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Efficient Screening Methods for Glucosyltransferase Genes in *Lactobacillus* Strains

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Limited information is available about homopolysaccharide synthesis in the genus *Lactobacillus*. Using efficient screening techniques, extracellular glucosyltransferase (GTF) enzyme activity, resulting in α-glucan synthesis from sucrose, was detected in various lactobacilli. PCR with degenerate primers based on homologous boxes of known glucosyltransferase (*gtf*) genes of lactic acid bacteria strains allowed cloning of fragments of 10 putative *gtf* genes from eight different glucan producing *Lactobacillus* strains (five *Lactobacillus reuteri* strains, one *Lactobacillus fermentum* strain, one *Lactobacillus sake* strain and one *Lactobacillus parabuchneri* strain). Sequence analysis revealed that these lactobacilli possess a large variation of (putative) *gtf* genes, similar to what has been observed for *Leuconostoc* and *Streptococcus* strains. Homologs of GTFA of *Lb. reuteri* 121 (synthesizing reuteran, a unique glucan with α-(1→4) and α-(1→6) glycosidic bonds) (Kralj *et al.*, 2002) were found in three of the four other *Lb. reuteri* strains tested. The other *Lactobacillus* GTF fragments showed the highest similarity with GTF enzymes of *Leuconostoc* spp.

Keywords: Glucosyltransferase; *Lactobacillus*; Glucansucrase; Sucrose; Glucan; Polysaccharide

INTRODUCTION

Various lactic acid bacteria employ large extracellular enzymes, glucosyltransferases (GTFs, EC 2.4.1.5, commonly named glucansucrases), for the synthesis of high molecular weight α-glucans from sucrose. The GTF enzymes of oral streptococci and the dextran- and alternansucrases from *Leuconostoc mesenteroides* strains have been studied in most detail. All GTFs from lactic acid bacteria share a common structure and are composed of four distinct domains (Fig. 1): their N-terminal end starts with (i) a signal peptide of, followed by (ii) a highly variable stretch, (iii) a highly conserved catalytic or sucrose binding domain of about 1,000 amino acids (Fig. 2), and (iv) a C-terminal glucan binding domain, composed of a series of tandem repeats (Monchois *et al.*, 1999).

There are only a few reports about α-glucan synthesis in lactobacilli (Dunican *et al.*, 1963; Hammond 1969; Sidebotham, 1974; Van Geel-Schutten *et al.*, 1998, 1999, 2001, 2002a,b, 2003). Only the GTFA enzyme responsible for reuteran (a glucan with α-(1→4) and α-(1→6) glycosidic bonds) synthesis in *Lactobacillus reuteri* strain 121 has been subjected to biochemical and molecular characterization (Kralj *et al.*, 2002).

This paper describes the cloning, identification and characterization of (parts of) 10 *gtf* genes from various lactobacilli. The data show that a diversity of *gtf* genes is present in the genus *Lactobacillus*, as is the case in other genera of lactic acid bacteria (*Leuconostoc* and *Streptococcus*).

MATERIALS AND METHODS

Bacterial Strains, Plasmids, Media and Growth Conditions

Strains previously identified as homopolysaccharide or heteropolysaccharide producers (van Geel-Schutten *et al.*, 1998; unpublished information), *Lactobacillus reuteri* 121 (LB 121), *Lactobacillus reuteri* 180 (LB 180), *Lactobacillus reuteri* ML1 (LB ML1), *Lactobacillus reuteri* 104R (LB 104R), *Lactobacillus fermentum* Kg3 (LB Kg3), *Lactobacillus sake* Kg15 (LB Kg15), *Lactoba-*

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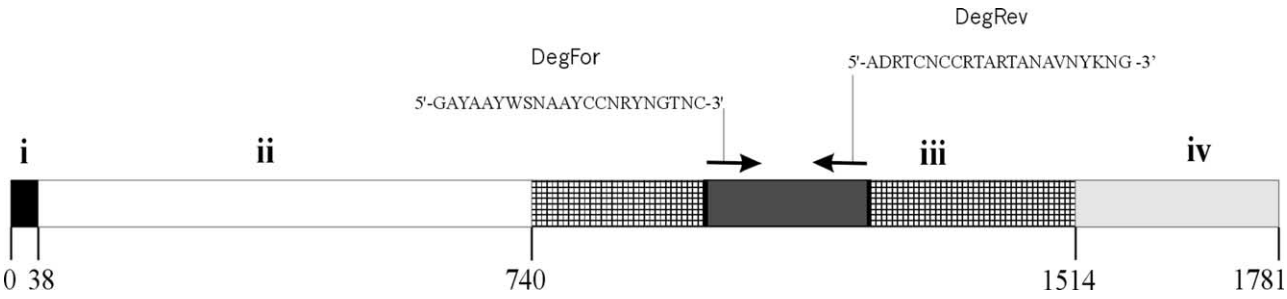

FIGURE 1 Degenerate primer sequences and their annealing sites in the catalytic domain of GTFA of *Lb. reuteri* 121. The four different domains shown are: i, N-terminal signal sequence; ii, variable region; iii, catalytic domain; iv, C-terminal (putative) glucan binding domain.

cillus parabuchneri 33 (LB 33), *Leuconostoc citreum* 86 (LN 86), *Lactobacillus* sp. 181 (LB 181), *Lactobacillus* sp. 182 (LB 182), were obtained from the culture collection of TNO Nutrition and Food Research, Zeist, The Netherlands. The taxonomic position of the various glucan producing strains was identified by 16sRNA analysis (Gendika, Veendam, The Netherlands).

Lactobacillus plantarum WCFS1 (LB WCFS1), with a recently completed genome sequence (Kleerebezem *et al.,* 2003), was obtained from the Wageningen Centre for Food Sciences (WCFS, Wageningen, The Netherlands). The *Lactobacillus reuteri* type strain DSM 20016 (LB DSM) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). All

Bacterial Strain	Main α-linkages in glucan polymer	Enzymes		I		II		III
(A)								
				⇓ ∇∇		⇓∇∇∇		♦⇓
S. mutans GS5	1→3	GTFB	443	ANFDSIRVDAVDNVDADLLQI	484	HLSILEAWSDNDTPY	555	YSFIRAHDSEVQDLI
S. mutans GS5	1→6	GTFD	457	ANFDGVRVDAVDNVNADLLQI	498	HLSILEAWSDNDPQY	577	YIFIRAHDSEVQTVI
S. downei MFe28	1→3	GTFI	404	ANFDSIRVDAVDNVDADLLQI	445	HVSIVEAWSDNDTPY	486	YSFARAHDSEVQDLI
S. salivarius ATCC 25975	1→3	GTFJ	463	ANFDGIRVDAVDNVDADMLQL	504	HISVLEAWSLNDNHY	605	YVFIRAHDNNVQDII
S. salivarius ATCC 25975	1→6	GTFK	453	AHFDGIRVDAVDNVSVDMLQL	494	NISILEAWSHNDPYY	575	YLFVRAHDSEVQTVI
S. downei MFe28	1→6	GTFS	388	ANFDGVRVDAVDNVNADLLQI	429	HLSILEAWSGNDNDY	470	YVFIRAHDSEVQTRI
Ln. mesenteroides NRRL B-1299	1→6	DSRB	484	ANFDGIRVDAVDNVDADLLQI	525	HLSILEDWSHNDPEY	566	YSFVRAHDSEVQTVI
Ln. mesenteroides NRRL B- 512F	1→6	DSRS	502	ANFDGIRVDAVDNVDADLLQI	543	HLSILEDWSHNDPLY	584	YSFVRAHDSEVQTVI
Ln. mesenteroides NRRL B-1355	1→6 / 1→3	ASR	585	ANFDGIRVDAVDNVDADLLKI	626	HLSILEDWNGKDPQY	667	YSFVRAHDYDAQDPI
Ln. mesenteroides NRRL B-1299	1→6	DSRE CD1	519	ANFDGYRVDAVDNVDADLLQI	560	HISILEDWDNNDSAY	631	YAFIRAHDSEVQTVI
Ln. mesenteroides NRRL B-1299	1→2	DSRE CD2	2202	ANFDSIRIDAVDFIHNDTIQR	2243	HISLVEAGLDAGTST	2315	YSIIHAHDKGVQEKV
(B)								
Lb. reuteri 121	1→4 / 1→6	GTFA	1016	ANFDSVRVDAPDNIDADLMNI	1056	HINILEDWNHADPEY	1126	YSFVRAHDNNSQDQI
Lb. reuteri 104R	ND	GTF104R		ANFDGIRVDAVDNVDVDLLSI		HINILEDWGWDDPAY		YNFVRAHDSNAQDQI
Lb. reuteri 180	ND	GTF180		ANFDGIRVDAVDNVDVDLLSI		HINILEDWGWDDPAY		YNFVRAHDSNAQDQI
Lb. reuteri ML1	ND	GTFML1		ANFDSIRVDAVDNVDADLLDI		HINILEDWGGQDPYY		YSFIRAHDNGSQDDI
Lb. parabuchneri 33	ND	GTF33		ANFDGYRVDAVDNVDADLLNI		HLSILEDWDNNDPAY		YTFIRAHDSEVQTII
Lb. sake Kg15	ND	GTFKg15		ANFDSVRVDAVDNVDADLLNI		HLSILEDWGHNDPLY		YSFVRAHDSEVQTVI
Lb. fermentum Kg3	ND	GTFKg3		ANFDAIRIDAVDNVDADLLQL		HLSILEDWSHNDPAY		YSFVRAHDSEVQTVI
Lb. reuteri ML1	ND	GTFML4		GNFDGFRVDAADNIDADVLDQ		HLSYNEGYHSGAAQM		WSFVTNHDQR-KNLI
Lb. reuteri 121	ND	GTFB		DNFDGFRVDAADNIDADVLDQ		HLSYNEGYHSGAAQM		WSFVTNHDQR-KNLI
Lb. reuteri DSM 20016	ND	GTFDSM		GNFDGFRVDAADNIDADVLDQ		HLVYNEGYHSGAARM		WSFVTNHDQR-KNVI
(C)								
Ln. citreum 86	ND	GTF86-1		ANFDEIRVDAVDNVDADLLQI		HLSILEDWSHNDPEY		YSFVRAHDSEVQTVI
Ln. citreum 86	ND	GTF86-5		ANFDSIRVDAVDNVDADLLDI		HISILEDWSGLDPNE		YSFVRAHDSEVQGII
Ln. citreum 86	ND	GTF86-8		ANFDGYRVDAVDNVDADLLQI		HISILEDWDNNDSAY		YAFIRAHDSEVQTVI
				:** *:** * : * :.		:: *		: : ** : :

FIGURE 2 Amino acid sequence alignment of highly conserved stretches (I, II, III) in catalytic domains of glucosyltransferases of lactic acid bacteria (see also Bozonnet *et al.,* 2002; Monchois *et al.,* 1999). A: Published sequences of dextran-, mutan- and alternansucrases of *Leuconostoc* and *Streptococcus* strains: GTFB, *S. mutans* GS5 (Shiroza *et al.,* 1987); GTFD, *S. mutans* GS5 (Honda *et al.,* 1990); GTFI, *S. downei* Mfe28 (Ferretti *et al.,* 1987); GTFJ, *S. salivarius* ATCC 25975 (Giffard *et al.,* 1991); GTFK, *S. salivarius* ATCC 25975 (Giffard *et al.,* 1993); GTFS, *S. downei* MFe28 (Gilmore *et al.,* 1990); DSRB, *Ln. mesenteroides* NRRL B-1299 (Monchois *et al.,* 1998); DSRS, *Ln. mesenteroides* NRRL B-512F (Monchois *et al.,* 1997); ASR, *Ln. mesenteroides* NRRL B-1355 (Arguello-Morales *et al.,* 2000); DSRE CD1 and CD2, *Ln. mesenteroides* NRRL-B1299 (Bozonnet *et al.,* 2002); B: Sequences of *Lactobacillus* glucosyltransferases, previously published or determined in this study: GTFA, *Lb. reuteri* 121 (Kralj *et al.,* 2002); GTFB, *Lb. reuteri* 121; GTF104R, *Lb. reuteri* 104R; GTF180, *Lb. reuteri* 180; GTFML1, *Lb. reuteri* ML1; GTF33, *Lb. parabuchneri* 33; GTFKg15, *Lb. sake* Kg15; GTFKg3, *Lb. fermentum* Kg3; GTFML4, *Lb. reuteri* ML1; C: Sequences of *Leuconostoc citreum* 86 glucosyltransferases determined in this study: GTF86-1; GTF86-5; GTF86-8. -, sequence gap; ⇓ , GTFDSM, *Lb. Reuteri* DSM80016; putative catalytic residue; ∇, residue possibly playing a role in binding of acceptor molecules and in the transfer of the glucosyl residue; ♦, putative residue stabilizing the transition state; ND, not determined.

strains were cultivated anaerobically at 37°C in MRS medium (Difco, Franklin Lakes, NJ) (De Man *et al.,* 1960) or in MRS-s medium (MRS-medium with 100 g l^{-1} sucrose instead of 20 g l^{-1} glucose). *Escherichia coli* DH5α (Phabagen, Utrecht, The Netherlands) (Hanahan 1983), and *E. coli* TOP 10 (Invitrogen, Carlsbad, USA) were used as hosts for cloning purposes. Plasmid PCR-XL-TOPO (Invitrogen) was used for cloning purposes. *E. coli* strains were grown aerobically at 37°C in LB medium (Ausubel *et al.,* 1987). *E. coli* strains containing recombinant plasmids were cultivated in LB medium with the appropriate antibiotic (50 μg ml^{-1} kanamycin). Agar plates were made by adding 1.5% agar to the LB medium.

Isolation of DNA

Total DNA was isolated according to Nagy *et al.* (1995), from MRS grown cells. Plasmid DNA of *E. coli* was isolated using a Wizard Plus SV plasmid extraction kit, according to the instructions of the manufacturer (Promega, Madison, WI).

Molecular Techniques

General procedures for cloning, *E. coli* DNA transformation, DNA manipulations, and agarose gel electrophoresis were as described (Sambrook *et al.,* 1989). Restriction endonuclease digestions were performed as recommended by the enzyme suppliers (New England Biolabs, Beverly, MA; Roche Biochemicals, Basel, Switzerland). Primers were obtained from Eurogentec (Seraing, Belgium). Sequencing was performed by GATC (Konstanz, Germany). DNA was amplified by PCR on a DNA Thermal Cycler PTC-200 (MJ Research, Waltham, Massachusetts) using Expand High Fidelity DNA polymerase (Roche Biochemicals). Fragments were isolated from agarose gels using a Qiagen gel extraction kit (Qiagen, Hilden, Germany) following the instructions of the supplier.

Identification and Nucleotide Sequence Analysis of the Glucosyltransferase (*gtf*) Genes

The *Lactobacillus gtf* genes were isolated by PCR amplification of chromosomal DNA from the different strains, using degenerate primers (DegFor 5′-GAYAAYWSNAAYCCNRYNGTNC-3′ and DegRev 5′-ADRTCNCCRTARTANAVNYKNG-3′; Y = T or C, K = G or T, W = A or T, S = C or G, R = A or G, N = inosine), based on conserved amino acid sequences present in the catalytic core (Fig. 1), deduced from the *gtf* genes of *Lb. reuteri* (*gtfA*), *Streptococcus downei* (*gtfS*), *Streptococcus mutans* (*gtfC*), *Streptococcus downei* (*gtfI*), *Streptococcus salivarius* (*gtfK* and *gtfM)*, and *dsrA* of *Leuconostoc mesenteroides* (Ferretti *et al.,* 1987; Giffard *et al.,* 1993; Gilmore *et al.,* 1990; Kralj *et al.,* 2002; Monchois *et al.,* 1996; Simpson *et al.,* 1995b; Ueda *et al.,* 1988;).

The PCR conditions for the amplification of the glucosyltransferase genes from the different bacterial strains were as follows: about 100 ng purified DNA as template, 125 pmol of each primer, 2mM dNTP, 10 × reaction buffer, 4.5 mM $MgCl_2$, 0.7 U Expand High Fidelity DNA polymerase (Roche Biochemicals), were used in the final reaction of 25 μl. The PCR reaction involved a denaturation step (95°C, 5 min), followed by denaturation (95°C, 30 s), annealing (42°C, 45 s) and elongation (72°C, 1 min), for a total of 35 cycles, and a final elongation step (72°C, 2 min).

Amplification products of *Lactobacillus* DNA with the expected size of about 660 bp were either directly sequenced, or ligated into pCR-XL-TOPO (Invitrogen) and transformed to *E. coli* TOP 10. From 10 random clones, plasmid DNA was isolated and analyzed by restriction using *Eco*RI and *Nsi*I. Subsequently five of the 10 clones (from each transformation) were sequenced. Determination of the different nucleotide sequences (GATC, Germany) and analysis confirmed *gtf* gene identities.

Dendrogram Construction

Amino acid sequences (∼ 200 amino acids of the catalytic core) were aligned with Clustal W 1.74 (Higgins *et al.,* 1988) with a gap opening penalty of 30 and a gap extension penalty of 0.5. Amino acid sequences were obtained from GenBank: DSRB of *Ln. mesenteroides* NRRL B-1299 (AAB95453), DSRS of *Ln. mesenteroides* NRRL B-512F (AAA53749), DSRE of *Ln. mesenteroides* NRRL B-1299 (AJ430204), GTFA of *Lb. reuteri* strain 121 (AX306822), ASR of *Ln. mesenteroides* NRRL B-1355 (CAB65910), GTFB of *S. mutans* GS5 (AAA88588), GTFS of *S. downei* Mfe28 (AAA26898), GTFK of *S. salivarius* ATCC 25975 (CAA77898), GTFI of *S. downei* Mfe28 (BAA0296), GTFJ of *S. salivarius* ATCC 25975 (CAA77900). The other sequences used were obtained during this study. Tree construction was performed using TreeCon 1.3b (no correction for distance estimation, 100 bootstrap samples, using the neighbor joining algorithm; Van de Peer *et al.,* 1994).

Activity Staining of *Lactobacillus* GTF Eenzymes

Aliquots of MRS-sucrose media (10 ml) were inoculated with 200 μl of overnight cultures of *Lactobacillus* strains (LB DSM, LB 121, LB 104R, LB ML1, LB 180, LB 33, LB Kg3, LB Kg15, LB 181, LB 182, LB WCFS1) or *Ln. citreum* 86, and incubated at 37°C for 8 h. Cells were removed by centrifugation 10,000 ×

g, and proteins in the supernatants were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (see below). After SDS-PAGE the gel was washed three times (1 min) with demineralized water and incubated overnight at 37°C in a 50 mM sodium acetate buffer, pH 5.5, containing 1% sucrose, 1% Tween 80 and 1 mM $CaCl_2$. Glucosyltransferase activity was detected by staining the gels for glucans produced with periodate Schiff stain (PAS) as previously described (Van Geel-Schutten *et al.,* 1999).

Gel Electrophoresis

SDS-PAGE was performed according to Laemmli (1970) using the Mini-PROTEAN II system (Biorad, Veenendaal, The Netherlands), with 7.5% polyacrylamide gels. After GTF activity staining, gels were stained for proteins with Coomassie BioSafe (BioRad). A High Molecular Weight marker was used as standard (Amersham Pharmacia Biotech, Piscataway, NJ).

RESULTS AND DISCUSSION

Screening for GTF Positive *Lactobacillus* Strains

Supernatants from 11 different *Lactobacillus* strains, LB DSM, LB 121, LB ML1, LB 104R, LB 180, LB 33, LB Kg3, LB Kg15, LB 181, LB 182 and LB WCFS1, plus a single *Leuconostoc* strain (LN 86), were loaded on SDS-PAGE. After incubation in sucrose buffer, GTF protein activity bands were identified by PAS staining of the glucans produced (Van Geel-Schutten *et al.,* 1999). With the exception of LB WCFS1 (of which the genome sequence does not encode glucansucrase genes (Kleerebezem *et al.,* 2003)), and LB 181 and LB 182 (previously identified as heteropolysaccharide producers (Van Geel-Schutten *et al.,* 1998)), all strains were positive, showing one or more activity bands at approximately 180 KDa (Fig. 3).

Isolation and Nucleotide Sequence Analysis of Parts of the Putative *Lactobacillus gtf* Genes

Based on sequence similarity between conserved regions, located in the catalytic core of different *gtf* genes of lactic acid bacteria, degenerate primers were designed and used for PCR with chromosomal DNA of the 12 different strains as template. Except for strains LB 181, LB 182 and LB WCFS1, fragments of approximately 660 bp were obtained with all strains tested (Fig. 4). PCR products obtained with LB Kg3 and LB Kg15 chromosomal DNA as template were sequenced directly, using the same degenerate PCR primers. Sequencing of these two PCR products showed in both cases the presence of only one product. The PCR products from the other seven positive strains (LB DSM, LB 121, LB 104R, LB ML1, LB 180, LB 33, and LN 86) were first ligated in pCR-XL-TOPO (Invitrogen). Subsequently, the seven different ligation mixtures were transformed to *E. coli* TOP10. Plasmid DNA was isolated from ten random clones from each transformation. Several *Streptococ-*

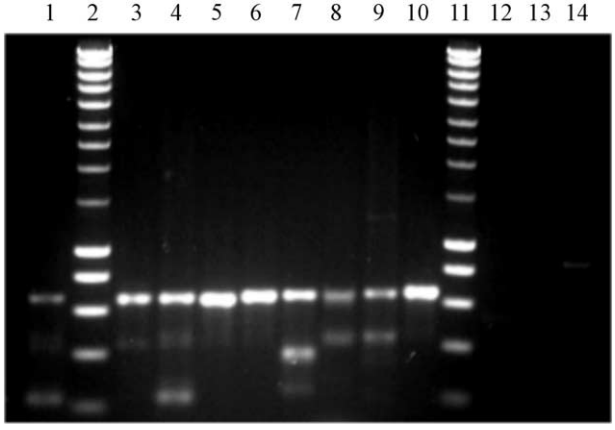

FIGURE 4 Agarose gel with PCR products obtained with DegFor-DegRev primers and DNAs from PAS-positive and PAS-negative lactobacilli as templates. Lanes 1, 3–9, glucan-positive lactobacilli: *Lb. reuteri* DSM 20016, 121, 104R, ML1 and 180; *Lb. parabuchneri* 33; *Lb. fermentum* Kg3, *Lb. sake* Kg15; Lane 10, *Ln. citreum* 86. Lanes 12, 13 and 14, glucan-negative strains, *Lactobacillus* sp. 181, *Lactobacillus* sp. 182 and *Lb. plantarum* WCFS1(Kleerebezem *et al.,* 2003; Van Geel-Schutten *et al.,* 1998). Lanes 2 and 11, smart ladder (Eurogentec, Seraing, Belgium).

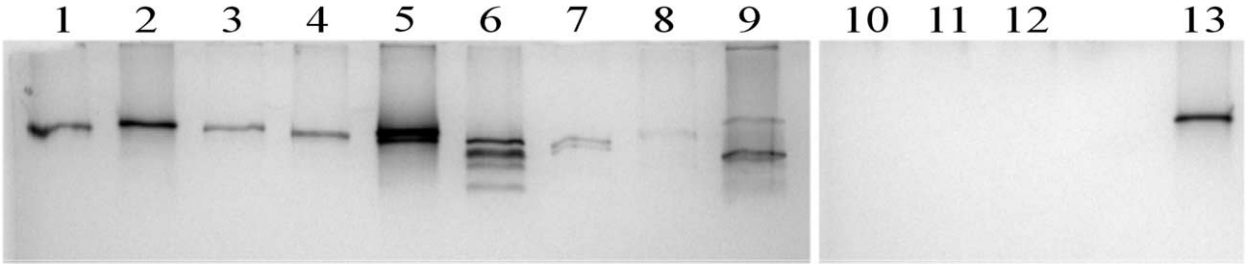

FIGURE 3 GTFs of different lactobacilli visualized by SDS-PAGE and PAs staining of glucans produced, Lanes 1–5, *Lb. reuteri* DSM, 121, 104R, ML1 and 180; Lane 6, *Lb. parabuchneri* 33; Lane 7, *Lb. fermentum* Kg3; Lane 8, *Lb. sake* Kg15; Lane 9, *Ln. citreum* 86; Lane 10, *Lactobacillus* sp. 181; Lane 11, *Lactobacillus* sp. 182; Lane 12, *Lb. plantarum* WCFS1; Lane 13, *Lb. reuteri* 121. Supernatants of the different strains (10 μl) were subjected to SDS-PAGE after overnight incubation at 37°C in MRS-s. Prior to application, supernatants of *Lb. fermentum* Kg3, *Lb. reuteri* DSM, and *Lb. sake* Kg15, *Lb. reuteri* 104R were concentrated 20 × and 50 × , respectively.

cis and *Leuconostoc* species have been shown to contain more than one *gtf* gene (Funane *et al.,* 2000; Simpson *et al.,* 1995a). Therefore, restriction of the different plasmids (70 in total), with *Nsi*I and *Eco*RI, was performed as a first screening to identify differences between the plasmids. Based on the restriction analysis, the inserts of five plasmids of each transformation were sequenced. Sequence analysis of five plasmids with chromosomal DNA inserts of LN 86 showed the presence of (parts of) three different (putative) *gtf* genes (*gtf*86-1, *gtf*86-5 and *gtf*86-8). Strains LB DSM, LB 104R, LB 180, and LB 33 most likely contain one *gtf* gene each. Strain 121 (*gtfA*, *gtfB*) (Kralj *et al.,* 2002; this study) as well as strain ML1 contained at least two *gtf* genes (*gtfML1*, *gtfML4*) (Fig. 2B, C).

The methods used thus allowed efficient identification of GTF positive *Lactobacillus* (and *Leuconostoc*) strains, and detection of one or more (putative) *gtf* genes per *Lactobacillus* (and *Leuconostoc*) strain.

GTF (Fragment) Sequence Comparisons

The amplified products from LB DSM, LB 121, LB 104R, LB ML1, LB 180, LB 33, LB Kg3, LB Kg15, and LN 86, were all identified as partial sequences of (the catalytic domains of putative) *gtf* genes (Fig. 2B, C). The fragments isolated from LB 104R, LB ML1 (GTFML1) and LB 180 showed the highest similarity at the amino acid level to GTFA of *Lb. reuteri* 121 (Table 1). The deduced amino acid sequences of the *gtf*180 and *gtf*104R encoded (putative) proteins showed very high homology to each other (99% similarity and 99% identity within 206 amino acids). A second (putative) GTF fragment was found in the *Lb. reuteri* strains ML1 (GTFML4) and 121 (GTFB). These fragments showed high homology to each other and to the fragment isolated from *Lb. reuteri* DSM 20016 (GTFDSM) ($\sim$ 80% identity and $\sim$ 90% similarity). GTF33 showed the highest homology with the first catalytic domain (CD1) encoded by *dsrE of Ln. mesenteroides* NRRL-B1299. GTFKg3 showed the highest homology with DSRB from *Ln. mesenteroides* NRRL-B1299. GTFKg15 showed the highest homology with DSRS of *Ln. mesenteroides* NRRL-B512F (Table 1).

Dendrogram

Construction of a dendrogram (Fig. 5), based on the partial amino acid sequences (approximately. 200 amino acids of the catalytic domains) of GTF enzymes of different lactic acid bacteria, revealed that the fragments of the following putative GTF enzymes (GTF180, GTF104R and GTFML1) isolated from different *Lb. reuteri* strains cluster closely together with GTFA of *Lb. reuteri* 121. Three other putative GTF fragments from *Lb. reuteri* strains (GTFDSM, GTFML4 and GTFB) formed a separate group. The GTF enzymes isolated from the various other lactobacilli cluster with *Leuconostoc* GTF enzymes.

CONCLUSIONS

SDS-PAGE activity staining for α-glucan synthesis from sucrose, and PCR based cloning of *gtf* gene fragments (catalytic domains), allowed fairly rapid identification of putative *gtf* genes in several *Lactobacillus* strains. Sequence analysis of the different *gtf* fragments confirmed their identity. Homologs of GTFA of *Lb. reuteri* 121 (Kralj *et al.,* 2002) were detected in three *Lb. reuteri* strains tested. Three other putative GTF fragments from *Lb. reuteri* strains (GTFDSM, GTFML4 and GTFB) formed a separate group. The other partial GTF sequences showed the highest similarity to glucosyltransferases from *Leuconostoc* sp. These results show that the large variation of glucosyltransferases previously reported for *Leuconostoc* and *Streptococcus* sp. also occurs within the lactobacilli. Conceivably, also such a range of different glycosidic bonds may be present in the glucans synthesized by the various GTF enzymes from lactobacilli. Currently, we are cloning and characterizing the full-length *gtf* gene sequences

TABLE 1 Similarities and identities of sequences of the newly isolated GTF fragments to GTF sequences shown in Fig. 5

Bacterial strain	(Putative) protein	Homology to	Genbank	Similarity (%)	Identity (%)
Lb. reuteri DSM 20016	GTFDSM	CD2 DSRE of *Ln. mesenteroides* NRRL B-1299	AJ430204	29	49
Lb. reuteri 104R	GTF104R	GTFA of *Lb. reuteri* 121	AX306822	83	63
Lb. reuteri ML1	GTFML1	GTFA of *Lb.* reuteri 121	AX306822	71	58
Lb. reuteri 180	GTF180	GTFA of *Lb.* reuteri 121	AX306822	83	63
Lb. parabuchneri 33	GTF33	CD1 DSRE of *Ln. mesenteroides* NRRL B-1299	AJ430204	88	79
Lb. fermentum Kg3	GTFKg3	DSRB of *Ln. mesenteroides* NRRL B-1299	AF030129	81	70
Lb. sake Kg15	GTFKg15	DSRS of *Ln. mesenteroides* NRRL B-512F	U81374	82	72
Lb. reuteri ML1	GTFML4	CD2 DSRE of *Ln. mesenteroides* NRRL B-1299	AJ430204	33	49
Lb. reuteri 121	GTFB	CD2 DSRE of *Ln. mesenteroides* NRRL B-1299	AJ430204	33	49
Ln. citreum 86	GTF86-1	DSRB of *Ln. mesenteroides* NRRL B-1299	AF030129	98	98
Ln. citreum 86	GTF86-5	ASR of *Ln. mesenteroides* NRRL B-1355	Q9RE05	62	49
Ln. citreum 86	GTF86-8	CD1 DSRE of *Ln. mesenteroides* NRRL B-1299	AJ430204	99	99

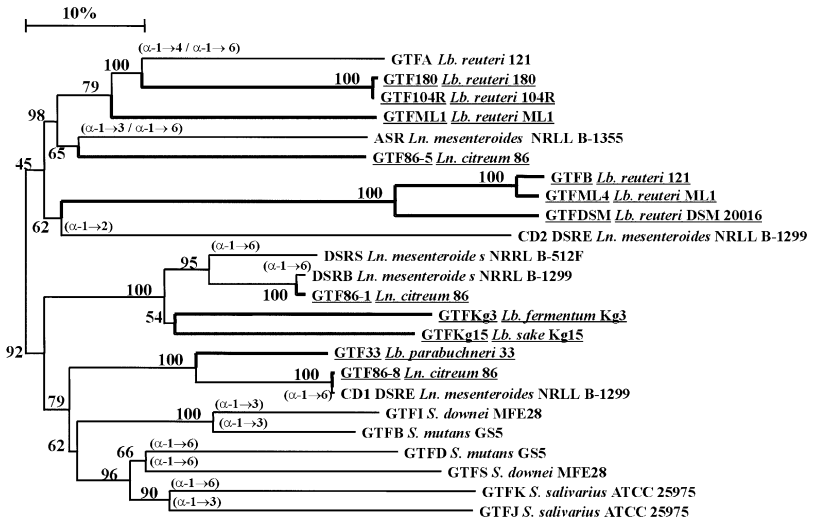

FIGURE 5 Dendrogram of glucosyltransferases of lactic acid bacteria. The horizontal distances are a measure for the differences at the amino acid level. The length of the upper bar indicates 10% difference. Bootstrap values are given at the root of each branch (in percentages). Sequences of glucosyltransferases determined in this study are indicated with a bold line and are underlined.

of the various lactobacilli. Many questions still remain to be answered, e.g. about expression, activity, and glucan synthesis of these (putative) GTF enzymes in their individual hosts, and about the number and nature of glucans (the type of glycosidic bonds present) produced by the various strains.

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