Bacilus sp. TKU004所生產耐有機溶劑蛋白?之純化及定性

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摘 要

由台灣中部土壤所篩選出之菌株Bacillus sp.TKU004能以 “ 烏賊軟骨粉末 ” 作為生產蛋白?之主要營養源。Bacillus sp.TKU004生產蛋白?之較適培養條件為: 將含有2%烏賊軟骨粉末、0.1%K2HPO4、0.05%MgSO4．7H2O之100mL液體培養基(pH7)於250mL之三角錐形中，於30℃振盪培養4天後，可得最大蛋白?活性。利用Bacillus sp.TKU004之蛋白質?較適生產條件大量培養，所得發酵液經硫酸銨沉澱、DEAE-Sepharose、CM-Sepharose離子交換層析及Sephacryl S-200膠體層析一連串分離步驟，所得蛋白質?之活性回收率與比活性分別為14%與0.062U/mL。TKU004 蛋白?之生化特性經分析結果，其最適反應溫度60℃、最適反應pH7、熱安定性小於50℃、pH安定性5-8及利用SDS-PAGE分析出來的分子量約為27kDa;活性受到EDTA的抑制，屬於金屬型的蛋白質?;金屬離子對酵素活性的影響，在Fe2+ 和Cu2+ (1.4mM)的存在下，蛋白質?只剩39%與62%的殘餘活性;酵素動力學結果，蛋白質?之Km，Vmax與Ea 分別為3.09 mg/mL、0.16U/mL與32kJ/mole。有機溶劑對酵素活性及安定性之影響方面，在甲醇、乙醇、丙酮與二甲基甲醯胺水溶性有機溶劑下，蛋白質?仍有50%以上的殘餘活性，而在丁醇和異戊醇非水溶性有機溶劑下，蛋白質?只剩約20%的殘餘活性;將酵素與有機溶劑於4℃與25℃下，預反應10天後，皆仍然保有50%以上的殘餘活性。利用TKU004進行烏賊軟骨去蛋白，可達73%之去蛋白率。

關鍵詞 : Bacillus sp. TKU004、蛋白質?、烏賊軟骨、去蛋白

目錄

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