A Comprehensive Review on Molecular Approaches for Enhancement of Bacterial Cellulase Production

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Abstract

Cellulases are the largest class of industrial enzymes produced worldwide because of their potential applications in cotton processing, paper recycling, juice extraction, detergent formulation, animal feed additives and their established uses in agricultural biotechnology and bioenergy production. Though many attempts have happened in the past few decades attempting to enhance the production and activity of cellulases by non molecular approaches (like Optimization of fermentative conditions and strain improvements) which are understood to have limited range of applications; molecular approaches have proved to be the best solution for many limitations faced by the other Biotechnological methods. The present review is an attempt to depict the recent advancements in the molecular approaches used to enhance the production of Bacterial cellulases.

Keywords - *Bacterial cellulases, molecular approaches, rational design*

I. INTRODUCTION

Cellulose is the major component of plant biomass and the most abundant renewable natural resource in the biosphere with an estimated annual production of $4.0x10^7$ tonnes [1]-[2]. Although cellulose is a linear homopolymer of repeated units of cellobiose, the β-1,4-glycosidic linkages make the crystalline structure with a few amorphous regions. Cellulases are the inducible bioactive enzymes produced by cellulolytic microbes during their growth on cellulosic materials[3].Cellulase transforms cellulose into glucose, which can be fermented to ethanol [4]-[6]. Although several chemical and biochemical methods have been successfully employed to convert cellulosic waste materials into useful products, the enzymatic hydrolysis is the most preferred method [7].

Molecular approaches for the modification of cellulase made wide application in the field of protein engineering and inturn in the activity of cellulase enzyme a successful computational design to convert non-active ribose binding protein to triose phosphate isomerase was based on 18–22 mutations and exhibited a 10^5–10^6-fold activity enhancement [8].

II. MOLECULAR APPROACHES

As no single enzyme is completely suitable as it is, for the hydrolysis of cellulose in the biorefining industry, improving cellulases could help to achieve the cost-effective biofuel production. Protein engineering or molecular approach through the mutagenesis of catalytic domain aminoacid is the main method for cellulase improvement. The two major strategies for the improvement of a cellulases are rational design and directed evolution. Tables 1 and 2 contain a list of different methods used to alter the properties of various cellulolytic bacterial strains by rational design and directed evolution respectively.

III. RATIONAL DESIGN

Rational design involves choice of a suitable enzyme, identification of the amino acid sites to be changed (structure based molecular modeling), site directed mutagenesis, transformation, expression and characterization of the mutants (Table 1). Recently, a successful computational design to convert non-active ribose binding protein to triose phosphate isomerase was based on 18–22 mutations and exhibited a 10^5–10^6-fold activity enhancement. Unfortunately, the success of computational models is often limited to well-understood reactions and enzymes.

IV. DIRECT EVOLUTION

In direct evolution there is no need of understanding the enzyme 3D structure and interaction between enzyme and substrate. Direct evolution utilizes the DNA techniques such as error-prone PCR and DNA shuffling techniques to randomly generate a library of large number of variants (Table 2). Major challenge of this method is developing tools to accurately evaluate and select high-performance mutants generated by recombinant DNA techniques [24]. Screening usually involves CMC-Congored staining or incorporation of

chromogenic or flurogenic substrates. In direct evolution larger the gene library variants, larger the chance of mutants with desired property.

V. CLONING AND EXPRESSION

Many fungal and bacterial cellulase have been characterized and their genes have been cloned [34]. Cloning and expression of cellulase gene in heterologous host is based on the facts that cellulase genes from eukaryotic fungal hosts cannot rely on direct expression in a prokaryotic cell because of the differences in the translation mechanism in the two groups and since the eukaryotic genomes are much larger than those of prokaryotes, pBR322 based vectors cannot give satisfactory results. The recombinant cellulolytic strategy for organism development is based on non-cellulolytic microorganisms having excellent product formation properties and involves heterologous expression of a functional cellulase system. Such heterologous expression has been undertaken for a variety of purposes. Main hetrologous expression systems were *Zymomonas mobilis* and *Sachromyces cerevisiae*. List of cellulase genes and their enzymes are listed in Table 3.

Table 1: Rational design for cellulase improvement

Strain	Enzyme	Method	Altered property	Reference
Bacillus sp. KSM 330	EndoK	SDM	Decrease inactivity to CMC	[8]
A. cellulolyticus	Endo	SDM	Type of products Released	[9]
C. cellulovorans	Endo	CBDE	Soluble form CBD	[10]
Bacillus sp. KSM 64	Endo	SDM	Thermostability Increased	[11]
T. maritime	Endo	SDM, CBDE	Incresed (enzyme activity, pH tolerance, activity to Avicel)	[12]
T. fusca	Endo	SDM	Incresed CMCase activity	[13]
C. fimi	Endo	Surface residue replacement	Change in pH tolerance	[14]
C. thermocellum	Endo(Cel8A)	Consensus Mutagenesis	Thermostability	[15]
C. thermocellum	Endo(Cel A)	Saturation mutagenesis	Incresed half life	[16]
B. subtilis JA18	Endo	C-terminal deletion	Half life increased	[17]
P. polymyxa	Blg	SDM	Thermostability	[18]
T. aurantiacus	Endo	SDM	Hydrolytic activity	[19]
Agrobacterium sp.	Blg	Codon optimization	Expression level	[20]
Aspergillus sp.	Endo, β - glucosidase	Cyclic mutagenesis	Expression level	[21]
P.piceum	Exo, β - glucosidase	Single point mutation	Thermostability	[22]
T. maritima	Endo(Cel12B)	SDM	Thermostability	[23]

*Endo, Endoglucanase; Exo, Exoglucanase; SDM, Site directed mutagenesis; CBDE, cellulose binding domain engineering

Table 2 Direct evolution for cellulase improvement

Strain	Enzyme	Method	Altered property	Reference
B. subtilis	Endo	DNA shuffling	Activity	[25]
C. cellulovorans	Endo	DNA recombination	Thermal stability	[26]
Agrobacterium sp.	β-glucosidase	error prone PCR	Activity	[27]
B. subtilis	Endo	error prone PCR DNA shuffling	Activity Thermostability	[28]
P. furiosus	β-glucosidase	DNA shuffling	Cold adaption	[29]
P. polymyxa	β-glucosidase	error prone PCR+ DNA shuffling	Thermal stability	[30]
T. fusca	β-glucosidase	DNA shuffling	Thermal stability SDM+ Saturation mutagenesis	[31]
C.phytofermentans	Endo	ND	Enhanced hydrolytic performance	[32]
T. reesei	Endo	Mutagenesis	Thermostability	[33]

Table 3 List of cellulase genes and their enzymes

Strain	Gene	Enzyme	Size (kDa)	pH	Temp (°C)	Host	Reference
Bacillus sp. N4	*PNK-1*	CMCase	50	5-10.9	60	*E. coli*	[35]
B. subtilis	*Endo*	CMCase	33	5.5	60	*B. megaterium*	[36]
P. fluorescens var. *cellulosa*	*eglX*	Endo	ND	ND	ND	*Z. mobilis*	[37]
C. fimi	*exg*	Exo	116	12	60-75	*S. cerevisiae*	[38]
E. chrysanthemi	*celZ*	Endo	45	ND	ND	*Z. mobilis*	[39]
B. polymyxa+ *B. circulans*	*celB*	Endo	44	ND	ND	*E.coli*	[40]
B. lautus	*celB*	Endo	56	ND	ND	*B. subtilis*	[41]
Bacillus sp.KSM330	*celA*	Endo	51.88	ND	ND	*E. coli*	[42]
Bacillus sp. D04	*cel*	Endo+Exo	55	ND	ND	-	[16]
B. subtilis	*CMCase*	CMCase	36	ND	ND	*B. subtilis*	[43]
Bacillus sp. BP23	*celA*	Endo	44.8	4.0	40	*E. coli*	[44]
B. subtilis	*celR*	Endo+Cbhase	12.9	ND	ND	*E. coli*	[45]
B. pumilus	*EglA*	Endo	71.3	5-8	60	*E. coli*	[46]
C. biazotea	*bglA*+*BglB*+ *BglC*	β-glucosidase	ND	ND	ND	*S. cerevisiae*	[47]
Paenibacillus sp. BP23	*celB*	Endo	106.9	5.5	53	*E. coli*	[48]
E. chrysanthemi	*celY*+ *celZ*	Endo	23.6	ND	ND	*Klebsiella*	[49]
A. acidocaldarius	*celA*	Endo	30	5.5	70	*E. coli*	[50]
A. acidocaldarius	*celB*	Endo	100	4	80	*E. coli*	[51]
C. biazotea	*bgl*	β-glucosidase	ND	ND	ND	*E. coli*	[52]
C. flavigena	*celcflB*	Endo	58	ND	ND	*E. coli*	[53]
Paenibacillus sp. BP23	*cel48C*	Exo	118	6	48	*E. coli*	[54]
B. licheniformis	*cel12A*	Endo	29.067	ND	ND	*E. coli*	[55]
B. licheniformis	*celW*	Endo	55	6	60	*E. coli*	[56]
B. licheniformis	*cel5A*	Endo	62	6	65	*E. coli*	[26]
B. subtilis	*celDR*	Endo	55	ND	50	*E. coli*	[57]
B. subtilis	*celI15*	Endo	52	6	60	*E. coli*	[58]
A. cellulolyticus	*GH12*+ *E1*	Endo	ND	ND	ND	*Z. mobilis*	[59]
Pseudomonas sp. BME14	*cel9p*	Endo	60	6.5	35	*E. coli*	[60]
M. thermophila	MtEG7a	Endo	65	ND	ND	*P. pastoris*	[61]
B. subtilis JS2004	ND	Endo	63	9	50	*E. coli*	[62]
B. subtilis IARI-SP-1	ND	Endo	55	8	50-60	*E. coli*	[63]
T. reesei	EG2	Endo	51	ND	ND	*Y. lipolytica*	[64]
	CBH	Exo	62				
T.halotolerans YIM 90462	thcel6A	Endo	45.9	8.5	55	-	[65]
Paenibacillus sp.	EG5C	Endo	63.5	5	40	*E. coli*	[66]
B. subtilis UMC7	EG1	Endo	56	6	60	*E. coli*	[67]
T. reesei TrEGI	Cel7B	Endo	46	ND	50	*S. cerevisiae*	[31]
C. thermocellum	CenC	Endo	137.11	6	70	*E. coli*	[67]
Actinomyces sp.	EG1	Endo	57.1	6	55	*E. coli*	[68]

Endo, Endoglucanase; Exo, Exoglucanase; ND, not determined

VI. CONCLUSION

The mixture of cellulase, hemicellulase and pectinase or their individual components have wide range of application in research. These enzymes are involved in plant defence and hence, have wide range of applications in agriculture. Cellulose binding domains (CBD) of cellulase have been successfully used as affinity tags for the purification and immobilization of proteins [64]. Biotinylated CBDs have been successfully used for the purification of antibodies [65].

Unutilized celluloses generated from forest, agricultural fields and agroindustries cause environmental pollution. Cellulase enzymes play an important role in degrading cellulosic waste materials. Cellulosic wastes have been utilized for the preparation of valuable products such as sugars, enzymes, biofuels, chemicals and improved animal feeds and human nutrients [66]-[68].

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