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Construction of a novel chimeric dextranucrase fused to the carbohydrate-binding module CBM2a

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Abstract: The lactic acid bacteria (LAB) have great potential to produce homoexopolysaccharides (HoPS), have been the subject of extensive research efforts, given their health benefits and physico-chemical properties. The HoPS functional properties are determined by structural characteristics of varied molecular weights, types of glycosidic linkages, degrees of branching and chemical composition. The dextransucrases (DSases) are responsible of the synthesis of a kind of HoPS (dextran polymers), which are among the first biopolymers produced at industrial scale with applications in medicine and biotechnology. The concept of glycodiversification opens additional applications for DSases. In that sense the design and characterization of new DSases is of prime importance. Previously, we described the isolation and characterization of a novel extracellular dextranucrase (DSRF) encoding gene. In this study, from DSRF, we design a novel chimeric dextranucrase DSRF∆SP∆GBD-CBM2a, where DSRF-∆SP-∆GBD is fused to the carbohydrate-binding module (CBM2a) of the β-1,4 exoglucanase/xylanase Cex (Xyn10A) of Cellulomonas fimi ATCC 484. This dextranucrase variant is active and without alteration in its specificity. The DSRF-∆SP-∆GBD-CBM2a is purified by cellulose affinity chromatography for the very first time. Our results indicate that new hybrids and chimeric DSases with novel binding capacity to cellulose can be designed to obtain glyco-biocatalysts from renewable lignocellulosic materials.

Keywords: dextransucrases; GH70; lactic acid bacteria; sucrose-active enzymes; carbohydrate binding module; glucansucrase; cellulose binding domain; Leuconostoc

1. Introduction

Homoeopolsaccharides (HoPS) produced by lactic acid bacteria (LAB) have received great research efforts into their physicochemical and bioactive properties [1]. The individual functional properties of HoPS are determined by their chemical composition, molecular weights, types of glycosidic linkages as well as degree and arrangement of branches [2]. The structural diversity of HoPS is a result of the unmatched variety of possible osidic bonds between sugar monomers, offering an extensive range of functionalities of interest for food, feed, pharmaceuticals, cosmetics and chemicals industries [3-6].

Dextran are among the first microbial HoPS produced at industrial scale [7]. Microorganisms of the genera Lactobacillus, Streptococcus, Weissella, Leuconostoc, Pediococcus, Oenococcus and Acetobacter [8] produce these polysaccharides. The Leuconostoc mesenteroides NRRL B-512F is used for the synthesis of the most common and widespread commercial dextran. The biopolymer’s main chain contains α(1-6) linked glucosyl residues with only 5% of α(1-3) linked branches [7]. The dextran fractions of controlled molecular weight and their numerous derivatives are mainly used in medicine, pharmaceuticals and fine chem-
The extracellular glucansucrase (dextranucrase) DSR-S, a 6-α-D-glucosyltransferase (EC 2.4.1.5) is responsible for the polymer production [11]. This enzyme belongs to the glycoside hydrolase family 70 (GH70) according to the CAZy classification (http://www.cazy.org/) [12]. 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 substrates [13,14].

The catalytic domain of GH70 presents the typical (α/β)8 barrel of glucansucrases, the three amino acids (D551, E589, D662, DSR-S numbering) forming the catalytic triad are highly conserved in the GH70 family [15-17].

The study of GH70 enzymes with different specificities from new LAB strains and from mining genome data sets could provide new insights in structure-function relationships of glucansucrases as well as enlarge the natural dextranucrase repertoire available for industrial application [18]. The Cuban Research Institute on Sugarcane By-products (ICIDCA) has a collection of LAB strains isolated from sugarcane and sugarcane derivatives. Some of them have already been characterized to some extent [19-21]. In the present study, a chimeric dextranucrase fused to the carbohydrate-binding module (CBM2a) of the exoglucanase/xylanase Cex (Xyn10A) of Cellulomonas fimic ATCC 484 was obtained. This variant, fully active, was purified by cellulose affinity chromatography and partially characterized.

2. Results and Discussion
2.1. Design of a Chimeric Dextranucrase (DSR-F-∆SP-∆GBD-CBM2a) Fused to the Carbohydrate Binding Module CBM2a

Based on the 3D structural model of DSR-F-∆SP-∆GBD (Fig. 1B), a chimeric dextranucrase fused to the carbohydrate binding module CBM2a (DSR-F-∆SP-∆GBD-CBM2a) was constructed (Fig. 1A and 1C). Deleting the two APY repeat units from the C-terminal end of DSR-F and adding the CBM2a, allowed further investigation of this domain fusion on the specificity of the chimeric fused variant.
Figure 1. (A) Primary structure representation of the dextranase DSR-F of *Leuconostoc citreum* B/110-1-2 and its truncated variant DSRF-ΔSP-ΔGBD. (B) 3D structure model of DSRF-ΔSP-ΔGBD, five different domains are highlighted, in red (domain V), yellow (domain IV), green (domain B), blue (domain A), and magenta (domain C) (C). Linear schematic representation of DSRF-ΔSP-ΔGBD domain organization with CBM2a fused to the C-terminal end.

The recombinant DSR-F enzyme was previously produced without part of its C-terminal glucan binding domain (Fig. 1A, DSRF-ΔSP-ΔGBD). This deletion of the C-terminal domain V including a cell wall (CW) repeat and the two APY repeats did not affect the DSRF-ΔSP-ΔGBD specificity and efficiency [19]. Other examples of APY repeats that have been deleted without adverse effect on other GH70 and related enzymes have been observed in alternansucrase, inulosucrase, and branching sucrase BSR-B [22-24].

2.2. Subcloning of DSR-F-ΔSP-ΔGBD to Obtain DSR-F-ΔSP-ΔGBD-CBM2a in the Expression Vector pdsrF-CBM2a. Purification of the Fusion Protein DSR-F-ΔSP-ΔGBD-CBM2a

The construction of plasmid pdsrF-CBM2a allows the inducible expression of DsrF-ΔSP-ΔGBD fused in its carboxyl end region with the carbohydrate binding module CBM2a of Cex, a β-1,4-exo-glucanase of *Cellulomonas fimi* ATCC 484. The fusion of the CBM2a to a dextranase is reported for the very first time. The presence of the CBM2a module seems to not affect the ability to form dextran polymer by the fusion enzyme DsrF-ΔSP-ΔGBD-CBM2a (Fig. 2_A and B), and it will permit its purification for further characterization and future immobilization on cellulosic or lignocellulosic materials. A success-
ful dextranucrase fusion for immobilization is the case of DSR-S from *Leuconostoc mesenteroides* B-512FMC to glutathione S-transferase (GST), resulting in a novel and completely active fused truncated variant [25].

The purification of DsrF-ΔSP-ΔGBD-CBM2a from the soluble fraction of the *E. coli* DH10B (pdsrF-CBM2a) was performed using regenerated amorphous cellulose (RAC) as an affinity matrix. The purified protein migrated as a single band with an apparent molecular mass of 155 kDa and a 90-95% purity estimated by densitometry (Fig. 2_B). This is the first report of the use of a cellulose-based matrix for the purification of a dextranucrase by affinity chromatography. The levels of purified protein are greater than those achieved by the immobilized metal ion affinity chromatography (IMAC) (data not shown). This may be because RAC has a higher binding capacity (365 mg of protein per gram of RAC) than any other commercial resin used to purify proteins (10-40 mg of proteins per gram of resin) [26].

![Image](image.png)

**Figure 2.** Production of DsrF-ΔSP-ΔGBD-CBM2a in *E. coli* DH10B. (A) Detection of dextranucrase activity through the formation of dextran on LBT agar plate. (B) SDS-PAGE of DsrF-ΔSP-ΔGBD-CBM2a purification, stained combining two detection methods. Zymography of the proteins with dextranucrase activity (colored bands), followed by detection of total proteins by negative staining with imidazole and zinc sulfate salts (transparent bands). The formation of the dextran polymer demonstrates the activity of the fusion. (1) Molecular Weight Marker (ColorBurst™, Sigma-Aldrich Co.). (2) Soluble fraction of *E. coli* DH10B (pSE380) cell lysate as negative control. (3) Soluble fraction of *E. coli* DH10B (pdsrF-CBD) cell lysate. (4) Flowthrough fraction, not retained by the RAC. (5) Fraction of the 1st washed of column (8 Column Volumes, CV). (6) - Fraction of the 2nd washed of column (12 CV). (7 and 8) - Fractions eluted.

### 2.3. Action of DSR-F-ΔSP-ΔGBD-CBM2a in Polymerization and Acceptor Reactions

A polymerization reaction with sucrose was carried out to ensure the chimeric DSR-F-ΔSP-ΔGBD-CBM2a behave in the same way as the DSR-F-ΔSP-ΔGBD variant. As dextranucrase GTF180 from *Lb. reuteri* 180 [27] and alternansucrase (ASR) from *L. citreum* NRRL B-1355 [28], DSR-F-ΔSP-ΔGBD-CBM2a and DSR-F-ΔSP-ΔGBD are also striking examples of α-transglucosylases among GH70 glucansucrases. They catalyze a bi-modal population of glucan, comprising a high molar mass (HMM, 2x10^7 g.mol^-1) dextran and a low molar mass gluco-oligosaccharides (LMM, DP<8, around 1300 g. mol^-1) from sucrose, as estimated by size exclusion chromatography (Fig. 3-I). This result corroborates the dextran formation observed in Fig. 2A and 2B. In addition, it suggests a semi-processive elong-
gation mechanism, as for other GH70 enzymes, where smaller oligosaccharides are produced in a non-processive mode at the beginning of a reaction. When a critical length is reached a processive mechanism starts helped by glucan-binding domains [29].

Figure 3. (I)-Analysis of HPSEC chromatograms synthesized using the chimeric dextranuclease DSR-F-ΔSP-ΔGBD-CBM2a (from sucrose 580 mM and 725 mM) and DSR-F-ΔSP-ΔGBD (from sucrose 292 mM and 435 mM) with 50 mM sodium acetate buffer, pH 5.5 and 1 U.mL⁻¹ of enzyme. Enzymatic reactions were stopped after 24 h. Peak identification: HMM dextran high molecular mass dextran (2.10⁷ g.mol⁻¹ as estimated by HPSEC-MALLS), LMM dextran low molecular mass dextran (oligosaccharides of DP<8), DP1 Fructose. (II)-Analysis of HPAEC-PAD chromatograms products synthesized using DSR-F-ΔSP-ΔGBD-CBM2a and DSR-F-ΔSP-ΔGBD from sucrose (292 mM) and maltose (146 mM) with 50 mM sodium acetate buffer, pH 5.5 and 1 U.mL⁻¹ of enzyme. Enzymatic reactions were stopped after 24 h. Arrows indicate two extra peaks immediately after the products with degrees of polymerization (DP) of 7 and 8. Peak identification: G Glucose, F Fructose, S Sucrose, M Maltose, P Panose (DP3), I4-I8 isomaltooligosaccharides of DP4–DP8. The line with the highest signal of S and M and no detection of isomaltooligosaccharides corresponds to the enzymatic reaction at the initial time (t = 0 min).

In the acceptor reaction (maltose as acceptor molecule) performed with DSR-F-ΔSP-ΔGBD-CBM2a, a series of analog isomaltdextrins very similar to the ones produced by the recombinant dextranuclease DSR-F-ΔSP-ΔGBD from L. citreum B/110-1-2 (Fig. 3-II) were synthesized. The isomalto-oligosaccharides (IMOs) products with 7 and 8 glucose units show two extra peaks (Fig. 3-II, arrows) indicating they were decorated at least with a branched α(1-4) glucose unit. These IMOs products correspond to previously characterized oligodextrins [20]. From these observations, it seems that the cellulose binding domain of DSR-F-ΔSP-ΔGBD-CBM2a does not affect the synthesis of HMM and LMM glucans from sucrose and IMOs products from sucrose/maltose, respectively.

2.4. Action of DSR-F-ΔSP-ΔGBD-CBM2a from Sucrose and Linear Oligodextrans (1500 and 6000 g.mol⁻¹)

A reaction using sucrose (292 mM) and 1500 g.mol⁻¹ dextran (66.6 mM), a linear α(1-6) glucan with a mean degree of polymerization of 9, was performed. Analyses of the
reaction products showed that a low amount of leucrose could be detected due to the action of fructose as an acceptor (Fig. 4-I). This kind of transglycosylation has been reported to be favored at the start of the dextransucrase reactions [29, 30]. It has been recently suggested that leucrose serves as the substrate for further dextran formation or elongation of IMOs [31]. The IMOs from DP8 to DP12 were glucosyl decorated in the same way by the chimeric dextransucrase DSR-F-ΔSP-ΔGBD-CBM2a and DSR-F-ΔSP-ΔGBD, leading to formation of products clearly observable on HPAEC-PAD chromatograms (Fig. 4-I, arrows). Such chromatographic patterns resulting from the acceptor reaction (oligodextrins as acceptor molecules) is reported for the first time for a DSR-F variant enzyme.

According to the specificity of both analyzed enzymes, these modified IMOs could be products being glucosylated to some extent and probably α(1-4) branched glucose units. As a specific population of IMOs seems to act as acceptor molecules to be branched, two factors could be involved with this observation. Structural analyses of dextrans have identified a relation between the degree of branching and the rms radii of the polymers where a comparatively higher proportion of more branched and more compact dextran molecules is increasingly formed. On the other hand branching may also depend on the steric properties of the glucosyl-donor in the active site of the enzyme [31]. However, the structure of these products (DP8-DP12) must be elucidated in more detail.

**Figure 4.** (I) Analysis of HPAEC-PAD chromatograms products synthesized using the chimeric dextransucrase DSR-F-ΔSP-ΔGBD-CBM2a, DSR-F-ΔSP-ΔGBD and native dextransucrase DSR-S from _Leuconostoc mesenteroides_ NRRL B-512F from sucrose (292 mM) and dextran 1500 g.mol\(^{-1}\) (66.6 mM) with 50 mM sodium acetate buffer, pH 5.5 and 1 U.mL\(^{-1}\) of enzyme. Enzymatic reactions were stopped after 24 h, nC, nanocoulombs. **I-(A)**- Enzymatic reaction using DSR-S from _L. mesenteroides_ NRRL–B512; **I-(B)**- Enzymatic reaction using DSR-F-ΔSP-ΔGBD recombinant; **I-(C)**- Enzymatic reaction using chimeric dextransucrase DSR-F-ΔSP-ΔGBD-CBM2a; **I-(D)** - Enzymatic reaction using chimeric dextransucrase DSR-F-ΔSP-ΔGBD-CBM2a at the initial time (t = 0 min). Arrows indicate four extra peaks immediately after the products with degrees of polymerization (DP) of 8-12, respectively. **(II)**- Analysis of HPAEC-PAD chromatogram products synthesized using the chimeric dextransucrase DSR-F-ΔSP-ΔGBD-CBM2a and DSR-F-ΔSP-ΔGBD sucrose (292 mM) and dextran 6000 g.mol\(^{-1}\) (66.6 mM) with 50 mM sodium acetate buffer, pH 5.5 and 1 U.mL\(^{-1}\) of enzyme. Enzymatic reactions were stopped after 24 h. **II-(A)**- Enzymatic reaction using chimeric dextransucrase DSR-F-ΔSP-ΔGBD-CBM2a at the initial time (t = 0 min); **II-(B)**- and **II-(C)**- Enzymatic reaction using DSR-F-ΔSP-ΔGBD-CBM2a and DSR-F-ΔSP-ΔGBD, respectively. Arrows indicate peaks corresponding to modified dextrans from
the initial reaction time. Peak identification: G Glucose, F Fructose, L Leucrose, S Sucrose, DP2-DP12 Isomaltooligosaccharides with a degree of polymerization (DP) from 2 to 12.

A reaction using sucrose (292 mM) and 6000 g.mol\(^{-1}\) dextran (66.6 mM), a linear α(1-6) glucan or oligodextran with a mean degree of polymerization of 37 was performed. As seen on HPAEC-PAD chromatograms, the product profile after 24 hours of reaction was different from the initial reaction profile. As in the previous reaction, a low amount of leucrose was also detected. Analyses of the final reaction products showed the intensity of several peaks corresponding to isomaltooligosaccharides decreased, suggesting those products were modified in a similar way by both enzymes DSR-F-∆SP-∆GBD-CBM2a and DSR-F-∆SP-∆GBD (Fig. 4-II, arrows). So far, only a few studies reported disproportionation reactions of glucansucrases [32,33] which were already studied in detail in other polymerizing enzymes such as levansucrases and inulosucrases [34]. Previous modification of linear α(1-6) short dextrans have only been reported on branching sucrases [13,24]. Therefore, further studies are needed to corroborate a potential utilization of short IMOs as glucosyl donors during dextran synthesis and dextran modifications by dextranucrase-α-transglucosylases enzymes.

From the products analysis of the reactions performed in this study, it seems that on dextranucrase DSR-F variants some kind of interactions with IMOs are taking place. Recently, the first functional surface binding site (SBS-A1) for a GH70 family enzyme was described, it binds isomaltose, isomalto triose, isomaltononaose, panose and oligoalternan [35]. An alignment sequence of SBS-A1 and the equivalent residues in dextranucrase DSR-F is shown in Fig. 5. Several residues are very conserved in both sequences which could be associated with a similar functionality in DSR-F.

![Figure 5](image-url)
3. Materials and Methods

3.1. Bacterial Strains and Culture Media

*Escherichia coli* DH10B was used for the subcloning steps and for protein expression with the pSE380 vector (Invitrogen, USA). The screening of *E. coli* colonies expressing functional dextranucrase enzymes was done [20]. LBT medium was used instead of Lu-ria-Bertani (LB) medium [11]. 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. *E. coli* was grown and maintained on LB medium supplemented when needed with ampicillin (100 μg mL⁻¹). All strains were stored at -80 °C in 15% glycerol.

3.2. Subcloning to Express the dsrF-ΔSP-ΔGBD-CBM2a Gene in *E. coli*

The 3.7 kb fragment encoding the truncated variant of DsrF (DsrF-ΔSP-ΔGBD) was digested with the restriction enzymes NcoI-EcoRI from the pSEdsrF plasmid and inserted into the plasmid pET38b (+) (Novagen), also digested with the same restriction enzymes, to create the 9.5 kb pETdsrF plasmid (Kmr) after the ligation of both fragments. The latter contains the truncated variant dsrF-ΔSP-ΔGBD under the inducible promoter PT7lac, fused at the 3’ end to the signal peptide of the exo-β-1,4-glucanase Cex from *Cellulomonas fimi* ATCC 484, at the 5’ end of the cellulose binding module (CBM2a) of said enzyme, and to the His tag. Likewise, the truncated variant is also fused to the 5’-terminus of the transcription terminator of bacteriophage T7. The strain of *E. coli* DH10B was transformed with the plasmid pETdsrF, which was subsequently purified and digested with the restriction enzymes NcoI-AvrII releasing the dsrF-ΔSP-ΔGBD fused with the CBM2a and the His tag. This fragment was ligated to the equally digested pSE380 plasmid NcoI-AvrII, giving rise to the 8.4 kb pdsrF-CBM2a plasmid (Ap°) containing the dsrF-ΔSP-ΔGBD-CBM2a fragment under the inducible Ptrc promoter, fused at the end 3’ to the terminator of the transcription of *E. coli* rrnBt1-t2. The strain of *E. coli* DH10B was transformed with this plasmid.
### Table 1. Plasmids used in this work.

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Description</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>pET38b(+)</td>
<td>Replicon colE1, vector used for protein production in E. coli, fused to the signal secretion Cex downstream the PT7lac promoter. It harbours the encoding region for the Carbohydrate-Binding Module (CBM2a) and a His tag, Km(^r), size: 5.8 kb.</td>
<td>Novagen</td>
</tr>
<tr>
<td>pSE380</td>
<td>Replicon colE1, vector used for protein production in E. coli downstream the PTrc promoter, Ap(^r), size: 4.4 kb.</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>pSEdsrF</td>
<td>pSE380 NcoI-EcoRI, fused to the 3.7 kb amplicon NcoI-EcoRI obtained from pGEMdsrF. Harbours the truncated variant DsrF-∆SP-∆GBD, Ap(^r), size: 8.1 kb.</td>
<td>[19]</td>
</tr>
<tr>
<td>pETdsrF</td>
<td>pET38b(+) NcoI-EcoRI, fused to the 3.7 kb (DsrF-∆SP-∆GBD) NcoI-EcoRI from pSEdsrF, Km(^r), size: 9.5 kb.</td>
<td>This work</td>
</tr>
<tr>
<td>pdsrF-CBM2a</td>
<td>pSE380 NcoI-AvrII, fused to the 4.2 kb DNA fragment (DsrF-∆SP-∆GBD-CBM2a) NcoI-AvrII from pETdsrF, Ap(^r), size: 8.6 kb.</td>
<td>This work</td>
</tr>
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#### 3.3. Inducible production of DSR-F-∆SP-∆GBD-CBM2a in E. coli

For the production of the recombinant dextranulase DsrF-∆SP-∆GBD-CBM2a, a colony of the recombinant strain of E. coli DH10B (pdsrF-CBM2a), was inoculated in 100 mL invaginated flasks with 10 mL of 2xYT medium supplemented with 100 mM Tris/HCl, pH 6.4, 100 µg.mL\(^{-1}\) ampicillin at 30 °C, and grown overnight at 175 r.min\(^{-1}\). The next day the cultures were diluted 1:100 with the same medium, supplemented with ampicillin 200 µg.mL\(^{-1}\) and grown at 30 °C to an OD(600nm) 0.5 with shaking at 175 r.min\(^{-1}\). Isopropyl-β-D-thio-β-D-galactopyranoside (IPTG) (inducer of the PTrc promoter) was added at a final concentration of 2.4 mmol.L\(^{-1}\) and incubated 12-14 h at 20 °C with shaking at 175 r.min\(^{-1}\). The cells were collected by centrifugation (Eppendorf 5804 R centrifuge) at 10,000 x g for 15 min at 4 °C and resuspended in the rupture buffer [50 mmol.L\(^{-1}\) NaAc, pH 5.4, Triton X 100 0.1% (v/v), CaCl\(_2\) 0.05 gL\(^{-1}\), cocktail of protease inhibitors (Roche)] up to an OD(600nm) of 80. Cell disruption was performed by ultrasound in a disruptor (MSP, England). The sample (always kept on ice) was exposed to seven cycles of 1 min, at a constant amplitude of 28 microns, with one minute of rest between cycles. The lysate extract was centrifuged (Eppendorf 5804 R centrifuge) at 21 390 x g for 40 min, and the soluble and insoluble fraction of each cell extract was recovered. The samples were stored at -20 °C until they were used in other analyses.

#### 3.4. Cellulose Affinity Chromatography (CAC)

The chimeric DSR-F-∆SP-∆GBD-CBM2a was purified by cellulose affinity chromatography (CAC). For this, a matrix of regenerated amorphous cellulose (RAC) obtained from Microgranular Cellulose CC31 (Whatman) was used [36]. The soluble fraction of the sonicate extract (10 mL) was mixed by slow agitation with 5 mL of RAC for 2 hours at 4 °C. The enzyme bound to the matrix was recovered by centrifugation (Eppendorf 5804 R) at 3 500 x g for 5 min and packed in a 10 mL column (10 cm X 2 cm 2, Bio-Rad). The protein was eluted with 1 mL of glycerol 99 %.

#### 3.5. Determination of Enzymatic Activity Dextranulase

The enzymatic activity was performed at 30 °C and 40 °C, in 50 mmol.L\(^{-1}\) sodium acetate buffer (pH 5.4), 0.05 gL\(^{-1}\) CaCl\(_2\), 1 gL\(^{-1}\) NaN\(_3\), and 100 gL\(^{-1}\) sucrose. The saccharolytic...
activity was determined by detecting the levels of free reducing sugars with the dinitrosalicylic acid (DNSA) method [37]. One unit of activity was defined as the amount of enzyme that catalyzes the release of 1 μmol.min\(^{-1}\) of fructose under the conditions tested. The concentration of proteins was detected according to [38] using BSA as a standard. All determinations were made in triplicate.

3.6. SDS-PAGE and Zymograms

Protein electrophoresis under denaturing conditions (SDS-PAGE) was performed with the XCell SureLockTM Mini-Cell system, with NOVEX Tris-Acetate gels of 1.5-mm thickness, NuPAGE® anti-oxidant and NuPAGE® Tris-Acetate SDS Running Buffer (for Tris-Acetate gels) from Invitrogen. The NuPAGE® Sample Reducing Agent (3 ml) and NuPAGE®LDS Sample Buffer (X4) sample buffer (7.5 ml) were mixed with 20 μl of sample and heated at 70 °C for 10 min prior to being loaded onto gels. Samples containing suspended cells were centrifuged at 10,000 \(\times\) g in a microcentrifuge before being applied to the gels. Approximately 2 mU of enzyme was loaded onto the gels and electrophoresis was carried out for 1 h at 150 V. The gels were then stained for dextranucrase activity \textit{in situ} according to the procedure of [39] in combination with a reversible negative protein staining method [40]. Precision Plus Protein™ All Blue Standard was included in all electrophoresis runs.

3.7. Production of High Molecular Weight Dextrans, Maltooligosaccharides, and Linear Dextran Modification

High-molecular-weight dextran synthesis was performed at 40 °C for 24 h, in buffer 20 mmol.L\(^{-1}\) NaAc (pH 5.4), CaCl\(_2\) 0.05 g.L\(^{-1}\), NaN\(_3\) 1 g.L\(^{-1}\), sucrose 100 g.L\(^{-1}\) and 1 U.ml\(^{-1}\) of enzyme. Malto-oligosaccharides were produced under the same conditions, except maltose 50 g.L\(^{-1}\) was added to the reaction. Linear dextrans were modified under the same conditions but 66.6 mM of α-1,6 dextrans (1500 g.mol\(^{-1}\) and 6000 g.mol\(^{-1}\); Sigma) was added.


Synthesized oligosaccharides were analyzed by high HPAEC-PAD using a Dionex Carbo-Pack PA100 4x250 mm column at room temperature. A gradient of sodium acetate (from 6 to 300 mmol.L\(^{-1}\) in 28 min) was applied in 150 mmol.L\(^{-1}\) NaOH at a flow rate of 1 mL.min\(^{-1}\). Detection was performed with an ED40 Dionex module gold electrode and a reference pH Ag/AgCl. Standards used were glucose, fructose, sucrose, panose, leucrose, prepared as 10 mg.mL\(^{-1}\) in buffer 20 mmol.L\(^{-1}\) NaAc (pH 5.4). The samples were diluted 10 times in water and were filtered through membranes with pores of 0.20 μm (Sartorius) before injection.

3.9. High-Performance Size Exclusion Chromatography (HPSEC)

Glucan molecular weight distributions were determined by HPSEC. For dextran analyses, two Shodex OH-Pack SB-805 and SB-802.5 columns were maintained in series, using an eluent containing 0.45 M of NaN\(_3\) and 1% of ethylene glycol at a flow rate of 0.3 ml min\(^{-1}\). Columns and guard columns were maintained at 70 °C, and samples were filtered through a 0.45-μm-pore-size filter (Sartorius) before injection [29]. The reaction was stopped after 24 h by heating 5 min at 95 °C in a boiling water bath. Calibration standards of commercial dextrans of 2.10\(^6\) g.mol\(^{-1}\), 530.103 g.mol\(^{-1}\), 70x10\(^3\) g.mol\(^{-1}\), and 10x10\(^3\) g.mol\(^{-1}\) (Sigma-Aldrich) were used.

4. Conclusions
Both enzymes were able to produce HMM and LMM polymers from sucrose. They were also able to modify at least two kinds of linear dextrans (1500 and 6000 g/mol), making DSR-F-∆SP-∆GBD-CBM2a and DSR-F-∆SP-∆GBD biocatalysts to act as efficient α-transglucosidases in the presence of sucrose and linear oligodextrans. The additional cel lulose-binding domain does not affect the modification capacity on linear oligodextrans of the fusion enzyme. These findings give new insights into the versatile reactions catalyzed by dextranases. Investigation of the relationship between structure and function of DSR-F variants will undoubtedly improve understanding of the polymerization mechanism of this enzyme and of GH70 glucansucrases in general. Considering its specificity, this fusion variant hold a great potential for the production of novel functional foods.

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