BACILLUS SUBTILIS V656所生產微生物抑制物質之研究

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摘 要

本研究主要係以農、水產廢棄物為主要碳源，經由微生物發酵後生產真菌抑制劑，並加以純化及進行生化性質分析。 經由台灣中部土壤篩選出一株菌，其發酵液具有抑制真菌生長之效果，此株菌經鑑定結果命名為BACILLUS SUBTILIS V656 (以下簡稱V656)。其最適培養基中以含有2% 蝦蟹殼粉、0.1％K2HPO4、0.05％MGSO4×7H2O ，填充100ML/250ML三角錐瓶，於PH7、30℃下，振盪培養24小時後，針對抑制FUSARIUM OXYSPORUM而言，可得最大之抑制活性(0.4 U/ML)。而V656所產之粗酵素液其PH值安定性為6~9，但不具有良好的100℃熱穩定性，其抑制活性與菌體生長有正向相乘之關係。 從V656所得發酵液經硫酸銨沉澱、DEAE-SEPHAROSE CL-6B等步驟分離結果，純化出分子量(SDS-PAGE及HPLC)分別為14 KDA及17 KDA之兩種酵素(FⅠ及FⅡ)，並發現此兩種酵素均具有抑制活性及幾丁質?活性，故幾丁質?可能在生物製劑上扮演一重要角色。酵素FⅠ及FⅡ之幾丁質?最適反應PH、最適反應溫度、抑制劑之PH值安定性、100℃熱安定性及等電點，分別為(7、40℃、6-9、3 MIN、5.8)及(6、40℃、5-9、3 MIN、5.3)，且針對FⅠ來說ZN2+可促進酵素活性，但CU2+卻強烈抑制酵素活性，而就FⅡ來說FE2+可促進酵素活性，但NA+卻強烈抑制酵素活性，且FⅠ及FⅡ均受ACETONE及HG2+幾乎完全抑制。 V656發酵液與分離純化後FⅠ及FⅡ對於病原性真菌FUSARIUM OXYSPORUM作用機制經顯微鏡觀察後發現，均會對FUSARIUM OXYSPORUM 之菌絲末端呈現膨大且破裂的現象，亦有不完整之菌絲外觀，與控制組的菌絲於外觀上有極大的差異。 將V656 所生產之真菌抑制劑實際應用於田間實驗及其他菌株之抑制方面，亦均有良好之效果，期日後能量產，應用於我國之農業上。

關鍵詞 : 真菌抑制劑、幾丁質?、生物防治、蝦蟹殼粉、枯草桿菌。

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