O2-1	**Fruit juice clarification using free and immobilized xylanase from *Pseudomonas* sp.**

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INTRODUCTION AND OBJECTIVES

Xylanases (E.C.3.2.1.8) is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose and also commonly known with synonymous terms viz. endoxylanase, 1,4- β -D-xylan-xylanohydrolase, endo-1,4-b-D-xylanase, b-1,4-xylanase and b-xylanase (Collins et al. 2005). These are interesting member of glycoside hydrolase family (mainly GH 10 and GH 11) which catalyzes the hydrolysis of 1,4 β-D-xylosidic linkages in xylan, which is the second most abundant polysaccharide and a major component of plant cell wall (Butt et al. 2008). Depending on the source, the xylopyranoside units are substituted with mainly acetyl, arabinosyl, and glucuronosyl residues (Yin et al. 2010). In industries xylanases are employed either alone or in combination with other enzymes, in various processes like pretreatment of forage crops and lignocellulosics biomass, improvement of nutrient utilization of cereal-based diets in pig and poultry, flour improvement for bakery products, saccharification of agricultural, industrial and municipal wastes, processing pulp and fibers, enhanced bleaching of cellulose pulps while decreasing consumption of chlorine containing chemicals and as an alternative to treatment with sulphuric acid of the textile-cellulosic waste and clarification of fruit juices (Beg et al. 2001; Ninawe & Kuhad 2006).

Fruit juice clarification is of utmost importance as raw juice is highly turbid and settles during storage, and therefore it must be clarified before commercialization. In present paper we have successfully described a method for fruit juice clarification using free and immobilized xylanase from *Pseudomonas* sp. XPB-6.

MATERIALS AND METHODS

Microorganism, culture conditions and production of extracellular enzyme

The bacterial isolate *Pseudomonas* sp. XPB-6 has been procured from the culture collection of the Department of Biotechnology, H. P. University, Shimla-5, India. Precultures were prepared by inoculating a loop full of culture from the slant to 4 ml of seed medium containing 0.5% peptone, 0.3% beef extract, 0.1% yeast extract and 1% glucose (pH 7.0) at 30 ˚C, 160 rpm for 24 h. These 24 h precultures were added to 50 ml of production medium and 0.25% xylan (birch wood) as an inducer, followed by incubation at 30 ˚C for 24 h in an incubator shaker at 180 rpm. Cells were harvested by centrifuging the cultures at 10,000 g for 20 min at 4 ˚C. The supernatant was designated as membrane free crude enzyme and assayed for xylanase activity.

The xylanase activity was determined spectrophotometrically by the method described by Miller (1959). If not stated otherwise, the assay was performed in 100mM sodium phosphate buffer (pH 7.0) at 55˚C for 5 min. The absorption was measured at 540 nm and related to the amount of xylose formed in reaction by comparison with a standard.

Optimization of culture and reaction conditions for xylanase

The optimization of cultural conditions was carried out with stepwise modifications of the production medium as carbon sources, nitrogen source, pH, temperature, inoculum's size etc. Optimization for juice clarification included independent variable viz. temperature, incubation time and enzyme dose to investigate the dependent variables such as yield, transmittance and filterability were studied. In reaction conditions viz. buffer system, substrate concentration, incubation time were optimized. Shelf life of xylanase was also examined at 4˚C and at room temperature.

Immobilization of xylanase

Immobilization of enzyme was done by dissolving 1gm of dry silica matrix in minimal buffer (sodium phosphate) and kept overnight. Swelleability was calculated as Wet-Dry matrix/dry matrix. For immobilization 4 ml of enzyme with 1gm matrix was incubated for 2 hours in shaking incubator. Enzyme activity from both supernatant and immobilized enzyme was calculated.

Clarification of fruit juice

For fresh juices, fruit pulp was macerated using a hand blender. Minimum amount of water was added to facilitate the maceration process as well as to extract maximum juice from the pulp to get a smooth textured puree which was further strained through a cheese cloth to separate the debris from the pulp and pH was adjusted 7.5 to get optimum effect of xylanase. Juice clarification (crude fresh and commercial i.e. apple Tropicana, Real, Minchy's and orange Tropicana and Real) was carried out at 30 and 55 ºC by measuring transmittance at 650 nm. Enzyme dose in the range of 5-20 IU/ml and time interval 6-54 hours were also tested for optimum juice clarification.

Juice clarification was carried out using free as well as immobilized enzyme (silica matrix) at 30ºC and 55ºC. A

sample of 50 ml of each juice was supplemented with 12.5 gm of matrix (250 IU of xylanase/gm of matrix with swelling capacity of 3.01 w/w).

RESULTS AND DISCUSSION

Optimization of culture and reaction conditions for xylanase

Maximum xylanase production was achieved in a medium containing 1% xylan, 4% dextrose, 0.5% peptone and 0.2% meat extract. The enzyme was most active in 100 mM sodium phosphate buffer (pH 7.5) at 55 ºC. Shelf life of xylanase was studied at both room temperature and at 4˚C. The results obtained are shown in Fig. 1. The enzyme was stable both at 4˚C for 25 and at 25˚C for 16 days. After that the enzyme activity gradually started decreasing.

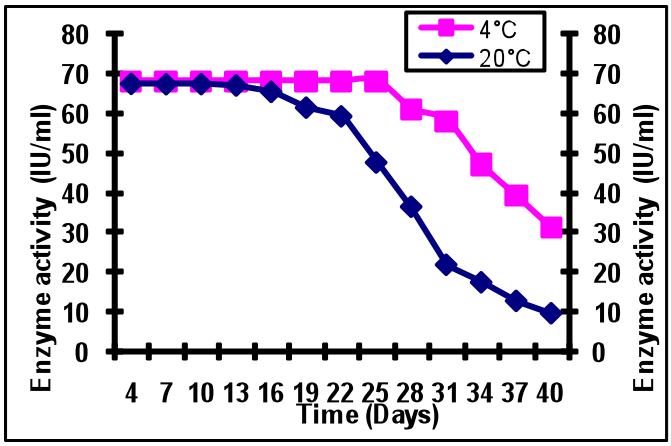


Figure 1: Shelf life of xylanase from *Pseudomonas* sp. XPB-6

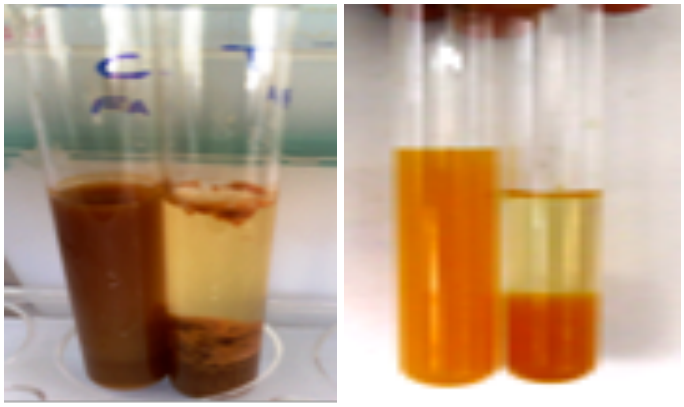
The results for juice clarification are summarized in the Table 1.

Table 1: Enhancement of transmittance (T_{650}) of juice after clarification with xylanase (55ºC, 6 hrs)

Juice used	T_{650} of juice sample	
	Untreated	Treated
Crude (Fresh Apple)	0.85	52.31
Apple Real	86.06	86.09
Apple Tropicana	84.12	84.23
Apple Minchy's	63.12	78.91
Crude (Orange Fresh)	0.74	16.45
Orange Real	11.63	40.21
Orange Tropicana	11.62	47.01

It is evident that xylanase from *Pseudomonas* sp. XPB-6 had efficiently clarified both fresh as well as commercial fruit juices. The optimum temperature, incubation time and enzyme dose were 55ºC, 6 hours and 10 IU/ml respectively.

The results obtained were similar to free as well as immobilized enzyme.



(A) (B)

Figure 2: Fresh apple juice before and after clarification (A) and fresh orange juice before and after clarification (B)

CONCLUSIONS

The results obtained in this investigation show that *Pseudomonas* sp. XPB-6 efficiently expresses xylanase activity that has been successfully used for the clarification of apple and orange juice. Biotransformation at commercial scale has a very high potential in contrast to chemical processes for the clarification of fruit juice.

Moreover, to the best of our knowledge, no one has so far reported this much (75%) clarification of fresh apple juice. Therefore, we conclude that *Pseudomonas* sp. XPB-6 or the appropriate enzymes extracted from this microbe could be successfully used for the clarification of fruit juice at commercial level.

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