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# Molecular Modeling and Docking of Ribitol Dehydrogenase Exploring Enzyme NAD$^+$ and D-psicose Interaction

**Hinawi A. M. Hassanin, Wanmeng Mu, Marwa Y. F. Koko, Tao Zhang, Ammar Alfarga, Mandour H. Abdelhai, Bo Jiang***

State Key Laboratory of Food Science and Technology, Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, Wuxi, China

## Email address:

bjiang@jiangnan.edu.cn (Bo Jiang)

*Corresponding author

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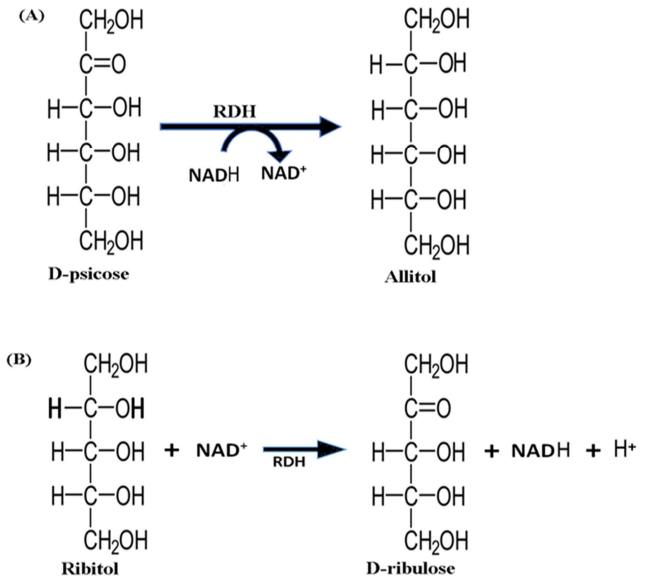
**Abstract:** Allitol is an alcohol monosaccharide, is a reduction of D-psicose. It is functions as a cross linking of D- and L-hexoses. It existed in too small quantities in commercial sugars and is difficult to synthesize by using chemical methods. It has a hypoglycemic function, and can use as Laxative in treating of constipation, which can exploit in production of diabetes drugs. The present report investigates about the production of allitol by ribitol dehydrogenase (RDH), its action of the enzyme through homology and molecular docking studies. We have investigated ribitol dehydrogenase (RDH) from *providencia alcalifaciens* RIMD 1656011. The protein sequence of RDH was conducted for homology modeling through Swiss model. 3D structure revealed was docked with NAD$^+$ and D-psicose using AutoDock Vina software version 5.6. The results of homology modeling and docking studies revealed that the conserved residues of RDH were Tyr 153, Tyr 92, Ser 17 and Lys157 with NAD$^+$, while conserved residues with D-psicose were GLN67 and ASP61. NAD$^+$ has good interaction with RDH showing grid score of -49.84, which is a good score for binding.

**Keywords:** Homology Modeling, Docking, AutoDock Vina, Ribitol Dehydrogenase

## 1. Introduction

Allitol is rare sugar alcohol naturally exist in *Itea virginica* plant and Fungus *Tylopilus plumbeoviolaceus* [1, 2]. Rare polyols are useful as low caloric sweetener, raw material for production of chemical compounds and research reagents [3]. Azasugars display potential medicines against diabetes, cancer and viral infections, which allitol may be use as intermediate for preparation of azasugars [4]. In addition, allitol has activity in increasing the water content of small intestine and speed up the small intestine transit and may lead to use in treating of constipation [5]. Moreover, allitol can use as an anti-crystallization agent similar as the known agents used and as effective saturation enhancer for vacuum lyophilization (freeze-drying) [6].

In Izumori strategy (Izumoring) for bio-production of hexoses, allitol located at the center of the strategy and its function as linking between D- and L-hexoses [7]. It is difficult to produce by chemical method [8]. According to Izumoring strategy, allitol can prepare by reduction of D-psicose, which ribitol dehydrogenase (RDH, EC1.1.1.56) is the enzyme responsible for reduction of D-psicose to allitol as shown in scheme 1(A) [3]. Moreover, RDH plays an important role in the production of D-ribulose from ribitol (scheme 1(B)) [9]. This enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with NAD$^+$ or NADP$^+$ as acceptor. RDH was characterized from various microorganisms, such as RDH from *Aerobacter aerogenes*, *Enterobacter agglomerans*, *Gluconobacter oxydans*, *Klebsiella aerogenes*, *Rhodobacter sphaeroides*, *Klebsiella oxytoca* and *Zymomonas mobilis* [9-15]. In a recent report, a new RDH from *Providencia alcalifaciens* RIMD 1656011 was cloned and characterized by our group and it exploit in production of allitol from D-psicose [16].




***Scheme 1.*** *(A) Allitol synthesis from D-psicose (B) D-ribulose synthesis from ribitol. The both reactions catalyzed by ribitol dehydrogenase.*

The three dimensional (3D) structure of enzyme's determination is an important to understand the biological function and mechanism of enzymes. Homology modeling is currently the most accurate method to generate reliable three dimensional (3D) protein structure models and routinely used in many practical applications. It is aimed constructed the amino acid in form of protein structure. Recently, homology modeling has been the most precise method comparing to other methods [17]. The structure achieved by homology modeling is similar to nuclear magnetic resonance (NMR) spectroscopy or X-ray crystallography [18].

Here in this study, we presented homology and structure modeling of RDH from *providencia alcalifaciens* RIMD 1656011, which was used in production of allitol from D-psicose, and docking of substrates into the active site to understand the enzyme-substrate interaction by using various homologies, docking and verification software. A genome comparison was conducted, which useful information was retrieved.

# 2. Materials and Methods

### *2.1. Sequence Similarity*

RDH from *providencia alcalifaciens* RIMD 1656011 amino acid sequence was retrieved from National Center for Biotechnology Information (NCBI) and compared with similar enzymes from other organisms using the NCBI web site tool (BLAST) and the sequence alignment tool ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html).

### *2.2. Protein Homology Modeling*

A three-dimensional homology model of RDH from *P. alcalifaciens* was generated through Swiss model [19-23]. Swiss model is a structural bioinformatics web-server dedicated to homology modeling of protein 3D structures. It is consists of three tightly integrated components; 1) The SWISS-MODEL Pipeline - a suite of software tools and databases for automated protein structure modeling; 2) the SWISS-MODEL Workspace - a web-based graphical user workbench; 3) The SWISS-MODEL Repository - a continuously updated database of homology models for a set of model organism proteomes of high biomedical interest. The method based on searching of high identity templates protein structures to build models for evolutionary related target's proteins. The Sequence of RDH was submitted to Swiss model and the options of model construction were selected according to enzyme structure (Tetramer, monomer). The template protein structure was then retrieved from the protein data bank (PDB) through the template search on Swiss model, which the high similar protein structure to the RDH was selected as template.

### *2.3. Three Dimensional (3D) Structure Verification*

The modeled structure revealed from Swiss model was verified through various verification methods. The structure was checked through PROCHEK to calculate the favoured regions, additional allowed regions, generously allowed regions and disallowed regions to predict the protein fold quality. Further, the structure was verified using Errat, which the error values was calculated based on the statistics of non-bonded atom-atom interactions in the structure (compared to a database of reliable high-resolution structures). In addition the structure was checked through Verify 3d to determines the compatibility of an atomic model (3D) with its own amino acid sequence by assigning a structural class based on its location and environment (alpha, beta, loop, polar, non-polar etc) and comparing the results to good structures.

### *2.4. Docking Analysis*

The 3D model generated by Swiss was used in docking studies. The docking studies were performed by AutoDock Vina software version 5.6. AutoDock is molecular modeling simulation software. It is especially effective for Protein-ligand docking. 3D structure of RDH and ligend structure ($NAD^+$ and D-psicose) were firstly processed using AutoDock tool software, which grid box was added to the RDH structure and PDBQT file were created for vina software. The best-docked model was selected according to results of Vina. The output results of AutoDock vina were analyzed by accelary studio and chimra program to understand and observe the molecule at an atomic structure.

# 3. Results and Discussion

RDH is responsible for conversion of ribitol to D-ribulose in addition its active in a wide range of sugar alcohols depend on the microorganism source. In a previous study by our group, we characterized a new RDH from *Providencia alcalifaciens* RIMD 1656011, and we found that the activity of the RDH was specific to ribitol and allitol. The RDH from *Providencia alcalifaciens* has not been crystallized. To

modeling the RDH, we used the crystal structure of clavulanic acid dehydeogenase (Cad) from *Streptomyces Clavuligerus* PDB ID 2JAH. As shown in figure 1 sequences similarity between 2JAH and RDH was 37.29 %.

As in a previous study for characterization of *Providencia alcalifaciens* RDH, the molecular mass of the purified enzyme was noted ~25 kDa in each monomer. The result of native molecular mass was104 kDa for *P. alcalifaciens* RDH, these results suggested that the enzyme functioned as a tetramer [16]. The result of molecular mass for RDH was taken into consideration when the structure modeled by using Swiss as shown in figure 2 the RDH was modeled into tetramer and monomer.

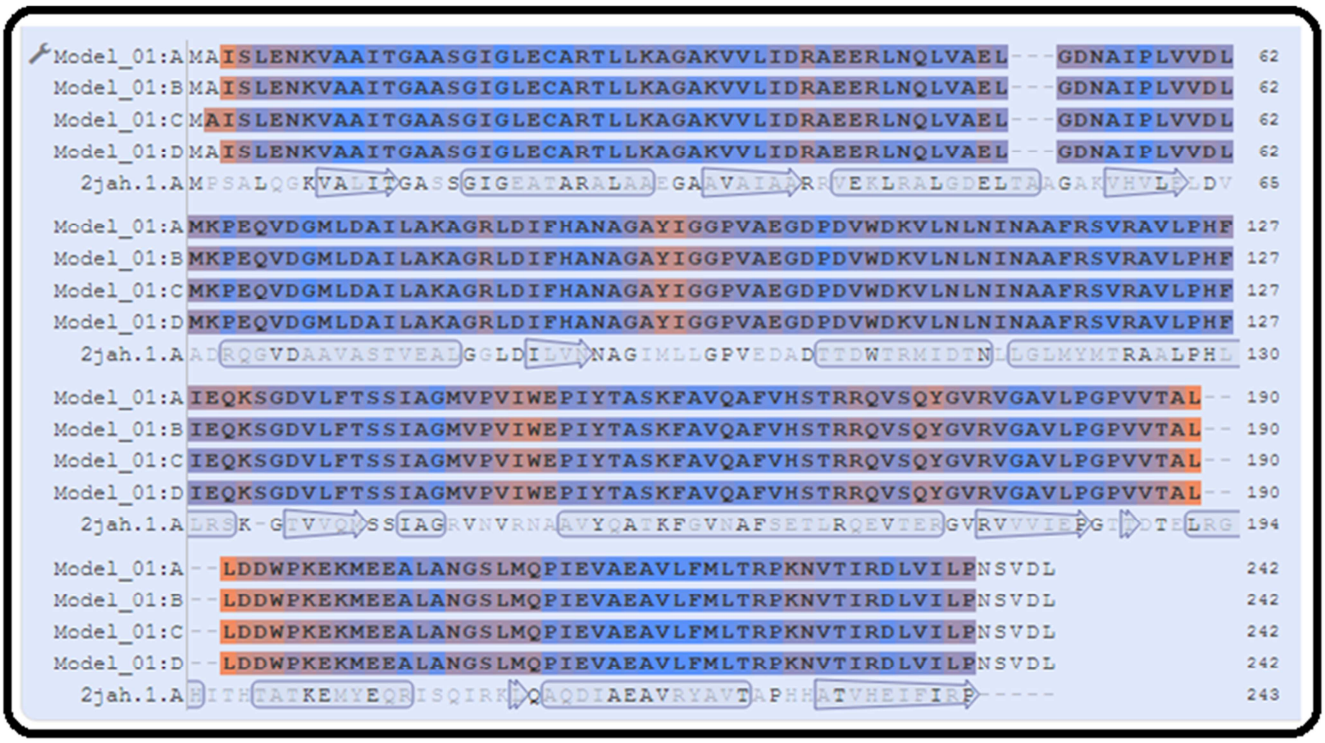



***Figure 1.*** *Alignment of amino acid sequences of RDH from providencia alcalifaciens and template Cad from Streptomyces Clavuligerus through Swiss model.*

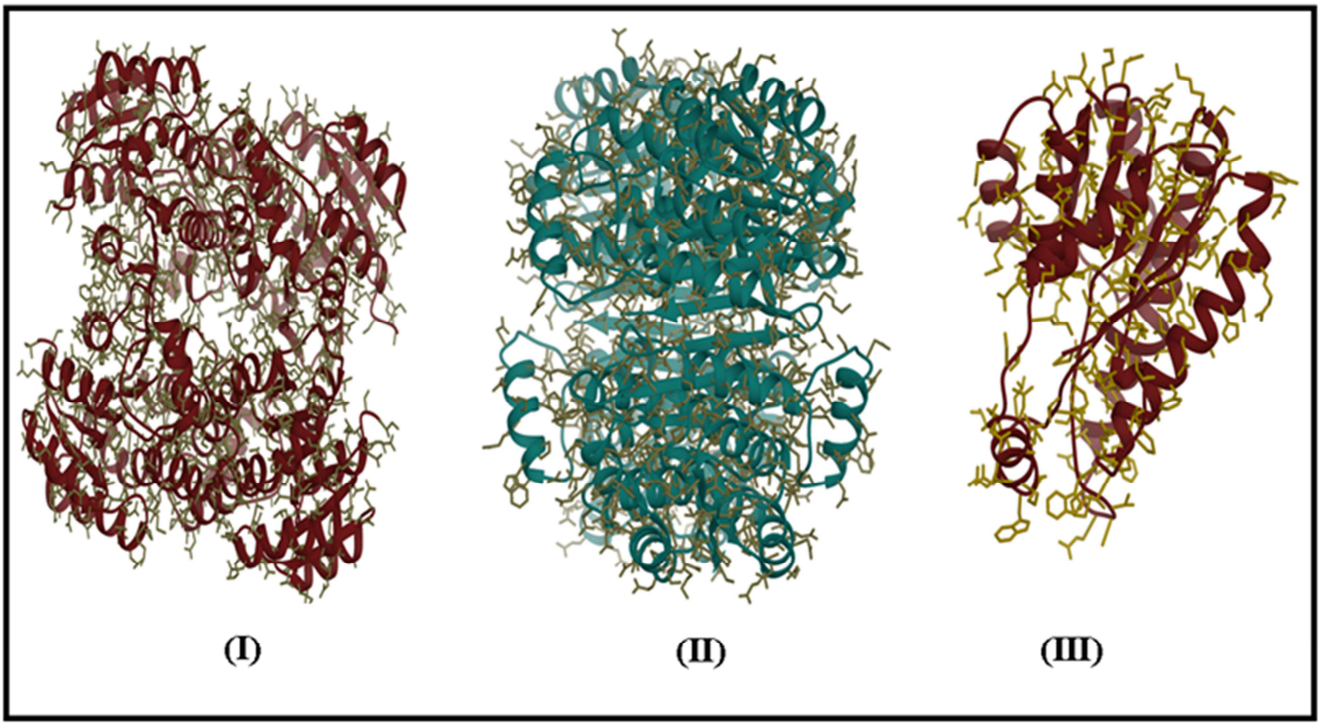



***Figure 2.*** *Complete 3D model structure of RDH from P. alcalifaciens. (I) Tetramer RDH (II) Tetramer side view (III) Monomer RDH.*

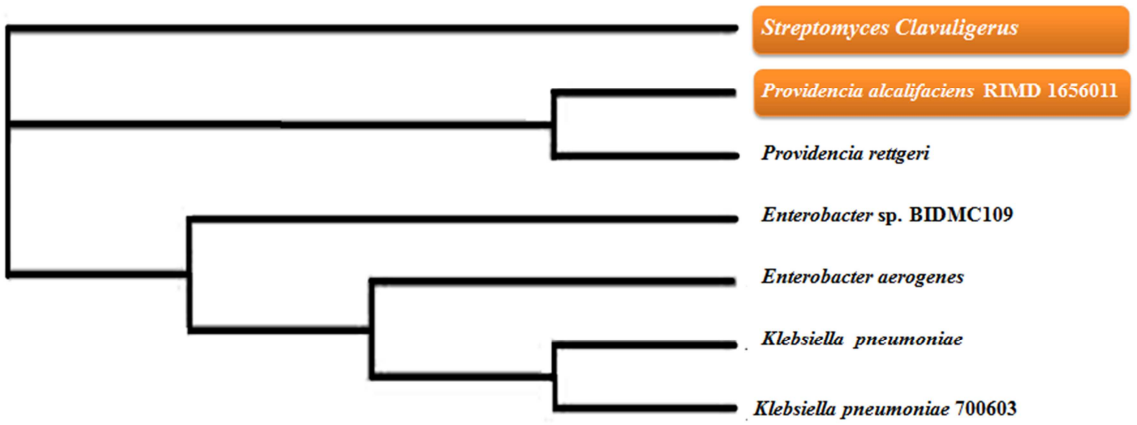



***Figure 3.*** *Phylogenetic analysis of different species producing RDH has been shown in Phenogram form developed.*

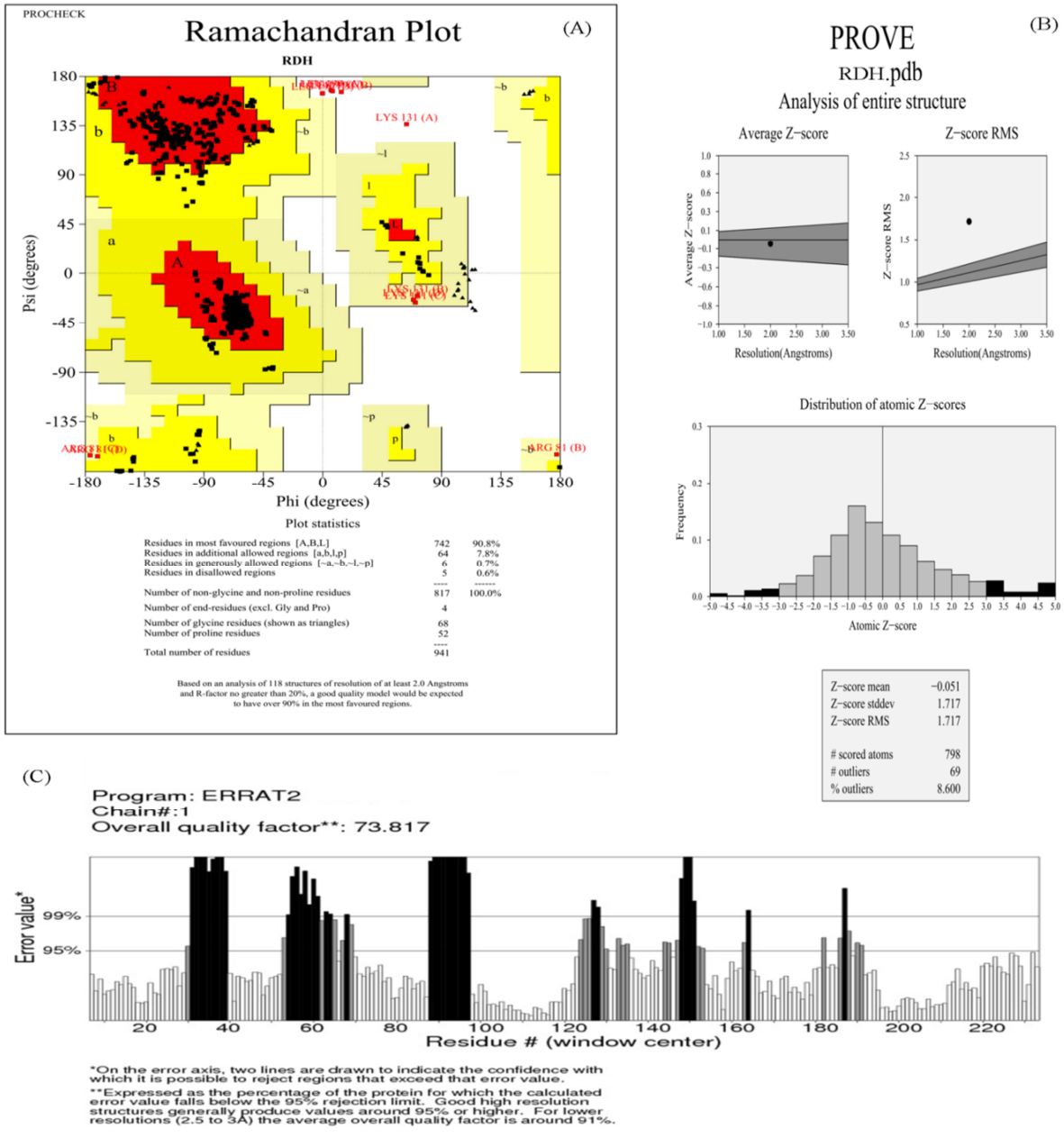



***Figure 4.*** *(A) Structure prediction and validation result of RDH from providencia alcalifaciens by homology modeling, a Ramachandran plot showing 90.8% of the atom residing in the most favored region, 7.8% in allowed region and 0.7% in generously allowed region and 0.6% was in disallowed region. (B) Prove analyzing the whole structure for the Z score and it was 8.6% for outliers. (C) Overall quality of structure using Errat was 73.817%.*

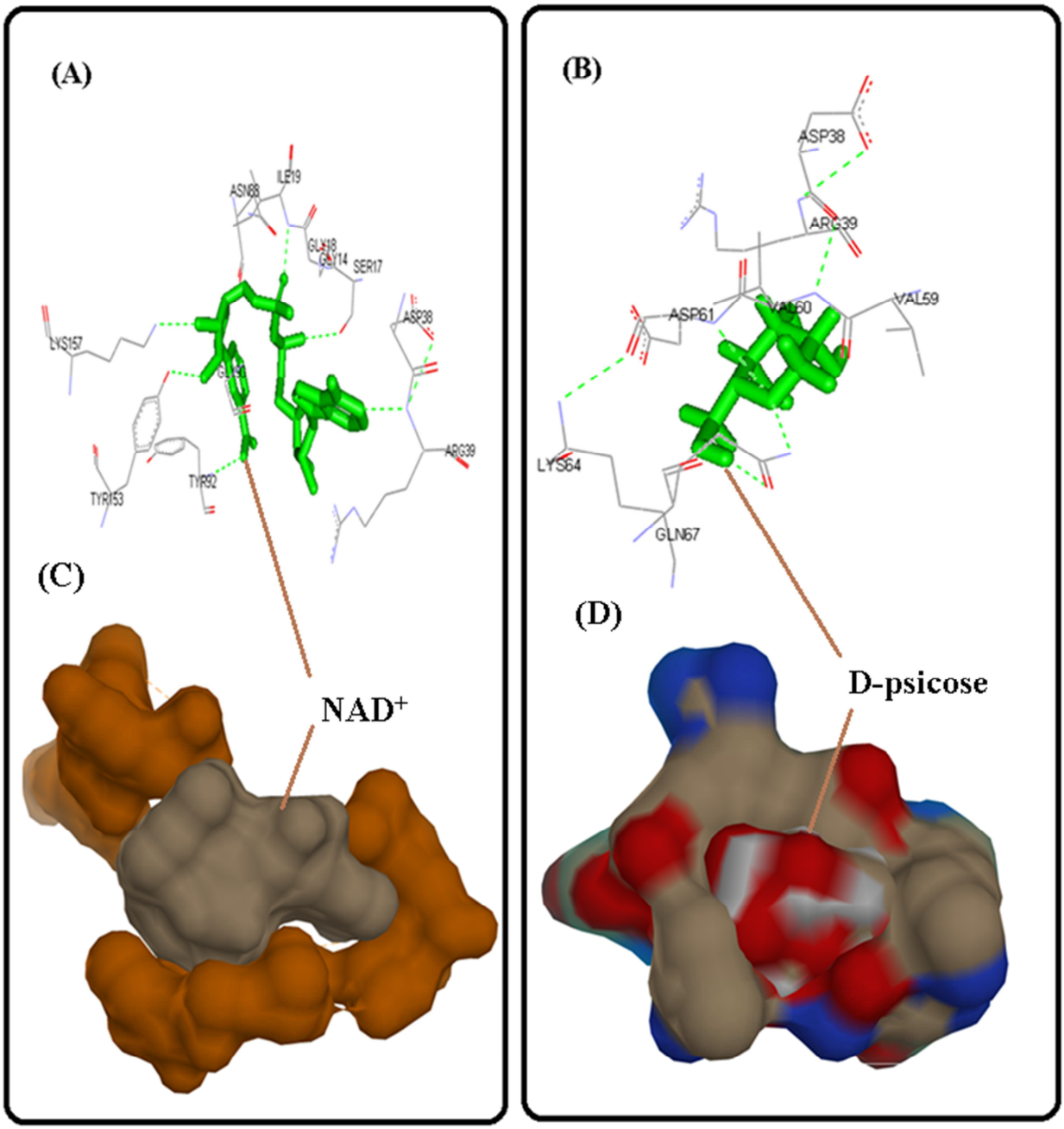



***Figure 5.*** *(A) Interaction between RDH and NAD$^+$. Tyr 153, Tyr 92, Ser 17 and Lys157 can be seen interacting with the NAD$^+$ molecule. (B) Interaction between RDH and D-psicose. GLN67 and ASP61 can be seen interacting with the D-psicose molecule. (C) NAD$^+$ molecule binds into the pocket of the funnel shape active site. (D) D-psicose molecule binds into the pocket of the funnel shape active site.*

The sequences were retrieved from NCBI database and submitted to ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) for sequences alignment similarity. The similarity of different species producing RDH and the template 2JAH were compared with *P. alcalifaciens* RDH and reveled 94%, 83%, 83%, 83%, 82% and 37.29 to RDH from *Providencia rettgeri*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Enterobacter* sp. BIDMC109, *Klebsiella pneumoniae* 700603 and template Cad From *Streptomyces Clavuligerus*, respectively. The glycine-rich consensus sequences have a structural role in coenzyme binding of all SDRs. The phylogenetic study was performed for seven different spices, which revealed that the RDH form *provendincia* and template Cad from *Streptomyces Clavuligerus* was nearly related (figure 3).

The sequence of RDH was submitted to Swiss model for structure modeling. The Swiss similarity result for selecting the template revealed that the most similar structure to RDH was Cad from *Streptomyces Clavuligerus* PDB ID 2JAH. The modeled structure was then checked through PROCHECK, Verify 3d and Errat (http://services.mbi.ucla.edu/ERRAT/) (figure 4). The Ramachandran plot for RDH structure suggested 90.8%, 7.8%, 0.7% and 0.6% for residues in most favoured regions, additional allowed regions, generously

allowed regions and disallowed regions, respectively (Figure 4 (A)). The combine favored and allowed categories with the high percentage for model structure prospective to appear a good protein fold. The combined favored and allowed categories for RDH were 98.6%. The results of PROCHECK for fold validation of our structure indicate that high quality of RDH structure derived from *Streptomyces Clavuligerus* Cad (PDB ID 2JAH) template in term of protein fold. Moon et al. they reported that the results of Ramachandran plot for RDH from *Zymomonas mobilis* structure were 91.9%, 7.0%, and 0.9% of residues in derived model are in most favored, additional allowed, generously allowed, and disallowed region, respectively and the combined favored and allowed categories was 99.0% [9]. Figure 4 (C) showed that the Errat overall quality for RDH was 73.817%. By validation of structures through Verify 3d 89.79% of the residues had an averaged 3D-1D score >= 0.2 for RDH.

In the docking analysis, the 3D structure of RDH revealed from Swiss was submitted to AutoDock tools as PDB file to create the PDBQT file required for Vina software to conduct docking analysis. Hydrogen atoms were added to the structure. The grid box was added to the structure, which grid parameters were put for the number of protein in x, y and z dimension and center grid box. The PDBQT files of protein structure and ligend were submitted to Vina software. Vina software was generated number of coenzyme ($NAD^+$) and D-psicose replications distributed around center of the active site, which the best docking model of RDH with $NAD^+$ was selected with the affinity of -9.1 kcal/mol, rmsd lower bound 0.0 and rmsd upper bound 0.0. For the RDH with D-psicose the affinity, rmsd lower bound and rmsd upper bound were -5.2 kcal/mol, 0.0 and 0.0 respectively.

RDH is member of the short-chain dehydrogenases / reductases family (SDR). This family is a functionally diverse family of oxidoreductases, but has a structurally conserved Rossmann fold (alpha/beta folding pattern with a central beta-sheet). Classical SDRs are typically about 250 residues in length, while extended SDRs are ~350 residues. Classical SDRs have a cofactor-binding motif and an active site motif, with the Tyr residue of the active site motif serving as a critical catalytic residue. In addition to the Tyr and Lys residues, there is often an upstream Ser and/or an Asn contributing to the active site, and substrate binding is in the C-terminal region, which determines specificity. The most important residue in SDRs is Tyr that plays an important role in the dehydrogenation reaction and may ease the sharing of the hydroxyl group [9]. The $NAD^+$ showed hydrogen bound with *P. alcalifaciens* RDH. The catalytic residues were Tyr 153, Tyr 92, Ser 17 and Lys157, which interacted properly with coenzyme $NAD^+$ (Figure 5(A)). There was interaction between oxygen of $NAD^+$ with Tyr 92 and Tyr 153 with distances of 3.011Å and 3.15 Å, respectively. The SDR observed structurally conserved conformations, forming a catalytic Tyr-Lys-Ser triad RDH important [24, 25]. Furthermore, the D-psicose was docked into the active site of the RDH and the catalytic residues that properly interact with the substrate (D-psicose) were GLN67 and ASP61 with distance of 1.6 and 3.08 Å, respectively (Figure 5(B)). GLN67 has good interacting distance with oxygen of D-psicose. To best of our knowledge, this is the first time for docking of D-psicose into the active site of RDH. Using Pose and Rank web service to calculate the interaction energy, which $NAD^+$ was indicated has good interaction with RDH showing grid score of -49.84 is a good score for binding. While D-psicose grid score was -21.97, which is also consider as appropriate score binding. The surface shape of the active site interacting with $NAD^+$ and D-psicose in forms of the funnel interaction shape of active site, figure 5 (C) and (D) demonstrated the shape interaction of $NAD^+$ and D-psicose.

# 4. Conclusion

The homology modeling and docking studies were investigated and reported in this study for Ribitol dehydrogenase from *providencia alcalifaciens* RIMD 1656011. The results revealed that RDH has an effective interaction toward $NAD^+$ with grid score of -49.84. The D-psicose interaction with RDH grid score was -21.97, which is also considered as appropriate score for binding. We observed that the active site region in the 3D-structure of RDH enzymes interacted with $NAD^+$ and D-psicose in forms of a funnel interaction shape. The form shape of RDH comprised with Tyr 153, Tyr 92, Ser 17 and Lys157 with $NAD^+$ and GLN67 and ASP61 with D-psicose.

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