Purification of naringinase produced by *aspergillus niger* $B_1{}^3$

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Naringinase has found wide application in debittering of grapefruit juices and pulps. This study was aimed at the purification of naringinase produced by *Aspergillus niger* $B_1{}^3$. *Aspergillus niger* was cultured in the liquid medium containing (gl^{-1}) naringin, 2.0: glucose, 2.0; soy broth, 20, yeast extract, 20.0; $MgSO_4.7H_2O$, 0.1: KH_2PO_4, 0.5 and 100ml mineral solution ($ZnSO_4.7H_2O$, 0.7, $CuSO_4.5H_2O$, 0.7 and $FeSO_4.7H_2O$, 0.7). The organism produced the highest naringinase activity ($28Uml^{-1}$) on 6th day at 30°C. The crude enzyme contained 28 Uml^{-1} naringinase acitvity and 23.22$mgml^{-1}$ protein. To purify the enzyme it was subjected to fractional precipitation by adding solid $(NH_4)_2SO_4$ to 40, 60 and 80% saturation. The activities found in the fractional precipitates were 28.84, 54.96 and 14.75 Uml^{-1} respectively. Hence for the purification studies the enzyme was precipitated with 60% saturation of $(NH_4)_2SO_4$. The precipitate was dissolved in 50mM phosphate buffer (pH 7.0) and dialysed against the same buffer. The residue contained naringinase activity of 87.64 U ml^{-1} and 18.46 mg ml^{-1} protein. The dialyzed enzyme was added to ion-exchange (DEAE-Sepharose™ CL-6B) column (11.5 x 2cm) and equilibrated with 50mM phosphate buffer (pH 7.0). The enzyme was eluted with the same buffer containing 0.5M - 1M NaCl. The purified enzyme sample contained 35.3 U ml^{-1} enzyme activity and 2.45mg ml^{-1} protein content. In another experiment the $(NH_4)_2SO_4$ precipitated enzyme was dialysed against 50mM phosphate buffer (pH 7.0) containing 1.5M $(NH_4)_2SO_4$. The dialyzed enzyme was loaded into Phenyl-Sepharose™ 6 Fast flow column (11.5 x 2cm) and equilibrated with 50mM phosphate buffer (pH 7.0) containing 1.5M $(NH_4)_2SO_4$. The adsorbed enzyme was eluted with 50mM phosphate buffer (pH 7.0). The purified enzyme showed 83.21Units ml^{-1} enzyme activity and had 6.15mg ml^{-1} protein. The recovery of naringinase by ion-exchange and hydrophobic interaction chromatographies were 81 and 98% respectively showing the specific activity of 13.9 and 12.12 Ug^{-1} protein respectively.

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The enzyme samples purified by ion-exchange chromatography and hydrophobic interaction chromatography were separated by SDS- polyacrylamide gel electrophoresis. The electrophorogram gave two clear bands. To confirm that the two bands are of that of naringinase, purified enzyme sample was mixed with naringin ($2gl^{-1}$) at pH 5.0 and incubated for 0 to 120 min at pH 5.0 and 50°C. The products were analyzed by thin layer chromatography. The chromatogram of the hydrolysate of naringin by the purified enzyme showed bands parallel to the positions of naringin, prunin, rhamnose, naringenin and glucose. Further studies are in progress to improve the purification of naringinase.