum. Transductants and transformants were scored after incubation at 37 C for 48 hr. For differentiation into the various recombinant classes, transductants were replicated by means of sterile toothpicks to supplemented and unsupplemented minimal agar plates. Linkage values were essentially the same whether transductants were scored by selective or replica plating. Transformants were usually scored by selective plating. Experiments designed to compare transformation linkages obtained by replica and selective plating showed essentially no differences in the results obtained by the two procedures.

The percentage of cotransformation of linked pairs of markers was calculated by the method of Barat et al. (4). The percentage of cotransduction was calculated by the following formula:

$$\frac{\frac{A^+B^+}{A^+B^- + A^+B^+} + \frac{A^+B^+}{A^-B^+ + A^+B^+}}{2} \times 100$$

These two methods of calculation are directly comparable.

Phage antisera. High-titer antisera against SP-15, PBS-1, and SP-10 were prepared in rabbits by subcutaneous injections of the concentrated phages.

Materials. Amino acids and vitamins were purchased from Calbiochem, Los Angeles, Calif. NTG was obtained from Aldrich Chemical Co., Milwaukee, Wis. Deoxyribonuclease, ribonuclease, and lysozyme were obtained from Worthington Biochemical Corp., Freehold, N.J.

RESULTS

Because SP-15 appeared to transfer long pieces of the *B. licheniformis* genome (28), we felt that this phage could be identical or at least closely related to PBS-1. This view was strengthened by electron microscopy studies done in collaboration with S. Vaituzis (University of Maryland) and H. G. Aaslestad by using an RCA EMU3-F electron microscope. SP-15 closely resembles PBS-1 in size (Fig. 1; Table 2). However, SP-15 and PBS-1 differ in host range, serological specificity, and physical characteristics of their DNA (Table 2). It is noted that the guanine plus cytosine content of SP-15 DNA calculated from the buoyant density does not agree with the value obtained from the melting temperature, indicating the presence of an unusual base.

It is clear from the data presented in Table 2 that the three generalized transducing phages we used in our studies are quite different from each other. However, it is of interest that the adsorption of SP-15, like PBS-1, is limited to flagellated cells (J. Mele and C. B. Thorne, *personal communication*).

Table 3 shows the results of experiments in which the frequencies of joint transfer of genetic markers in *B. licheniformis* by SP-15, SP-10, and transforming DNA were compared. The origins of the mutants used are shown in Table 1. Similar values to those shown were reported previously (28) for cotransduction with SP-15 and for cotransformation. Transforming DNA was used at levels well below saturation to avoid simultaneous

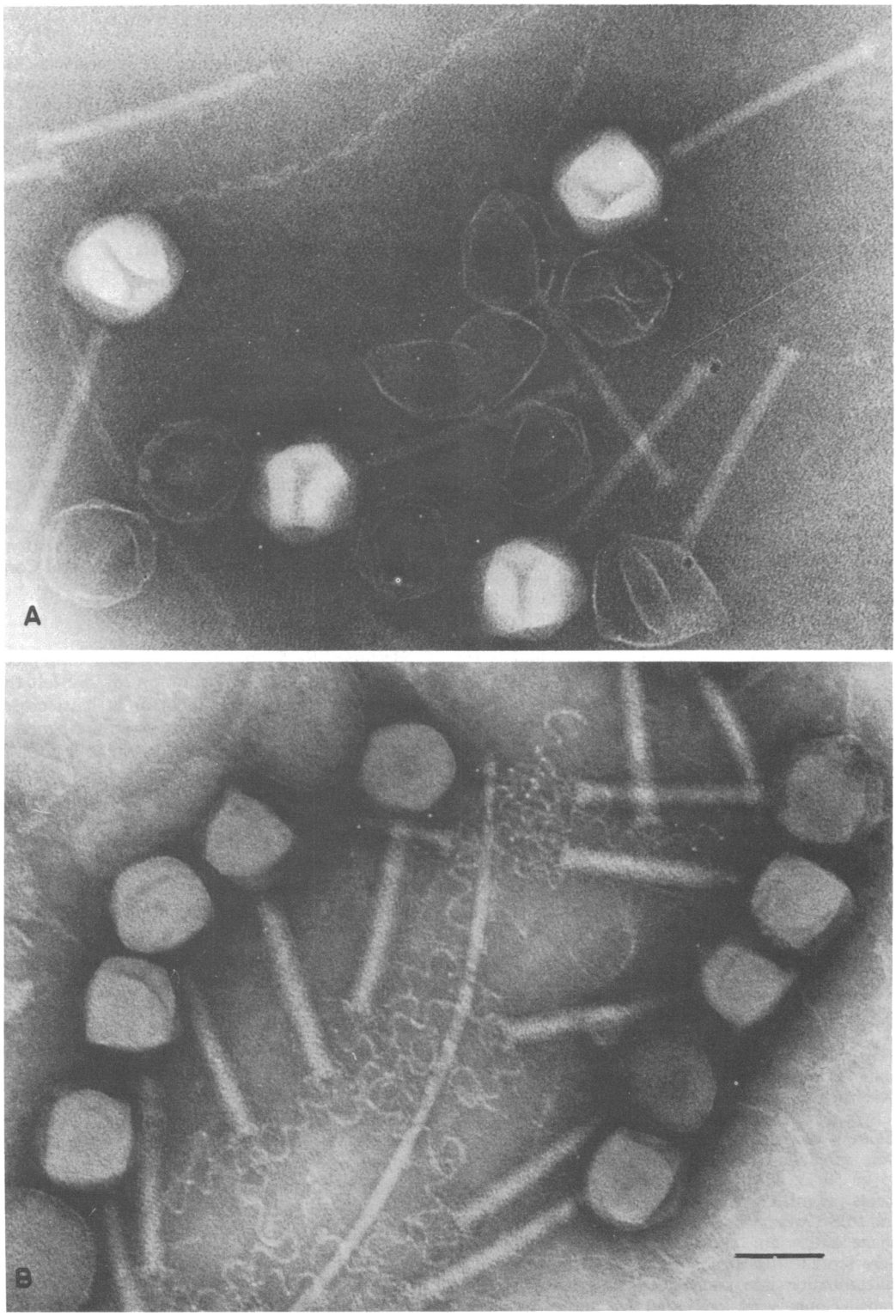

FIG. 1. *(A) Bacteriophage SP-15; (B) bacteriophage PBS-1. Phosphotungstic acid. ×167,500; marker represents 100 nm. Figure supplied by Z. Vaituzis.*

TABLE 2. *Properties of bacteriophages SP-15, PBS-1, and SP-10*

Properties	Bacteriophage		
	SP-15	PBS-1	SP-10
Size (nm)			
Head	120	120 (20)[a]	90 (17)
Tail	250	250	160
Host range (plaque formation)			
B. subtilis W-23	+	+	+
B. subtilis 168	−	+	−
B. licheniformis 9945A	+	−	+
Inactivation by antiserum against:			
SP-15	+	−	−
PBS-1	−	+	−
SP-10	−	−	+
Native DNA buoyant density in CsCl (g/cm³)	1.762	1.722 (22)	1.719 (5)
T_m (C)	61	76.5 (22)	81.5 (5)
$S_{20,w}$	85.6	73.5	NT[c]
Molecular weight[b]	2.5×10^8	1.7×10^8	NT

[a] Numbers in parentheses refer to literature citations.
[b] Calculated from formula of Eigner and Doty (8): $S_{20,w} = 0.034\ M^{0.405}$.
[c] NT = not tested.

TABLE 3. *Demonstration of various linkage groups in Bacillus licheniformis*

Recipient	Transformants		Co-transformation (%)	Transductants[a]					Cotransduction [(2)/(1) × 100]			
	Selected markers	Colonies[b] per ml		Selected marker	Colonies tested (1)[c]		No. of doubles obtained (2)			Per cent		Avg per cent	
					SP-15	SP-10	Class	SP-15	SP-10	SP-15	SP-10	SP-15	SP-10
FD52	*trp*⁺	7,900		*trp*⁺	278	288	*trp*⁺*tyr*⁺	251	259	90.3	89.9	93.5	89.9
	tyr⁺	10,700		*tyr*⁺	238	208	*tyr*⁺*trp*⁺	230	187	96.6	89.9		
	trp⁺*tyr*⁺	6,230	68.5										
FD55	*trp*⁺	20,800		*trp*⁺	177	338	*trp*⁺*met*⁺	110	158	62.1	46.7	63.9	47.1
	met⁺	24,200		*met*⁺	160	527	*met*⁺*trp*⁺	105	250	65.6	47.4		
	trp⁺*met*⁺	1,170	5.2										
FD59	*lys*⁺	3,850		*lys*⁺	347	533	*lys*⁺*tyr*⁺	181	167	52.2	31.3	63.0	29.5
	tyr⁺	2,650		*tyr*⁺	244	339	*tyr*⁺*lys*⁺	180	94	73.8	27.7		
	lys⁺*tyr*⁺	250	8.0										
FD28	*leu*⁺	24,600		*leu*⁺	454	416	*leu*⁺*arg*⁺	184	38	40.5	9.1	42.2	7.3
	arg⁺	23,500		*arg*⁺	330	203	*arg*⁺*leu*⁺	145	11	43.9	5.4		
	leu⁺*arg*⁺	350	1.5										
FD60	*his*⁺	20,700		*his*⁺	236	224	*his*⁺*pep*⁺[d]	161	110	68.2	49.1	68.2	49.1
	gly⁺	30,300		*gly*⁺	85	228	*gly*⁺*pep*⁺	25	0	29.4	0	29.4	0
	his⁺*pep*⁺[d]	1,170	5.7				*his*⁺*gly*⁺	19	0	15.2	0	15.2	0
	gly⁺*pep*⁺	0											
FD084	Not tested			*his*⁺	1,037	160	*his*⁺*pep*⁺	587	71	56.6	44.4	56.6	44.4
				gly⁺	394	138	*gly*⁺*pep*⁺	145	0	36.8	0	36.8	0
							his⁺*gly*⁺	55	0	9.6	0	9.6	0

[a] Donor strain = FD01.
[b] The donor DNA concentrations varied from 0.05 to 0.1 μg/ml.
[c] Scored by replica plating.
[d] For FD60 and FD084, *his*⁺*pep*⁺ and *gly*⁺*pep*⁺ were distinguished phenotypically.

transformation of unlinked markers. In most cases in *B. licheniformis*, the percentage of cotransduction calculated on the basis of marker A equalled the value obtained when the calculation was based on marker B. In the case of the *lys tyr* mutant, there was a notable difference. Markers that were closely linked as indicated by the high frequency of cotransduction with SP-15 were also cotransduced by SP-10, but at a somewhat lower frequency. However, markers that were further apart, e.g., *gly pep*, *gly his*, and *leu arg*, were cotransduced by SP-15, but at a much reduced frequency or not at all by SP-10. These results can be interpreted to mean that SP-15 transfers a larger segment of the *B. licheniformis* genome than does SP-10. In all cases, the frequency of cotransformation was much lower than the frequency of cotransduction by SP-10. Thus, the situation in *B. licheniformis* is apparently different from that reported by Ephrati-Elizur and Fox (9) for *B. subtilis*. Their results can be interpreted to mean that the same size fragment is transferred by both SP-10-mediated transduction and by transformation. However, the markers used by these investigators were very closely linked.

It would have been of advantage to be able to compare the frequency of joint transfer of *B. licheniformis* markers by SP-15 with another phage believed to transfer large DNA fragments, such as PBS-1. Unfortunately, although PBS-1 adsorbs to *B. licheniformis*, it neither plaques nor propagates on this strain. However, PBS-1, SP-15, and SP-10 all propagate on and transduce markers to *B. subtilis* W-23. Initially we attempted to obtain markers in W-23 by transfer from auxotrophic strains of *B. subtilis* 168. These interstrain crosses were done with PBS-1 that had been propagated on the 168 derivatives. It was necessary to use transductions because of the poor transformability of W-23. Control experiments designed to determine the feasibility of interstrain transduction between 168 and W-23 proved this approach to be more difficult than we had anticipated. The number of W-23 transductants obtained with PBS-1 propagated on 168 was always much lower than the number obtained with PBS-1 propagated on W-23 (Table 4). In many experiments, the interstrain transductants were 10^{-2} times the intrastrain transductants. Essentially the same results were obtained when the crosses were run in the opposite direction (Table 4).

The low numbers of interstrain transductants compared with intrastrain transductants could have been the result of differences in efficiency of plating or adsorption depending upon the strain on which the PBS-1 had been propagated. To test these possibilities, PBS-1 was propagated on W-23-S^r, and the lysate was tested for the ability to plaque on and adsorb to both 168 and W-23 auxotrophs. PBS-1 propagated on W-23-S^r adsorbed equally well to the 168 and W-23 auxotrophs (Table 5). Although the phage plaqued on 168 at a somewhat lower efficiency than on W-23 (Table 5), this slight difference could not account for the large discrepancy between intrastrain and interstrain transduction.

TABLE 4. *Interstrain transduction between B. subtilis 168 and W-23*[a]

Phage	Recipient	
	168 *lys*$^-$*ind*$^-$ (1.4×10^9/ml) *lys*$^+$ transductants/ml	W-23-S^r *lys*$^-$*thi*$^-$ (6.5×10^8/ml) *lys*$^+$ transductants/ml
PBS-1 propagated on 168 *ind*$^-$	3×10^2	<10
PBS-1 propagated on W-23-S^r	<10	2.1×10^3

[a] Recipient cells were grown for 6 hr in Penassay broth. Phage was added at a multiplicity of approximately 0.5 to 1.0. Incubation was at 37 C for 45 min. Transductants were scored after 48 hr at 37 C on minimal 1 medium supplemented with the appropriate requirements.

It was also possible that the transducing lysates produced on W-23-S^r contained particles that drastically reduced the viability of the recipient 168 cells, while having much less or no effect on W-23 viability. Auxotrophs of 168 and W-23, grown under the same conditions as for transduction, were incubated with various concentrations of PBS-1 that had been propagated on W-23-S^r. After 45 min of incubation at 37 C, the survivors were assayed. Although the survival of the 168

TABLE 5. *Comparison of B. subtilis 168 and W-23 auxotrophs in terms of adsorption and efficiency of plating (EOP) with PBS-1 propagated on W-23-S^r*

Strain	EOP[a]	Adsorption[b]
		%
168 *lys*$^-$*ind*$^-$	0.6	>99
W-23-S^r *lys*$^-$*thi*$^-$	1.0	>99

[a] Plaque-forming units were assayed in PA agar as described, by using 10^9 spores as seed. The EOP on W-23-S^r *lys*$^-$*thi*$^-$ was arbitrarily set at 1.0.

[b] Adsorption was determined after incubation of PBS-1 with 7-hr cells for 45 min at 37 C. The mixtures were filtered through membrane filters (0.65 μm pore size; Millipore Corp.), and the free phage were assayed as described for footnote *a*.

strain was slightly higher than the W-23 strain, 25-fold more transductants were obtained with the W-23 strain as recipient. Heavy ultraviolet irradiation (10^{-5} to 10^{-6} decrease in plaque-forming units) of transducing lysates propagated on W-23-S^r increased the number of transductants obtained with W-23 recipients by two- to sevenfold with slight, if any, concurrent increase in the number of transductants obtained with 168 recipients. The results (Tables 6 and 7) suggest that differential killing activity cannot explain the low number of transductants obtained in interstrain crosses. Perhaps differences in homology between 168 and W-23 are responsible. It is also possible that a restriction mechanism active against bacterial but not phage DNA may be involved.

Because of the difficulties encountered in transferring markers from 168 to W-23, we decided to obtain multiply marked strains of W-23 by mutagenesis. In most cases, we started with singly marked strains that had been obtained by ultraviolet irradiation and then introduced subsequent mutations by mutagenesis with NTG. Pairs of markers were chosen that, by analogy to the map of 168 (4, 7), we felt should show various degrees of linkage. These multiple auxotrophs were transduced with SP-15, PBS-1, and SP-10 propagated on W-23-S^r, and the mixtures were plated simultaneously on singly supplemented and unsupplemented agar plates. In the case of the triple auxotroph, the selective platings were done on doubly and singly supplemented as well as unsupplemented plates. Final differentiation into classes was accomplished by replication to singly (and doubly, in the case of the triple auxotroph) supplemented and unsupplemented minimal media. Each transduction was repeated several times; typical results are shown in Table 8. Because the percentage of cotransduction calculated as the number of doubles among the cells transduced for marker A differed in many cases from the percentage of cotransduction calculated on the basis of marker B, we have presented both calculations as well as the average percentage of cotransduction.

TABLE 6. *Effect of PBS-1 lysate propagated on W-23-S^r on viability of 168 and W-23 auxotrophs; survival*[a]

Dilution of phage	Survivors/ml	
	168 *lys⁻ind⁻*	W-23-S^r *lys⁻thi⁻*
Undiluted	9.5×10^8	2.1×10^8
1:2	1.7×10^9	5.4×10^8
1:4	1.4×10^9	7.6×10^8
1:16	1.5×10^9	5.5×10^8
Control (no phage)	1.5×10^9	6.6×10^8

[a] Cells (0.9 ml of 6-hr culture) were incubated with 0.1 ml of various dilutions of phage (stock = 3.7×10^9 plaque-forming units/ml) for 45 min at 37 C. A multiplicity of infection of approximately 1.0 was used for the undiluted phage-cell mixture. Survivors were plated on NBY agar. Control cells were incubated with 0.1 ml of Penassay broth.

TABLE 7. *Effect of PBS-1 lysate propagated on W-23-S^r on viability of 168 and W-23 auxotrophs; transduction*[a]

Recipient	Transduced to	Transductants/ml
168 *lys⁻ind⁻*	*ind⁺*	7.0×10^1
W-23-S^r *lys⁻thi⁻*	*lys⁺*	1.8×10^3

[a] Cells (same as in Table 6) were incubated with 0.1 ml of undiluted phage for 45 min at 37 C. The 168 transductants were scored on minimal 1 + lysine, and the W-23 transductants were scored on minimal 1 + thiamine.

SP-15 and PBS-1 are able to transfer much larger fragments of DNA than can SP-10 (Table 8). With loci that were not very tightly linked (e.g., *ile tyr*, *ile lys*, *leu arg*), SP-15 and PBS-1 exhibited a much higher frequency of cotransduction than did SP-10. As expected, with more closely linked loci (e.g., *lys tyr*, *leu phe*), the differences were not as great. These results are in agreement with those of Takahashi (21), who showed that in *B. subtilis* 168 there was little difference between the degree of linkage conferred by transformation and PBS-1-mediated transduction with tightly linked markers, but that there was a great difference with loosely linked markers.

SP-15 also cotransduced linked markers at a significantly higher frequency than did PBS-1 (Table 8), suggesting that SP-15 transfers a larger fragment of the *Bacillus* genome than any phage heretofore described.

DISCUSSION

SP-15 transfers linked markers in *B. licheniformis* at a higher frequency than does SP-10 or isolated DNA. With SP-15, we were able to demonstrate linkages (*gly* with *pep*, *his* with *gly*) that are not detectable by transformation or transduction with SP-10. In this respect, SP-15 functions in the *B. licheniformis* system as PBS-1 (4, 7, 21) does in *B. subtilis*. PBS-1 cannot be used in mapping studies of *B. licheniformis* 9945A because of its inability to propagate on this strain. We demonstrated a gradation among the means of transfer in *B. licheniformis* in terms of the degree

TABLE 8. *Cotransduction of various markers in Bacillus subtilis W-23 by bacteriophages SP-15, PBS-1, and SP-10*

Recipient	Transductants[a]								Cotransduction [(2)/(1) × 100]					
	Selected marker	No. of colonies tested (1)			No. of doubles obtained (2)				Per cent			Avg per cent		
		SP-15	PBS-1	SP-10	Class	SP-15	PBS-1	SP-10	SP-15	PBS-1	SP-10	SP-15	PBS-1	SP-10
leu-1 phe-2	*leu*$^+$	462	122	178	*leu*$^+$*phe*$^+$	369	92	120	79.9	75.4	67.4	84.3	75.4	68.7
	phe$^+$	434	130	177	*phe*$^+$*leu*$^+$	385	98	124	88.7	75.4	70.0			
lys-1 tyr-1 ile-1	*lys*$^+$	206	445	298	*lys*$^+$*tyr*$^+$	163	305	129	79.1	68.5	43.3	75.7	69.6	49.5
	tyr$^+$	235	468	268	*tyr*$^+$*lys*$^+$	170	331	149	72.3	70.7	55.6			
	tyr$^+$	235	468	268	*tyr*$^+$*ile*$^+$	172	251	75	73.2	53.6	28.0	60.6	49.6	32.2
	ile$^+$	372	528	278	*ile*$^+$*tyr*$^+$	178	241	101	47.9	45.6	36.3			
	lys$^+$	206	445	298	*lys*$^+$*ile*$^+$	113	140	0	54.9	31.5	0	44.1	32.0	0
	ile$^+$	372	528	278	*ile*$^+$*lys*$^+$	124	171	0	33.3	32.4	0			
leu-1 arg-1	*leu*$^+$	307	840	504	*leu*$^+$*arg*$^+$	164	394	36	53.4	46.9	7.1	51.4	39.8	7.3
	arg$^+$	344	538	442	*arg*$^+$*leu*$^+$	170	176	33	49.4	32.7	7.5			
arg-1 phe-2	*arg*$^+$	212	1001	578	*arg*$^+$*phe*$^+$	76	162	1	35.9	16.2	0.2	32.8	19.6	0.5
	phe$^+$	239	801	396	*phe*$^+$*arg*$^+$	71	184	3	29.7	23.0	0.8			
met-2 ile-1	*met*$^+$	300	300	300	*met*$^+$*ile*$^+$	0	0	0	0	0	0	0	0	0
	ile$^+$	300	300	300	*ile*$^+$*met*$^+$	0	0	0	0	0	0			

[a] Donor strain was W-23-S^r.

of linkage revealed, i.e., SP-15 > SP-10 > transforming DNA. Thus, *B. licheniformis* 9945A appears to be especially amenable to mapping studies.

We considered three hypotheses to explain the low frequency of joint marker transfer by transformation in *B. licheniformis*. (i) The transforming fragments of *B. licheniformis* DNA may be small —smaller perhaps than those of *B. subtilis* DNA. However, the DNA was prepared by the method of Kelly and Pritchard (15), which should yield DNA that is less fragmented than that obtained by more severe extracting procedures. (ii) Transforming DNA carrying linked loci may be fragmented during uptake by competent cells. Kelly (14) suggested that linkage values in *B. subtilis* can be decreased by two recipient cells taking up a single DNA fragment. (iii) The competent state in *B. licheniformis* may be transient, so that individual cells do not remain competent long enough to complete the uptake of large pieces of DNA. Thorne and Stull (27) suggested that *B. licheniformis* populations are heterogeneous with respect to competence, so that, during incubation in the transformation medium, some cells lose competence as others gain it. It is of interest that, to our knowledge, no one has succeeded in transfecting *B. licheniformis*, although we and others have tried. We are unable at the present time to choose from among these three possibilities.

We have been able to directly compare SP-15, PBS-1, and SP-10 in terms of their ability to cotransduce linked markers. Our data can be interpreted to mean that SP-15 and PBS-1 are both able to transfer larger fragments of the genome than does SP-10 (Table 8). In all cases, SP-15 transferred linked markers at higher frequencies than did PBS-1. Mean frequencies of cotransduction with SP-15 were from 6 to 13% higher than those obtained with PBS-1. This difference compares favorably with the higher molecular weight for SP-15 DNA estimated from boundary sedimentation (Table 2). Our interpretation that SP-15 is able to transfer a larger fragment of DNA than does PBS-1 is complicated, however, by the asymmetry of the cotransduction percentages observed when the calculation was made separately for each marker of a pair (Table 8). Thus, for example, an examination of the data for the *lys ile* pair revealed a 23% difference in percentage of cotransduction between SP-15 and PBS-1 when the calculation was based on the frequency of doubles among the *lys* transductants, but no difference in percentage of cotransduction when the calculation was based on only the *ile* transductants. This situation holds true for most of the linked pairs of W-23 markers that we have studied; the differences between SP-15 and PBS-1 in percentage of cotransduction reflect differences in the frequency of the doubles obtained among the transductants for only one of the two markers of a pair. As can be seen from the numbers of transductants (Table 8), the asymmetry usually results from a paucity in one class of single transductants. Therefore, the asymmetry could be the result of a polarity occurring during recombination (3, 21) or a polarity in the fragmentation of the donor DNA, or conceivably it could result from a specific chemical inhibition of one of the two classes of single transductants (13). The latter hypothesis is improbable, because the degree of asymmetry observed for a given pair of markers varies among the phages used for transduction.

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