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Global Analysis of *Saccharomyces cerevisiae* Strains Expressing a Recombinant Human Protein

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Yeast is a suitable host organism for a high-level production of secreted soluble proteins of human origin. Indeed, many kinds of pharmaceutically important proteins have been expressed in yeast. Especially, *Saccharomyces cerevisiae*, the molecular and cellular biology of which has been intensively studied, has been exploited as a host for heterologous protein production (Romanos *et al.*, 1992). Moreover, its use in food fermentation for thousands of years proved that *S. cerevisiae* causes no harm to human beings and the processes for the production of therapeutic proteins using *S. cerevisiae* acquired GRAS (generally recognized as safe) status. Altogether, these features make *S. cerevisiae* one of the most suitable organisms for heterologous protein expression.

To develop a protein super-secreting strain of *S. cerevisiae*, overall cellular metabolism governing protein secretion, protein degradation, stress response, and energy should be comprehensively modified at the genome level. In this study, we constructed several mutants in the backgound of the *S. cerevisiae* 2805 strain through gene disruption and UV mutagenesis and analyzed their ability to secrete a recombinant protein, the tissue inhibitor of metalloprotease-2 protein fused to the C-terminus of HSA (HSA-TIMP-2, 87 kDa). Although TIMP-2, which is known to act as a key inhibitor molecule in angiogenesis and cancer metastasis (Seo et al. 2003), is barely secreted in yeast, the fusion protein HSA-TIMP-2 is secreted in tens of milligram quantity in *S. cerevisiae* 2805.

It was found that one of the mutant strains generated in this study, Y28G1H2UV74, was able to secrete much higher level of the recombinant protein than other mutant and wild type strains. Analysis of culture supernatant revealed that Y28G1H2UV74 produced less ethanol than the other strains, suggesting that Y28G1H2UV74 may have increased respiratory activity during respiro-fermentative growth in the presence of glucose.

To investigate the reason why Y28G1H2UV74 has the improved physiology for heterologous protein production compared to other *S. cerevisiae* strains, whole genome microarray analyses of Y28G1H2UV74 and other *S. cerevisiae* strains expressing HSA-TIMP-2 were carried out using Affymetrix yeast chip. One of the interesting findings made by the microarray analysis was that genes involved in iron transport and homeostasis were remarkably up-regulated in Y28G1H2UV74, which suggests that efficient respiration may be related with the improved ability of Y28G1H2UV74 strain to secrete recombinant proteins. Since

the Y28G1H2UV74 strain appears to possess several of the desirable properties of a host strain for the production of heterologous proteins using the *GAL* promoters, Y28G1H2UV74 is expected to be exploited as a host strain for the production of valuable heterologous proteins, or as a platform strain for further development.

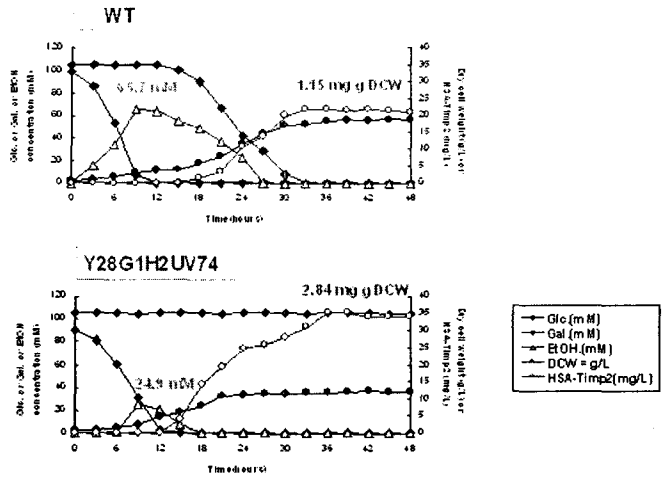

Figure 1. Fermentation kinetics of the wild type (2805) and Y28G1H2UV74 mutant strains during batch culture.

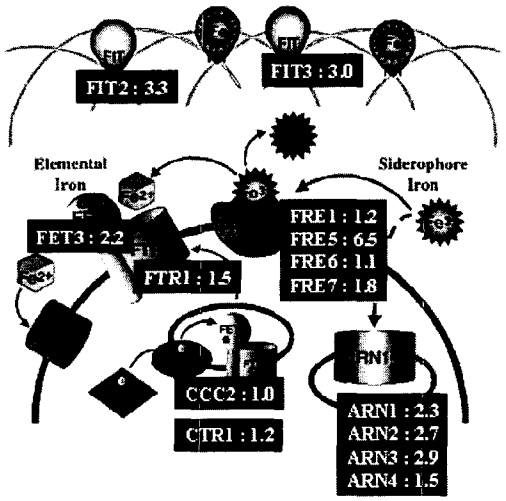

Figure 2. Up-regulated genes in the Y28G1H2UV74 mutant strain expressing HSA-TIMP-2.

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