SCREENING FOR YEASTS WITH ENHANCED PROTEIN SECRETION EMPLOYING FUNCTIONAL COMPLEMENTATION OF CELL-WALL DEFECTIVE PHENOTYPE

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Introduction. Gas1 protein is a beta(1,3)-glucanosyl transglycosylase playing an essential role in the assembly of cell wall as localized on the yeast surface through a glycosylphosphatidylinositol (GPI) anchor (1). When *GAS1* gene was disrupted in several yeasts including *Saccharomyces cerevisiae* and *Pichia pastoris*, the resulting mutant strains were reported to exhibit hypersensitivity to cell wall-perturbing reagents and temperature-sensitive phenotype together with increased capability of protein secretion due to the loosened cell wall structure (2-3). Functional complementation of this cell wall-defctive phenotype of *GAS1*-deletion mutant using recombinat expression of Gas1 fusion protein was employed to generate a screening system for a strain with improved capability of protein secretion.

Methods. We constructed the expression vectors encoding fusion proteins with *N*-terminal secretory protein of interest (glucocerebrosidase and alpha-galactosidase) linked to Gas1 proteins without signal sequence (Fig. 1). After these vectors were transformed into *GAS1*-deletion mutant, the growth of the resulting transformants was checked on the agar plates containing cell wall-perturbing reagents.

Results. *S. cerevisiae Gas1* null mutant displayed hypersensitivity to cell wall-perturbing reagents such as Calcofluor White and Congo Red. Recombinant expression of Gas1 protein restored the cell wall-defective phenotype of *Gas1* mutant (Fig 2). When Gas1 fusion proteins containing a secretory protein in N-terminal part were expressed in *Gas1* mutant, their resistances to cell-perturbing reagents correlated with the secretion ability of expressed protein. For example, glucocerebrosidase-Gas1 fusion protein (GCase-Gas1), which showed poor secretion ability, hardly restored cell-wall defective phenotype while alpha-galactosidase-Gas1 (GLA-Gas1) showed better restoration (Fig 2). Currently, we are exploring the possiblity that this system can be used to enrich yeasts with improved secretion capability after genome-wide random mutagenesis, which would lead to the development of Gas1-based screening system for super secretory yeasts.

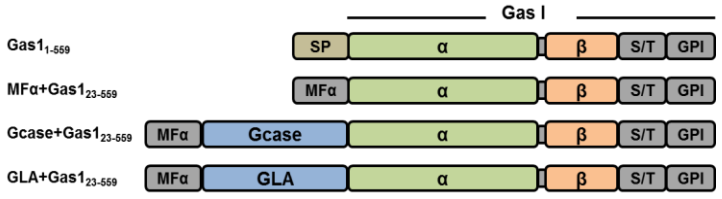

Fig.1 Schematic representation of Gas1 fusion proteins

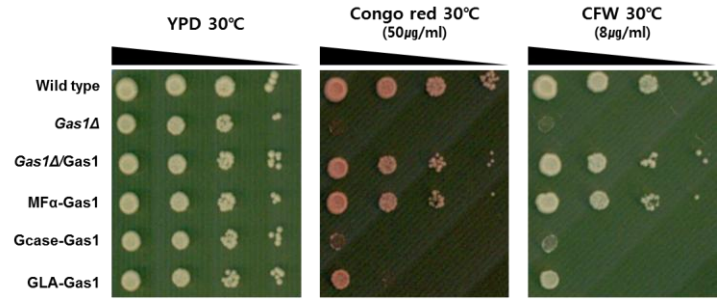

Fig 2. Growth phenotypes of *S. cerevisiae Gas1*-disrupted strains expressing Gas1 and fusion proteins.

Conclusions. We developed the screening system for yeasts with enhanced protein secretion using the cell-wall defective phenotype of *Gas1*-disrupted mutant and its functional complementation using the expression of Gas1 fusion protein.

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