利用嗜甲醇酵母菌產製重組牛乳鐵蛋白素三元體胜

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摘 要

本試驗主要為探討利用 Pichia pastoris 酵母菌產製重組牛乳鐵蛋白素（Lactoferricin）的單元體（Monomer）、二元體（Dimer）與 三元體（Trimer）的可行性。首先，選擇牛乳鐵蛋白素的其中 20（18-37）個胺基酸片段，由人工合成核?酸片段、經黏合作用後，將此片段基因利用接合作用，構築於酵母菌表現載體 pGAPZαC中，經由自動核酸定序確認後，利用電穿孔的方式將構築好的B-LFcin/ pGAPZα C 單元體、二元體及三元體之質體 DNA，轉殖進入 Pichia pastoris GS115 酵母菌菌株中，經由高倍數抗生素篩選後，選擇有較高拷貝數的轉型株做為實驗菌株，因 pGAPZα C 質體上有 α-factor 序列可將重組牛乳鐵蛋白素釋泌於細胞外，先行使用具有三角凹槽之搖瓶小量發酵重組酵母菌轉型株牛乳鐵蛋白素三元體 B-LFcin/ pGAPZα C Trimer 29，依不同時間點收集上清液，利用分子篩過濾濃縮，進行 Tricine SDS PAGE 和 Western Blot 分析，發現在 10.8kD 的位置上有蛋白質條帶的呈色，因此利用發酵槽產製重組酵母菌轉型株牛乳鐵蛋白素三元體 B-LFcin/ pGAPZα C Trimer 29，收集大量的上清液，經由快速蛋白質液相層析系統經由Hitrap Heparin 管柱，利用不同鹽梯度緩衝液沖提出目標蛋白，進行 Tricine SDSD PAGE 分析後在預期的 10.8 kD 位置上有蛋白質條帶顯示，進而利用 Western Blot 經載體上的 6 個 His tag 和 c-myc 辨識，於預期的位置上亦有蛋白質條蛋顯示，此純化策略應可分離出重組酵母菌轉型株牛乳鐵蛋白素三元體 B-LFcin/ pGAPZα C Trimer 29，但目前在發酵與純化方面仍有待修改條件，以利產製更多的重組酵 母菌轉型株牛乳鐵蛋白素三元體 B-LFcin/ pGAPZα C Trimer 29。

關鍵詞 : 乳鐵蛋白素、嗜甲醇酵母菌

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