生產N-乙醯幾丁寡醣菌株之篩選與幾丁質?之分離純化

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摘 要

N-乙醯幾丁寡醣具有抗癌、增強免疫力及抑菌等生理功效，應用價值相當高，卻受限於製備方法，使得生產成本過高，目前多種的N-乙醯幾丁寡醣製備方法各有其優缺點，而本研究採微生物發酵生產N-乙醯幾丁寡醣，考量其低污染與作用專一性。 自苗栗水尾溪篩選出幾丁質分解菌株，初步命名為DYU-Too 11，經新竹食品工業發展研究所生物資源保存及研究中心鑑定為Aeromonas hydrophila，故命名為Aeromonas hydrophila DYU-Too 11。分別以幾丁質與膠態幾丁質為碳源，比較N-乙醯幾丁寡醣與幾丁質?之差異，結果顯示，幾丁質碳源培養可生成N-乙醯葡萄糖胺與聚合度2 ~ 5的N-乙醯幾丁寡醣，最高產量分別為0.79、0.94、0.4、0.16及0.26 g/L，而幾丁質?活性最高為370 U/L，膠態幾丁質碳源培養可生成N-乙醯葡萄糖胺與聚合度3和4的N-乙醯幾丁寡醣，最高產量分別為15.8、0.37及0.06 g/L，而幾丁質?活性最高為310.4 U/L，因此生產N-乙醯幾丁寡醣之碳源以幾丁質為最佳。 Aeromonas hydrophila DYU-Too 11菌株以不同濃度幾丁質培養，結果1%幾丁質可生成N-乙醯葡萄糖胺與聚合度2、4、5的N-乙醯幾丁寡醣，2%幾丁質濃度，可生成N-乙醯葡萄糖胺與聚合度2 ~ 6的N-乙醯幾丁寡醣，而3%、4%及5%幾丁質所生成的水解產物，其聚合度偏高，因此可依目標產物選擇適合的幾丁質濃度。 發酵培養Aeromonas hydrophila DYU-Too 11，並取其上清液以硫酸銨沉澱蛋白質，收集硫酸銨飽和百分比0 ~ 80的沉澱區間，將沉澱物復溶於50 mM Tris-HCl buffer (pH 7.8)中，經DEAE-Sepharose CL-6B與Sephacryl S-100膠體管柱純化後，可獲得單一幾丁質?活性波峰，將此具活性樣品收集並進行活性電泳染色分析，發現具幾丁質?活性之分子量分別為60與43 kDa，此酵素之最適反應溫度與pH值分別為40°C與pH 8.0，而溫度穩定性則於10 ~ 40°C時，具有70 %的殘留活性，於50°C下僅剩13.8 %的酵素活性；pH值穩定性則於pH 6 ~ 9具有50%以上的殘留活性。金屬離子對幾丁質?活性影響之測定，Ca2+、Zn2+、Mn2+及Hg2+離子對幾丁質?活性有抑制作用，以Zn2+離子的抑制效果最大，殘留活性僅6.5%。

關鍵詞 : 幾丁質 ; N-乙醯幾丁寡醣 ; 幾丁質? ; 酵素分離純化

目錄

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