245 Enhancement of Xylanase Production by Supplement of Mono-sodium glutamate in Recombinant *Bacillus subtilis*

(Department of Biological Sciences, KAIST, Korea) Chang Hoon Rhee, Jung Hoe Kim

[Object] Massproduction of xylanase from recombinant *Bacillus subtilis* and the effect of glutamate supplement on cellular physiology

[Methods and Result] Production of xylanase was studied using a recombinant *Bacillus subtilis* harbouring pJHKJ4 plasmid. As a gene source of xylanase, *Bacillus* sp. originated gene was used because it mainly produced a xylobiose from xylan as a hydrolysis product. In semi-defined medium containing 20 g/L of glucose, 260 IU/mL of xylanase was obtained in 48 hours of cultivation. However, 530 IU/mL of xylanase was obtained when 0.3% (w/v) of glutamate was supplemented to the above medium, which corresponded to 20-30 times increase as compared to 10-20 IU/mL of xylanase with wild type strains in the literature. So the effect of glutamate supplement on cellular physiology was further investigated on the transcription and translation levels. For this purpose, plasmid copy number, mRNA content, rRNA content, and concentration of protein secretion were quantitatively determined as a function of culture time during the fermentation. As results, mRNA content of the cells remained similar level between the culture with/without supplement of sodium glutamate, even though plasmid copy number was significantly decreased in the culture without glutamate supplement after the exponential growth phase. However, by supplementation of sodium glutamate, total cellular RNA level was increased almost twice, especially ribosomal RNA. Extracellular protein expression including xylanase was proportionally with increase of rRNA. It was concluded that glutamate supplement seemed to increase the translational rate by increasing the cellular rRNA content.

[Key words] Xylanase, *Bacillus subtilis*, mono-sodium glutamate

246 麹菌 *A.oryzae* による発芽ﾀﾞｲｽﾞﾌﾟﾛﾃｱｰｾﾞ D3 の分泌発現

○二宮大記 小寺智博 丹尾式希 上田要一 三輪哲也 (味の素㈱ 食品研)

【目的】ﾀﾞｲｽﾞ発芽時に貯蔵ﾀﾝﾊﾟｸ質の分解を担うﾌﾟﾛﾃｱｰｾﾞを探索したところ、ｼｽﾃｲﾝﾌﾟﾛﾃｱｰｾﾞ D3 の単離、精製に成功した[1]。D3 は難分解性の未変性ﾀﾞｲｽﾞﾀﾝﾊﾟｸ質を効率良く分解する活性を有しており、特徴ある食品用酵素の可能性が示唆された。我々はこれまでに D3 の c DNA をｸﾛｰﾆﾝｸﾞし、*E.coli* での発現に成功しているが[2]、菌体内でｲﾝｸﾙｰｼﾞｮﾝﾎﾞﾃﾞｨｰとして発現する為、refolding、活性化の操作が必要となり、結果得られる D3 の活性体は非常に少なかった。そこで本研究では、麹菌 *A.oryzae* で活性体 D3 を大量発現させることを検討した。

【方法、結果】直接発現、並びにｸﾞﾙｺｱﾐﾗｰｾﾞ A との融合発現による２通りの方法を検討した。*TEF-1* ﾌﾟﾛﾓｰﾀｰの下流に D3 遺伝子等を導入し、*argB* 遺伝子をﾏｰｶｰとして *A.oryzae* M-2-3 株に導入した。形質転換体を YPD 培地で培養し、その培養上清中の D3 活性、並びに抗 D3 抗体を用いたｳｴｽﾀﾝﾌﾞﾛﾃｨﾝｸﾞを行った。結果、分泌されたﾌﾟﾛ体 D3 は培養液中で活性型に変化し、培養開始２日目にその蓄積量は最大に達した。収量は直接法で約 1mg/L、融合発現法で約 5mg/L であった。

1)鈴木ら、第 69 回日本生化学会大会要旨集、p757 (1996)
2)小寺ら、1999 年度日本農芸化学会大会要旨集 p48

Expression and secretion of germinating soybean protease D3 in *Aspergillus oryzae*.

○Daiki Ninomiya, Tomohiro Kodera, Noriki Nio, Youichi Ueda, Tetsuya Miwa
(Food Research & Development Laboratories, Ajinomoto, Co., Inc.,)

【Key words】 *Aspergillus*, soybean, protease, recombinant, expression