第一型抗凍蛋白之產製與生物活性分析

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摘 要

摘要 抗凍蛋白（antifreeze proteins, AFPs）最早於極區魚類之血液及組織發現中，其具有降低水結冰溫度及抑制冰晶生成之作用，使魚類能存活於極區低溫下而防止其凍結。本研究之目的為(1)將已構築於表現載體pET-28b(?)之比目魚第一型抗凍蛋白基因序列送入大腸桿菌，並以isopropylthio-b-D-galactoside (IPTG)誘導其表現而產製此蛋白，經純化後將其應用於斑馬魚胚之冷凍保存，以探討此蛋白之產能及生物活性；(2)將比目魚第一型抗凍蛋白基因序列構築於pEGFP-N1及pAAV-CMV-IRES-hrGFP-Neo載體中，並利用顯微注射法導入斑馬魚受精卵內，以探討其表現，另為往後評估斑馬魚之抗寒能力奠定基礎。利用0.4 mM IPTG誘導AFP產製之結果顯示，經誘導2小時後其產製量較1小時者增加4倍(33.1 mg vs. 7.4 mg)，且其產量隨培養之時間延長而有增加之趨勢，然於3小時至24小時其產量並無顯著差異(39.9～65 mg, p＞0.05)。經誘導6小時後，所測得之AFP濃度平均為65 mg/1L broth。另將純化之AFP以不同濃度(0, 1, 5, 10, 15 mg AFP / mL)添加及5% glycerol等6組作為冷凍保護劑，進行降溫至-10?C，並經螢光染劑判定細胞存活之結果分別為70.2%、82.4%、81.5%、83.4%、84.3%及90.2%，其中以5% glycerol組之存活率較佳，但其與AFP各組間並無顯著差異(p＞0.05)。抗凍蛋白之濃度及冷凍速率需配合以提高冷凍保存之成功率，期望能將此擴展至其他產業之應用。另將構築於不同載體之AFP序列經顯微注射至斑馬魚胚後，於注射pEGFP-N1 (control)、pEGFP-N1-AFP、pAAV-CMV-IRES-hrGFP-Neo (control)及pAAV-CMV-IRES-hrGFP-Neo-AFP組之存活率分別為：58.1%、60.6%、43.8%及34.3%；而表現率則分別為：40.6%、52.9%、9.6%及6.1%，pEGFP-N1 (control)及pEGFP-N1-AFP組之表現率較pAAV-CMV-IRES-hrGFP-Neo (control)與pAAV-CMV-IRES-hrGFP- Neo-AFP者顯著為佳(p＜0.01)。於轉基因斑馬魚基因組中可選殖出似AFP約276 bp大小之片段，經定序確認後，約120 bp大小之片段吻合，相似度為43.4%。期以此建立抗凍蛋白基因轉殖斑馬魚品系，並以此模式應用於其他魚種，為漁業寒害之防治提供對策。

關鍵詞 : 抗凍蛋白、顯微注射、冷凍保存、斑馬魚 抗凍蛋白、顯微注射、冷凍保存、斑馬魚 抗凍蛋白、顯微注射、冷凍保存、斑馬魚 抗凍蛋白、顯微注射、?

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