Yeast cell surface display: An efficient strategy for improvement of bioethanol fermentation performance

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ABSTRACT
The cell surface serves as a functional interface between the inside and the outside of the cell. Within the past 20 y the ability of yeast (Saccharomyces cerevisiae) to display heterologous proteins on the cell surface has been demonstrated. Furthermore, S. cerevisiae has been both developed and applied in expression of various proteins on the cell surface. Using this novel and useful strategy, proteins and peptides of various kinds can be displayed on the yeast cell surface by fusing the protein of interest with the glycosylphosphatidylinositol (GPI)-anchoring system. Consolidated bioprocessing (CBP) using S. cerevisiae represents a promising technology for bioethanol production. However, further work is needed to improve the fermentation performance. There is some excellent previous research regarding construction of yeast biocatalyst using the surface display system to decrease cost, increase efficiency of ethanol production and directly utilize starch or biomass for fuel production. In this commentary, we reviewed the yeast surface display system and highlighted recent work. Additionally, the strategy for decrease of phytate phosphate content in dried distillers grains with solubles (DDGS) by display of phytase on the yeast cell surface is discussed.

KEYWORDS
anchoring protein; biocatalyst; bioethanol production; phytase; Saccharomyces cerevisiae; surface display system

Introduction
Glycosylphosphatidylinositol (GPI) anchors not only play critical roles in the surface expression of cell surface proteins but they are also essential for the viability of the yeast.1,2 Many glucanase-extractable proteins contain these GPI anchors, such as agglutinin (Ago1p and Aga1p) and flocculin (Flo1p, Sed1p).2 Among these, Ago1p, Aga1p and Flo1p have been studied extensively and used successfully for development of a yeast display system. In S. cerevisiae cells the localization of GPI-anchored proteins on the cell surface is accomplished through the general secretory pathway, release from the plasma membrane and transferring to the outermost surface of the cell wall.3 Three surface display systems using α-agglutinin, a-agglutinin and Flo1p as GPI anchors are illustrated in Fig. 1, respectively.

α-Agglutinin (Ago1p) exists in mating type α cells of S. cerevisiae. α-Agglutinin is the most frequent anchor in N-terminal fusion displays, in which the N terminus of the anchor protein is genetically fused to the C terminus of the target protein (Fig. 1a). Moreover, when there are differences in the size of the C-terminal region of α-agglutinin the capability of surface displays of foreign protein is affected. Comparably, a-agglutinin consists of 2 subunits, Aga1p and Aga2p. Aga1p is incorporated into the cell wall through a GPI anchor, and the secretion-type protein Aga2p is conjugated to Aga1p via a disulfide bond (Fig. 1b). Generally, Aga2p is used as anchor for C-terminal fusion to display foreign protein in yeast surface.4,5 The heterologous proteins are fused to the C-terminus of the 69 amino acid binding subunit Aga2p (Fig. 1b). Both Ago1p and Aga1p contain a secretion-signal region, an active region, a support region rich in serine and threonine and a putative GPI anchor-attachment signal. These proteins presumably exist in heavily O-glycosylated forms.6,7

Flocculin Flo1p plays an important role in flocculation. Flo1p is used for N-terminal fusion displays, in which a heterologous protein is fused to the C-terminal region of Flo1p. This consists of a
GPI-attachment signal with various anchor lengths (Fig. 1c). Also, truncated forms of Flo1p (FL and FS) were used as anchors for C-terminal fusion experiments.7

*S. cerevisiae* has the ability to produce and express many of the functional proteins necessary for post-translational modification and in a range of different sizes. This property lends *S. cerevisiae* to be uniquely useful among the various display systems currently available. Additionally it is capable of conferring novel additional abilities upon living cells. As cell-surface engineering enters a new era of combinatorial bioengineering in the field of biotechnology there are more options for use of *S. cerevisiae* to play a significant role.

This commentary describes molecular display using *S. cerevisiae* and its applications in bioethanol production. We also highlight recent studies pertaining to anchoring phytase on *S. cerevisiae* cell surface for ethanol production.

**Applications in expanding substrates for bioethanol production**

Due to environmental pollution and the depletion of oil reserves, bioethanol has become one of the most promising alternatives to conventional fossil fuels because of its high octane value and combustion efficiency.7 Therefore, low production cost, high ethanol fermentation yield and expanding substrates are very important for industrial bioethanol refinery. In recent years, bioethanol production from different substrates using *S. cerevisiae* surface display system has been

Table 1. Applications of yeast cell surface display during bioethanol production.

| Anchor protein | Strategy* | Products and substrates | Reference |
|----------------|-----------|-------------------------|-----------|
| *α*-Agglutinin | Co-display of EG and BGL for ethanol production | Ethanol production from β-glucan | 16 |
| *α*-Agglutinin | Co-display of EG, BGL, CBH for ethanol production | Ethanol production from amorphous cellulose | 17 |
| *α*-Agglutinin | Co-display of xylanase and β-xylosidase | Ethanol production from xylan | 18 |
| *α*-Agglutinin | Display of BGL | Ethanol production from xylose/cellobiose | 19 |
| *α*-Agglutinin | Co-display of BGL and EG from *A. oryzae* | Ethanol production from β-glucan | 10,11 |
| *α*-Agglutinin | Co-display of α-amylase, glucoamylase, EG, BGL, CBH | Ethanol production from cassava pulp | 20 |
| *α*-Agglutinin | Display of phytase | Ethanol production from starch | 9 |
| Aga2 | Display of trifunctional minicellulosomes | Ethanol production from phosphoric acid-swollen cellulose | 5 |
| Aga2 | Display of a trifunctional scaffoldin using a synthetic yeast consortium | Ethanol production from trifunctional minicellulosome | 21 |
| Aga2 | Display of minicellulosomes (2 individual minicellulosins) | Ethanol production from trifunctional minicellulosome | 4 |
| Flo1p | Co-display of glucoamylase and *α*-amylase | Production of ethanol from raw corn starch | 22 |
| Flo1p | Co-display of glucoamylase and *α*-amylase | Ethanol production from starch | 23 |

*EG indicates endoglucanase; CBH indicates cellobiohydrolase I; BGL indicates β-glucosidase.*
studied extensively. Table 1 summarizes some prominent bioethanol production strategies using cellulosic materials and starch. Lignocellulose is particularly attractive in this context because of its widespread abundance and low cost. However, the central technological impediment to more widespread utilization of lignocellulose is the absence of a low cost technology to break down its major component, cellulose. Degradation of cellulose requires cellulase, which includes endoglucanase, cellobiohydrolase and β-glucosidase. Cellulase is the primary cost for lignocellulosic biofuel production. However, *S. cerevisiae* does not produce sufficient amounts of cellulase. To develop an efficient bioethanol production process using cellulosic materials as substrates, different groups developed novel biocatalysts (Table 1).

Recently, *S. cerevisiae* was engineered through display of minicellulosomes on the cell surface to directly convert the microcrystalline cellulose into bioethanol. The resulting strain could produce 1,412 mg/L ethanol in fermentation of carboxymethyl cellulose. Cellulase-displaying *S. cerevisiae* was also used as whole-cell biocatalysts for bioethanol production from other substrates (Table 1). Kotaka et al. constructed transformants to co-display both β-glucosidase and endoglucanase from *Aspergillus oryzae*. The co-displaying strain could produce 7.94 g/L from 20 g/L barley β-glucan, in which the conversion ratio of ethanol from β-glucan was 69.6% of the theoretical ethanol concentration. After then, they constructed another recombinant *S. cerevisiae* strain that expresses glucoamylase from *A. oryzae*; this construct can produce 18.5 g/L ethanol from 50 g/L liquefied starch with a 64.9% ethanol conversion efficiency. More recently, Liu et al. engineered a *S. cerevisiae* strain that is capable of co-displaying β-glucosidase, endoglucanase and cellobiohydrolase I. The resulting strain could produce 2.9 g/L ethanol from 10 g/L phosphoric acid swollen cellulose. Similarly, an engineered strain of *S. cerevisiae* was developed to co-display heterologous α-amylase and glucoamylase; the resulting strain yielded 22.5 g/L of ethanol from 100 g/L of raw starch after 120 h of fermentation.

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No potential conflicts of interest were disclosed.

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