# 利用獸疫鏈球菌於發酵槽中生產透明質酸及其流變學特性探討 = Study of production of hyaluronic acid from streptococcus ...


## 賴韋光、吳建一

E-mail: 9708313@mail.dyu.edu.tw



**摘 要**

透明質酸(Hyaluronic acid, 簡稱HA)為一高分子量聚合物，是由glucuronic acid和N-acetyl-glucosamine以β-1,3和β-1,4鍵重複鍵結而成。HA是一種高價值的聚合物，可廣泛應用在醫學與化妝品上。HA可以從公雞的雞冠純化出來或者是利用微生物發酵生產得之。從雞冠分離出的HA是利用proteoglycans聚合而成且具有高純度與高分子量的特性。取自於動物的HA對於人體來說是不具有免疫上的問題，因此可應用在人類疾病的治療上，然而利用此方法所得到的HA易被外來的病毒與其他藥劑污染。因此目前普遍上HA的生產來源是利用微生物發酵生產得之的。因此本篇研究主要是探討溫度、攪拌速率與曝氣量對突變Streptococcus zooepidemicus菌株(將之命名為S. zooepidemicus var. HAWU)發酵生產HA之影響及HA發酵液與HA水溶液之流變學性質。本研究主要可分成四個部分，分別如下： 第一部份為利用搖瓶與5-L發酵槽，探討不同培養條件對S. zooepidemicus var. HAWU菌株發酵生產HA及其分子量的影響。在搖瓶部分，在溫度為37°C的條件下，可得最佳HA產量0.78 g/L。在5 L發酵槽中，由實驗結果發現當攪拌速度和曝氣速率同時增加時，可得最大HA產率(0.6 g/h/L)；在曝氣量為1 vvm與攪拌速率為300 rpm的條件下可得最大HA產量6.7 g/L。另外，於20-L發酵槽中利用曝氣速率1 vvm、攪拌速率300 rpm進行發酵生產HA亦具有相同結果。 第二部分為以動力學模式解析S. zooepidemicus var. HAWU生長、HA生產、葡萄糖消耗和氧氣消耗。根據Monod和Michaelis-Meten model的結果顯示，當初始葡萄糖濃度高於最適濃度值( > 20 g/L)時，對S. zooepidemicus var. HAWU來說可觀察到基質抑制生產HA的現象。此外，logistic model除了可合理且精確地模擬S. zooepidemicus var. HAWU生長、HA生產、葡萄糖消耗和氧氣消耗之情形，並證實HA生產行為屬於混和相關模式。 第三部分則是探討在不同的溫度(4-70 ℃)、pH值(1-11)與轉速(10-250 rpm)下，含有不同含量之HA發酵液與不同濃度之純HA溶液之流變學特性。利用修飾後之Power law model可合理解析HA發酵液與純HA溶液的流體行為，並可精確模擬流體的行為曲線。最後利用Arrhenius方程式計算出活化能，並發現到HA的活化能隨著HA含量的增加而減少。 第四部分則是以熱力學解析HA水解反應。在不同HA濃度下，利用不同pH與溫度計算出HA一階降解反應常數。當pH < 5時，會因網狀聚合物內部鍵結作用力的降低而導致HA溶液黏度的降低；而在pH > 5的環境裡，黏度的降低是因為在鹼性環境下，氫氧基會破壞HA網狀結構裡的氫鍵，因而導致HA結構的堅硬度降低。最後，利用反應常數計算出HA水解的activation entropy (△S)與rnthalpy (△H)。



關鍵詞 : S. zooepidemicus var. HAWU ; 透明質酸 ; 發酵動力學 ; 流變學


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