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NOTES

Plasmid Transfer via Transduction from *Streptococcus thermophilus* to *Lactococcus lactis*[▽]

Andreas Ammann, Horst Neve, Arnold Geis, and Knut J. Heller*

Department of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute for Nutrition and Food, Hermann-Weigmann-Str. 1, D-24103 Kiel, Germany

Using *Streptococcus thermophilus* phages, plasmid transduction in *Lactococcus lactis* was demonstrated. The transduction frequencies were 4 orders of magnitude lower in *L. lactis* than in *S. thermophilus*. These results are the first evidence that there is phage-mediated direct transfer of DNA from *S. thermophilus* to *L. lactis*. The implications of these results for phage evolution are discussed.

Bacteriophages of *Streptococcus thermophilus* and *Lactococcus lactis*, two important starter strains in the dairy industry (5, 25), share significant sequence homology (3, 6). Therefore, horizontal gene transfer between the two genera has to be supposed. *S. thermophilus* has recently been shown to be capable of natural transformation (1), but *L. lactis* lacks natural competence for DNA uptake (29). Conjugal transfer of plasmids from *L. lactis* to *S. thermophilus* has been demonstrated (14); however, no native conjugative plasmids have been found in *S. thermophilus* yet. Transduction of several plasmids with different phages of *S. thermophilus* within this species has been shown to occur (17; A. Ammann, H. Neve, A. Geis, and K. J. Heller; unpublished); however, transduction between the two species has not been reported previously, nor have phages of one species been demonstrated to be able to infect the other species.

In this paper, we describe for the first time plasmid transduction with three *S. thermophilus* phages beyond the genus into *L. lactis*. Three virulent *cos*-type *S. thermophilus* phages were selected for this study (Table 1), which had different host ranges for three *S. thermophilus* strains (strains a10, 55n, and St11) (18, 21). Phage P1109 could infect all three hosts, while phage P53 could be propagated in strains a10 and 55n. Phage a10/J9 had a narrow host range (only strain a10). For transduction assays, plasmid pAG106AE was introduced into the plasmid-free host strains a10 and St11 either by electroporation or by transduction. This plasmid construct is based on a native *S. thermophilus* plasmid (8) that has been cloned in a charomid vector conferring erythromycin resistance (Table 1). For preparation of plasmid-transducing lysates, 0.1 to 0.3 ml of phage lysate was added at low multiplicities of infection (MOI) (between 10^{-4} and 1) to 0.1- to 0.3-ml portions of overnight

cultures of plasmid-bearing *S. thermophilus* strains in the presence of 10 mM $CaCl_2$. After 10 min of adsorption at 40°C, each mixture was diluted with 10 ml of prewarmed thM17 medium (containing 10 mM $CaCl_2$) (15). The infected cultures were incubated at 40°C aerobically without shaking until cell lysis occurred. In cases of premature lysis at low optical densities at 620 nm (OD_{620}) (OD_{620}, <0.2), the lysates were inoculated again with 1 ml of the same host culture grown to the early logarithmic phase (OD_{620}, approximately 0.5). Residual bacterial cells and cell debris were finally removed by membrane filtration (pore size, 0.45 μm).

For transduction assays with plasmid-free strains *S. thermophilus* a10 and *L. lactis* Bu2-60 (20), cultures grown in thM17 (strain a10) or in GM17 (28) (strain Bu2-60) were harvested in the logarithmic phase (OD_{620}, 0.5) and resuspended in 0.2 volume of 10 mM ice-cold $MgSO_4$ (approximately 1×10^9 CFU/ml). Phage lysates (150 μl) were added at an MOI of 0.01 (*S. thermophilus*) or 0.1 (*L. lactis*) to 300 μl of bacteria. $CaCl_2$ was added at a final concentration of 10 mM (*S. thermophilus*) or 100 mM (*L. lactis*) before the sample volume was increased to 1 ml by addition of fresh thM17 or GM17 medium. Phage adsorption was performed for 10 min at 42°C (*S. thermophilus*) or for 30 min at 30°C (*L. lactis*). Phage adsorption was stopped on ice by addition of 1 ml of ice-cold thM17 or GM17 broth containing 20 mM (*S. thermophilus*) or 200 mM (*L. lactis*) trisodium citrate. Transduction mixtures were subsequently incubated for 50 min at 42 or 30°C. All transduction assay mixtures were finally plated on GM17 agar (*L. lactis*) or thLM17 agar (*S. thermophilus*) supplemented with 10 μg/ml erythromycin. Control assays either without phage or without cells were also included.

Plasmid pAG106AE was transduced in *L. lactis* Bu2-60 using phage P1109 grown on *S. thermophilus* St11 (harboring pAG106AE) and phages P53 and a10/J9 grown on *S. thermophilus* a10 (harboring pAG106AE) (Table 1). For all phages tested, the transduction frequencies (calculated by determining the number of erythromycin-resistant CFU per added PFU) were ca. 4 orders of magnitude lower in *L. lactis* Bu2-60 than in *S. thermophilus* a10 (Table 2).

* Corresponding author. Mailing address: Department of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute for Nutrition and Food, P.O. Box 6069, D-24121 Kiel, Germany. Phone: 49 431 609 2340. Fax: 49 431 609 2306. E-mail: knut.heller @mri.bund.de.

TABLE 1. Strains, phages, and plasmid used

Strain(s), phage, or plasmid	Properties[a]	Source or reference
Strains		
S. thermophilus a10	Host for phages P1109, P53, and a10/J9	FRCNF-Kiel strain collection
S. thermophilus 55n	Host for phages P1109 and P53	FRCNF-Kiel strain collection
S. thermophilus St11	Host for phage P1109	18
L. lactis Bu2-60	Plasmid-cured derivative of *L. lactis* Bu2, Sm^r Rm^r	20
L. lactis Bu2-60-STD1, Bu2-60-STD2, and Bu2-60-STD3	Bu2-60 transductants containing pAG106AE obtained by transduction with *S. thermophilus* phage P1109, Sm^r Rm^r Em^r	This study
Phages		
P1109	Virulent *S. thermophilus* phage, *cos* type	FRCNF-Kiel phage collection
P53	Virulent *S. thermophilus* phage, *cos* type	21
a10/J9	Virulent *S. thermophilus* phage, *cos* type	21
P008	Virulent *L. lactis* phage, *cos* type, 936 species	12
Plasmid		
pAG106AE	9.2 kb, pSt106(AatII)::charomid 9-36, Δspacer and Δcos of charomid 9-36, Em^r	8

[a] Sm^r, streptomycin resistance; Rm^r, rifampin resistance; Em^r, erythromycin resistance.

L. lactis Bu2-60 transductants were easily identified and distinguished from *S. thermophilus* host cells phenotypically by their growth characteristics (e.g., no growth at 45°C; resistance to streptomycin and rifampin [20]) and by their sensitivity to lactococcal virulent phage P008 (26) (data not shown). Hence, contamination of *S. thermophilus* plasmid donor cells could be ruled out.

L. lactis has recently been shown to harbor an incomplete set of genes involved in natural competence (2). To exclude the possibility that natural transformation by contaminating plasmid DNA was the cause of the gene transfer observed, we used polyclonal phage antiserum raised against phage P53 for neutralization of the phage particles (19). Phage suspensions were mixed with the same volume of a 1:100 dilution of antibodies in phosphate-buffered saline and incubated for 10 min at room temperature before the transduction experiments were carried out. Inactivation of phage particles was determined by spotting dilutions of phage-antibody suspensions on soft agar plates inoculated with *S. thermophilus* a10. A reduction in the transduction frequency corresponded with a reduction in phage infectivity (Table 2), showing that intact phage particles and not naked DNA was responsible for plasmid transfer. As a second control, *S. thermophilus* phage lysates were replaced by 0.5 μg of intact plasmid pAG106AE DNA in the transduction assays. No transformants were detected (data not shown).

For another demonstration of the efficient transductional gene transfer of plasmid pAG106AE from *S. thermophilus* to *L. lactis*, phage P1109 was propagated in its plasmid-bearing *S. thermophilus* host strain and purified by CsCl buoyant density gradient centrifugation (24). The phage-containing fraction was used for transduction assays with the lactococcal recipient strain Bu2-60 at different MOI (0.1, 0.01, and 0.001). The number of *L. lactis* transductants obtained correlated well with the serial dilutions of the phages used (634, 41, and 5 transductants per ml), while the calculated transduction frequency remained remarkably stable (2×10^{-5} to 4×10^{-5}) (Table 3). When the purified phages were neutralized by using phage P53-specific antiserum (99.9% inactivation), no transductants were obtained (data not shown). Preincubation of these puri-

fied phages with DNase (0.2 to 1 mg/ml) did not affect the transduction frequency in *L. lactis* Bu2-60 (data not shown). DNase activity was confirmed by spiking transduction assay mixtures with purified pAG106AE DNA and then performing agarose gel electrophoresis and demonstrating hydrolysis of pAG106AE DNA (not shown).

Plasmids were extracted from recipient cells to confirm transduction events. Plasmid pAG106AE was isolated from *L. lactis* Bu2-60 transductants in a high-molecular-weight concatemeric form (Fig. 1A). This was most likely due to reduced expression in *L. lactis* of the resolvase gene pSt106, the plasmid on which the theta-replicating *S. thermophilus* plasmid pAG106AE was based (8). Concatemers were also observed when pAG106AE was transferred via electroporation (data not shown). The StyI restriction enzyme profiles of the plasmid DNA extracted from the *L. lactis* transductants Bu2-60-STD1, -STD2, and -STD3 (obtained by P1109-mediated transduction) were in most cases identical to the corresponding pattern of the plasmid extracted from the *S. thermophilus* a10 (pAG106AE) donor strain (Fig. 1B). However, we sometimes observed restriction fragments that did not fit the control profile. These fragments may have been due either to incomplete hydrolysis with StyI or to inhomogeneities in the plasmids created by incorrect resolution of concatemers. It should be noted that the plasmid was packaged into the phage as concatemers, as demonstrated by restriction analysis of packaged DNA followed by Southern blot hybridization with plasmid DNA as the probe (data not shown).

In order to elucidate the efficiency of adsorption of phages to *S. thermophilus* or *L. lactis*, we analyzed reversible and irreversible binding to the recipient cells. As has been demonstrated previously mostly for phages of gram-negative bacteria, reversible binding may considerably enhance adsorption (9) and may thus increase the efficiency of infection when there is poor irreversible binding (10). Cells were harvested from GM17 or thM17 early-log-phase broth cultures (OD_{620}, 0.5) and resuspended in 0.02 volume of 10 mM MgSO$_4$. The adsorption assays were performed as described by Østergaard Breum et al. (23) at a low MOI (0.01). After 20 min of ad-

TABLE 3. Transduction of plasmid pAG106AE in *L. lactis* Bu2-60 with *S. thermophilus* phage P1109 purified by CsCl density gradient centrifugation[a]

P1109 titer (PFU/ml)	MOI	Transductants/ml	Transduction frequency (CFU/PFU)
1.8×10^7	0.1	634 ± 10	$3.5 \times 10^{-5} \pm 0.1 \times 10^{-5}$
1.8×10^6	0.01	41 ± 3	$2.3 \times 10^{-5} \pm 0.1 \times 10^{-5}$
1.8×10^5	0.001	5 ± 4	$2.5 \times 10^{-5} \pm 1.9 \times 10^{-5}$

[a] Data from two experiments.

sorption at either 30°C (*L. lactis* assays) or 40°C (*S. thermophilus* assays), the samples were diluted 1:100 in ice-cold 0.25× Ringer buffer (supplemented with 1 M NaCl) for determination of irreversible adsorption. For determination of reversible adsorption, assay mixtures were centrifuged immediately after 20 min of adsorption, followed by dilution (1:100) of the supernatant with 0.25× Ringer buffer-1 M NaCl and determination of the number of PFU. The three *S. thermophilus* phages adsorbed to *S. thermophilus* a10 cells equally well with high efficiency (98 to 99% reversible adsorption and 96 to 98% irreversible adsorption). Furthermore, phage P1109, which exhibited the highest transduction efficiency in *L. lactis* Bu2-60 (Table 2), adsorbed significantly (46% reversible adsorption and 43% irreversible adsorption) to *L. lactis* Bu2-60, while adsorption of the two remaining phages was either not detectable or close to the limit of detection (Table 4). This correlated well with their low transduction efficiencies in *L. lactis*. The fact that there was basically no difference between reversible adsorption and irreversible adsorption indicates that binding of *S. thermophilus* phages to the *S. thermophilus* or *L. lactis* cell surface either is a single-step process with no reversible binding or is a two-step process with irreversible binding that occurs so fast that differentiation between reversible binding and irreversible binding is not possible.

In order to visualize adsorption of P1109 phage particles to *L. lactis* Bu2-60 cells, transduction assays were performed at higher MOI (approximately 1 to 10). Samples were taken for

TABLE 2. Transduction of plasmid pAG106AE in *S. thermophilus* and *L. lactis* with *S. thermophilus* phages

Phage	*S. thermophilus* host strain	Phage neutralization by P53-specific antiserum (%)[a]	*S. thermophilus* a10[b]			*L. lactis* Bu2-60[b]		
			Transduction frequency (CFU/PFU)	Transduction frequency after phage neutralization (CFU/PFU)	Reduction in transduction frequency (%)	Transduction frequency (CFU/PFU)	Transduction frequency after phage neutralization (CFU/PFU)	Reduction in transduction frequency (%)
P1109	St11(pAG106AE)	53 ± 8	$1.9 \times 10^{-2} \pm 0.6 \times 10^{-2}$	$1.4 \times 10^{-2} \pm 0.1 \times 10^{-2}$	~26	$3.4 \times 10^{-5} \pm 1.5 \times 10^{-5}$	$1.9 \times 10^{-5} \pm 0.6 \times 10^{-5}$	~44
P53	a10(pAG106AE)	79 ± 14	$6.8 \times 10^{-3} \pm 3.1 \times 10^{-3}$	$9.8 \times 10^{-4} \pm 0.3 \times 10^{-4}$	~86	$9.5 \times 10^{-6} \pm 1.6 \times 10^{-6}$	$1.4 \times 10^{-6} \pm 0.3 \times 10^{-6}$	~85
a10/J9	a10(pAG106AE)	99 ± 0	$6.3 \times 10^{-5} \pm 3.2 \times 10^{-5}$	ND ($<3.1 \times 10^{-5}$)[c]	NA[d]	$5.6 \times 10^{-8} \pm 2.6 \times 10^{-8}$	ND ($<3.2 \times 10^{-8}$)[e]	NA

[a] Data from two experiments.
[b] Data from four experiments.
[c] ND, not detectable (0.2 ml from a 2-ml transduction mixture was plated).
[d] NA, not applicable.
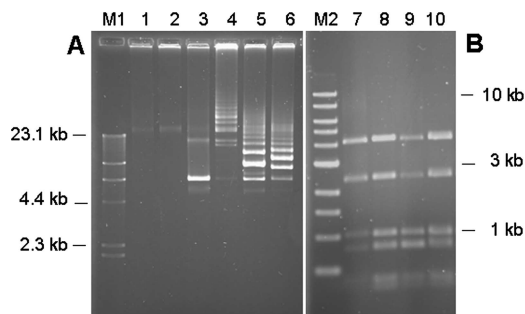[e] ND, not detectable (1 ml from a 2-ml transduction mixture was plated).

FIG. 1. (A) Agarose gel electrophoresis of intact plasmid DNA extracted from plasmid-free recipient strains *S. thermophilus* a10 (lane 1) and *L. lactis* Bu2-60 (lane 2), from *S. thermophilus* donor strain a10(pAG106AE) (lane 3), and from three *L. lactis* Bu2-60 transductants, Bu2-60-STD1, Bu2-60-STD2, and Bu2-60-STD3 (lanes 4 to 6). (B) Agarose gel electrophoresis of StyI digests of plasmid DNA extracted from *S. thermophilus* donor strain a10(pAG106AE) (lane 7) and from the three *L. lactis* Bu2-60 transductants (lanes 8 to 10). Phage lambda cut with HindIII (lane M1) and a 1-kb ladder (lane M2) were used as size references.

TABLE 4. Reversible and irreversible adsorption of *S. thermophilus* phages to *S. thermophilus* a10 and *L. lactis* Bu2-60 cells at a low MOI $(0.01)^a$

Strain	Adsorption of *S. thermophilus* phages (%)					
	P1109		P53		a10/J9	
	Reversible	Irreversible	Reversible	Irreversible	Reversible	Irreversible
S. thermophilus a10[b]	99	98	99	98	98	96
L. lactis subsp. *lactis* Bu2-60[c]	46 ± 2	43 ± 4	ND[d]	4.1 ± 0.2	2.8 ± 0.2	4.0 ± 0.9

[a] Adsorption was performed for 20 min at 30°C (*L. lactis*) or at 40°C (*S. thermophilus*).
[b] Data from one experiment.
[c] Means ± standard deviations for two experiments.
[d] ND, not detectable.

negative staining with 1% (wt/vol) uranyl acetate and analyzed with a Tecnai 10 transmission electron microscope (FEI Company, Eindhoven, The Netherlands) as described previously (23, 26). Figure 2 shows specific adsorption of intact *S. thermophilus* P1109 phage particles (Fig. 2a and c) and of a phage ghost (Fig. 2b) to the *L. lactis* Bu2-60 cell surface; no free phages were detected in the space between cells. Most of the adsorbed phage particles analyzed appeared to be intact, indicating that DNA injection had not occurred yet. It is notable that adsorption did not occur randomly but occurred preferably at regions involved in cell division.

Our experiments provide evidence that plasmids can be transferred via transduction from *S. thermophilus* to *L. lactis*. We demonstrate that the *S. thermophilus* phages tested are able to adsorb to *L. lactis* cells and to inject their DNA into these cells, albeit at a low frequency. So far, no *S. thermophilus* phage able to propagate on *L. lactis* has been described. Our experiments indicate that the failure of *S. thermophilus* phages to propagate on *L. lactis* may be due to their low efficiency of adsorption, as shown for phages P53 and a10/J9 (Table 3). If we assume, on the basis of the reduced transduction efficiency, that the adsorption rate is 10^3-fold lower for *L. lactis* than for *S. thermophilus*, this would (for a phage producing approximately 10^2 progeny phage per infected cell, as is the case for the *S. thermophilus* phages described here) result in the failure of the phage to form plaques. However, we cannot rule out the possibility that, in addition, intracellular multiplication proceeds inefficiently, if at all. An in-depth analysis of host range mutants of two *S. thermophilus* phages revealed previously that

at least three structural tail proteins may be involved in the phage-host interactions in *S. thermophilus* (7). The mutants were obtained at a low frequency (10^{-6}), and host range extensions were shown to be due to single nucleotide mutations in the structural genes. Host range phage mutants had expanded host ranges and were still able to adsorb to the original host cells. On the other hand, two prophages of *L. lactis* IL-1403 (phages bIL286 and bIL309) possess structural proteins with high levels of similarity to proteins of *S. thermophilus* phages (portal protein, capsid protein, tail protein, tail host specificity protein). These data also indicate that *S. thermophilus* phages can interact with the surface of lactococcal cells.

The high levels of similarity of genes of *S. thermophilus* and *L. lactis* phages support the hypothesis that horizontal gene transfer occurs between these species belonging to two different genera (4). Exchange of genetic modules between *S. thermophilus* and *L. lactis* phages appears to be one of the motors of the evolution of these organisms (16, 22). In principle, recombination between two phage genomes can occur by two mechanisms following phage infection: (i) recombination may take place between the genome of an infecting phage and a prophage residing in the genome of the infected cell; and (ii) recombination may take place between the genomes of two phages simultaneously infecting a host cell. Since we have shown that transduction by *S. thermophilus* phages occurs in *L. lactis*, this implies that at least for the *S. thermophilus* phages, which are capable of transduction in *L. lactis*, infection of *L. lactis* is possible. Thus, recombination and exchange of genetic modules by either of the two mechanisms indicated above
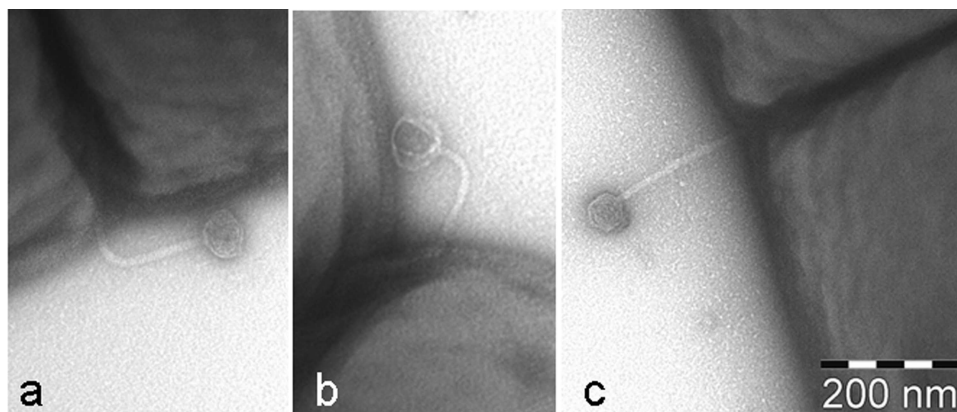

FIG. 2. Transmission electron micrographs of *S. thermophilus* P1109 phage particles adsorbed to *L. lactis* Bu2-60 cells.

appear to be a realistic option. As a consequence, a mixture of recombined lactococcal and streptococcal progeny phages (with respect to host specificity) is produced, which carry newly acquired genetic modules. The phages with *S. thermophilus* host specificity are able to transfer the newly acquired genetic modules back into *S. thermophilus*. It remains to be determined whether genetic exchange involving *L. lactis* phages and *S. thermophilus* recipient cells is also possible. Comparison of the genome sequences of two *S. thermophilus* yoghurt strains clearly showed that their genomes contain more than 100 "nonstreptococcal" genes potentially acquired by horizontal gene transfer. Approximately 30 of these genes exhibit the highest levels of similarity to *L. lactis* genes (11). It is also notable that a number of these *L. lactis*-related genes code for putative membrane proteins. Two findings have indicated that phage-related traits are functional in both genera. First, the AbiA lactococcal abortive infection mechanism, when introduced into an *S. thermophilus* host, has been shown to be effective at 30°C in *S. thermophilus* cells against six *S. thermophilus* phages. As observed with lactococcal phages, AbiA affected *S. thermophilus* phages by also interfering with DNA replication (27). Second, the *S. thermophilus* TP-J34 phage protein Ltp, involved in superinfection exclusion, has been shown to be functionally active in *L. lactis* against phage of this host. In both hosts, Ltp interfered with the process of DNA injection (26).

In conclusion, we present for the first time evidence that phage-mediated horizontal gene transfer occurs in gram-positive dairy starter cultures from *S. thermophilus* to *L. lactis*. To our knowledge, horizontal transductional gene transfer by broad-host-range phages infecting different species has been demonstrated previously only for gram-negative phages (13). Our results support the hypothesis that some *S. thermophilus* and *L. lactis* bacteriophages have evolved by direct exchange of genetic modules.

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