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ORIGINAL RESEARCH

Molecular interaction of linear and branched fructans of different sizes with levansucrase and inulosucrase from *Lactobacillus gasseri*: modeling and computational analysis

Interacción molecular de fructanos lineales y ramificados de distintos tamaños con levansacarasa e inulinsacarasa de *Lactobacillus gasseri*: modelado y análisis computacional

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ABSTRACT

Human beings consume probiotics and prebiotics because of the health benefits they provide. *Lactobacillus gasseri* is a probiotic microorganism native to humans which produces glycolytic enzymes directed at the hydrolysis of soluble fibers as prebiotic fructans. In this work, levansucrase (LevG) and inulosucrase (InuGB) from *L. gasseri* were used to predict the ability of *L. gasseri* to interact with linear and branched fructans prebiotics (inulin and agavins). AlphaFold and SWISS-MODEL were the servers used for tertiary structure prediction of LevG and InuGB, and fructans with different degrees of polymerization (DP2, DP4 DP6, DP8, DP12 and DP20) were used to generate ligand-enzyme molecular dockings by AutodockVina, GlycoTorchVina, and Autodock FR software. The best affinity energies obtained by molecular docking were obtained with agavin and inulin with a DP of 6 and 8 units. Aspartic acid, glutamic acid, asparagine and arginin are the main amino acids involved in the interaction with these substrates. At the same time, the binding pocket shows hydrophilic characteristics; GlycoTorchVina was the best software for protein-ligand docking. These results demonstrate the ability of *L.*

gasseri enzymes to interact with different molecular structures of fructans; levansucrase with better affinity to agavin and inulosucrase with better affinity to inulin. The above helps to understand the structure-functionality relationship of the prebiotic effect, both in symbiotic formulations and in the human digestive tract.

Keywords: *Lactobacillus gasseri*, Inulosucrase, levansucrase, agavin, inulin, molecular docking.

RESUMEN

Los seres humanos consumimos probióticos y prebióticos por los beneficios que aportan a la salud. *Lactobacillus gasseri* es un microorganismo probiótico nativo del ser humano que produce enzimas glicolíticas dirigidas a la hidrolisis de fibras solubles (prebióticos tipo fructanos). En este trabajo se utilizaron levansacarasa e inulinsacarasa de *L. gasseri* para predecir su capacidad de interacción con prebióticos lineales y ramificados (inulina y agavina). AlphaFold y SWISS-MODEL fueron los servidores utilizados para la predicción de la estructura terciaria de LevG e InuGB, y se utilizaron sacáridos con diferentes grados de polimerización (DP2-DP20) para generar acoplamientos moleculares usando AutodockVina, GlycoTorchVina y Autodock FR. Las mejores energías de afinidad obtenidas fueron con agavinas e inulinas (DP6 y DP8). Los principales aminoácidos implicados en la interacción con estos sustratos son el ácido aspártico, ácido glutámico, asparagina y arginina. A su vez, el bolsillo de unión muestra características hidrofílicas necesarias para la interacción. GlycoTorchVina fue el mejor software para el acoplamiento de carbohidratos. Estos resultados demuestran la capacidad de las enzimas de *L. gasseri* para interactuar con diferentes estructuras moleculares de fructanos. Lo anterior ayuda a comprender la relación estructura-funcionalidad del efecto prebiótico, tanto en formulaciones simbióticas como en el tracto digestivo humano.

Palabras clave: *Lactobacillus gasseri*, Inulinsacarasa, levansacarasa, agavina, inulina, molecular docking

1. INTRODUCCIÓN

Probiotics are live microorganisms that directly impact the gut microbiome through selective delivery, and when administered in adequate amounts to the host, they confer health benefits (Plaza-Díaz *et al*., 2019; Żółkiewicz *et al*., 2020). They have often been administered in fermented milk products, which have the faculty to occupy niches in the mucosa of humans, such as the oral cavity, gastrointestinal tract, and genitalia, among others (Selle & Klaenhammer, 2013). *Lactobacilli* is a genus composed of more than 170 species, and some of these species are used in producing fermented foods derived from animals and plants. The major *Lactobacillus* genus found in the microbiota from the adult gastrointestinal tract are *L. gasseri, L. reuteri, L. crispatus, L. salivarius, L. ruminis*; in infant feces, *L. plantarum, L. salivarius, L. rhamnosus, L. paracasei, L. fermentum, L. delbrueckii, L. reuteri* and *L. gasseri* (Zhang *et al.,* 2018).

The *L. gasseri* is a probiotic native to the human intestine studied for its several beneficial properties for humans; it can improve the intestinal environment, tolerance to bile salts, deconjugation of bile acids and cholesterol-binding, and cholesterol-lowering. Also, benefic effects in humans with hypercholesterolemia, preventive effect in ulcerative colitis in rats, bacteriocin production and anti-obesity effect, and antiInflammatory bowel disease (Han *et al*., 2022; Pan *et al*., 2020; Nishida *et al.,* 2019; Oh *et al*., 2018). *L. gasseri* can act on many polysaccharides and can be delivered as a complementary symbiotic (probiotics plus prebiotics working together to achieve one or more health benefits) or as a synergistic symbiotic, where the selected substrate is used to potentiate specific health benefits provided by the co-administered live microorganisms to humans (Swanson *et al*., 2020; Gibson *et al*., 2017).

A prebiotic is a dietary fiber that resists hydrolysis by digestive enzymes, reaching the colon whole, where they are selectively fermented by beneficial intestinal microflora (Trowell *et al*., 1976), in which *L. gasseri* is present. Fructans (fructose-based polymers), extracted from vegetal sources, have been recognized as prebiotic ingredients because they stimulate the growth of bacteria in the colon, benefit gastrointestinal health, and have several beneficial metabolic effects (Alvarado-Jasso *et al*., 2020; Huazano-García *et al*., 2017; Moreno Vilet *et al*. 2016).

Inulin belongs to the group of fructans and is mainly composed of β-D-fructosyl subgroups linked by glycosidic bonds (2→1), and the molecule usually ends with an attached α-D-glucosyl group (1↔2); thus, it has linear molecular structure. Agavin-type fructans, also known as agave fructans, are extracted from plants of the *Agave* genus and present a branching structure characterized by two different bonds between fructose monomers, β (2→1) and β (2→6) (Buitrago-Arias *et al*., 2021). Both inulin and agavin are mixtures of fructans of different chain sizes, represented by the number of fructose units attached or the degree of polymerization (DP). The average DP for inulin has been reported around 21-26, while for agavin, 15-19.5 (Moreno Vilet *et al.* 2017). However, short-chain fructans, also known as fructooligosaccharides (FOS) with DP between 3 and 12, are of particular interest due to their greater prebiotic effect (Mueller *et al.* 2016).

To be able to use the carbohydrate polymers, the microorganisms produce two prominent enzyme families to synthesize and hydrolyze glycosidic bonds: glycosyltransferases (GTFs, EC 2.4.1-) and glycosyl hydrolase (EC 3.2.1-). In *L. gasseri*, three fructansucrases (InuGA-RM, InuGB-R, and LevG-R) have been reported (Anwar *et al.,* 2010) and are catalytically active in hydrolysis and synthesis of fructan polymers. Some amino acids involved in catalysis are mainly residues D266 and D251, as they function as nucleophiles in the inulosucrase and levansucrase of *L. gasseri,* respectively. Other amino acids responsible for the enzyme-substrate interaction are R418, D419, E516, I517 and E518 for InuGB, while S320, R403, D404, E503 and R523, for LevG. However, their structural characteristics are not well known.

The exponential growth rate of the volume, variety, and velocity of data creates "big data" (Pal *et al.,* 2020). The big biological data and the development of bioinformatics tools imply predictive power, reproducibility, and simulation of biological systems (Gauthier *et al.,* 2018). Thanks to these bioinformatics advances, genetic information on *L. gasseri* is available in different databases, which provide information about the enzymes involved in

carbohydrate transformation. Furthermore, with the technologies of structural modeling of proteins (SWISS-MODEL) or AlphaFold, it is possible to generate three-dimensional models of proteins (David *et al*., 2022; Whaterhouse *et al.,* 2018) and thereby study the protein-ligand interaction using bioinformatic tools of dockings methods such as AutodockVina (Vina), GlycoTorchVina (GlycoT) or AutoDock FR (ADFR) (Boittier *et al.*, 2020; Ravindranath *et al*., 2015; Trott & Olson 2010).

The study of probiotics and prebiotics have been extensively studied; however, the enzymatic mechanisms they interact are poorly understood. Due to *L. gasseri* being a microorganism widely used as a probiotic, studying hydrolase/transferase enzymes is essential to know how it interacts with different prebiotic molecules of different sizes. Because of this, the objective of the present study is to know the interaction of levansucrase (LevG) and inulosucrase (InuGB) from *L. gasseri* with inulin and agavin with different DP (6, 8, 12, and 20) as well as sucrose (DP: 2) and nystose (DP: 4). Different molecular docking methodologies were used, and thus set a precedent for the software to be used with linear and branched carbohydrates such as inulins and agavins. To our knowledge, this is the first work in which the enzyme-ligand interaction is carried out using inulin and agavin of various degrees of polymerization.

2. MATERIALS AND METHODS

2.1 Collection of enzymes from *L. gasseri*

The sequences of two *L. gasseri* enzymes were collected from the UNIPROT database (https://www.uniprot.org/) with accession numbers D3WYW0 and D3WYV9 corresponding to levansucrase (LevG) and Inulosucrase (InuGB), respectively.

2.2. Phylogenetic analysis

The phylogenetic tree was constructed using an amino acid alignment of bacterial fructansucrases from the genera *Bacillus*, *Geobacillus*, *Paenibacillus*, *Evansella*, *Streptococcus*, *Frutilactobacillus*, and *Leuconostocaceae*. as outgroup pepsin from *Homo sapiens* was used. The sequences were aligned using MUSCLE using MEGA X software (Kumar *et al*., 2008) with the following configuration: Maximum likelihood method with 1000 bootstrap replicates based on the Jones-Taylor-Thornton (JTT) matrix.

2.3 Modeling and evaluation of LevG and InuGB structures from *L. gasseri*

Protein modeling was performed with the SWISS-MODEL (SM) online server (https://swissmodel.expasy.org), using the structure of the levansucrase from *Lactobacillus johnsonii* NCC533 (PDBID: 2YFS.1.A) as a template (Pijning *et al*., 2011). Three-dimensional models with sequence identity greater than 62.75% and monomer confirmation with the uncovered binding pocket site were used. Protein models of LevG and InuGB were generated with AlphaFold (AF) via the google colab platform (Mirdita *et al*., 2022).

The evaluation and validation of the three-dimensional models were performed with the PDB format files of the enzymes. The ProSA-Web server (https://saves.mbi.ucla.edu/) was

used for quality assessment. With the SAVES v6.0 metaserver (Structure Analysis and Verification Server), the Verify3D tool was used for compatibility analysis between the atomic model and its amino acid sequence. The MOLPROBITY (http://molprobity.biochem.duke.edu/) for validating the protein structure by the Ramachandran graph.

2.4 Molecular docking

The inulin and agavin structures used in this work were generated using ACD/ChemSketch version 2020.2.0, and Chem3D version 15.0.0.106 (Fig. 1); the sucrose, nystose, and glycerol structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) with code 5988, 166775, 753 respectively.

Carbohydrate polymers were optimized with Avogadro software (Hanwell *et al*., 2012), using MMFF94. Molecular docking analysis was performed with AutoDockVina software (Trott & Olson, 2010) in conjunction with AutoDockTools (Morris *et al*., 2009), GlycoTorchVina (Boittier *et al*., 2020), and Autodock FR (Ravindranath *et al.,* 2015). Molecular docking software considered the conformation of the target receptor as a rigid molecule unit. In contrast, the ligands and nucleophiles from LevG and InuGB (Asp251 and Asp266, respectively) were flexible and adaptive to the target. Vina searched for conformations with the lowest binding affinity and was run using an exhaustivity of 12 and a grid box with 25 × 35 × 35 Å in the x, y, and z directions (Supplementary Table S1). Redockings were performed with 2YFS using glycerol and sucrose as ligands. The interaction of the ligand with the enzyme was visualized in Discovery Studio visualizer V21.1.0.20298 and UCSF Chimera software.

2.5 Electrostatic potential of LevG and InuGB from *L. gasseri*

Adaptive Poisson-Boltzmann Solver (APBS) analysis was performed using the APBS-PDB2PQR server (https://server.poissonboltzmann.org/) (Jurrus *et al.,* 2018) at pH 5.5.

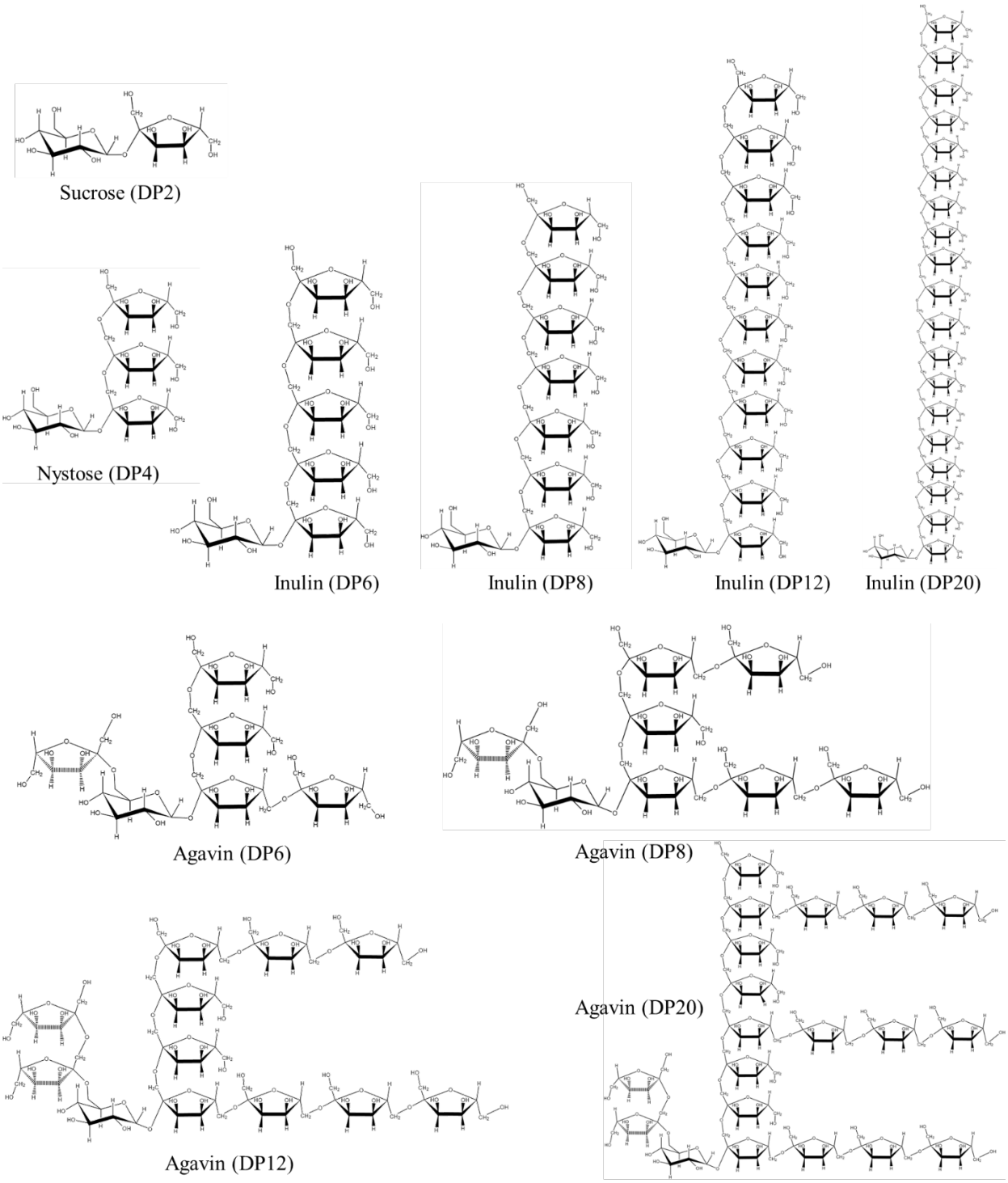

Fig. 1. Carbohydrate structures of inulins and agavins modeled for molecular docking analysis. The degree of polymerization is shown in parentheses.

3. RESULTS

Glycosyltransferases and glycosyl hydrolases are two large families of enzymes that constitute the main catalytic motor for synthesizing and hydrolysis of glycosidic bonds. A phylogenetic tree was constructed to know the evolutionary affiliations between enzymes from different microorganisms. Three distinctive clades were observed: one dominated by enzymes belonging to the genus *Bacillus*, another by *Lactobacillus,* and a third by *Streptococcus*. The LevG and InuGB of *L. gasseri* (Fig. 2) are found within the clade corresponding to the genus Lactobacillus, sharing similarities with the inulosucrase of *L. johsonii*, and the levansucrase of *Fructilactobacillus sanfranciscensis*.

The data obtained by evaluating the quality of the three-dimensional models of LevG and InuGB from SWISS MODEL with ProSA-web show negative values -9.11 and -10.04, and AlphaFold models LevG and InuGB showed -9.54 and -8.94 values, respectively. The Z-score value indicates the degree of nativeness of the modeled protein structures; if it is a negative value, it means that it has native folding, while positive values indicate erroneous or problematic models (Wiederstein & Sippl, 2007). Therefore, these values indicate proper protein folding (Supplementary figure; Fig. S1). The overall quality of the models shows a small region sequence representing positive energy values (Fig. S1); this indicates that this sequence segment presents an unreliable structure compared to the rest of the amino acid fragments, whose values are within the allowed negative energy values.

Fig. 2. Phylogenetic tree. The phylogenetic tree is based on the sequences of glycosyltransferases and glycosylhydrolases from microorganisms. Pepsin from *Homo sapiens* was used as an outgroup. The neighbor-joining algorithm, 1000 bootstrap method were used. The black boxes indicate the microorganisms used in this study.

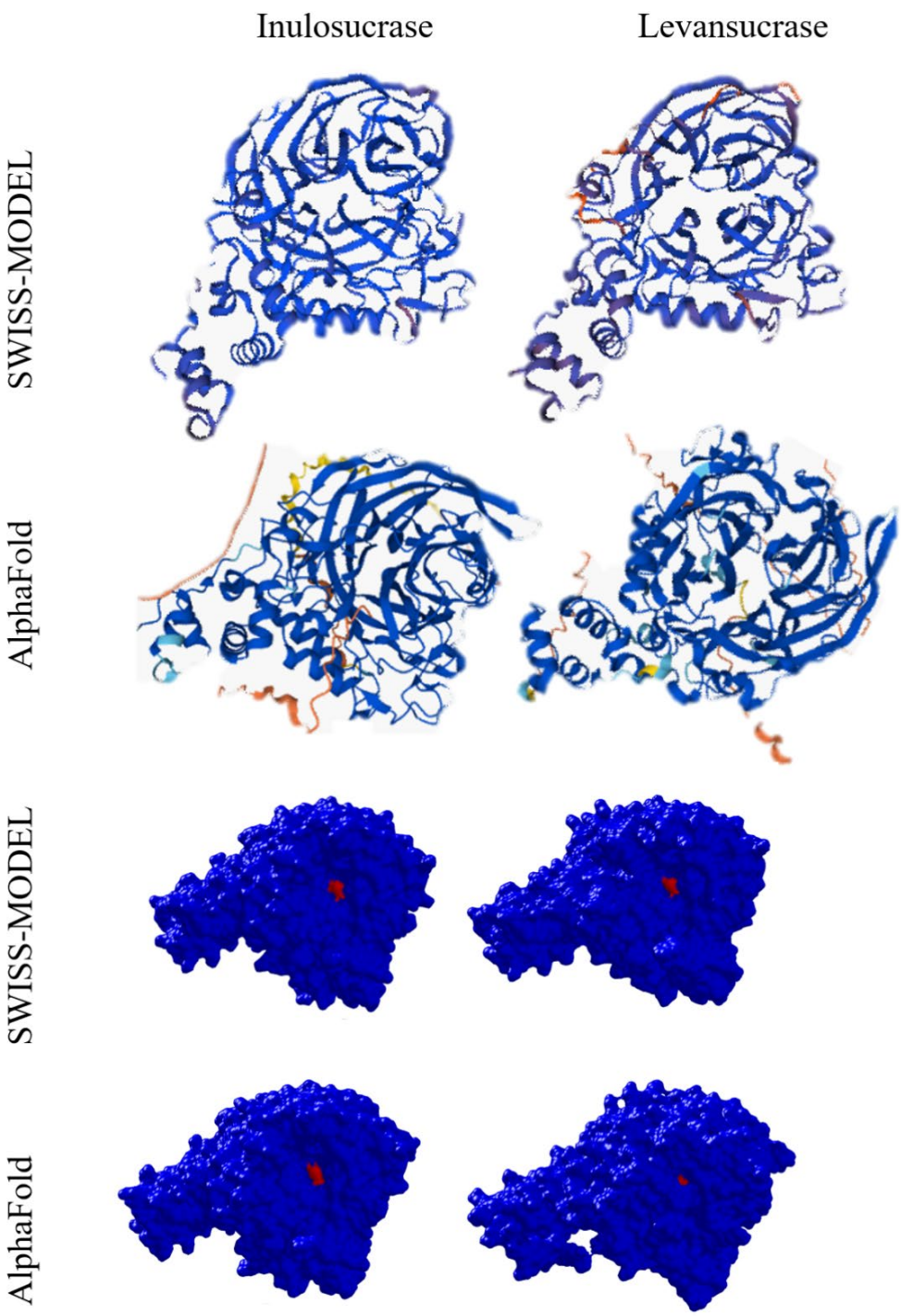

Fig. 3. Structure of levansucrase and inulosucrase enzymes from *Lactobacillus gasseri*. A: Cartoon models with a confidence score, in blue: very high (pLDDT > 90), confidence in cyan: confident (90 > pLDDT > 70), yellow: confidence low (70 > pLDDT > 50), and orange: very low (pLDDT < 50), where pLDDT correspond to per-residue confidence score (pLDDT) between 0 and 100. B: Surface models. The red surface indicates the active site of LevG and InuGB.

Another quality analysis of the three-dimensional models is the Ramachandran plot, using the PROCHECK server to confirm the quality of the protein structures. According to the Ramachandran plot generated for SWISS-MODEL structures of LevG and InuGB, 95.62.5% and 94.65% of the residues are observed within the three allowed zones, respectively, while 0.56% and 0.19% of the amino acids are in the non-allowed regions (lower right side). For AlphaFold models, the Ramachandran Plots from LevG and InuGB 94.19% and 95.51% of residues are observed within the three allowed zones, while 0.91% and 0.56% residues are shown in non-allowed regions (Fig. S1). A typical Ramachandran plot of a good model should contain very few residues in the disallowed regions and report many residues in the most favorable regions (Kleywegt & Jones, 1996). On the other hand, it is suggested that values greater than 90% of residues in favored regions indicate the quality of the protein model (Laskowski *et al.* 2012).

Using Verify3D analysis, we observed that at least 95.68% of the residuals have a mean 3D-1D score ≥ 0.2 for the LevG structure, while InuGB showed that 99.62% of the residuals have a mean 3D-1D score ≥ 0.2; however, InuGB and LevG from AlphaFold models showed a 3D/1D profile of score ≥ 0.2, 98.92% and 96.64% of residues have averaged, respectively. All models were found to be above the ratio allowed (>80%) by the program, indicating a good quality of the models (Fig. S2).

On the other hand, the construction of the three-dimensional models generated by SWISS-MODEL using the 3D model of 2YFS.1.A as a template resulted in 62.57% and 92.71% sequence identity for LevG and InuGB, respectively (Fig. 3). Structural and confidence comparison between models from SM and AF show in Fig. 3 and supplementary Fig. S3, where, similar structures are observed in both modeling software. No confidence scores less than 50 were found located in the substrate binding pocket, indicating that both modeling software can be used for molecular docking; however, in levansucrase from AlphaFold an alpha helix blocks the binding pocket marginally (Fig. 3 and Fig. S3-D).

The redocking performed with 2YFS showed the best binding affinity towards sucrose compared to glycerol (-6.9 and -4.0 kcal/mol, respectively), and a shorter distance between the catalytic aspartic acid and glycosidic bond of sucrose when redocking was performed with Autodock Vina. These findings indicate that it is plausible to use the models described above for subsequent molecular docking (Supplementary Table S2).

With the data obtained from the quality of the models, molecular docking was carried out. The substrates used in molecular dockings, sucrose, nystose, and the polysaccharides agavin and inulin of different DP (6, 8, 12, and 20) show that LevG and InuGB have higher affinity energy with DP6 and DP8 substrates (< -7.0 kcal/mol) than with DP2 and DP12 when AutodockVina was used (SM-Vina) (see Table 1). On the other hand, nystose (DP4) was the second substrate with the best affinity energy (< -7.0 kcal/mol for LevG and InuGB). Analysis with GlycoT shows similar results at molecular dockings with AutodockVina; however, the best affinity energy ranged the -6.2 to 7.9 kcal/mol with DP6 and DP8 substrates and protein models of SM and AF. The carbohydrate affinity using GlucoT showed preferences for agavins when LevG and InuGB were tested. Although the ADFR can generate flexible ligands, it was inefficient in generating carbohydrate-

protein dockings; only sucrose can interact with enzymes showing affinity energy of -3.2 and -3.8 for InuGB and LevG, respectively (Table 1).

Substrates of polymerization degree DP12 and DP20 did not bind efficiently as smaller substrates, indicating the preference of LevG and InuGB for substrates of short chains (DP2-DP8). The selectivity of the enzymes for a substrate is also notable, where LevG and InuGB show a greater affinity for agavin (Table 1). Also, the selectivity of the enzymes for a substrate shows the best affinity for agavin than inulin, considering the binding energies with flexible ligand models SM-GlycoT and AF-GlycoT.

Although the analyses with the different molecular docking (AutodockVina and GlycoT) programs worked to generate similar affinity energies in the protein-ligand interaction, it is necessary to consider that AutodockVina shows a standard error of 2.85 kcal/mol (Trott & Olson, 2010). Also, GlycoTorchVina is a VinaCarb-based molecular docking tool that quantifies the glycosidic torsion angle preferences in carbohydrates (Nivedha *et al.,* 2016); therefore, the best software to generate polysaccharide interactions (agavin and inulin) was GlicoT.

The molecular interactions observed between agavin or inulin with LevG show more interaction with acidic amino acids and arginine. When the agavin DP8 interacts with LevG, we observed an interaction of the Asp251-catalytic and Arg523, with fructose from agavin and Trp250 when interacting with inulin, generating hydrogen bridges to stabilize the binding of the substrate to the protein. Asn346 forms a hydrogen bridge in the fructose-fructose glycosidic bond of agavin while this same mechanism occurs with inulin in the amino acids Asn399 and Arg604 (Fig. 4 G-H).

The molecular interaction of agavin DP8 with InuGB shows hydrogen bridge-type bonds between Arg418 and Glu516, amino acids involved in the substrate-binding site. When inulin DP8 interacts with the enzyme, hydrogen bonds are observed between Glu518, related to the substrate-binding site, transition, and proton acceptor/donor nucleophilic attack (Anwar *et al.,* 2010) (Fig. 4). Also, in InuGB, arg617 forms hydrogen bridges with the glycosidic bond between fuctose-fructose and fructose-glucose with agavin and inulin, respectively (Fig. 4 A-B), In inulin, also Asn360 forms a hydrogen bond.

The calculation of the electrostatic surface potential from the structures of LevG and InuGB was elaborated according to pH 5.5. The surface charge distribution of the two enzymes shows alkaline cavities at the substrate-binding site (Fig. 5). The regions surrounding the active site "carbohydrate binding cavity" on the electrostatic surface of LevG and InuGB are relatively well conserved (Fig. 5). Acidic patches are found on the surface of the proteins. However, there are more alkaline patches that allow the interaction of the substrate with the protein.

Table 1. Comparison of binding affinity energies with different molecular docking software and substrates with different degrees of polymerization and branching.

Enzyme	Substrate				Binding affinity (kcal/mol)				
	Sucrose	Nystose	Agavin	Inulin	SM-Vina	AF-Vina	SM-GlycoT	AF-GlycoT	AF-ADFR
InuGB	DP2	-	-	-	-6.0	-6.8	-5.6	-6.7	-3.2
		DP4	-	-	-7.4	-7.1	-6.1	-7.3	-0.5
	-	-	DP6	-	-7.0	-8.1	-6.4	-7.9	4.5
	-	-	DP8	-	-8.4	-7.7	-6.2	-8.0	10.7
	-	-	DP12	-	-7.2	7.0	-5.3	-7.7	18.5
	-	-	DP20	-	-5.4	-5.3	-4.2	-6.0	36.6
	-	-	-	DP6	-7.0	-7.2	-6.8	-7.4	3.6
	-	-	-	DP8	-7.9	-8.4	-6.3	-7.7	10.2
	-	-	-	DP12	-5.9	-7.7	-5.8	-7.9	20.3
	-	-	-	DP20	-5.2	-6.0	-4.0	-5.3	48.1
LevG	DP2	-	-	-	-6.1	-6.0	-6.0	-5.7	-3.8
	-	DP4	-	-	-7.3	-4.7	-6.9	-5.4	-0.2
	-	-	DP6	-	-8.6	-6.4	-6.8	-5.3	6.4
	-	-	DP8	-	-8.1	-5.4	-6.7	-5.1	9.5
	-	-	DP12	-	-7.1	-4.8	-4.5	-3.9	20.9
	-	-	DP20	-	-5.5	NA	-3.4	NA	37.2
	-	-	-	DP6	-7.4	-5.3	-6.3	-5.2	7.3
		-	-	DP8	-7.4	-5.7	-5.8	-4.0	11.5
	-	-	-	DP12	-3.2	-5.6	-5.4	-4.3	20.4
	-	-	-	DP20	NA	NA	1.9	NA	39.5

SM-VINA: Molecular docking with Autodock Vina and 3D Swiss Model, AF-VINA: Molecular docking with AutodockVina and AlphaFold 3D model, AF-ADFR: Molecular docking with Autodock FR and AlphaFold 3D model, -GlycoT: Dockings with GlycoTorchVina and NA: not available.

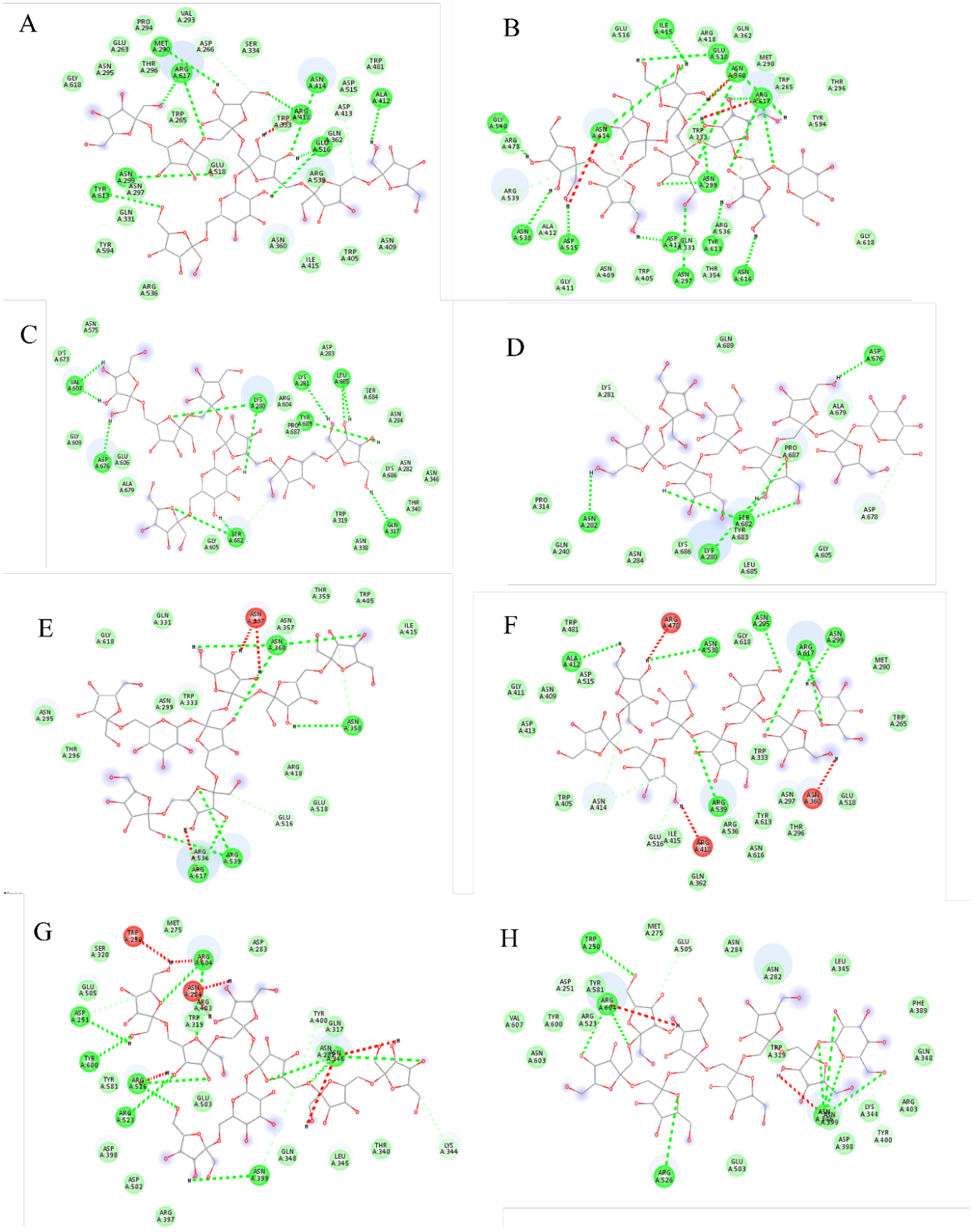

Fig. 4. Protein-ligand molecular interaction using GlycoT. The green dotted lines represent conventional hydrogen bonds, and the yellow dotted lines represent carbon-hydrogen bonds. The molecular modeling figures were generated with Discovery Studio software. A: InuGB

AlphaFold with Agavina (DP8), B: InuGB from AlphaFold with inulin (DP8), C: LevG from AlphaFold and Agavin (DP8), D: LevG from AlphaFold with inulin (DP8), E: InuGB from SWISS-MODEL with agavin (DP8), F: InuGB from SWISS-MODEL with inulin (DP8), G: LevGB from SWISS-MODEL with agavin (DP8) and LevG from SWISS-MODEL with inulin (DP8).

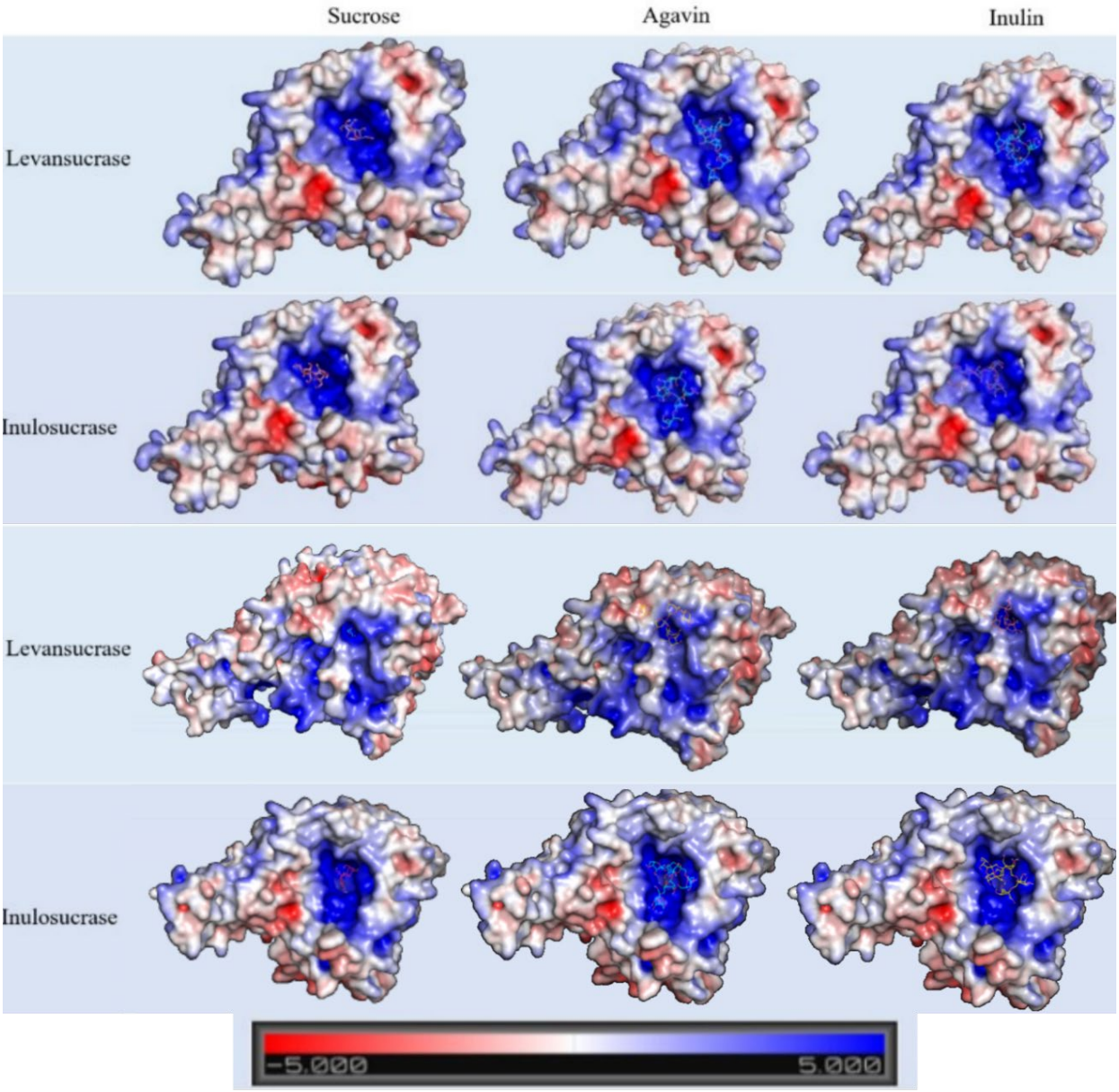

Fig. 5. Structure of LevG and InuGB from *L. gasseri*. Molecular docking with levansucrase and inulosucrase. The Adaptive Poisson-Boltzmann Solver (APBS) analysis is shown in colors, the red color indicates a negative potential (excess of negative charges, -5), and the blue color indicates a positive potential (excess of positive charges: 5). The agavin and inulin (DP6) were used as an illustrative ligand. The two upper rows correspond to the SWISS-MODEL and the two lower rows to the AlphaFold models

4. DISCUSSION

Probiotics are microorganisms that confer a health benefit to the host, as long as they are administered in adequate doses and have the following characteristics: preferably of human origin, free of vectors that could lead to antibiotic resistance, ability to survive in intestinal conditions, antagonism against pathogens and stimulation of the immune system, and finally, have demonstrable beneficial effects on the host (Plaza-Diaz *et al*., 2019). Analyses of the human microbiome have found *Lactobacillus gasseri* in the gastrointestinal tract and vaginal fluids (Pan *et al*., 2020). Several studies have been conducted to know its potential for human health (Gao *et al*., 2022; Nishida *et al*., 2021; Oh *et al*., 2018). Due to the growth of the probiotic industry (estimated at 7% per year), a 12.7% increase in the probiotic industry is expected in the next eight years (Cunningham *et al.,* 2021). In this context, prebiotic fructans such as inulin (linear structure) and agavin (branched structure) have received much attention for consumption in humans or animals. A lot of studies have used inulin or agavin in symbiotic formulations of functional foods (Dutra Rosolen *et al.* 2019; Santiago-García *et al.,* 2021), and more studies evidenced the health effects of including inulin and agavin in the human diet (Espinosa- Andrews *at al.* 2021; Tawfick *at al.* 2022) or aquaculture nutrition diets (Ochoa-Romo *et al*., 2022). The beneficial effect of fructans, can be via direct or indirect mechanisms (Moreno-Vilet *et al.* 2016). Indirect mechanisms involve stimulating probiotic growth and producing short-chain fatty acids that trigger a series of metabolic mechanisms. The influence of structure and DP of fructans on their prebiotic effect has been studied with different strains, in which the growth enhancement was higher with fructans with lower DP and branching characteristics (Muller *et al.* 2016; García Gamboa *et al.,* 2018).

This study performed protein modeling and docking to understand the molecular interaction of linear and branched fructans with LevG and InuGB of *Lactobacillus gasseri*. Molecular dockings with the enzymes LevG and InuGB from *L. gasseri* had a percentage of sequence identity of 2YFS.1.A greater than 62%, and couplings with binding energies greater than -7.0 kcal/mol for agavins of a low degree of polymerization. Compared with this study, tridimensional structures of levansucrase from proteobacteria *Sphingobium chungbukense* DJ77, *Zymomonas mobilis* and *Bacillus amyloliquefaciens* levansucrase KK9 with 34%, 54% and 89% of sequence identity have been employed (Xu *et al.,* 2021). When *B. amyloliquefaciens* KK9 levane-type fructooligosaccharides were used, affinity energy of -7.7 kcal/mol was obtained (Phengnoi *et al.,* 2020). In addition to favorable affinity energies (negative values of kcal/mol), the interaction of acidic amino acids (Asp and Glu) is indispensable for catalysis by levansucrases and inulosucrases. The importance of acidic aminoacids resides in the possibility of carrying out a nucleophilic attack on the glycosidic bonds and hydroxyl groups of fructoses. In levansucrases from *Bacillus subtilis*, an aspartic acid is a binding site of the fructose; similar interactions occur in the inulosucrase from *B. gasseri* (Chambert & Gonzy-Treboul, 1976; Ni *et al.,* 2018).

The results obtained from molecular docking indicate that the interaction of LevG and InuGB with linear and branched fructans is possible. However, the selectivity of the enzymes for a specific substrate is also notable, where LevG shows a greater affinity for agavin and InuGB with a better affinity to inulin. Moreover, the preference of these

enzymes is directed towards substrates with low degrees of polymerization (six to eight units), and the fructan with the best binding energy to the catalytic site was a branched structure with DP6 and DP8. These findings are congruent with the growth of *L. gasseri* on agave fructans of *A. salmiana spp* crassipina with a degree of polymerization between 2-10 (García Gamboa *et al.,* 2018). Similar results are reported by Muller *et al.,* 2016; inulin and agave fructans tested their prebiotic effect in seven probiotic strains, which is favored with branched and low DP fructans; mentioning that inulin is more often cleaved extracellularly into mono or disaccharides prior fermentation, whereas agavin, a branched structure with better solubility, cleaved intracellularly. Also, Kilua *et al.,* 2021 indicate that agavin mainly induces intestinal proliferation of *Bifidobacterium* and *Lactobacillus* compared to maltodextrin and cellulose.

In conclusion, the analyses show the similarity of the enzymes studied with the genus *Lactobacillus*, which can act on fructans of different molecular structures such as agavin (branched) and inulin (linear). The results suggest that LevG and InuGB prefer substrates with low degrees of polymerization (six to eight monomers). In addition, the best software to generate molecular dockings with agavins and inulins was GlycoTorchVina. The molecular dockings (protein-ligand) occur mainly by the interaction of acidic amino acids (Asp, Glu and Asn) through hydrogen bonding due to the hydrophilic conditions of the substrate-binding site of both enzymes. Therefore*, L. gasseri* may interact with prebiotics such as inulin and agavin. These results contribute to understanding the prebiotic effect's structure-functionality relationship.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Supplementary material

Table S1. Localization of boxes for molecular dockings.

		Box localization		
Enzyme	Coordinates	SWISS-MODEL	GlycoT	ADFR
InuGB	x	-14.46	48.00	-17.33
	y	-2.11	67.00	-1.39
	z	-9.12	61.00	-9.65
LevG	x	16.89	16.00	20.92
	y	7.89	6.00	6.19
	z	7.02	-1.00	4.66

Table S2. Comparative molecular docking with 2YSF from PDB database and redocking.

Enzyme	Substrate	Binding affinity (kcal/mol)	Distance (Å)
2YSF*	Glycerol	-4.0	NA
	Sucrose	-6.9	5.1
2YSF**	Secrose	NA	5.8

*Molecular docking with 3D clean structure.
**Clean structure used to molecular docking in Autodock Vina

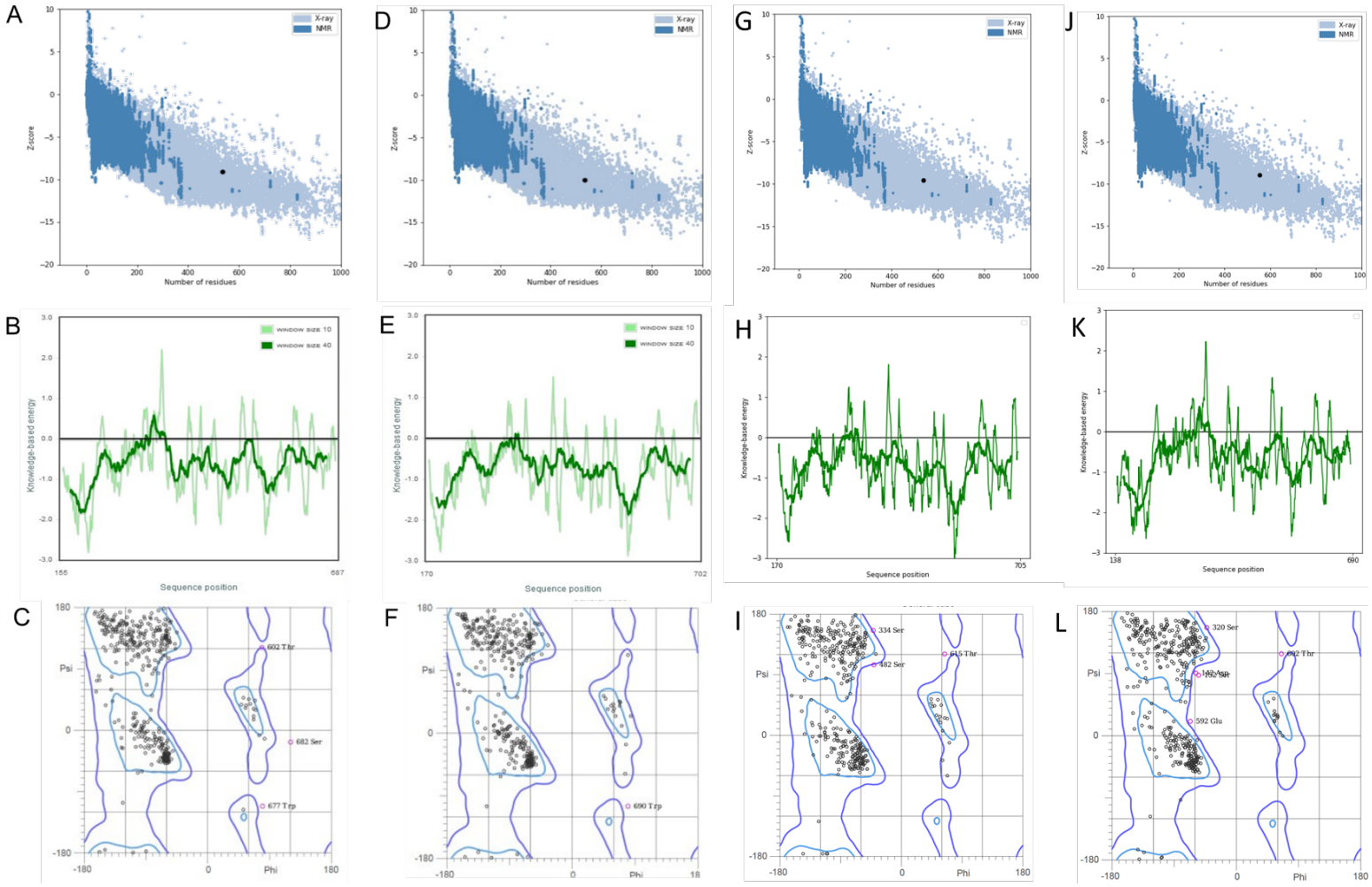

Fig. S1. Quality analysis of LevG and InuGB three-dimensional models. A, D, G, and J, the overall protein quality model generated by ProSA-web; B, E, H, and K, the local protein quality model generated by ProSA-web, and C, F I and L, Ramachandran graph for protein models generated by MOLPROBITY. A-C, for models from SWISS-MODEL from LevG; D-F: SWISS-MODEL from InuGB; G-I: AlphaFold model from InuGB and J-L: AlphaFold Models from LevG.

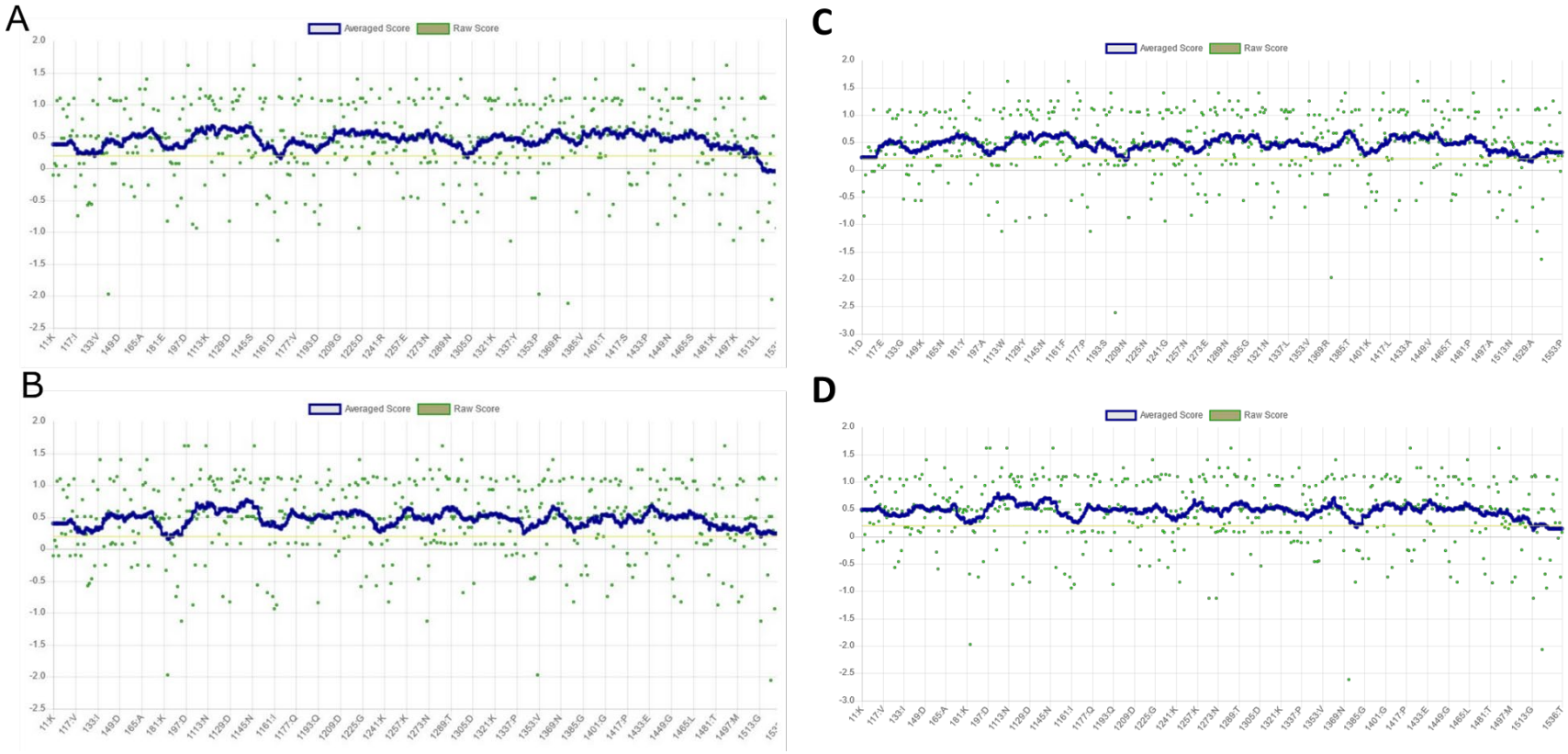

Fig. S2. Quality of three-dimensional models of LevG and InuGB by Verify3D. A: for LevG structure and B: for InuGB structure from SWISS-MODEL; C and D: structures from LevG and InuGB from AlphaFold models.

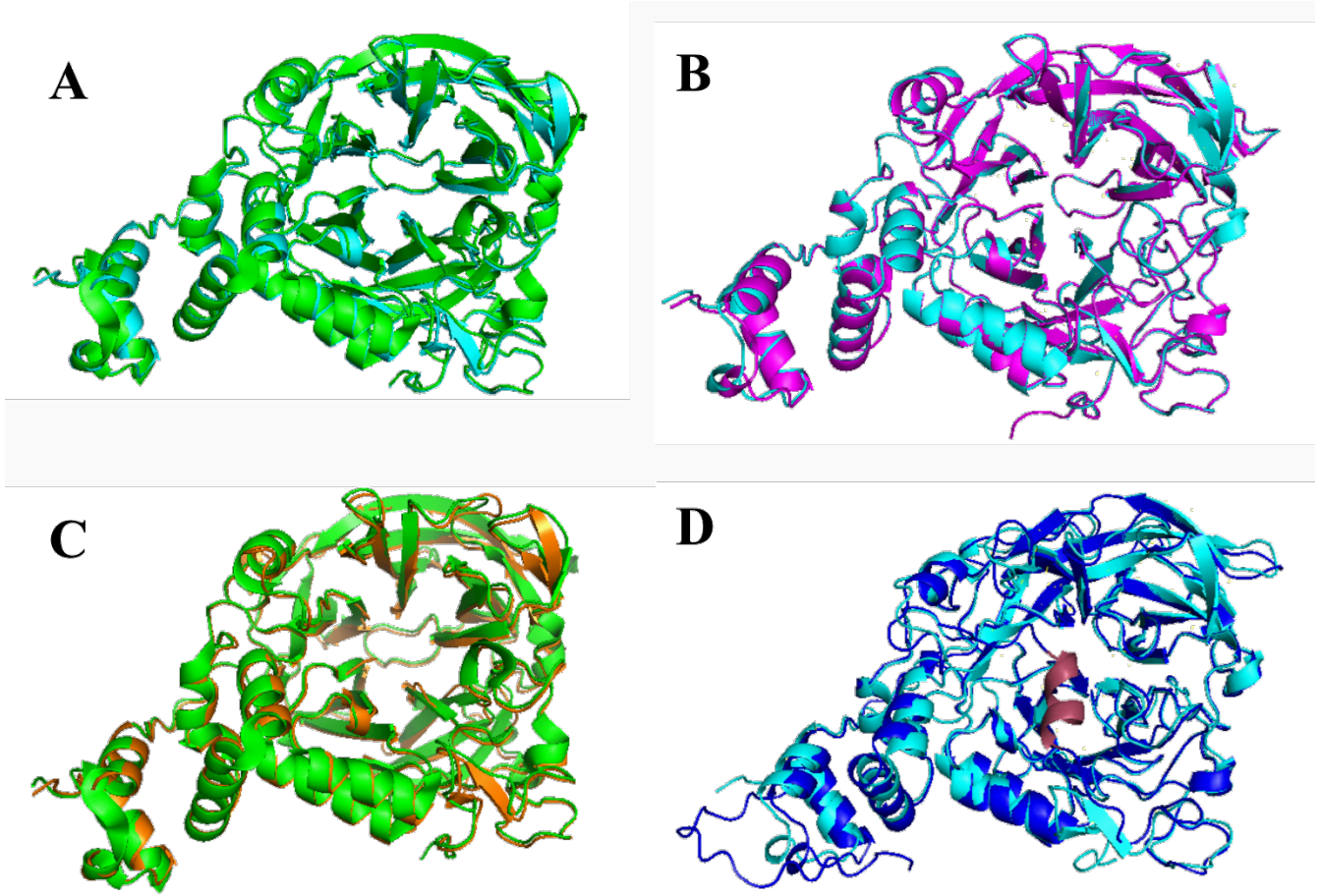

Fig. S3. Structural alignment of 3D models produced with SWISS-MODEL an AlphaFold with 2YFS. A: InuGB with 2YFS, B: InuGB from AlphaFold with InuGB from SWISS-MODEL. C: LevG from SWISS-MODEL with LevG from AlphaFold and D: LevG from SWISS-MODEL with 2YFS. Green color represent to 2YFS, Cyan: InuGB from SWISS-MODEL, Pink: InuGB from AlphaFold, yellow: LevG from SWISS-MODEL and navy blue: LevG from AlphaFold.