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Research Article

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INDUCTION OF β-GLUCANASE FROM WHEY YEAST

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ABSTRACT

β-glucanase enzyme was isolated from whey yeast. The semipurified enzyme showed substrate specificity towards insoluble β-glucan isolated from S. cerevisiae, with 70 % of relative activity, whereas no activity was observed for CM cellulose, Barley glucan, Sorghum glucan and starch. The enzyme activity obtained in the supernatant after cell homogenization was refereed as first intracellular enzyme (I) whereas the activity observed with residual fraction was termed as second (II) intracellular enzyme. The activity of I intracellular enzyme was comparatively higher than that for II. The activity of both I & II intracellular β-glucanase enzyme was increased after semipurification. The activity of β-glucanase enzyme goes on increasing with increase in glucose concentration from 2 % to 8 % w/v. The enzyme action on β-glucans, an alkali insoluble mass isolated from yeast, resulted in its solubilisation by liberating glucose residues those can be estimated later on.

KEYWORDS

β-glucan, glucanase, induction, whey yeast, Yeast.

INTRODUCTION

β- glucanases are hydrolytic enzymes capable of causing lysis of cell walls. They are active on insoluble substrates, such as beta glucan components of fungal cell walls, laminarin and pachyman. β-glucanases has been of great interest because of their action on yeast cell walls, a very useful ability for studies on the structure and disposition of β-polymers along the outer yeast cell envelope. β- Glucanases have been found in higher plants as well as fungi and bacteria. Six different beta 1, 3-Glucanases have been purified from *Saccharomyces cerevisiae* [1]. Strains of *Bacillus* and *Streptomyces* species are reported to be highly active in producing extracellular beta-1, 3 -glucanases. Non specific beta glucanase i.e. beta glucosidase and beta-1, 3- Glucanase enzymes have been purified from *Penicilium ochro-chloron* [2]. *Fungi imperfecti* species [3] have been found to be responsible to produce (1→3)-β-D glucanase activity which on incubation with yeast glucan causes its solubilisation with rapid reduction in turbidity. In case of *Cyptophaga johnsonii* [4]; both (1→3) and (1→4) - β-D-glucanase activities are found to be present in the cultural filtrate. ***O**erskovia xanthineolytica* [5] produces endo (1→3)-β-D- glucanase. Endo (1→6)-β-D glucanase produced by *Penicillium brefeldianum* [6] is inducible in nature. β-glucanases are components of lytic enzyme system active against cell wall of fungi like yeast. β-glucanases are hydrolytic enzymes which may be exo or endo hydrolases that are active on insoluble substrates like β-glucan components of fungal cell wall. Yeast lytic enzyme system includes a mixture of several enzymes such as β-glucanase, protease, mannanase, and chitinase. β-glucanases are further classified as β- (1→3) glucanase , β-(1→6) glucanase and β-(1→4) glucanase on the basis of the type of glycosidic linkage cleaved by them. Glucanase from Arthrobacter [7] hydrolyzes only insoluble laminarin indicating that the enzyme requires long uninterrupted segments of (1→3)-β-D linked glucose units for binding. *Bacillus circulans* WL – 12 has two types of (1→6)-β-D glucanases [8]. β-glucanases due to their specificity have significant role in structural analysis of polysaccharides. β-glucans are major structural

components of fungal cell wall like yeast and impart rigidity and mechanical strength to wall architecture. In yeast, β-1, 3-Glucanases are involved in morphogenetic events, such as cell budding, conjugation and sporulation. So, yeast cell wall glucan must be considered as the only natural substrate for these enzymes. β-glucanase enzymes may produce architectural changes in the cell wall during yeast and fungal morphogenesis. The cell wall of yeast undergoes continuous rearrangement of beta glucans during growth period. The process involves making and breaking of bonds between wall polymers, manipulated by beta glucanases through controlled hydrolysis. As a consequence, different glucanases are required at different stages during cell life cycle. For their higher specificity and selective hydrolysis of cell wall polymers, beta glucanases are employed in the preparation of protoplasts. The present study deals with isolation, purification of β-glucanase using yeast sp. isolated from whey, a by-product obtained during manufacturing of cheese.

MATERIALS AND METHODS

Isolation of yeast sp. from whey:

Whey samples are collected from local market. The pure culture of yeast strain was isolated by serial dilution method using Sabouraud Dextrose Agar (SDA) (P^H 7.0). The plates were incubated at 27^0C for 24 h. The purified isolates preserved on SDA slants at 4^0C. The pure culture of the isolates was Gram stained for microscopic morphology to check bacterial contamination.

Yeast propagation:

Sterile basal medium containing ammonium sulphate (0.3 %), yeast extract (0.5%), peptone (0.5 %) and glucose (2 %, 4%, 6%, 8%) was prepared with P^H 7.0. The inoculums was grown at 25 ± 2°C for 24 h and then transferred to major SD broth under sterile conditions. Fermentation was carried out at 25 ± 2°C for 48 h.

Harvesting:

After completion of 48 h of fermentation, yeast cells were harvested from media by centrifugation under cold conditions (+4°C) at 10,000 rpm for 10 min. Cells were washed repeatedly with chilled distilled water. These cells were used further for extraction of β-glucanase enzyme.

Enzyme extraction:

Isolated yeast cells were washed twice with distilled water and once with phosphate buffer (0.1 M, PH 7.0). The cell mass collected was suspended in phosphate buffer (0.1 M, PH-7.0) and crushed using mortor – pestle along with glass beads under cold conditions. The resultant homogenate was centrifuged at 10,000 rpm for 10 min at +4°C. The supernatant obtained was used as the source of β-glucanase enzyme. This was termed as 1st intracellular enzyme. Pellet was further subjected to crushing using mortar-pestle. The resultant homogenate was incubated for overnight period and then it was subjected to centrifugation at 10,000 rpm for 10 min at +4°C. The supernatant obtained was used as the source of β-glucanase enzyme. This was termed as 2nd intracellular enzyme [9]. β glucanase assay was done with crude β-glucan substrate.

Preparation of β- glucan substrate:

20 gm of yeast cake or yeast granules were added to approx.70 ml warm distilled water to prepare cell suspension. The resultant suspension was centrifuged at 8,000 rpm for 10 min. The cell mass was weighed and 2ml of 20 % KOH was added per gram of cell mass. Then alkali digestion was carried out using boiling water bath for 1 hour. The reddish brown liquid obtained was termed as yeast digest. The yeast digest was cooled in ice bath and neutralized by using 0.5 N HCl. The neutralized yeast digest was subjected to centrifugation at 10,000 rpm for 10 min. The supernatant was discarded and the residue containing insoluble yeast β-glucans was repeatedly washed using distilled water till it gives negative phenol sulphuric acid test. The residue was suspended in distilled water and the resultant suspension was used as a β-glucan substrate.

Enzyme assay:

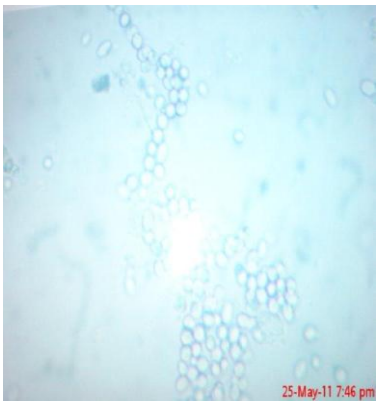
β- glucanase assay was performed with β- glucan as a substrate isolated from yeast. The assay mixture containing 0.5 ml crude substrate, 0.5 ml acetate buffer (P^H-5.5), and 1ml of enzyme extract was incubated at 45°c for 1 h. A suitable control tube was also prepared along with the sample tube. After 1 hr incubation the reaction

mixture was centrifuged at 6,000 rpm 10 min. 1ml reaction mixture was taken from both sample & control tubes and incubated along with GOD-POD reagent at 37° C for 30 min. The glucose released in the reaction mixture was measured colorimetrically at 540 nm using glucose oxidase peroxidise method [10].

Ammonium sulphate fractionation:
Crude enzyme was semipurified using 70% salt fractionation and was subjected to dialysis using acetate buffer (10 mM, PH-5.5). The dialysed enzyme was further used to check its activity and protein content using Lowry method [11].

RESULTS AND DISCUSSION

Isolation of yeast:



Yeast sp. isolated

From the **figure1** it is displayed that the activity of β-glucanase enzyme goes on increasing with increase in glucose concentration from 2 - 8 % w/v. Thus glucose acts as carbon source for the production of maximal yeast cell biomass and thus ultimately maximum β-glucanase enzyme.

From **figures 2 & 3** it indicats that the activity of both I & II intracellular β-glucanase enzyme gets increased after semipurification. Since the activity of I intracellular enzyme was comparatively higher on yeast β-glucan substrate than that for II; enzyme I was further used to determine the activity of β-glucanase.

Fig.1 Effect of Glucose concentration on the activity of β-Glucanase

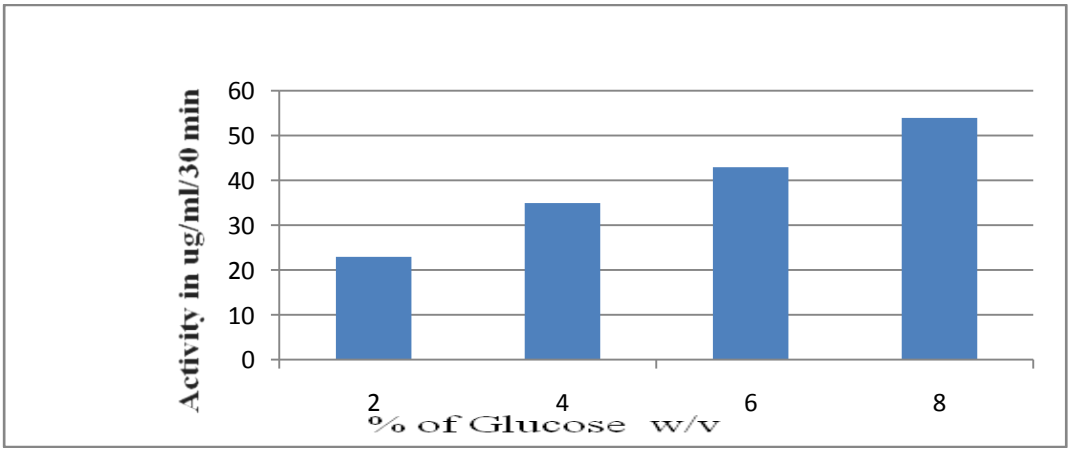


Fig.2 Activity of the β-glucanase I intracellular enzyme before and after semi-purification

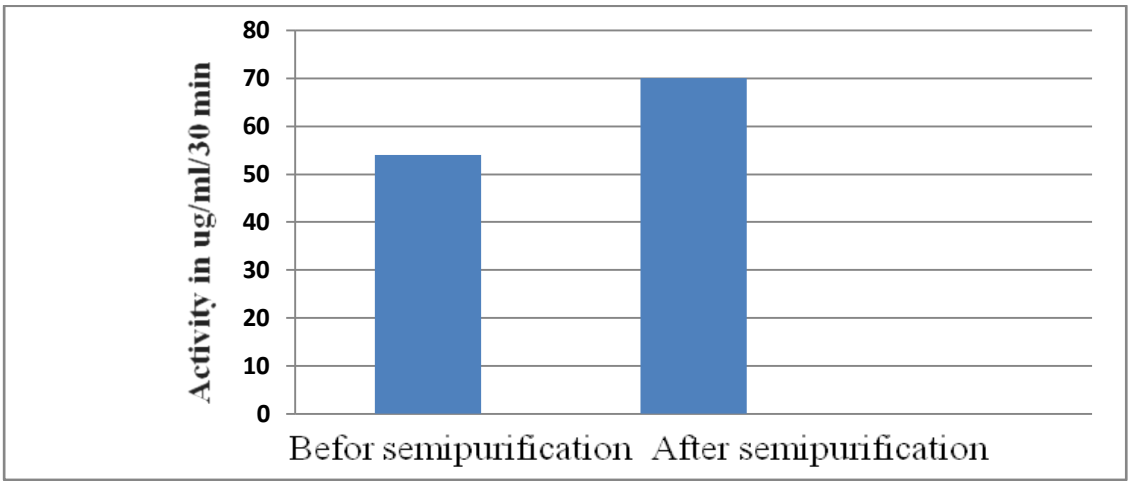
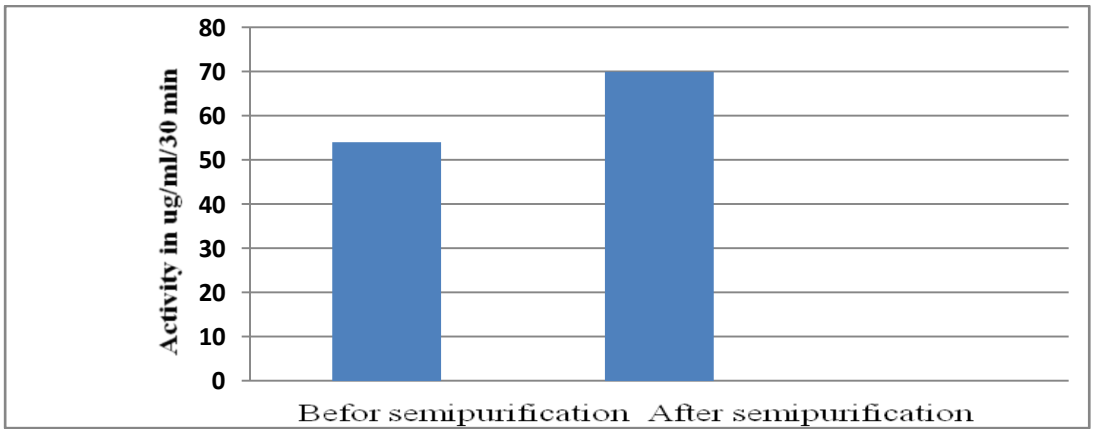


Fig.3 Activity of the β-glucanase II intracellular enzyme before and after semi- purification



From **Table 1** it indicates that the semipurified enzyme shows substrate specificity towards insoluble β-glucan isolated from *S. cerevisiae*, with 70 % of relative activity, whereas no activity was observed for CM cellulose, Barley glucan, Sorghum glucan and starch.

Table 1 Substrate Specificity of semipurified β-glucanase toward various glucans

Substrate	Major Linkage types	Relative activity (%)
Insoluble yeast glucan (*S. cerevisiae*)	β-(1-3)	70
CM-cellulose	β-(1→4)	0
Barley glucan	β -(1→3, 1→4)	0
Sorghum glucan	β -(1→3, 1→4)	0
Starch	α-(1→4)	0

Table 2 Amount of total carbohydrate released after the action of β-Glucanase on whole yeast cells

Incubation time	Total carbohydrate (µg/ml/30 min)
4	Nil
12	30
24	54

Action of semipurified enzyme on whole yeast cells was carried out. **Table 2** indicates that prolonged action of β-glucanase on yeast causes lysis of cell walls releasing glucose as a source of carbohydrate.

CONCLUSION

Thus yeast sp. isolated from whey exhibits β-glucanase activity which is intracellular enzyme.The activity of β-glucanase was found to be higher in case of IInd intracellular enzyme released after incubation of yeast cells already subjected to the process of homogenization for overnight duration. The enzyme activity was enhanced after the partial purification using the salt fractionation process. The enzyme specifically acts on crude β-glucan substrate isolated from yeast cake, but is inactive for substrates like CM cellulose, Barley glucan, Sorghum glucan and starch. The semipurified enzyme is able to cause partial lysis of whole intact yeast cells showing the release of glucans.

ACKNOWLEDGEMENT

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