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Cloning and Partial Characterization of an Extracellular Dextransucrase Coding Region (DSR-V) from *Leuconostoc citreum* M-3

11

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Abstract

The dextransucrase enzymes synthesize dextran, a glucose polymer with broad industrial applications, making the search for new dextransucrases of great interest. The work described aimed at the partial characterizing of a recombinant dextransucrase enzyme from *Leuconostoc citreum* M-3. From the genomic DNA of strain M-3, an amplicon containing a coding region of a dextransucrase called DSR-V was isolated, and deposited in the GenBank™ (Accession number: KF724950). The amino acid sequence alignment of DSR-V with other dextransucrases demonstrated that it shares a 94% identity with the DSR-D of *L. mesenteroides* Lcc4 and the DSR-S of *L. mesenteroides* NRRL B-512F. The DSR-V was cloned and expressed in *Escherichia coli* JM109 facilitating the formation and detection of DSR-

V specific dextran. The SDS-PAGE soluble fraction zymography of *E. coli* DSR-V and the ^{13}C-NMR spectra of dextran polymers synthesized by this clone confirm that *L. citreum* M-3 *dsrV* gene codes for a different dextransucrase synthesizing linear dextrans with mainly α(1-6) linkages.

Introduction

Lactic acid bacteria (LAB) have great potential to produce exopolysaccharides (EPS) and oligosaccharides, which have been the subject of extensive research efforts due to their physicochemical and bioactive properties (Pu et al., 2001). EPS represent a group of very diverse polymers, whose individual functional properties are determined by their chemical composition, molecular weights, types of glycosidic linkages as well as degree and arrangement of branches (Zannini et al., 2016). As a result of the unmatched variety of possible osidic bonds between sugar monomers, the structural diversity of these polysaccharides offers an extensive range of functionalities of interest for food, feed, pharmaceuticals, cosmetics and chemicals industries (Xu and Zhang, 2016).

Among the first microbial polymers produced at industrial scale are the dextrans (Naessens et al., 2005). These polysaccharides are produced by microorganisms of the genera *Lactobacillus*, *Streptococcus*, *Weissella*, *Leuconostoc, Pediococcus and Oenococcus* (Daudé et al., 2014). The most common dextran of commercial use is synthesized by *Leuconostoc mesenteroides* strain NRRL B-512F. The biopolymer contains a(1-6) linked glucosyl residues in the main chain with only 5% of a(1-3) linked branches (Naessens et al., 2005). Dextran fractions of controlled molecular weight and their numerous derivatives are mainly used in medicine, pharmaceuticals and fine chemistry (Zdolsek et al., 2011). The polymer production is attributed to the extracellular glucansucrase (dextransucrase) DSR-S, a 6-a-D-glucosyltransferase (EC 2.4.1.5) (Monchois et al., 1997). This enzyme belongs to glycoside hydrolase family 70 (GH70) according to the CAZy classification (http://www.cazy.org/) (Lombard et al., 2014). The GH70 family consists of a large and diverse group of polymerases and branching enzymes, some of them being mainly active on sucrose and others on starch substrate (Passerini et al., 2015, Gangoiti et al., 2017). In the catalytic domain of GH70 enzymes can be identified three regions which contain the amino acids involved in the catalysis (D551, E589, D662, DSR-S numbering) forming

the catalytic triad and the domain presents the typical $(b/a)_8$ barrel of glucansucrases (Macgregor et al., 1996, Vujicić-Zagar and Dijkstra 2006 , Pijning et al., 2008).

Biochemical and molecular studies of these enzymes have allowed identifying some of determinants of enzyme specificity and are promising to increase the prospects of their practical applications (Daudé et al., 2014). The study of GH70 enzymes with different specificity from new LAB strains could provide new insights in structure-function relationships of glucansucrases as well as enlarge the dextransucrase toolbox available for industrial application. In this sense, the Cuban Research Institute on Sugarcane By-products (ICIDCA) has a collection of LAB strains. Some of them have already been characterized (Fraga et al., 2011a, Fraga et al., 2011b). They have been isolated from sugarcane and sugarcane derivatives which includes the *Leuconostoc citreum* strain M-3. In the present study, we describe the cloning, sequencing, and heterologous expression in *Escherichia coli* of a novel extracellular dextransucrase coding region (*dsrV*) from *L. citreum* M-3, and the partial characterization the polymer produced by the recombinant dextransucrase. The alignment of the deduced amino acid sequence with other dextransucrase sequences is presented as well as a 3D structural model of DSR-V.

Materials and methods

Bacterial strains and culture media

The *Leuconostoc citreum* strain M-3 was obtained from the Cuban Research Institute on Sugarcane By-products (ICIDCA) culture collection. It was isolated from sugar cane juice. Stock cultures were maintained at 80^{o}C in 15% (v/v) glycerol. The cultures were grown in a rotary shaker at 30^{o}C, 175 cycles min^{-1} in standard medium M1 (20 g.L^{-1} sucrose, 20 g.L^{-1} potassium hydrogen phosphate, 20 g.L^{-1} yeast extract, 0.2 g.L^{-1} $MgSO_4$ $7H_2O$, 0.01 g.L^{-1} $MnSO_4$ H_2O, 0.01 g.L^{-1} NaCl, 0.02 g.L^{-1} $CaCl_2$, 0.01 g.L^{-1} $FeSO_4$ $7H_2O$) with an adjusted pH of 6.9. Glucose instead of sucrose was added in the standard medium for chromosomal DNA purification purposes.

Dextransucrase enzyme activity assays

The enzyme activity was determined by measuring the release of reducing sugars with the dinitrosalicylic acid method (Sumner and Howell, 1935). One unit of glucansucrase enzyme (U) is defined as the amount of enzyme that catalyzes the formation of 1 mmol.min^{-1} fructose at 30^{o}C in 20 mmol.L^{-1} (pH 5.4) sodium acetate

buffer, 2 mmol.L^{-1} $CaCl_2$, with 100 g.L^{-1} sucrose and 1 mL of enzymatic preparation in a total reaction volume of 5 mL.

SDS-PAGE and zymogram analysis

Protein electrophoresis under denaturing conditions (SDS-PAGE) was performed with the Mini-Cell SureLock™ XCell system with polyacrylamide gels (8%, v/v) - SDS (Novex Tris-Acetate 1.5 mm thick, Invitrogen Corp.) NuPAGE antioxidant, and NuPAGE running buffer Tris-acetate - SDS (for Tris-Acetate gels) from Invitrogen Corp. The NuPAGE reducing agent (DTT 1 mol.L^{-1}) 3mL and 7.5 mL of loading buffer NuPAGE®LDS (4X) were mixed with 20 mL of the sample and heated to 70°C for 10 min prior to loading on the gel. Samples containing resuspended cells were centrifuged (Eppendorf centrifuge 5804 R) 10,000 x *g* for 5 minutes before being applied. About 2 mU of enzyme was applied to the gels and electrophoresis was performed for approximately one hour at 150 volts. Gels were treated for in situ detection of glucansucrase-dextransucrase activity (zymogram) using sucrose as the substrate (Miller and Robyt, 1986) in combination with the technique for reversibly negative staining protein (Fernandez-Patrón et al., 1995). High Molecular Weight Marker (Amersham Biosciences) was included in the electrophoresis runs.

DNA extraction and purification

Leuconostoc citreum M-3 DNA was purified using the “Blood and Cell Culture DNA Maxi kit” (Qiagen, Germany), and plasmid DNA purification was done using the “QIAprep Spin Plasmid kit” (Qiagen, Germany). LA-PCR (Long and Accurate PCR) amplification products and gel extraction were performed using “QIAquick gel extraction kit” (Qiagen, Germany).

PCR amplification and cloning of the dextransucrase encoding region

Primers were designed based on the sequence of the dextransucrase gene *dsrS* deposited in GenBank™ (accession number: I09598). The recognized sequence of the restriction endonuclease *Cfr9*I (*Sma*I) was inserted in both primers to flank the amplicon. DNA fragments were generated by LA PCR (Long and Accurate PCR) using the Expand High Fidelity PCR System (Roche Applied Science) with a Perkin Elmer Gene Amp PCR System 2400 thermocycler and 50 ng of genomic DNA, and 10 µmol.L^{-1} of forward and reverse primers:

dsrS-dir-PS (5'-CCCGGG*ATG*CCATTTACAGAAAAAGTAATGCGGA-3')
dsrS-inv-PS (5'- CCCGGG*TTA*TGCTGACACAGCATTTCCATTATT-3')

(*ATG* and *TTA* in italic type represent, respectively, the start and stop codons. Nucleotides underlined represent mismatches with the sequence and the cleavage site of restriction enzyme of *Cfr9*I.

The thermal cycling was 1 cycle at 94^{o}C for 2 min, ten cycles of 94^{o}C for 15 s, 50^{o}C for 30 s, 68^{o}C for 10 min, 20 cycles of 94^{o}C for 15 s, 50^{o}C for 30 s, 68^{o}C for 10 min plus 5 s for each cycle, and 1 cycle 68^{o}C for 15 min.

The amplicon was digested with the restriction endonuclease *Cfr9*I (MBI Fermentas) and cloned in the vector pGEM-3Zf(+) (Promega). The transformation of *Escherichia coli* JM109 with the construction was achieved by the procedure of Hanahan, (1983). The screening of *E. coli* colonies producing functional dextransucrase enzymes was done basically as described by Hernández et al. (2002). LBT medium (10 g tryptone, 5 g yeast extract, and 10 g NaCl, 100 mmol.L^{-1} Tris-HCl, pH 6,4) with ampicillin 100 µg.mL^{-1} was used instead of Luria-Bertani (LB) medium (Monchois et al., 1997). The method relies on the addition of the pH indicator bromothymol blue to LBT media, supplemented with 5% sucrose and 1% glycerol as extra carbon sources 2.4 mM isopropyl-1-thio-b-D-galactopyranoside (IPTG). *E. coli* was grown and maintained on LB medium supplemented when needed with ampicillin (100 µg.mL^{-1}). All *E. coli* strains were stored at -80°C in 15% glycerol.

Amplicon DNA sequence analysis

Amplicons were sequenced in both directions by Genome Express, France. Nucleotide sequence analysis was performed with Vector NTI 8.0 software (Invitrogen- InforMax, USA). The amino acid sequence encoded by the amplicon was subjected to a BLAST 2.2.7 search (Altschul et al., 1997). COBALT – constraint-based multiple alignment tool (Papadopoulos and Agarwala 2007) was run on BLAST search result and was used to build the phylogenetic tree using the neighbor joining method with a maximum sequence difference (0.85) and the distance estimated according the model of Kimura (1980). Final edition of the phylogenetic tree was conducted using MEGA version X (Kumar et al., 2018). The MultAlin (http://multalin.toulouse.inra.fr/multalin/) internet software of Corpet (1988) was used to

perform the sequence alignments of the dextransucrases and the result was visualized using the EsPript 3.0 software (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi) (Robert and Gouet 2014). The cleavage site of the peptidase signal was predicted by the SignalP 4.1 software (Petersen et al., 2011).

Inducible production of dextransucrase in Escherichia coli JM109

For the expression of recombinant dextransucrase, a single colony of a clone was inoculated into 10 ml of 2xYT/T medium (16 g tryptone, 10 g yeast extract, 5 g NaCl, complete to 1L with 100 mmol.L^{-1} Tris/HCl, pH 6.4 buffer) supplemented with 100 µg.mL^{-1} of ampicillin and grown at 30ºC, 175 cycles.min^{-1} overnight. The cultures were then diluted 1:100 with 2xYT/T medium supplemented with 200 µg.ml^{-1} ampicillin, and grown at 30ºC and 175 cycles.min^{-1} until the OD_{600} reached 0.5. Then IPTG was added to a final concentration of 2.4 mmol.L^{-1} and the cultures were incubated 12-14 h at 20ºC at 175 cycles.min^{-1}. The cells were centrifuged at 10,000 x *g* 15 min, 4ºC and resuspended in a rupture solution [50 mmol.L^{-1} NaAc, pH 5.4, Tritón X-100 0.1 % (v/v), $CaCl_2$ 0.05 g.L^{-1}, inhibitors cocktail of proteases (Roche)] until the OD_{600} reached 80. Finally the cells were disrupted by sonication (MSP, England). The crude extract was centrifuged at 21,390 x *g* for 40 min. The soluble and insoluble fractions of every crude extract were separated, recovered, and kept at -20ºC until they were analyzed.

Isolation of the exopolysaccharide produced by the recombinant dextransucrase in Escherichia coli JM109

A positive colony was spread on solid LBT medium and grown at 30°C for one week. The cells and exopolysaccharides were recovered, resuspended in 1 mL of deionized (Mili Q) water and mixed by vortexing. The cellular and the supernatant fractions were separated by centrifugation (Eppendorf 5804 R) at 10,000 x *g* at 4°C for 15 min. The soluble exopolysaccharide of the supernatant fraction was precipitated in 60 % (v/v) ethanol at room temperature. The precipitate was recovered by centrifugation (Eppendorf 5804 R) at 7,000 x *g* 10 min at 20°C and was resuspended in deionized (Mili Q) water, and this step was repeated two times. The polymer was finally resuspended in deuterium oxide (D_2O).

^{13}C-NMR analyses of dextran polymers

All NMR spectra were obtained from solutions of 20 mg.mL^{-1} in deuterium oxide (D_2O). The NMR 1D spectra were obtained using inverse sequences taken from the Bruker pulse sequences library and using 90^{o} pulse, 25,000 Hz sweep width, 2.5 s of relaxation delay and 0.63 s acquisition time. A total of 30,000 scans were recorded. Signal assignments were made by the comparison of spectra from commercially available dextran, inulin and levan, (Sigma-Aldrich). Some signals of dextran were assigned according to Maina et al. (2008).

Results and discussions

Amplification, cloning and expression to a coding DNA fragment for DSR-V dextransucrase

Primers were designed based on the nucleotide sequence of the N-terminal and C-terminal regions of the dextransucrase gene *dsrS* from *L. mesenteroides* NRRL B-512F. This method was previously used in the isolation and cloning of the gene *dsrF* and its truncated variant *dsr*F-ΔSP-ΔGBD from *L. citreum* B/110-1-2 (Fraga et al., 2011a). Briefly, a DNA fragment of 4602 bp containing an open reading frame (ORF) of 4587 bp putatively encoding a glucansucrase was amplified by PCR and cloned into pGEM3Zf (+) (Figure 1A, lane 2).

E. coli JM109 clones expressing glucansucrase activity (DSR-V) were selected using a simple phenotypic screening procedure. First, the positive clones turned the medium color from the initial green (pH 6.5–7.0) to yellow (pH 6.0) due to medium acidification resulting from the consumption of the glucose and fructose released from sucrose (Hernández et al., 2002). In addition, the positive clones showed the typical dextran polymer formation phenotype and negative clones appeared as flat colonies without dextran (Figure 1B). The dextransucrase activity in the soluble fraction of sonicated cell extracts was low (0.032 U.mL^{-1}) as compared with other production levels of recombinant dextransucrase in *E. coli* (Moulis et al., 2006, Yalin et al., 2008). A possible cause of these levels may be the presence of 53 rare codons in the *dsrV* sequence including 2 Arginine-AGG (2), AGA (4); Isoleucine-AUA (9); Leucine-CUA (12); Proline-CCC (2); Glycine-GGA (24) and two in tandem (AGAAUA). This tandem codon is especially unfavorable because it may cause an early end of the translation process (Rogulin et al., 2004). In other study, an *E. coli* clone expressing the dextransucrase DSR-F was supplemented with the pRARE

vector, which carries rare RNAt genes, resulting in an increase in enzymatic activity of 66%, indicating that rare codons have a negative effect on the production of dextransucrase enzymes (Fraga 2015).

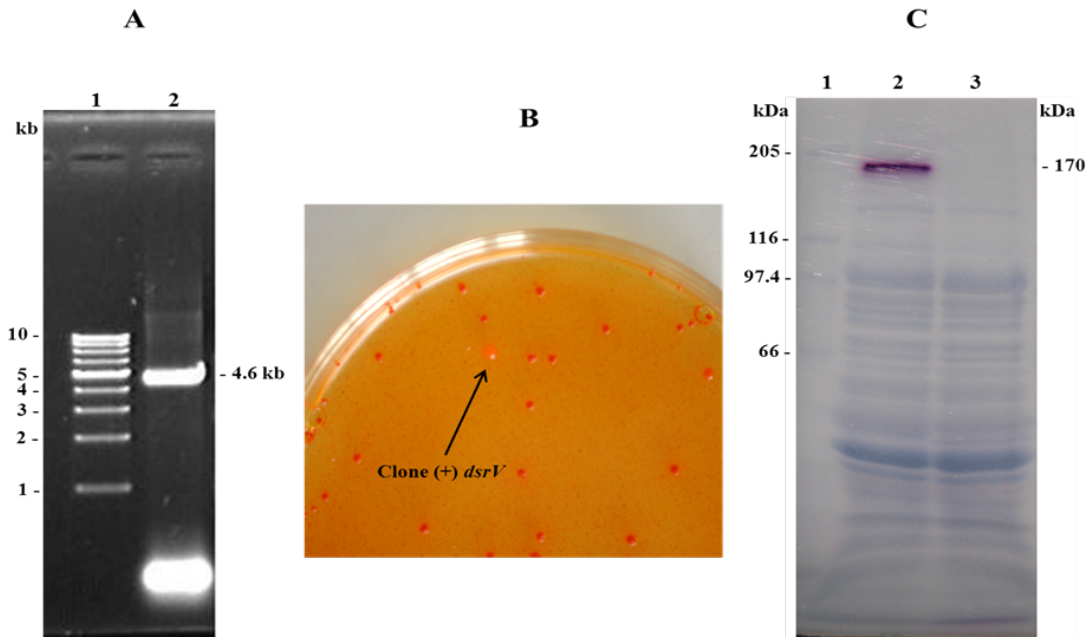

Figure 1. Cloning and detection of glucansucrases in *E. coli* JM109. (A): Lane 1: Protein marker 1 Kb DNA Step Ladder (Promega). Lane 2: DNA electrophoresis of 4.6 kb amplicons encoding for glucansucrase enzyme amplified by LA PCR. (B) Histochemical detection of dextransucrase enzyme activity in *E. coli.* (C) Zymogram for detection of dextransucrase activity in the clone pGEM-3Zf(+) *dsr*V. Lane 1: Protein marker HMW Maker. Lane 2: *E. coli* JM109 pGEM-3Zf(+) *dsr*V. Lane 3: *E. coli* JM109 pGEM-3Zf(+).

Amino acid sequence analysis of DSR-V dextransucrase

The *dsr*V gene, whose sequence was deposited in GenBank™ data base (accession number: KF724950), encoding the protein DSR-V (GenBank™ accession number: AHC31982) of 1530 amino acids, has a theoretical molecular weight of 170466.27 Da (~ 170 kDa) and an isoelectric point of 4.25 according to information provided by

the Vector NTI 8.0 software. The deduced amino acid sequence of DSR-V showed a high level of identity of 94% with several characterized dextransucrases of *L. mesenteroides* strains like: DSR-D, a dextransucrase of 1527 aa of *L. mesenteroides* Lcc4 (Neubauer et al., 2003), with the DSR-S of *L. mesenteroides* NRRL B-512F (Moulis et al., 2006), with DexYG, a dextransucrase of 1527 aa of *L. mesenteroides* 0323 (Zhang et al., 2008) (Figure 2). The highest level of identity of DSR-V (99%) was with a recently reported uncharacterized hydrolase of 1525 aa from an annotated genome of *L. mesenteroides* subsp. *suionicum* deposited in GenBank™: API72687.1. Sequence alignment showed that DSR-V mainly differed from DSR-S in 89 amino acids all over the sequence. Concerning *L. citreum*, the highest identity was 70% with an uncharacterized dextransucrase of 1381 aa deposited in GenBank™: CDX65712. The characterized dextransucrase of 1477 aa from *L. citreum* strain HJ-P4, showed an identity of 67% (Yi et al., 2009).

Furthermore, the three amino acids (D551, E589, D662, DSR-S and DSR-V numbering) forming the catalytic triad are highly conserved in GH70 family. It has been proposed that the first conserved residue D551 (Asp) operates in the nucleophilic attack, upon forming the intermediate enzyme substrate and activity transferase (Macgregor et al., 1996). The second amino acid E589 (Glu) is involved in the acid/general base catalysis (Macgregor et al., 1996, Vujicić-Zagar and Dijkstra 2006, Pijning et al., 2008). The conserved residue D662 (Asp) is the catalytic residue stabilizing the transition state intermediate (Macgregor et al., 1996, Vujicić-Zagar and Dijkstra 2006, Yi et al., 2009). Located downstream of the catalytic triad amino acid D662 is the dextransucrase consensus sequence SEV (Ser663-Glu664-Val665) which seems to be the responsible for a(1-6) linkage formation (Moulis et al., 2006). All the characteristic motifs responsible for a(1-6) linkages specificity, were actually observed in the predicted amino acid sequence of DSR-V (Figure 3). The highly conserved regions (II, III, IV) (Vujicić-Zagar and Dijkstra, 2006) of the catalytic domain of DSRV are identical to DSR-S dextransucrase ones (Figure 3).

Figure 2. Phylogenetic tree of DSR-V (arrow) with members of the GH-70 family. *Enterococcus* (Ent.), *Lactobacillus* (Lc.), *Leuconostoc mesenteroides* (Leu. mesent.), *Oenococcus* (O.), *Weissella* (W.).

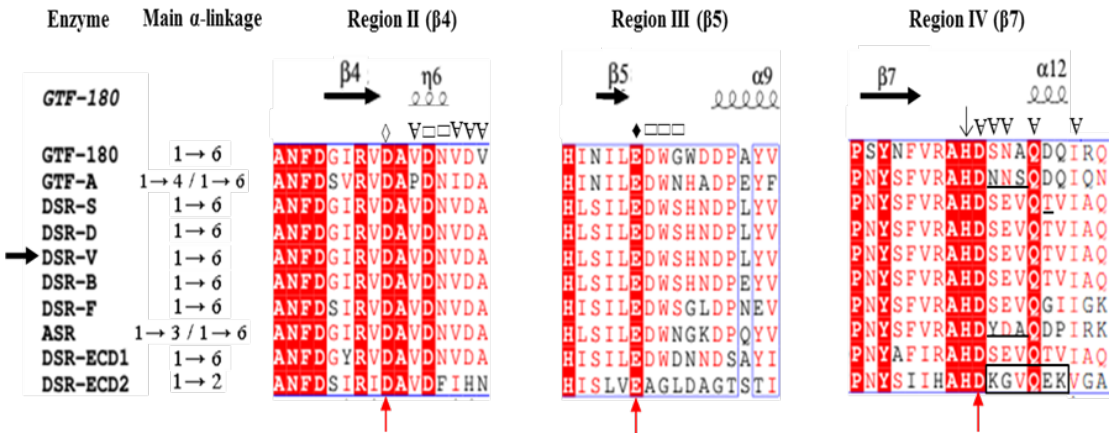

Figure 3. Amino acid sequence alignment of highly conserved regions (II, III, IV) in the catalytic domains of dextran-, mutan-, alternan-, and reuteransucrase enzymes of lactic acid bacteria (including DSR-V) and residues with functional roles identified by site directed mutagenesis. Key: GTF-180 *Lb. reuteri* 180, GTF-A *Lb. reuteri* 121, Dsr-S, *L. mesenteroides* NRRL B-512F; Dsr-D *L. mesenteroides* Lcc4; DSR-V *L. citreum* M-3; Dsr-B 742, *L. citreum* NRRL B-742; Dsr-F, *L. citreum* B/110-1-2; ASR, *L. citreum* NRRL B-1355; Dsr-E CD1 y CD-2, *L. citreum* NRRL B-1299. Symbols → dextransucrase from *L. citreum* strain M-3, ↑ catalytic triad amino acid, ◊ putative catalytic nucleophile, ♦ putative acid/base catalyst, ↓ putative residue stabilizing the transition state intermediate (Macgregor et al., 1996, Ito et al., 2011), ∀ putative sugar-binding/glycosyl transfer sites (Devulapalle et al., 1997), ∀ residues involved in linkage specificity and glucan solubility. Amino acids involved in enzyme linkage specificity (Corpet 1988, Kralj et al., 2005) are in bold underlined type.

Primary structure analyses revealed that DSR-V displays the same organization as that of all the dextransucrases from family GH-70 (EC 2.4.1.5) (Figure 4).

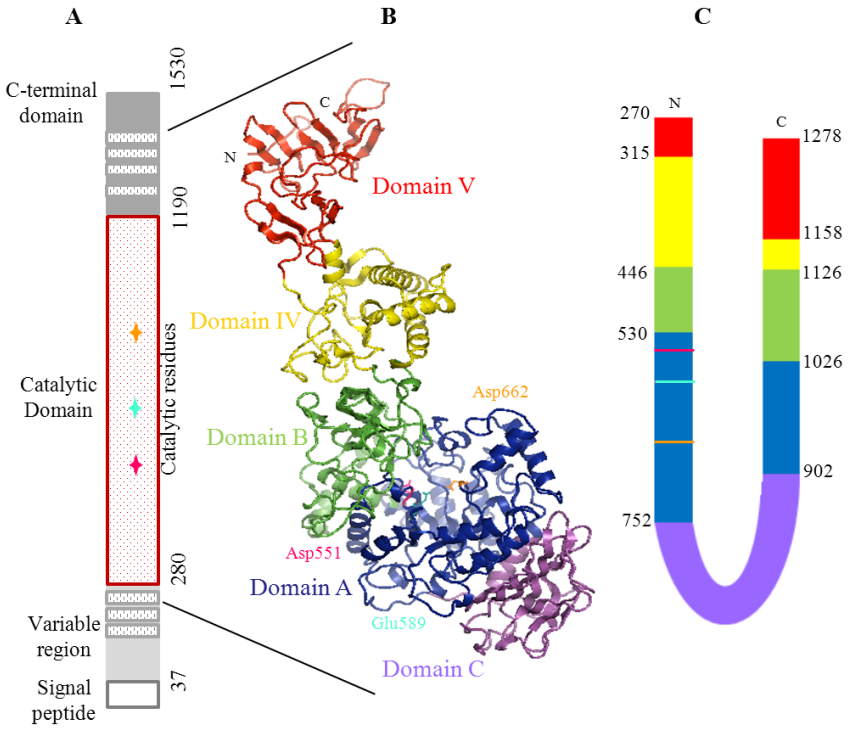

Figure 4. Representation of DSR-V from *L. citreum* M-3**.** (A): Schematic representation of the primary structure organization of DSR-V from *L. citreum* M-3. Repeat regions CW are shown. (B): Model of the 3D structure to DSR-V based in the 3D structure of GTF-180 from *Lactobacillus reuteri* 180 (3HZ3) obtained by SWISS-MODEL, https://swissmodel.expasy.org. (C): "U" conformation based on the 3D structure.

In the N-terminal region, a Gram-positive signal peptide with a cleavage site followed by the amino acid 37 was identified. These are typical regions of Gram positive bacteria dextransucrases. Previous deletion studies of this region in DSR-B of *L. citreum* NRRL B-1299 have shown that its absence does not affect the enzyme activity (Monchois et al., 1998). Following the signal peptide it was identified the variable region consisting of 243 aa with three conserved regions corresponded to sequences binding to the cell wall (CW) consisting of repeating units YG-consensus. These regions have homology with other proteins binding to the cell wall; available three-dimensional structures of such proteins show a b-solenoid folding where the repeating units for binding to the cell wall are binding sites of choline like the choline binding domain of LytA autolysin from *Streptococcus pneumoniae* (Fernandez-

Tornero et al., 2001) or a trisaccharide (C-terminal domain of *Clostridium difficile* toxin A [Greco et al., 2006]) present in the cell wall components.

Adjacent to the variable region is the catalytic domain that has 910 aa and is the region of greatest homology with other known dextransucrases as the DSR-S of *L. mesenteroides* NRRL B-512F and DSR-D of *L. mesenteroides* Lcc4 (Figure 3). Amino acid alignment sequence analysis of DSR-V compared to other dextransucrases of the GH70 family was performed to detect the correspondence between the predicted secondary structure elements of DSR-V and those determined from the 3D structure of GTF-180 (PDB: 3HZ3). From sequence alignment, DSR-V can be predicted to adopt the typical U-shape fold adopted by GH70 glucansucrases which comprises five domains: the catalytic A domain, domain B and C that are related to GH13 family enzymes and the domains IV and V specific of GH70 family (Figure 4).

Finally, the C-terminus, also named glucan binding domain (GBD), is 340 residues in size. Once again four putative CW units can be localized at the N-terminal region of this domain. Truncation and deletion studies showed that GBD is involved in binding to the polymerization products, constituting functional domains that can bind to the glucans themselves, but they are not directly involved in catalysis (Suwannarangsee et al., 2007, Brison et al., 2012). The GBD contributes to increasing the concentration of the carbohydrate chain to the vicinity of the active site and plays a role in the presentation of these chains to the catalytic site for elongation and branching (Brison et al., 2016). Truncations in the GBD of DSR-S, synthesizes dextrans with lower molar mass than complete enzymes, having an effect on the elongation of the polymer without affecting the catalysis reaction (Moulis et al., 2006).

^{13}C-NMR analyses of dextran polymers synthetized by recombinant DSR-V

The polymer formed by the recombinant DSR-V was analyzed and compared to the dextran T70 synthesized by dextransucrases DSR-S from *L. mesenteroides* NRRL B-512F (ATCC 10830), as shown in Figure 5.

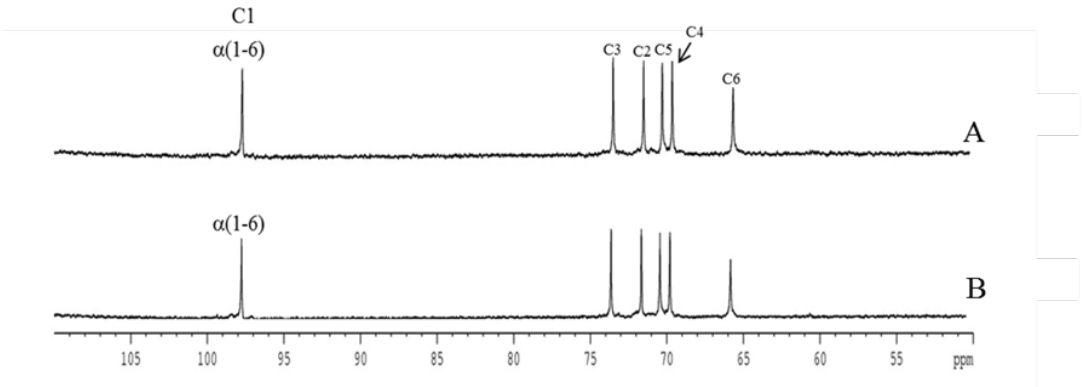

Figure 5. ^{13}C-NMR spectra of dextran polymers. (A) Dextran polymers synthetized by recombinant DSR-V in *E. coli* JM109. (B) Dextran T-70 produced from *L. mesenteroides* NRRL B-512F DSR-S dextransucrase.

Dextrans have their signals ^{13}C NMR anomeric with chemical shifts after 90 ppm, while at C-2, C-3, C-4 and C-5 the chemical shift appears in the range of 70-85 ppm, which is the resonance region known for branched links, and the C-6 is normally before 60 ppm (Gorin, 1981, Ahmed et al., 2012).

The ^{13}C-NMR spectrum of the dextran produced by recombinant dextransucrase DSR-V in the presence of sucrose, has 6 prominent resonances, characteristic of linear dextrans (Maina et al., 2008), showing a very similar maximum spectrum to dextran T-70. In addition to these six peaks, the spectrum doesn't contain peaks indicative of lower branches. The maximum resonance 98.4 ppm indicates the presence of α(1−6) links. This has also been described for DSR-B (Monchois et al., 1998) and DSR-M (Passerini et al 2015), both of the *L. citreum* NRRL B-1299. Overall, these results strongly support the proposal that *L. citreum* M-3 it is probably the first *L. citreum* strain that possesses a DSR-S like protein (DSR-V) with specificity like DSR-B and DSR-M.

Conclusions

On the basis of its amino acid sequence and specificity, it is concluded that DSR-V, is a novel dextransucrase enzyme (EC 2.4.1.5), which must be classified in family GH70. The data obtained in this study enhances the understanding of this dextransucrase and enriches the great biodiversity of this group of enzymes. Further

studies of new chimeric fusion enzymes obtained by the substitution/addition of different Carbohydrate Binding Modules (CBM) will permit the design of rationally/ semi-rationally engineered dextransucrases producing a wide range of glucose polymers with novel physicochemical properties.

Conflict of interest

No conflict of interest exist since the authors declare no competing financial interest, and manuscript is approved by all authors for publication

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