Effect of stress responsible transcription factor Msn 2 on xylose-fermentative yeast Pichia stipitis

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ABSTRACT

With the development of modern industry, fossil fuel consumption is dramatically increasing. New energy research and development are urgent than ever. Biomass energy has some advantages, they are recycles and they are less CO2 is emitted. Both bioethanol fuel and gasoline mixed with ethanol are used worldwide. Currently, there are two so called ethanol crops which are corn and sugar cane. The consumption of corn and sugar in ethanol production results in the raising of the crops price. Therefore, wood fiber alcohol has become a major development. The wood fiber contains about 24 % to 45 % of cellulose (β -polymer of glucose), 22 % ~ 31 % of hemicellulose (contains 80 ~ 85% xylose) and others. The wine yeast, used to ferment the ethanol, use glucose as carbon source easily. However, it cannot directly use the xylose as carbon source. This will leave xylose as waste and contaminate our environment. If any organism can ferment both the cellulose and hemicelluloses, it will help lower the cost, produce more ethanol and leave less waste. In these criterions, P. stipitis is good candidate yeast for this wood sugar fermentation. But P. stipitis alcohol tolerance is poor and the accumulation of ethanol during fermentation causes the death of P. stipitis. This may the reason for poor ethanol production by P. stipitis fermentation. To solve this dilemma, we try to find out the ethanol stress response transcription factors, and transfected into the P. stipitis, expectedly ethanol tolerance will be increased in P. stipitis. In wine yeast, several stress transcription factors have been reported, they are Msn 2, Msn 4 and Skn 7. By semi-quantitative RT-PCR analysis, Msn 2 and Msn 4 have increased 40 % while Skn7 was not detected when 3% ethanol were included in the media. Since the Msn 2P can form either homodimer or heterodimer with Msn4, Msn 2 was cloned into pGAPZ vector, which contains a constitutive promoter. The pGAPZ-Msn2 plasmid was transformed into P. stipitis. The experimental results showed that P. stipitis transformants had higher ethanol tolerance compared to wild-type and pGAPZ transformant. Keywords: bioethanol, xylose, P. stipitis 目錄 封面內頁 簽名頁 授權書iii 中文摘要iv 英文摘要v 誌謝vii 目錄xiii 圖目錄xvi 表目錄xvii 1. 前言1 1.1 木質纖維組成及發酵限制3 1.2 利用六碳糖發酵菌種之比較分析4 1.3 利用五碳糖發酵菌種之比較分析4 1.4 發酵菌種的篩選與改良5 1.5 轉錄因子6 2. 實驗架構及方法8 3. 材料與方法9 3.1 實驗材料9 3.1.1 實驗菌種9 3.1.2 實驗載體9 3.2 實驗方法9 3.2.1 菌種取得與活化9 3.2.2 前培養10 3.2.3 菌體之凍存10 3.2.4 P. stipitis 逆境轉錄因子表現量之半定量判定11 3.2.4.1 P. stipitis RNA萃取11 3.2.4.2 反轉錄聚合?連鎖反應 (Reverse transcription; RT-PCR)11 3.2.4.3 P. stipitis逆境轉錄因子引子之設計12 3.2.4.4 P. stipitis逆境轉錄因子表現量之半定量分析12 3.2.5 逆境轉錄因子Msn 2基因之選殖與定序13 3.2.5.1 P. stipitis染色體DNA萃取13 3.2.5.2 選殖引子之設計13 3.2.5.3 聚合?連鎖反應 （polymerase chain reaction, PCR）14 3.2.5.4 膠體電泳分析14 3.2.5.5 膠體萃取（gel extraction）15 3.2.5.6 連接反應（ligation reaction）15 3.2.5.7 勝任細胞之製備16 3.2.5.8 轉形作用16 3.2.5.9 小量質體抽取17 3.2.5.10 限制?切割作用（Digestion）18 3.2.6 表現載體的製備18 3.2.7 表現載體之構築18 3.2.8 宿主轉形作用19 3.2.9 基因重組菌體培養19 3.3 分析方法19 3.3.1 重組菌酒精耐受度20 3.3.2 西方墨點（Western blot）分析20 3.3.2.1 細胞蛋白質之萃取20 3.3.2.2 蛋白質定量20 3.3.2.3 配置SDS-膠體21 3.3.2.4 SDS膠體電泳分析21 3.3.2.5 電轉印（electroblotting）21 3.3.2.6 西方墨點反應（western blot）22 4. 結果與討論24 4.1 P. stipitis之酒精耐受性24 4.2 逆境轉錄因子Msn 2, Msn 4, Skn 7之表現半定量分析24 4.3 Msn 2 PCR之基因選殖25 4.4 基因定序及序列比對25 4.5 表現載體pGAPZ α ABC之 α -factor剔除26 4.6 基因於表現載體pGAPZ之構築26 4.7 宿主轉型作用27 4.8 轉殖株中Msn 2基因於RNA程度表現之表現PCR分析27 4.9 高濃度酒精下重組菌株的生長情形28 5. 未來展望29 6. 結論31 ?考文獻51 附錄58 圖目錄 圖1. P. stipitis生長之酒精耐受性圖32 圖2. 逆境轉錄因子Msn 2及Msn 4 之mRNA表現量膠圖33 圖3. 逆境轉錄因子Msn 2 mRNA表現量之半定量分析34 圖4. 逆境轉錄因子Msn 4 mRNA表現量之半定量分析35 圖5. 逆境轉錄因子Skn7 表現量之RT-PCR分析36 圖6. 基因Msn 4, Msn 2 PCR擴增圖37 圖7. 基因Msn 2 T/A連接反應之篩選38 圖8. 基因Msn 2序列與NCBI基因庫 之Msn 2序列比對序列3839 圖9. 表現載體pGAPZ α ABC之 α -factor剔除定序圖40 圖10. Msn 2基因自TA-Msn 2純化分離41 圖11. 表現載體pGAPZ-Msn 2之PCR確認圖42 圖12. 表現載體pGAPZ-Msn 2 之構築確認43 圖13. 轉殖株於Msn 2基因於mRNA程度表現 之RT-PCR分析44 圖14. P. Stipitis轉形株之酒精耐受性45 表目錄 表1. 葡萄糖醱酵菌種之比較46 表2. 木糖醱酵菌種之比較47 表3. pGAPZ α ABC載體之圖48 表4. pGAPZ α ABC之序列49 表5. 各類引子表50 ?考文獻 1. 王秀華。2002。木材化學及其應用。台北，國立編譯館。 2. 周楚洋。1998。農業廢棄物處理知回顧與前瞻。高知武教授紀念研討會文輯。第六篇。國立台灣大學農業機械系編印。 3. 鄭宏德、江國瑛、蔡永興、張耀民、黃傳安、洪怡芳、邱文琳、李佩玲。2005。農業廢棄物產出、再利用情形調查與管理制度建立計畫報告。環保署編印。278頁。 4. 林俊義。2007。國際推動生質能源作物之展望。林業研究專訊, Vol. 14,No.3, 35-40。 5. 洪永杰、許博爾。2005。纖維素轉換生質酒精技術專利檢索與分析報告。元智大學 6. 張容慈

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