Characterization of Thermophilic β-Glucosidase of Rhizospheric Bacterial Strain (LSKB15) Isolated from Cholistan Desert, Pakistan

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Abstract Fifty thermophilic bacterial strains isolated from rhizospheric soil of Cholistan desert, Pakistan, and designated as LSKB01-LSKB50 were screened for β-glucosidase gene (*bgl*) belonging to glycoside hydrolase family 1 (GH 1) using PCR technique. Subsequently, the same strains were screened for extracellular β-glucosidase production using esculin as substrate. All fifty strains were shown to be amplified for conserved region of *bgl* gene and to secrete extracellular β-glucosidase. One strain (LSKB15) secreted relative high amount of this enzyme as indicating by size of ferric-esculetin precipitate. This strain was further cultivated on cellulose containing media and β-glucosidase was purified by ammonium sulfate, dialysis and gel filtration chromatography. The purified enzyme showed an optimal temperature of 60°C and an optimal pH of 7. It also showed excellent temperature and pH stability retaining > 90% activity after incubation for 2 h at pH 5-8 and 40-60°C. Finally, the purified enzyme was run on Native-PAGE and subsequently incubated in phosphate buffer containing 5 mM of 4-methylumbelliferyl-β-D-glucoside (4-MUG) for 15 min at 50°C and visualized by UV light as white band. We concluded that thermophilic LSKB15 β- glucosidase may work with other cellulase to degrade available cellulose synthesized by plant and the properties exhibited by it such as high temperature and pH stability pointed out its potential industrial importance.

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1. Introduction

Cellulose is the most abundant biopolymer on the biosphere and the major constituent of plant biomass. Cellulase is a group of enzymes that synergistically degrade cellulose polymer into glucose unit. These enzymes are endoglucanase (EC. 3.2.1.4), exoglucanase (E.C. 3.2.1.91), and β-glucosidase (EC. 3.2.1.21) [1,2]. Cellulose degradation is involved the random attack of the interior of cellulose chains especially at the amorphous regions by endoglucanase which hydrolyzes glucosidic bonds to generate oligosaccharides chains of different length. Subsequently, exoglucanase/cellobiohydrolase processively hydrolyzes these chains from their reducing and nonreducing ends releasing glucose, cellobiose, and short oligosaccharides. These two enzymes act synergistically and are usually inhibited by cellobiose. Finally, β-glucosidase breaks down cellobiose and short oligosaccharides into glucose units thus alleviating cellobiose inhibitions on endoglucanase and cellobiohydrolases [3,4,5].

β-Glucosidase catalyzes, in addition to its role on cellulose degradation, the hydrolysis of nonreducing glucosyl terminal from variety of alkyl-, aryl-β-glucosides, disaccharides, and short cello-oligosaccharides [5].

β-Glucosidases are found in all living organisms and play a fundamental role in many biological processes. For example, in plants, β-glucosidase is involved in defense, β-glucan chain synthesis, cell wall metabolism, lignification, phytohormone activation, secondary metabolism and fruit ripening [3,6,7]. In microorganisms, it plays roles in cellulose hydrolysis, carbon recycling and cellulase gene induction [8]. In mammals, β-glucosidase is involved in hydrolysis of glucosyl ceramides and in humans its defect causes Gaucher's disease [9]. Biotechnologically, β-glucosidase are used in many applications such as biofuel production, flavor enhancement in wine and fruit juices industry, isoflavone hydrolysis, cassava detoxification, and debittering of fruit juices [10,11]. Furthermore, β-glucosidase, under certain circumstances of high substrate or product concentration, can synthesize various alkyl β-glycoside, aryl-β-glycoside, oligosaccharides which have a wide

range of applications such as diagnostic tools, probiotics and antimicrobial agents [12,13]. Researchers are therefore focusing on characterizing this enzyme from different microbial sources for its applications. Bacterial β-glucosidases are of interest because of short production time and the novel properties they tend to exhibit such as thermostability and glucose tolerances.

Cholistan desert is one of the largest deserts in the Subcontinent with an area of 26000 Km^2. It is located on the south border of Punjab province, Pakistan [14]. This desert confronts with high temperature in summer and low in winter and has generally poor soil lacking organic matters with high alkalinity [15]. The microbial inhabitant of this desert is largely unexplored and could be expected to be a good source of novel microorganisms e.g., thermophilic bacteria which could be then used as source of thermostable enzymes. We are interested in exploring the microbial community of this desert for various industrial enzymes like amylases, cellulases, β-glucosidase etc. In the present work, fifty thermophilic bacterial strains designated as LSKB01-LSKB50 isolated from rhizospheric soil of *Lasiurus sindicus* plant from Cholistan desert were screened for β-glucosidase gene belonging to Glycoside Hydrolase family 1 (GH 1) and subsequently were screened for extracellular β-glucosidase. One strains (LSKB15) showed relatively higher activity was purified and further characterized for its biochemical properties. The findings of this study reflect the diversity of microbiome inhabiting this desert and its potential biotechnological utilization.

2. Methods and Materials

2.1. Materials and Bacterial Strains

Bacterial strains (LSKBs) were isolated from the rhizospheric soil of *Lasiurus sindicus* plant of Cholistan desert and stored at -70°C. Four pairs of oligonucleotides primers were synthesized by Macrogen, Inc (Korea). *p*-Nitrophenol-β-D-glucoside (*p*-NPG), esculin and 4-methylumbelliferyl-β-D-glucoside were purchased from Sigma-Aldrich, (USA). All other chemicals and salts were of analytic grade.

2.2. DNA Extraction

LSKBs bacterial strains were cultured on nutrient broth media (1.3%) for 20 h and 2 ml of overnight culture taken in Eppendorf tube was centrifuged at 6000 rpm for 10 min at 4°C. Supernatant was discarded and pellet was suspended in lysis buffer pH 8.0 (100 mM Tris-HCl, 1.5 M NaCl and 20% SDS) and incubated in water bath at 68°C for 1 h with frequent vigorous mixing. The mixture was centrifuged at 10000 rpm for 10 min at room temperature (RT) and supernatant was transferred into new properly labeled Eppendorf tube and cell debris was

discarded. An equal volume of phenol-chloroform was added, mixed, and then centrifuged at 10000 rpm for 10 min at RT. Subsequently, the upper aqueous layer was withdrawn carefully into a new labeled Eppendorf tube and further purified through isopropanol precipitation and washing with 70% ice cold ethanol. Finally, DNA pellet was air-dried, resuspended in 150 µl low TE buffer pH 8.0 (100 mM Tris-HCl, 0.4 mM EDTA), and stored at 4°C for subsequent analysis. The extracted DNA was visualized in 1% agarose gel to check its integrity and its quantity and purity was determined UV light spectrophotometer at 260 nm and 280 nm, respectively.

2.3. Polymerase Chain Reaction

Two degenerate oligonucleotides primers reported by Li *et al.* (2013) were used to synthesize four pairs of primers to amplify the conserved region of β-glucosidase belonging to GH 1 from bacteria [16]. The sequences of degenerate primers are BGH1F: 5'CCT ACC AGA TYG ARG G-3' BGH1R: 5'GAG GAA GRT CCC ART G-3' (where Y= C/T R= A/G). Among the four pair of primers synthesized, third pair with BGH1F3 5'CCT ACC AGA TTG AAG G-3' and BGH1R3 5'GAG GAA GAT CCC AGT G'3 was shown to specifically amplify the conserved sequence of *bgl* gene. The amplification of 16S rRNA was used as positive control using universal primers sequence: 16S rRNA-F: 5'AACACATGCAAGTCGAAC3', 16S rRNA-R: 5'ACGGGCGGTGTGTACAAG3'. PCR conditions were optimized as in Table 1. The standard PCR reaction mixture contains 2.5 µl 1X PCR buffer (10 mM Tris-HCl, pH 8.3 at 25 °C, 50 mM KCl), 0.5 µl (200 µM dNTP), 2 µl (2 mM MgCl$_2$), 1 µl (20 pico mole of each primer) and 20 ng DNA template for 25 µl PCR reaction mixture. PCR products were run on 1% agarose gel electrophoresis and visualized using UV Pro documentation system.

2.4. Screening of Extracellular β-Glucosidase

The LSKB bacterial strains stored at -70°C were cultured individually in 100 ml culture flasks containing 30 ml of nutrient broth medium supplemented with 1% cellulose at 50°C in an incubator shaker at 230 rpm for 24 h. The culture supernatant obtained by centrifugation at 6000 rpm at 4°C for 10 minutes was stored at 4°C and used for screening of β-glucosidase production using esculin as substrate. In this test, β-glucosidase hydrolyzes esculin to esculetin and glucose. Esculetin then chelates ferric ions to form a complex which eventually precipitate [6]. Briefly, 1 ml esculin (1%) was taken in a test tube and 1 ml crude supernatant was added. This mixture was incubated at 50°C for 1 h. Then, 1 ml ferric chloride (1%) was added to the test tube and left at RT for 1 h for the precipitate formation. Different controls were used for validate our results [17,18,19].

Table 1. PCR conditions for amplification of conserved regions of *bgl* gene and 16s rRNA

PCR amplification step	*bgl* conserved region		16S rRNA	
	Temp (°C)	Time	Temp (°C)	Time
Initial denaturation	94	5 min	94	5 min
Denaturation	94	1 min	94	30 sec
Annealing	50	1 min	50	1 min
Extension	72	1 min	72	1 min
Final extension	72	10 min	72	6 min
Number of cycles	35		30	

2.5. Enzyme Assay

Enzyme activity was measured using *p*-NPG as substrate. Briefly, 150 µl of 5 mM *p*-NPG was added to 100 µl of 50 mM phosphate buffer (pH 7). Then 150 µl of enzyme solution was added and the mixture was incubated for 20 min at 60°C. The reaction was stopped by adding 100 µl of 2 M Na_2CO_3 solution and the absorbance of the released *p*-nitrophenol was measured at 410 nm. Mixture containing *p*-NPG, buffer and heat-inactivated enzyme was used as a control. One unit of the enzyme was defined as the amount of enzyme required to release 1 µmol of *p*-nitrophenol per min per mg protein [20].

2.6. Purification of β-Glucosidase

LSKB15 culture supernatant (500 ml) was saturated with ammonium sulfate salt $[(NH_4)_2SO_4]$ to 40% saturation with continuous stirring at 0°C and then placed overnight at 4°C. Next morning, this equilibrated solution was centrifuged at 16000 rpm for 20 mins at 4°C and the protein precipitate was dissolved in 20 mM phosphate buffer pH 7. The supernatant was subjected to repeated ammonium sulfate precipitation to attain 80% and 100% saturation following the same procedure. The precipitated protein was suspended in 20 mM phosphate buffer pH 7. Protein fractions were dialyzed against the same buffer overnight with continuous stirring and changing the buffer every 4 h to remove NH_4SO_4 traces and other impurities [21]. The enzyme activity was measured in all three fractions (40%, 80%, and 100%) and the fraction in which the activity was detected was further purified through gel filtration chromatography on sephadax G-100 column in 0.05 M phosphate buffer, pH 7. The dialyzed enzyme fraction (3 ml) was loaded into the packed column and eluted with 0.05 M phosphate buffer, pH 7. The eluted fractions of 1 ml were collected and the enzymes activity was measured using the standard enzyme assay mentioned above. Proteins were quantified in each fraction, and purified enzyme was used to characterize β-glucosidase for biochemical properties.

2.7. Protein Quantification

Protein content of the culture supernatant and purified fractions was quantified by Bradford assay. Hen egg albumin was used as standard [22].

2.8. Effect of pH and Temperature on Enzyme Activity

The effect of pH on enzyme activity was evaluated by measuring enzyme activity in pH range of 3-11 using 50 mM of the following buffer: citrate buffer (pH 3-5), phosphate buffer (pH 6-8), and glycine-NaOH buffer (pH 9-11) at 60°C. To examine the effect of temperature on purified enzyme activity, it was measured at a temperature range of 30-90°C at pH 7.

2.9. pH and Temperature Stability

For pH stability, purified enzyme was incubated in different buffers pH of 3-10 and incubated at 4°C for 2 h. The residual activity was then measured using *p*-NPG as substrate at pH 7 and 60°C. Purified enzyme mixed with pure deionized water and incubated at 4°C was used as a control and its activity was taken as 100% and residual activity was calculated.

For thermostability test, purified enzyme was incubated at different temperatures of 40-90°C for 2 h. The residual activity was then measured at pH 7 and 60°C. Enzyme kept at 4°C for 2 h prior to measuring its activity was used as a control and its activity was regarded as 100% activity and residual activity was calculated.

2.10. Zymography

The purified native enzyme was electrophoresed on 4% stacking and 10% resolving native polyacrylamide gel for 2 h at 100 V. Subsequently, the gel was removed and incubated in 50 mM sodium phosphate buffer (pH 7) containing 5 mM of 4-methylumbelliferyl-β-D-glucoside (4-MUG) for 15 min at 50°C. The gel was documented under UV light and appearance of white fluorescent bands indicates the release of methylumbelliferone due to enzyme activity.

2.11. Metal Ions Effect

The following metal ion Co^{2+}, Ni^{2+}, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} were used to check their effect on enzyme activity at two different concentrations i.e., 1 and 10 mM. The enzyme was incubated with metal ions solution for 15 mins after which the enzyme activity was measured as described in enzyme assay section. All metal ions used were in chloride form except copper which was used in sulfate form.

3. Results and Discussion

3.1. PCR Amplification

In the present work, 50 bacterial strains isolated from rhizospheric soil of *Lasiurus sindicus*, a Cholistan Desert plant, and designated as LSBK01-LSKB50 were used to screen them for *bgl* gene belonging to Glycoside Hydrolase family (GH) 1. GH 1 has reported in bacteria and fungi, plant and animals. We synthesized 4 pairs of primers (BGH1F1:BGH1R1-BGH1F4:BGH1R4) reported by Li *et al* (2013) to amplify the conserved region of *bgl* belong to GH 1. The genomic DNA was extracted from overnight culture of these strains using standard manual protocol optimized in our laboratory. The genomic DNA was separated on 1% agarose gel electrophoresis to check for its integrity. Similarly, the quantity and purity of DNA was determined through spectrophotometer at 260 nm and 280 nm, respectively, and appropriately diluted to obtained equal concentration of DNA for PCR. Twenty Nano grams (ng) of genomic DNA was used for PCR amplification using the different pairs of oligonucleotides primers. Only third pair (BGH1F3:BGH1R3) readily amplify internal *bgl* conserved sequence from these strains without nonspecific amplification under stringent optimized conditions with amplicon of 320 bp as shown on 1.5% agarose gel electrophoresis. Interestingly, all fifty

strains were amplified for conserved region of *bgl* gene by this pair reflecting the conservation of these sequence and phylogenetic relatedness of these strains and suggesting that this gene may be a housekeeping gene playing some conservative functions (Figure 1 & Figure 2). We hypothesized that these rhizospheric bacterial strains may synthesize and secrete this enzyme extracellularly along with other cellulase enzymes to degrade cellulose available to them by plants. Therefore, we further screened these strains for the production of extracellular β-glucosidase.
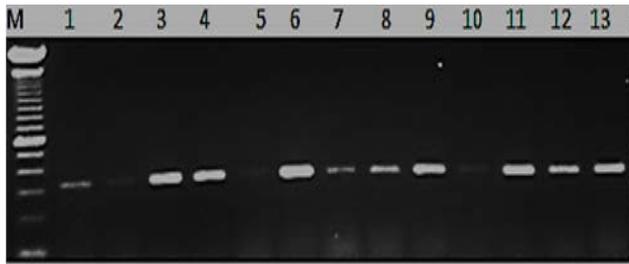
Figure 1. PCR amplification. Lane M: DNA ladder (100bp), Lane 1: LSKB08, Lane 2: LSKB09, Lane 3: LSKB10, Lane 4: LSKB11, Lane 5: LSKB12, Lane 6: LSKB13, and Lane 7: LSKB14, Lane8: LSKB15, Lane 9: LSKB16, Lane 10: LSKB17, Lane 11: LSKB18, Lane 12: LSKB19, and Lane 13: LSKB20
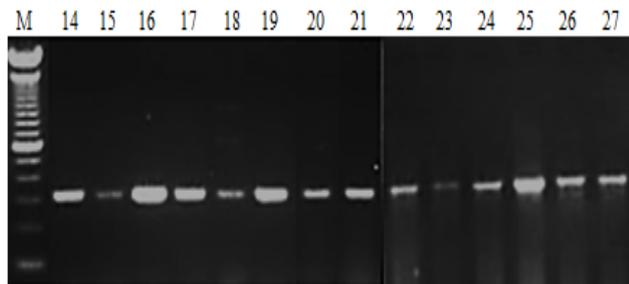
Figure 2. PCR amplification. Lane M: DNA ladder (100bp), Lane 14: LSKB21, Lane 15: LSKB22, Lane16: LSKB23, Lane 17: LSKB24, Lane 18: LSKB25, Lane 19: LSKB26, and Lane 20: LSKB27, Lane21: LSKB28, Lane 22: LSKB29, Lane 23: LSKB30, Lane 24: LSKB31, Lane 25: LSKB32, Lane 26: LSKB33, and Lane 7: LSKB34 (remaining figures are not shown)

3.2. Screening of β-Glucosidase

The same fifty thermophilic bacterial strains, LSKBs, were also screened for extracellular β-glucosidase using esculin as substrate. It was observed that all fifty strains formed ferric-esculiten precipitate indicating the presence of extracellular β-glucosidase (Figure 3). Previously this assay has been carried out using agar plate by growing

microbes on agar medium supplemented with esculin and ferric salt e.g., ferric ammonium citrate. Here, we carried out the same test on liquid medium using culture supernatant as enzyme source. We found that this method is simple, rapid and interference of ferric salt with bacterial growth is eliminated [23]. LSKB15 was found to form the esculetin-ferric precipitate rapidly probably reflecting high enzyme activity secreted by this strain therefore we selected this strain for purification of β-glucosidase enzyme and subsequent characterization.
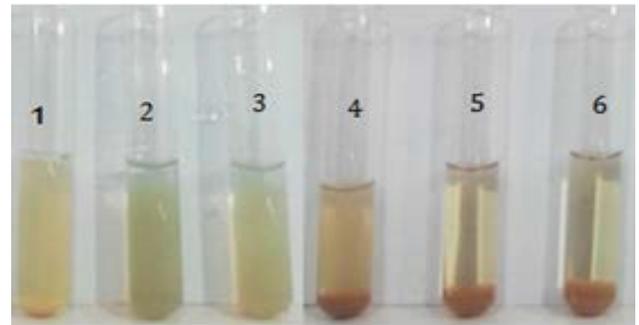
Figure 3. Screening for extracellular β-glucosidase using esculin as substrate. Tube 1: simple broth media, esculin and ferric chloride, Tube 2: boiled supernatant from LSKB09, esculin, and ferric chloride, Tube 3: supernatant, water and ferric chloride, Tube 4-6: supernatants from LSKB09, LSKB12, and LSKB15, respectively, esculin and chloride

3.3. Purification of β-Glucosidase

The culture supernatant of 500 ml culture was subjected to ammonium sulfate precipitation at 40, 80 and 100% saturation. Precipitated protein was re-suspended in 20 mM phosphate buffer pH 7 and dialyzed overnight against the same buffer. Subsequently, the enzyme activity in each fraction was measured using *p*-NPG as substrate. Enzyme activity was detected in 100% ammonium sulfate fraction whereas no or very minor activity was detected in 40% and 80% fraction. Therefore we proceeded with this fraction for further purification through gel filtration chromatography achieving 4.1 purification fold and a yield of 31% of the total activity (Table 2). A β-glucosidase was purified from Sporothrix schenckii *using* hydroxyapatite (HAp) adsorption chromatography and Sephacryl S200-HR size exclusion chromatography upto 3.2 purification fold [24]. In another study, β-glucosidase was purified from *Bacillus subtilis* strain PS using ammonium sulfate precipitation and size exclusion chromatography up to 18.04 fold [25].

Table 2. Purification of β-glucosidase from LSKB15

Methods	Total protein (mg)	Total activity (U)	Sp. activity (U/mg)	Yield (%)	Purification (fold)
Crude extract	53	32	0.60	100	1
NH2SO4 (100%)	21	23	1.1	69	1.8
Dialysis	11.6	15	1.3	47	2.1
Gel filtration chromatography	4	10.1	2.5	31	4.1

3.4. Effect of pH on Enzyme Activity

The activity of purified enzyme was measured using p-NPG as substrate at 60°C and pH of 3-11. Purified enzyme exhibited maximum activity at pH 7 and maintains significant activity at pH of 4 and 9 (Figure 4). Bacterial β-glucosidase with pH optima of 7 has been reported from *Terrabacter ginsenosidimutans* [26], *Pseudonocardia* sp [27], *Paenibacillus* sp Strain C7 [28], *Exiguobacterium antarcticum* B7 [29] and *Bacillus subtilis* strain [30]. β-Glucosidase from different sources showed different optimal pH, for instance, β-glucosidase with pH of 5 has been reported from the hyperthermophilic archaeon *Pyrococcus furiosus* [31], *Caldicellulosiruptor saccharolyticus* [32], *Trichoderma reesei* [33] and that of pH 6 has also been identified in *Bacillus licheniformis* [34], *T. aotearoense [35]*, *T. thermosaccharolyticum* [36]. Alkali-active β-glucosidase with pH of 8 and 10 has been reported from *Bacillus halodurans* [37], and alkaline *Klebsiella pneumoniae* [38], respectively. Alkali-active β-glucosidase has also been reported from agricultural soil metagenome [39] and alkaline soil metagenome [40]. The diversity of this enzyme pointed out the crucial function played by this enzyme in different microorganisms, however, the mechanism by which pH affect the enzyme activity is not understand and call for further investigations.
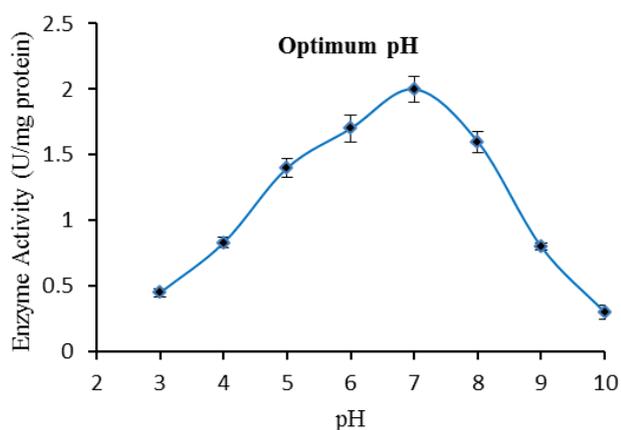
Figure 4. Effect of pH on LSKB15 β-glucosidase activity. For determination of optimal pH, β-glucosidase activity at 60°C was determined by assaying the activity at various pH values (pH 3-10) using different buffers. The values represent means ± standard error of three independent experiments.

3.5. Effect of Temperature on Enzyme Activity

The activity of purified enzyme was measured using p-NPG as substrate at pH of 7 and different temperature 30-90°C. Purified enzyme exhibited maximum activity at 60°C and maintains significant activity at 50°C and 70°C (Figure 5) indicating its thermostabilty and it is not surprising since these bacterial strains have been isolated from Cholistan desert where the temperature in summer exceeds 50°C. The optimal temperature reported in the present work is similar to those reported in literature. For instances, β-glucosidase showing optimal activity at 60°C has been reported from *Aspergillus fumigatus* Z5 [41], *Phoma sp.* KCTC11825BP [42], *Monascus sanguineus* [43], and *thermoanaerobacterium aotearoense [35]*,

although, β-glucosidases with optimal activity at 50°C has been reported from *Flammulina velutipes* [44], *Monascus purpureus* [45], and *Bacillus licheniformis [34]*. β-Glucosidase with optimal activity at 37°C has been reported from *Neocallimastix patriciarum* W5 [46], *Pseudonocardia* sp. Gsoil 1536 [27], and *Proteus mirabilis VIT117* [47]. β-Glucosidases obtained from metagenome with an optimal temperature of 38°C and 45°C, respectively, have been reported [48,49]. The optimal temperature exhibited by reported enzyme in this study suggests its potential applications such as biofuel production.
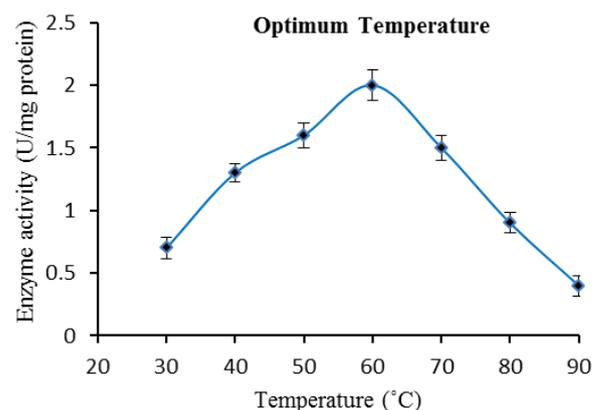
Figure 5. Effect of Temperature on LSKB15 β-glucosidase activity. For determination of optimal temperature, β-glucosidase activity was determined by assaying the activity at various temperature values (30-90°C) at phosphate buffer of pH 7. The values represent means ± standard error of three independent experiments

3.6. pH Stability

The purified enzyme was incubated at different pH ranging from 3-10 at 4°C for 2 h after which the activity was measured at pH 7 and 60°C using p-NPG as substrate. This enzyme from LSKB15 retained 100% activity at pH of 5, 6, and 7 and significant activity was retained at pH of 4 and 9 (Figure 6). A variety of β-glucosidases stable at different pH range have been reported from *Tolypocladium cylindrosporum* Syzx4 [50], *Penicillium simplicissimum* H-11 [51], marine *Streptomycete [52]*, and *T. aotearoense [35]*.
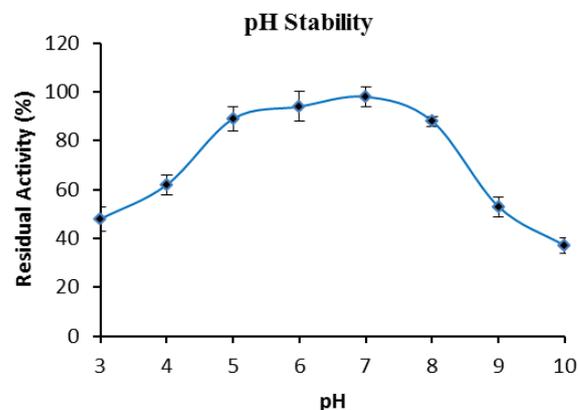
Figure 6. pH stability of purified β-glucosidase of LSKB15. For determination of pH stability, purified enzyme was incubated in different pH of 3-10 for 2 h. Then enzyme activity was determined at phosphate buffer of pH 7 and 60°C using p-NPG as substrate. The values represent means ± standard error of three independent experiments

3.7. Thermostability

The purified β-glucosidase from LSKB15 was incubated at temperature of 40-90°C for 2 h and then measured at pH 7 and 60°C. It maintained 99% activity after incubation at 50°C and 60°C for 2 h. Moreover, these crude β-glucosidases retained more than 50% and 30% activity at 80°C and 90°C, respectively (Figure 7). β-Glucosidase from *Aspergillus saccharolyticus* retained 90% activity after incubation for 2 h at 60°C with half-life of 6 h at the same temperature has also been reported [53]. A high glucose tolerant β-glucosidase from *A. oryzae* showed good stability for 4 h at 45°C and was completely inactivated at 60°C [54]. β-Glucosidase from *Fomitopsis palustris* retained 100% of its activity after incubation at 45°C for 50 h and 50% of its activity after incubation at 65°C for 15 h [55]. This pronounced thermostability exhibited by this enzyme suggests its biotechnological applications and its worth of further investigation.
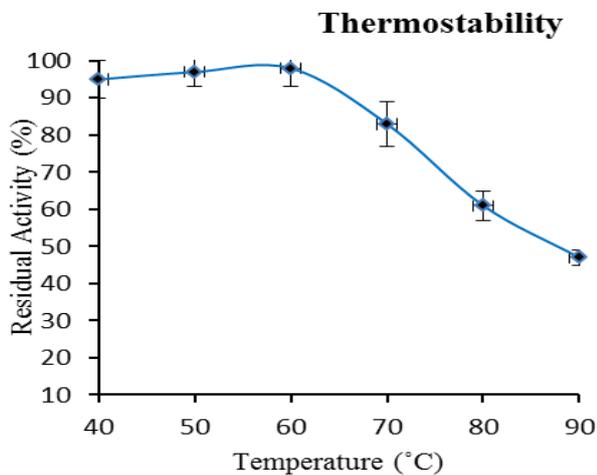
Figure 7. Temperature stability of purified β-glucosidase of LSKB15. For determination of temperature stability, purified enzyme was incubated in different pH of 40-90°C for 2 h. Then enzyme activity was determined at phosphate buffer of pH 7 and 60°C using p-NPG as substrate. The values represent means ± standard error of three independent experiments

3.8. Zymography

Purified β-glucosidase activity was run on native polyacrylamide gel for 2 h at 100 V. Subsequently, the gel was removed and incubated in 50 mM sodium phosphate buffer (pH 7) containing 5 mM of 4-methylumbelliferyl-β-D-glucoside (4-MUG) for 15 min at 50°C. Then gel was visualized under UV light to detect fluorescence due to the 4-MU released which appeared as white band as shown in in Figure 8.
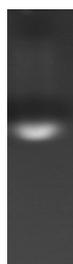
Figure 8. Zymography: detection of purified β-glucosidase from LSKB15 with 4-MUG

3.9. Metal ions effect

The enzyme activity pre-incubated with 1 and 10 mM of Co^{2+}, Ni^{2+}, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} salt and its activity was measured using p-NPG substrate. The enzyme activity was enhanced by 1 mM of Ni^{2+}, K^+, Ca^{2+}, Mn^{2+}, and Cu^{2+} (Figure 9). In line with our findings, β-glucosidase is known to be metal-independent and differential effect of metal ion has been reported by many researchers [35,56]. For example, β-glucosidase from *Stachybotrys* strain was activated by Co^{2+}, Cu^{2+}, Ca^{2+}, and Zn^{2+} at 10 mM and inhibited by Fe^{2+} at the same concentration [57]. Cold active β-glucosidase reported in *Exiguobacterium oxidotolerans* A011 was inhibited by Co^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, K^+, Ca^{2+}, and Na^+ [58]. Glucose-tolerant β-Glucosidase reported from marine *Streptomycete* was activated by Na^{2+}, K^{2+}, Mg^{2+}, and Ca^{2+} and inhibited by Zn^{2+} and Cu^{2+} [52], and that reported from *Exiguobacterium antarcticum* B7 was activated by Mg^{2+}, Na^+, Li^{2+} and K^+ and was strongly inhibited by Cu^{2+}, Fe^{3+}, and Zn^{2+} [29]. The mechanism by which these metal ions affect the enzyme activity is poorly understood. Although, inactivation of an enzyme by Hg^{2+} indicates that thiol groups are required for function of the enzyme or for maintaining 3D structure of the protein. Inhibition by Cu^{2+}, Co^{2+} and Zn^{2+} suggests that basic (Arg, Lys, His) and acidic (Asp, Glu) amino acids may have important roles in the active site and stimulation of enzyme activity by the cations Ca^{2+}, K^+, Co^{2+}, Mn^{2+}, Mg^{2+} and Na^+ suggest that it may enhance the structural stability of the enzyme
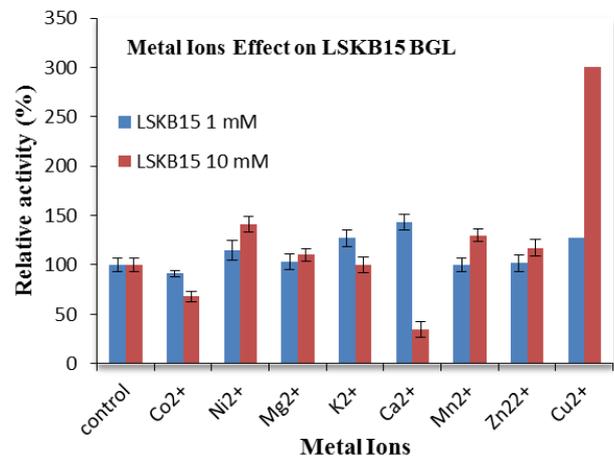
Figure 9. Metal Ion effect of LSKB15 β-glucosidase

4. Conclusion

The present work describes screening of bacterial strains isolated from rhizospheric soil of Cholistan desert, Pakistan at molecular and biochemical level for β-glucosidase enzyme. One strains designated as LSKB15 was investigated further for production and biochemical characterization of this enzymes upon cultivation of on cellulase containing media. The enzyme was purified and characterized for some biochemical and molecular properties. The properties exhibited by this enzyme such as high optimal temperature and neutral pH indicate its potential applications in biofuel industries. However,

further studies are needed to investigate other properties of this enzyme such as substrate specificity, and application to synergistic degradation of biomass and biofuel production. Cloning, sequencing and expression of the gene encoding for this enzyme may be carried out in order to produce this enzyme in large quantities for industrial applications and mechanistic studies and protein engineering studies may be performed to enhance the catalytic efficiency of this enzyme and improve its properties for industrial purposes.

Acknowledgements

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