幾丁質分解酵素之生產與其基因選殖

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摘 要

本研究首先探討不同基質對於Aeromonas sp. DYU-Too7生合成幾丁質分解酵素之影響，並純化經誘導所產生之幾丁質分解酵素。結果顯示，除幾丁質粉末外，葡萄糖胺亦可誘導試驗菌株生合成幾丁質分解酵素，其中皆以36 kDa幾丁質分解酵素(Chi36)為主，而幾丁質粉末與葡萄糖胺之最適誘導濃度分別為2%與0.1%。後續分別利用蛋白質純化技術分離此兩種基質所誘導之Chi36，而所得之純化酵素於特性上並無顯著差異，如：最適反應pH皆為pH 5.0，於溫度10-80°C或pH 5.0-8.0下酵素具高穩定性。而以純化酵素水解幾丁質所得產物皆為N-乙醯幾丁二醣，因此推判此酵素為外切型幾丁質分解酵素。然而，以幾丁質培養基所生產的幾丁質分解酵素經純化後，於10 mM Hg2+離子中反應1 h後，其活性仍保有55%；以葡萄糖胺培養基所生產的幾丁質分解酵素經純化後，於10 mM Hg2+離子中反應1 h後，則仍保有67%的活性。 此外，利用奈米化之工業鑽石(100 nm)為蛋白質之吸附載體，以提高蛋白質收集與濃縮的效率。首先以先前所純化之Chi36為試驗蛋白質，探討蛋白質經奈米化之工業鑽石吸附與脫附後之影響。結果得知，最適蛋白質吸附的條件為75 mg/mg-diamond，最適吸附pH為4.1，其吸附效率約為86%，且經工業鑽石吸附後之酵素特性並無顯著的改變。於蛋白質的脫附試驗中發現，緩衝液pH 8.0或9.0可脫附大部分(? 100%)載體上之蛋白質，而緩衝液pH 7.0之脫附效率則僅為76%。後續將奈米化之工業鑽石應用於蛋白質純化程序中，藉以取代傳統硫酸銨沈澱法。結果顯示，此法可吸附發酵液中87%的總蛋白質，再以緩衝液pH 8.0脫附工業鑽石上的蛋白質後，得知，脫附後之蛋白質仍保有脫附前的88%，且脫附後之蛋白質仍保有脫附前的活性。本研究亦利用聚合?鏈反應選殖Aeromonas sp.DYU-Too7所生合成之36 kDa幾丁質分解酵素基因，結果得知，此基因的可譯框架(open reading frame)為1,080 bp，共編碼360個胺基酸，其中前27個胺基酸為訊息胜?(signal peptide)，而胺基酸序列中的137F- DGIDIDLE145符合醣?水解酵素之第18族的共有催化區。此外，於蛋白質表現與幾丁質水解試驗中得知，含有能產生訊息胜?之chi36基因，其重組蛋白質可表現幾丁質分解酵素活性，而幾丁質水解產物以N-乙醯幾丁二醣為主，反之，缺少產生訊息胜?之chi36基因，其重組蛋白質無顯著的幾丁質分解酵素活性，且無水解幾丁質之能力。

關鍵詞 : 幾丁質 ; 葡萄糖胺 ; N-乙醯幾丁二醣 ; 幾丁質分解酵素 ; 工業鑽石 ; 聚合?鏈反應

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