本土性Aeromonas sp.幾丁質 之純化與特性分析

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摘 要

本研究從台灣各地篩選具生產幾丁質?〕孝茜錥A初步篩選出11株，其中以DN15與DN23生產的還原醣量較高，此二菌株經NCBI比對DNA序列後，分別命名為Aeromonas hydrophila DYU-Too15與Aeromonas punctata DYU-Too16。藉由改變CB (chitin broth) 培養基中α-幾丁質含量與氮源種類，探討N-乙醢幾丁寡醣生成之培養條件。 菌株DN15與DN23於CB培養基中，改變α-幾丁質含量，對於A. hydrophila DYU-Too15與A. punctata DYU-Too16生產N-乙醢寡醣種類並無影響，但寡醣隨著幾丁質含量上升而增加。以α-幾丁質含量4%培養A. hydrophila DYU-Too15時，在培養96 h時可得到最大量之N-乙醢葡萄糖胺，約3.65 g/L；若以5%之α-幾丁質培養A. punctata DYU-Too16於96 h時，N-乙醢幾丁三醣可得到約1.22 g/L之最高產率。於CB培養基中，以不同氮源 (yeast extract + peptone 、yeast extract、peptone、tryptone、NH4Cl) 種類，對於A. hydrophila DYU-Too15生產N-乙醢寡醣種類並無影響，主要產物仍以N-乙醢葡萄糖胺為主；A. punctata DYU-Too16培養於氮源yeast extract+peptone時，產物以N-乙醢葡萄糖胺與N-乙醢幾丁三醣為主，異於其它四種氮源，產物只以N-乙醢葡萄糖胺為主。將上述兩菌株與實驗室先前篩到之菌株Aeromonas hydrophila DYU-Too14就N-乙醢幾丁寡醣種類與含量作一比較，發現，使用氯化銨為氮源，培養菌株A. hydrophila DYU-Too14時，可生成N-乙醢幾丁五醣與六醣，因五醣與六醣具有增強免疫力、抑制腫瘤細胞生長等生理活性，其價值高於N-乙醢葡萄糖胺或三醣，而決定以此為培養條件，並純化其酵素。以4% α-幾丁質為碳源與0.7 g/L氯化銨為氮源培養菌株A. hydrophila DYU-Too14，其粗酵素液經硫酸銨沉澱、透析、陰離子膠體 (DEAE-Sepharose)層析後，發現於膠體DEAE-Sepharose層析之第90-93與94-98管之酵素液具有幾丁質?′〝坻i峰，因此，將其加入膠態幾丁質溶液，進行水解，離心後凍乾上清液，以HPLC分析其成分，發現水解產物以N-乙醢幾丁五醣與六醣為主。經由電泳分析具幾丁質?′〝坻i峰(粗酵素液經DAEA-Sepharose層析之89-99管)，酵素分子量以25 kDa為主。

關鍵詞 : 幾丁質?｛ BN-乙醢葡萄糖胺、N-乙醢幾丁三醣、N-乙醢幾丁五醣、N-乙醢幾丁六醣

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