New fungal sources for α -L-Rhamnosidase: an important enzyme used in the synthesis of drugs and drug precursors

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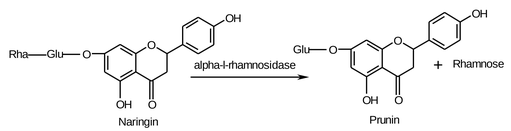
Introduction

Alpha-L-Rhamnosidase [EC 3.2.1.40] enzyme belongs to the group of hydrolases, which hydrolyses the terminal non reducing L-Rhamnose from the natural and synthetic rhamnosides.



The production of alpha-L-Rhamnosidase by a number of mammalian tissue, plants, bacteria and fungi has been reported. This enzyme has been used in the structural determination of polysaccharides, glycosides, and glycolipids **(Kamiya et al., 1985**), debittering of citrus juice **(Puri et al., 2000)**, improving the aromas in wine, in the metabolism of gellan (**Hashimoto, 1999**), in production of pruning (**Roitner, 1984**) which possesses anti-inflammatory and antiviral activity against DNA/RNA viruses (**Yu et al., 1973 and Kaul et al., 1985**) and for the derhamnosylation of many L-rhamnose containing steroids *for e.g.* diosgene desglucoruscin, ginsenosides Rg2 etc. whose derhamnosylated products have their clinical importance (**Elujoba et al., 1987, Monti et al., 2004, Yu et al., 2002**). The glycopeptide antibiotic chloropolysporin C is prepared from the related compound chloropolysporin B **(Sankyo, 1988**) by enzymatic hydrolysis, using rhamnosidase. Chloropolysporin C exhibits antibacterial activity and is useful in the treatment and prophylaxis of infections, and as a growth-promoting agent for animals. The derhamnosylated product quercetin-3-glucoside of flavonoid rutin increases the antioxidant activity of asparagus juice (**Sun et al., 2007**). These informations are indicated that Alpha-L-Rhamnosidase is a very important enzyme having due to its wide application in Parma industries. So there is need to identify new sources of this enzyme having novel properties.

Methodology

Media composition:

The liquid culture medium in which α -L-rhamnosidases production by above given fungal strains have been observed has composition: Water (Double Distilled)1000ml,CaCl2 1 gm, MgSO4.7H2O 3 gm, KH2PO4 20gm, N(CH2COONa)3 1.5 gm, MnSO4 1 gm, ZnSO4.7H2O 0.1gm , CuSO4.5H2O 0.1gm, FeSO4.7H2O 0.1 gm, H3BO3 10.0mg, Sucrose 40.0 gm, Ammonium Tarterate 8.0 gm.

Characterization of α -L-rhamnosidase :

pH Optima: The enzyme activity of the crude α -L-rhamnosidase was determined by the reported method given by Romero et al. (1985). The method consist of adding 0.5ml of 3.5 ml p-nitrophenyl α -L-rhamnopyranoside dissolved in water to 0.5 ml of the different buffers of 0.1M in the pH range of 1-13 . Steady state velocity of the reaction medium was plotted against pH.

pH Stability: the pH stability of the enzyme was studied by exposing the enzyme to buffers of different pHs for 24 hrs at 20°C. The residual activities were assayed and plotted in the form of percent residual activity versus pH.

Temperature optima: The enzyme activity for the optimum temperature was determined by assaying the activity of enzyme at different temperature in the range 10-80°C.

Temperature Stability: Thermal stability of the enzyme was tested by incubating an enzyme aliquot at a particular temperature for 1 hour assaying its residual activity and plotting the percent residual activity against temperature.

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Results and Discussion

Two fungal strains were isolated from decaying fruits and seeds. They were tentatively identified as *Penicillium* VY and *Aspergillus* VY. All the isolated species show the maximum production on third day in a liquid culture media. The enzyme unit per ml observed for *Aspergillus* VY without inducer was 1805.1 IU/ml where as in the presence of inducer naringin was 1980.99 IU/ml and other inducer rutin was 5940 IU/ml. The enzyme unit per ml observed for *Penicillium* VY without inducer was 3341.25 IU/ml where as in presence of naringin was 3073.95 IU/ml and in presence of rutin was 9919.8 IU/ml. The temperature and pH optima of α -L-rhamnosidase produced by *Penicillium* VY and *Aspergillus* VY have been determined. The pH optimum was found to be 10.0 for *Penicillium* VY and 11.0 for *Aspergillus* VY. The temperature optima were 50ºC in both the cases. The enzyme produced by *Penicillium* VY was found to be stable in the pH range 3.0-7.0 for 24 hours and 3.0–6.0 in case of *Aspergillus* VY at 25 ºC. The enzyme does not loose activity up to 40º C in case of *Penicillium* VY and 40ºC in case of *Aspergillus* VY if exposed for 1 h. As per our knowledge α -L-rhamnosidase enzyme obtained from different sources have highest activity at acidic or neutral pH. On the other hand α -L-rhamnosidase produced by *Aspergillus* VY and *Penicillium* VY have pH optimum 11 and 10 respectively. As mentioned by Roitner et al (1984) prunin is obtained from naringin by the action of naringinase. They pretreated the immobilized naringinase with highly alkaline buffer to remove the glucosidase activity. As in this case as it described that both the α -L-rhamnosidases have alkaline pH optima hence there is no need to treat them by alkaline buffer if they are used to synthesise prunin. So by using α -L-rhamnosidase from *Aspergillus* VY and *Penicillium* VY it will be more easier and economic to produce prunin from naringin as compared to method mentioned by Roitner et al (1984).