Süleyman Demirel Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
Cilt 21, Sayı 1, 279-285, 2017

Süleyman Demirel University
Journal of Natural and Applied Sciences
Volume 21, Issue 1, 279-285, 2017

DOI: 10.19113/sdufbed.81065

Structural Modelling and Structure-Function Analysis of *Zymomonas mobilis* Levansucrase

Bahar BAKAR[1], Burcu KAPLAN-TÜRKÖZ[*1]

[1]Ege University, Faculty of Engineering, Department of Food Engineering, 35100, İzmir

(Alınış / Received: 13.12.2016, Kabul / Accepted: 28.02.2017, Online Yayınlanma / Published Online: 23.03.2017)

Keywords
Levansucrase,
Zymomonas mobilis,
Protein structure modelling,
Structural bioinformatics

Abstract: Levansucrases are bacterial enzymes which produce fructan polymers from sucrose via hydrolysis and transfructosylation activities. These polymers; levan and fructooligosaccharides are valuable for food and pharmaceutical industries. Levansucrases from Gram-positive bacteria such as *Bacillus subtilis* tend to produce levan, while those from Gram-negative bacteria preferentially produce fructooligosaccharides. *Zymomonas mobilis* is an efficient levansucrase producer and its extracellular levansucrase can produce both fructooligosaccharides and levan depending on the reaction parameters. In this study, the structure of *Z. mobilis* levansucrase was modeled in order to help to understand the structure-function relationship of the enzyme. Furthermore, amino acids previously reported to be important for levansucrase activity were mapped on the model. The structural model presents a five-bladed propeller with a deep, negatively charged central pocket, similar to other bacterial levansucrases. Mapping showed that amino acids which previously reported to affect fructan length are located on the periphery of the structure covering the active site central pocket. Thus it is showed that, for the first time, that hydrolysis and transfructosylation reactions are catalyzed on different parts of *Z. mobilis* levansucrase structure. The structural location of the critical amino acids will pave the way to identify other residues which control fructan length by site directed mutagenesis without altering the overall fold of the enzyme.

Zymomonas mobilis Levansükrazının Yapısal Modellemesi ve Yapı-Fonksiyon Analizi

Anahtar Kelimeler
Levansükraz
Zymomonas mobilis,
Protein yapı modelleme
Yapısal biyoinformatik

Özet: Bakteriyal enzimler olan levansükrazlar, hidroliz ve transfruktosilasyon aktiviteleri ile sakkarozdan fruktan polimer oluşumunu katalizlerler. Bu polimerler; levan ve fruktooligosakkaritler, gıda ve ilaç endüstrileri için değerlidir. *Bacillus subtilis* gibi Gram-pozitif bakterilerden elde edilen levansükrazlar levan üretme eğilimindeyken, Gram-negatif bakteriler tercihen fruktooligosakkaritleri üretirler. *Zymomonas mobilis* etkili bir levansükraz üreticisi olup, hücre dışı levansükraz enzimi, tepkime parametrelerine bağlı olarak hem fruktooligosakkaritleri hem de levanı üretebilir. Bu çalışmada, enzimin yapı-fonksiyon ilişkisini anlamaya yardımcı olmak için *Z. mobilis* levansükrazın yapısı modellenmiş ve daha önce enzim aktivitesi için önemli olduğu bildirilen amino asitler model üzerinde haritalanmıştır. Elde edilen yapısal model, diğer bakteriyel levansükrazlara benzer şekilde derin, negatif yüklü bir merkezi cebe sahip beş bıçaklı pervane yapısına sahiptir. Amino asit haritalaması, daha önce fruktan uzunluğunu etkilediği bildirilen amino asitlerin, aktif bölge merkez cebini çevreleyecek şekilde yapının yüzeyinde bulunduğunu göstermiştir. Böylece, bu çalışma ile ilk kez, hidroliz ve transfruktosilasyon tepkimelerinin *Z. mobilis* levansükraz yapısının farklı kısımlarında katalizlendiği gösterilmiştir. Kritik amino asitlerin yapısal konumunun bilinmesi, fruktan uzunluğunu kontrol eden diğer amino asitlerin mutajenez yoluyla ve protein yapısına zarar vermeden belirlenmesine yardımcı olacaktır.

*Corresponding author: burcu.kaplan.turkoz@ege.edu.tr

1. Introduction

Levansucrases are bacterial extracellular enzymes which are grouped under the glycoside hydrolase family GH68 [1] based on the structural and functional similarity of β-fructofuranosidases, inulosucrases and levansucrases. The main reaction products of levansucrases are fructooligosaccharides (FOS) and/or levan.

FOS are non-digestible short fructose chains. FOS are three times less sweet than sucrose, have prebiotic properties since they are fermented by Bifidobacteria and they provide a positive effect on the absorption of minerals in the colon. Thus FOS are valuable additives in food industry. FOS can be produced from inulin degradation or by transfructosylation reactions [2, 3, 4]. Levans are longer fructose polymers, which can be used in food, cosmetic, pharmaceutical and medicine industries [5, 6]. In the food industry, levan can be used as an emulsifier, flavour modifier, stabilizer, thickener and encapsulation agent [7]. Levan is also important due to its anti-inflammatory, anti-oxidant and anti-bacterial properties [6]. FOS and levan are natural products of levansucrase enzymes and production of them is increasingly gaining interest due to their commercial and health benefits.

Levansucrases are dual-activity enzymes; the hydrolysis of a fructosyl donor releases a fructosyl unit and the fructosyl unit is transferred to an acceptor with the transfructosylation activity. Levansucrases follow a Ping-Pong mechanism, where a fructosyl-enzyme intermediate is formed by covalent bonds. In the first step of the levansucrase reaction, fructose unit from the donor substrate is attached to the enzyme. In the second step, the fructose unit bound to the enzyme is transferred to the fructosyl acceptor molecule [8]. Depending on the fructosyl acceptor, levansucrase will produce different products. Several molecules may play role as an acceptor such as sucrose, FOS and the growing levan polymer [9].

Crystal structures of levansucrases from *Bacillus subtilis* [10], *Gluconacetobacter diazotrophicus* [11], and *Erwinia amylovora* [12] were solved and all have five-bladed β–propeller architecture with a deep, negatively charged central pocket (Figure 1). The central pocket is the binding site for the substrate sucrose, with the fructosyl unit placed at the bottom and the glucosyl unit on the top of the cavity [10, 12]. Comparison of *B. subtilis* SacB and *E. amylovora* Lsc structures, highlight the conservation of overall fold in both structures (Figure 1).

Multiple alignment of bacterial levansucrases highlight the presence of five conserved regions (Figure 2). Two of these regions; RDP and DE/QXER, contain the active site residues [13].

Levansucrases from both Gram-positive and Gram-negative bacteria share the conserved amino acid regions, including the active site. Nevertheless, levansucrases of different origins have considerably different biochemical properties and product spectrum. Levansucrases of Gram-positive bacteria (such as *B. subtilis, Bacillus megaterium* and *Streptococcus salivarius*) produce generally levan without accumulating the intermediate FOS [14, 15, 16], while levansucrases from some Gram-negative bacteria (such as *G. diazotrophicus, E. amylovora* and *Pseudomonas syringae*) produce high amounts of FOS with small amounts of levan [17, 18, 19]. Structural studies recently pointed out that the main differences between FOS and levan-producing enzymes are located on the surface loops [12, 20]. Levansucrase from *Z. mobilis*; on the other hand, can produce both levan and FOS depending on the reaction conditions [9,21,22, 23]. There are several studies investigating the *Z. mobilis* levansucrase product specificity based on amino acid mutagenesis approaches. Thus several amino acids were shown to be critical for levan or FOS production including His296, Cys244, Glu117 and P340 [13, 24, 25]. Yet the location of these amino acids is not known since the atomic structure of *Z. mobilis* levansucrase is not solved up to date. Following these, we modeled the structure and mapped the critical amino acids retrieved from literature on our model. The results of this study will help to design site directed mutagenesis studies without altering the overall fold of the protein.

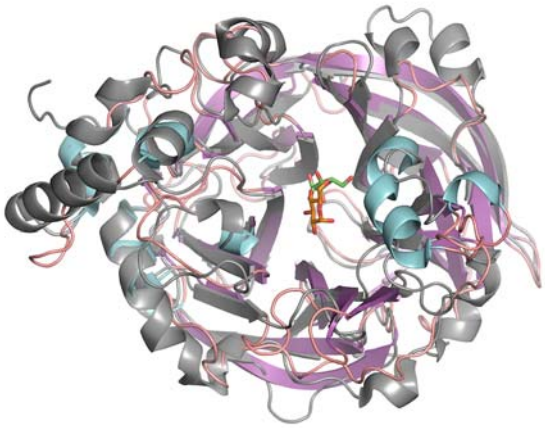

Figure 1. Crystal structures of *B. subtilis* SacB and *E. amylovora* Lsc. BsSacB (pdb:1oyg) is colored in gray and EaLsc (pdb:4d47, chain A) is colored according to secondary structure; helices (cyan), sheets (magenta) and loops (wheat). Glucose (orange) and fructose (green) in the active site from the structure of EaLsc are shown. Structures are viewed from top [10,12].

2. Material and Method

2.1. Multiple sequence alignment

Z. mobilis levansucrase sequence is retrieved from UniProtKB (SACB_ZYMMO) and is a 423 amino acid protein with an estimated molecular weight of 47

kDa. Multiple structural alignment was done using T-Coffee, Expresso [26] and ESPRIPT [27] was used for display.

2.2. Structural modeling

The structural models were built using Raptor X and I-TASSER web tools. RaptorX uses template based modeling program; the query sequence is partitioned into units based on template structures in PDB. RaptorX generates some quality criteria about the model, which are score, P-value, GDT and uGDT (un-normalized GDT) of the model [28].

I-TASSER (Iterative Threading ASSEmbly Refinement) server is an online integrated platform for prediction of protein structure and function from their amino acid sequences. I-TASSER creates 3D models from multiple threading alignments and iterative structural assembly simulations. The function of the protein is predicted by structurally matching the 3D models with other known proteins. Structure templates are identified from the PDB library [29, 30]. The server presents several different predictions. 'The Top 5 Models Predicted by I-TASSER' and 'Prediction function using COACH – Ligand Binding Sites' were used in our study for evaluating 3D structure prediction of the levansucrases. COACH is a meta-server approach that predicts ligand binding sites [31]. Quality of the predicted model is evaluated with C-score (confidence score), which is calculated based on the significance of threading template alignments and structure assembly from threading templates [29, 30]. The models were displayed using the PyMOL Molecular Graphics System.

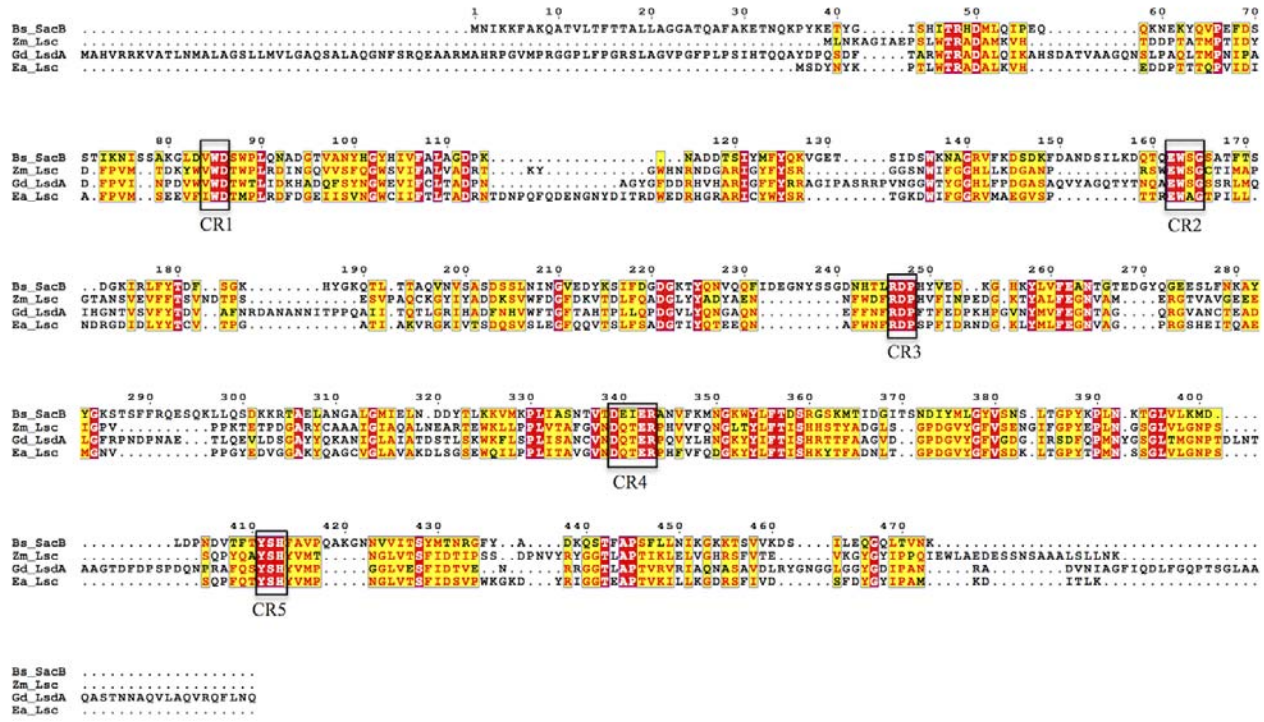

Figure 2. Conserved regions of bacterial levanscurases**.** *B. subtilis* SacB, Z. *mobilis* Lsc, *G. diazotrophicus* LsdA and *E. amylovora* Lsc amino acid sequences were structurally aligned. Five conserved regions (CR) are shown in black boxes.

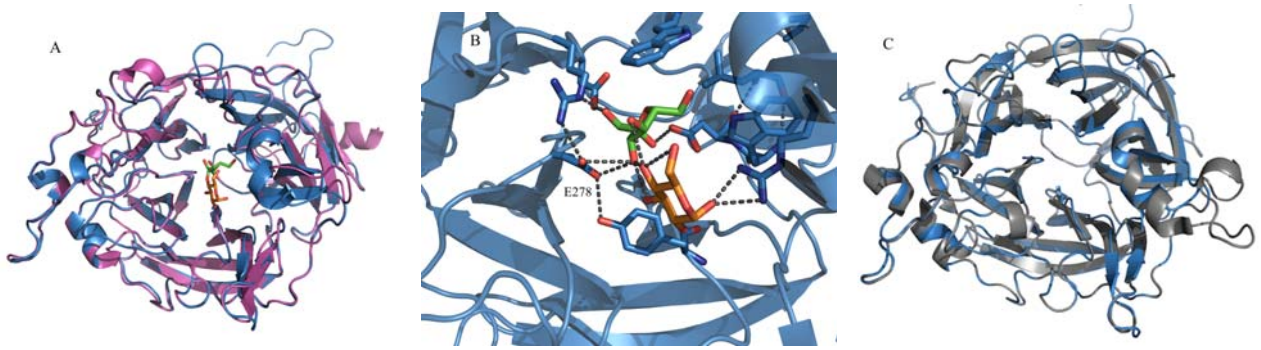

Figure 3. Structural model *Z. mobilis* levanscurase (ZmLsc). A) Superposition of 3D structure predictions of ZmLsc by RaptorX (blue) and I-TASSER (pink). B) Ligand binding prediction of ZmLsc. Interactions between glucose (orange), fructose (green) and the residues of ZmLsc are shown with gray lines. C) Structural comparison of ZmLsc (blue) model and crystal structure of *E. amylovora* Lsc (gray).

3. Results

3.1. Structural models of *Z. mobilis* levansucrase

The structural model of *Z. mobilis* levansucrase is given in Figure 3A. Two models were built using one template based (Raptor X) and one threading assembly (I-TASSER) modeling programme. The models were superimposable with an root mean square deviation between the location of atoms of 0.548 (Figure 3A). Raptor X models were generated based on the best template *E. amylovora* Lsc, all 423 residues were modeled and five positions were predicted as disordered. The model had a p-value of $8.95e^{-31}$; smaller than the 10^{-4} threshold; and a score of 387, indicating good model quality. The top model generated by I-TASSER had a C-score of 0.97.

Z. mobilis levansucrase structural model had the levansucrase fold; five-bladed beta-propeller architecture with a deep central pocket; similar to other bacterial levansucrases (Figure 3B and 3C). Ligand binding residues were predicted by COACH as W47, D48, L71, R83, W118, S119, R193, D194, E278, Y344 (Figure 3B).

3.2. Mapping of important amino acids on the model

There are many biochemical data about *Z. mobilis* levansucrase based on mutagenesis studies, which highlight the role of several different amino acids in hydrolysis or transfructosylation reactions. After a detailed investigation of mutagenesis studies in literature, we selected residues D194, E278, Cys121, Cys151, Cys244, W80, P340 and His296 as important amino acids for enzyme activity [13, 24, 25]. Since the structure of *Z. mobilis* levansucrase is not solved yet, specific locations of these amino acids are not revealed totally. In an attempt to structurally interpret the importance of these amino acids, we mapped these residues on our 3D model.

3.2.1. Active site residues; D194 and E278 are located in central pocket

The active site for hydrolysis reaction is composed of three acidic amino acids which are located in the central pocket of the structure [10, 12]. These amino acids are D86, D247 and E342 for *B. subtilis* and according to sequence alignments, correspond to D48, D194 and E278 for *Z. mobilis* Lsc (Figure 2). Yanase et al., (2002) mutated these residues and showed that E278D had a 30-fold smaller k_{cat} value for sucrose hydrolysis when compared with the wild type enzyme [13]. Mutation to a positively charged residue (E278H) had remarkable effects on sucrose hydrolysis activity, causing a 210-fold decrease in k_{cat} value. Therefore, E278 is an essential amino acid for hydrolysis [13]. Similarly, the D194N mutant enzyme nearly lost its hydrolysis activity, however, a small amount of highly polymerized fructan were produced, indicating that the transfructosylation activity remained intact. Further mutations of D194 (Glu, His, Gln, Ser, Ala) resulted in loss of sucrose hydrolysis activity which supports that D194 is essential for hydrolysis of sucrose-fructoside bond [13].

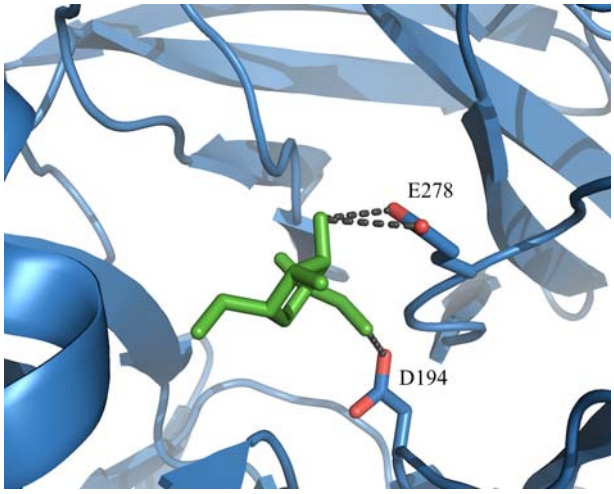

Figure 4. Structural locations of residues important for hydrolysis are shown on the model of ZmLsc. D194 and E278 are shown as sticks and labeled; their interactions with fructose (green) are shown with gray lines.

D194 and E278 have a direct role in the hydrolysis reaction and both are located in the central pocket, directly interacting with fructose (Figure 4) like their counterparts in other bacterial levansucrases [12]. Thus we structurally confirmed that these amino acids directly affect the hydrolysis activity due to their critical location in the active site.

3.2.2. His296 is related with both hydrolysis and transfructosylation activity

His296 is conserved in all levansucrases except *B. subtilis* SacB, where the corresponding amino acid is Arg360 (Figure 2). Mutation of Arg360 abolished the production of levan [32]. Similarly H296 mutations of *Z. mobilis* levansucrase resulted in increased K_M values and reduced transfructosylation activities [13, 25]. The H296R mutant lost its ability to produce levan, but not FOS. H296S and H296L mutants completely lost their transfructosylation activity although they retained hydrolysis activity [13]. In summary, H296 is an important residue for holding the fructose after hydrolysis and its transfer to the growing acceptor molecule. [13, 25]. According to our model, His296 is located on the surface relatively close to central pocket (Figure 5).

3.2.3. Transfructosylation activity is controlled by surface located amino acids

Senthikumar et al., (2003) mutated the cysteines of *Z. mobilis* Lsc to identify the effect of sulphydryl groups on enzyme activity [24]. All three cysteines were

mutated individually (C121, C151 and C244) to serines. Sucrose hydrolysis activities of mutants were decreased by two-fold, while levan-forming activities were almost abolished. Cys121 and Cys151 are located in beta strands according to our structural model, thus mutations of these amino acids might cause structural disorders in the protein, affecting enzymatic activity. Cys244; on the other hand; is located on a surface loop (Figure 6A) and so it is not expected to alter the overall fold of the protein. Thus the activity loss of Cys244Ser of mutant shows the direct involvement of Cys244 in product type.

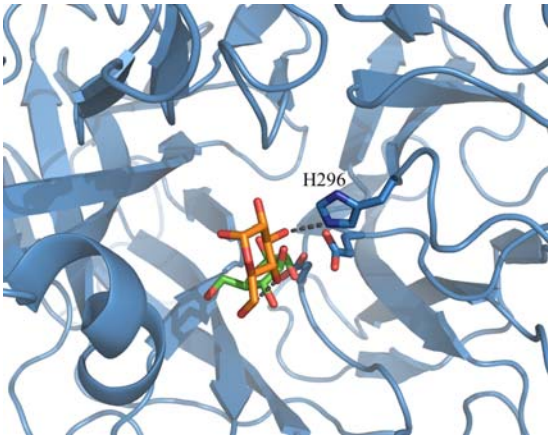

Figure 5. Structural location of H296 is shown on the model of ZmLsc. Top view of ZmLsc (blue) structure. H296, D194 and E278 are shown as sticks. H296 is labeled and its interactions between glucose (orange) are shown with gray lines.

W80 and P340, which were reported to play a role in transfructosylation, were also found on the surface of the structure (Figure 6A). W80R and double mutation Q339H-P340A had no effect on hydrolysis activity, but the transfructosylation products were different; the mutants produced only lower molecular weight fructans of size 170 and 50 kDa, respectively [13].

According to our structural models, the amino acids; W80, Q339,P340, C244 and H296; which were previously reported to affect transfructosylation activity of the enzyme are located on the surface loops of *Z. mobilis* levansucrase, surrounding the active site cavity (Figure 6B).

4. Discussion and Conclusion

In this study, the structure of the *Z. mobilis* levansucrase was modeled and our model verified the presence of the levansucrase fold; five-bladed beta-propeller architecture with a deep central pocket; similar to other bacterial levansucrases [10, 11, 12]. Furthermore, information from mutagenesis studies in literature were mapped on our structural model. Our results show that, amino acids, which are located on the top surface of the structure, affect transfructosylation activity and those located in the central pocket affect hydrolysis activity. Thus polymerization and hydrolysis reactions probably are catalyzed on different parts of structure. The distant location enabling a control mechanism of two catalytic reactions were recently proposed from the atomic resolution structure of *E. amylovora* levansucrase [12] and our results point out a similar mechanism for *Z. mobilis* levansucrase. Current research is underway to obtain the atomic resolution structure of the enzyme using X-ray crystallography. Crystal structure will provide the atomic location of substrate and/or product bound to levansucrase. Following that, it will be possible to identify the amino acids which are critical for polymerization and hydrolysis reactions. This way, we will be able to verify our structural bioinformatics analysis on the experimental structure. Furthermore obtaining the atomic resolution crystal structure will pave the way to identify other residues which control fructan length by site directed mutagenesis without altering the overall fold of the enzyme.

Acknowledgment

This work is part of the research project supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK), Grant No. TOVAG (214O174).

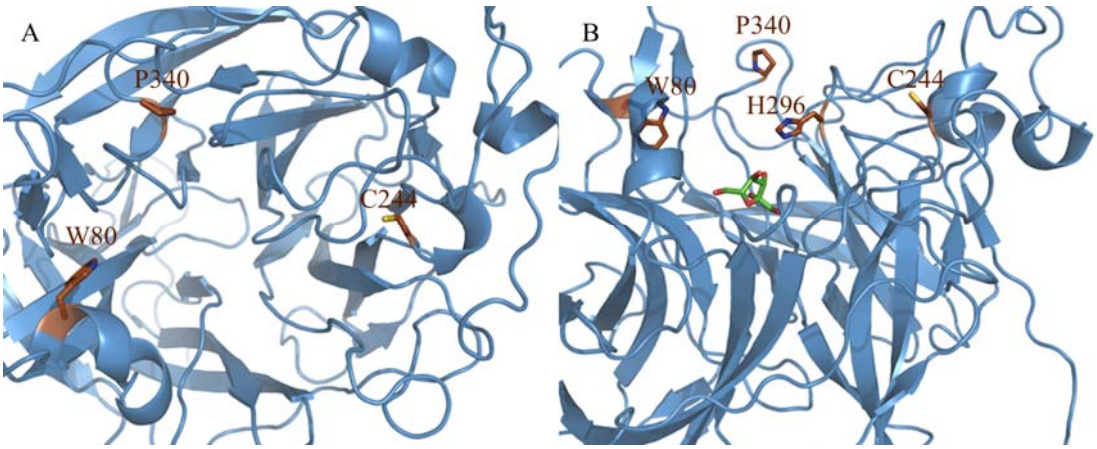

Figure 6. Structural locations of residues important for transfructosylation are shown on the model. A) Top view of ZmLsc (blue) structure. W80, C244 and P340 are shown as brown sticks. B) Side view of ZmLsc structure. H296, W80, C244 and P340 are shown as brown sticks. Fructose (green) is shown to highlight the active site central pocket.

References

[1] Henrissat, B. 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochemical Journal, 280(2), 309-316.

[2] Sabater-Molina, M., Larquee, E., Torrella, F., Zamora, S. 2009. Dietary fructooligosaccharides and potential benefits on health. Journal of Physiology and Biochemistry, 65(3), 315-328.

[3] İnanç, N., Şahin, H., Çiçek, B. 2005. Probiyotik ve Prebiyotiklerin Sağlık Üzerine Etkileri. Erciyes Tıp Dergisi, 27(3), 122-127.

[4] Yun, W. Y. 1996. Fructooligosaccharides-Occurrence, preparation, and application. Enzyme and Microbial Technology, 19(2), 107-117.

[5] Kim, K. H., Chung, C . B., Kim, Y. H., Kim, K. S., Han, C. S., Kim, C. H. 2005. Cosmeceutical Properties of Levan Produced by *Zymomonas mobilis*. Journal of Cosmetic Science, 56(6), 395-406.

[6] Öner, E. T., Hernandez, L., Combie, J. 2016. Review of Levan polysaccharide: From a century of past experiences to future prospects. Biotechnology Advances, 34(5), 827–844.

[7] Byun, B. Y., Lee S. J., Mah, J. H. 2014. Antipathogenic activity and preservative effect of levan (β-2,6-fructan), a multifunctional polysaccharide. International Journal of Food Science & Technology, 49(1), 238–245.

[8] Chambert, R., Gonzy-Treboul, G. 1976. Levansucrase of *Bacillus subtilis*: Kinetic and Thermodynamic Aspects of Transfructosylation Process. European Journal of Biochemistry, 62(1), 55-64

[9] Yanase, H., Iwata, M., Nakahigashi, R., Kita, K., Kato, N., Tonomura, K. 1992. Purification, crystallization and properties of the extracellular levansucrase from *Zymomonas mobilis*. Bioscience Biotechnology and Biochemistry, 56 (8), 1335– 1337

[10] Meng, G., Fütterer, K. 2003. Structural framework of fructosyl transfer in *Bacillus subtilis* levansucrase. Nature Structural Biology, 10(11), 935–941

[11] Martinez-Fleites, C., Ortíz-Lombardía, M., Pons, T., Tarbouriech, N., Taylor, E. J., Arrieta, J. G., Davies, G. J. 2005. Crystal structure of levansucrase from the Gram-negative bacterium *Gluconacetobacter diazotrophicus*. The Biochemical Journal, 390(1), 19–27.

[12] Wuerges, J., Caputi, L., Cianci, M., Boivin, S., Meijers, R., Benini, S. 2015. The crystal structure of *Erwinia amylovora* levansucrase provides a snapshot of the products of sucrose hydrolysis trapped into the active site. Journal of Structural Biology, 191(3), 290-298.

[13] Yanase, H., Maeda, M., Hagiwara, E., Yagi, H., Taniguchi, K., Okamoto, K. 2002. Identification of functionally important amino acid residues in *Zymomonas mobilis* levansucrase. Journal of Biochemistry, 132(4), 565–572.

[14] Chambert, R., Treboul, G., Dedonder, R. 1974. Kinetic studies of levansucrase of *Bacillus subtilis*. European Journal of Biochemistry, 41(2), 285–300.

[15] Song, D. D., Jacques, N. A. 1999. Purification and enzymic properties of the fructosyltransferase of *Streptococcus salivarius* ATCC 25975. Biochemical Journal, 341(2), 285–291.

[16] Homann, A., Biedendieck, R., Götze, S., Jahn, D., Seibel, J. 2007. Insights into polymer versus oligosaccharide synthesis: mutagenesis and mechanistic studies of a novel levansucrase from *Bacillus megaterium*. Biochemical Journal, 407(2), 189–198.

[17] Hernandez, L., Arrieta, J., Menendez, C., Vazquez, R., Coego, A., Suarez, V., Selman, G., Petit-Glatron, M. F., Chambert, R. 1995. Isolation and enzymic properties of levansucrase secreted by *Acetobacter diazotrophicus* SRT4, a bacterium associated with sugarcane. Biochemical Journal, 309(1), 113–118.

[18] Caputi, L., Nepogodiev, S. A., Malnoy, M., Rejzek, M., Field, R. A., Benini, S. 2013. Biomolecular characterization of the levansucrase of *Erwinia amylovora*, a promising biocatalyst for the synthesis of fructooligosaccharides. Journal of Agricultural and Food Chemistry, 61(50), 12265–12273.

[19] Visnapuu, T., Mardo, K., Alamae, T. 2015. Levansucrases of a *Pseudomonas syringaepathovar* as catalysts for the synthesis of potentially prebiotic oligo- and polysaccharides. New Biotechnology, 32(6), 597–605.

[20] Lammens, W., Le Roy, K., Schroeven, L., Van Laere, A., Rabijns, A. and Van den Ende, W. 2009. Structural insights into glycoside hydrolase family 32 and 68 enzymes: functional implications. Journal of Experimental Botany, 60(3), 727–740

[21] Tanaka T., Oi, S., Yamamoto T. 1980. The molecular structure of low and high molecular weight levans synthesized by levansucrase, Journal of Biochemistry, 87(1), 297–303.

[22] Vigants, A., Upite, D., Scherbaka, R., Lukjanenko, J., Ionina, R. 2013. An influence of ethanol and temperature on products formation by different preparations of *Zymomonas mobilis* extracellular levansucrase. Folia Microbiologica, 58(1), 75–80.

[23] Santos-Moriano, P., Fernandez-Arrojo, L., Poveda, A., Jimenez-Barbero, J., Ballesteros, A. O., Plou, F. J. 2015. Levan versus fructooligosaccharide synthesis using the levansucrase from *Zymomonas mobilis*: effect of reaction conditions. Journal of Molecular Catalysis B: Enzymatic, 119, 18–25.

[24] Senthikumar, V., Bushby S. J. W., Gunasekaran, P. 2003. Serine substitution for cysteine residues in levansucrase selectively abolishes levan forming activity. Biotechnology Letters, 25(19), 1653–1656

[25] Li, S. Y., Chen, M., Li, G., Yan, Y. L. , Yu, H. Y., Zhan, Y. H., Peng, Z. X., Wang, J., Lin, M. 2008. Amino acid substitutions of His296 alter the catalytic properties of *Zymomonas mobilis* 10232 levansucrase. Acta Biochimica Polonica, 55(1), 201-206.

[26] Armougom F., Moretti, S., Keduas, V., Notredame, C. 2006. APDB: a web server to evaluate the accuracy of sequence alignments using structural information. Bioinformatics, 22(19), 35-39.

[27] Robert, X., Gouet, P. 2014. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Research, 42(1), 320-324.

[28] Kallberg, M., Wang, H., Wang, S., Peng, J., Wang, Z., Lu, H., Xu, J. 2012. Template-based protein structure modeling using the RaptorX web server. Nature Protocols, 7(8) 1511–1522.

[29] Roy, A., Kucukural, A., Zhang, Y. 2010. I-TASSER: a unified platform for automated protein structure and function prediction, Nature Protocols, 5(4), 725-738.

[30] Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., Zhang. Y. 2015. The I-TASSER Suite: Protein structure and function prediction. Nature Methods, 12(1), 7-8.

[31] Yang, J., Roy, A., Zhang, Y. 2013. Protein-ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. Bioinformatics (Oxford Journals), 29(20), 2588–2595.

[32] Chambert, R., Petit-Glatron, M. F. 1991. Polymerase and hydrolase activities of *Bacillus subtilis* levansucrase can be separately modulated by site-directed mutagenesis. Biochemical Journal, 279(1), 35-41.