

利用重組酵母菌生產玻尿酸

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摘要

玻尿酸(hyaluronan或hyaluronic acid, 簡稱HA)又稱透明質酸, 由葡萄糖醛酸-N-乙醯氨基葡萄糖為雙醣單位組成的直鏈聚合物, 是一種應用廣泛且深具潛力的生物高分子。其具有相當高的保水性、生物相容性及粘性等特性, 使得玻尿酸目前廣泛的應用於眼科、關節炎、整形手術及美容保養品。由於玻尿酸應用廣泛, 市場需求量越來越高, 但傳統的生產方法以從動物組織(如牛眼及雞冠)萃取的方式, 由於取得原料來源有限造成產量低且生產成本極高, 因此近年來發展出了以微生物發酵生產玻尿酸的方法。而所利用的微生物有Lancefield氏A群及C群鏈球菌, 在這種菌株中的玻尿酸是以莢膜(capsule)的型式存在, 而莢膜與菌體的致病性有很大的關係。因此鏈球菌應用於大規模生產時均篩選不具致病力的突變株進行培養。但是不具致病力的突變菌株, 生產玻尿酸的能力會有所降低; 此外發酵過程中是否殘留外毒素、內毒素等有害物質亦不容忽視, 且動物中可能含有透明質酸分解酵素(hyaluronidase), 因而降低萃取之透明質酸之分子量。因此科學界希望找尋其他適合的菌種來生產玻尿酸。因此本研究使用了長時間被人利用生產重組蛋白的啤酒酵母菌(*Saccharomyces cerevisiae*)來改善以上缺點; 分別送入hasB、hasC、IRES、has2及ADH1 promoter構築於兩個表現載體, 並將此兩個表現載體送入酵母菌中經由篩選之菌株希望可生產玻尿酸, 並探討酵素輔因子、受質及短鏈玻尿酸為引子對於產量之影響。實驗結果得知構築完成之啤酒酵母胞內的glucuronic acid產量為42.5 mg/L, 高於未構築之啤酒酵母菌胞內的glucuronic acid產量32.9 mg/L, 而當於培養基中添加入輔因子MgCl₂與受質glucosamine時, 於隨機挑選之菌株中有最高的glucuronic acid產量57.6 mg/L, 而短鏈玻尿酸為引子並未使延長合成玻尿酸分子量, 因此可知於培養基中加入MgCl₂與glucosamine對於玻尿酸產量是有幫助的。

關鍵詞: 玻尿酸, 啤酒酵母菌

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