Construction of acid-tolerant yeast by artificial improvement of cell surface

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Cell surface is only a place contacting with surrounding and all organisms have improved their cell surfaces for many times for the survival in evolutionary history. Development of "yeast molecular display system" enables artificial improvement of the cell surface. This system enables the display of many functional proteins and peptides on the yeast cell surface, and is expected as an innovative technology to create novel functional proteins and cells. We have already succeeded in creating an attractive protein displayed on the yeast cell surface that endows yeasts with organic-solvent tolerance [1]. This research is designed to create novel peptides displayed on the cell surface that endow yeasts with acid-tolerance. Construction of acid-tolerant yeast would enable wide applications of yeasts as biocatalysts for bioremediation and efficient production of chemical compounds under acidic conditions.

The multi-copy plasmid, pKRD1 for the display of combinatorial peptides using molecular information of α-agglutinin was constructed. This plasmid consists of GAPDH promoter, signal sequence of glucoamylase, multi-cloning site, red fluorescent protein (DsRed2) gene, FLAG epitope tag-encoding gene, and 3'-half of α-agglutinin-encoding gene. DNA fragments encoding combinatorial peptides consisting of 25 amino acid residues were inserted into multi-cloning site of pKRD1. These plasmid libraries were introduced into yeasts and the transformants were screened under the acidic condition that wild-type yeast strain cannot survive. As a result, several clones showing acid-tolerance were obtained. Cell surface display of screened peptides was confirmed by immunofluorescence labeling. Subsequently, these peptides displayed on the yeast cell surface were analyzed. The DNA sequences encoding these peptides were determined by DNA sequencer. From the results, the amino acid sequences were deduced, and their secondary structures and properties were predicted using computer programs (SOSUI, SSThread, and BLAST). These peptides were hydrophobic overall, and had polar amino acid regions and nonpolar amino acid regions separately. Consequently, there are two possibilities: nonpolar amino acid regions protect yeasts from acidic environment by covering the yeast cell surface, or displayed peptides interact with cell surface proteins such as ion channels in the cell membrane.

[1] Zou, W., Ueda, M., and Tanaka, A. (2002) *Appl. Microbiol. Biotechnol.* **58**, 806-812