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In silico characterization of a novel β-1,3-glucanase gene from *Bacillus amyloliquefaciens*—a bacterial endophyte of *Hevea brasiliensis* antagonistic to *Phytophthora meadii*

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Abstract We report the molecular characterization of β-1,3-glucanase-producing *Bacillus amyloliquefaciens*—an endophyte of *Hevea brasiliensis* antagonistic to *Phytophthora meadii*. After cloning and sequencing, the β-1,3-glucanase gene was found to be 747 bp in length. A homology model of the β-1,3-glucanase protein was built from the amino acid sequence obtained upon translation of the gene. The target β-1,3-glucanase protein and the template protein, endo β-1,3-1,4-glucanase protein (PDB ID: 3o5s), were found to share 94 % sequence identity and to have similar secondary and tertiary structures. In the modeled structure, three residues in the active site region of the template—Asn52, Ile157 and Val158—were substituted with Asp, Leu and Ala, respectively. Computer-aided docking studies of the substrate disaccharide (β-1, 3-glucan) with the target as well as with the template proteins showed that the two protein-substrate complexes were stabilized by three hydrogen bonds and by many van der Waals interactions. Although the binding energies and the number of hydrogen bonds were the same in both complexes, the orientations of the substrate in the active sites of the two proteins were different. These variations might be due to the change in the three amino acids in the active site region of the two proteins. The difference in substrate orientation in the active site could also affect the catalytic potential of the β-1,3 glucanase enzyme.

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Introduction

Bio-control—the use of organisms to combat disease and pests—has the potential to complement or become an alternative to traditional chemical treatments and is more eco-friendly than the use of chemicals. Anti-biosis through the production of antifungal compounds (proteins or small molecules) is probably the best known and most important mechanism used by bio-control bacteria to limit pathogen invasion in host plant tissues [1].

Rubber trees (*Hevea brasiliensis*) are perennial crops of Amazonian origin that have been spread over the whole tropical belt to guarantee worldwide production of natural rubber [2]. *Phytophthora*, the 'plant destroyer', is one of the most destructive genera of plant pathogens in temperate and

tropical regions, causing damage to rubber trees, and hence reducing the production of natural rubber. There are about 60 species in the genus *Phytophthora* proved to act as plant pathogens. In addition to causing multiple diseases on the same host, *Phytophthora* can also attack a wide range of host plants. *Phytophthora* causes severe leaf fall, shoot, root and growth retardation in rubber holdings [3].

Glucans are important structural compounds in the cell walls of fungi. The role of β-glucanase enzyme in preventing fungal disease is well studied [4] and microbes producing β-1,3-glucanase can act as potent bio-control agents. Search for a novel β-1,3-glucanase-producing microbes from new sources is of great significance in this regard. Furthermore, the identification of conserved residues essential for catalysis and structural domains responsible for the regulation of enzyme activity can be identified using bioinformatics tools. Such information is a major step towards understanding the function of the protein of interest. It is clear that computer-based homology modeling and docking studies has greatly aided research into enzyme function and has become an essential tool in protein studies. Studies on the structural and interaction characteristics of β-1,3-glucanase may give an insight into the potency of its antagonistic activity, and such studies facilitate the screening of β-1,3-glucanase-producing bio-control agents with different potentialities and unique characteristics.

Here, we report a novel β-1,3-glucanase gene from an antagonistic *B. amyloliquefaciens*, isolated as an endophyte from petiole tissue of *H. brasiliensis*. Homology modeling of the predicted β-1,3-glucanase protein was also carried out with the aim of understanding the interactions of this enzyme with the substrate. Homology modeling was performed using the online SWISS-MODEL work space, and docking studies used the program Autodock. The results were compared with the template protein endo β-1,3-1,4-glucanase protein (PDBID: 3o5s) from *Bacillus subtilis* (strain 168).

Materials and methods

Isolation of β-1,3-glucanase-producing endophytic bacteria

Freshly collected leaf, petiole and root samples of *H. brasiliensis* were cut into sections (1 g) and surface sterilized by 2 % sodium hypochlorite (Merck, Mumbai, India) for 2–3 min followed by five rinses in sterilized distilled water. All samples were homogenized with a mortar and pestle, serially diluted with sterile 0.85 % NaCl, plated on to Tryptic Soy Agar (Hi Media, Mumbai, India) and incubated for 48 h at 28±2 °C. Individual colonies of differing morphologies were picked and re-streaked on fresh plates to obtain pure

cultures [5]. Exo-β-1,3-glucanase production was checked in peptone media with 0.2 % laminarin inoculated with 1 % bacterial inoculum. The cell-free supernatant was collected after incubation at 37 °C for 48 h and was taken as the crude enzyme extract of β-1,3-glucanase. Glucanase activity was assayed by the DNS method [6]. One unit of enzyme activity was defined as the amount of enzyme required for the production of 1 μmol glucose in 1 min under the defined conditions. Protein was estimated according to Lowry's method [7] and the specific activity was determined.

In vitro evaluation of endophytic bacteria against *Phytophthora meadii*

The selected endophytic bacterial isolate was screened for its activity to inhibit the growth of the pathogen, *Phytophthora meadii*. The isolate was assessed by the dual culture technique using Potato Dextrose Agar (PDA) plates. A bacterial isolate was streaked on one half of the plates and *P. meadii* bits were placed on the other half of the plate parallel to the bacterial streak. PDA plates inoculated with *P. meadii* alone served as the control. After 7 days of incubation at 28±2 °C, colony diameters and inhibition zones were measured. The percent growth inhibition was calculated using the formula $n=(a-b)/a\times100$, where n is the percent growth inhibition, a the colony area of uninhibited *P. meadii* and b the colony area of treated *P. meadii*.

Molecular characterization of endophytic bacteria

Genomic DNA was prepared [8], and the following conserved eubacterial primers were used for the amplification of 16S ribosomal DNA: (1) pA- 5′-AGAGTTTGATCCTGGCTCAG-3′, (2) pH- 5′-AAGGAGGTGATCCAGCCGCA-3′. Each reaction mixture contained *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania), magnesium chloride at a concentration of 1 mM, deoxynucleoside triphosphate at a concentration of 200 μM, primers at a concentration of 100 pmol and 50 ng DNA per 20 μl reaction mixture. The PCR reaction was carried out in an Eppendorf AG22331 Thermal Cycler with the following PCR Cycle: 1 cycle at 94 °C for 2 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, followed by a final 2-min incubation at 72 °C. The PCR products were size-fractionated on a 1 % agarose gel and the bands were excised from the gel and purified using a GenElute™ Gel Extraction Kit (Sigma–Aldrich, Steinheim, Germany). Purified 16S rDNA sequence was cloned in pGEMT Easy vector (Promega, Madison, WI), transformed in *Escherichia coli* JM 109 cells (Promega) and sequenced at Macrogen (Seoul, South Korea). Sequence similarity was analyzed by sequences available in the National Center for Biotechnology Information (NCBI) database using

BLAST analysis, and isolates were identified on the basis of the best match in the database. Sequences of antagonistic bacterial endophyte and reference sequences from NCBI Gen-Bank were aligned using the multiple sequence alignment program ClustalW2. Using the alignment file generated by ClustalW2, phylogenetic analysis was performed in MEGA4 [9]. UPGMA (unweighted pair group method with arithmetic mean) [10] was used to infer phylogeny across the data. Bootstrap analysis (1,000 replicates) was also performed to check the reliability of the phylogram [11].

Cloning and sequencing of β-1,3-glucanase-encoding gene

The following primers were used for the amplification of β-1,3-glucanase gene from genomic DNA: (1) Forward primer: 5′-AGGGGATCCATGAAACGAGTGTTGCTA- 3′ (2) Reverse primer: 5′-TTGCCAGTAGTCTGTGCTAG CTTTTTTTGTATAGCGCAC-3′. The PCR reaction was carried out in an Eppendorf AG22331 Thermal Cycler as mentioned above. Purified PCR product was cloned in pGEMT Easy vector (Promega), transformed in JM 109 cells (Promega) and sequenced at Macrogen. The sequences obtained were compared to those in the NCBI GenBank database by BLAST searching (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

In silico studies of β-1,3-glucanase protein

Amino acid sequence and physico-chemical properties of β-1,3-glucanase protein

The amino acid sequence of β-1,3-glucanase protein was obtained by translation of the nucleotide sequence of the β-1,3-glucanase gene using the EXPASY tool (http://web.expasy.org/translate/). Physico-chemical properties like molecular weight, isoelectric point (pI), half-life, aliphatic index, amino acid property, instability index and Grand Average Hydropathy (GRAVY) were obtained by the EXPASY tool ProtParam (http://web.expasy.org/protparam/). Proteins with an in vivo half-life of less than 5 h show an instability index of more than 40, whereas those with an in vivo half-life of more than 16 h have an instability index of less than 40. The aliphatic index of a protein is a measure of the relative volume occupied by aliphatic side chain of the following amino acids: alanine, valine, leucine, and isoleucine. The GRAVY of the linear polypeptide sequence was calculated as the sum of hydropathy values of all amino acids, divided by the number of residues in the sequence; increasing positive score indicates greater hydrophobicity. Secondary structure elements of β-1,3-glucanase protein were predicted using the PredictProtein Server (http://web.expasy.org/predictprotein/).

Amino acid sequences of β-1,3-glucanase from related strains of *Bacillus* sp. were aligned with our own sequence using the multiple sequence alignment program ClustalW2. Using the alignment file generated by ClustalW2, phylogenetic analysis was performed in MEGA4 [9]. UPGMA [10] was used to infer the phylogeny across the data. Bootstrap analysis (1,000 replicates) was performed to check the reliability of the phylogram [11]. The extracellular nature of the protein was predicted by the program Signal-P [12].

Homology modeling of β-1,3-glucanase protein

The three-dimensional (3D) structure of β-1,3-glucanase was homology modeled using the SWISS-MODEL workspace, which is an integrated web-based modeling tool [13]. Homology modeling is used to build 3D protein structure models using experimentally determined structures of related family members as templates. The template used for modeling β-1,3-glucanase was the endo β-1,3-1,4-glucanase protein (PDB ID: 3o5s) from *Bacillus subtilis* (strain 168) with 94 % sequence identity. Energy minimization of the built model was performed using the GROMOS96 implemented in the Swiss PDB viewer program [14]. The quality of the predicted model was analyzed by PROCHECK [15]. Visualization and analysis of the model were performed using the Swiss PDB viewer and PyMOL [16] programs. The active site was identified using the online Q-site finder tool [17] to predict possible binding sites.

Docking studies

Computer-aided molecular docking of the substrate disaccharide (β-1,3-glucan) to the active site of the modeled structure as well as the template protein (PDB ID: 3o5s) was done using the Autodock4.2 program [18]. The program AutoDock allows consistent computational docking of flexible ligands and receptor with a maximum 32 torsional degrees of freedom. For the best placement of the ligand, the Lamarckian genetic algorithm was used in the program. Initial co-ordinates of the substrate were obtained from the PDB database (PDB ID 2w39). The modeled structure of the β-1,3-glucanase and the crystal structure of the template protein (PDB ID: 3o5s) were taken as receptor proteins. Prior to docking, the bound ligand and water molecules were removed from the template crystal structure, and missing hydrogens were added to the ligand and receptors. Grid maps were prepared using the AutoGrid utility with $62 \times 82 \times 58$ points and grid spacing was set to 0.375. The distance-dependent dielectric constant was set to +80. Docking parameters modified from the defaults were: number of individuals in the population (set to 300), maximum number of energy

Table 1 Production of β-1,3-glucanase from bacterial endophytes. Enzymatic and specific activities of the enzyme from six isolates are given. Means within columns followed by the same letter are not significantly different at P<0.01 according to Duncan's multiple range test

Isolate	Enzyme activity (IU/ml)	Specific activity
REB8	70.83 f	60.78 b
REB 19	90.50 b	60.76 b
REB 20	100.00 a	76.15 a
REB 24	86.20 c	57.76 b
REB 29	77.57 e	53.04 c
REB 35	81.17 d	60.84 b

evaluations (set to 2,500,000), maximum number of generations (set to 27,000) and number of hybrid GA-LS runs (set to 100). The ligand was initially placed next to the active site of the receptor. A flexible docking strategy was applied in which all the binding site residues were set flexible. All rotatable bonds in the ligands were allowed to rotate during the docking trials. For each of the substrate–receptor complexes, 100 independent docking runs were calculated and the lowest energy pose with acceptable geometry was selected, for further analysis. Hydrogen bond interactions were determined using the following criteria: (1) distance between proton donor (A) and acceptor (B) atoms ≤3.2 Å; and (2) the A–H···B angle=120°. van der Waals contacts between the heavy atoms were determined within a distance limit of ≤4.0 Å.

Results

Isolation of β-1,3-glucanase-producing bacterial endophytes and in vitro screening against *Phytophthora meadii*

Endophytic bacteria were obtained from surface-disinfected tissues of *H. brasiliensis*. A total of 48 morphologically different bacterial endophytes were isolated on the bases of colony morphology. Screening of β-1,3-glucanase (Table 1) resulted in the selection of the strain REB20, which could give 100 units enzyme activity in 100 ml broth with a specific activity of 76. The bacterial endophyte showing maximum glucanase production was screened, and the isolate showed 35 % inhibition against *P. meadii* (Fig. S1 in the Supplementary Material).

Molecular characterization of endophytic bacterium

Data from molecular and phylogenetic analyses were used to characterize taxonomically the β-1,3-glucanase producing bacterial endophyte from *H. brasiliensis*. PCR amplification of the 16S rDNA generated a fragment of approximately 1.6 kb and the sequence of the isolate was compared to the sequences of organisms in the GenBank database. The isolate showed 99 % identity to *Bacillus amyloliquefaciens* (GenBank ID: HQ641261). The highest score sequences were recovered from the database as reference sequences and aligned with the 16S rDNA sequence of the endophytic isolate from *H. brasiliensis*. Phylogenetic analysis was conducted in MEGA4 based on UPGMA method. Evolutionary distances were computed using the maximum composite likelihood method and were in the units of the number of base substitutions per site. The phylogenetic tree constructed using 16S rDNA sequences demonstrated that the β-1,3-glucanase-producing endophytic *B. amyloliqufaciens* was considered as the root clade, and that there was least divergence among others in group clades when comparing to the root clade. The isolated strain showed a higher rate of divergence when compared to other *B. amyloliquefaciens* strains (Fig. S2, in the Supplementary Material).

Cloning and sequencing of a β-1,3-glucanase encoding gene

DNA isolated from *Bacillus amyloliquefaciens* was selectively amplified with primers designed for β-1,3-glucanase.

Table 2 Physico-chemical properties of the target β-1,3-glucanase enzyme and the template protein used for homology modeling. *GRAVY* Grand average of hydropathicity

Properties	Target β-1,3- glucanase	Template endo β-1,3-1, 4- glucanase (3o5s)
Number of amino acids	248	238
Molecular weight	27.79 kDa	26.84 kDa
PI	5.93	6.58
Total number of negatively charged residues	22	20
Total number of positively charged residues	19	18
Instability index	19.69	12
Aliphatic index	58.23	48.36
GRAVY	−0.476	−0.659

Fig. 1 Dendrogram representing the amino acid sequence homology of β-1,3-glucanase from endophytic *Bacillus amyloliquefaciens* with other *Bacillus* sp. The amino acid sequence of β-1,3-glucanase from endophytic *B. amyloliquefaciens* showed divergence from other sequence. Bootstrap values are given to the left of the respective nodes
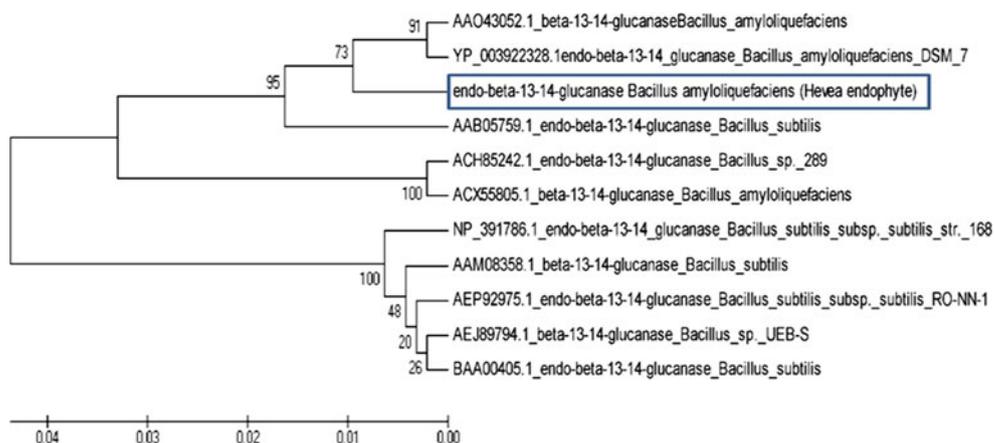
The PCR amplification yielded a ~0.8 kb band in an agarose gel (Fig. S3, in the Supplementary Material). BLAST analysis of the amplified gene sequence showed identity with other β-1,3-glucanase genes. The open reading frame (ORF) in the β-1,3-glucanase gene was 747 bp. The gene was deposited with NCBI GenBank under the accession no. JQ710746.

In silico studies of β-1,3-glucanase protein

Amino acid sequence and physico-chemical properties of the new β-1,3-glucanase protein

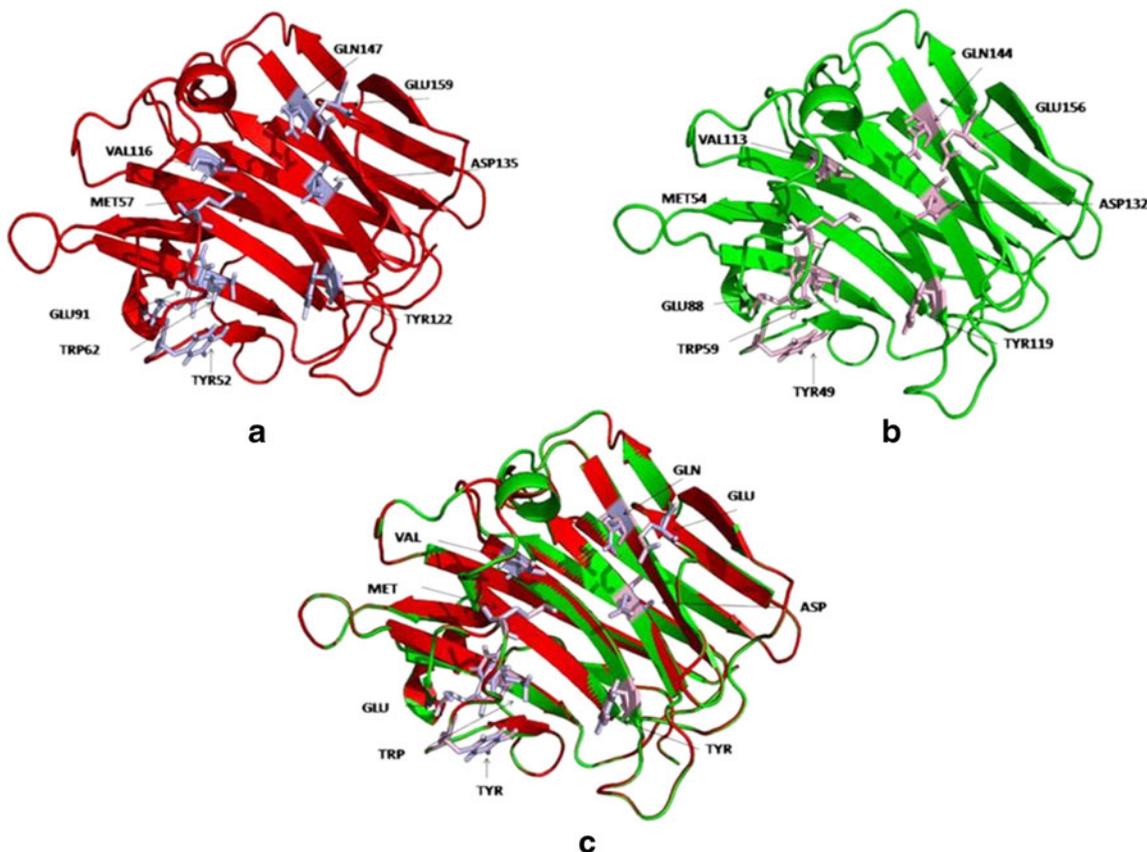The amino acid sequence obtained by translation of the nucleotide sequence of β-1,3-glucanase gene has 248 amino

Fig. 2 **a** Homology modelled structure of the new β-1,3-glucanase protein. Active site residues, identified by the Q-site finder, are labelled. **b** Crystal structure of the template protein, endo β-1,3-1,4-glucanase protein (PDB ID: 3o5s). Active site residues, identified by Q-site finder, are labelled. **c** Superposition of the structures shown in **a** and **b**
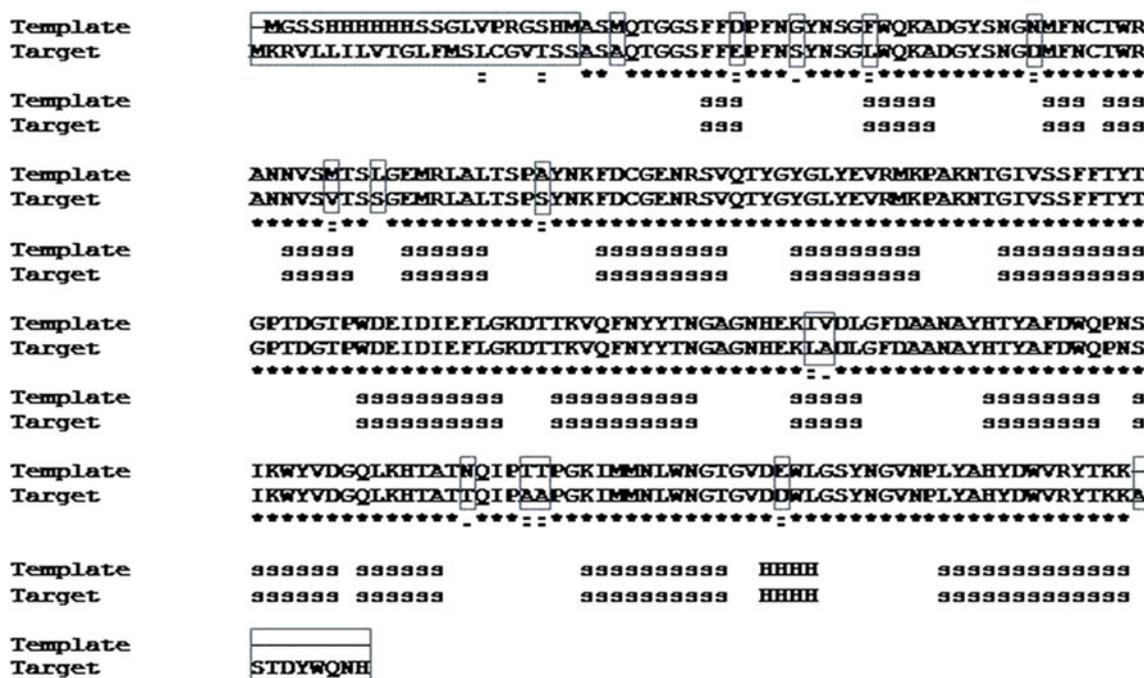
Fig. 3 Amino acid sequence alignment between the new β-1,3-glucanase protein (target) and the template, endo β-1, 3-1,4-glucanase protein. Mismatched amino acids are *boxed*. The secondary structural elements in both the proteins are also given. *S* β-sheet structure, *H* helical structure

acids (Fig. S4, in the Supplementary Material). The physico-chemical properties, predicted by ProtParam tool, of the new β-1,3-glucanase protein and that of the template protein used in homology modelling (PDB ID:3o5s) are listed in Table 2. The secondary structure prediction showed 6.45 % α-helix, 39.52 % β-sheet and remaining percentage of random coil conformation (54.03 %). The SignalP-4.0 prediction showed that the β-1,3-glucanase protein was secretory

in nature, and that a cleavage site exists between amino acid positions 25 and 26. An unrooted phylogenetic tree constructed on the basis of amino acid sequence alignment revealed the similarity of sequence with other bacterial β-1,3-glucanases. The tree consists of two major groups which again sub-divide into many sub-groups. The amino acid sequence from the endophytic isolate was grouped among the major clade of the tree. The amino acid sequence from

Fig. 4 Hydrogen bond interactions of β-1,3-glucan with catalytic residues in the active site of **a** template protein (3o5s) and **b** modeled β-1,3-glucanase. **c** Superposition of the two structures shown in **a** and **b**. In the modeled protein all the three H-bonds are to the same ligand atom, and in the template H-bonds are to different ligand atoms. Orientations of the substrate molecule in the active sites are different for the two proteins
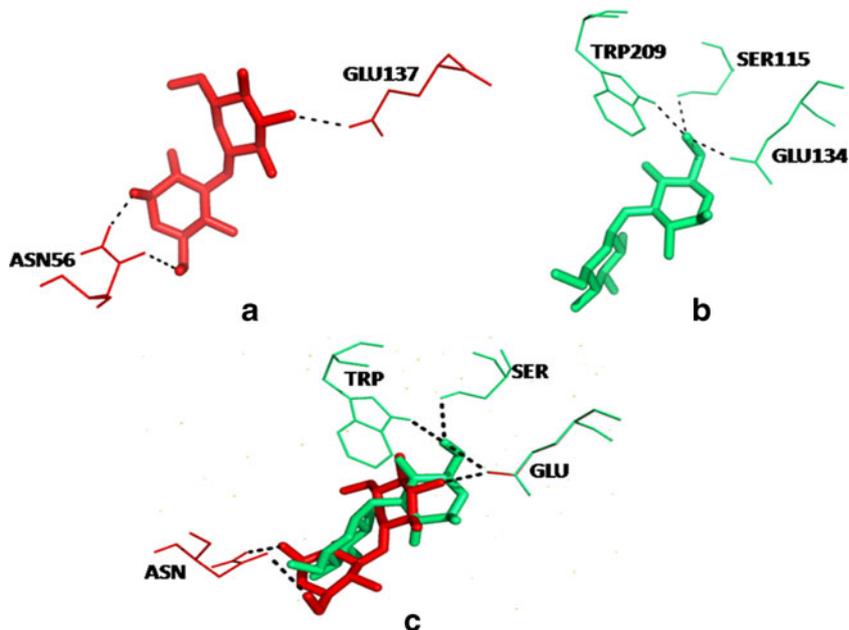
Table 3 Docking features of modeled β-1,3-glucanase and template proteins with substrate

Property	Modeled β-1,3-glucanase	Template protein (3o5s)
Binding energy (kcal/mol)	−20.20	−20.06
No. of hydrogen bonds	3	3
Residues forming hydrogen bonds	Glu134, Ser115,Trp209	Glu137, Asn 56, Asn 56
No. of van der Waals interactions	82	50

endophytic *B. amyloliquefaciens* strain showed a prominent divergence from the other sequences (Fig. 1).

Homology modeling

The 3D structure model of β-1,3-glucanase protein from *Bacillus amyloliquefaciens* obtained by homology modeling using SWISS-MODEL workspace is given in Fig. 2a. The template endo β-1,3-1,4-glucanase protein (PDB ID: 3o5s) from *Bacillus subtilis* (strain 168) has 94 % sequence similarity with the target protein (β-1,3-glucanase) and also an E-value of 2.72837e-110. A Q MEAN Z-score value of −0.602 showed the reliability of the model. The stereochemistry of the modelled structure was checked by the Ramachandran plot in which 92.2 % of the total amino acids were present in most favored regions and 7.2 % in allowed regions (Fig. S5, in the Supplementary Material). The active site residues in the modelled structure obtained by the Q-site finder tool are Tyr49, Met54, Trp59, Glu88, Val113, Tyr119, Asp132, Gln144 and Glu156, as shown in Fig. 2a. Comparison of the 3D structure and active site residues showed good matching with the template protein (Fig. 2b,c). The position and length of secondary structure elements in the modeled structure match that of the template protein exactly although they share only 94 % sequence similarity. Amino acid residues that are not conserved between the target and template proteins sequences are highlighted in Fig. 3. The three residues in the active site region of the template Asn52, Ile157 and Val158 are substituted with Asp, Leu and Ala respectively, in the modeled structure.

Docking studies

Docking studies showed that the substrate disaccharide (β-1,3-glucan) has almost comparable binding energies with the modeled protein (−20.20 kcal mol^{-1}) as well as the template protein (−20.06 kcal mol^{-1}). The substrate–receptor complexes for the target and template proteins are shown in Fig. 4a and b, respectively. The list of hydrogen bonding interactions between the substrate and the receptor proteins are given in Table 3. The numbers of van der Waals contacts (heavy atoms only) for the target and template proteins with the substrates are 82 and 50, respectively. Superposition of the two docked structures showed that the orientation of the substrate within the active site was different between the

target and the template proteins (Fig. 4c). In the modeled protein (Fig. 4b), residues Trp209, Ser115 and Glu134 form hydrogen bonds with the ligand, and these residues are conserved with those of the template sequence. In the template protein (Fig. 4a), residues Asn56 and Glu137 form hydrogen bonds with the ligand. Residue Glu137 is conserved with that of the modeled sequence, but Asn56 is not conserved and is replaced by Asp.

Conclusions

Abnormal leaf fall (ALF) is the most destructive disease of *H. brasiliensis* in India and caused by *Phytophthora* sp. Extensive defoliation during ALF disease results in considerable latex yield loss of 38–56 % in different clones. There are no previous promising reports of bio-control agents against *P. meadii*. Several microorganisms produce lytic enzymes that can degrade the cell walls of other organisms [19]. Expression and secretion of these enzymes by different microbes can sometimes result in the suppression of plant pathogen activities directly. The oligosaccharides derived from fungal cell walls by the action of these lytic enzymes are known to be potent inducers of plant host defences [20]. These endophytic microorganisms occupy a relatively unexplored source for the selection of microorganisms with novel bio-control potentialities.

In this study, bacterial endophytes were isolated from *H. brasiliensis* against *P. meadii* and were screened for β-1,3-glucanase enzyme production. A total of 48 isolates were collected from RRII campus, of which 6 showed β-1,3-glucanase production. The isolate showing maximum β-1,3-glucanase production was evaluated for its antagonistic activity against *P. meadii* and showed 35 % inhibition under in vitro conditions. Since the cell wall of *Phytophthora* sp. consists largely of β-1,3-glucans it can be inferred that β-1,3-glucanase produced by antagonists were involved in pathogen suppression. β-1,3-glucanase contributes significantly to bio-control activities of *Lysobacter enzymogenes* strain C3 [4]. The β-1,3-glucanase producing antagonistic endophyte from *H. brasiliensis* was characterized by 16S rRNA gene sequencing, and studies confirmed that the strain showed maximum identity to *Bacillus amyloliquefaciens*. In this study, we report this β-1,3-glucanase-producing endophytic microorganism from the tissues of *H. brasiliensis* for the first

time, and phylogenetic analysis showed the uniqueness of the isolated *B. amyloliquefaciens* strain.

We have elucidated the gene sequence of the novel β-1,3-glucanase protein from *B. amyloliquefaciens* and investigated the structural and interaction features of the protein (with the substrate) using computation biology tools. The new β-1,3-glucanase has 248 amino acids. Prediction of the physico-chemical properties showed that its isoelectric point (pI) was 5.93, which will be useful for developing buffer systems for precipitation in pH-based separation. Our protein under study had an in vivo half-life of more than 16 h, indicated by an instability index value of 19.69. An increase in the aliphatic index is known to increase the thermostability of globular proteins [21]. The relatively high aliphatic index value (58.23) of β-1,3-glucanase from this endophyte indicated that it was stable over a large range of temperatures. The GRAVY value of −0.476 indicated the water soluble nature of the β-1,3-glucanase protein. Analysis of the phylogenetic tree of β-1,3 glucanase from endophytic *Bacillus amyloliquefaciens* against NCBI database indicated the uniqueness of the sequence. Secondary structure prediction showed a higher β sheet content (39.52) over other regular secondary structures, which is a common feature of β-1,3 glucanases.

To build the 3D structure of the β-1,3 glucanase of endophytic *B. amyloliquefaciens* we have used homology modeling in SWISS PDB Workspace. The template protein, 3o5s, has slightly different physico-chemical properties (Table 2). The secondary as well as tertiary structures of the target β-1,3 glucanase were similar to that of the template protein with a sequence similarity of 94 %. From the Q-MEAN Z Score value and the results of procheck it was clear that the model was reliable. The possible binding sites in the modeled structure were identified using the online Q-site finder tool. The active site of the template protein was identified from its crystal structure complexed with the ligand and was used for docking study. The binding site in the modeled structure, corresponding to the ligand bound active site in the template, was taken as the active site for docking study. A comparison of the active site residues in the target protein with that of the template showed that they were similar despite the fact that the three residues in the active site region of the template Asn52, Ile157 and Val158 were substituted with Asp, Leu and Ala, respectively, in the modelled structure. This slight difference in the active site region might affect the nature of substrate binding.

In order to understand the nature of the subtrate interactions with the target β-1,3 glucanase as well as the template (3o5s), we carried out computer-aided docking studies. The substrate–protein complex was stabilized by three hydrogen bonds (Table 3) and many van der Waals interactions in both proteins. Although the binding energies and the number of hydrogen bonds were the same in both complexes, the residues involved in hydrogen bonding were different. When we superimposed the two complex structures, it was clear that the orientation of the substrate in the active site of the two proteins differed (Fig. 4c). This difference in orientation might be due to the difference in the three amino acids in the active site region of the two proteins. After all, the binding energy remained the same in both substrate–protein complexes. The change in substrate orientation in the active site might also affect the catalytic potential of the β-1,3 glucanase enzyme. Computational methods have been widely employed to predict the structure of enzymes and to simulate the docking structure of the enzyme-substrate complex as well as to analyze the resulting mutant enzymes. Missense mutations located at the ligand-binding surfaces, catalytic site, regulatory sites or allosteric sites can be regarded as functional. Functional mutations disrupt specific interactions between the ligand and binding domain affecting specificity and activity, without necessarily causing large variations into the structure. According to Noorbatcha et al. [22], a single point mutation in the *Aspergillus niger* polygalacturonase II enzyme affects its binding to polysaccharide pectin and affects the formation and disruption of hydrogen bonds between residues in the active site and the ligand as well as between residues within the active site, which has significant consequences for ligand binding. The mutation study of active site residues in the chitin-binding domain from chitinase A1 of *Bacillus circulans* alters substrate specificity [23]. An in silico mutagenesis study of six amino acids (Agr17, Glu28, Gly39, Ala40, Trp76, and Trp81) in *Ralstonia solanacearum* lectin and docking studies with the substrate showed that both polar (Arg17 and Glu28) and non polar (Trp76 and Trp81) residues are crucial for binding. The Ala40 residue was found to be the most interesting residue for mutagenesis and can affect selectivity and affinity. Gly39 may also influence ligand binding because any mutations at this position lead to a change in binding pocket shape [24]

The β-1,3-glucanase-producing *B. amyloliquefaciens* from a new source, *H. brasiliensis*, provided a novel gene sequence. Prediction of the protein structure of the new β-1,3 glucanase via homology modeling offered an alternative means of studying structure–function relationships and is of great importance especially in unlocking the full potential of β-1,3 glucanase enzymes. Our study clearly indicates that the sequence variation in the β-1,3 glucanase gene from an endophytic *B. amyloliquefaciens* affects its functional properties and may reflect the antagonistic potential of this isolate.

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