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Purification and Characterization of Extracellular β-Glucosidase from *Sinorhizobium kostiense* AFK-13 and Its Algal Lytic Effect on *Anabaena flos-aquae*

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Abstract A β-glucosidase from the algal lytic bacterium *Sinorhizobium kostiense* AFK-13, grown in complex media containing cellobiose, was purified to homogeneity by successive ammonium sulfate precipitation, and anion-exchange and gel-filtration chromatographies. The enzyme was shown to be a monomeric protein with an apparent molecular mass of 52 kDa and isoelectric point of approximately 5.4. It was optimally active at pH 6.0 and 40°C and possessed a specific activity of 260.4 U/mg of protein against 4-nitrophenyl-β-D-glucopyranoside (*p*NPG). A temperature-stability analysis demonstrated that the enzyme was unstable at 50°C and above. The enzyme did not require divalent cations for activity, and its activity was significantly suppressed by Hg^{+2} and Ag^{+}, whereas sodium dodecyl sulfate (SDS) and Triton X-100 moderately inhibited the enzyme to under 70% of its initial activity. In an algal lytic activity analysis, the growth of cyanobacteria, such as *Anabaena flos-aquae*, *A. cylindrica*, *A. macrospora*, *Oscillatoria sancta*, and *Microcystis aeruginosa*, was strongly inhibited by a treatment of 20 ppm/disc or 30 ppm/disc concentration of the enzyme.

Keywords: β-Glucosidase, *Sinorhizobium kostiense*, extracellular, algal lytic

The control of water blooms has become a serious issue that has attracted extensive research owing to the adverse impact on public health, plus economic and natural resources. The use of biological controls, such as algal lytic microorganisms, can effectively mitigate algal blooms, as a number of algal lytic bacteria inhibit algal growth through direct or indirect attacks [10, 11]. Several recent studies [4, 12] have shown that algal lytic bacteria degrade the algal cell wall by producing extracellular substances, such as protease [14], hydroxylamine production [1], antibiotics [11], aminophenol [29], and rhamnolipid biosurfactants [28]. In particular, for aquatic environments (viz., water and sediments), algal lytic bacteria are the main producer of glycosidase [24]. Ectoenzymes and extracellular enzymes can play a significant role in aquatic ecosystems, as they are responsible for the degradation process through which decomposing products enter the food chain. Therefore, algal extracellular polysaccharides (EPS) are vulnerable to attack by ectoglycosidases and/or extracellular glycosidases either endogenously or exogenously, and depending on the position of the attacked linkages in the polymeric chain, this leads to a rapid or slow breakdown [12].

Anabaena flos-aquae is a filamentous cyanobacterium with an outer cell wall that consists of a wide polysaccharide capsule [12]. *A. flos-aquae* is usually present in eutrophic lakes and reservoirs, as a great proportion of its biomass is produced *via* photosynthesis. Moreover, when huge blooms are formed and remain for a long period of time, this causes low water turbulence in lakes and reservoirs. Cyanobacteria also produce large amounts of EPS, which are not easily utilized by bacteria, and thus accumulated in the environment, plus these recalcitrant high-molecular-weight substances are pooled in aquatic ecosystems [25]. However, heterotrophic bacteria, such as algal lytic microorganisms, degrade such undesirable compounds, which essentially become a substrate.

Accordingly, the present study isolated and characterized an extracellular β-glucosidase possessing algal lytic activity from the algal lytic bacterium *Sinorhizobium kostiense* AFK-13. The effect of different carbon sources on the enzyme biosynthesis in the supernatant fluid of *S. kostiense* AFK-13 was evaluated because of the differential β-glucosidase synthesis according to the strain [26]. The effect of pH, temperature, stability, substrate specificity, and the influence of metal ions and compounds were all investigated and the potential efficacy of the β-glucosidase confirmed as a bioagent to control algal blooms in aquatic environments.

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MATERIALS AND METHODS

Growth and β-Glucosidase Production

S. kostiense AFK-13 is an algal lytic bacterium that was isolated from surface water taken from the Paldang Reservoir, where cyanobacteria blooms often occur in Korea [12]. The *S. kostiense* AFK-13 cells were maintained at 4°C and recovered by growth at 30°C on a malt-extract agar (Oxoid CM59) for 4 days. An Erlenmeyer flask (1 l) was filled to 500 ml with YTG (5.0 g of yeast extract, 10.0 g of tryptone yeast, and 10.0 g of glucose) and YTC (5.0 g of yeast extract, 10.0 g of tryptone yeast, and 10.0 g of cellobiose) media at pH 6.8±0.2 for the production of β-glucosidase. The flask was inoculated with a 24-h-old culture to an initial OD_{650} of 0.5, and then incubated in a rotary incubator at 200 rpm and 30°C for 72 h. The cell growth of the algal lytic bacterium and enzyme activity of the culture supernatants were evaluated at certain intervals.

Algal Culture

The *Anabaena flos-aquae* NIES-75 used as the host for the algal lytic bacteria was kindly supplied by the National Institute for Environmental Studies, Japan. A clonal axenic culture was cultivated and maintained in a BG-11 medium, including 1.5 g of $NaNO_3$, 0.04 g of K_2HPO_4, 0.075 g of $MgSO_4 \cdot 7H_2O$, 0.036 g of $CaCl_2 \cdot 2H_2O$, 0.001 g of EDTA-disodium, 0.02 g of Na_2CO_3, 0.006 g of citric acid, 0.006 g of ferric ammonium citrate, and 1 ml of micronutrients per liter under the continuous illumination of cool white fluorescent lamps, giving an incident light intensity of 35 μE/m^2/s, and at 25±0.2°C with an agitation of 150 rpm in a rotary shake incubator. The composition of the micronutrients was H_3BO_3 2.86 g, $MnCl_2 \cdot 4H_2O$ 1.81 g, $ZnSO_4 \cdot 7H_2O$ 0.222 g, $Na_2MoO_4 \cdot 2H_2O$ 0.39 g, $CuSO_4 \cdot 5H_2O$ 0.079 g, and $Co(NO_3)_2 \cdot 6H_2O$ 0.0494 g per liter [3].

Enzyme and Protein Assays

The total proteins in the enzyme samples were determined using a Commassie brilliant blue G-250 dye according to the manufacturer's instructions (Bio-Rad, Hercules, CA, U.S.A.), and bovine serum albumin used as the calibration standard. The routine β-glucosidase activities were determined in duplicate using 2 mM 4-nitrophenyl-β-D-glucopyranoside (*p*NPG) as the substrate according to the method of Gonzalez *et al.* [7] with modifications. The assays were performed by adding 0.1 ml of an enzyme solution to 4.9 ml of a 50 mM sodium acetate buffer (pH 5.5) containing 5 mM *p*NPG (final concentration) and incubated at 40°C for 30 min, after which time NH_4OH was added to a final concentration of 1 M. The amount of 4-nitrophenol (*p*NP) released was estimated by the absorbance at 400 nm using a *p*NP standard curve. One unit (U) of enzyme activity was defined as the amount of enzyme releasing 1 μmol of *p*NP from the substrate per min. The protein was determined using the method of Bradford [2], with bovine serum albumin as the standard.

Purification of β-Glucosidase

All the steps were carried out at 4°C. The *S. kostiense* AFK-13 cells were incubated at 30°C in 500-ml Erlenmeyer flasks containing 150 ml of the YTC medium on a rotary shaker at 150 rpm, and harvested in the late exponential phase (for 48 h). The cells were removed from the culture broth by centrifugation at 12,000 ×*g* and 4°C for 20 min. The resulting supernatant solution was then used as the crude enzyme preparation for the subsequent enzyme purification. The crude enzyme preparation was concentrated to about 100 ml by ultrafiltration using a 10 kDa molecular cut membrane (Ultrafilter-MC, Millipore, MA, U.S.A.). The concentrated extracts were then fractionated by the slow addition of solid $(NH_4)_2SO_4$ at pH 6.0 to give the desired degree of saturation. The precipitates were collected by centrifugation at 22,000 ×*g* for 30 min, resuspended in a 20 mM Bis-Tris buffer, pH 6.0, containing phenymethylsulfonyl fluoride (PMSF), 2-mercaptoethanol, and ethylenediaminetetraacetate (EDTA) at the above concentrations, centrifuged again (22,000 ×*g*, 30 min) to remove any undissolved material, and desalted in the same buffer using a PD-10 desalting column (Pharmacia LKB, Biotechnology AB, Uppsala, Sweden). The extracts were stored at −70°C prior to further use. All the chromatographic procedures were performed using an FPLC system and pre-packed columns (Pharmacia LKB, Biotechnology AB, Uppsala, Sweden). For the anion-exchange chromatography, a Mono-Q column HR5/5 (5 mm×50 mm) equilibrated with a 20 mM Bis-Tris buffer (pH 6.0) was used. The prepared extracts were filtered first through Millex-GV filters (0.22 μm, filter pore size; Millipore, Bedford, MA, U.S.A.), and then loaded onto the column in the equilibration buffer. The absorbed protein was eluted using a linear NaCl gradient (0–0.6 M) in the same buffer with a flow rate of 1 ml/min, and 5 ml fractions were collected. The active fractions were further purified by FPLC gel-filtration chromatography using a Superose 12HR 10/30 column (10 mm×300 mm) equilibrated with a 50 mM sodium acetate buffer, pH 5, containing 150 mM NaCl. The samples (1 ml) were applied and eluted with the equilibration buffer at a flow rate of 0.5 ml/min, and collected in 2 ml fractions. In addition, the relative molecular mass of the native enzyme was estimated by gel filtration on a calibrated column.

Polyacrylamide Gel Electrophoresis (PAGE), Zymograms, and Isoelectric Focusing of β-Glucosidase

The β-glucosidase-positive fractions were pooled and the protein present analyzed by SDS-PAGE [13]. A separation gel (15%, w/v) and stacking gel (5%, w/v) were both used. The proteins were revealed using the silver staining method described by Merril *et al.* [16], and the electrophoretical

migration of the proteinase compared with broad range protein markers (Sigma, St. Louis, MO, U.S.A.). For the zymogram analysis, a nondenaturing 15% gel was used containing 10 mg/ml of the extracellular polysaccharide (EPS) from *A. flos-aquae*. The gel was stained with Commassie brilliant blue R-250 (Sigma, St. Louis, MO, U.S.A.) and scanned using an Image Reader FLA-5000 (Fuji Photo Film Co. Ltd., Japan). The isoelectric point (pI) was estimated by PAGE with 6.35% Ampholine (pI 3.5–10) in a gel rod (0.5×10 cm) using a kit for Isoelectric Focusing Calibration (Phamacia LKB) according to the recommendations of the manufacturer.

Influence of Temperature and pH on β-Glucosidase Activity

The influence of temperature on the β-glucosidase activity was investigated between 20 and 70°C in a 50 mM phosphate buffer (pH 6.0). The effect of pH on the activity of the purified enzyme was investigated using the following 50 mM buffers: citric acid/Na_2HPO_4 for pH 3–5, $NaHPO_4$/Na_2HPO_4 for pH 6–7, Tris-HCl for pH 8–9, and glycine/NaOH for pH 10–11.

Determination of Substrate Specificity

The activity of the purified β-glucosidase was measured at the optimal temperature and pH with various substrates at two different concentrations, 1 and 10 mM, unless otherwise noted. Several substances containing glucoside bonds, such as aesculin, laminarin, 2-(hydroxymethyl)phenyl-β-D-glucopyranoside (salicin), pustulan, 4-nitrophenyl-β-D-glucopyranoside (*p*NPG), and 4-methylumbelliferyl-β-D-glucopyranoside (4-MUG), were employed as substrates for the enzyme. The hydrolysis of *p*NPG was assayed using standard procedures, as described previously, and for all the other substrates, the β-glucosidase activity was determined by analyzing the reducing power using the method of Somogyi [23], as modified by Nelson [18], with glucose as the standard.

Preparation of Extracellular Polysaccharide (EPS) of *A. flos-aquae*

The extracellular polysaccharide (EPS) of *A. flos-aquae* was extracted according to the method of Colombo *et al.* [5]. Cyanobacterial cells from a 10-l batch culture were removed from the culture medium at the end of the exponential growth phase (15 days old) by tangential filtration in hollow fiber cartridges with 0.65 μm pore-size membranes (A/G Technology Corporation, Needham, MA, U.S.A.). The medium, which contained the released EPS, was further concentrated (circa 1,000 ml) by tangential filtration in hollow fiber cartridges with a 30 kDa pore size and washed three times with three volumes of deionized water to eliminate any low-molecular-weight compounds. The concentrated medium was then freeze-dried to obtain the EPS and kept at −20°C until further use.

Effect of Metal Ions and Compounds

To evaluate the effect of different cation compounds on the purified β-glucosidase activity, 1 or 10 mM of various divalent or monovalent ions and compounds, such as $AgNO_3$, $CaCl_2$, $CoCl_2$, $CuSO_4$, $HgCl_2$, $MgCl_2$, $MnCl_2$, and $ZnCl_2$, plus EDTA, SDS, 1,10-phenanthroline, and Triton X-100 were added to the reaction mixtures prior to incubation at 40°C. All the experiments were repeated in triplicate, and the given results are the mean and standard deviation of the raw data.

Assay for Algal Lytic Activity of β-Glucosidase

The algal lytic activity of the purified β-glucosidase was assessed using a modified soft-agar over-layer technique [20]. To generate an algal lawn, *A. flos-aquae* was grown in BG-11 for 15 days, harvested by centrifugation at 18,000 ×*g* for 20 min, spread on a liquid BG-11 soft agar (0.8% agar), and equilibrated at 50°C. A fixed quantity (20 ml) of the algal mixture was poured onto a BG-11 bottom agar (1.5% agar) and solidified for 2 days under the algal growth conditions described above. A paper disc (diameter=6.25 mm) containing 5, 10, or 20 ppm of the purified enzyme was placed at the edge of the plates, which were then incubated for 2 h under the algal culture conditions, and the clear zone formed by the purified enzyme evaluated. Furthermore, to examine the host range, the algal lytic activity of the enzyme was also tested with *Anabaena cylindrica*, *A. macrospora*, *Oscillatoria sancta*, *Microcystis aeruginosa*, *Chlorella vulgaris*, *Chlamydomonas tetragama*, *Stephanodiscus hanzschii*, and *Cyclotella* sp. A disc treated with sterilized distilled water was used as the control.

RESULTS AND DISCUSSION

Growth and β-Glucosidase Production

The effect of two different carbon sources on the enzyme biosynthesis in the culture of *S. kostiense* AFK-13 is shown in Fig. 1. The bacterial cells grew well on both substrates, although the cell growth in the cellobiose-containing medium was lower than that in the glucose-containing media. However, distinct differences were observed in the total enzyme activity, and not correlated with the differences in the cell growth. In previous studies, the production of β-glucosidase reached the maximal level during the aerobic growth of *Pseudomonas* sp. ZD-8 [30] and the yeast *Debaryomyces pseudopolymorphus* [27] in a growth medium with cellobiose as the sole carbon source. In the present study, as shown in Fig. 1, although the bacterial cell growth reached a maximum after 36 h of culture in the YTC medium, the β-glucosidase activity in the supernatant showed a low algal lytic activity, yet this gradually increased following the exponential growth phase. Thus, the supernatant after 52 h of culture exhibited the highest activity, implying that the extracellular products

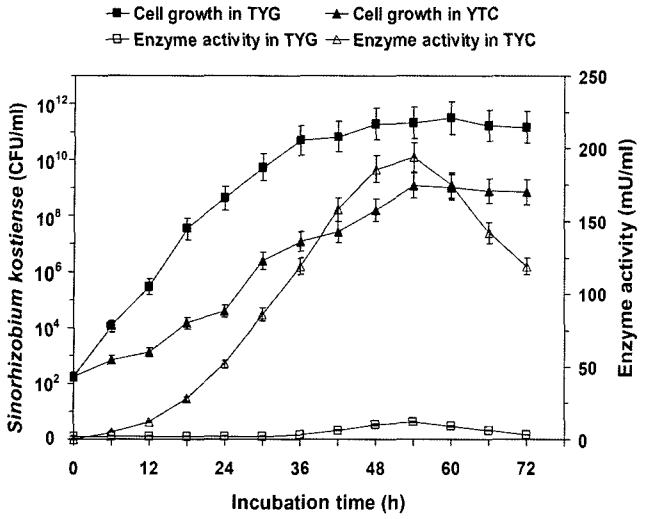

Fig. 1. Time course of growth and β-glucosidase biosynthesis in *S. kostiense* AFK-13.
YTG, Yeast Tryptone Glucose medium; TYC, Yeast Tryptone Cellobiose medium.

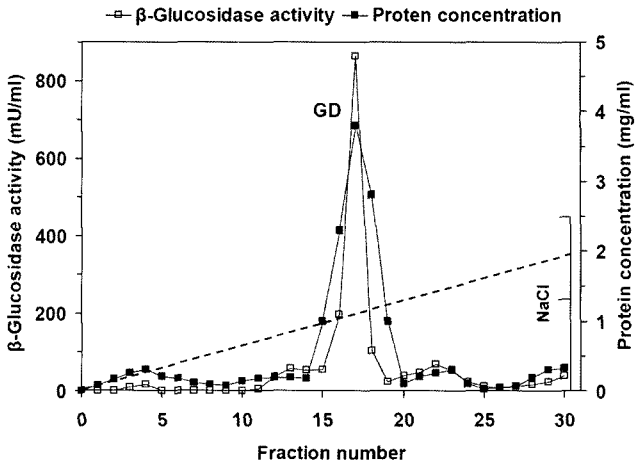

Fig. 2. Anion-exchange chromatography by FPLC.
The 75%-saturated $(NH_4)_2SO_4$ fraction of the crude enzyme filtrate was desalted and loaded on a Mono-Q column HR5/5 with a 20 mM Bis-Tris buffer (pH 6.0) and linear gradient of 0–0.6 M NaCl (--). GD is the β-glucosidase activity peak.

were only released little by little during the lag phase, and then mainly released after the stationary phase [12]. In contrast, the enzyme production during aerobic growth in the medium containing 2% glucose as the sole carbon source was less than 1% of the maximum value in the YTC medium. Thus, cellobiose was confirmed as a good carbon source for extracellular β-glucosidase production from *S. kostiense* AFK-13. High glucose levels (higher than 2%, w/v) have previously been found to have a repressive effect on catabolic repression enzyme synthesis, including the synthesis of β-glucosidase from *Debaryomyces hansenii* [19] and lactic acid bacteria [31], along with amylases [9], xylanases, and cellulases [6]. However, in contrast to the present results, the extracellular β-glucosidase isoforms from *Pseudomonas* sp. ZD-8 [30] and *D. pseudopolymorphus* [27] are not suppressed by glucose. Therefore, the inhibition of the extracellular β-glucosidase from AFK-13 may result from a different mechanism.

β-Glucosidase Purification

A β-glucosidase was purified from the culture supernatant of *S. kostiense* AFK-13 using ammonium sulfate precipitation, FPLC anion-exchange chromatography on a Mono-Q column, and gel filtration on a Superose 12HR 5/30 column. The purification data are summarized in Table 1. Ammonium sulfate fractionation of the concentrated culture filtrate showed that 95% of the β-glucosidase activity was recovered in the 75%-saturated $(NH_4)_2SO_4$ fraction. For anion exchange, the chromatography of the desalted 75%-saturated $(NH_4)_2SO_4$ fraction loaded on a Mono-Q column HR5/5 showed a single peak related to the β-glucosidase activity (GD in Fig. 2). The peak corresponding to fractions 13–18 contained a considerable amount of β-glucosidase (Fig. 2). The fractions from the Mono-Q column with β-glucosidase activity were applied to a Superose 12HR 10/30 column. The enzyme was purified 45.7-fold to a specific activity of 260.4 U/mg protein from cells with only 14.3% total activity in the crude culture filtrate. The purified enzyme gave a single band in SDS-PAGE. Moreover, activity staining on a zymogram gel also produced a single band that corresponded to the band after silver nitrate staining in SDS-PAGE, indicating that the purified sample was electrophoretically homogeneous under dissociating conditions. The molecular mass of the purified enzyme, as estimated by an SDS-PAGE analysis, was approximately 53 kDa (Fig. 3). The relative molecular mass of the native enzyme, as estimated by gel filtration on a calibrated column of Superose 12HR 10/30, was around

Table 1. Purification and yield of extracellular β-glucosidase from *S. kostiense* AFK-13.

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification factor
Crude culture filtrate	47.91	273.09	5.70	100.0	1.0
Ultrafiltrate	35.90	269.25	7.50	98.3	1.3
$(NH_4)_2SO_4$ precipitation	8.49	245.53	28.92	89.9	5.1
Mono-Q HR5/5	0.36	57.20	158.89	20.9	27.9
Superose 12 HR 10/30	0.15	39.06	260.40	14.3	45.7

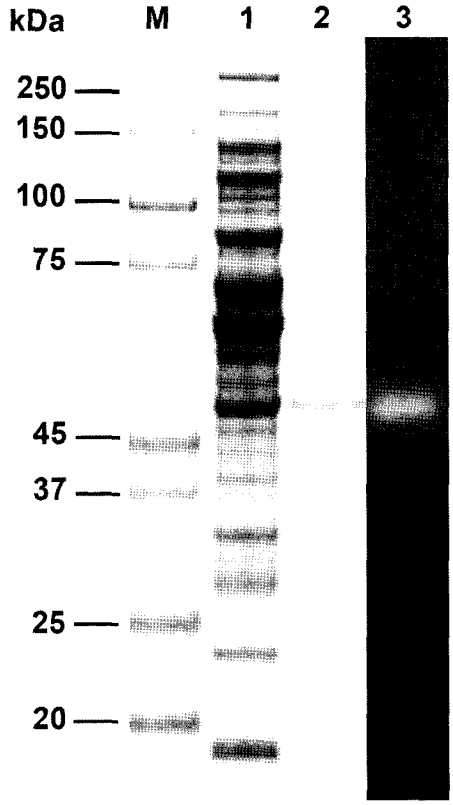

Fig. 3. SDS-PAGE of crude enzyme preparation (lane 1) and purified β-glucosidase activity (lane 2) of *S. kostiense* AFK-13, plus zymogram analysis (lane 3).
The molecular masses (lane M), in kilodaltons, are indicated on the left.

52 kDa and the pI value estimated to be 5.4 (data not shown). Hence, it was assumed that the native β-glucosidase was a monomer, which is similar to several other β-glucosidases that have been isolated from microorganisms [17] and found to have a molecular mass of 50 and 51 kDa, respectively.

Effect of pH and Temperature on Enzyme Activity

Using a range from 20 to 70°C, the optimal temperature for the enzyme activity was determined as 40°C (Fig. 4). The enzyme activity remained somewhat stable up to 45°C, but only 44% of the initial activity was left at 50°C and the enzyme became almost inactivated at 65°C. The half-life time of activity $t_{1/2}$ for the enzyme at 35, 40, 45, and 50°C was observed to be 194, 52, 9 h, and 23 min, respectively. The optimal temperature of 40°C was similar to that previously identified for the wine β-glucosidase from yeast [26] and other bacterial β-glucosidases from *Pseudomonas* sp. ZD-8 [30] isolated from plant roots. Using a pH range from 3 to 11, the pH-activity profile for the enzyme showed the maximum value at pH 6.0 (Fig. 4), which also matches the values previously reported for other microorganisms from fungi [17], yeasts [22], and bacteria [30]. The enzyme retained more than 80% of its initial

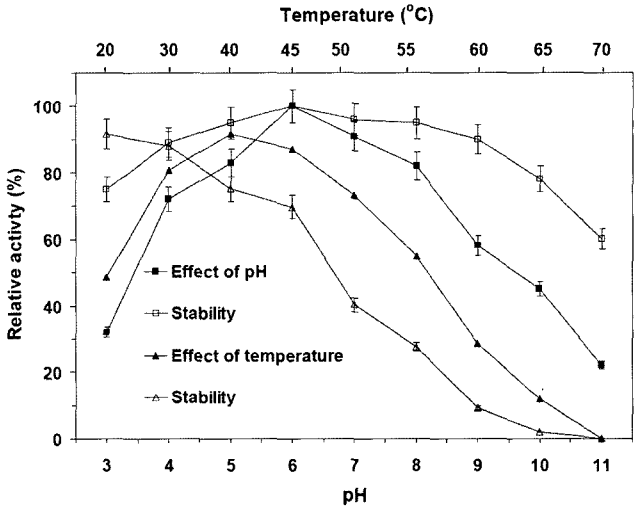

Fig. 4. Effect of pH and temperature on enzyme activity and stability of β-glucosidase from *S. kostiense* AFK-13.

activity when kept within a range of pH 4.0–9.0 at 30°C, which is a broad interval and similar to that reported for other enzymes from *Streptomyces albaduncus* [8] and the hyphomycete *Chalara paradoxa* CH32 [15].

Substrate Specificity

The substrate specificity of the purified β-glucosidase was determined using several different polymers containing either β-1,4, β-1,3, α-1,4 linkages or β-aryl-glycosides. The enzyme hydrolyzed *p*NPG and cellobiose efficiently, whereas salicin, pustulan, laminarin, aesculin, and 4-MUG liberated lower quantities of reducing sugars (Table 2). The soluble polysaccharide laminarin was only hydrolyzed at 35% of the level of the hydrolysis of *p*NPG, suggesting that the enzyme activity may be confined to the endo activity of the purified peptide. 4-MUG was only hydrolyzed 46% of the maximum hydrolysis rate. The enzyme displayed little (≤15%) activity against the other substrates: salicin, pustulan, and aesculin. Therefore, the β-glucosidase was able to hydrolyze all four β-linked glucose dimers, where the hydrolysis rate improved in the following descending

Table 2. Relative activity of β-glucosidase from *S. kostiense* AFK-13 on various β-glucosides.

Substrate	Configuration of glycoside linkage	Relative initial rate of hydrolysis (%)
Cellobiose	β-(1,4)-Glc	100
*p*NPG	β-(1,4)-Glc	100
Salicin	β-(1,2)-Glc	14
Pustulan	β-(1,6)-Glc	9
Laminarin	β-(1,3)-Glc	35
Aesculin	β-(1,6)-Glc	5
4-MUG	β-(1,4)-Glc	46

Table 3. Effect of different cations and compounds on β-glucosidase from *S. kostiense* AFK-13.

Substances	Relative activity (%)[a]
None	100
1 mM $AgNO_3$	24±0.9
1 mM $CaCl_2$	93±2.1
1 mM $CoCl_2$	117±5.8
1 mM $CuSO_4$	105±4.3
1 mM $HgCl_2$	6±0.6
1 mM $MgCl_2$	93±5.8
1 mM $MnCl_2$	94±6.4
1 mM $ZnCl_2$	94±4.8
10 mM EDTA	95±3.9
10 mM SDS	54±1.9
10 mM 1,10-phenanthroline	96±3.6
10 mN Triton X-100	68±2.2

[a]Data are mean±SD of triplicate experiments.

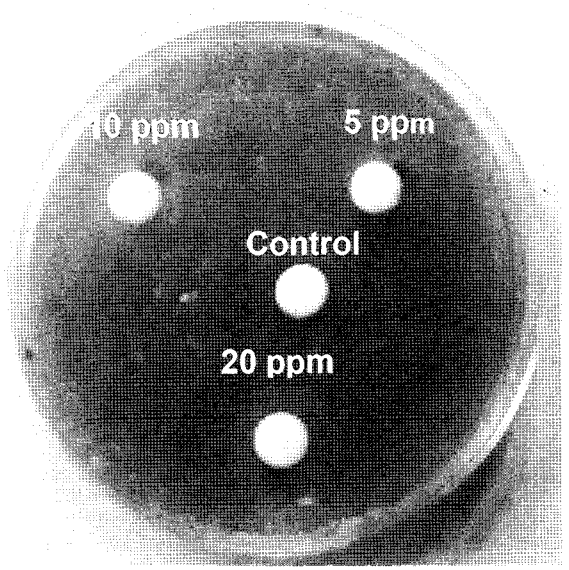

Fig. 5. Growth inhibition of *A. flos-aquae* NIES-75 after 2 h of treatment with purified β-glucosidase from *S. kostiense* AFK-13.

order: β-1,4>β-1,3>β-1,2>β-1,6. β-Glucosidases can be divided into three groups based on their substrate specificity: (i) aryl-β-glucosidases, which only hydrolyze aryl-β-glucosides, (ii) cellobiases, which only hydrolyze oligosaccharides, and (iii) broad-specificity β-glucosidases, which exhibit activity against both substrate types, and are the most common form [21]. Hence, the β-glucosidase from *S. kostiense* AFK-13 is a broad-specificity type β-glucosidase, as it hydrolyzed both β-diglucosides and β-glucosides (Table 2).

Effect of Metal Ions and Compounds on Enzyme Activity

Several metal ions and compounds were examined for their effect on the activity of the purified β-glucosidase. The results are shown in Table 3. The enzyme was completely inhibited by 1 mM $HgCl_2$ and 1 mM $AgNO_3$, showing only 6% and 24% of the maximum activity, respectively. At the same concentration, the enzyme was only slightly inhibited by the other divalent cations. However, the enzymatic activity was slightly enhanced by 1 mM $CoCl_2$. Furthermore, the chelating agents, such as ethylenediamine tetraacetate (EDTA) and 1,10-phenanthroline, had no influence on the activity. As such, the purified enzyme was significantly inhibited by sulfhydryl oxidant metals, such as Hg^{+2} and Ag^{+}, and the other metal ions had no remarkable effect on its activity, implying that thiol may be involved in the catalytic activity site and divalent cations are not required for the enzyme activity (Table 3). The moderate inhibition of the enzyme activity by both sodium dodecyl sulfate (SDS) and Triton X-100 suggests that the integrity of the enzyme's three-dimensional structure was significant for its catalytic activity.

Algal Lytic Activity of β-Glucosidase

After incubating at 27°C for 2 h, an inhibition zone, referred to as the algal lytic activity, formed around the paper discs (Fig. 5). The purified enzyme β-glucosidase exhibited activity at 5 ppm/disc and especially strong activity over 20 ppm/disc. The β-glucosidase was also subjected to

Table 4. Algal lytic effect of purified enzyme β-glucosidase on several algal strains.

Strain		β-Glucosidase concentration (ppm/disc)	Inhibition zone (mm)
Anabaena flos-aquae	Blue-green algae	20	19
A. cylindrica	Blue-green algae	30	18
A. macrospora	Blue-green algae	30	15
Microcystis aeruginosa	Blue-green algae	30	17
Oscillatoria sancta	Blue-green algae	30	16
Chlorella vulgaris	Green algae	50	-
Chlamydomonas tetragama	Green algae	50	-
Cyclotella sp.	Diatom	50	-
Stephanodiscus hanzschii	Diatom	50	-

-, No activity.

an algal lytic assay against several strains of cyanobacteria, green algae, and diatoms. Despite showing algal lytic activity against the cyanobacteria *Anabaena cylindrica*, *A. macrospora*, *Oscillatoria sancta*, and *Microcystis aeruginosa* at a concentration of 30 ppm/disc, the purified enzyme β-glucosidase did not show any activity at a concentration of 30 ppm/disc and above against the green algae *Chlorella vulgaris* and *Chlamydomonas tetragama*, and the diatoms *Stephanodiscus hanzschii* and *Cyclotella* sp. (Table 4).

In conclusion, this study found that *S. kostiense* AFK-13, which produces an algal lytic enzyme β-glucosidase, is a useful algicidal agent. Thus, further work is needed to determine its application in lakes and reservoirs.

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