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Glucan synthesis in the genus *Lactobacillus*: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains

S. Kralj,[1,2] G. H. van Geel-Schutten,[1,3] M. M. G. Dondorff,[1,2] S. Kirsanovs,[1,2] M. J. E. C. van der Maarel[1,4] and L. Dijkhuizen[1,2]

[1]Centre for Carbohydrate Bioengineering (CCB), TNO-University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

[2]Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Kerklaan 30, 9751 NN, Haren, The Netherlands

[3]Innovative Ingredients and Products Department, TNO Nutrition and Food Research, Utrechtseweg 48, 3704 HE, Zeist, The Netherlands

[4]Innovative Ingredients and Products Department, TNO Nutrition and Food Research, Rouaanstraat 27, 9723 CC, Groningen, The Netherlands

Correspondence
L. Dijkhuizen
L.Dijkhuizen@biol.rug.nl

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Members of the genera *Streptococcus* and *Leuconostoc* synthesize various α-glucans (dextran, alternan and mutan). In *Lactobacillus*, until now, the only glucosyltransferase (GTF) enzyme that has been characterized is *gtfA* of *Lactobacillus reuteri* 121, the first GTF enzyme synthesizing a glucan (reuteran) that contains mainly α-(1→4) linkages together with α-(1→6) and α-(1→4,6) linkages. Recently, partial sequences of glucansucrase genes were detected in other members of the genus *Lactobacillus*. This paper reports, for the first time, isolation and characterization of dextransucrase and mutansucrase genes and enzymes from various *Lactobacillus* species and the characterization of the glucan products synthesized, which mainly have α-(1→6)- and α-(1→3)-glucosidic linkages. The four GTF enzymes characterized from three different *Lb. reuteri* strains are highly similar at the amino acid level, and consequently their protein structures are very alike. Interestingly, these four *Lb. reuteri* GTFs have relatively large N-terminal variable regions, containing RDV repeats, and relatively short putative glucan-binding domains with conserved and less-conserved YG-repeating units. The three other GTF enzymes, isolated from *Lactobacillus sakei*, *Lactobacillus fermentum* and *Lactobacillus parabuchneri*, contain smaller variable regions and larger putative glucan-binding domains compared to the *Lb. reuteri* GTF enzymes.

INTRODUCTION

Many lactic acid bacteria employ large extracellular glucosyltransferase (GTF) enzymes (EC 2.4.1.5, commonly named glucansucrases) for the synthesis of high-molecular-mass α-glucans from sucrose. Although high similarity exists between these glucansucrase enzymes, they are able to synthesize α-glucans with different types of glucosidic linkages. These glucans can be divided into the following five groups: (i) reuteran, which contains large amounts of α-(1→4)-glucosidic bonds (*Lactobacillus reuteri* 121) (Kralj *et al.*, 2002), (ii) dextran, which contains predominantly α-(1→6)-linked glucopyranosyl units in the main chain (Cerning, 1990), (iii) mutan, a polyglucose with mainly α-(1→3) linkages (various streptococci) (Hamada & Slade, 1980), (iv) alternan, which has alternating α-(1→6)- and α-(1→3)-linked D-glucopyranosyl units (*Leuconostoc mesenteroides*) (Arguello-Morales *et al.*, 2000), and (v) glucan polymers containing large amounts of α-(1→2) linkages (mainly α-(1→2,6) branching points), as produced by *Ln. mesenteroides* strain NRRL-B1299 and mutant strain (R510) of NRRL B-1355 (Bozonnet *et al.*, 2002; Smith *et al.*, 1998). Within these five distinct groups, the glucans may further differ in the degree of branching, the nature and amount of other glucosidic linkages present, e.g. of the α-(1→2), α-(1→3), α-(1→4) and α-(1→6) types, the type

Abbreviations: GBD, glucan-binding domain; GTF, glucosyltransferase; HPSEC, high performance size exclusion chromatography; iPCR, inverse PCR.

The GenBank/EMBL/DDBJ accession numbers for the sequences of the *Lactobacillus gtf* genes *gtf180*, *gtfML1*, *gtf33*, *gtfKg3*, *gtfKg15* and *gtfB* and their flanking regions are AY697430, AY697431, AY697432, AY697433, AY697434 and AY697435.

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of branching point, e.g. with α-(1→2,6) to α-(1→3,6)- or α-(1→4,6)-glucosidic linkages, their molecular mass, and the length of the branching chains and their spatial arrangement (Monchois *et al.*, 1999c).

The distribution of glucosidic linkages has been elucidated for the glucans synthesized by heterologously produced GTF enzymes including: (i) 13 GTFs from seven *Streptococcus* strains (Monchois *et al.*, 1999c; Hanada *et al.*, 2002; Konishi *et al.*, 1999), (ii) seven GTFs from four *Leuconostoc* strains (Monchois *et al.*, 1999c; Bozonnet *et al.*, 2002; Neubauer *et al.*, 2003; Funane *et al.*, 2001; Arguello-Morales *et al.*, 2000) and (iii) from *Lb. reuteri* strain 121 (Kralj *et al.*, 2002). Only *gtf* genes encoding either dextran- or mutansucrase enzymes have been characterized in the genus *Streptococcus* (Monchois *et al.*, 1999c; Hanada *et al.*, 2002; Konishi *et al.*, 1999). *Leuconostoc* strains carry *gtf* genes encoding mainly dextransucrase enzymes, but an alternansucrase-encoding gene and one gene encoding a glucansucrase that synthesizes large amounts of α-(1→2)-branch linkages have also been characterized (Arguello-Morales *et al.*, 2000; Bozonnet *et al.*, 2002; Monchois *et al.*, 1999c).

The GTF enzymes of *Streptococcus* species are generally produced constitutively (Kim & Robyt, 1994). GTF enzymes of *Leuconostoc* species are specifically induced by sucrose. This is disadvantageous for several applications, and therefore some constitutive mutants were constructed (Kim & Robyt, 1994; Kitaoka & Robyt, 1998). Reuteransucrase from *Lb. reuteri* 121 is produced constitutively (van Geel-Schutten *et al.*, 1999). The GTF enzymes from *Lb. reuteri* 180 and *Lb. parabuchneri* 33 are also produced constitutively (unpublished data).

The only streptococcal species that is associated with food technology is *Streptococcus thermophilus*, which is used in the manufacture of yoghurt. The genus *Streptococcus* contains several well-known pathogens (e.g. *Streptococcus pneumoniae*) (Axelsson, 1998; Leroy & De Vuyst, 2004). Furthermore, glucans produced by oral streptococci play a key role in the cariogenesis process, by enhancing the attachment and colonization of cariogenic bacteria (Loesche, 1986). *Leuconostoc* strains play an important role in vegetable fermentations (Axelsson, 1998; Leroy & De Vuyst, 2004).

Lactobacilli are widespread in nature, and many species have found applications in the food industry (e.g. dairy products, sourdough) (Axelsson, 1998; De Vuyst & Degeest, 1999). Several *Lb. reuteri* strains are able to produce antimicrobial metabolites (e.g. reutericyclin, reuterin and reutericin), which delay the growth of some food-borne pathogens (Kabuki *et al.*, 1997; Ganzle *et al.*, 2000; Talarico *et al.*, 1988). Furthermore, some *Lb. reuteri* strains have probiotic properties, as has been demonstrated in humans and various animals (Casas *et al.*, 1998; Valeur *et al.*, 2004). The range of glucans and oligosaccharides produced by GTF enzymes present in lactobacilli (Kralj *et al.*, 2004) may potentially act as prebiotics by stimulating the growth of probiotic strains or of beneficial endogenic strains of the gastrointestinal tract (Monsan & Paul, 1995; Olano-Martin *et al.*, 2000; Chung & Day, 2002).

Lactobacillus reuteri strains producing glucans thus possess the following general advantages: (i) constitutive GTF enzyme production, (ii) safe (GRAS status) and (iii) potential pro- and prebiotic properties. Glucans and gluco-oligosaccharides from lactobacilli are therefore interesting and feasible alternatives to the additives currently used in the production of foods (e.g. sourdough, yoghurts, health foods). Although different *Lactobacillus* strains are able to produce glucans (Kralj *et al.*, 2003; Tieking *et al.*, 2003; Sidebotham, 1974; van Geel-Schutten *et al.*, 1998), only the *Lb. reuteri* GTFA enzyme has been characterized thus far. This enzyme synthesizes a highly branched glucan (reuteran) containing α-(1→4) and α-(1→6) linkages (Kralj *et al.*, 2002). The same types of glucosidic linkages were synthesized in its oligosaccharide products (Kralj *et al.*, 2004). Recently, we have shown that lactobacilli, in fact, contain DNA sequences of other putative glucansucrase genes (Kralj *et al.*, 2003).

This paper describes the characterization of the glucans produced by six different *Lactobacillus* strains, and the isolation and characterization of the corresponding *gtf* genes and GTF enzymes. The data show that members of the genus *Lactobacillus* contain the same variety of *gtf* genes, GTF enzymes and glucan products as *Leuconostoc* and *Streptococcus* species.

METHODS

Bacterial strains, plasmids, media and growth conditions. *Lb. reuteri* strains 121 (LB 121; LMG 18388), ML1 (LB ML1; LMG 20347) and 180 (LB 180; LMG 18389), *Lactobacillus sakei* Kg15 (LB Kg15), *Lactobacillus fermentum* Kg3 (LB Kg3) and *Lactobacillus parabuchneri* 33 (LB 33; LMG 20349) were obtained from the culture collection of TNO Nutrition and Food Research. All strains were cultivated as described previously (Kralj *et al.*, 2003). *Escherichia coli* TOP 10 (Invitrogen) was used as host for cloning purposes. Plasmids pET15b (Novagen) and pET-101-D-TOPO (Invitrogen) were used for expression of the different *gtf* genes in *E. coli* BL21 Star (DE3) (Invitrogen). *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 (100 μg ampicillin ml^{-1} or 50 μg kanamycin ml^{-1}). Agar plates were made by adding 1·5 % agar to the LB medium.

Isolation of DNA. *Lactobacillus* total DNA was isolated according to Nagy *et al.* (1995). Plasmid DNA of *E. coli* was isolated using the alkaline lysis method (Birnboim & Doly, 1979) or with a Wizard Plus SV plasmid extraction kit (Promega).

Molecular techniques. General procedures for gene cloning, *E. coli* DNA transformations, DNA manipulations, and agarose gel electrophoresis were as described by Sambrook *et al.* (1989). Restriction endonuclease digestions and ligations with T4 DNA ligase were performed as recommended by the enzyme suppliers (New England Biolabs; Roche Biochemicals). Primers were obtained from Eurogentec. Sequencing was performed by GATC. DNA was amplified by PCR on a DNA Thermal Cycler PTC-200 (MJ Research) using *Pwo*

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DNA polymerase (Roche Biochemicals) or Expand High Fidelity polymerase (Roche Biochemicals). For inverse PCR (iPCR) the Expand High Fidelity PCR system (Roche Biochemicals) was used as described by the supplier. Fragments were isolated from agarose gels using a Qiagen gel extraction kit following the instructions of the supplier.

Identification and nucleotide sequence analysis of the glucansucrase genes. A first fragment of the glucansucrase genes was isolated by PCR amplification of chromosomal DNA from the different *Lactobacillus* strains using degenerate primers (DegFor and DegRev) which were based on sequence similarity between conserved regions, located in the catalytic core, of different GTF enzymes of Gram-positive bacteria (Kralj *et al.*, 2003). The ~660 bp amplified fragments were used to identify appropriate restriction sites and to design primers for subsequent iPCR reactions (Triglia *et al.*, 1988) (Table 1).

Construction of plasmids. Appropriate primer pairs and template DNA were used to create eight different expression constructs for complete and/or N-terminally truncated and (C-terminally) His-tagged GTF enzymes (Table 2).

Expression and purification of GTF proteins. Cells of *E. coli* BL21 Star (DE3) harbouring different pET15b or pET-101-D-TOPO derivatives (Table 2) were harvested by centrifugation (10 min at 4 °C at 10 000 ***g***) after 16 h of growth. The pellet was washed with 50 mM phosphate buffer (pH 8·0) and the suspension was centrifuged again (10 min at 4 °C at 10 000 ***g***). Pelleted cells were resuspended in 50 mM sodium phosphate buffer (pH 8·0) containing 250 mM NaCl, 5 mM β-mercaptoethanol and 10 mM imidazole. Cells were broken by sonication (7 × 15 s at 7 micron with 30 s intervals) and centrifuged (10 min at 4 °C at 10 000 *g*). The clear lysate containing GTF activity was loaded on a Ni-NTA column (Qiagen). Binding was achieved using 50 mM sodium phosphate buffer (pH 8·0) containing 250 mM NaCl, 5 mM β-mercaptoethanol and 10 mM imidazole, followed by washing using the same buffer. The His-tagged protein(s) were eluted using 50 mM sodium phosphate buffer (pH 8·0) containing 250 mM NaCl, 1 mM β-mercaptoethanol and 200 mM imidazole.

Enzyme assays. His-tag-purified GTF enzymes from *E. coli* extracts and culture supernatants of *Lactobacillus* strains grown on MRSs were used as a source of enzyme for the enzyme assays. GTF total activity was measured by determining the release of fructose from sucrose at 37 °C in 50 mM sodium acetate buffer (pH 5·5) containing 1 mM $CaCl_2$ and 100 mM sucrose (van Geel-Schutten *et al.*, 1999).

SDS-PAGE followed by activity staining. Gel electrophoresis and GTF activity staining of gels with periodic acid Schiff reagents was performed as described previously (Kralj *et al.*, 2003).

Production of glucans and analysis

(i) Polymer production. Glucans synthesized by cultures of the *Lactobacillus* strains and glucans synthesized by the His-tag-purified GTF enzymes from *E. coli* were produced and isolated by ethanol precipitation as described previously (Kralj *et al.*, 2002).

(ii) Methylation. Polysaccharides were permethylated using methyl iodide and dimsyl sodium ($CH_3SOCH_2^-$-Na^+) in DMSO at room temperature (Hakomori, 1964). After hydrolysis with 2 M trifluoroacetic acid (1 h, 125 °C), the partially methylated monosaccharides were reduced with $NaBD_4$ (Harris *et al.*, 1984). Mixtures of partially methylated alditol acetates obtained were analysed by GLC on a CP Sil 5 CB column (25 m × 0·53 mm; Chrompack) and by GLC- MS on an RTX 5 Sil MS (30 m × 0·25 mm; Restek) column (Chaplin, 1982; Jansson *et al.*, 1976).

(iii) Molecular masses of the glucans. Molecular mass analysis was performed as described previously, using high-performance size-exclusion chromatography (HPSEC) coupled on-line with multi-angle laser light scattering (MALLS) and differential refractive index detection (Kralj *et al.*, 2002).

RESULTS AND DISCUSSION

Isolation and nucleotide sequence analysis of six putative *Lactobacillus* glucansucrase genes

Previous work showed that a second putative *gtf* gene was located upstream of *gtfA* in *Lb. reuteri* 121 (Kralj *et al.*, 2002). Part of this putative *gtfB* gene was isolated from *Lb. reuteri* 121 chromosomal DNA using degenerate primers (Kralj *et al.*, 2003). Using iPCR, the complete *gtfB* sequence was obtained in the present study (Table 1, Fig. 1).

From five other *Lactobacillus* strains, parts of six putative *gtf* genes (*gtfML1*, *gtf180*, *gtfKg15*, *gtfKg3*, *gtf33* and *gtfML4*) were isolated (Kralj *et al.*, 2003; van Geel-Schutten, 2003). In subsequent steps the complete nucleotide sequences of the different *gtf* genes (except *gtfML4*) were obtained using the iPCR method (Fig. 1, Table 1).

The *gtf* genes in the different *Lactobacillus* species/strains appear to have different chromosomal locations, with a relatively high frequency of transposase homologues flanking the different *gtf* genes (Fig. 1, Table 3).

Amino acid sequence analysis of the six isolated GTFs

Alignment of the deduced amino acid sequence of the different GTF enzymes with other glucansucrases using BLAST (Altschul *et al.*, 1990) revealed clear similarities to other GTF enzymes of lactic acid bacteria (Table 3). The four GTF enzymes characterized from three different *Lb. reuteri* strains were highly similar (Table 3, Fig. 2) (Kralj *et al.*, 2002). *Lb. sakei* GTFKg15 and *Lb. fermentum* GTFKg3 displayed highest identity and similarity with *Ln. mesenteroides* Lcc4 DSRD. *Lb. parabuchneri* GTF33 was found to have highest similarity with CD1 of DSRE from *Ln. mesenteroides* NRRL B-1299. Analysis of the deduced GTF amino acid sequences encoded by the six completely isolated *gtf* genes revealed the presence of: (i) a signal peptide, (ii) a highly variable stretch, (iii) a highly conserved catalytic domain. and (iv) a C-terminal domain often referred to as a glucan-binding domain (GBD; Fig. 2) (Monchois *et al.*, 1999c).

Signal peptides

Consistent with the extracellular location of GTF enzymes, all the sequences contained a typical Gram-positive signal peptide ranging in size from 37 to 46 amino acids (Fig. 2). The predicted cleavage sites were located using the SignalP server (http://www.cbs.dtu.dk/services/SignalP/).

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Table 1. Primers used for iPCR reactions to obtain the 5′ or 3′ nucleotide sequences of the different *gtf* genes and surrounding regions

Indicated are restriction enzymes used to digest and ligate the chromosomal DNA of the different *Lactobacillus* strains, yielding circular DNA molecules, which were subsequently used as template in iPCR reactions.

Gene	Primer	Sequence direct primer (5′→3′)	Primer	Sequence reverse primer (5′→3′)	Restriction enzyme used	Size of fragment obtained (bp)
gtfB						
5′	GTFBF	GTTCCGCAAGTTTACTATGGTGACCTTTATAATG	GTFBR	GTTACAAATGACCAGTTTGGCGTAGCTTCATTC	*Sac*I*	5·0
gtfML1						
5′	ML1F	ATTAGATATAGCTGGTGATTACTTTAATGCAG	ML1R	CACGAATGCTATCAAAATTAGCATCAGGATC	*Cla*I	3·5
3′	ML1F2	TTGAAGCATACATTGCAGATCAAAGTAATG	ML1R2	CCCTTTTGCTCTTGCTCAAAGTTAAACTTCG	*Bal*I†	2·0
gtfML4						
5′	ML4F	GTAATTTTGATGGCTTCCGAGTTGATGCTGCTG	ML4R	CTGGATTATACCCCATTAACTTACCATAATTTAG	*Ssp*I‡	0·5
3′	ML4F2	GTATGCAATTCTTTTGAGCAATAAAGATACGG	ML4R2	GCCGGAACATTATATTGATCATATTGTTTATTAG	*Ssp*I	2·5
5′	ML4NF	GGCACCGATCAGCTCATGAGAAACTTGGTTGC	ML4NR	CCGACCAGTAATCAACGCGGTTCCCTTCACC	*Ssp*I	1·0
3′	ML4CF	GGTATTGGTTTAGCTGTAAAATTAGCTAATGG	ML4CR	GCAATGATGTCCCATTTTCGTATTTAGCAGACC	*Eco*RI	1·2
gtf180						
5′	180F1	CATGGAGCAAAGTGATGCCAGTGCTAATAAGC	180R1	GTCAACATCTACATTATCAACAGCATCCACTCG	*Kpn*I	1·5
3′	180F2	GCTTATACTATTCTTCTAACAAATAAAGATTCAG	180R2	GGCATATTGTAAAGATTCCACTTCTTATTAG	*Ssp*I	4·5
5′	1802F1	GAAGCAATATGGTAATTTATTGCCGGCTAG	1802R1	GCTTGTGTAACAGTATTTGGCCACCATACC	*Eco*RI	4·0
gtfKg15						
5′	Kg15F1	GGCCACAATGACCCATTATATGTCAAGGACC	Kg15R1	GCGGCTGTAATGTTTAGCAGGTCGGCATCC	*Nsi*I	2·5
3′	Kg15F2	GCTTTGTCCGTGCACACGATAGTGAAGTTC	Kg15F2	GGAATTGCTGGATCAGACGTATTGTCCTTAGC	*Bcl*I	2·0
3′	Kg15CF	AATGGCGATGGTATCGCGTTGAAGGGTTGG	Kg15CR	GTAGAACCAATCCCCGTTTCGGTTTACTGC	*Ase*I	1·5
gtfKg3						
5′	Kg3F1	CGGGATGCCTATGGCATGGCTACAACTGAC	Kg3R1	GAAGTAAATCAGCATCGACATTATCGACCGC	*Ase*I§	0·9
3′	Kg3F2	GGCGCAACCTAGTTACTCGTTTGTGCGTGC	Kg3R2	CATCATTAGCACGGTTGGTGAGATAGAAGTCC	*Bcl*I	2·0
5′	Kg3CF	GGCCAACCGGTGATGACCGGATTCTATCAC	Kg3CR	GCATTTGTACCATTATCACGCAGCACATAGCC	*Sph*I	2·5
3′	Kg3NF	CGACCACTGTTAGCAACTTGGTGGCCAAC	Kg3NR	GGTTGCGATTCGCGCCACGTCTCACCATTTTCC	*Ase*I	1·8
gtf33						
5′	33F1	CGAGTGGACGCTGTGGACAATGTCGATGC	33R1	GCGGTGGGATCATTGGCAGTAATGCTACC	*Bgl*II	6·0
3′	33F2	CCCTAACTCTGACGGATTAACAGTTACTCCCG	33R2	GCGATGATTGTTTGCACTTCACTATCGTGAGCC	*Hpa*I	6·0

*For *gtfB* ~2·4 kb of 3′ nucleotide sequence information was already available (Kralj *et al.*, 2002).

†Another iPCR product obtained, using *Pst*I-digested and ligated chromosomal DNA as template, completed the 3′ sequence.

‡Another iPCR product obtained, using *Nsi*I-digested and ligated chromosomal DNA as template, yielded additional 5′ sequence, which was used to design the primers ML4CF and ML4CR.

§Non-specific iPCR product obtained, providing new sequence information used to design the primers Kg3NF and Kg3NR.

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Table 2. Primers and expression vectors used for amplification, cloning and expression of the different full-length and N-terminally truncated GTF enzymes in *E. coli* BL21 Star (DE3)

*Sac*I and *Nco*I restriction sites are shown by underlining and italics, respectively. *Apa*I and *Bam*HI restriction sites are shown in bold type and underlined italics, respectively. *Bgl*II restriction sites are shown in bold type italics. Sequences encoding His-tags are shown in bold type, underlined italic. Stop codons are shown in lower case font. For cloning in pET15b, *Nco*I and *Bam*HI/*Bgl*II restriction sites were used. For directional cloning the pET-101-D-TOPO expression vector was used.

Construct	Primer	Sequence direct primer (5′→3′)	Primer	Sequence reverse primer (5′→3′)	Expression vector
GTFB–CHis	GTFBexpF	GGATGCA*CCATGG*ATAC-AAATACTGGTGATCAG-CAAACTGAAC	GTFBexpR	CCTCCTTTCT***AGATct***atta***GTGATGGT-GATGGTGATG***GTTGTTAAAGTTTAA-TGAAATTGCAGTTGG	pET15b
GTFML1–ΔN–CHis	ML1expCF	GATGCATGAGCTC*CCAT-GG*GCATTAATGGTCAT-CAATATTATATTGACCC	ML1expCR	ATATCGAT**GGGCCC**C***AGATct***atta***GTG-ATGGTGATGGTGATG***CTTATTAGTA-CCACTTAAATCCTGTTGAGtaattg	pET15b
GTF180–CHis	180expF	GATGCATGAGCTC*CCAT-GG*ATCAACAAGTTCAG-TCTTCCACAACCC	180expR	ATATCGAT**GGGCCC**C*GGATCc*tatta***GT-GATGGTGATGGTGATG***TTTTTGGCC-GTTTAAATCACCAGGTTTTAATGG	pET15b
GTF180–ΔN–CHis	180expCF	GATGCATGAGCTC*CCAT-GG*GCATTAACGGCCAA-CAATATTATATTGACCC	180expR	ATATCGAT**GGGCCC**C*GGATCc*tatta***GT-GATGGTGATGGTGATG***TTTTTGGCC-GTTTAAATCACCAGGTTTTAATGG	pET15b
GTFKg15–ΔN–CHis	Kg15expCF	CACCATGATTGCTGGTA-AGACGTATTACTTTGA-CAAAG	Kg15expCR	ATCTTTTTTATGCTTTTTACTTGTAA-ACAACAGACC	pET-101-D-TOPO
GTFKg3–ΔN–CHis	Kg3expF1	CACCATGATTGCAAACA-AGGTTTATGATTTTGA-TGAG	Kg3expR1	AATCGTCACCAACGTACCAGCACCA-GTTTTC	pET-101-D-TOPO
GTF33–CHis	33expF	CACCATGGCCGGCAAT-AATGATCCGCAGCAG-ACC	33expR	CTTGATATTGGTTTTAATTACTTTAG-TTAATGC	pET-101-D-TOPO
GTF33–ΔN–CHis	33expCF	CACCATGATCAATGGAC-AATCACTATATTTCAA-TAAAAC	33expR	CTTGATATTGGTTTTAATTACTTTAG-TTAATGC	pET-101-D-TOPO

N-terminal variable regions

The protein structures of GTFB, GTF180 and GTFML1 found in three different *Lb. reuteri* strains were very similar to that of GTFA of *Lb. reuteri* 121 (Fig. 2). All three GTFs contained a relatively large and highly similar variable region (~700 amino acids) with 5 RDV repeats (sequence R(P/N)DV-x_{12}-SGF-$x_{19–22}$-R(Y/F)S, where x represents a non-conserved amino acid residue), as previously observed in GTFA of *Lb. reuteri* 121 (Kralj *et al.*, 2002) (Fig. 2). The variable domains of the other three isolated GTFs were smaller and contained different repeating units from the *Lb. reuteri* GTFs. In their variable region, GTFKg3 and GTFKg15 contained, respectively, five and six conserved and less-conserved YG-repeats (sequence NDGYYFxxxGxxH°x(G/N)H°H°H°, where x represents a non-conserved amino acid residue and H° represents a hydrophobic amino acid residue) (Fig. 2) (Giffard & Jacques, 1994). In the variable region of GTF33, nine short unique repeating units, designated 'TTQ', were found. These repeats were 15 amino acids long, with TTTQN(A/T)(P/A)NN(S/G)N(D/G)PQS as their sequence, and showed no significant similarity to any protein motif present in databases (Fig. 2). Different repeating units also could be identified in the N-terminal variable domain of other glucansucrases: A-repeats in alternansucrase and dextransucrases of *Ln. mesenteroides* (Janecek *et al.*, 2000); motif T, TDD**KA**(A/T)**TT**A(A/D)**T**S (bold type indicates conserved amino acids) in DSRT of *Ln. mesenteroides* NRRL B-512F (Funane *et al.*, 2000); motif S, PA(A/T)**DKA**VD**TT**P(A/T)**T**, bold type indicates conserved amino acids) in DSRE of *Ln. mesenteroides* NRRL B-1299 (Bozonnet *et al.*, 2002) and RDV repeats in GTFA of *Lb. reuteri* 121) (Kralj *et al.*, 2002). However, deletion studies of the variable domain showed that it did not determine the type of glucosidic linkages present or the size (only determined for GTFA) of the synthesized glucans (Monchois *et al.*, 1999a; Kralj *et al.*, 2004).

Catalytic domains

The catalytic domains of the putative GTF enzymes range in size from 741 to 774 amino acids (Fig. 2). Within all the catalytic domains the three completely conserved amino acids already identified as being essential for enzymic activity in other GTF enzymes (Asp1024, Glu1061 and

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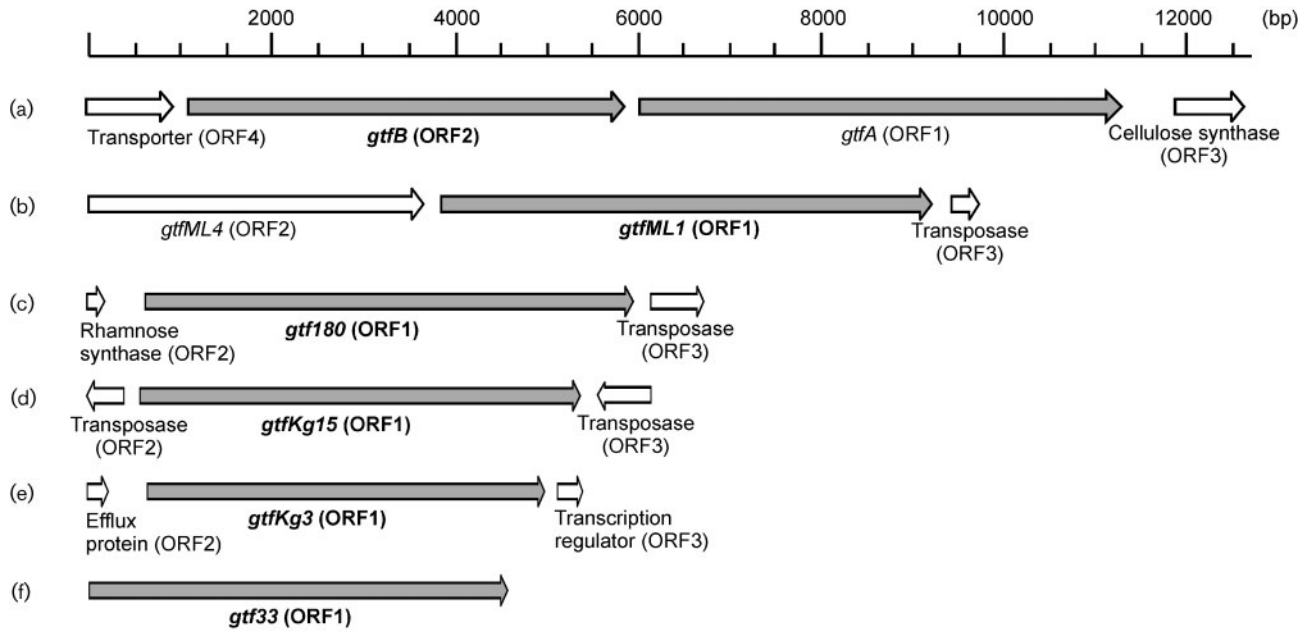

Fig. 1. Overview of the size and organization of DNA fragments, isolated by iPCR, carrying the different *gtf* genes and surrounding regions from six different *Lactobacillus* strains characterized in this study (shown in bold type): (a) *gtf*A (characterized previously, Kralj *et al.*, 2002) and *gtfB* from *Lactobacillus reuteri* 121; (b) *gtfML1* and (partly) *gtfML4* from *Lactobacillus reuteri* ML1; (c) *gtf180* from *Lactobacillus reuteri* 180; (d) *gtfKg15* from *Lactobacillus sakei* Kg15; (e) *gtfKg3* from *Lactobacillus fermentum* Kg3; (f) *gtf33* from *Lactobacillus parabuchneri* 33. Partial ORFs are indicated with open arrows.

Asp1133; GTFA *Lb. reuteri* 121 numbering) could be identified (Devulapalle *et al.*, 1997; Kralj *et al.*, 2003; Kralj *et al.*, 2004).

Putative glucan-binding domains (GBDs)

The C-terminal domain of *Streptococcus* and *Leuconostoc* GTF enzymes consists of a series of different tandem repeats, which have been divided into four classes: A, B, C and D repeats. Within the A–D repeats, a repeating unit designated YG can be distinguished (Giffard & Jacques, 1994). GTFB, GTFML1 and GTF180 possessed a relatively short GBD of 134–263 amino acids, comparable with the GBD from GTFA of *Lb. reuteri* 121 (Fig. 2) and consisting of several conserved and less-well-conserved YG-repeats (Kralj *et al.*, 2002). Characterization of sequential C-terminal deletion mutants of GTFA revealed that the C-terminal domain has an important role in glucan binding (Kralj *et al.*, 2004).

The putative GBDs of the other isolated GTFs were approximately twice as large as the *Lb. reuteri* GBDs. They contained a varying number of conserved and less-well-conserved YG-repeating units (Fig. 2) and no A, B, C or D repeats could be identified. GTF33 contained, besides the 17 YG-repeats, two unique repeating units, designated 'KYQ' [49 amino acids, AVK(T/A)A(K/Q)(A/T)(Q/K)-(L/V)(A/N)K(T/A)KAQ(I/V)(A/T)KYQKALKKAKTTKAK-(A/T)QARK(S/N)LKKA(E/N)(T/S)S(F/L)(S/T)KA] that showed no significant similarity to any protein motif present in databases. GTFKg15 possessed, at the end of its putative GBD, an additional stretch which shows similarity to part of a putative extracellular matrix binding protein from *Streptococcus pyogenes* M1 (AE006525; 44 % similarity and 56 % identity within 75 amino acids) (Fig. 2).

Expression of the *gtf* genes in *E. coli*

Based on the nucleotide sequence information obtained, six different *gtf* genes were cloned and expressed in *E. coli* (Table 2). The *gtfB* gene was expressed as a full-length protein. The *gtf180* and *gtf33* genes were expressed as proteins with and without their N-terminal variable regions. The *gtfML1*, *gtfKg3* and *gtfKg15* genes were expressed as proteins without their N-terminal variable regions (Table 2). Except for GTFB from *Lb. reuteri* 121, the His-tag-purified GTF proteins all showed enzymic activity, as measured by fructose release from sucrose. SDS-PAGE showed that, in all cases, protein was present as a band corresponding to the molecular mass of the different truncated and full-length enzymes (data not shown). Staining with Schiff reagents for polymer-synthesizing activity was positive, under the conditions examined, for all recombinant enzymes (except for GTFB, data not shown).

In the supernatant of *Lb. reuteri* 121 cultures only the GTFA enzyme was found (under the growth conditions tested). Furthermore, the glucan synthesized by *Lb. reuteri*

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Table 3. Overview of the highest identity and similarity scores of the different GTF enzymes and surrounding ORFs, isolated from the various *Lactobacillus* strains, to proteins present in databases (see also Fig. 1)

Lactobacillus strain	(Putative) proteins	Highest similarity to	GenBank no.	Identity (%)	Similarity (%)	No. of amino acids*
Lb. reuteri 121	GTFA	ASR from *Ln. mesenteroides* NRRL B-1355	AJ250173	47	60	1261
	GTFB	GTFA from *Lb. reuteri* 121	AX306822	45	60	1677
	ORF3†	Cellulose synthase from *Populus tremuloides*	AY095297	28	47	87
	ORF4†	Putative transporter from *Streptococcus thermophilus*	AF454500	26	48	333
Lb. reuteri ML1	GTFML1	GTFA from *Lb. reuteri* 121	AX306822	78	86	1775
	GTFML4†	GTFB from *Lb. reuteri* 121	This study	90	92	1231
	ORF3†	Transposase from *Lb. reuteri* plasmid	AF449484	98	99	102
Lb. reuteri 180	GTF180	GTFA from *Lb. reuteri* 121	AX306822	78	87	1768
	ORF2†	dTDP L-rhamnose synthase from *Streptococcus pneumoniae*	AF246897	64	74	56
	ORF3†	Putative transposase from *Enterococcus faecium*	U63997	37	56	181
Lb. sakei Kg15	GTFKg15	DSRD from *Ln. mesenteroides* Lcc4	AY017384	57	69	1336
		GTFA from *Lb. reuteri* 121	AX306822	45	57	1111
	ORF2†	Putative transposase from *Streptococcus criceti* E49	BAB64015	36	51	132
	ORF3†	Putative transposase from *Leuconostoc lactis*	JC4048	49	69	188
Lb. fermentum Kg3	GTFKg3	DSRD from *Ln. mesenteroides* Lcc4	AY017384	51	65	1475
		GTFA from *Lb. reuteri* 121	AX306822	40	54	1303
	ORF2†	Putative efflux protein from *Streptococcus pyogenes* SSI-1	AP005145	29	56	57
	ORF3†	Alkaline phosphatase synthesis transcriptional regulatory protein from *Staphylococcus aureus* MW2	AP004827	50	73	86
Lb. parabuchneri 33	GTF33	CD1 from DSRE from *Ln. mesenteroides* NRRL B-1299	AJ430204	68	76	1261
		GTFA from *Lb. reuteri* 121	AX306822	49	65	1029

*No. of amino acids within which the identity and similarity applies.
†Partial open reading frames.

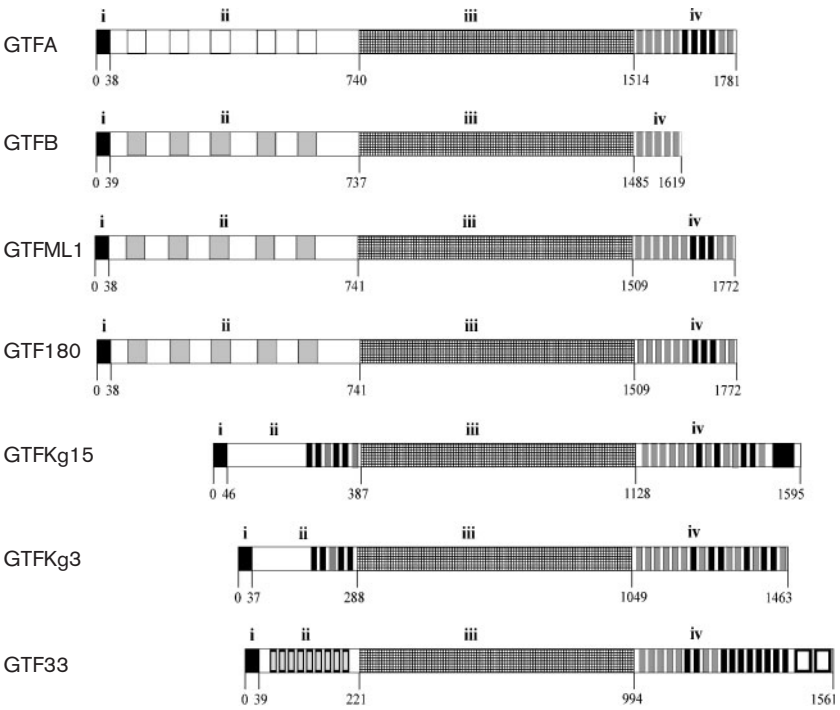

Fig. 2. Schematic representation of the organization of GTFA (Kralj *et al.*, 2002) and GTFB from *Lb. reuteri* strain 121, GTFML1 from *Lb. reuteri* ML1, GTF180 from *Lb. reuteri* 180, GTFKg15 from *Lb. sakei* Kg15, GTFKg3 from *Lb. fermentum* Kg3 and GTF33 from *Lb. parabuchneri* 33, showing the four different domains: (i) N-terminal signal sequence; (ii) variable region (RDV repeats are indicated by grey squared boxes, TTQ repeats in GTF33 are indicated by grey boxes with a bold outline); (iii) catalytic domain; (iv) C-terminal (putative) GBD (KYQ repeats in GTF33 are indicated with white squared boxes with a bold outline; the domain showing similarity to an extracellular matrix binding protein in GTFKg15 is indicated by a black square box). Conserved YG repeats, according to the definition of Giffard and Jacques (1994), in the N-terminal variable region and in the GBD are indicated by black boxes; less-conserved YG-repeats are shown as grey boxes.

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121 is identical to the glucan produced by GTFA (Kralj *et al.*, 2002). This suggests that GTFB of *Lb. reuteri* 121 either synthesizes a similar glucan product or (most likely) is not active under the growth conditions tested. GTFB has a relatively small GBD, compared to GTFA (Fig. 2). However, deletion studies with GTFA showed that this enzyme was still active after truncation of the GBD to the size (6 YG repeats deleted) of the GBD in GTFB (Kralj *et al.*, 2004). GTFB also possesses the three catalytically important residues (Devulapalle *et al.*, 1997; Kralj *et al.*, 2004). Its inactivity may be caused by the aberrant amino acid sequence at the start of its catalytic core. The highly conserved motif 'IN**G**QY**Y**', indicating the start of the catalytic core in GTF enzymes, is absent in GTFB. This GTFB region also contains many gaps and overall very poor similarity when compared to other GTF enzymes. Truncations in this region of GTFI from *Streptococcus downei* Mfe28 resulted in drastic loss of enzyme activity (Monchois *et al.*, 1999b).

GTFML4 showed high similarity with GTFB of *Lb. reuteri* 121 (Table 3), including the differences with other GTF enzymes listed above. Furthermore, the organization of *gtfML1* and *gtfML4* on the genome of *Lb. reuteri* ML1 was similar to that of *gtfA* and *gtfB* from *Lb. reuteri* 121 (Fig. 1). Consequently, no further efforts were made to clone the full-length *gtfML4* gene. The identical nature of the glucans produced by *Lb. reuteri* ML1 and by the purified recombinant GTFML1 was confirmed by methylation analysis (Table 4). It appears likely that under the conditions tested *gtfML4* also is not active, as is the case with GTFB.

Analysis of glucans produced by *Lactobacillus* GTFs, N-terminally truncated GTFs and full-length recombinant GTFs

Supernatants of sucrose-grown cultures of the different *Lactobacillus* strains, His-tag-purified truncated and full-length recombinant GTFs from *E. coli* extracts were incubated with sucrose and the soluble glucans produced were purified. Methylation analysis showed that the soluble glucans produced by the five *Lactobacillus* strains and the corresponding recombinant GTF enzymes were virtually identical (Table 4). The polymers produced by the different *Lactobacillus* strains were large in size, ranging from $0{\cdot}2\times10^6$ to 50×10^6 Da (Table 4). Previous work showed that deletion of the N-terminal variable domain of GTFA from *Lb. reuteri* 121 and GTFI from *S. downei* MFe28 has no effect on polymer size (only determined for GTFA) and linkage-type distribution (Monchois *et al.*, 1999a; Kralj *et al.*, 2004). To facilitate cloning and reduce enzyme sizes, some of the GTF enzymes were produced without this variable region (ΔN, Table 2). This yielded active GTF enzymes, which synthesized virtually the same glucans as the wild-type *Lactobacillus* strains (Table 4). As previously reported (Kralj *et al.*, 2002), GTFA of *Lb. reuteri* 121 synthesized a reuteran [mainly α-(1→4) linkages]. Three *Lactobacillus* strains, Kg3, Kg15 and 33, and their GTF enzymes were characterized as producing dextran- [mainly α-(1→6) linkages] like polymers. Strain *Lb. reuteri* ML1 and the GTFML1 enzyme produced a highly branched mutan- [mainly α-(1→3) linkages] like polymer. Strain *Lb. reuteri* 180 and GTF180 produced a polymer containing large amounts of α-(1→6)-glucosidic linkages and lower

Table 4. Methylation analysis and molecular masses of the glucans produced from sucrose by GTF enzymes in supernatants of *Lactobacillus* strains (LB) and by His-tag-purified complete (rec) or N-terminally truncated (tru) GTF enzymes from *E. coli*

GTFB from *Lb. reuteri* 121 was inactive under the conditions used in this study.

Linkage type	121*			ML1		180			Kg15		Kg3		33		
	LB	rec	tru	LB	tru	LB	rec	tru	LB	rec	LB	rec	LB	rec	tru
	Methylation (%)														
Terminal	9	11	9	18	17	10	7	8	4	5	3	5	6	3	4
α-(1→3)				47	48	26	32	26	1	1	1	0	9	18	17
α-(1→4)	49	46	46												
α-(1→6)	26	26	34	10	10	51	52	54	86	85	89	87	75	71	70
α-(1→3,6)				26	25	13	10	12	9	9	7	7	9	8	9
α-(1→4,6)	15	17	12												
	$10^{-6}\times$ Molecular mass (Da)														
	40	45	50	8	ND	36	ND	ND	27	ND	24	ND	0·2	ND	ND

ND, Not determined.

*GTFA from *Lb. reuteri* 121 was used as a reference (Kralj *et al.*, 2002, 2004).

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amounts of α-(1→3)-linked glucosyl units (most likely a dextran with large amounts of α-(1→3 linkages).

Conclusions

This paper reports the first examples, we believe, of isolation and characterization of dextransucrase and mutansucrase genes/enzymes, and dextran/mutan products from *Lactobacillus* species. The genus *Lactobacillus* thus contains the same variety of *gtf* genes, GTF enzymes and glucan products as found within the genera *Leuconostoc* and *Streptococcus*, plus the ability to synthesize reuteran (reuteransucrase of *Lb. reuteri* 121). GTFA, GTF180 and GTFML1 are highly similar (Table 3) but synthesize glucans with different glucosidic linkages. Thus these enzymes are very interesting candidates for structure/function studies aiming to identify amino acid residues responsible for glucosidic-bond specificity.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Arguello-Morales, M. A., Remaud-Simeon, M., Pizzut, S., Sarcabal, P. & Monsan, P. (2000). Sequence analysis of the gene encoding alternansucrase, a sucrose glucosyltransferase from *Leuconostoc mesenteroides* NRRL B-1355. *FEMS Microbiol Lett* **182**, 81–85.

Ausubel, F. M., Brent, R. E., Kingston, D. D., Moore, J. G., Seidman, J. G., Smith, J. A. & Struhl, K. (1987). *Current Protocols in Molecular Biology*. New York: Wiley.

Axelsson, L. (1998). Lactic acid bacteria: classification and physiology. In *Lactic Acid Bacteria: Microbiology and Functional Aspects*, pp. 1–71. Edited by W. Salminen & A. von Wright. New York: Marcel Dekker.

Birnboim, H. C. & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* **7**, 1513–1523.

Bozonnet, S., Dols-Laffargue, M., Fabre, E., Pizzut, S., Remaud-Simeon, M., Monsan, P. & Willemot, R. M. (2002). Molecular characterization of DSR-E, an α-1,2 linkage-synthesizing dextransucrase with two catalytic domains. *J Bacteriol* **184**, 5753–5761.

Casas, I. A., Edens, F. W. & Dobrogosz, W. J. (1998). *Lactobacilus reuteri*: an effective probiotic for poultry and other animals. In *Lactic Acid Bacteria: Microbiology and Functional Aspects*, pp. 475–518. Edited by W. Salminen & A. von Wright. New York: Marcel Dekker.

Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiol Rev* **7**, 113–130.

Chaplin, M. F. (1982). A rapid and sensitive method for the analysis of carbohydrate components in glycoproteins using gas-liquid chromatography. *Anal Biochem* **123**, 336–341.

Chung, C. H. & Day, D. F. (2002). Glucooligosaccharides from *Leuconostoc mesenteroides* B-742 (ATCC 13146): a potential prebiotic. *J Ind Microbiol Biotechnol* **29**, 196–199.

Devulapalle, K. S., Goodman, S. D., Gao, Q., Hemsley, A. & Mooser, G. (1997). Knowledge-based model of a glucosyltransferase from the oral bacterial group of mutans streptococci. *Protein Sci* **6**, 2489–2493.

De Vuyst, L. & Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol Rev* **23**, 153–177.

Funane, K., Mizuno, K., Takahara, H. & Kobayashi, M. (2000). Gene encoding a dextransucrase-like protein in *Leuconostoc mesenteroides* NRRL B-512F. *Biosci Biotechnol Biochem* **64**, 29–38.

Funane, K., Ishii, T., Matsushita, M., Hori, K., Mizuno, K., Takahara, H., Kitamura, Y. & Kobayashi, M. (2001). Water-soluble and water-insoluble glucans produced by *Escherichia coli* recombinant dextransucrases from *Leuconostoc mesenteroides* NRRL B-512F. *Carbohydr Res* **334**, 19–25.

Ganzle, M. G., Holtzel, A., Walter, J., Jung, G. & Hammes, W. P. (2000). Characterization of reutericyclin produced by *Lactobacillus reuteri* LTH2584. *Appl Environ Microbiol* **66**, 4325–4333.

Giffard, P. M. & Jacques, N. A. (1994). Definition of a fundamental repeating unit in streptococcal glucosyltransferase glucan-binding regions and related sequences. *J Dent Res* **73**, 1133–1141.

Hakomori, S. (1964). A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *J Biochem* **55**, 205–208.

Hamada, S. & Slade, H. D. (1980). Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiol Rev* **44**, 331–384.

Hanada, N., Fukushima, K., Nomura, Y., Senpuku, H., Hayakawa, M., Mukasa, H., Shiroza, T. & Abiko, Y. (2002). Cloning and nucleotide sequence analysis of the *Streptococcus sobrinus gtfU* gene that produces a highly branched water-soluble glucan. *Biochim Biophys Acta* **1570**, 75–79.

Harris, P. J., Henry, R. J., Blakeney, A. B. & Stone, B. A. (1984). An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr Res* **127**, 59–73.

Janecek, S., Svensson, B. & Russell, R. R. (2000). Location of repeat elements in glucansucrases of *Leuconostoc* and *Streptococcus* species. *FEMS Microbiol Lett* **192**, 53–57.

Jansson, P.-E., Kenne, L., Liedgren, H., Lindberg, B. & Lönngren, J. (1976). A practical guide to the methylation analysis of carbohydrates. *Chem Commun Univ Stockholm* **8**, 1–74.

Kabuki, T., Saito, T., Kawai, Y., Uemura, J. & Itoh, T. (1997). Production, purification and characterization of reutericin 6, a bacteriocin with lytic activity produced by *Lactobacillus reuteri* LA6. *Int J Food Microbiol* **34**, 145–156.

Kim, D. & Robyt, J. F. (1994). Production and selection of mutants of *Leuconostoc mesenteroides* constitutive for glucansucrases. *Enzyme Microb Technol* **16**, 659–664.

Kitaoka, M. & Robyt, J. F. (1998). Use of microtiter plate screening method for obtaining *Leuconostoc mesenteroides* mutants constitutive for glucansucrase. *Enzyme Microb Technol* **22**, 527–531.

Konishi, N., Torii, Y., Yamamoto, T. & 8 other authors (1999). Structure and enzymatic properties of genetically truncated forms of the water-insoluble glucan-synthesizing glucosyltransferase from *Streptococcus sobrinus*. *J Biochem* **126**, 287–295.

Kralj, S., van Geel-Schutten, G. H., Rahaoui, H., Leer, R. J., Faber, E. J., van der Maarel, M. J. & Dijkhuizen, L. (2002). Molecular characterization of a novel glucosyltransferase from *Lactobacillus reuteri* strain 121 synthesizing a unique, highly branched glucan with α-(1→4) and α-(1→6) glucosidic bonds. *Appl Environ Microbiol* **68**, 4283–4291.

Kralj, S., van Geel-Schutten, G. H., van der Maarel, M. J. & Dijkhuizen, L. (2003). Efficient screening methods for glucosyltransferase genes in *Lactobacillus* strains. *Biocatal Biotransform* **21**, 181–187.

Kralj, S., van Geel-Schutten, G. H., van der Maarel, M. J. & Dijkhuizen, L. (2004). Biochemical and molecular characterization of *Lactobacillus reuteri* 121 reuteransucrase. *Microbiology* **150**, 2099–2112.

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Leroy, F. & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol* **15**, 67–78.

Loesche, W. J. (1986). Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* **50**, 353–380.

Monchois, V., Arguello-Morales, M. A. & Russell, R. R. (1999a). Isolation of an active catalytic core of *Streptococcus downei* MFe28 GTF-I glucosyltransferase. *J Bacteriol* **181**, 2290–2292.

Monchois, V., Vignon, M. & Russell, R. R. (1999b). Isolation of key amino acid residues at the N-terminal end of the core region *Streptococcus downei* glucansucrase, GTF-I. *Appl Microbiol Biotechnol* **52**, 660–665.

Monchois, V., Willemot, R. M. & Monsan, P. (1999c). Glucansucrases: mechanism of action and structure-function relationships. *FEMS Microbiol Rev* **23**, 131–151.

Monsan, P. & Paul, F. (1995). Oligosaccharide feed additives. In *Biotechnology in Animal Feeds and Animal Feeding*. pp. 233–245. Edited by R. J. Wallace & A. Chesson. Weinheim: VCH.

Nagy, I., Schoofs, G., Compernolle, F., Proost, P., Vanderleyden, J. & de Mot, R. (1995). Degradation of the thiocarbamate herbicide EPTC (S-ethyl dipropylcarbamothioate) and biosafening by *Rhodococcus* sp. strain NI86/21 involve an inducible cytochrome P-450 system and aldehyde dehydrogenase. *J Bacteriol* **177**, 676–687.

Neubauer, H., Bauche, A. & Mollet, B. (2003). Molecular characterization and expression analysis of the dextransucrase DsrD of *Leuconostoc mesenteroides* Lcc4 in homologous and heterologous *Lactococcus lactis* cultures. *Microbiology* **149**, 973–982.

Olano-Martin, E., Mountzouris, K. C., Gibson, G. R. & Rastall, R. A. (2000). In vitro fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. *Br J Nutr* **83**, 247–255.

Sambrook, J., Fritsch, E. F. & Maniates, T. (1989). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sidebotham, R. L. (1974). Dextrans. *Adv Carbohydr Chem Biochem* **30**, 371–444.

Smith, M. R., Zahnley, J. C., Wong, R. Y., Lundin, R. E. & Ahlgren, J. A. (1998). A mutant strain of *Leuconostoc mesenteroides* B-1355 producing a glucosyltransferase synthesizing $\alpha(1\rightarrow2)$ glucosidic linkages. *J Ind Microbiol Biotechnol* **21**, 37–45.

Talarico, T. L., Casas, I. A., Chung, T. C. & Dobrogosz, W. J. (1988). Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. *Antimicrob Agents Chemother* **32**, 1854–1858.

Tieking, M., Korakli, M., Ehrmann, M. A., Ganzle, M. G. & Vogel, R. F. (2003). In situ production of exopolysaccharides during Sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Appl Environ Microbiol* **69**, 945–952.

Triglia, T., Peterson, M. G. & Kemp, D. J. (1988). A procedure for *in vitro* amplification of DNA segments that lie outside the boundaries of known sequences. *Nucleic Acids Res* **16**, 8186.

Valeur, N., Engel, P., Carbajal, N., Connolly, E. & Ladefoged, K. (2004). Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol* **70**, 1176–1181.

van Geel-Schutten, G. H. (2003). Novel glucans and novel glucansucrases derived from lactic acid bacteria. Patent number: WO 03/008618.

van Geel-Schutten, G. H., Flesch, F., ten Brink, B., Smith, M. R. & Dijkhuizen, L. (1998). Screening and characterization of *Lactobacillus* strains producing large amounts of exopolysaccharides. *Appl Microbiol Biotechnol* **50**, 697–703.

van Geel-Schutten, G. H., Faber, E. J., Smit, E., Bonting, K., Smith, M. R., Ten Brink, B., Kamerling, J. P., Vliegenthart, J. F. & Dijkhuizen, L. (1999). Biochemical and structural characterization of the glucan and fructan exopolysaccharides synthesized by the *Lactobacillus reuteri* wild-type strain and by mutant strains. *Appl Environ Microbiol* **65**, 3008–3014.

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