International Journal of Advanced Biotechnology and Research(IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol5, Issue4, 2014, pp708-712
http://www.bipublication.com

Isolation, Purification and Characterization Of B-Glucosidase from Seed of *Hordeum vulgare*

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[Received 05/10/2014, Accepted-05/11/2014]

ABSTRACT:

β-glucosidase (EC 3.2.1.21) was extracted from seeds of *Hordeum vulgare* and was purified using ammonium sulphate fractional precipitation, Acetone precipitation and Sephadex G-75 chromatography. The molecular weight of enzyme was found to be 60kd .The enzyme β-glucosidase has optimum pH 5.0 and the optimum temperature was found at 60ºC. The enzyme activity was also characterized in different carbon, nitrogen, metal ions and inhibitors. Bioethanol was produced from seeds of *Hordeum vulgare.*

Keywords: *Hordeum vulgare, β-Glucosidase, Purification, Characterization, etc.*

[I] NTRODUCTION

β-Glucosidase (EC 3.2.1.21) occurs widely in prokaryotes and eukaryotes and is shown to be implicated in at least three important biological processes [1]. First, in fungi and bacteria, β-glucosidases are involved in cellulose and cellobiose catabolism as part of the cellulase complex and thus play a role in the process of biomass conversion [2]. Second, specific plant β-glucosidases are involved in chemical defense against pathogens and herbivores via the process of cyanogenesis, whereby HCN and other toxic compounds are released upon hydrolysis of cyanogenic glucosides [3]. Third, other plant β-glucosidases are implicated in regulating the biological activity of plant phytohormones such as cytokinin, gibberellin, and auxin by releasing active forms from inactive hormone-glucoside conjugates [4]. β-Glucosidases are enzymes that transfer a glycosyl group between oxygen nucleophiles. They are, therefore, accountable for the hydrolysis of β-glycosidic linkages in amino-, alkyl-, or aryl- β-D-glucosides, cyanogenic glycosides, and di- and short chain oligo-saccharides [5]. β-glucosidases can be used in the production of aromatic compounds, in the stabilization of juices and beverages, and in the improvement of the organoleptic properties of food and feed products; they are also used in biomass degradation, in the production of fuel ethanol from cellulosic agricultural residues, and in the synthesis of alkyl- and arylglycosides from natural polysaccharides or their derivatives and

alcohols, by reversed hydrolysis or trans-glycosylation, leading to products with applications in pharmaceutical, cosmetic, and detergent industries [6][7]. In the present study the alkaline protease β-glucosidase was extracted from barley seeds then the enzyme was purified and optimized at different parameters.

[II] MATERIALS AND METHODS

2.1 Collection of Seed sample

The seed of *Hordeum vulgare* was for the isolation of β-glycosidase enzyme. The seeds were ground to fine powder in a chilled mortar and pestle using liquid nitrogen and extraction buffer (1 ml buffer/1g tissue) was mixed to it. After that the extract was centrifuge at 12000 rpm 4^oC/30 minutes. Then the supernatant was ultra-filtered to obtain crude enzyme solution.

2.2 Precipitation of Enzyme

2.2.1 Precipitation by Ammonium sulphate

The crude extract was precipitated by adding ammonium sulfate at different saturation levels (30%, 50% and 70%) and kept overnight in refrigerator. Then centrifugation was done at 12000 rpm for 10 min at 4□C. Thereafter the pellet was collected and dissolved in minimum volume of citrate buffer for enzyme activity determination.

2.2.2 Precipitation by Acetone

Chilled acetone (-20□C) at different saturation (40%, 60%, 80% and 100%) was added to the crude extract 50% (V/V), and the mixture was stirred and incubated overnight at 4□C. The precipitate obtained from the crude extract by centrifugation at 10000 rpm for 10 min. Thereafter the pellet was collected and dissolved in minimum volume of citrate buffer for enzyme activity determination.

2.3 Determination of β-glycosidase activity

The β-glycosidase activity was determined against p-nitrophenyl β-D-glucopyranoside (p-NPG) as its substrate in citrate buffer at room temperature and the activity was estimated using double beam spectrophotometer at wavelength 405 nm.

2.4 Partial purification and SDS-PAGE

The isolated enzyme was partially purified by gel filtration chromatography viz. sephadex G-75 and the molecular weight was determined through SDS-PAGE technique.

2.4 Characterization of purified β-glucosidase

The effect of different pH (3. 5, 7, 9 & 11), temperatures (20^oC, 40^oC, 60^oC, 80^oC & 100^oC), metal ions ($CaCl_2$, NaCl, KCl, $ZnSO_4.7H_2O$ & $MgSO_4.7H_2O$), carbon source (mannitol, maltose, sucrose, lactose & glucose), nitrogen source (ammonium sulphate, ammonium nitrate, ammonium chloride, sodium carbonate & hydroxylamine hydrochloride) and inhibitors (copper sulphate, SDS, mercaptoethanol, ferrous sulphate & EDTA) was studied on the activity of β-glucosidase enzyme.

2.5 Bioethanol production from *Hordeum vulgare* seeds

Seeds of *Hordeum vulgare* were used for the bioethanol production. The bioethanol produced was poured into a petri dish and lighten with matchstick. After that its ethanol content was compared to the lab grade ethanol.

[III] RESULTS AND DISCUSSION

3.1 Isolation and activity determination of β-glucosidase

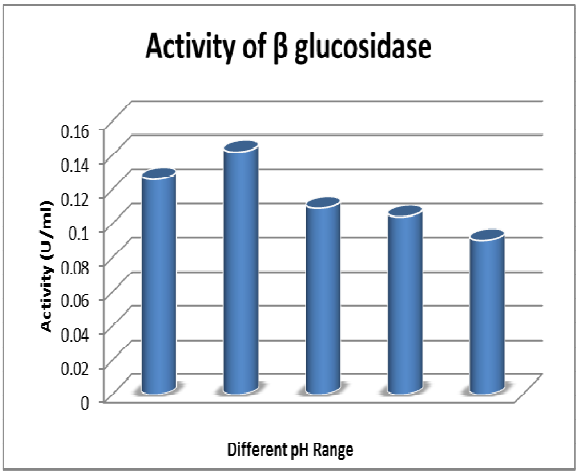
The enzyme was isolated from the seeds of *Hordeum vulgare*, precipitated by ammonium sulphate and acetone to get the partially purified extract of enzyme (without cell debries). After precipitation the enzyme's activity was determined using p-nitrophenyl β-D-glucopyranoside as substrate at 404nm which was found to be 0.055u/ml.

3.2 Characterization of purified β-glucosidase activity

3.2.1. Effect of pH

The β-glucosidase activity was characterized at different pH range, and the maximum activity

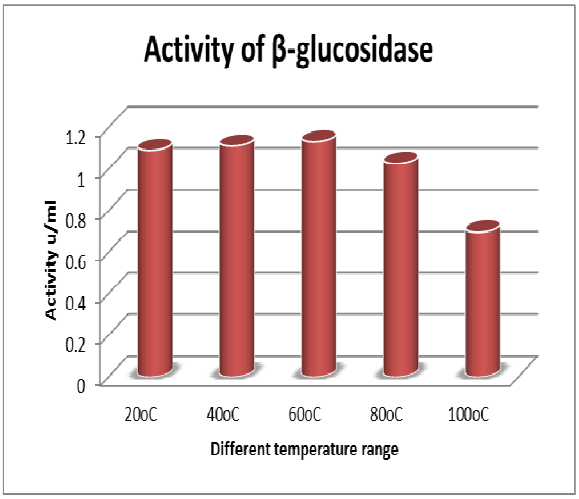
was observed at pH 5, which was found to be 0.142±0.02U/ml (Graph 1).



Graph 1: Optimization of β-glucosidae production from *Barley seeds* in different pH range (3, 5, 7, 9 & 11) (a) Maximum yield was observed at pH 5 (b) Minimum yield at pH 11.

3.2.2. Effect of temperature

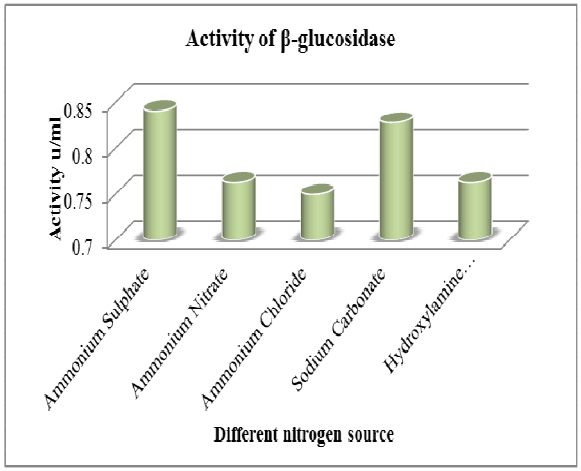
The β-glucosidase activity was characterized at different temperatures, and the maximum activity was observed at 60°C, which was found to be 1.132±0.17U/ml (Graph 2).



Graph 2: Optimization of β-glucosidae production from *Barley seeds* in different temperature range (20°C, 40°C, 60°C, 80°C & 100°C) (a) Maximum yield was observed at 60°C (b) Minimum yield at 100°C.

3.2.3. Effect of nitrogen source

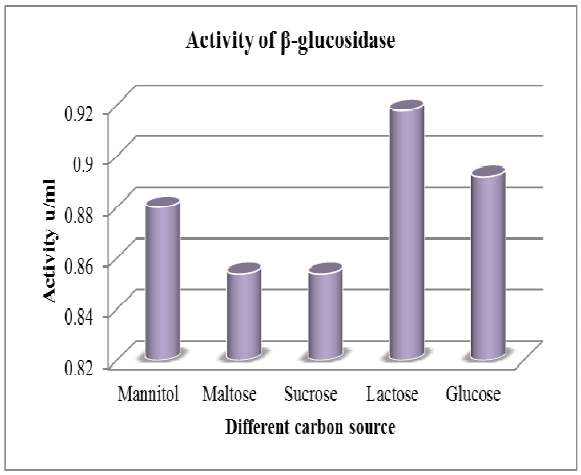
The β-glucosidase activity was characterized in different nitrogen source, and the maximum activity was observed in ammonium sulphate, which was found to be 0.841±0.04U/ml (Graph 3).



Graph 3: Optimization of β-glucosidae production from *Barley seeds* by different nitrogen source (ammonium sulphate, ammonium nitrate, ammonium chloride, sodium carbonate & hydroxylamine hydrochloride) (a) Maximum yield was observed in ammonium sulphate (b) Minimum yield in ammonium chloride.

3.2.4. Effect of carbon source

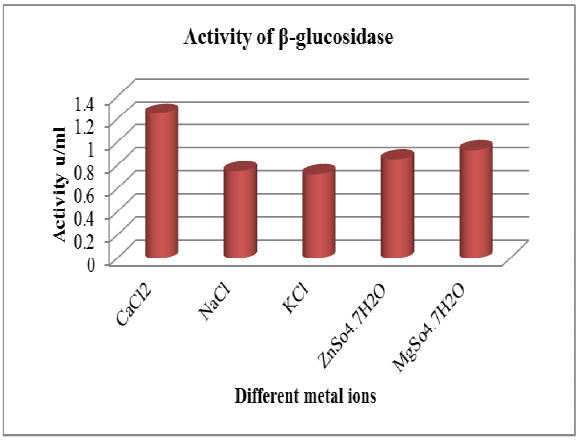
The β-glucosidase activity was characterized in different carbon source, and the maximum activity was observed in lactose, which was found to be 0.918±0.02U/ml (Graph 4).



Graph 4: Optimization of β-glucosidae production from *Barley seeds* by different carbon source (mannitol, maltose, sucrose, lactose & glucose) (a) Maximum yield was observed in lactose (b) Minimum yield in maltose & sucrose.

3.2.5. Effect of metal ions

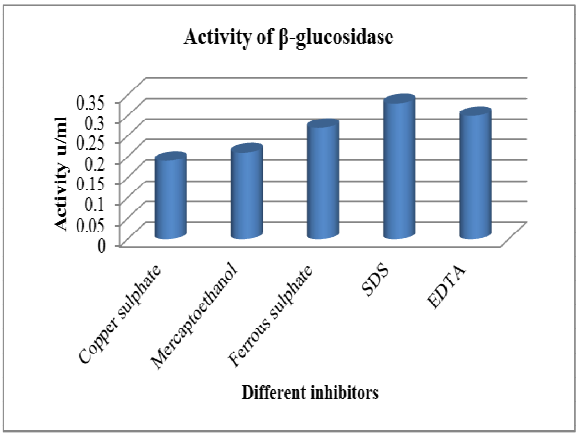
The β-glucosidase activity was characterized at different metal ions, and the maximum activity was observed in calcium chloride, which was found to be 1.255±0.21U/ml (Graph 5).



Graph 5: Optimization of β-glucosidae production from *Barley seeds* by different metal ions (mannitol, maltose, sucrose, lactose & glucose) (a) Maximum yield was observed in calcium chloride (b) Minimum yield in potassium chloride.

3.2.6. Effect of inhibitors

The β-glucosidase activity was characterized in different inhibitors, and the maximum inhibitory effect was observed in SDS, which was found to be 0.33±0.05U/ml (Graph 2).



Graph 6: Optimization of β-glucosidae production from *Barley seeds* by different metal ions (copper sulphate, SDS, mercaptoethanol, ferrous sulphate & EDTA) (a) Maximum yield was observed in SDS (b) Minimum yield in copper sulphate.

3.3 Bioethanol production from *Hordeum vulgare* seeds

Seeds of *Hordeum vulgare* were used for the bioethanol production. By comparing the alcohol content of bioethanol produced from the seeds of *Hordeum vulgare* was found to be higher than absolute alcohol (Figure 1).

Figure 1: Bioethanol extracted after distillation from *Hordeum vulgare* seeds.

[IV] CONCLUSION

β-glucosidase (EC 3.2.1.21) was extracted from seeds of *Hordeum vulgare* and was purified using ammonium sulphate fractional precipitation, Acetone precipitation and Sephadex G-75 chromatography. The molecular weight of enzyme was found to be 60kd .The enzyme β-glucosidase has optimum pH 5.0 and the optimum temperature was found at 60ºC. The maximum activity was also observed in lactose, ammonium sulphate and calcium chloride as carbon, nitrogen and metal ions as sources respectively. The SDS showed the maximum inhibitory effect. Bioethanol was produced from seeds of *Hordeum vulgare*.

ACKNOWLEDGEMENT

I am highly obliged and thankful to my guide Dr. O. P. Verma under whose constant sharp and creative guidance, I have completed my thesis. I really admire him for his gravity and maturity and in giving out his hoard of cognizance.

I wish to express my deep sense of gratitude and sincere thanks to Er. Sujeet Kumar Singh, Director (Admin) of CytoGene Research & Development for providing me with all necessary requirements during my thesis and for his kind support.

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