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# Yeast as a Protein Factory for Biocatalysts and Pharmaceutical Proteins

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Recombinant production of valuable proteins limited in nature has been recognized as an essential field not only for human biopharmaceuticals and industrial biocatalysts but also for the functional genomics in the post-genome era. It is necessary to develop a stable and easy-to-use expression system to express and purify in large-scale of useful proteins. Yeast *Saccharomyce cerecvisiae* as a eukaryotic microorganism has long been generally recognized as safe (GRAS) organism for human and has several advantages over other expression system such as *Escherichia coli* and mammalian cells. *S. cerevisiae* has an excellent pathway of secretion and post-translational modifications of protein which are similar to those of higher eukaryote. Thus, it can produce various complex proteins originated from higher eukaryotes including human, which are often expressed insoluble forms in *E. coli*. Furthermore, secretory production of foreign proteins using yeast greatly simplifies the purification procedure and reduces the production cost of proteins.

Although *S. cerevisiae* has advantages for the production of foreign proteins, a current obstacle as a general host seems to be the secretory productivity of proteins. Generally, yeast has a secretion capacity of 50 to 200 mg/liter of proteins. But it varies according to the type of proteins. For example, human serum albumin (HSA) could be secreted up to several g/liter in *S. cerevisiae*. In contrast, secretory productivity of human pharmaceutical proteins, such as interleukin-2 (IL-2), granulocyte-colony stimulating factor (G-CSF) and platelet-derived growth factor (PDGF) were extremely low, less than several mg/liter. Due to such discrepancy between proteins, the secretory productivity of a foreign protein can not be estimated until it is measured. Therefore, for the development of yeast secretion pathway as a general process for protein factory, such limitations must be overcome.

To minimize the inconsistency of secretion level between proteins and improve the secretion level of rarely secretable proteins, we have developed a high throughput screen to obtain an optimal translational fusion partner (TFP) leading to efficient secretion of each protein, from yeast genome. An invertase-based autoselection system has been designed for the positive selection of cells secreting a rarely secretable protein through a translational fusion of a random peptide generated from genomic DNA library. Using this system, 4 different TFPs(TFP1 to 4), rendering an efficient secretion of IL-2, were obtained. To evaluate the TFPs obtained in this study, several recombinant strains producing pharmaceutical proteins (IL-2 and

G-CSF) and industrial enzymes (lipase B of *Candida antarctica* (CalB), lipase of *Rhizopus oryzae* (ROL), xylanase of *Bacillus subtilis* and CGTase of *Brevibacillus brevis*), were developed. Rarely secretable small proteins, IL-2 and G-CSF could be efficiently secreted into culture supernatant using TFP1 as a secretion partner. In contrast, no secreted IL-2 and G-CSF were found when commonly adopted yeast secretion signal, mating factor a pre-pro leader (MFa) were used. In case of industrial enzymes, TFP3 were commonly efficient for CalB, ROL, xylanase and CGTase. Fed batch fermentation of a recombinant yeast producing CalB resulted in the secretion of around 1.5-2.0 g/liter into culture supernatant.

In conclusion, several translational fusion partners obtained by invertase-based autoselection system could improve the secretion level of IL-2 up to several hundred folds. TFPs were also found to be useful for the secretion of other human cytokines and industrial enzymes. Accordingly, TFPs obtained in this study will be valuable for the secretory production of many other protein resources in yeast. Furthermore, there are still numerous uncovered TFPs in the yeast genome which will be optimal for the secretion of various proteins. High throughput screen developed in this study can search an optimal TFP rapidly for various kind of protein. The TFP technology will give a new chance for yeast to be a protein factory for biocatalysts and pharmaceutical proteins.