Fermentation optimization of cellulase production from sugarcane bagasse by *Bacillus pseudomycoides* and molecular modeling study of cellulase

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**A R T I C L E   I N F O**

Keywords:
Cellulase  
Sugarcane bagasse  
Fermentation  
*Bacillus pseudomycoides*  
Homology modeling

**A B S T R A C T**

Degradation of cellulosic carbon, the most important natural carbon reservoirs on this planet by cellulase is very essential for valuable soluble sugars. This cellulase has potential biotechnological applications in many industrial sectors. Thus the demand of cellulase is increasing more frequently than ever. Agro industrial byproducts and suitable microbes are of an important source for the production of cellulase. *Bacillus pseudomycoides* and sugarcane bagasse were used for the production of cellulase and different process parameters influencing the production of cellulase were optimized here. The bacterium showed maximum cellulase production in the presence of sugarcane bagasse, peptone and magnesium sulfate at pH 7, 40 °C in 72 h of incubation. Primary structures of the cellulase is consists of 400 amino acid residues having molecular weight 44,790 Dalton and the theoretical PI is 9.11. Physiochemical properties of cellulase indicated that the protein has instability index 25.77. Seven hydrogen bonds were observed at multiple sites of the cellulase enzyme; His269, Asp237, Asn235, Tyr271, Ser272, Gln309, Asn233. This protein structure may play first hand in further development of exploring cellulase and cellulose interaction dynamics in *Bacillus* sp. Thus this bacterium may be useful in various industrial applications owing to its cellulase producing capability.

1. **Introduction**

Cellulose is an organic compound which consists of thousands of carbon, hydrogen and oxygen atoms. The primary cell wall of green plants contains cellulose as an important structural molecule, which is also secreted by many forms of algae, oomycetes and some species of bacteria to form biofilms. Cellulose is a biodegradable compound, which is tasteless and odorless as well as cellulose is not soluble in water and maximum organic solvents (Bishop and Murphy, 2011). Simple sugars such as beta-glucose, or shorter polysaccharides and oligosaccharides can be produced through the breakdown of the polymer cellulose by the enzyme cellulase (Knowles et al., 1987). Cellulose is an important element in the diet as fiber but human body lacks cellulose-digesting enzyme. Abundant quantities of cellulose containing raw materials and waste products found in nature may be used efficiently by the cellulose breakdown process, that is why these have many economic importance (Bhat, 2000). In the agricultural industries sugarcane bagasse is a waste product but it contains 45–55% cellulose. For that reason, it can be used as a substrate to produce cellulase, microbial product and glucose. The use of cellulose as a microbial substrate has been increased in the past few years. Microbial degradation of bagasse depends on some bacteria and fungi (Aiello et al., 1996). Generally, 280 kg of bagasse are generated from the 1 ton of sugarcane, and 5.4 × 108 dry tons of sugarcane is processed annually throughout the world (Cardona et al., 2010). 150,000 tons of sugar, 100,000 tons of molasses and 800,000 tons of bagasse are produced in Bangladesh every year (Mahamud and Gomes, 2011). Different types of economical products for example, pressed building board, acoustical tile, biodegradable plastics, furfural, nylons, solvents, medicines etc. can be produced from the sugarcane bagasse. In agricultural industries bagasse is a waste product containing high amount of sugar that can be used as potential alternative to corn and as a source of the biofuel ethanol (Rainey, 2009). Cellulolytic microorganisms possess the ability of degrading carbohdrates but they cannot utilize lipids and proteins as source of energy for their metabolism and growth. Among the microorganisms, bacteria, ctyophaga, cellulosomans are the most important microorganisms that are used for producing cellulase (Mawadza et al., 2000). Thus, the present study was designed to optimize fermentation condition for maximum production of cellulase from sugarcane bagasse by *Bacillus pseudomycoides*.  

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2. Materials and methods

2.1. Isolation, screening and identification of cellulase producing bacteria

 Sugarcane bagasse was collected from Rajshahi Sugar Mills Ltd. Sugarcane bagasse was cut into small pieces and suspended in 50.0 mL sterile distilled water, agitated for 30 min and 0.1 mL suspension was spread over CMC agar plates (pH 7.0) containing, 2.0%, CMC: 0.5%, ammonium sulfate and 2%, agar. The inoculated plates were incubated at 37 °C, till sufficient growth appeared. After sufficient growth incubated plates were overlaid with iodine solution (1%) for 15 min and then washed with 1 N NaOH solution for de-staining. Selection was done as per colonies with clear and transparent zone as cellulase producing strain. Bacterial colonies with clear zones were selected, streaked again on CMC agar plates for purification and maintained as pure culture over CMC agar slants (pH 7.0, 4 °C). The isolates having clearing zone were selected for further studies. The selected bacterial isolates were identified by morphological and biochemical characterization as per the Bergey’s Manual of Systematic Bacteriology.

2.2. Optimisation for maximum cellulase production

Various process parameters that influence cellulase production were optimized individually and independently of the others. For optimization, the CMC medium was inoculated and incubated at different temperature viz., 25–45 °C under the standard assay conditions. To study the effect of incubation period samples were withdrawn at every 24 h interval up to 144 h. The effect of pH on enzyme production was determined by varying the pH (ranging from 4.0–9.0) of the broth in different flasks. The enzyme activity at different pH values was measured using the appropriate buffers (citrate buffer) under standard assay conditions. The growth medium was supplemented with different carbon sources viz., CMC, sugar, glucose, fructose, dextrose (at the level of 1%, w/v). Different nitrogen sources viz, yeast extract, peptone, urea, ammonium sulfate and sodium nitrate (1%, w/v) were also used for enzyme production. Thereafter, optimization of different metal ions (5 mM) viz, MnCl₂, KCl, CuSO₄, FeSO₄, MgSO₄, CaCl₂, NiCl₂, NaCl and HgCl₂ were used for enhanced enzyme production.

2.3. Determination of protein concentration

Protein concentrations were measured using the Bradford method with bovine serum albumin as a standard.

2.4. Enzyme assay

Cellulase activity was measured by using the following method. Briefly, a reaction mixture composed of 0.2 mL of crude enzyme solution plus 1.8 mL of 0.5% carboxymethyl cellulose (CMC) in 50 mM sodium phosphate buffer (pH 7) was incubated at 40 °C in a water bath for 30 min. The reaction was terminated by adding 3 mL of DNS reagent. The reaction was terminated by boiling the mixture for 5 min. OD of samples was taken at 540 nm. Enzyme activity was measured as u/mg.

2.5. Homology modeling

The crystal structure of cellulase enzyme was not available for Bacillus pseudomycoides and amino acid sequence of cellulase protein of this bacterial strain still unreviewed and computationally annotated. So, we took sequence of endoglucanase from Bacillus sp. BP-23 (Uniprot ID: O08342) for further protein modeling and docking study as they have evidence and reviewed data at protein level. Furthermore, alignment between unreviewed sequence cellulase from Bacillus pseudomycoides and reviewed sequence were conducted to observe the conservancy among them. Here, fasta format of endoglucanase was extracted from Uniprot database (Bateman et al., 2017) and used as entry system of SWISS-MODEL server (Schwede et al., 2003). The best and most structurally correct protein model was selected on the basis of GMQE (Global Model Quality Estimation) and QMEAN (Quality Model Energy Analysis). The sequence similarity between model protein and template was done by Discovery Studio (BIOVIA, 2016).

2.6. Model validation and energy minimization

The model quality was checked by Ramachandran Plot, ERRAT (Verdonk et al., 2001), Verify 3D (Eisenberg et al., 1997). After that, the protein model was subjected to molecular dynamics simulation in Yasara. AMBER14 force field was employed for energy minimization. For initial minimization conducted by simulated annealing method using steepest gradient approach (5000 cycles). The hydrogen bonding system was optimized and a cubic cell of 20 Å was formed by maintaining periodic conditions. TIP3P or transferable intermolecular potential 3 potential 3 points was applied with Na/Cl ions and the cell density was 1.012 gm/cm³. The Particle Mesh Ewald method was employed with a distance of 8 Å. The total physiological condition of the system was set as (298 K, pH 7.4, 0.9% NaCl). Then molecular dynamics simulation was conducted for 10 ns to analyze RMSD and RMSF of the protein.

2.7. Primary structure prediction

Physicochemical characteristics of the protein is calculated by Expasy’s Protparam tools (Negahdaripour et al., 2017). Several parameters such as molecular weight, Isoelectric point, total number of positive and negative residues, extinction coefficient, aliphatic index, grand average hydrophobicity (GRAVY), instability index were evaluated by this server.

2.8. Secondary structure analysis

Self-optimized prediction method with alignment (SOPMA) was used to determine secondary structure of the protein. Secondary structural properties like as alpha helix, pi helix, beta Bridge, extended strand, Bend region, Beta turns, Random coil, Ambiguous states and other states were calculated by SOPMA. It is a valuable alternative to neural network-based method, which accuracy is based on different principles available for the experimentalist and used for joint prediction, allowing cross-validation. These tools also proved the agreement between predicted and observed length of helical structure of several protein and can recognize folding of protein in the best way (Geourjon and Deléage, 1995).

2.9. Ligand preparation

The ligand structure of cellulase was downloaded from Pubchem database in sdf format and minimized by employing mmff94 force field with steepest descent algorithm (Kim et al., 2015).

2.10. Docking

Molecular docking plays a crucial role for the identification of best chemical compound against receptor molecules on the basis of docking energy. We used Auto Dock Vina (Pyx) tools to evaluate the inhibitory potential of the selected compound against the hypothetical protein structure. Energy minimization of this system was carried out by using Universal Force Field and conjugant gradient algorithm. Total number of steps and number of steps for update for our system was set as default. Finally, we converted our protein and ligand into PDBQT format since it is the only acceptable format in Auto Dock Vina. A grid box of about (X:26.5680 x Y:14.6187 x Z: 8.2399)Å and box size was designed as 52.5160 × 54.054 × 52.9585 for X,Y,Z axis respectively.
and nine binding modes were generated for the most favorable binding (Morris et al., 2008). Details analysis of non-bonded interaction of snake venom protein and ligand complex were evaluated by Accelrys Discovery Studio (BIOVIA, 2016) and Pymol software (DeLano, 2002).

2.11. Statistical analysis

All experiments were carried out in triplicates and the results are presented as the mean of three independent observations. Graphs were prepared using Graph Pad Prism Software version 8.0 (Graph Pad Software, San Diego, CA, USA).

3. Results

3.1. Isolation, screening and identification of cellulose producing bacterial strains

Thirty bacterial isolates were produced on CM agar plates. Among these isolates, only two (Isolate A and Isolate B) were selected by further staining in iodine solution (Fig. S1). The efficient cellulase producing strain (Isolate A) was rod-shaped, Gram-positive, aerobic, motile, with positive TSI, methyl red, catalase, urea hydrolysis, BSA and EMB test. On the other hand, isolate B was gram positive, non-motile, with positive methyl red, catalase, urea hydrolysis, starch hydrolysis test. Both the isolates grew over a wide range of pH (4.0–8.0) and temperature (25–50 °C). On account of morphological, biochemical and molecular characteristics, the efficient strains were identified as Bacillus pseudomycoides and Bacillus massiliorenexius (Table S1). Only Bacillus pseudomycoides was used for the production of cellulase from sugarcane bagasse in this study.

3.2. Effect of incubation periods on cellulase production

Incubation periods plays an important role on enzyme production by bacterial strain thus various periods ranging from 24–144 h (Fig. 1A) were used to optimize the incubation periods for higher enzyme production. The strain reported a wide range of incubation periods (48–96 h) for cellulase production, but maximum (4.25 mg/ml) enzyme was produced within 72 h of incubation.

3.3. Optimization of pH on enzyme production

The media comprising various pH ranging from 4.0 to 8.0 (Fig. 1B) was used to study the effect of pH on crude cellulase production. In all the pH, Bacillus pseudomycoides produced cellulase enzyme, but maximum enzyme concentration (4.7 mg/ml) was achieved at pH 7.0 (Fig. 1B).

3.4. Effect of temperature on enzyme production

Enzyme concentration and activity were recorded at different temperatures ranging from 25 to 50 °C. It was revealed that Bacillus pseudomycoides showed maximum cellulase activity (2.24 U/mg) with an enzyme concentration (4.01 mg/ml) at 40 °C (Fig. 1C). Here bacterial strains showed better enzyme production from 35–50 °C, but 40 °C was found to be the most effective temperature for enzyme production by Bacillus pseudomycoides.

3.5. Effect of carbon sources

Various carbon sources such as sugar, glucose, fructose, dextrose, maltose, and bagasse were used in growth media. Different carbon sources had both stimulating and inhibitory effects on cellulase production. Bacillus pseudomycoides reported maximum cellulose production with sugarcane bagasse (Fig. 1D). It was confirmed from the result that sugarcane bagasse could be an effective for production of cellulase by Bacillus pseudomycoides.

3.6. Effect of nitrogen sources on enzyme production

For determining the effect of nitrogen sources on cellulase production different nitrogen sources such as yeast extract, peptone, urea, ammonium sulfate and sodium nitrate were used in the growth medium. Among the various nitrogen sources tested, yeast extract was found to be the best nitrogen source for cellulase production and the highest concentration (4.22 mg/ml) was estimated in the medium contained peptone (Fig. 1E).

3.7. Role of carbon and nitrogen sources

After selecting best carbon (sugarcane bagasse) and nitrogen (yeast extract) sources, we used both of them to observe their combined effect on optimum cellulase production (Fig. 1F). When 1% and 1.5% sugarcane bagasse and yeast extract were mixed respectively the strain produced maximum cellulose (5.6 mg/ml) with higher (3.5 U/ml) cellulase activity.

3.8. Effect of metal ions on enzyme production

The effect of different metal ions on cellulase production by Bacillus pseudomycoides was also investigated in this study (Fig. 1G). Bacillus pseudomycoides produced maximum cellulose (4.91 mg/ml) in the presence of magnesium sulfate with an activity of 2.84 U/mg.

3.9. Homology modeling

SWISS-MODEL webserver employed to get three dimensional (3D) structure of the targeted protein (Fig. 2). The model protein was run in molecular dynamics simulation for refinement process. The hypothetical protein structure runs in the different server (Verify 3D, ERRAT), to check the quality and accuracy (Figs. S2 and S3).

3.10. Primary and secondary structure prediction

Several parameters related to primary structure of the protein evaluated from ProtParam server (Table 1). Protein function and stability is largely depend on these criteria. Primary structures of the protein obtained from ProtParam showed that the protein is consisting of 400 amino acid residues, molecular weight 44,790 and the theoretical PI is 9.11. The secondary structure information derived from SOPMA server of the endoglucanase enzyme depicted in Table 2.

3.11. Molecular docking

The investigation of binding affinity of cellulose and endoglucanase complex is accomplished by molecular docking studies through Auto Dock Vina. Table 3 displayed the molecular interactions and binding affinities of the complexes. The docked structure was computed by calculating RMSD (Root Mean Square Deviation) between coordinates and cluster formation based on RMSD profile. The binding energy consists of total internal energy, total intermolecular energy, and torsional free energy minus energy of the unbound system. Among the top nine generated conformers, the -8.7 kcal/mol found as a lower docking energy value of the system.

4. Discussion

Cellulose is the most abundant natural biomass on earth and most dominating agricultural residue. In the present study cellulase production by Bacillus pseudomycoides from sugarcane bagasse was optimized. This bacterium showed a broad range of incubation periods for the cellulase production and maximum cellulase was produced in 72 h of incubation. Selvankumar et al. (2011) reported that Bacillus amylobiformfaciens produced maximum cellulase within 72 h of incubation. Tiwari et al.
Fig. 1. Optimization of various process parameters for maximum cellulase production and higher cellulase activity by *Bacillus pseuodomycoides*. Error bars presented mean±standard deviation of triplicates of three independent experiments. A. Influence of incubation periods; B. Impact of pH; C. Effect of temperature; D. Impact of carbon sources; E. Effect of nitrogen sources; F. Combined effect of carbon and nitrogen sources; G. Effect of metal ions.

Table 1

| Parameter                        | Value   |
|----------------------------------|---------|
| Molecular Weight                 | 44,790  |
| Extinction co-efficient Abs 0.1%(=1 g/l) assuming all pairs of Cys-residues form cysteine| 101,760 |
| Theoretical PI                   | 9.11    |
| Total number of negatively charged residue(Asp+Glu) | 37      |
| Total number of positively charged residue(Arg+Lys) | 33      |
| Instability index                | 25.77   |
| Grand average of hydropathicity (GRAVY) | -0.404 |
| Aliphatic index                  | 67.58   |

Table 2

Secondary structure prediction from SOPMA webserver.

| Parameter   | Values |
|-------------|--------|
| Alpha helix | 43.50% |
| 3_10 helix  | 0.00%  |
| Pi helix    | 0.00%  |
| Beta bridge | 0.00%  |
| Extended strand | 14.00% |
| Beta turn   | 4.5%   |
| Bend region | 0.00%  |
| Random coil | 38.00% |
| Ambiguous states | 0.00% |
| Other states | 0.00% |

(2017) also reported that *Streptococcus* and *Bacillus* sp. produced maximum cellulase from sugarcane bagasse within 72 h of incubation. This was due to its late stationary phase. Production of enzymes is usually initiated during the log phase of the growth and reaches maximum levels during the initial stationary phase (Sudharhsan et al., 2007).

The temperature can change the physical properties of the cell membrane and thus influence extracellular enzyme secretion. When fermentation was carried out at 45 °C minimum cellulase production was observed while maximum yield was obtained at 40 °C by *Bacillus pseuodomycoides*. These results were closed to the report of Ray et al. (2007).

Acharya and Chaudhary (2012) reported that *Bacillus licheniformis* MVS1 and *Bacillus* sp. MVS3 produce maximum cellulase at pH 6.5 and 7.0, respectively. In this study, the bacterium was allowed to grow in media of different pH ranging from 4.0 to 8.0, but maximum enzyme production and concentration was achieved at pH 7.0 (Fig. 1B). This result was correlated with the findings of other workers for different *Bacillus* strains (Sudharhsan et al., 2007).

Agro-industrial residues such as rice bran, rice straw, sugarcane bagasse, wheat straw and wheat bran could be used as carbon sources for cellulase production. *B. subtilis* CBTK 106, *B. subtilis* BC 62 and *B. pumilus* produced maximum cellulase when wheat bran, banana fruit stalk and soybean were supplemented to the production media. When CMC...
was used as a carbon source Bacillus sp. produced maximum cellulase (Das et al., 2010). Here, we used sugarcane bagasse as a carbon source and Bacillus pseudomycoides produced maximum cellulase after 72 h of incubation.

Among different nitrogen sources, yeast extract enhanced the cellulase production. B. licheniformis produced maximum cellulase in the presence of ammonium sulfate substituted medium than the other organic and inorganic nitrogen sources. Some other researchers have also been found that ammonium sulfate gave maximum cellulase production by B. pumilus, Ruminococcus albus, Bacillus sp. B21, Streptomyces sp., BRC2 (Wood et al., 1982).

Production of extracellular cellulase has been shown to be sensitive by different carbohydrate and nitrogen sources. Thus we used different carbon, nitrogen and their combinations to observe the cellulase activity produced by B. pseudomycoides. The maximum cellulase activity of Bacillus sp. Y3 was 7.82 IU/mL when CMC was used as a carbon source in the basal media (Lugani et al., 2015), Sethi et al. (2013) reported that Bacillus sp produced maximum cellulase activity when used fructose and ammonium sulfate as a carbon and nitrogen source respectively. When CMC was used as a sole carbon source, Enhydrobacter sp. ACC2A exhibited its maximum (2.61 U/mL) cellulase activity (Premalatha et al., 2015). Here, Bacillus pseudomycoides produced maximum cellulase activity 2.89 u/mg and 2.92 u/mg when sugarcane bagasse and yeast extract were used as carbon and nitrogen sources respectively. But in case of sugarcane bagasse (1%) and yeast extract (1.5%) combination, the screened strain exhibited its highest activity (3.5 u/mg). Yang et al. (2014) reported that the maximum CMCase activity (2.91 U/mL) of Bacillus subtilis BY-2 was observed when a mixture of 1% corn powder and 1% CMC was used as the sole carbon source. Nitrogen is one of the major cell proteins and stimulation of cellulase activity by ammonium sulfate salt might be due to their direct entry in protein synthesis (Mandels, 1975). The substrate not only serves as a carbon and nitrogen source but also produces the necessary inducing compounds for the organism (Haltrich et al., 1996).

Some microbes produce higher enzyme in presence of some metal ions. Here magnesium sulfate stimulated the bacterium for producing maximum cellulase from sugarcane bagasse. Lee et al. (2008) reported that K+ and Mn+ activated cellulase production by Bacillus thuringiensis. Tiwari et al. (2017) used magnesium sulfate for maximum cellulase production by Streptococcus and Bacillus sp. by using sugarcane bagasse as a carbon source. Metal ions such as Ca, Mg, Fe, Co and Zn were necessary for cellulase synthesis by Trichoderma viride QM6a (Mandels and Reese, 1999). The major action of these metal ions is to work as cofactor of the enzyme.

Physicochemical properties obtained from ProtParam indicated the target protein has instability index 25.77 which suggest the protein will be stable in vitro condition. As instability index higher than 40.00 denotes that the protein will be unstable, whereas our protein score 25.77 in instability index (Guruprasad et al., 1990). Higher aliphatic index is expected for increasing thermo stability where our protein showed 67.58 aliphatic profile. The sequence had 14 negatively charged residues and 15 positively charged residues. Grand average of hydropathicity (GRAVY) score was -0.404 which indicates the soluble and hydrophilic nature of the protein (Table 1). Secondary structure generated tools SOPMA showed the protein is occupied with alpha helix 43.50%, extended strand 14.00% and random coil 38.00%. The stability and higher conservation ability of the protein confirmed by higher coil region area (Table 2).

Therefore, homology modeling has been using for finding protein function, mutation assessment, role in cell signaling, druggable targets and understanding of the binding mode. Nowadays, it is time consuming to get experimental structure and in few cases hard to get the structure due to numerous factors like; solubility, large size, low expression (Berman et al., 2000). The sequence alignment in Bacillus pseudomycoides confirms high similarity with Bacillus sp. denotes (Fig. S4) the functional similarity (Carpentier and Chomilier, 2019; Higdon et al., 2010). The crystal structure 2JEQ with 1.94 Å resolution was selected for template where a sequence identity of 84.30% and sequence similarity of 0.58. The sequence alignment between template and model structure showed that template sequence covers 42–400 sequences of Bacillus sp. Therefore, the three dimensional structure of the protein was subjected to quality checking by different program. The GROMACS scoring program is ranked between 0 to 1 where higher score denote more reliability (Biasini et al., 2014). However, in low sequence homology, packing of secondary structure and folding may be incorrect. Even in high homology sequence error may be present in variable which may prevent the structure for further usages. Therefore, short dynamic simulation can be useful to refine homology models (Fan, 2004). molecular dynamics simulation study, the hypothetical model was run for 5 ns. During the whole simulation time, the RMSD profile of the protein was way below to 2 Å and did not fluctuate too much. Therefore, in

**Table 3**

| Compound name | Binding energy Kcal/mol | Hydrogen bond Ligand atom-amino acid distance in A | Hydrophobic bond Interaction-amino acid distance in A |
|---------------|-------------------------|-----------------------------------------------|-----------------------------------------------|
| Cellulose     | -8.7                    | H-His265:O(1.98), H-Asp237:O(2.22), HD21-Asn235:O(2.32), HN-Tyr271:O(1.89), H-Glu237:O(2.45), H22-Gln309:O(3.00), H18-Asn233:O(3.04) | |
whole simulation time the protein showed stable behavior. On the other hand, root mean square fluctuation of the amino acid residues in protein was stable and only little fluctuation were seen (Fig. 3). In our study the model protein structure exhibited 0.88 in GMQE which indicated model protein accuracy. On the other hand, degree of nativeness and per residue quality checked by QMEAN (Benkert et al., 2009) score where -4 or below indicate low quality model. Our protein had -0.60 QMEAN-Z score, which depicts structural preciseness. The Ramachandran plot (Fig. S3) of the protein showed that 95.2% (352) of amino acid are in the allowed region indicating higher stability of the model protein (Gopalakrishnan et al., 2007). Furthermore, ERRAT (Fernando et al., 2016) and Verify3D (Eisenberg et al., 1997) (Fig. S2) were employed to analyze the accuracy where the structure should score >80% in ERRAT and our protein structure scored 97.157%. On the other hand, 99.44% residues have average 3D-1D score which is the indicator of better structure.

Non covalent interaction between protein target or enzyme and ligand plays a key role in blocking the active cavity which ultimately liable for causing certain diseases or molecular targets. Non-bonded interaction like halogen, hydrogen, anion-pi interaction, T-shape interaction, π-π stacking has been seen in protein-ligand systems (De Hoog et al., 2004). It is interesting to observe that, the complex did not show any hydrophobic bond rather than multiple hydrogen bonds. Moreover, seven hydrogen bond were observed at multiple site of the enzyme; His269, Asp237, Asn235, Tyr271, Ser272, Gln309, Asn233 (Fig. 4). The activity of this enzyme can be improved by the presence of Mg and Ca with an optimum conditions (Blanco et al., 1998).

5. Conclusion

Based on the above study, it can be concluded that sugarcane bagasse can be very good source for the maximum production of cellulase and it can be used in industrial purpose. We are optimistic to set up a protocol for the production of cellulase enzyme in industrial scale with promising cost-effective procedure.

Declaration of Competing Interest

No potential conflict of interest was reported by the author(s).

Author contributions

SKP and MAS formulated the present hypothesis. SKP and SM performed most of the experiments. GKP, TJ and KN helped during the research work and also helped for drafting the manuscript. MSU and SZ revised the manuscript and provided advice. MAS prepared the draft manuscript and supervised the whole work.

Acknowledgments

We express our deepest gratitude and thanks to Almighty Allah and all those who have helped and supported us along the way. The authors received no funding from an external source.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crcmicr.2020.100013.

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