Screening of lactic acid bacteria from Indonesia reveals glucansucrase and fructansucrase genes in two different Weissella confusa strains from soya

Amarila Malik1, Maksum Radji1, Slavko Kralj2,3 & Lubbert Dijkhuizen2,3

1Laboratory of Microbiology and Biotechnology, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, Depok, Indonesia; 2Centre for Carbohydrate Bioprocessing (CCB), TNO-University of Groningen, Haren, The Netherlands and; 3Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Kerklaan, Haren, The Netherlands

Correspondence: Amarila Malik, Laboratory of Microbiology and Biotechnology, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia. Tel.: +62 21 727 0031; fax: +62 21 786 3433; e-mail: amarila.malik@ui.ac.id

Present address: Slavko Kralj, Genencor, A Danisco Division, Archimedesweg 30, 2333 CN Leiden, The Netherlands.

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Abstract
Homopolysaccharide (glucan and fructan) synthesis from sucrose by sucrase enzymes in lactic acid bacteria (LAB) has been well studied in the genera Leuconostoc, Streptococcus and Lactobacillus. This study aimed to identify and characterize genes encoding glucansucrase/glucosyltransferase (GTF) and fructansucrases/fructosyltransferase (FTF) enzymes from genomic DNA of ‘rare’ Indonesian exopolysaccharide-producing LAB. From a total of 63 exopolysaccharide-producing LAB isolates obtained from foods, beverages and environmental samples, 18 isolates showing the most slimy and mucoid colony morphologies on sucrose were chosen for further study. By comparing bacterial growth on De Man, Rogosa and Sharpe (MRS)-sucrose with that on MRS-raffinose, and using the results of a previous PCR screening study with degenerate primer pairs targeting the conserved catalytic domain of GTFs, various strains were identified as producers of fructan (13), of glucan only (five) or as potential producers of both glucan and fructan (nine). Here, we report the characteristics of three gtf genes and one ftf gene obtained from Weissella confusa strains MBF8-1 and MBF8-2. Strain MBF8-1 harbored two putative gtf genes with high sequence similarity to GTFB of Lactobacillus reuteri 121 and GTF180 of L. reuteri 180, respectively. Strain MBF8-2 possessed single gtf and ftf genes with high sequence similarity to GTFKg3 of Lactobacillus fermentum Kg3 and DSRWC of Weissella cibaria, and FTF levansucrase of L. reuteri 121, respectively.

Introduction
Homopolysaccharides produced by lactic acid bacteria (LAB) are made up of one monosaccharide type, for example glucose (α-glucans) or fructose (β-fructans), and are synthesized from sucrose by a single, extracellular or cell-associated enzyme, depending on the monosaccharide, known as glucosyltransferases (GTFs) (E.C. 2.4.1.5; common name glucansucrase) and fructosyltransferases (FTFs) (E.C. 2.4.1.10; common name fructansucrase), respectively (Monsan et al., 2001; Van Hijum et al., 2006). The LAB genera Leuconostoc and Streptococcus are well-known producers of α-glucans (and to a lesser extent also of β-fructans). In recent years, members of the genus Lactobacillus have also been identified as producers of a range of α-glucans (Van Geel-Schutten et al., 1998; Van Hijum et al., 2006; Van der Meulen et al., 2007), as well as of fructans (Van Hijum et al., 2006; Anwar et al., 2008).

At present, five different groups of glucans (glucose as monomer) can be distinguished, i.e. dextran (with α-1,6 glucosidic bonds), mutan (with α-1,3 glucosidic bonds), alternan (with α-1,3 and 1,6 alternating glucosidic bonds), reuteran (with α-1,4 glucosidic bonds) and glucan polymers containing large amounts of α-1,2 linkages (Bozonnet et al., 2002; Van Hijum et al., 2006). In addition, two fructan types are known, i.e. levan [with β-2,6 glycosidic bonds, produced by levansucrases (Lev, E.C. 2.4.1.10; van Hijum et al., 2004)] and inulin (with β-2,1 bonds), synthesized by inulosucrase (Inu, E.C. 2.4.1.9; Van Hijum et al., 2003, 2006).
Forty-seven different GTFs have been classified in the glycoside hydrolase family 70 (http://www.cazy.org) to date. These glucansucrase enzymes display high sequence similarity. Nevertheless, they are able to synthesize glucans that differ considerably in the type of glucosidic linkages present, the degree and type of branching and molecular mass. There are indications that glucan and fructan synthesis, as reported previously for Leuconostoc, Streptococcus and Lactobacillus spp., occurs more widely in LAB genera (Van Hijum et al., 2006), for example in Weissella cibaria/Weisella confusa strains (Tieking et al., 2003; Di Cagno et al., 2006; Van der Meulen et al., 2007; Kang et al., 2009). Attempts to identify the sucrase enzyme-encoding genes involved, and the linkage types of the glucans and fructans produced, have remained limited (Kang et al., 2009).

Weisella strains may thus produce novel exopolysaccharide products, potentially with new combinations of glycosidic bonds, molecular mass and degree of branching, providing excellent opportunities for new applications in the pharmaceutical, health, cosmetic and food industries. Dextran and inulin are widely applied in industry, for example in pharmaceutical technology to stabilize therapeutic proteins/peptides during drying and subsequent storage (Hinrichs et al., 2001).

Weisella strains have been isolated from a variety of sources, for example fresh vegetables, silage fermentation (Dellaglio et al., 1984; Dellaglio & Torriani, 1986), and have been commonly associated with meat or meat products (Niven & Evans, 1957; Milbourne, 1983; Collins et al., 1993), as well as being detected in sugar cane, carrot juice and occasionally in raw milk and sewage (Hammes & Vogel, 1995). They have also been isolated from the sludge of milking machines, and from traditionally fermented foods (Kandler et al., 1983; Ampe et al., 1999; Paludan-Muller et al., 1999), and as contaminants in nonfermented foods from Southeast Asia (Leisner et al., 1997, 1999). Here, we report the molecular characteristics of various sucrase-encoding genes (gtf, ftf) harbored by two Weisella strains isolated from homemade soya by screening a collection of Indonesian exopolysaccharide-producing LAB. In a previous study, using degenerate PCR primers for gtf, we observed that these Weisella strains harbored gtf gene/s on their genomic DNA (Malik et al., 2008). Here, we show that, by visual inspection for exopolysaccharide production on indicator media, as well as by molecular characterization of partial gene fragments, strains of LAB potentially producing glucan or fructan only, or glucan plus fructan, can be distinguished. This clearly increases our knowledge of the diversity of sucrase-encoding LAB genes available for genetic engineering and production of novel polysaccharides. Further work is in progress on the characterization of these Weisella GTF and FTF enzymes and their exopolysaccharide products.

Materials and methods

Bacterial strains and growth conditions

All LAB strains used in this study were obtained from the culture collection of the Laboratory of Microbiology and Biotechnology, Department of Pharmacy, Universitas Indonesia (Depok, Indonesia). They were isolated previously from various sources, i.e. foods, beverages and environmental samples collected from Indonesia (Malik et al., 2007). Solid samples were weighed and resuspended in sterile water and were subsequently diluted $10^{-3}$, $10^{-4}$ and $10^{-6}$ times, whereas liquid samples were directly diluted $10^{-3}$, $10^{-4}$ and $10^{-6}$ times as well. Both samples were spread onto a De Man, Rogosa and Sharpe (MRS) modified agar medium with 1% sucrose supplemented with 0.01% cycloheximide.

All LAB strains were cultivated at 32 °C in MRS (De Man et al., 1960) medium (Difco, Franklin Lakes, NJ) or in MRS-s medium (i.e. MRS-medium with 100 g L$^{-1}$ sucrose instead of 20 g L$^{-1}$ glucose) for exopolysaccharide producer screening. Agar plates were prepared by adding 1.5% agar to the MRS medium. Escherichia coli TOP 10 was used as the host for cloning and for DNA sequencing, and was grown aerobically at 37 °C in Luria–Bertani (LB) medium, and 1.5% agar was added for LB agar medium (Difco). Strains containing recombinant plasmids were cultivated in LB medium with the appropriate antibiotic (50 µg mL$^{-1}$ kanamycin).

DNA extraction, PCR and DNA sequencing

All genomic DNA extractions were performed according to Kralj et al. (2002). Other standard DNA manipulation protocols, i.e. digestion, ligation and transformation, were performed as described (Sambrook et al., 1989). All oligonucleotide primers used in this study are listed in Table 1. Degenerate oligonucleotides used in this study (Table 1), targeting regions encoding catalytic domains of known bacterial gtf and ftf genes, were designed as described (Van Hijum et al., 2002). Degenerate oligonucleotide primers were used for cloning gtf and ftf gene segments encoding catalytic domains, and 16S rRNA gene of the bacterial isolates.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DegFor</td>
<td>5’-GAYAYWSNAAYCNCNYNCTC-3’</td>
<td>Kralj et al. (2002)</td>
</tr>
<tr>
<td>DegRev</td>
<td>5’-ADRTCNCCRTARTAVNAYKNG-3’</td>
<td>Kralj et al. (2002)</td>
</tr>
<tr>
<td>5FTF</td>
<td>5’-GAYGNTGGGAYWSNNTGCCC-3’</td>
<td>Van Hijum et al. (2002)</td>
</tr>
<tr>
<td>6FTFi</td>
<td>5’-GNTGNCWNCCNWCCAYTSYTG-3’</td>
<td>Van Hijum et al. (2002)</td>
</tr>
<tr>
<td>LABfw</td>
<td>5’-AGAGHTTGGATYDTGCGCTCAG-3’</td>
<td>Heilig et al. (2002)</td>
</tr>
<tr>
<td>LABrv</td>
<td>5’-CACCGCTACATGAGGAG-3’</td>
<td>Heilig et al. (2002)</td>
</tr>
</tbody>
</table>

Y=T or C, K=G or T, W=A or T, S=C or G, R=A or G, D=A or C, N=inosin.
Hijum et al., 2002; Kralj et al., 2003). In a previous study, DegRev and DegFor were used to screen a collection of exopolysaccharide-producing LAB strains for the presence of gtf genes (Malik et al., 2008). Both gtf and ftf gene segments obtained were cloned into the XL-TOPO cloning system (Invitrogen, Carlsbad, CA) using E. coli TOP 10 as the host for cloning and DNA sequencing purposes. PCR conditions for identification of gtf genes have been described by Kralj et al. (2003). PCR for amplification of ftf genes was performed using the following conditions: denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (58 °C for 30 s) and elongation (72 °C for 45 s), and a final elongation step of (72 °C for 3 min). The PCR reaction was performed by incubation for 5 min at 95 °C, 5 U EX Taq DNA polymerase (TaKaRa, Japan) was added, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (58 °C for 30 s) and elongation (72 °C for 45 s). The PCR mixture contained 10 mM Tris/HCl (pH 8.5), 3 mM MgCl₂, 2 mM of each dNTP, 0.25 µg genomic DNA from each LAB strain as a template, 10 pmol of the primers 5FTF and 6FTFi and 5 U High Fidelity PCR Enzyme Mix (Fermentas, Canada) (Table 1). From randomly chosen clones, recombinant plasmids were isolated and as a first analysis digested with Nsil and NotI. Based on the difference in restriction pattern, plasmids were picked and analyzed further by DNA sequencing (GATC, Konstanz, Germany). The DNA sequence information obtained was analyzed by BLAST. In addition, the sucrase genes were further studied using CLONE MANAGER SUITE® to identify ORFs and for translation purposes, followed by aligning with known sucrase enzymes, downloaded from databases (http://www.cazy.org/), using CLUSTALX.

Nucleotide sequence accession numbers

Eighteen 0.7-kb sequences of the 16S rRNA gene determined in this study were deposited with the GenBank database under accession numbers GQ456930–GQ456942 and GQ466165–GQ466169. The sucrase sequences obtained in this study are available under GenBank accession numbers FJ436354 (gtf8-1A), FJ460018 (gtf8-1B), FJ478145 (gtf8-2) and FJ485940 (ftf8-2).

Visual inspection for FTF activity on MRS-raffinose

MRS medium modified with 5% raffinose (MRS-r), i.e. MRS-medium with 50 g L⁻¹ raffinose (Merck), was used to visualize FTF enzyme activity [raffinose, (galactose–glucose–fructose) is not a substrate for glucansucrase enzymes] apparent from the synthesis of exopolysaccharides (fructan). Also, MRS modified medium supplemented with 1% glucose and 5% raffinose (MRS-raff+glu) was used because both Weissella strains showed poor growth on MRS-raffinose. Agar plates were prepared by adding 1.5% agar to the MRS medium. After 24 h of incubation at 32 °C, the production of exopolysaccharide was apparent from the slimy and mucoid colonies on the plate; Lactobacillus reuteri 121 was used as a positive control (Van Hijum et al., 2002, 2004).

Species identification by 16S rRNA gene PCR

A pair of primers highly specific for targeting the gene coding for the 16S rRNA gene of LAB were used for PCR: forward primer LABfw (5'-D-Bact-0011-a-S-17) 5'-AGA GTT GTA TYD TGG CTC AG-3' and reverse primer LABrv (5'-G-Lab-0677-a-A-17) 5'-CAC CGC TAC ACA TGG AG-3' (corresponding to region 677–693 of E. coli 16S rRNA gene numbering) (Heilig et al., 2002) (Table 1). The PCR reaction was performed by incubation for 5 min at 95 °C, 5 U Taq polymerase (TaKaRa, Japan) was added, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (58 °C for 30 s) and elongation (72 °C for 45 s). The PCR mixture contained 10 mM EX Taq buffer, 3 mM MgCl₂, 2 mM of each dNTP, 0.25 µg genomic DNA from each LAB strain (genomic DNAs obtained previously were used) and 20 pmol of each primer. Approximately 700-bp amplicons were obtained and were purified for DNA sequencing using LABfw and LABrv primers. The DNA sequence information obtained was analyzed by BLAST http://www.ncbi.nlm.nih.gov/BLAST) for species identification.

Results and discussion

A total of 63 exopolysaccharide-producing LAB isolates from foods, beverages and environmental samples from Indonesia, i.e. Jakarta and Tangerang, were obtained in our previous study (Malik et al., 2007). Out of 29 isolates that possessed the most slimy and mucoid colonies on sucrose, 18 isolates were randomly chosen for determination of glucan and/or fructan production (Table 2). All 18 isolates were identified at the species level by PCR of 16S rRNA gene using primer pairs LABfw 5'-AGA GTT GTA TYD TGG CTC AG-3' and LABrv 5'-CAC CGC TAC ACA TGG AG-3' (Heilig et al., 2002; Malik et al., 2007) (Tables 1 and 2). From visual inspection on MRS-s, MRS-r and MRS-r+g, we concluded that 13 out of 18 strains possessed FTF activity. Interestingly, nine of these strains also encode a glucansucrase, apparent from PCR screening with degenerate primer pairs targeting a conserved catalytic domain of GTFs, DegFor and DegRev (Table 2). Five other strains only encode a glucansucrase, apparent from a positive gtf score (Table 2). Because these five strains are FTF negative, but score positive for exopolysaccharide formation on MRS-s, they most likely produce an α-glucan polymer. Two interesting strains, MBF8-1 and MBF8-2, identified as W. confusa, were studied in more detail. These W. confusa strains had been isolated from homemade soy from Pamulang home industry, Tangerang, Indonesia. Both strains formed ropy and slimy colonies on MRS-sucrose. Only strain MBF8-2 scored...
positive for the presence of an active FTF enzyme (MRS-r; Table 2). From the screening with degenerate PCR for gtf genes, we concluded that these two W. confusa strains harbored at least one gtf gene on their genomic DNA (Table 2; Malik et al., 2008). We subsequently attempted to clone gtf and ftf gene fragments for further confirmation of their presence and identity.

The PCR products obtained by degenerate primers for glucansucrase and fructansucrase genes using genomic DNA of both strains MBF8-1 and MBF8-2 were first ligated into pCR-XL-TOPO (Invitrogen), and then transformed to E. coli TOP10. Plasmid DNA was isolated from four random clones from each transformation. Restrictions of the different recombinant plasmids were carried out with NsiI and NotI as first screening to identify differences between the plasmids. DNA sequence analysis confirmed the presence of partial putative gtf and ftf genes. Strain MBF8-1 showed at least two gtf genes, while MBF8-2 showed only one gtf gene and one ftf gene. DegFor and DegRev primers, as listed in Table 1, for gtf gene screening used in this study were shown to allow the identification of more than one gtf gene in a single strain (Kralj et al., 2003).

Three partial sequences of putative gtf genes, and one of a putative ftf gene, were identified in these W. confusa strains and characterized by DNA sequencing, followed by BLAST analysis. Strain MBF8-1 harbored two putative gtf genes, the predicted amino acid sequences of their products, designated as GTF8-1A (FJ436354) and GTF8-1B (FJ460018), possessed high sequence identity (99% identity, 100% similarity) to the GTF8-1B enzyme of L. reuteri 180 and to GTFB (83% identity, 87% similarity) of L. reuteri 121 (Kralj et al., 2004) (see the alignment in Fig. 1). Only a single gtf gene was detected in strain MBF8-2; the predicted amino acid sequence of its product, designated as GTF8-2 (FJ478145), had high sequence identity to GTFKg3 of Lactobacillus fermentum Kg3 (90% identity, 94% similarity) (see the alignment in Fig. 1). Also, the recently characterized W. cibaria glucansucrase exhibited the highest similarity to L. fermentum GTFKg3 (Kang et al., 2009).

Only a single partial ftf gene was obtained from strain MBF8-2 using ftf degenerate primers 5FTF and 6FTFi (Table 1). Alignment of the predicted amino acid sequence of its product, designated as FT8-2 (FJ485940), with FTF proteins of other LAB revealed the highest similarity (100% identity within 64 amino acids) to levansucrase of L. reuteri 121 (Van Hijum et al., 2004) (Fig. 2). The available FTF amino acid sequence information does not yet allow straightforward identification of FTF enzymes as inulosucrase or levansucrase enzymes (Van Hijum et al., 2006; Anwar et al., 2008). Strain MBF8-2 FTF thus needs to be subjected to further analysis of products synthesized from sucrose/raffinose. Identification and characterization of inulosucrase/levansucrase enzymes from various alternative sources (as initiated in the present study), combined with

### Table 2. Screening of LAB strains by visual inspection for exopolysaccharide production on MRS agar plates*, and by PCR for conserved regions in gtf genes

<table>
<thead>
<tr>
<th>Strains1</th>
<th>% Identity</th>
<th>MRS-s*</th>
<th>MRS-r/</th>
<th>MRS-r+g*</th>
<th>PCR gtf (Malik et al., 2008)</th>
<th>Origin (Malik et al., 2007; unpublished data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. ps. mesenteroides MBF2-1</td>
<td>99</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Mixed pickles (fd)</td>
</tr>
<tr>
<td>L. mesenteroides MBF2-2</td>
<td>92</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Mixed pickles (fd)</td>
</tr>
<tr>
<td>L. mesenteroides MBF2-5</td>
<td>99</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Mixed pickles (fd)</td>
</tr>
<tr>
<td>L. mesenteroides MBF3-1</td>
<td>100</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Sekoteng (bev.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF3-2</td>
<td>100</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Sekoteng (bev.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF3-5</td>
<td>80</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Sekoteng (bev.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF4-2</td>
<td>96</td>
<td>+</td>
<td>+</td>
<td>NI</td>
<td>+</td>
<td>Soil (env.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF4-4</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>NI</td>
<td>+</td>
<td>Soil (env.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF5-4</td>
<td>99</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Soil (env.)</td>
</tr>
<tr>
<td>L. pseudo mesenteroides MBF6-9</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Soil (env.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF7-5</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>NI</td>
<td>+</td>
<td>Soil (env.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF7-8</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>NI</td>
<td>+</td>
<td>Soil (env.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF7-17</td>
<td>99</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Soil (env.)</td>
</tr>
<tr>
<td>Weissella confusa MBF8-1</td>
<td>90</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Soya (bev.)</td>
</tr>
<tr>
<td>Weissella confusa MBF8-2</td>
<td>96</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Soya (bev.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF9-2</td>
<td>89</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Tofu waste (env.)</td>
</tr>
<tr>
<td>Streptococcus macedonicus MBF10-2</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Tofu waste (env.)</td>
</tr>
<tr>
<td>L. mesenteroides MBF11-2</td>
<td>100</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Soy sauce waste (env.)</td>
</tr>
</tbody>
</table>

*Visual inspection for appearance of slamy and mucoid colonies on MRS-s agar plates indicating glucansucrase and/or fructansucrase activity, and on MRS-r and MRS-r+g indicating fructansucrase activity exclusively.

1Species were named according to their closest relative.

+/−, positive or negative score for production of exopolysaccharide on MRS agar plate; NI, not identified; fd, food; bev., beverage, env., environment.
high-resolution protein structural information for inulosucrase/levansucrase proteins, appear to be excellent further steps to provide such structure/function information in the future.

The presence of an FTF activity in MBF8-2 was supported by the visual observation of exopolysaccharide formation using raffinose as a substrate; in comparison, MBF 8-1 scored as an FTF-negative strain. However, these strains showed relatively poor growth on MRS-raffinose. Using a modified MRS-raffinose supplemented with 1% glucose, the strains grew well and we were able to observe the exopolysaccharide product formation. This exopolysaccharide formation was raffinose dependent, i.e. was not observed with MRS-g. Besides sucrose, FTF (i.e. levansucrase) enzymes also utilize this trisaccharide D-raffinose \[a\text{Gal(1-6)}a\text{Glc}\] as a fructosyl donor by cleaving the glycosidic bond between glucose and fructose and using the energy to couple a fructosyl unit to (1) a growing fructan (polyfructan) chain, (2) to sucrose, (3) to water (hydrolysis) or (4) to other acceptors (various mono- and disaccharides) (Meng & Futterer, 2008). The products resulting from levansucrase-catalyzed hydrolysis of raffinose are melibiose \[a\text{Gal(1-6)}a\text{Glc}\] and free fructose (Hernandez et al., 1995).

**Fig. 1.** Amino acid sequence alignment of putative GTF enzymes (partial) from *Weissella confusa* strains MBF8-1 and MBF8-2 (bold) to various glucansucrase enzymes of LAB. * Identical residue; (:), highly conserved residue; (.), conserved residue. Putative catalytic residues are shown in gray scale. GenBank protein accession numbers of the different glucansucrase enzymes are indicated.

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### Bacterial strain Enzyme GenBank

| W. confusa MBF8-1 | GTF8-1A | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| L. reuteri 180 | GTF180 | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| W. eibarisa CMU | GTFML1 | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| L. reuteri 121 | GTF1A | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| L. reuteri ATCC 55730 | GTF1A | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| W. confusa MBF8-2 | GTF8-2 | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| L. fermentum Kg3 | GTFKg3 | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| W. eibarisa CMU | GTFML1 | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| L. reuteri 121 | GTF1A | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |
| L. reuteri ATCC 55730 | GTF1A | AEELWLYLYNGFPDTTTTQPIAENGFDVRAVNVDDVTVLIAKRDHYANAYAAYMQQSLN | AC64929 |

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**W. confusa MBF8-1**

- **GTF8-1A**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929
- **GTF8-1B**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929

**L. reuteri 180**

- **GTF180**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929
- **GTFML1**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929

**L. reuteri 121**

- **GTF1A**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929
- **GTFML1**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929

**W. confusa MBF8-2**

- **GTF8-2**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929
- **GTFML1**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929

**L. fermentum Kg3**

- **GTFKg3**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929
- **GTFML1**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929

**W. eibarisa CMU**

- **GTFML1**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929

**W. confusa MBF8-1**

- **GTF8-1B**: ANKNIHILEDWMMPPAYNKNQQNLNLCLMNDQMAQLSSKSLNMLSTSLTQLSNL | AC64929

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**W. confusa MBF8-1**

- **GTF8-1A**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929
- **GTF8-1B**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929

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**W. confusa MBF8-2**

- **GTF8-2**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929
- **GTFML1**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929

**W. fermentum Kg3**

- **GTFKg3**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929
- **GTFML1**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929

**W. eibarisa CMU**

- **GTFML1**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929

**W. confusa MBF8-1**

- **GTF8-1B**: RNACNTANAV1PSYNVFRAHNSNQDIKIQAA7TCPYCO----EFNLDI4564 | AC64929

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**W. confusa MBF8-1**

- **GTF8-1A**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929
- **GTF8-1B**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929

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**W. confusa MBF8-2**

- **GTF8-2**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929
- **GTFML1**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929

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**W. fermentum Kg3**

- **GTFKg3**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929
- **GTFML1**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929

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**W. eibarisa CMU**

- **GTFML1**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929

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**W. confusa MBF8-1**

- **GTF8-1B**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929
- **GTFML1**: INDQDSNKNWNYMPAAYTIL1TNXSDY | AC64929

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**Fig. 1.** Amino acid sequence alignment of putative GTF enzymes (partial) from *Weissella confusa* strains MBF8-1 and MBF8-2 (bold) to various glucansucrase enzymes of LAB. * Identical residue; (:), highly conserved residue; (.), conserved residue. Putative catalytic residues are shown in gray scale. GenBank protein accession numbers of the different glucansucrase enzymes are indicated.

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Sucrase enzymes responsible for exopolysaccharide production have remained relatively unexplored in the genus Weissella. Recently, Kang et al. (2009) reported cloning and heterologous expression of the glucansucrase dsrWC-encoding gene from W. cibaria. This glucansucrase was characterized as an extracellular enzyme that produces glucans with only α-1,6-linkages.

Screening and identification of GTF and FTF enzymes for exopolysaccharide synthesis by representatives of less well-studied LAB genera, with GRAS status, from the large biodiversity of the Indonesian biosphere, will provide a rich source of further types of homopolysaccharides (and genes/enzymes involved in their synthesis), for example the inulin type or dextran type of exopolysaccharide. Characterization of these novel GTF/FTF enzymes and their exopolysaccharide products will provide further information about the evolution (i.e. phylogeny and conserved sequence motifs), biochemistry (pH and temperature-optima; hydrolysis vs. transglycosylation activities), structure/function relationships of these enzymes contributing to, or determining, substrate/product specificities.

Here, we have shown that screening for exopolysaccharide polymers by an array of wild-type exopolysaccharide-producing LAB on modified media is a rapid and inexpensive preliminary method to analyze the potential to harbor either single gtf and ftf genes, or both. The concomitant synthesis of glucan and fructan, and the presence of their sucrase enzymes, has been described for the genera Streptococcus, Lactobacillus and Leuconostoc, as well as for the genus Weissella. Among other LAB strains that have been reported as exopolysaccharide producers, the genus Weissella has received little attention. This may be due to the origin of this organism that is still regarded as relatively unique and unexplored; it has been isolated within a number of developing countries; for example Thai fermented food (Paludan-Müller et al., 1999), Malaysian fermented food (tapay) (Bjorkroth et al., 2002) and now from Indonesian homemade soya. Here, our results revealed that W. confusa strains MBF8-1 and MBF8-2 potentially synthesize glucans and both glucan and fructan, respectively, using glucansucrase and fructansucrase enzymes. An ftf gene in Weissella has not yet been reported; therefore, this is the first report of a gene for a fructansucrase type of enzyme in Weissella. In future work, this will lend further diversity to the exopolysaccharide molecules available for both fundamental and applied studies. These Weissella sucrase enzymes will allow a further increase in carbohydrate product diversity for a range of applications, and for studies of protein structure and function.

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References


