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Research review paper

Cell surface engineering of industrial microorganisms for biorefining applications

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ABSTRACT

In order to decrease carbon emissions and negative environmental impacts of various pollutants, biofuel/biochemical production should be promoted for replacing fossil-based industrial processes. Utilization of abundant lignocellulosic biomass as a feedstock has recently become an attractive option. In this review, we focus on recent efforts of cell surface display using industrial microorganisms such as *Escherichia coli* and yeast. Cell surface display is used primarily for endowing cellulolytic activity on the host cells, and enables direct fermentation to generate useful fuels and chemicals from lignocellulosic biomass. Cell surface display systems are systematically summarized, and the drawbacks/perspectives as well as successful application of surface display for industrial biotechnology are discussed.

Contents

1. Introduction

Utilization of biomass as a feedstock for the production of biofuels and bio-based chemicals has recently become an attractive option, due to the depletion of fossil fuels and with the environmental issues posed by fossil fuels. One of the main feedstocks for biofuel/chemical

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production is starch-rich biomass, which is easily depolymerized by amylases to generate high yields of glucose. Other feedstocks are lignocellulosic biomass such as sugar cane bagasse, corn stover, rice and wheat straw, and switchgrass. Lignocellulosic biomass is considered an especially promising starting material for biofuel and chemical production, given these feedstocks' abundance, low expense, renewability, and favorable environmental properties. Lignocellulose is composed primarily of cellulose, hemicellulose, and lignin. Cellulose, the main framework of lignocellulose, is composed of chains of glucose moieties linked by β-1,4 bonds. These chains are in turn linked by hydrogen bonds, resulting rigid structure. The cellulose chains are enclosed by hemicellulose and lignin, which are more complex than that of cellulose and contain xylose, and other monomeric sugar components. Lignin is composed of phenylpropane units such as p-coumaryl (Van Dyk and Pletschke, 2012). Conversion of lignocellulose is much more time- and cost-intensive than that of starchy grains, due to the need for extensive pretreatment and introduction of relatively large amounts of cellulases for effective hydrolysis (Yamada et al., 2013). Thus, efficient and cost-effective methods for the degradation of lignocellulose are required for lignocellulosic biorefining.

The most advanced integrated process for lignocellulose-based industrial biotechnology is called consolidated bioprocessing (CBP) (Hasunuma and Kondo, 2012; Olson et al., 2012). In CBP, all biological processes (including enzyme production, saccharification, and hexose and pentose fermentation) are conducted in a single reactor by a single microorganism, requiring only a single operation. Although optimization of CBP and development of ideal microorganisms are challenging issues, the cost of facilities and operation is expected to be drastically reduced compared to separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes. Recent research has focused considerable attention on process optimization and/or development of microorganisms for CBPs designed for efficient production of biofuels and chemicals (la Grange et al., 2010; Lynd et al., 2005; Tamaru et al., 2010; Xu et al., 2009).

For efficient CBP, both the degradation of lignocellulose into monosaccharides (e.g., glucose and xylose) and the production of large amounts of biofuels or chemicals from these monosaccharides are required. Metabolic engineering and synthetic biology tools have directly contributed to the improved productivity of useful chemicals, fuels, pharmaceuticals, polymers, and proteins. One successful approach has been to endow native cellulolytic microorganisms with the ability to produce high-value products; these processes have been reviewed previously (Maki et al., 2009; Parisutham et al., 2014; Peterson and Nevalainen, 2012; Tamaru et al., 2010). In the present review, we focus on cell surface display in several industrial microorganisms and the application of this technology for biofuel/chemical production. Cell surface display endows host cells with biomass-degrading activity, rendering organisms suitable for CBP (Fig. 1). Displaying cellulase(s) on the cell surface can expand the availability of carbon sources and several kinds of display systems have been developed each kinds of host microorganisms. We discuss recent achievements, perspectives, and limits that concern the cell surface engineered microorganisms for industrial applications.

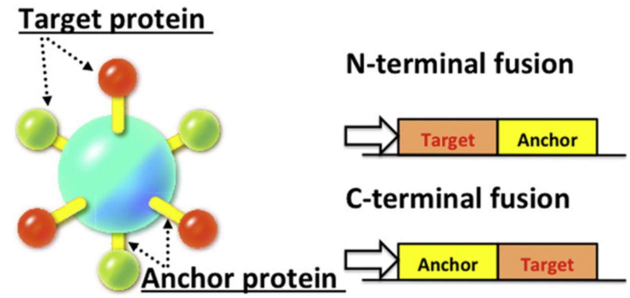

Fig. 2. Schematic illustration of N-terminal or C-terminal cell surface display system.

2. Overview of cell surface display system

Cell surface display is a powerful tool for endowing novel functions on the host cell by displaying functional proteins on the cell surface. Amylase-displaying yeasts have amylolytic activity, and cellulose-displaying yeast and bacteria have cellulose-depolymerizing activity. In surface display, different types of enzymes are located in close proximity, and this proximity could improve their synergism. In nature, the specific activity of the cellulosome, a structure in which cellulases are located in close proximity, was reported to be 50 times higher than that of the *Trichoderma reesei*-secreted (freely-diffusing) cellulases (Schwarz, 2001). In producing ethanol from hydrothermally pretreated rice straw, the display of cellulases on the yeast cell surface allowed a

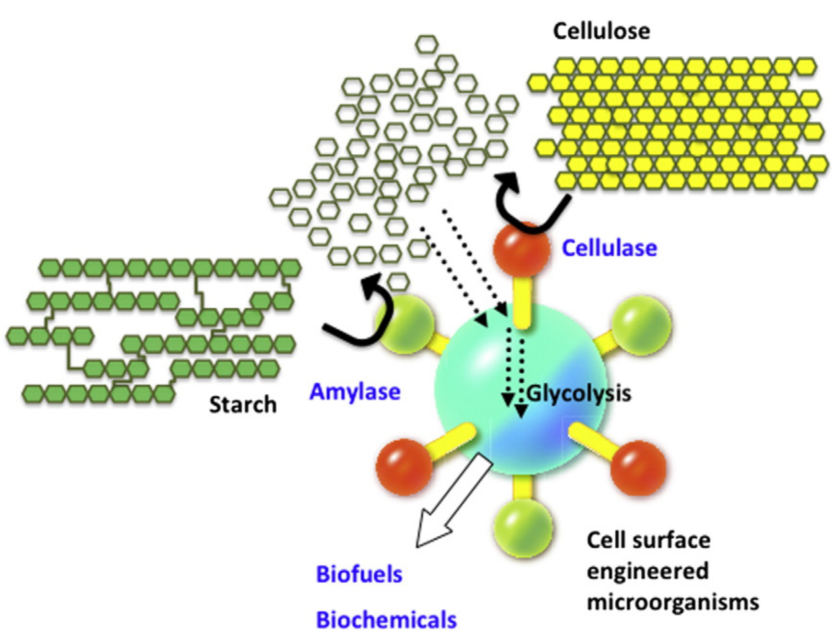

Fig. 1. Schematic illustration of cell surface engineered microorganisms for biorefinery.