1019 Cloning and characterization of Δ 9-fatty acid desaturase genes from *Pichia*, *Yarrowia* and *Kluyveromyces*

Sarintip Anamnart[1], Tetsushi Tomita[2], Fumio Fukui[2], Ko Fujimori[1], Satoshi Harashima[1], Yasuji Oshima[1] and Yasuhiro Yamada[1]

[1]*Department of Biotechnology, Faculty of Engineering, Osaka University, 2-1 Yamadaoka, Suita-shi, Osaka 565, Japan;* [2]*Research and Development Center, Showa Sangyo Co., Ltd., 20-2 Hinode 2-Chome, Funabashi, Chiba 273, Japan*

In the area of research on lipid biotechnology, yeasts as well as other microorganisms are considered to be one of potential lipid producers. Although *S. cerevisiae* synthesizes only monounsaturated fatty acids when grown under normal laboratory conditions, some other yeasts such as *Candida*, *Pichia*, *Yarrowia*, *Lipomyces* and *Kluyveromyces* species produce polyunsaturated as well as monounsaturated fatty acids. We are interested in genetic engineering of yeasts for production of high-value unsaturated fatty acids and the regulation of fatty acid desaturation. In this study, we investigated homologues of *S. cerevisiae OLE1* gene encoding Δ9-fatty acid desaturase which catalyzes the insertion of the first double bond to saturated fatty acids. We found that *P. angusta*, *K. thermotolerans* and *Y. lipolytica* have *OLE1*-like genes, which we designated *P-OLE1*, *K-OLE1*, and *Y-OLE1*, respectively. *P-OLE1* was cloned and sequenced. An open reading frame of *P-OLE1* encodes 451 amino acid residues, which shows high identity (62%) and similarity (89%) to that deduced from the *OLE1* nucleotide sequence. Transcription of *P-OLE1* was repressed by oleic acid in the growth medium and *P-OLE1* ORF driven by *GAP* promoter as well as *P-OLE1* complemented the *S. cerevisiae ole1* mutation. We concluded that *P-OLE1* and possibly *K-OLE1* and *Y-OLE1* encode Δ9-desaturase.

Keywords: Acyl-CoA desaturase; gene expression; *Kluyveromyces thermotolerans*; *OLE1*; oleic acid; *Pichia angusta*; *Yarrowia lipolytica*; yeasts

1020 シグナルペプチド非依存型酵素分泌遺伝子(*zliS*)の大腸菌内での発現

（鳥取大・生物応用，*福山大・食品工学）
簗瀬英司，○岩佐恵一郎，岡本賢治，喜多恵子，*外村健三

【目的】グラム陰性のアルコール醗酵性細菌である*Zymomonas mobilis*が生産する菌体外レバンスクラーゼとインベルターゼはN末領域にシグナルペプチド様配列が認められず，これら酵素はGSP (General Secretory Pathway)とは異なる機構で内膜や外膜を透過して分泌されていると推定した．今回は，シグナルペプチド非依存型分泌機構の解明を目的として，*Z. mobilis*由来染色体DNAからクローン化した分泌促進遺伝子(*zliS*)の大腸菌内での発現とその分泌促進機能を検討した．
【方法と結果】*zliS*(推定ORF:553 bp)をpKK223-3の*Ptac*下流に挿入して，pKKZS1を作製した．*zlis*を大腸菌内で発現させたところ，その90%以上のタンパク質が細胞膜画分に局在した．次に，大腸菌内で，*Z. mobilis*由来の菌体外レバンスクラーゼやインベルターゼ遺伝子と*zlis*を共存させて，*zlis*の発現に伴うこれら酵素の大腸菌内での細胞内局在性を検討した．その結果，*zliS*が大腸菌においても酵素分泌促進遺伝子として機能することが明らかになった．

Expression of *Zymomonas zlis* gene that enhances secretion of an enzyme *via* non-GSP in *Eschrichia coli*.

Hideshi Yanase, ○Keiichiro Iwasa, Kenji Okamoto, Keiko Kita, Kenzo Tonomura* (Dept. Biotechnol., Tottori Univ., *Dept. Food Sci., Fukuyama Univ.)

【Key Words】 secretion, ABC transportor, *Zymomonas mobilis*