Recent Advances in Selection and Engineering of Biomass Degrading Enzymes

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Plant biomass represents a renewable and practically inexhaustible bio resource for production of fuels and chemicals, and in the coming years it is considered to play the same role in human civilization as oil in 20th and beginning of the 21st century. For the last three decades, the production of first-generation liquid biofuels (mainly, ethanol from food crops in the USA and that from sugarcane in Brazil) has continuously grown [1]. Since 2012 an era of full-scale commercial facilities for production of second-generation biofuels, derived from lignocellulosic feedstock's, began [1,2]. Enzymes, including cellulases, hemicellulases and auxiliary activities, are the most essential part of the second-generation biofuel technology [2,3]. Because of the recalcitrant nature of lignocellulosic biomass, demanding high enzyme loadings, much attention is paid in the laboratories worldwide to finding and selection of novel enzymes with higher activity, thermostability, less susceptibility to inhibition, etc., as well to engineering of already known enzymes in order to improve their properties.

Cellulases and hemicellulases produced by mutant strains of the filamentous fungus *Trichoderma reesei* (synonym *Hypocrea jecorina*) are the most studied and widely used biomass-degrading enzymes [2,3]. More active and thermostable cellulases than those secreted by *Trichoderma* have been discovered recently [2-9]. In particular, cellobiohydrolases from *Myceliophthora thermophila* (formerly name *Chrysosporium lucknowense*) [4],

Acremonium thermophilum, Thermoascus aurantiacus, Chaetomium thermophilum [6,7] and *Penicillium* species (*P. funiculosum, P. verruculosum, P. canescens*) [5,8,9] display higher activity against microcrystalline cellulose (Avicel) and lignocellulosic substrates than cellobiohydrolase I of *T. reesei* (TrCel7A) at its optimal temperature (45-50°C). The last mentioned enzyme represents a major enzyme of *T. reesei* multienzyme cellulase system, a kind of a reference golden standard in the field of fungal cellulases [3]. Cellobiohydrolases from *M. thermophila, T. aurantiacus, C. thermophilum* are also characterized by higher thermostability than TrCel7A, and their superiority over TrCel7A becomes even more pronounced at temperatures above 50°C [4,6,7].

One of the most exciting recent events in the field of lignocellulose microbial degradation is a discovery of metal-dependent lytic polysaccharide monooxygenases that cleave cellulose via an oxidative mechanism [10,11]. These enzymes have recently been classified into family 9 of Auxiliary Activities (AA9) [12].The AA9 enzymes act as enhancers of cellulase activity, and now they become a standard component of commercial multienzyme cocktails for conversion of lignocellulosic biomass [13]. So, finding and selection of novel lytic monooxygenases that can effectively cleave cellulose and hemicelluloses, acting in synergism with true glycoside hydrolases, is now a very hot topic amongst researchers working in this area.

In parallel with finding more active cellulases and enzymes belonging to a new class of auxiliary activities, the good old enzymes of *T. reesei* have been subjected to engineering by site-directed mutagenesis or directed evolution in order to improve their activity/thermostability [14-16]. Using a combination of stabilizing mutations identified from homologous recombination, consensus design and structure-based computational approaches, the engineered *T. reesei* endoglucanase TrCel5A was obtained, having an optimal temperature higher by 17°C than the wild type enzyme [16]. Together with previously engineered thermo stable variants of cellobiohydrolases (TrCel6A and TrCel7A), TrCel5A was successfully applied for cellulose hydrolysis at 70°C over 60 h. The thermostable mixture produced 3-fold higher concentration of sugars than the best mixture of the wild type enzymes operating at its optimum temperature (60°C) [16].

An interesting approach for engineering of cellobiohydrolases with higher activity toward cellulose is adding or removing N-linked glycosylation motifs in the enzyme catalytic domain by site-directed mutagenesis [17]. Depending on the position of glycosylated Asn residue on the surface of enzyme molecule, the N-linked glycan may either reduce or stimulate cellulase activity. So, mutation of certain Asn residues to Ala in cellobiohydrolases from *T. reesei* and *P. funiculosum*, thus removing N-glycosylation at the respective sites, resulted in up to 60% increase in conversion degree of bacterial crystalline cellulose after 120-h hydrolysis. Introducing a new glycosylation motif at N194 in *P. funiculosum* enzyme by applying A196S mutation also led to 70% increase in the enzyme activity [17].

Other engineering approaches have been focused on changing other properties of cellulases, such as pH-optimum of activity or susceptibility to inhibition. For example, using site-directed mutagenesis, the D98N mutant of endoglucanase III from *P. verruculosum* was obtained; as a result, the pH-optimum of enzyme activity was shifted from pH 4.0 to 5.1 [18]. As another case, chemical modification (succinylation) of *T. reesei* cellulases resulted in reduced inhibition of the enzymes by lignin; thus, more than 2-fold increase in the conversion of 1% Avicel in the presence of 1% lignin after 170-h hydrolysis was achieved [19]. The modified cellulases also displayed higher tolerance to ionic liquids.

The limits of this article do not permit more detailed analysis of the topic discussed. However, even a few examples presented above show that significant progress has been achieved in the last few years in the field of bioconversion of lignocellulosics through finding and selection of novel enzymes as well as engineering of enzymes, which had been discovered and studied previously.

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