A specific immunological method to detect and quantify bacterial 2-substituted (1,3)-β-D-glucan

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ABSTRACT

Exopolysaccharides synthesized by lactic acid bacteria have prebiotic properties and contribute to the rheology and texture of fermented foods. Here, we have standardized an immunological method for the specific detection of 2-substituted (1,3)-β-D-glucans. The method allows direct detection and quantification of this exopolysaccharide in culture supernatants containing other mono- and poly-saccharides. Moreover, it allows specific detection of the biomolecules synthesized in vitro in enzymatic reactions. Thus, this method allows the fast identification of producing bacteria, as well as biochemical characterization of the glycosyltransferases responsible for their synthesis.

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1. Introduction

Most commercialized probiotic microorganisms are lactic acid bacteria (LAB) and some produce exopolysaccharides (EPS) (Ruas-Madiedo, Abraham, Mozzi, & de los Reyes-Gavilán, 2008; Salminen et al., 1998; Ventura, Canchaya, Fitzgerald, Gupta, & van Sinderen, 2007). In addition, some LAB strains belonging to the *Pediococcus*, *Lactobacillus* and *Oenococcus* genera, isolated from cider and wine, are able to produce a 2-substituted (1,3)-β-D-glucan EPS (Dols-Lafargue et al., 2008; Dueñas-Chasco et al., 1997, 1998; Ibarburu et al., 2007; Llaubères, Richard, Lonvaud, Dubourdieu, & Fournet, 1990). Analysis of the rheological properties of the 2-substituted (1,3)-β-D-glucan synthesized by *Pediococcus parvulus* 2.6R showed that it has potential utility as a biothickener (Lambo-Fodje et al., 2007; Velasco et al., 2009). *Pediococcus* (Fernández de Palencia et al., 2009) and *Lactobacillus* (Garai-Ibabe et al., 2010) strains that produce this EPS have increased adherence to Caco-2 cells.

P. parvulus 2.6R is able to immunomodulate macrophages (Fernández de Palencia et al., 2009) and production of the 2-substituted (1,3)-β-D-glucan confers to the intestinal *Lactobacillus paracasei* NFBC 338 higher resistance to gastrointestinal and technological stresses (Stack et al., 2010). In addition, human consumption of oat-based food prepared with *P. parvulus* 2.6R resulted in a decrease of serum cholesterol levels, boosting the effect previously demonstrated for (1,3)(1,4)-β-D-glucans of oat-based products (Mårtensson et al., 2005). Moreover, the production of yogurt and various beverages with 2-substituted (1,3)-β-D-glucan producing LAB indicates the advantageous techno-functional properties of these strains (Elizaquível et al., 2011; Kearney et al., 2011). Furthermore, the purified EPS from *P. parvulus* 2.6R increased the growth, viability and adherence capability of probiotic LAB (Russo et al., 2012). Therefore, LAB producing this EPS could have potential as probiotic strains and the 2-substituted (1,3)-β-D-glucan itself appears to have prebiotic properties.

The specific detection and quantification of EPS produced by LAB, especially in a background that contains other carbohydrates, is laborious and time-consuming. The colorimetric phenol–sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) to quantify the total polysaccharide content of samples, requires the removal of any disaccharides or monosaccharides present in the media by ultrafiltration, dialysis or ethanol precipitation (Ruas-Madiedo & de los Reyes-Gavilán, 2005; Notararigo et al., 2013). A complete characterization of the polysaccharide
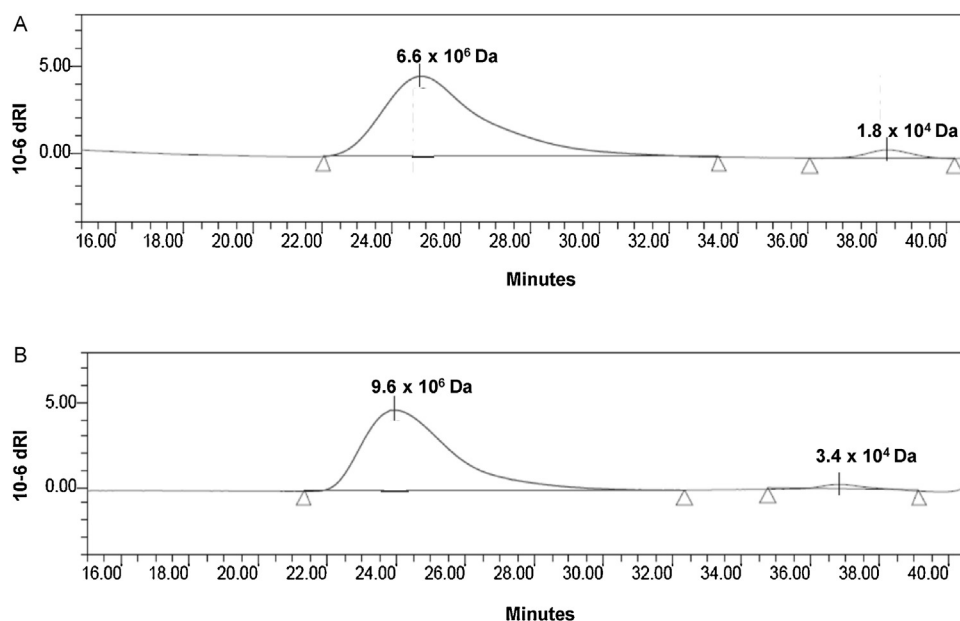
Fig. 1. Analysis of purified EPS produced by *L. lactis* NZ9000[pNGTF] (A) and *P. parvulus* 2.6R (B) by high-performance size exclusion chromatography using as molecular weight standards dextrans of 10^4, 4×10^4, 7×10^4, 1.5×10^5, 2.7×10^5, 4.1×10^5, 6.7×10^5, 1.4×10^6 and 2×10^6 Da (Fluka).

then usually requires further purification, followed by relatively sophisticated, derivatization, degradation and analytical techniques. Therefore a simple direct method for the specific and quantitative detection of 2-substituted (1,3)-β-D-glucan synthesized by bacteria would facilitate the identification of new potential probiotic strains, and the quantification of 2-substituted (1,3)-β-D-glucan production.

LAB 2-substituted (1,3)-β-D-glucans are synthesized by GTF glycosyltransferase (Werning et al., 2006, 2008), which has significant identity (33%) only with the Tts glycosyltransferase of *Streptococcus pneumoniae* serotype 37 (Llull, Muñoz, López, & García, 1999). This latter enzyme synthesizes this organism's capsule (Llull, García, & López, 2001), which is a β-D-glucan similar to that synthesized by *Pediococcus* and *Lactobacillus* strains. Anti-serotype 37 antibodies also agglutinate *Lactococcus lactis* and *Lb. paracasei* NFBC 338 strains that over-produce GTF glycosyltransferase (Dols-Lafargue et al., 2008; Stack et al., 2010; Werning et al., 2008) as well as LAB strains naturally carrying this gene (Dols-Lafargue et al., 2008; Werning et al., 2006). Therefore, in this work, we report on the design and validation of a competition enzyme linked immuno-sorbent assay (ELISA) method for the specific detection of 2-substituted (1,3)-β-glucans, based on *S. pneumoniae* serotype 37 antibodies.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The following bacteria were used in this work. The 2-substituted (1,3)-β-D-glucan-producer strains isolated from Basque natural cider: *Lactobacillus diolivorans* G-77 (Dueñas-Chasco et al., 1998), *Oenococcus oeni* I4 (Ibarburu et al., 2007), *P. parvulus* 2.6R, and its isogenic non-producing strain (2.6NR) (Dueñas-Chasco et al., 1997). The 2-substituted (1,3)-β-D-glucan-producing *L. lactis* NZ9000[pNGTF] recombinant strain, which carries in the pNGTF plasmid, based on the pNZ8048 vector, the *P. parvulus* 2.6R *gft* gene under control of the PnisA promoter, and its isogenic β-D-glucan non-producing strain *L. lactis* NZ9000[pNZ8048] (Werning et al., 2008).

The *Pediococcus* strains were routinely grown at 30 °C in Man Rogosa Sharpe (MRS) broth (De Man, Rogosa, & Sharpe, 1960) supplemented with 0.5% glucose and 0.1% Tween 80. *O. oeni* I4 was routinely grown at 30 °C under anaerobic conditions in MRS supplemented with 0.5% fructose and 1% glucose. *Lb. diolivorans* G-77 was routinely grown at 30 °C in MRS supplemented with 0.5% glucose. The *Lactococcus* strains were routinely grown at 30 °C in M17 broth (Terzaghi & Sandine, 1975) supplemented with chloramphenicol ($5 \, \mu g \, ml^{-1}$).

For EPS production, batch fermentations without pH control were carried out in a semi-defined medium (SDM) supplemented with glucose (2%) (Dueñas-Chasco et al., 1997) or MRS for *P. parvulus* 2.6R and *Lb. diolivorans* G-77 as well as in the same media supplemented with fructose (1%) for *O. oeni* I4 or in a defined medium (DM) (Sánchez et al., 2008) or M17, both supplemented with glucose (0.5%) and chloramphenicol ($5 \, \mu g \, ml^{-1}$) for *L. lactis* NZ9000[pNGTF]. *Pediococcus*, *Lactobacillus* and *Oenococus* strains were grown to stationary phase to increase production of the EPS. *L. lactis* strains were grown in the indicated media until $OD_{600} = 0.6$. Then, for *L. lactis* NZ9000[pNGTF] only, expression of the *gtf* gene encoding the GTF glycosyltransferase was induced with nisin ($0.25 \, ng \, ml^{-1}$) 24 h prior to testing. The strains were stored at −80 °C in either MRS or M17 media supplemented with 20% (v/v) glycerol.

2.2. Isolation, purification and characterization of EPS synthesized by LAB

The 2-substituted (1,3)-β-glucan samples from *P. parvulus* 2.6R and *L. lactis* NZ9000[pNGTF] were isolated and purified from culture supernatants by ethanol precipitation and dialysis as previously described (Notararigo et al., 2013). Stock solutions of both purified EPS were prepared at $1 \, mg \, ml^{-1}$ in phosphate buffered saline (PBS) pH 7.0 and stored at −20 °C. For ELISA assays, EPS stock solutions were diluted in PBS pH 7.0 prior to analysis. The molecular masses of the EPS were determined by high-performance size exclusion chromatography (HP-SEC, GPCV 2000, Waters), as previously described (Velasco et al., 2009).
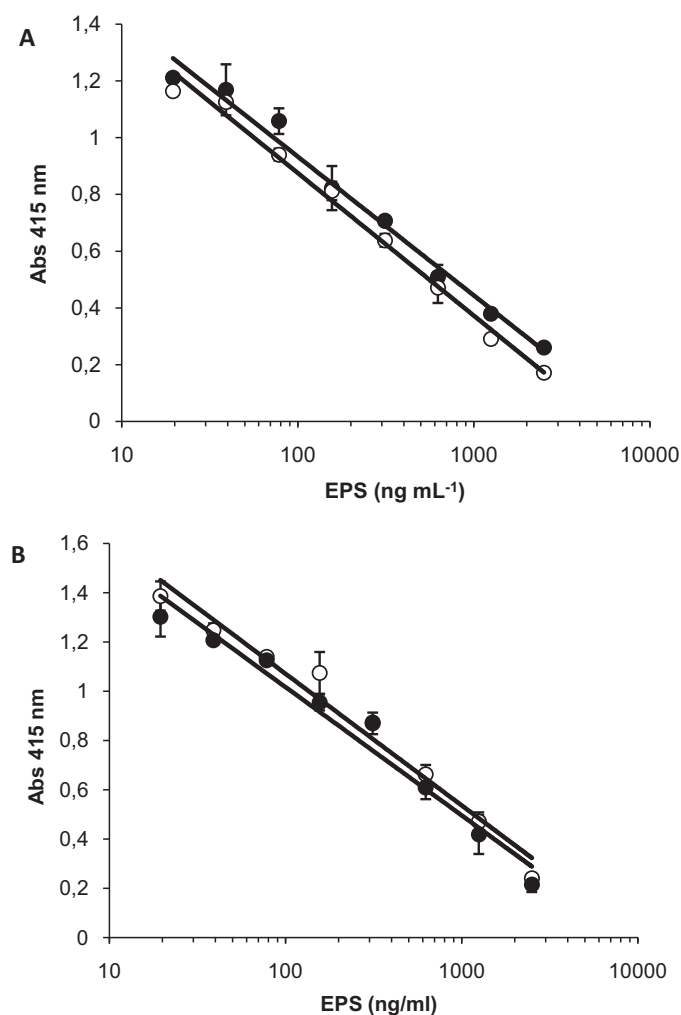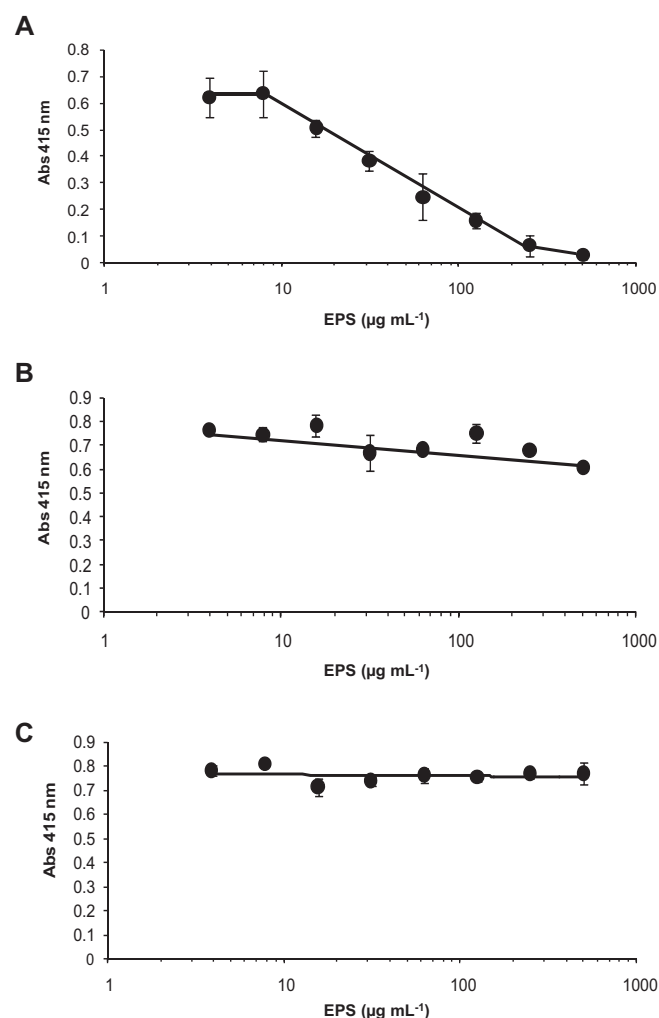
Fig. 2. Range of detection of purified 2-substituted (1,3)-β-D-glucan by the immunological method. EPS produced by *L. lactis* NZ9000[pNGTF] (A) and *P. parvulus* 2.6R (B) immobilized in microtitre plates and purified EPS from *L. lactis* NZ9000[pNGTF] (●) and *P. parvulus* 2.6R (○) were tested as competitors. The values are the mean of three independent experiments. Standard deviations are indicated as vertical bars over dots.

Fig. 3. Specificity of the immunological method. Laminarin (A), curdlan (B) and xanthan (C) were tested as competitors. The values are the mean of three independent experiments. Standard deviations are indicated as vertical bars over dots.

2.3. ELISA assays

ELISA assays were carried out in 96-well Nunc Maxisorp microtitre plates (Thermo Fisher Scientific, USA). The samples (cultures supernatants or purified polysaccharides) and a negative control without biopolymers were tested in duplicate in each assay. In the standard assay the 2-substituted (1,3)-β-D-glucan synthesized by *L. lactis* NZ9000[pNGTF] or by *P. parvulus* 2.6R was used at $62.5\,ng\,ml^{-1}$ as immobilized EPS as well as at various concentrations as competitor for binding to the primary antibody (dilution 1:800 of anti-serotype 37, Statens Serum Institut, Denmark). In addition, to test specificity of the method, laminarin from *Laminaria digitata*; curdlan from *Alcaligenes faecalis* and xanthan from *Xanthomonas campestris* (all from Sigma-Aldrich, Germany) were used as competitors at concentrations from $3.9\,\mu g\,ml^{-1}$ to $2.5\,mg\,ml^{-1}$.

To optimize the ELISA assay, various 2-substituted (1,3)-β-glucan solutions (31.25, 62.5, 125, 250 and $500\,ng\,ml^{-1}$) were used to coat the wells of microtitre plates ($200\,\mu l$/well) by incubation for 16 h at 4 °C. The wells were washed twice with $200\,\mu l$ of solution I (PBS pH 7.0 and 0.05% Tween 20), and then $300\,\mu l$ of blocking solution (solution I plus 0.5% gelatin) were added, followed by 3 h of incubation at 21 °C. To perform the competition assay $150\,\mu l$

of each dilution of the primary antibody (1:200, 1:400, 1:800 or 1:1600) was incubated for 30 min at 21 °C with $150\,\mu l$ of each EPS dilution (from $2.4\,ng\,ml^{-1}$ to $5.0\,\mu g\,ml^{-1}$). Then, $200\,\mu l$ of the mixture were added to each activated well of the microtitre plates and after 1.5 h of incubation at 21 °C (to allow binding of free antibody to the immobilized 2-substituted (1,3)-β-glucan) the wells were washed three times with solution I. The amount of primary antibody bound to the wells was detected by addition of $200\,\mu l$ of the secondary antibody (polyclonal Anti-IgG against rabbit alkaline phosphatase, Sigma-Aldrich, Germany) diluted 1:25,000 in blocking solution, incubation for 2 h at 21 °C, four washes of the wells with solution I followed by the addition of $200\,\mu l$ of developing solution (p-nitrophenylphosphate at $1\,mg\,ml^{-1}$) in diethanolamine buffer (0.2 M diethanolamine pH 9.8 and 5 mM MgCl$_2$, Sigma) and a final incubation for 30 min at 37 °C. Then, the reactions were stopped by addition of 2 N NaOH ($50\,\mu l$) and the absorbance at 415 nm was measured in a microtitre plate reader (BioRad model 680).

2.4. Phenol–sulphuric method

To determine the 2-substituted (1,3)-β-D-glucan concentration by this method, two replicates of 0.5 ml culture supernatants were precipitated overnight with 1 ml of ethanol at −20 °C and sedimented by centrifugation ($13,600 \times g$, 20 min 4 °C). The

Table 1
Comparative analysis of exopolysaccharide production by ELISA and phenol–sulphuric methods.

Bacteria	Method				
	ELISA			Phenol–sulphuric	Phenol–sulfuric/ELISA
	EPS concentration				
	Total[a] (μg ml^{-1})	Precipitated[b] (μg ml^{-1})	Precipitated[b]/total[a] (%)	Precipitated[b] (μg ml^{-1})	Precipitated[b]/precipitated[b] (%)
P. parvulus 2.6R	322 ± 15	200 ± 35	62	117 ± 21	58
L. lactis NZ9000/pNGTF	235 ± 35	183 ± 19	78	117 ± 8	64
O. oeni I4	118 ± 7	94 ± 9	80	59 ± 5	63
L. diolivorans G-77	211 ± 40	166 ± 24	79	64 ± 6	39

[a] Total: concentration of the EPS was determined directly in culture supernatants.

[b] Precipitated: concentration of the EPS present in culture supernatants was determined after ethanol precipitation.

precipitates were twice resuspended in 0.5 ml of distilled water and ethanol reprecipitated and resedimented as described above. Finally, after resuspension in distilled water, the concentration of the biopolymer present in the precipitates was determined by the phenol–sulphuric method as reported by Dubois et al. (1956).

2.5. Analysis of GTF glycosyltransferase activity

Membrane vesicles displaying GTF glycosyltransferase activity, were prepared from *L. lactis* NZ9000[pNGTF] as previously described (Werning et al., 2008) and the enzymatic reactions were carried out as follows. Duplicate 100 μl reactions were prepared containing 12.5, 25 or 50 μg of membrane proteins as well as a range of UDP-glucose (Sigma) concentrations (from 0.04 to 25 mM), 1 mM CaCl$_2$, 9 mM MgCl$_2$ and 50 mM NaCl. These were incubated for 30 min at 30 °C then the reactions were stopped by addition of 900 μl of 100% ethanol. After 2 h incubation at 4 °C, samples were centrifuged at 13,226 × g at 4 °C for 20 min, the precipitates were air dried, and resuspended in 500 μl of PBS buffer pH 8.0. The reaction product was quantified by the ELISA method. The kinetic parameter, apparent K_m and apparent V_{max} were determined from Lineweaver Burk plots.

2.6. Chemical analysis of the enzymatic reaction product

The neutral sugar composition and linkage type was determined using polysaccharide synthesized in duplicate 1 ml reactions containing membrane vesicles (50 mg of total proteins) with or without 25 mM UDP-glucose. The ethanol precipitates, after air drying, were resuspended in 1 ml of distilled H$_2$O plus 5 μl of 10% SDS, incubated for 15 min at 37 °C and dialyzed (molecular mass cut-off 3.5 kDa) against distilled H$_2$O for 24 h. Then, 100 μl aliquots containing the membrane vesicles and the reaction product were hydrolyzed, acetylated and analyzed for the presence of neutral sugars by gas–liquid chromatography (GLC) or after methylation, hydrolysis, reduction and acetylation for the determination of the structural unit of the polysaccharide by gas–liquid chromatography–mass spectrometry (GLC–MS) as previously described (Werning et al., 2008).

3. Results

3.1. Sensitivity of the ELISA assay

To determine the sensitivity of the method two samples of 2-substituted (1,3)-β-glucans synthesized by either *L. lactis* NZ9000[pNGTF] or *P. parvulus* 2.6R were purified and their molecular masses determined by HP-SEC. The average molecular masses of the two main fractions were 6.6×10^6 and 1.8×10^4 Da for *L. lactis* (Fig. 1A) and of 9.6×10^6 and 3.4×10^4 Da for *P. parvulus* (Fig. 1B).
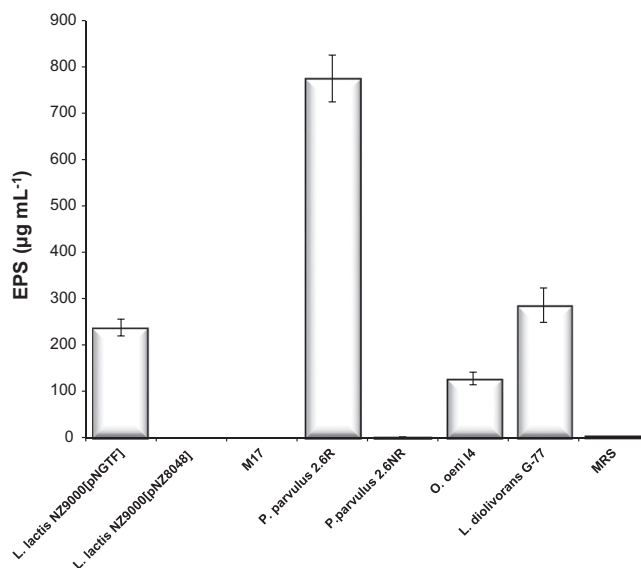

Fig. 4. Detection of 2-substituted (1,3)-β-D-glucan in culture supernatants. (A) The indicated *L. lactis* strains were grown and expression of the EPS induced in either M17 medium. *P. parvulus* 2.6R and 2.6NR, *Lb. diolivorans* G-77 and *O. oeni* I4 were grown in MRS medium. Triplicates of these cultures were then centrifuged and the supernatants were directly used for quantification of the β-D-glucan present by the ELISA assay. The values are the mean of three independent experiments. Standard deviations are indicated as vertical bars over bars.

The higher molecular mass fraction was the most abundant for both strains constituting, respectively, 96% and 92% of the total polymer population. Both EPS preparations were used for testing as immobilized β-glucans and as competitors for binding to the primary antibody. Both EPS preparations were immobilized efficiently (Fig. 2A *versus* B) and both purified β-D-glucans could be quantified within a range of 20 to 2500 ng ml^{-1}, independently of the origin of the immobilized polymer (Fig. 2).

3.2. Specificity of the assay

Using the purified β-D-glucan from *L. lactis* (described above) as the immobilized substrate, we tested the specificity of the method using three polysaccharides as competitors: (i) laminarin, a 6-substituted (1,3)-β-D-glucan (Fig. 3A); (ii) curdlan, a linear (1–3)-β-D-glucan (Fig. 3B) and (iii) xanthan, a branched heteropolysaccharide (Fig. 3C). Xanthan was unable to bind to the primary antibody even at very high concentration (500 μg ml^{-1}) and curdlan also showed a very low affinity for the antibody at 250 μg ml^{-1}. Laminarin was able to compete with the immobilized 2-substituted (1,3)-β-D-glucan in a linear mode, when tested in the range 10 to 250 μg ml^{-1}.
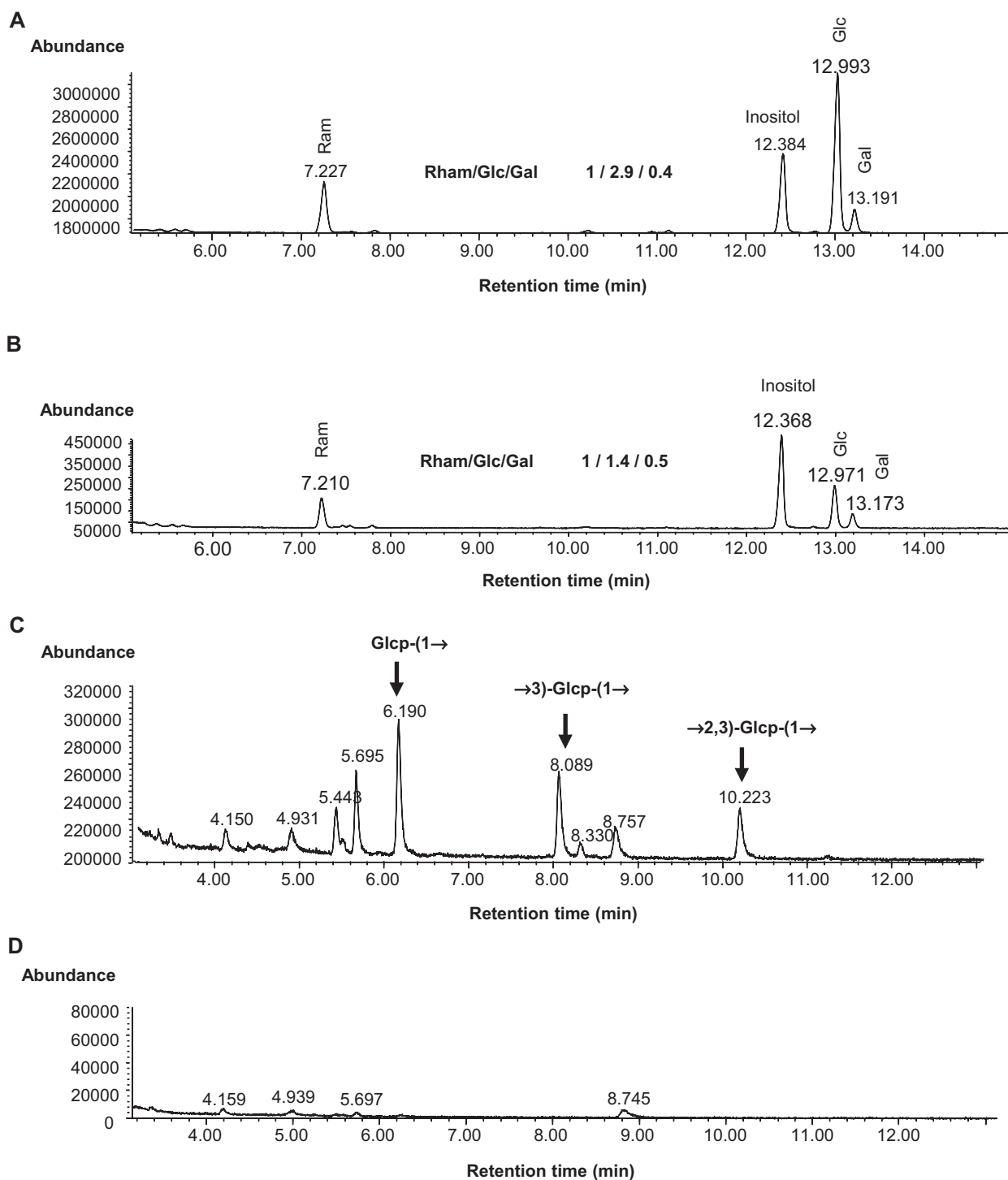
Fig. 5. Analysis by GLC of monosaccharides (A and B) and by GLC–MS (C and D) of linkage types between glucose units of the EPS synthesized *in vitro* by GTF glycosyltransferase. Analysis of reactions containing (A and C) or lacking (B and D) UDP-glucose.

3.3. Quantification of EPS in growth media by using the ELISA assay or the phenol–sulphuric method

The suitability of the immunological method for directly quantifying (1–3)-β-D-glucan in LAB growth media was tested as follows. The β-D-glucan purified from *L. lactis* NZ9000[pNGTF] was dissolved in either the DM (defined) or SDM (semi-defined) medium or in M17 and MRS complex media, and the EPS concentration was determined by the competition ELISA assay. In all cases the method was able to quantify EPS in the range of 30 to 500 ng ml^{-1}

(Supplemental material S1). However, examination of the different slopes of the curves (see panels A; B, C and D of Supplemental material S1) in comparison with Fig. 2, indicated that for correct quantification of 2-substituted (1,3)-β-D-glucan in different buffers or growth media, the corresponding standard curve should be prepared using the corresponding solvent. Therefore, the method used to quantify β-D-glucan present in culture supernatants of LAB grown in the above mentioned media always used the appropriate standard curve. Thus, *L. lactis* NZ9000[pNGTF] and the isogenic β-D-glucan non-producing *L. lactis* NZ9000[pNZ8048] were grown

in M17 medium as described previously (Werning et al., 2008). *P. parvulus* 2.6R and the isogenic β-ᴅ-glucan non-producing strain (2.6NR) as well as *O. oeni* I4 and *Lb. diolivorans* G-77, and two other 2-substituted (1,3)-β-ᴅ-glucan producers, were grown in MRS medium to stationary phase as described above. The cultures were then centrifuged and, after removal of the supernatants, the EPS still bound to the cells were released by resuspension into one volume of PBS and the bacterial cells were removed by centrifugation. Then, the supernatants, after appropriate dilution in PBS, were directly used in the ELISA assay. The results revealed that the 2-substituted (1,3)-β-ᴅ-glucan secreted by the four producing-LAB could be detected in the media used, whereas only background levels were observed in the supernatants of the non-producing strains (Fig. 4).

To compare the inhibition enzyme immunoassay and the phenol–sulphuric method, culture supernatants of *L. lactis* NZ9000[pNGTF] grown in DM medium and *P. parvulus* 2.6R, *Lb. diolivorans* G-77 and *O. oeni* I4 grown in SDM medium were analyzed using both methods. Only these media were tested for detection of the 2-substituted (1,3)-β-ᴅ-glucan, because the presence of other polysaccharides in M17 and MRS media impairs the usage of the phenol–sulphuric method. The concentration of 2-substituted (1,3)-β-ᴅ-glucan was determined directly in the supernatants by the ELISA test or in their ethanol precipitates by the ELISA and phenol–sulphuric methods (Table 1). Determination of the EPS concentration in the precipitates showed that for all the strains tested the ELISA assay revealed a higher level of production (from 61% for *Lb. diolivorans* G-77 to 36% for *L. lactis* NZ9000[pNGTF]). In addition, comparison of EPS detection by the ELISA method prior to, or after, precipitation demonstrated that indeed precipitation results in a moderate loss of 2-substituted (1,3)-β-ᴅ-glucan in a range from 38% to 20% for *P. parvulus* 2.6R and *O. oeni* I4 biopolymers. These differences were probably due to low molecular weight EPS that was not precipitated by ethanol. Therefore, these results indicate that the ELISA method allows a more reliable determination of the biopolymer after its synthesis by bacteria.

3.4. Detection of 2-substituted (1,3)-β-ᴅ-glucan produced in an enzymatic reaction

Membrane vesicles containing the GTF glycosyltransferase, were prepared from *L. lactis* NZ9000[pNGTF] and the enzymatic activity was analyzed *in vitro* using UDP-glucose as substrate. The reaction product was detected and quantified by the ELISA method. Kinetic analysis of the results (Supplemental material S2) indicated that the GTF glycosyltransferase has an affinity for UDP-glucose of $K_m = 123 \pm 3\,\mu M$ and a $V_{max} = 18 \pm 0.8$ ng of EPS/mg of total protein/min. This K_m is almost identical to that reported for the cellulose synthase BscA (125 μM) (Ross, Mayer, & Benziman, 1991) which, like GTF, belongs to the GT-2 family of glycosyltransferases.

To confirm the nature of the enzymatic reaction product, reactions performed with membrane vesicles and containing UDP-glucose (test) or lacking the substrate (control) were analyzed. In the presence or absence of the substrate, the GLC analysis detected rhamnose, glucose and galactose, in the ratios 1:2.9:0.4 and 1:1.4:0.5 for the test (Fig. 5A) and control (Fig. 5B) reactions, respectively. Thus, the *L. lactis* membrane vesicle preparations contained three monosaccharides, whose origin could be due to polysaccharides bound to them and as expected only glucose was highest in the test reaction. The GLC–MS analysis (Fig. 5C and D) detected, in the test reaction only (Fig. 5C), the three peaks that are characteristic of 2-substitute ᴅ-(1,3)-β-glucan, corresponding to terminal glucopyranose, 3-*O*-substituted glucopyranose, and 2-di-*O*-substituted glucopyranose. Thus, the product synthesized *in vitro*

by the GTF glycosyltransferase was indeed 2-substituted (1,3)-β-ᴅ-glucan, confirming the ELISA detection of the product.

4. Discussion

ELISA tests have been developed previously for the detection of both linear and 6-substituted (1,3)-β-ᴅ-glucans (Douwes, Doekes, Montijn, Heederik, & Brunekreef, 1996; Sander, Fleischer, Borowitzki, Brüning, & Raulf-Heimsoth, 2008) as well as for the specific detection of 6-substituted (1,3)-β-ᴅ-glucans (Milton, Udeni Alwis, Fisette, & Muilemberg, 2001; Rao et al., 2007; Sander et al., 2008). However, as far as we know, this is the first instance of an ELISA test developed for the detection of 2-substituted (1,3)-β-ᴅ-glucan. In the inhibition enzyme immunoassay developed in this work the commercially available antibody (anti-*S. pneumoniae* serotype 37) has been used. Our results show that this primary antibody specifically detects 2-substituted (1,3)-β-ᴅ-glucan, has very low binding affinity for 6-substituted (1,3)-β-ᴅ-glucan and does not bind linear (1,3)-β-ᴅ-glucan even at a concentration as high as 500 μg ml^{-1}. The ELISA method allows detection of 30 ng ml^{-1} of the biopolymer in growth medium, a level of sensitivity similar to that observed for other β-ᴅ-glucan, such us (1,6)-β-ᴅ-glucan (Vink et al., 2004) or (1,3)-β-ᴅ-glucan (Douwes et al., 1996), when polyclonal antibodies were used for their detection. This method is less sensitive than that obtained for the detection of other polysaccharides with monoclonal antibodies (in the range of pg ml^{-1}) or than the amoebocyte lysate assays for which commercial kits, such as Glucatell® (Associates of Cape Cod, USA) or Fungitic-G (Seikagaku, Japan) are available. However, the first mentioned type of assay cannot be applied to the detection of 2-substituted (1,3)-β-ᴅ-glucan and the second type does not discriminate between different types of (1,3)-β-ᴅ-glucans. In addition, our results show that the sensitivity of our ELISA test permits the detection of novel 2-substituted (1,3)-β-ᴅ-glucan-producing bacteria by direct analysis of culture supernatants even after growth in complex media. Moreover, the method can be used for the biochemical characterization of the glycosyltransferases involved in their synthesis. These findings will facilitate the identification of new probiotic strains and the optimization of the production of this EPS.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.06.072.

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