Concomitant Conjugal Transfer of Reduced-Bacteriophage-Sensitivity Mechanisms with Lactose- and Sucrose-Fermenting Ability in Lactic Streptococci†

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Ten previously reported lactose-positive (Lac^+) transconjugants from *Streptococcus lactis*, *S. cremoris*, and *S. lactis* subsp. *diacetylactis* and one sucrose-positive (Suc^+) transconjugant from *S. lactis* were examined for their sensitivity to prolate- and small isometric-headed bacteriophages. Four of the Lac^+ transconjugants showed a 10- to 100-fold reduction in the efficiency of plating (EOP) as well as a reduced plaque size for the prolate phage c2 and were insensitive to the small isometric phage 712. A fifth Lac^+ transconjugant demonstrated a similar reduced sensitivity to phage c2; however, this transconjugant was able to plaque phage 712, but with a reduced plaque size and EOP. The other five Lac^+ transconjugants were sensitive to both c2 and 712 phages. The Suc^+ transconjugant plaqued phage 712 with a reduced plaque size and EOP, but no reduction in plaque size or EOP was observed for phage c2. The Lac^+ and reduced bacteriophage sensitivity (Rbs^+) phenotypes were correlated with specific plasmids in the Lac^+ transconjugants. As four of the Lac^+ transconjugants exhibited a phenotypically indistinguishable Rbs^+ , one (AB001) was selected for further study. The Rbs^+ in AB001 for both small isometric- and prolate-headed phages was not related to adsorption, and the reduced EOP for phage c2 was not related to the presence of a restriction and modification system. The latent period for phage c2 was unchanged, but the burst size was reduced 80%. The presence of the plasmid coding for Rbs^+ retarded the lysis of a mitomycin C-induced prophage-containing strain. The Rbs^+ mechanism appears to be abortive phage infection. This study supports previous observations that Rbs^+ and conjugal transfer ability are physically linked among some group N streptococci. The results presented have implications in the identification of plasmids coding for Rbs^+ and may also aid in explaining the dissemination of Rbs^+ genes among lactic streptococci.

Recent reports have linked reduced bacteriophage sensitivity (Rbs^+) to conjugally transferred plasmids in several group N streptococci (3, 4, 14, 17, 30, 31). Chopin et al. (4) identified a restriction and modification (R^+/M^+) system encoded on a 28-megadalton (MDa) plasmid in *Streptococcus lactis* IL594. McKay and Baldwin (17) identified a conjugally transferred 40-MDa plasmid in *S. diacetylactis* DRC3 that coded for a temperature-dependent mechanism effective against prolate-headed phage at 21 and 32°C but not at 37°C. Klaenhammer and Sanozky (14) identified a self-transmissible 30-MDa plasmid coding for a temperature-sensitive Rbs^+ mechanism that caused a reduced burst size with prolate-headed phage at 32°C. Further reports (10, 25, 28) demonstrated that this 30-MDa plasmid inhibited the proliferation of small isometric-headed phage on *S. cremoris* at both 32 and 37°C. A 50-MDa plasmid from *S. cremoris* UC653 coded for an Rbs^+ mechanism that was effective against prolate- and small isometric-headed phages (3). Vedamuthu and Neville (30, 31) isolated transconjugants possessing two conjugally transferred plasmids, one coding for mucoidness and the other coding for lactose metabolism. These transconjugants demonstrated an Rbs^+ phenotype; however, further analysis of this system showed that a residual Rbs^+ phenotype remained following curing of the plasmids.

We previously reported the conjugal transfer of plasmid DNA for lactose-fermenting (12, 18, 26, 32) and sucrose-fermenting (27) (Lac^+ and Suc^+ , respectively) ability among group N streptococci. Whether these conjugally transferred plasmids also carried genes for Rbs^+ was not determined. These previously reported transconjugants were examined in this study for their ability to plaque both prolate- and small isometric-headed phages. If these transconjugants also gained Rbs^+ mechanisms, the genetic material coding for Rbs^+ would be available for strain construction strategies.

MATERIALS AND METHODS

Bacterial strains. The strains examined were maintained by biweekly transfer at 32°C in M17 broth containing 0.5% glucose or 0.5% lactose (29). Table 1 lists the strains of *S. lactis* used in this study.

Phage assay. The following five lytic phages active on *S. lactis* C2 and *S. lactis* 712 were used: two small isometric-headed phages, 712 (M. Gasson, personal communication) and sk1 (W. D. Sing and T. R. Klaenhammer, personal communication), and three prolate-headed phages, c2 (10), stl5 (20), and eb1 (unpublished data). Phage lysates were prepared by the addition of a single plaque to an actively growing culture of *S. lactis* MG1614 and incubation at 32°C in M17-glucose broth supplemented with 100 μl of 0.185 M CaCl_2 per 10 ml until lysis was complete. The lysates were filter sterilized through a 0.45- μm -pore syringe filter. The phage titer was determined by dilution in 0.85% saline and plaquing with the appropriate host on M17-glucose agar (29). Plates were incubated at 21, 30, and 37°C. Cultures for

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TABLE 1. *S. lactis* strains used in this study

Strain	Derivation ^a	Plasmid content (MDa)	Source or reference
AB001	Lac ⁺ transconjugant; <i>S. cremoris</i> UC653 × MG1614	50, 26	3
AB002	Lac ⁻ derivative of AB001	50	This study
WW4	Lac ⁺ transconjugant; <i>S. diacetylactis</i> WM4 × LM2301	88, 33	24
JS30	Lac ⁺ transconjugant; <i>S. diacetylactis</i> 11007 × LM2336	88, 32	Unpublished data
CC101	Lac ⁺ transconjugant; <i>S. cremoris</i> C3 × LM2301	34, 27	26
EB101	Lac ⁺ transconjugant; <i>S. cremoris</i> EB7 × LM3302	56, 27, 5.5, 2.0, 1.0	26
JS21	Suc ⁺ transconjugant; <i>S. lactis</i> 11454 × LM2306	None detectable ^b	27
KC1	Lac ⁺ transconjugant; <i>S. lactis</i> C20 × LM2301	Two plasmids	18
PW1	Lac ⁺ transconjugant; <i>S. lactis</i> ML3 × LM2301	60	32
RM108	Lac ⁺ transconjugant; <i>S. cremoris</i> R1 × LM3302	34, 27, 5.5, 2.0, 1.0	26
ZM803	Lac ⁺ transconjugant; <i>S. cremoris</i> Z8 × LM3302	30, 27, 5.5, 2.0, 1.0	26
GK4101	Lac ⁺ transconjugant; <i>S. diacetylactis</i> 18-16 × LM2301	41	12
LM2201	Lac ⁻ Str ^r derivative of <i>S. lactis</i> C2	18, 12, 5, 2.0, 1.0	J. L. Steele ^c
Pro16	Lac ⁺ transconjugant; <i>S. cremoris</i> UC653 × LM2201	50, 26, 18, 12, 5, 2.0, 1.0	This study
JS13	Suc ⁺ transconjugant; <i>S. lactis</i> 11454 × LM2201	18, 12, 5, 2.0, 1.0	J. L. Steele ^c
MG1614	Plasmid-free Str ^r Rif ^r derivative of <i>S. lactis</i> 712	None	8

^a Lac⁺, Lactose fermenting; Lac⁻, lactose negative; Str^r, streptomycin resistant; Suc⁺, sucrose fermenting; Rif^r, rifampin resistant.

^b Although no plasmid DNA was detected, data suggesting the involvement of plasmid DNA with the Suc⁺ phenotype have been reported (27).

^c M.S. thesis, University of Minnesota, St. Paul, 1985.

plaque assays were grown overnight at 32°C in M17-glucose broth.

Curing trials. Curing of plasmids was accomplished by growth at an elevated temperature (37°C) or by growth in the presence of novobiocin (16) or acriflavine. Lactose-negative (Lac⁻) derivatives were detected on bromocresol purple-lactose indicator agar (19) and screened for susceptibility to phages by a spot test (3) followed by standard plaque assays (29). The plasmid content was determined as described by Anderson and McKay (1).

Phage adsorption. The adsorption of phages c2 and 712 to *S. lactis* MG1614 and *S. lactis* AB002 was described previously (3).

Determination of the latent period and burst size. The infective centers, latent period, and burst size of phage c2 on *S. lactis* MG1614 and *S. lactis* AB002 were determined as described by Keogh (13), except that M17-glucose broth was substituted for 11% skim milk.

Determination of R⁺/M⁺ activity. *S. lactis* AB002 was examined for the presence of an R⁺/M⁺ system as described by Pearce (22). Phage c2 was modified by growth on R⁺/M⁺ *S. cremoris* KH (22, 23). The modified phage, c2 · KH, was purified by two subsequent single-plaque isolations on KH to

ensure a homogenous phage population. This phage was then titrated on KH, AB002, and MG1614. Subsequent cycles were performed by picking single-plaque isolates on the restrictive host and propagating them as described above.

Prophage induction from *S. lactis* LM2201. Prophage induction of *S. lactis* LM2201, LM2306, JS13, and Pro16 by mitomycin C was conducted as previously described (21). The Lac⁺ transconjugant Pro16 and the Suc⁺ transconjugant JS13 each contained a genetic element coding for Rbs⁺ (Table 1).

RESULTS

Transconjugant derivation and plasmid content. Eleven previously reported transconjugants in which *S. cremoris*, *S. lactis*, or *S. diacetylactis* strains served as donors in conjugal matings were examined (Table 2). The transconjugants derived from *S. cremoris* included AB001 (3), CC101, EB101, RM108, and ZM803 (26). Transconjugants JS21 (27), PW1 (32), and KC1 (18) were from *S. lactis* donors, and transconjugants WW4 (24), JS30 (unpublished data), and GK4101 (12) were from *S. diacetylactis* donors. All transconjugants,

TABLE 2. Survey of various Lac⁺ or Suc⁺ transconjugants for Rbs⁺ to phages c2 and 712 at 30°C

Donor	Transferred phenotype	Transconjugant designation	Plasmid(s) ^a transferred (MDa)	EOP ^b of:		Plaque type ^c of:	
				712	c2	712	c2
<i>S. diacetylactis</i> 11007	Lac ⁺	JS30	88, 32	<6.0 × 10 ⁻⁷	5.5 × 10 ⁻²	NP	RPS
<i>S. diacetylactis</i> WM4	Lac ⁺	WW4	88, 33	<1.0 × 10 ⁻⁸	9.0 × 10 ⁻²	NP	RPS
<i>S. cremoris</i> UC653	Lac ⁺	AB001	50, 26	<3.9 × 10 ⁻⁷	9.2 × 10 ⁻³	NP	RPS
<i>S. cremoris</i> C3	Lac ⁺	CC101	34, 27	<1.0 × 10 ⁻⁸	8.6 × 10 ⁻²	NP	RPS
<i>S. cremoris</i> EB7	Lac ⁺	EB101	56	4.4 × 10 ⁻⁶	6.4 × 10 ⁻²	RPS	RPS
<i>S. lactis</i> C20	Lac ⁺	KC1	Two plasmids ^d	1.3	6.1 × 10 ⁻¹	N	N
<i>S. lactis</i> ML3	Lac ⁺	PW1	60	1.1	4.9 × 10 ⁻¹	N	N
<i>S. cremoris</i> R1	Lac ⁺	RM108	34	3.4 × 10 ⁻¹	2.8 × 10 ⁻¹	N	N
<i>S. cremoris</i> Z8	Lac ⁺	ZM803	30	5.6 × 10 ⁻¹	5.8 × 10 ⁻¹	N	N
<i>S. diacetylactis</i> 18-16	Lac ⁺	GK4101	41	5.9 × 10 ⁻¹	6.1 × 10 ⁻¹	N	N
<i>S. lactis</i> 11454	Suc ⁺	JS21	ND ^e	1.1 × 10 ⁻⁵	8.1 × 10 ⁻¹	RPS	N

^a Determined by comparing the plasmid profile of the transconjugant to the plasmid profile of the recipient.

^b Calculated for each transconjugant relative to the recipient from which it was derived.

^c NP, No plaques detected; RPS, reduced plaque size (<0.5 mm in diameter); N, normal plaque size (2.5 mm in diameter).

^d This transconjugant contains two plasmids of undetermined size.

^e No additional plasmid DNA was detected in this transconjugant.

TABLE 3. Effect of the Rbs⁺ mechanism on the ability of prolate- and small isometric-headed phages to form plaques at 21, 30, and 37°C

Strain	Plaques temp (°C)	PFU of the following phage/ml:				
		c2	eb1	stl5	sk1	712
<i>S. lactis</i> MG1614	21	5.6×10^8	8.0×10^8	2.2×10^8	7.7×10^8	8.1×10^8
	30	6.5×10^{8a}	1.1×10^{9a}	2.8×10^{8a}	9.5×10^{8a}	7.9×10^{8a}
	37	6.1×10^8	9.2×10^8	2.1×10^8	1.1×10^8	$<3.0 \times 10^1$
<i>S. lactis</i> AB002	21	$<3.0 \times 10^1$	2.3×10^{3b}	1.1×10^{6b}	$<3.0 \times 10^1$	$<3.0 \times 10^1$
	30	1.6×10^{6b}	1.0×10^{7b}	2.2×10^{6b}	$<3.0 \times 10^1$	$<3.0 \times 10^1$
	37	5.7×10^{5b}	5.9×10^{5b}	4.0×10^{5b}	$<3.0 \times 10^1$	$<3.0 \times 10^1$

^a Normal plaque size; average diameter, 2.5 mm.^b Reduced plaque size; average diameter, ≤ 0.5 mm. Ten percent of plaques were of an intermediate size; average diameter, 1.0 mm.

except JS21, were selected by their Lac⁺ ability. JS21 was selected on the basis of its Suc⁺ ability and contained no detectable plasmid DNA (27). The Lac⁺ transconjugants each gained one or two plasmids ranging in size from 26 to 88 MDa.

Rbs⁺ to prolate and small isometric phages exhibited by transconjugants. All 10 Lac⁺ transconjugants and the Suc⁺ transconjugant were screened for their sensitivity to the small isometric phage 712 and the prolate phage c2 at 30°C. Five Lac⁺ transconjugants, JS30, WW4, AB001, CC101, and EB101, exhibited a 100- to 1,000-fold decrease in the efficiency of plating (EOP) and a reduced plaque size for phage c2. However, when examined for sensitivity to phage 712, EB101 exhibited a decrease in EOP and plaque size, but JS30, AB001, WW4, and CC101 were totally resistant. The Suc⁺ transconjugant JS21 also exhibited Rbs⁺ that was phenotypically different from that expressed by JS30, AB001, WW4, CC101, and EB101. JS21 exhibited reduced EOP and plaque size with phage 712; however, JS21 was sensitive to phage c2 at a level equivalent to that of LM2306. The remaining Lac⁺ transconjugants did not show reduced bacteriophage sensitivity (Rbs⁻) to phage 712 and phage c2 (Table 2).

Curing trials. The Lac⁺ Rbs⁺ transconjugants contained plasmids of different sizes (Table 2). To determine whether the Rbs⁺ and Lac⁺ phenotypes were linked to plasmid DNA, we generated plasmid-cured derivatives. Rbs⁺ to phages c2 and 712 was associated with plasmids of 88, 50, 88, 34, and 56 MDa in JS30, AB001, WW4, CC101, and EB101, respectively. Curing results also indicated that the Lac⁺ phenotype was correlated with plasmids of 32, 26, 33, 27, and 56 MDa in JS30, AB001, WW4, CC101, and EB101, respectively.

Characteristics of the Rbs⁺ mechanism. The effect of phage assay temperature on the five Lac⁺ Rbs⁺ transconjugants was examined by plaquing at 21, 30, and 37°C with c2 or 712 phages. Phage assay temperatures exhibited the same effects on the Rbs⁺ phenotype (data not shown). Since four of the Lac⁺ transconjugants exhibited a phenotypically indistinguishable Rbs⁺ phenotype and since preliminary data had been reported for *S. lactis* AB001 (3), this transconjugant was selected for further characterization. To ensure that the 26-MDa Lac⁺ plasmid in AB001 was not affecting the Rbs⁺ phenotype, we examined a Lac⁻ derivative designated *S. lactis* AB002.

To determine whether the Rbs⁺ phenotype involved the inability of phage to adsorb, we examined the adsorption of phages 712 and c2 to *S. lactis* AB002 and *S. lactis* MG1614. Both strains adsorbed >99% of phage 712 and about 97% of phage c2.

Two small isometric-headed phages, 712 and sk1, and three prolate-headed phages, eb1, stl5 and c2, were examined for their ability to form plaques on AB002 and MG1614 at 21, 30, and 37°C (Table 3). Phages sk1 and 712 were unable to form plaques on AB002 at the three temperatures. Phages c2, eb1, and stl5 showed differences in sensitivity to the Rbs⁺ mechanism. All three had a notably reduced plaque size and a 10- to 1,000-fold decrease in titer at 30 and 37°C when compared with plaques on MG1614. Plaque size was reduced about 80%, and the pinpoint plaques observed had a diameter of ≤ 0.5 mm. However, 10% of the plaques formed on AB002 at 30 and 37°C were of an intermediate size and had an average diameter of 1.0 mm. At 21°C, phage c2 formed no detectable plaques on AB002. Phages eb1 and stl5 both formed plaques at 21°C; however, phage eb1 had a 10,000-fold decrease in titer when compared with the titer at 30°C. Both pinpoint and intermediate-sized plaques were seen at 21°C for phages eb1 and stl5.

To determine whether the 10- to 100-fold decrease in the EOP of the prolate phages on AB002 at 30°C was due to an R⁺/M⁺ system, we used phage c2 · KH (22). *S. cremoris* KH (R⁺/M⁺) served as the positive control. The effects of the 50-MDa plasmid in AB002, designated pCI750, were ascertained by comparing the plating efficiencies of AB002 and its isogenic plasmid-free strain, MG1614. Phage c2 · KH formed plaques at a high EOP on both KH and MG1614 but not on AB002 (Table 4). Propagation of phage c2 · KH · AB002 on AB002 or MG1614 resulted in a high EOP, but propagation of the same phage on KH resulted in a low EOP. Propagation of phage c2 · KH · AB002 · KH on KH or MG1614 resulted in a high EOP. However, AB002 did not exhibit the expected low EOP of phage c2 · KH · AB002 · KH, as was observed for phage c2 · KH. Nevertheless, AB002 did not plaque this phage as efficiently as did MG1614 or KH.

As demonstrated for phage c2 on AB002, phage c2 · KH also produced two plaque types on AB002. Ninety percent

TABLE 4. Assessment for R⁺/M⁺ with pCI750

Bacterial strain	EOP ^a of phage:		
	c2-KH ^b	c2-KH-AB002 ^c	c2-KH-AB002-KH ^d
KH	1.0	1.7×10^{-5}	1.0
AB002	1.3×10^{-3}	1.0	1.5×10^{-1}
MG1614	9.0×10^{-1}	5.2×10^{-1}	1.3

^a Relative to the strain on which the phage was last propagated.^b Two subsequent single-plaque isolations on *S. cremoris* KH were carried out to ensure a homogenous phage population.^c Propagated on *S. lactis* AB002.^d Propagated on *S. cremoris* KH.

FIG. 1. Induction of lysis in *S. lactis* strains by mitomycin C. An exponentially growing culture was harvested, washed, and suspended in broth containing 0.75 μ g of mitomycin C per ml, and the change in absorbance was measured. The *S. lactis* strains were LM2201 (○), JS13 (△), and Pro16 (□). LM2201 grown in the absence of mitomycin C served as an uninduced control (●). O.D.₆₅₀, Optical density at 650 nm.

were pinpoint, and 10% were intermediate. The average plaque size of c2 · KH on the plasmid-free host MG1614 was 2.5 mm. The pinpoint- and intermediate-plaque phages were purified and examined individually. Phage stocks were obtained by picking single plaques from both types and propagating them on AB002. The resulting phages were then plated on AB002, KH, and MG1614. No differences in phage titer or plaque morphology were observed between the two phage types. Both phage stocks exhibited an intermediate plaque size on AB002, with a few pinpoint plaques being observed. The plaque morphology of the two phage stocks on KH and MG1614 was similar to that of phage c2 · KH. The two types of plaques were only observed on AB002. Expression of the intermediate plaque type on AB002 was not lost by passage of the phage stocks on KH. These experiments were conducted with single-plaque isolations throughout and were repeated four times with consistent results.

Determination of the latent period and burst size of phage c2 on AB002. In two experiments, the 30-min latent period of phage c2 on AB002 was not significantly different from that of phage c2 on MG1614. The average burst size of phage c2 on MG1614 was 75, while on AB002 it was reduced by 80% to 15. The number of infective centers for phage c2 on AB002 was about 38% of that obtained on MG1614.

Induction of prophages from transconjugants exhibiting Rbs⁺. The prophage induction of *S. lactis* LM2201 by mitomycin C is illustrated in Fig. 1. The 50-MDa plasmid,

pCI750, from *S. cremoris* UC653 was introduced into LM2201 by conjugation, and the resulting Lac⁺ Rbs⁺ transconjugant was designated *S. lactis* Pro16. Attempts to induce prophage from Pro16 resulted in incomplete lysis of the culture (Fig. 1). Cell counts on the induced lysates at 300 min indicated that both the Pro16 and LM2201 lysates contained a low number of viable cells, 1.9×10^4 and 1.8×10^4 CFU/ml, respectively. However, when the noninduced control for Pro16 was diluted to the same optical density at 650 nm as the induced lysate, the cell count was 2.5×10^7 CFU/ml. Pinpoint plaques were observed in the induced lysates for both Pro16 and LM2201; however, it was not possible to determine titers, since the plaques formed by the temperate phage were nonquantifiable (2). Attempts to induce prophage from a Suc⁺ prophage-containing transconjugant, designated JS13, yielded a curve similar to that of Pro16 (Fig. 1).

DISCUSSION

Several laboratories have linked Rbs⁺ to conjugally transferred plasmids in group N streptococci (3, 4, 14, 17, 30, 31). In this study, 11 previously reported Lac⁺ or Suc⁺ transconjugants were examined for Rbs⁺ to prolate- and small isometric-headed phages. Of 10 Lac⁺ transconjugants examined, 4 (JS30, AB001, WW4, and CC101) demonstrated a similar Rbs⁺ phenotype; small isometric phages did not form any detectable plaques, whereas prolate phages had a reduced plaque size and a reduced EOP at 21, 30, and 37°C. One Lac⁺ transconjugant (EB101) and the Suc⁺ transconjugant (JS21) expressed a different level of Rbs⁺ when compared with the four similar Lac⁺ transconjugants in that both allowed small isometric phages to plaque at a reduced plaque size and a low EOP. The Rbs⁺ phenotype of JS21 was ineffective against prolate phages.

Plasmid curing experiments with JS30, AB001, WW4, CC101, and EB101 indicated that the Rbs⁺ phenotype could be linked to the 88-MDa plasmid in WW4 and JS30, the 50-MDa plasmid (pCI750) in AB001, the 34-MDa plasmid in CC101, and the 56-MDa plasmid in EB101. Curing of the Suc⁺ phenotype from JS21 resulted in a concomitant loss of the Rbs⁺ phenotype. This involvement of plasmid DNA with Rbs⁺ should facilitate the genetic analysis of this trait.

Some of the preliminary work on AB002, the Lac⁻ derivative of AB001, was reported by Daly and Fitzgerald (6). As indicated for AB001 (3), the presence of the Rbs⁺ phenotype in AB002 did not affect phage adsorption. This phenotype was unaffected by the phage assay incubation temperature for the two small isometric-headed phages sk1 and 712 and for the prolate-headed phage stl5. However, the phage assay temperature did affect the EOP of the prolate-headed phages c2 and eb1 (Table 3). The three prolate phages (c2, stl5, and eb1) exhibited a high plaquing ability on MG1614, yet at 21°C each was affected differently by the presence of pCI750. This result suggests diversity among morphologically similar phages.

AB002 did not exhibit an R⁺/M⁺ phenotype. Phage c2 propagated from either a pinpoint or intermediate-sized plaque formed plaques at a high efficiency on AB002 after propagation on AB002. Additionally, when the phage was grown on AB002, the plaque morphology changed. These changes were maintained even after subsequent propagation of the phage on KH, suggesting that a permanent change had occurred in the phage (15). Gautier and Chopin (9) also observed two plaque types (large and pinpoint) of phage 66 in the plasmid-mediated abortive infection described for *S. lactis* IL1403. They concluded that the large plaque type

represented a mutant phage. The frequency of isolation of the intermediate-sized plaque on AB002 was higher than the expected spontaneous mutation rate. This result suggests that an event responsible for allowing the phage to overcome the Rbs⁺ phenotype may involve an insertion sequence (5). Our observations are preliminary, and further work is needed to clarify this phenomenon.

The latent period of phage c2 was not significantly altered in the presence of pCI750; however, the burst size was reduced by 80%, an effect which was similar to the effect of pTR2030 on phage c2 at 32°C (14). The number of infective centers on AB002 was only about 38% of that on the plasmid-free control. The reduced plaque size would therefore appear to result from a decreased burst size and a reduced number of infective centers.

Normal prophage induction of LM2201 was inhibited by pCI750. The presence of pCI750 did not appear to inhibit mitomycin C induction of prophage, as growth was arrested after 90 to 120 min. However, the plasmid did appear to retard lysis of the induced cells, since no significant drop in the optical density at 650 nm was observed. Some lysis occurred, since nonquantifiable pinpoint plaques were observed. In the presence of pCI750, the optical density at 650 nm remained constant following induction, possibly as a result of a high proportion of nonviable cells relative to viable and lysing cells of Pro16. This possibility was supported by the observation that the viable counts after leveling off of the induction curve for Pro16 were approximately 1,000-fold lower than those for noninduced LM2201 adjusted to the same optical density at 650 nm. The viable counts at the end of induction were similar in the strains with or without pCI750.

In contrast, Jarvis and Klaenhammer noted that the presence of pTR2030 in *S. cremoris* R1 did not affect the normal prophage induction response (11). They proposed that the mechanism of insensitivity to small isometric phages acted at the cell surface or membrane to prevent DNA penetration. This does not appear to be the case for prolate phages, since plaques are formed. Although pTR2030 (10, 14) and pCI750 appear to be similar in their effect against different phage types, these observations indicate that the actual mechanisms of Rbs⁺ may be different. Alternatively, the different induction responses may be host dependent and hence unrelated to the Rbs⁺ mechanisms.

The genetic determinant in the Suc⁺ transconjugant was ineffective against prolate-headed phages, but allowed small isometric-headed phages to plaque with a reduced plaque size and EOP. Interestingly, novel Lac⁺ cointegrate plasmids isolated from matings between AB001 and plasmid-free recipients had an identical Rbs⁺ phenotype (unpublished data). The genetic determinant(s) for Rbs⁺ in JS13 was indistinguishable from pCI750 in its effect on prophage induction from LM2201, supporting the concept that a common genetic determinant for Rbs⁺ is involved.

The data presented further support the observation that Rbs⁺ can be associated with conjugally transferred plasmids in lactic streptococci (4, 14, 17). Phenotypic evidence suggests that some of the transconjugants exhibited a similar Rbs⁺ mechanism. Duckworth et al. (7) defined an abortive infection as the inhibition of phage infection following adsorption, DNA penetration, and the early stages of the phage lytic cycle. It is normal for most of the cells to be killed during an abortive infection. In the pIL105 system, Gautier and Chopin (9) observed that 97% of the infected cells demonstrated an abortive infection. Watanabe and Okada (33) also demonstrated that during an abortive infection of

Escherichia coli K-12 F⁺ cells by phage W31, the majority of the F⁺ cells were killed by infection with W31 without producing progeny phage, while most of the F⁻ cells produced progeny phage. Strains containing pCI750 also appear to exert an abortive infection, since absorption is normal, an R⁺/M⁺ system is not involved, the majority of infecting phage do not form infective centers, and induction of prophage in the presence of pCI750 results in a high proportion of nonviable cells. The evidence for a common genetic determinant coding for Rbs⁺ is only phenotypic. We are currently attempting to clone the DNA fragment responsible for the Rbs⁺ phenotype. Isolation of that fragment would yield the DNA probe necessary to determine if a common genetic determinant is involved in the transconjugants examined.

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