



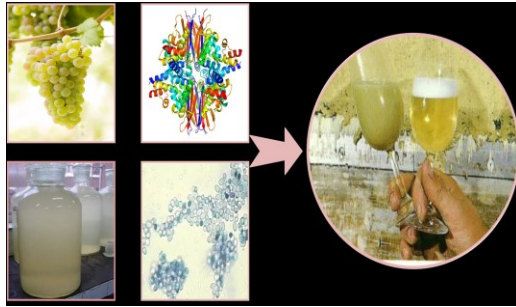
Study on Expression of Acid Protease Using *Saccharomyces cerevisiae* Surface Display Technology

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Introduction



In white wine, unstable proteins from fermented raw materials and excipients cause aggregation of protein and other components, resulting in turbidity and protein deposition. Acid protease can catalyze hydrolysis of protein under pH 2.5-4.5 conditions to generate amino acids and peptides, which is widely used in alcohol fermentation, beer, clarification of fruit wine and other industries.

Materials & Methods

In this study, *Aspergillus usarii* was used as the donor of acid protease gene (*pepA*), and the *pepA* of *A. usarii* was integrated into the gene site of *Saccharomyces cerevisiae* by yeast cell surface display technology (Fig. 2). In the fermentation process of *S. cerevisiae*, the unstable protein was hydrolyzed into amino acids and small peptides to solve the problem of protein turbidity in white wine and provide an absorbable nitrogen source for the growth of yeast.

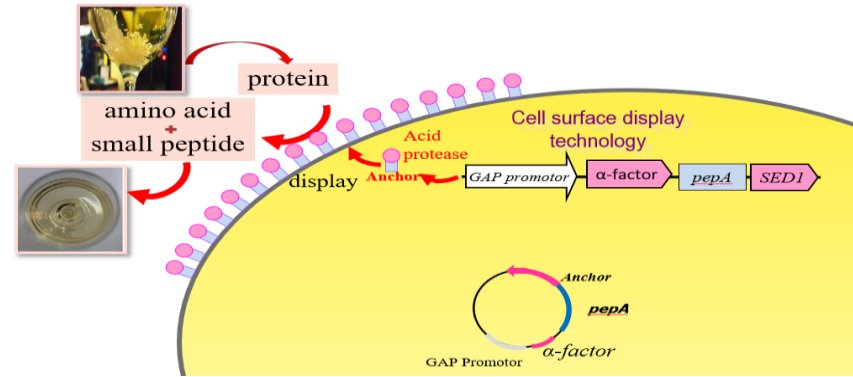


Fig.2. Schematic design of *pepA* displayed on yeast cell surface

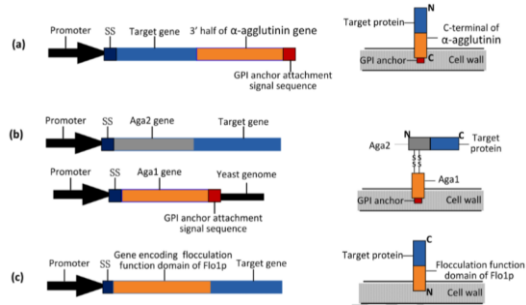


Fig.1. Schematic illustrations of yeast surface display systems using α -agglutinin (a), α -agglutinin (b) and N-terminus flocculation function domain of Flo1p (c). SS indicated signal sequence.

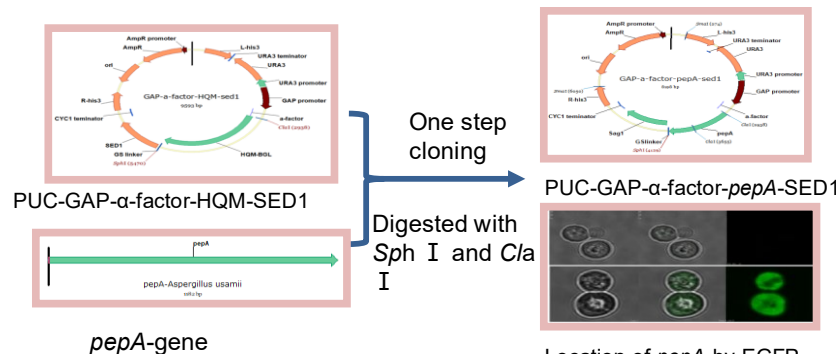


Fig.3. Construction process of recombinant plasmid

Determination of acid protease (*pepA*) activity

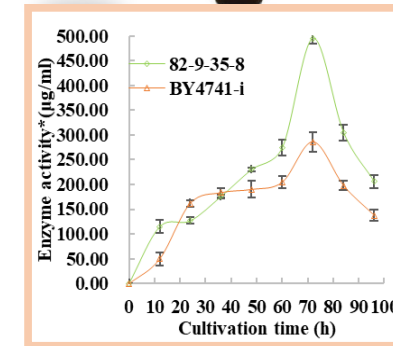
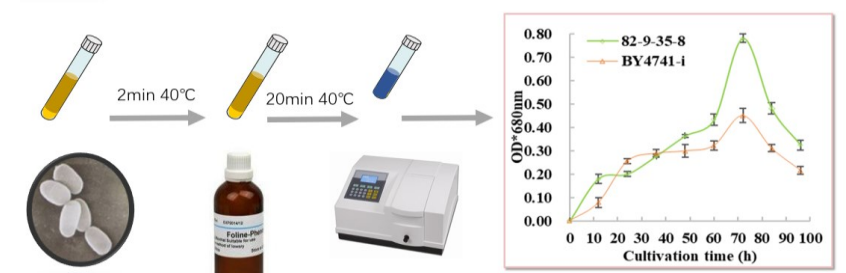


Fig.4. Changes of enzyme activity of BY4741-i and 82-9-35-8 on YPD medium

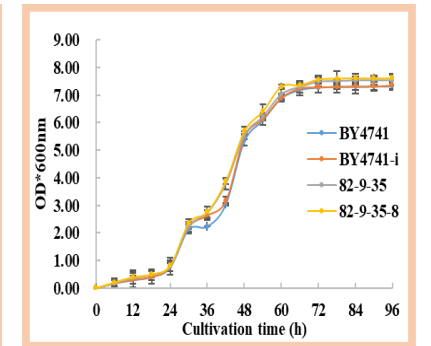


Fig.5. Growth curve of recombinant yeast on YPD medium.

Conclusions

- The α -lectin surface display system of *S. cerevisiae* was successfully constructed with SED1 as anchor protein and gap as promoter. Two strains of *S. cerevisiae* expressing acid protease by yeast cell surface display technology were obtained, namely haploid BY4741-i and diploid 82-9-35-8.
- In this study, acid protease gene (*pepA*) was cloned and integrated into *Saccharomyces cerevisiae* by homologous recombination. Haploid and diploid *S. cerevisiae* were obtained successfully, and the highest enzyme activity was 285.71u/ml and 495.24u/ml respectively.