1P-133 Bacterial surface display of functional enzyme using *Bacillus subtilis* spore

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Bacterial surface display technology has been acknowledged as a useful tool in various fields of biotechnological application such as in vitro evolved enzyme itself, enzymes or proteins involved in whole cell bioconversion, bioremediation, whole cell adsorption and live vaccine production. For the functional bacterial surface display of active enzyme of multimeric form, which is generally impossible due to molecular assembly of the monomer subunit subsequent to the secretion of displayed target protein outside the cell, a new surface display system based on B. subtilis spore should be developed.
Using cotE and cotG of *B. subtilis* as anchoring motives, beta-galactosidase, which is active in tetrameric form, was functionally displayed on the surface of *B. subtilis* spore. The surface localization of beta-galactosidase was verified by Miller assay of purified spore, protease accessibility test of purified spore, and flow cytometric analysis of spore expressing beta-galactosidase. While *B. subtilis* spore wall integrity, examined by lysozyme and heat treatments, was affected by the incorporation of CotE-LacZ fusion protein, it was not affected by the incorporation of CotG-lacZ fusion. Heat stability of displayed protein was similar with that of free enzyme.
In this study, we developed a general method for the efficient surface display system for the tetrameric protein using *B. subtilis* spore. Using the same anchoring motif (cotG), we could successfully surface displayed another tetrameric protein (streptavidin) and dimeric protein (w-transaminase) and GFP. One more advantage of this system is the size of functionally displayed beta-galactosidase (4*116 kDa = 464 kDa), of which size was not possible to be displayed with current bacterial surface display system. This spore display system also overcame the toxicity caused by fusion protein accumulation on the transport machinery or titration of export component.

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Key words *Bacillus subtilis*, spore, surface display, anchoring motif

1P-134 酵母によるタンパクの高効率細胞表層提示ならびに分泌生産のための新規分泌シグナル配列の検討

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【目的】近年、低炭素循環型社会実現の観点から、再生可能資源であるリグノセルロース系バイオマスからエタノールを製造する技術の導入が望まれている。その普及には植物原料をエタノールに変換するための多段階の工程を統合し、効率化した、低コスト型同時糖化発酵プロセスの構築が急務である。そこで我々は、セルラーゼやヘミセルラーゼを細胞表層に提示することで糖化能力を付与した酵母の作製と、その細胞表層における酵素活性の向上を試みている。本研究では、セルラーゼの高効率細胞表層提示を可能にする分泌シグナル配列の探索と、その特性解析を行った。
【結果】異なる遺伝子に由来するいくつかの分泌シグナル配列をβ- グルコシダーゼ（BGL）遺伝子の 5’ 末端にそれぞれ付与し、BGL を酵母の細胞表層に提示させた場合と細胞外へ分泌させた場合のそれぞれについて、酵素活性に対する影響を検討した。その結果、表層提示型、分泌型ともに、酵母の主要な細胞壁タンパクをコードする SED1 遺伝子由来の分泌シグナル配列を付与された株が最も高い BGL 活性を示した。また、SED1 分泌シグナル配列は、エンドグルカナーゼ（EG）を表層提示させた場合にも最も高い活性を示した。さらに、SED1 分泌シグナル配列は異なる複数のプロモーター配列との組合せにおいても安定して高い活性を得られることが明らかとなった。

Development of the novel secretion signal sequence for highly-efficient cell surface display and secretory production of proteins by yeasts

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Key words cell surface display, cellulase, *Saccharomyces cerevisiae*, secretion signal

1P-135 Improvement of weak acid-tolerance of a recombinant xylose-fermenting industrial *Saccharomyces cerevisiae* strain

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【Introduction】In production of bioethanol from lignocellulosic biomass, xylose-fermenting *Saccharomyces cerevisiae* strain with good tolerance to inhibitors is of utmost importance. In this study, the effect of *TAL1* (encoding transaldolase) overexpression and *PHO13* (encoding *p*-nitrophenylphosphatase) deletion on the tolerance of weak acid of xylose-fermenting strain NAPX37 was studied.
【Methods and Results】The effect of differential expression of *TAL1* was firstly investigated. Ethanol yields of all *TAL1* overexpressed strains increased in the absence or presence of weak acid inhibitors. Ethanol productivity of strain NAPX37/AHP1p at 60 mM formic acid or levulinic acid is 1.6-fold and 1.3-fold higher than that of the parental strain NAPX37, respectively, but ethanol productivity of strain NAPX37/UBI4p at 60 mM acetic acid exhibit 1.8-fold higher than that of strain NAPX37. Furthermore, the synergistic effects of *TAL1* overexpression and *PHO13* deletion on the improvement of ethanol production and the reduction of xylitol accumulation in the presence of weak acids were confirmed. Either *TAL1*overexpression or *PHO13* deletion could improve the efficiency of xylose fermentation in the presence of weak acids. Simultaneous overexpression of *TAL1* and deletion of *PHO13* had synergistic effects on the fermentation of xylose in the presence of weak acids. However, the improvement of weak acid-tolerance in this study is not as significant as those studies which laboratory yeast strains were used.

Improvement of weak acid-tolerance of a recombinant xylose-fermenting industrial *Saccharomyces cerevisiae* strain

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Key words lignocellulosic bioethanol, *TAL1* gene, *PHO13* gene, xylose fermentation