角蛋白?‘芠ㄤ酋鏊尿z選及酵素分析與應用

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摘 要

從彰化養雞場的羽毛廢棄物土壤中，篩選出六株初步命名為Wu1、Wu2、Wu3、Wu4、 Wu5及Wu6之具分泌角蛋白?能力的羽毛降解微生物，經由微生物菌種鑑定並命名為Bacillus megaterium Wu1、Bacillus cereus Wu2、Bacillus cereus Wu3、Brevibacillus parabrevis Wu4、Bacillus thuringiensis Wu5及Bacillus cereus Wu6。除了Wu3之外，其餘五株菌在培養基初始酸鹼值為pH 5.0時，有最大角蛋白?活性，Wu3則是pH 9.0。六株菌最適的生長溫度範圍在30-40oC之間。B. megaterium Wu1是唯一不需額外添加碳氮源，就具有較高的角蛋白?活性。將家禽廢棄羽毛作為六株菌生長時的碳源與氮源，均可產生胞外角蛋白?。將發酵液經過硫酸銨沉澱、Sephacryl S-200 HR膠體層析及DEAE Sephadex A-50離子交換樹脂的純化，角蛋白?Wu1、Wu3、Wu5及Wu6的純化倍率分別為7.63、19.48、2.23及4.71，合成率分別為13.59%、26.32%、16.60%及10.55%。以SDS-PAGE分析純化後酵素的分子量，分別為34、46、32及55與68 kDa，其中角蛋白?Wu6為二聚體。以偶氮酪蛋白為基質，B. megaterium Wu1角蛋白?於pH 4-12.0與溫度範圍為10-100oC下有活性，最適pH與溫度分別為pH 7與50oC。B. cereus Wu6角蛋白?於pH 6.0-11.0，10-100oC範圍內具有活性，最適pH與溫度分別為pH 8與50oC。金屬螯合劑EDTA和O-phenanthroline蛋白?抑制劑會對本研究角蛋白?之酵素活性造成抑制，因此此二種角蛋白?皆金屬型蛋白?。發現於B. megaterium Wu1 和B. cereus Wu6角蛋白?中添加Na+和Mg2+離子可增加酵素活性。B. megaterium Wu1 和B. cereus Wu6角蛋白?以粉末形式貯存於最具有穩定性，若以液態形式存在於室溫下，酵素會快速失活。此外，添加一些有機溶劑對Wu1可穩定酵素活性；添加還原劑則會對B. megaterium Wu1 和B. cereus Wu6的活性造成抑制效果。特別是B. megaterium Wu1 和B. cereus Wu6角蛋白?以偶氮酪蛋白為基質，Wu1和Wu6角蛋白?之Km值，分別為0.85和3.28 g/L。

關鍵詞 : 角蛋白?｛B羽毛廢棄物、金屬蛋白?

目錄

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