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NOTES

Conjugal Plasmid Transfer (pAMβ1) in *Lactobacillus plantarum*†

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The streptococcal plasmid pAMβ1 (erythromycin resistance) was transferred via conjugation from *Streptococcus faecalis* to *Lactobacillus plantarum* and was transferred among *L. plantarum* strains. *Streptococcus sanguis* Challis was transformed with pAMβ1 isolated from these transconjugants, and transformants harboring intact pAMβ1 could conjugate the plasmid back to *L. plantarum*.

Lactobacillus plantarum is commonly used as a starter culture in the manufacture and preservation of many fermented food products (10). Because of its industrial importance as a starter culture, there exists considerable interest in rendering *L. plantarum* amenable to genetic technology. However, very little is known about genetic transfer mechanisms in lactobacilli. The conjugal streptococcal plasmid pAMβ1 (3) was chosen for preliminary studies on conjugation in *L. plantarum* because it is reported to have a broad host range in five genera of gram-positive bacteria, including lactobacilli (5–7, 9, 11, 12, 14, 16–20). However, no evidence has been presented that pAMβ1 in these *Lactobacillus* transconjugants retained its conjugative ability for intrageneric transfers once the plasmid was introduced into these strains.

The initial donor in this study was *Streptococcus faecalis* JH2-2, which harbors the 17-megadalton erythromycin resistance plasmid pAMβ1. *L. plantarum* recipient strains for pAMβ1 include NC1, NC4, NC5, and NC8. All *L. plantarum* strains harbor cryptic plasmids except NC8, which is plasmidless. *Streptococcus sanguis* Challis was used in transformations. All strains were transferred monthly to LCM (4) broth containing 20 mM glucose (LCMG) and were stored at 4°C. Overnight cultures grown in LCMG broth were mixed in a 1:10 donor-to-recipient ratio. The cells were collected onto a filter with a vacuum, transferred to an LCMG agar plate, and incubated for 24 h at 37°C. Total counts of donor and recipient cells were determined on LCMG plates. The cells were washed off the filter with saline, and the suspension was plated onto a selective medium. DNase was incorporated in some filter matings to determine whether transformation was the mechanism of gene transfer. For this purpose, the procedure of Orzech and Burke (17) was used. The procedure of Gonzalez and Kunka (8) was used to determine whether transduction was involved in DNA transfer.

In the matings between *Streptococcus* and *Lactobacillus* spp., erythromycin-resistant (Em^r) lactobacilli were selected on *Lactobacillus*-selective medium (LBS) containing 50 μg of erythromycin per ml. The formula for LBS is as follows (grams per liter unless otherwise noted): Trypticase Peptone (BBL Microbiology Systems, Cockeysville, Md.), 10.0; yeast extract (Difco Laboratories, Detroit, Mich.), 5.0; monobasic potassium phosphate, 6.0; ammonium citrate, 2.0; Tween 80, 1 ml; sodium acetate (trihydrate), 34.0; glucose, 10.0; $MgSO_4 \cdot 7H_2O$, 1.2; $MnSO_4 \cdot H_2O$, 0.13; $FeSO_4 \cdot 7H_2O$, 0.6. The pH was adjusted to 5.5. Erythromycin was added to a final concentration of 50 μg/ml. The transconjugants isolated from intrageneric matings were selected on LCM containing 50 μg of erythromycin per ml. Selection of transconjugants was based on the capacity of recipients to ferment melezitose or their spontaneous resistance to 3 mg of streptomycin per ml (Sm^r). Melezitose fermentation was a property of selected recipient cells and not selected donor cells.

Transformations of *S. sanguis* Challis with plasmid DNA were performed as described by LeBlanc and Lee (13). *Lactobacillus* and *Streptococcus* plasmids were isolated by the method of Chassy et al. (1, 2). Plasmid content was analyzed by agarose gel electrophoresis. DNA from agarose gels was transferred to and hybridized on GeneScreen (New England Nuclear Corp., Boston, Mass.) membrane as directed by the manufacturer. Plasmid pVA1, a deletion mutant of pAMβ1 (15), was labeled with ^{32}P with a nick translation kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as directed by the manufacturer and was used as the probe for hybridization.

The results of these conjugations are presented in Table 1. Neither transformation nor transduction played a role in these transfers. DNA-DNA hybridization confirmed that these Em^r lactobacilli were indeed transconjugants (Fig. 1). ^{32}P-labeled pVA1 was used as a probe to hybridize the DNA isolated from *L. plantarum* NC1, NC1-C1, NC5, and NC5-C1. The results of this study (Fig. 1) show that the transconjugant strains contained plasmid DNA homologous to pVA1 (Fig. 1B, lanes b and d). The recipient strains exhibited no homology to pVA1 (Fig. 1B, lanes a and c). The plasmid profiles of these transconjugants were stable, and the strains retained their Em^r phenotype after growth in antibiotic-free LCMG broth. *L. plantarum* transconjugants were able to transfer pAMβ1 to other *L. plantarum* strains by conjugation (Table 1). Acquisition of pAMβ1 in these recipients was confirmed by agarose gel electrophoresis (data not shown). To further verify that pAMβ1 was transferred intact into *L. plantarum*, pAMβ1 was isolated from *L. plantarum* NC1-C1, Nc4-C1, and NC5-C1 and used to trans-

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TABLE 1. Inter- and intrageneric transfer frequencies of pAMβ1

Lactobacillus transconjugant strain	Cross	Selection medium	Transfer frequency[a]
NCl-Cl	*S. faecalis* JH2-2 × *L. plantarum* NCl	LBS-50[b]	1.5×10^{-7}
NC4-Cl	*S. faecalis* JH2-2 × *L. plantarum* NC4	LBS-50	1.1×10^{-7}
NC5-Cl	*S. faecalis* JH2-2 × *L. plantarum* NC5	LBS-50	2.0×10^{-7}
NC8-Cl	*S. faecalis* JH2-2 × *L. plantarum* NC8	LBS-50	4.5×10^{-8}
NCl-C3	*S. sanguis* Em^r transformant × *L. plantarum* NCl	LBS-50	1.4×10^{-7}
NCl-C5	NC5-Cl × *L. plantarum* NCl	Em^r Mel+[c]	7.5×10^{-9}
NC4-C2	NCl-Cl × *L. plantarum* NC4	Em^r Sm^r[d]	4.4×10^{-8}

[a] Transfer frequency is expressed as CFU/milliliter of transconjugant cells per CFU/milliliter of donor cells.
[b] LBS-50, LBS medium containing 50 μg of erythromycin per ml.
[c] Selection was made on LCM containing melezitose (Mel) and 50 μg of erythromycin per ml. +, Fermentation of Mel.
[d] Selection was made on LCMG containing 50 μg of erythromycin per ml and 3 mg of streptomycin per ml.

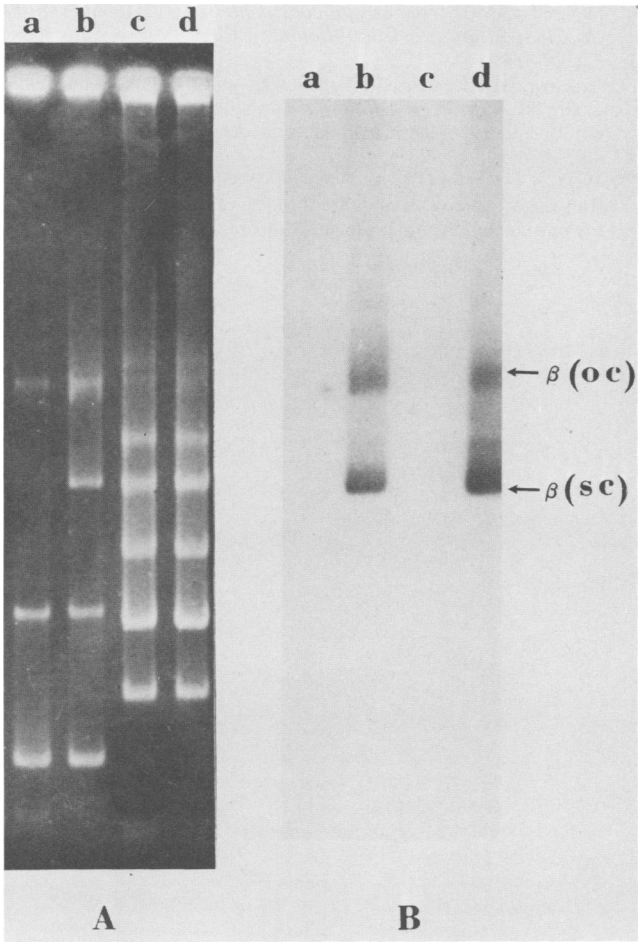

FIG. 1. Southern blot hybridization analysis of transconjugant *L. plantarum* strains by using ^{32}P-labeled pVA1 as a probe. (A) Agarose gel electrophoresis of the following donor, recipient, and Em^r transconjugant strains: lane a, *L. plantarum* NC1, recipient; lane b, *L. plantarum* NC1-Cl, transconjugant; lane c, *L. plantarum* NC5, recipient; lane d, *L. plantarum* NC5-C1, transconjugant. (B) Corresponding autoradiogram after transfer of the DNAs to a membrane and hybridization to the pVA1 probe. All transconjugant strains (lanes b and d) show homology to the probe, whereas the recipient strains (lanes a and c) show none. pAMβ1 (β) is present in these transconjugant samples as supercoiled (sc) and open circular (oc) forms.

form competent *S. sanguis* Challis cells. Em^r *S. sanguis* Challis transformants containing intact pAMβ1 were used as donors of pAMβ1 into *L. plantarum* via conjugation (Table 1). Conjugation was confirmed by agarose gel electrophoresis and DNA-DNA hybridization (data not shown).

These data show, as was previously reported for *Lactobacillus casei* (7), *Lactobacillus acidophilus*, *Lactobacillus reuteri*, and *Lactobacillus salivarius* (19), that pAMβ1 could be transferred by a conjugal process from *Streptococcus* spp. into *Lactobacillus* spp. During the preparation of this manuscript, West and Warner (20) reported the transfer of pAMβ1 into *L. plantarum* from streptococci. We confirmed their results with our data on intergeneric transfers. The observed frequency of transfer from *Streptococcus* spp. to *L. plantarum* was low (ca. 10^{-7}). However, we were able to demonstrate that *L. plantarum* carrying pAMβ1 stably maintained the plasmid, even with nonselective conditions. This observation illustrates the potential use of pAMβ1 as a cloning vector. We were also able to show, for the first time, that *L. plantarum* strains harboring pAMβ1 could transfer intact pAMβ1 to other *L. plantarum* strains. West and Warner (20) were not able to demonstrate this event in their *L. plantarum* transconjugants. The reasons for the low frequencies are difficult to assess. We evaluated the possibility that the environmental conditions for conjugal exchange were not optimal. Some variables tested to observe whether transfer could occur at higher frequency included (i) the age of the cells before mating, (ii) the dilution of cells in fresh broth before mating, (iii) anaerobiosis, and (iv) several donor-to-recipient ratios, such as 10:1, 1:1, 1:2, 1:20, and 1:50. Conditions i to iii did not alter the frequency (data not shown); condition iv produced no transconjugants. We believe that the DNA transfer cycle presented here (conjugation → transformation → conjugation) will be useful in future studies on *Lactobacillus* genetics. We are currently optimizing the transfer frequencies and developing plasmid vectors to use in the cycle presented in this communication.

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