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RESEARCH **P**APER

Isolation, partial purification, product formation and characterization of β- glucosidase from roots of *Hordeum vulgare* L.

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β-glucosidase (EC 3.2.1.21) was extracted from roots of *Hordeum vulgare* and was purified using ammonium sulphate fractional precipitation and sephadex G-25 chromatography. The molecular weight of enzyme was found in the range of 17-54KDa .The enzyme β-glucosidase has optimum pH 5.0 and the optimum temperature was found at 60ºC. Bioethanol was produced from roots of *Hordeum vulgare*.

Key words : *Hordeum vulgare*, β-Glucosidase, Purification, Characterization

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INTRODUCTION

β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) comprises a heterogeneous group of enzymes that are able to cleave the β-glucosidic linkages of di- and/or oligosaccharides, or other glucose conjugates. β-glucosidases are widely distributed and play pivotal roles in many biological processes, such as degradation of cellulosic biomass, hydrolysis of glycolipids, cyanogenesis, and modification of secondary metabolites.β-glucosidases can be used in cellulose conversion process but also have broad applications such as cellular signaling and oncogenesis (Bhatia *et al*., 2002), 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 aryl glycosides 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 (Bhat, 2002; Yeoman *et al*., 2000; Bhatia *et al*., 2002; Gargouri *et al.*, 2004 and Longo and Sanroman,2006). It is used in flavour industries as flavouring agent (Gueguen *et al.*,1996). In the present study the enzyme was extracted from *Hordeum vulgare* roots.

RESEARCH METHODOLOGY

Collection of root sample :

The roots of *Hordeum vulgare* were collected for the isolation of β- glycosidase enzyme. The roots were ground to fine powder in a chilled mortar and pestle using liquid nitrogen and extraction buffer (1 ml buffer/1g tissue) which was mixed with it. After that the extract was

centrifuged at 12000 rpm 4°C/30 minutes. Then the supernatant was ultra-filtered to obtain crude enzyme solution.

Precipitation of enzyme :

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.

Determination of –glucosidaseactivity :

The β-glucosidase 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.

Partial purification and SDS-PAGE :

The isolated enzyme was partially purified by gel filtration chromatography *viz.*, sephadex G-25 and the molecular weight was determined through SDS-PAGE technique.

Characterization of purified -glucosidase :

The effect of different pH (3,4,5,6,7,8 and 9), temperatures (20°C, 30°C, 40°C,50°C, 60°C, 70°C and 80°C) and varying concentration of substrate (p-nitro phenyl β-D- glucopyranoside in the range of 0.5- 3.0mM) was studied on the activity of β-glucosidase enzyme.

Bioethanol production from *Hordeum vulgare* roots:

Roots 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.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Isolation and activity determination of - glucosidase:

The enzyme was isolated from the roots of *Hordeum vulgare*, precipitated by ammonium sulphate to get the partially purified extract of enzyme (without cell debries). After precipitation the enzymes activity was determined using p-nitrophenyl β-D glucopyranosideas substrate at 405nm which was found to be 0.023u/ml. The purity of β-glucosidase was studied by purification table and found to be 26.1 per cent as shown in Table 1.

Characterization of purified - glucosidase activity:

Effect of pH :

The β- glucosidase extracted from *Hordeum vulgare* roots was optimized at different pH (3, 4,5,6,7,8 and 9) range using sodium hydrogen phosphate-citrate buffer. The maximum activity was observed at pH 5 (0.444U/ ml±0.02) (Fig. 1). The optimum pH for catalyzing β-glucosidase action of different plants ranged as strawberry 4.0 (Bothast and Saha, 1997), *Lodgepole Pine* - 5 to 6 in variety of substrate (Dharmawardhana *et al.*,1995).

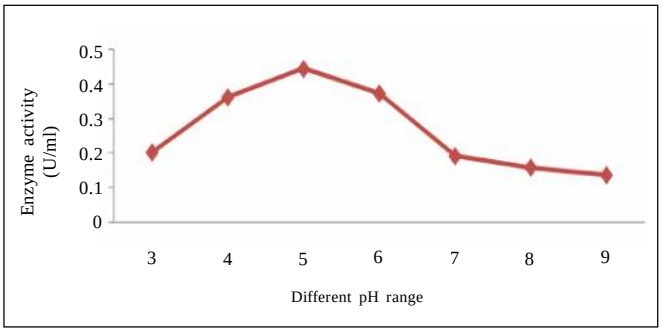

Fig.1: Effect of pH on -glucosidase activity from *Hordeum vulgare* roots (a) maximum activity was observed at pH 5 (b) minimum activity was observed at pH 9

Table 1: Fold purification of β-glucosidase via barley roots

Volume (cm^3)	Concentration	Total protein	Activity	Total activity	Specific activity	Purification fold	Overall yield
$15cm^3$	10.64	159.6	0.059	0.88	0.0055	1.00	100%
$7cm^3$	9.53	66.71	0.057	0.39	0.0058	0.83	44.3%
$4cm^3$	9.82	39.28	0.058	0.23	0.0058	1.00	26.1%

Table 2: Molecular weight from SDS (Coomassie staining) gel of *Hordeum vulgare* roots β- glucosidase

Sr. No.	M.W. of marker (kDa)	Distance of marker (cm)	Distance of protein band (cm)	M.W. of protein band (kDa)
1.	100 kd	1.5 cm	4.7 cm	54 kd
2.	75 kd	2.9 cm	5.6 cm	37 kd
3.	37 kd	3.5 cm	6.8 cm	29 kd
4.	15 kd	5.6 cm	7.4 cm	25 kd
5.	10 kd	7.0 cm	8.5 cm	17 kd

Effect of temperature :

The β-glucosidase extracted from *Hordeum vulgare* roots was optimized at different temperature ((20°C, 30°C, 40°C,50°C, 60°C, 70°C and 80°C) range using sodium hydrogen phosphate-citrate buffer. The maximum activity was observed at 60°C (1.598U/ml±0.17) (Fig. 2).The optimum temperatures in different plants were as strawberry 60°C (Poulton and Li,1994), *Leuconostoc mesenteroides* 50°C (Cicek and Esen, 1999).

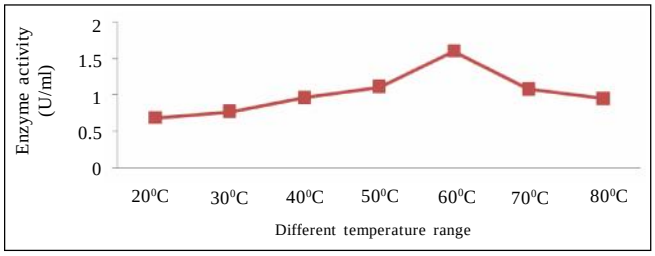

Fig.2: Effect of temperature on -glucosidase activity from *Hordeum vulgare* roots (a) maximum activity was observed at 60°C (b) minimum activity was observed at 20°C

Effect of substrate concentration :

The effect of different concentration of substrate was studied on production of β-glucosidase from *Hordeum vulgare* roots. The maximum activity was observed at1.5mM (Fig. 3). The effect of mono/ disaccharides (1 mg/ml) on β–glucosidase activity was studied using pNPG as a substrate (Kaur *et al.,* 2007).

Molecular weight determination of enzyme :

The molecular weight of enzyme isolated from *Hordeum vulgare* roots was determined by SDS-PAGE using specific markers of known molecular weight. The

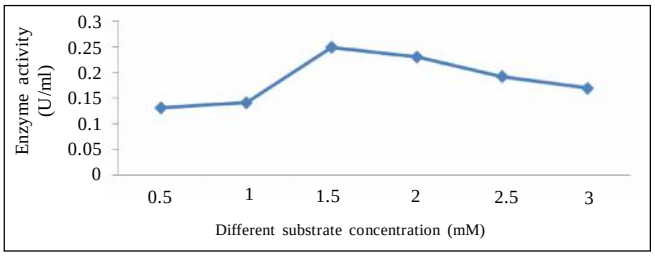

Fig.3: Effect of substrate concentration on -glucosidase activity from *Hordeum vulgare* roots (a) maximum activity was observed in 1.5 mM (b) minimum activity was observed in 0.5 mM

molecular weight of β-glucosidase from *Hordeum vulgare* roots was found to be 17-54kDa (Table 2 and Fig. 4).The molecular weight of different bands in *Rauvolfia serpentine* was found in the range of 19.48 KDa to 92.257KDa (Verma *et al.*, 2011).

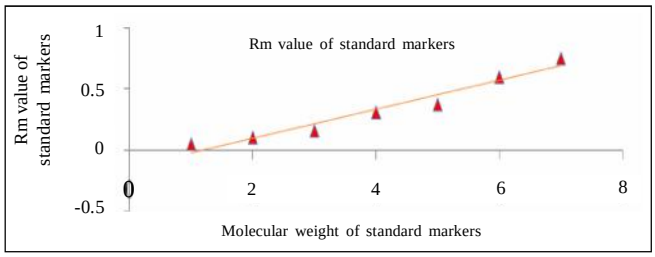

Fig.4: Determining the molecular weight of protei by SDS-PAGE

Bioethanol production from *Hordeum vulgare* roots:

Roots of *Hordeum vulgare* were used for the bioethanol production. Ethanol was produced by fermentation and then distillation of fermented product. By comparing the alcohol content of bioethanol produced from the roots of *Hordeum vulgare* was found to be lower than absolute alcohol.

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