

In silico Characterization of Cellulases from Genus *Bacillus*

Yogita Lugani¹, Balwinder Singh Sooch¹

'Enzyme Biotechnology Laboratory, Department of Biotechnology, Punjabi University, Patiala-147002, Punjab, India.

ABSTRACT

Background: Cellulases are hydrolytic enzymes which hydrolyze β -1,4-glycosidic linkage in cellulose and these are present in many microorganisms including bacteria, fungi and protozoa. The three types of cellulases involved in complete hydrolysis of cellulose are endoglucanase, exoglucanase and β -glucosidase. Various structural and functional domains are present in cellulose. Cellulases and among all these domains, cellulose binding and catalytic domains are found to be important for the hydrolysis of cellulose. Cellulases have showed promising applications in different industrial sectors like paper and pulp, textile, laundry, bioethanol production, brewing, detergent and waste management. A major focus has been given in the recent past by researchers to understand the functional domains and catalytic mechanism of this enzyme to make their effective use for industrial applications.

Material and Methods: The protein sequences of cellulases belonging to different *Bacillus sp.* were retrieved using Uniprot and then physicochemical properties were analyzed using ProtParam and Protscale. Multiple sequence alignment of retrieved sequences was performed using Clustal W and phylogenetic tree was constructed using Mega 6.0 software. SOPMA and GOR IV tools were used for the prediction of secondary structure. The tertiary structure of enzyme was computed using Raptor X.

Results: The molecular weight of cellulases were found to range between 49,263-94,682 Da with hydropathicity ranges between -0.292 to -0.580. The acidic amino acid glutamate was found at the active site and methionone was found at the N-terminal of enzyme. The results have shown that the sequence is highly diversed at N-terminus and C-terminus region between different types of cellulases with conserved sequences in the middle. The phylogenetic tree has showed high similarity amongst retrieved sequences. From the tertiary structure, a great degree of variability in α -helix, extended strand in β ladder, hydrogen bonded turn, bend and coil was observed between different types of cellulases.

Conclusion: This study provides insights about the physicochemical properties, hydrohobicity, structure and function of cellulases, which would help to exploit this enzyme at industrial level.

Key Words: Cellulase, Cellulose, Bacillus sp., Endoglucanase, Exoglucanase, β-glucosidase

INTRODUCTION

Cellulases are hydrolytic enzymes which can readily hydrolyze both crystalline and paracrystalline structures of cellulose, the largest component of plant residues enters terrestrial ecosystems. Cellulose is present in various lignocellulosic wastes generated from agricultural and industrial processes like sawdust, citrus peel waste, paper mill sludge, industrial waste, paper pulp and municipal solid waste (Maki et al., 2009). The synergistic action of three types of cellulases i.e. endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.74) and β -glucosidase (EC 3.2.1.21) have been involved for the complete hydrolysis of cellulose (Lugani et al., 2015). Cellulases are industrially important enzymes and are involved in the conversion of lignocellulosic residues for the production of single cell protein, ethanol, bleaching of pulp, fruit juice extraction and for the treatment of waste papers (Shankar and Isaiarasu, 2011). Cellulases are produced by all the microorganisms but mainly by bacteria, actinomycetes and fungi. Among all the microorganisms, members of bacteria have gained intense importance for commercial production of cellulases due to their high growth, wide genetic variability, adaptability and high amendability to genetic manipulations (Patagundi et al., 2014; Lynd et al., 2002). The structure of cellulase composed of carbohydrate binding domain (CBD) at C-terminal which is joined by a short poly- linker region to the N-terminal of catalytic domain. There is presence of two acidic amino acids at the active site of enzyme which catalyze the reaction by acid-base catalysis either through inversion or retention of the configuration of anomeric carbon (Maki

9136

Corresponding Author:

Balwinder Singh Sooch, Enzyme Biotechnology Laboratory, Department of Biotechnology, Punjabi University, Patiala-147002, Punjab, India; Ph: 0175-3046263; E-mail: soochb@yahoo.com

ISSN: 2231-2196 (Print)	ISSN: 0975-5241 (Online)	DOI: 10.7324/IJCRR.2017
Received: 03.05.2017	Revised: 23.05.2017	Accepted: 20.06.2017

et al., 2009). The current focus of most of the researchers has been towards the large scale production of this industrially important enzyme to meet the industrial needs by utilizing various novel bacterial strains. However, a great degree of variability have been observed between different bacterial strains like molecular weight, stability, amino acid composition, family and domain to which they belong, secondary and tertiary structure. Bioinformatics is an interdisciplinary field which is currently used for structural and functional analysis of proteins using various computations tools and databases (Prashant et al., 2010). The information which has been retrieved from available tools and databases about the protein might be useful for the selection of highly efficient bacterial strain for industrial production of enzyme. Moreover, this information may also be helpful for developing new microbial strains with enhanced enzyme production ability by adapting recombinant DNA technology. Keeping in view the above facts about industrial importance of cellulases and use of bioinformatics as an emerging field of molecular biology, the present study was aimed to utilize in silico tools for the characterization of cellulase enzymes from different Bacillus species for their physicochemical characteristics, ancestral relationship and structure determination at various levels.

MATERIAL AND METHODS

Sequence retrieval and alignment

The cellulase protein sequences from different species of *Bacillus* were retrieved from Uniprot (Universal Protein Resource). The retrieved sequences from *Bacillus sp.* were *Bacillus subtilis*, Accession number: P10475; *Bacillus akibai*, Accession number: P06564; *Bacillus thuringiensis*, Accession number: M1QQC9; *Bacillus pumilus*, Accession number: B2ZHC9; *Paenibacillus polymyxa*, Accession number: E3EEC5; *Paenibacillus macerans*, Accession number: A0A090Y895. Clustal Omega (version 1.2.4) algorithm was used for the alignment of retrieved protein sequences through multiple sequence alignment.

Physicochemical characterization

ProtParam tool was used to compute different physicochemical properties of retrieving sequences of cellulases like amino acid composition, aliphatic index (AI), pI, instability index (II), number of positive and negative charged residues, grand average of hydropathicity (GRAVY) and extinction coefficient (Kumar et al., 2012). The isoelectric point (pI) is determined based on the pK value of protein during protein migration under denaturation conditions (Bjellqvust et al., 1993). The concentration of purified protein in the sample is evaluated from the value of extinction coefficient (Umang et al., 2012). The stability of protein is calculated from its instability index (II) and the proteins are predicted as stable when their instability index is smaller than 40; however when the value of instability index is greater than 40, the protein is regarded as unstable (Guruprasad et al., 1990). The volume occupied by aliphatic amino acids side chain (alanine, valine, leucine and isoleucine) relative to total volume occupied is called aliphatic index and it determines the thermostability of a globular protein (Walker, 2005). The hydrophilicity or hydrophobicity of protein is determined by grand average of hydropathicity (GRAVY), which is the ratio of sum of hydropathy values of all the amino acids to total number of residues in the sequence (Umang et al., 2012). The hydropathy plots based on Kyte and Doolittle scale for all the retrieved sequences of cellulases were predicted using Protscale tool (Kumar et al., 2012; http://www.expasy.org/tools/).

Phylogenetic analysis

The ancestral relationship between retrieved protein sequences of cellulase for different species of *Bacillus* was estimated by constructing the phylogenetic tree using Mega 6.0 software (Gouripur et al., 2016). Neighbor joining (NJ) algorithm was used for distance tree building and bootstrap value was set at 1000. The bootstrap value denotes to generation of new data sets with replacements.

Secondary structure prediction

Self optimized prediction method with alignment (SOPMA) and GOR IV tools were used for the analysis of secondary structure and results obtained from these tools were also compared to determine α - helix, β - sheet, turns and loops (Geourjon and Deleage, 1994; http://npsa-pbil.ibcp.fr/cgi-).

Tertiary structure prediction

The tertiary structure of proteins were constructed by using RaptorX structure prediction server, which provides high quality structural model by using the template of primary protein sequence (http://raptorx.uchicago.edu/StructurePrediction/predict/).

RESULTS

Sequence retrieval and alignment

The protein sequences of cellulase enzymes belonging to different strains of *Bacillus sp.* were retrieved from Uniprot and these sequences were then characterized using Uniprot tool (Table 1). It has been analyzed from data that the molecular weight of enzyme lies between 54,681 to 94,682 Da and they belong to endoglucanase, β -glucosidase and exoglucanase, respectively. Different types of cellulases shows different catalytic mechanism due to variation in multienzyme complex formation.

Thereafter, clustal omega software was used for multiple sequence alignment of these proteins (Fig.1). The cellulase

sequences from species of *Bacillus* were found to be highly diverged at the N-terminal and C-terminal, respectively. However, conserved amino acid sequences with good similarity were found in the middle. The positions with absolute conservation are indicated with asterlink (*), whereas dots (.) represent the position of relative conservation.

Physicochemical characterization

The results of physicochemical properties like pI, number of positive and negative amino acids, extinction coefficient, instability index, aliphatic index, grand average of hydropathicity and total number of atoms for cellulase from different different species of Bacillu sare shown in Table 2. The isolectric point (pI) is the pH value at which mobility of protein becomes zero with more compact and stable conformation. The pI value of cellulase from Bacillus subtilis is more than 7, which means it contains more number of negatively charged amino acids. Whereas, for all the other species of Bacillus, the pI was found to be less than 7 and their cellulases were acidic in nature containing more number of positive charge residues. Expasy's Prot Param can compute the extinction coefficient for a range of 276, 278, 279, 280 and 282, however 280 nm is more preferred because proteins absorb this wavelength more strongly with minimum interference from other substances. Cellulases from all the selected Bacillus sp. were found to be stable with instability index less than 40. The GRAVY value is negative for all the cellulase sequences and this has showed better possibilities of aqueous interactions. The total number of atoms in different cellulases ranging from 7717 to 13113. The comparison of amino acid composition (%) in different cellulase sequences was also carried out (Table 3) and different amino acids were found to be dominant in different sequences. Protscale tool was used for the construction of Kyte and Doolittle hydropathy plots (Fig.2) and transmembrane region of cellulase from different Bacillus sp. was found to be rich in hydrophobic amino acids as many points lie above the zero baseline. The minimum and maximum hydrophobic position and score for each cellulase sequence was also predicted (Table 4) with minimum and maximum score of -3.100 and 3.489, respectively.

Phylogenetic analysis

Mega 6.0 software, which provides a subset of substitution model and neighbor joining algorithm for distance tree building, was used for the construction of phylogenetic tree (Fig. 3). Cellulases from *Bacillus subtilis* P10475, *Bacillus pumilus* B2ZHC9, *Paenibacillus polymyxa* E3EEC5, *Paenibacillus macerans* A0A090Y895 were found to be closely related compared to *Bacillus akibai* P06564 and *Bacillus thuringiensis* M1QQC9, which were diverged from many species of *Bacillus*.

Secondary structure prediction

The secondary structure of different cellulase sequences was estimated using GOR IV and SOPMA tools, the percentage of α -helix, extended strand and random coils incellulase from different *Bacillus sp.* are shown in Table 5. The presence of amino acid in the helix, strand or coil is depicted from the secondary structure (Ojeiru et al., 2010) and secondary structure of cellulase was depicted by SOPMA (Pradeep et al., 2012). From the results, it has been observed that alpha helix was dominant in *Bacillus pumilus* B2ZHC9 (46.52%), whereas extended strand and random coil was observed to be dominant in *Bacillus subtilis* P10475 (26.45%) and *Paenibacillus polymyxa* E3EEC5 (44.91%), respectively.

Tertiary structure prediction

The tertiary structures of different cellulase sequences were analyzed using RaptorX structure prediction server, which results in modelling of a protein in a step wise manner like template threading, alignment quality assessment and multiple template threading. Different sequences of cellulases showed variability in α -helix, extended strand in β ladder, hydrogen bonded turn, bend and coil (Fig. 4). A high degree of variation was found in the geometric shape and interaction between side chains of amino acids, which lead to differences in their functions.

DISCUSSION

The present study showed the industrial importance of cellulases in different sectors such as textile, laundry, bioethanol production, brewing, detergent, waste management, paper and pulp. There are different types of cellulases with different catalytic subunits which are involved in complete hydrolysis of cellulose. In silico studies are very promising tools in the current era for the characterization of industrially important enzymes for various properties for their selection for appropriate industrial application. The different types of cellulases from Bacillus sp. were studied for comparing their physicochemical characteristics, ancestral relationship and structure prediction at different levels. Various computational tools were used for the characterization of cellulases from different Bacillus sp. A great degree of diversity has been observed in molecular weight, family, domain, number of amino acids, positive and negative charged residues, secondary and tertiary structure between the different forms of cellulases. The phylogenetic analysis also showed the ancestral divergence of difference types of cellulases. This study will be helpful for the selection of industrially important bacterial strain with desirable characteristics for particular industrial processes. Moreover, this information can also be useful for designing new microbial strains by applying proteomics, system biology and microarray based strategies. Further, wet lab studies with reduced labour are required to design novel

cellulase producing bacterial strains by using *in silico* data output.

CONCLUSIONS

The present study concludes that cellulases are industrially important enzymes due to their ability to utilize agricultural wastes for the production of industrially important products. In recent years, various researchers have adapted genetic engineering approach to develop novel microbial strains with enhanced enzyme producing ability. However, such processes are very tedious and time consuming, which is the major obstacle for adapting such processes for commercial enzyme production. Such limitations can be overcome by initial screening through *in silico* studies to understand the structure, function and physicochemical properties of the enzyme. These computational studies would be promising tool to design enzymes with desirable characteristics for exploiting them at industrial level.

Conflict of interest: There is no conflict of interest between the authors regarding the publication of this article.

ACKNOWLEDGEMENTS

The authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this paper. The authors are grateful to al authors/ editors/ publishers of all those articles, papers, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

- Bjellqvust B, Hughes GJ, Pasquali C, Paquet N, Ravier F, Sanchez JC, Frutiger S, Hochstrasser D. The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. Electrophoresis 1993; 14(1):1023-1031.
- Geourjon C, Deleage G. 1994. SOPM: a self optimized method for protein secondary structure prediction. Protein Engineering 1994; 7(2):157-164.

- 3. Gouripur GC, Kaliwa RB, B.B. Kaliwal BB. *In silico* characterization of beta-galactosidase using computational tools. Journal of Bioinformatics and Sequence Analysis 2016; 8(1):1-11.
- Guruprasad K, Reddy B, Pandit MW. Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Engineering 1990; 4(2):155-164.
- Protein secondary structure prediction http://npsa-pbil.ibcp.fr/ cgi- [Accessed on 15/03/2017].
- 6. Protein tertiary structure prediction, [Accessed on 12/03/2017].
- Physicochemical characterization of proteins http://www.expasy.org/tools/ [Accessed on 19/03/2017].
- Kumar NV, Rani ME, Gunaseeli R, Kannan ND, Sridhar J. Modeling and structural analysis of cellulases using *Clostridium thermocellum* as template. Bioinformation. 2012; 8(22):1105-1110.
- Lugani Y, Singla R, Sooch BS. Optimization of cellulase from newly isolated *Bacillus sp.* Y3. Journal of Bioprocessing and Biotechniques 2015; 5(11):1-6.
- Lynd LR, Weimer PJ, Zyl, WH, Pretorius IS. Microbial cellulase utilization: fundamentals and biotechnology. Microbiology and Molecular Biology Reviews 2002; 66(3):506-577.
- Maki M, Leung KT, Qin, W. The prospects of cellulase producing bacteria for the bioconversion of lignocellulosic biomass. International Journal of Biological Sciences 2009; 5(5):500-516.
- Ojeiru FE, Kazuya T, Yuki H, Mohammed SM, Shunsuke M. Circular dichroism studies on C-terminal zinc finger domain of transcription factor GATA-2. Yonago Acta medica 2010; 53:25-28.
- Patagundi BI, Shivasharan CT, Kaliwal BB. Isolation and characterization of cellulase producing bacteria from soil. International Journal of Current Microbiology and Applied Sciences 2014; 3(5):59-69.
- Pradeep NV, Anupama, Vidyashree KG, Lakshmi P. *In silico* characterization of industrial important cellulases using computational tools. Advances in Life Science and Technology 2012; 4:8-14.
- Prashant VT, Uddhav SC, Madura SM, Vishal PD, Renuka RK. Secondary structure prediction and phylogenetic analysis of salt tolerant proteins. Global Journal of Molecular Sciences 2010; 5(1):30-36.
- Shankar T, Isaiarasu L. Cellulase production by *Bacillus pumilus* EWBCM1 under varying cultural conditions. Middle East Journal of Scientific Research 2011; 8(1):40-45.
- Umang M, Astha J, Aastha C, Neha A, Vibha R. Computational structural and functional characterization of protein family: Key for the hidden mystry. Journal of Pharmacy Research 2012; 5(7):3643-3649.
- 18. Walker JM. The Proteomics Protocols Handbook: Humana Press, 2005, Chap. 52.

S. No.	Organism	Accession number	Protein	Number of amino acids	Molecular weight (in Da)	Molecular function	Family	Domain
1.	Bacillus subtilis	P10475	Endoglucanase	499	55,287	CA CB	GH5	CBM ₃
2.	Bacillus akibai	Po6564	Endoglucanase	800	88,602	CA CB	GH5	CBM17 CBM28
3.	Bacillus thuring- iensis	M1QQC9	β-glucosidase	469	54,681	BGA	GH1	GHC
4.	Bacillus pumilus	B2ZHC9	β-glucosidase	488	56,352	HA	GH1	GHC
5.	Paenibacillus polymyxa	E3EEC5	Exoglucanase	717	77,018	CA HA	GH6	CBM6
6.	Paenibacillus macerans	AoAo9oY895	Exoglucanase	889	94,682	CB HA	GH6	CBM3

Table 1: Characterization of retrieved se	quences of cellulases for different	Bacillus sp.	using Unit	prot tool.
rubic i. churacterization of retricted se	quenees of centuluses for unrefen	. Ducinus spi	using only	100000

where, GH- Glycoside Hydrolase, CBM- Carbohydrate Binding Module, GHC- Glycoside Hydrolase Catalytic, CA-Cellulase activity, CB- Cellulose binding, BGA- β -galactosidase activity, HA- Hydrolase activity

Table 2: Cellulases parameters computed using Expasy's Prot Param tool.

S. No.	Organism	Accession number	pI	+ R	-R	EC (M ⁻¹ cm ⁻¹)	п	Stability	AI	GRAVY	Formula	TNA
1.	Bacillus subtilis	P10475	8.55	59	55	106925	26.16	Stable	73.91	-0.580	$C_{_{2459}}H_{_{3815}}N_{_{673}}O_{_{760}}S_{_{10}}$	7717
2.	Bacillus akibai	Po6564	4.3	59	133	147250	34.10	Stable	80.21	-0.456	$C_{_{3938}}H_{_{6000}}N_{_{1040}}O_{_{1263}}S_{_{15}}$	12256
3.	Bacillus thuringien- sis	M1QQC9	5.74	57	69	127785	30.86	Stable	73.37	-0.574	$C_{_{2510}}H_{_{3729}}N_{_{639}}O_{_{715}}S_{_{12}}$	7605
4.	Bacillus pumilus	B2ZHC9	4.99	56	78	91930	36.44	Stable	76.39	-0.506	$C_{_{2543}}H_{_{3856}}N_{_{650}}O_{_{761}}S_{_{20}}$	7830
5.	Paeni- bacillus polymyxa	E3EEC5	5.51	65	76	117480	23.73	Stable	70.95	-0.420	$C_{_{3422}}H_{_{5276}}N_{_{920}}O_{_{1079}}S_{_{14}}$	10711
6.	Paenibacil- lus macer- ans	AoAo9oY895	4.67	64	98	156330	23.15	Stable	73.35	-0.292	$C_{_{\!$	13113

where pI: Isoelectric point, +R: number of positive charged residues (Arg+ Lys), -R: number of negative charged residues (Asp+ Glu), EC: Extinction coefficient at 280 nm, II: Instability index, AI: Aliphatic index, GRAVY: Grand average of hydropathicity, TNA: Total number of atoms

S. No.	Amino acid	P10475	Po6564	M1QQC9	B2ZHC9	E ₃ EEC ₅	AoAo9oY895
1.	Ala (A)	7.0	8.4	5.5	7.4	10.0	11.9
2.	Arg (R)	2.8	3.1	3.6	3.9	2.5	2.5
3.	Asn (N)	7.6	8.4	5.1	4.3	6.8	6.3
4.	Asp (D)	7.2	7.4	6.2	7.8	7.5	7.1
5.	Cys (C)	0.6	0.0	0.6	1.0	0.6	0.4
6.	Gln (Q)	4.2	2.2	2.3	3.9	2.6	2.8

Lugani et.al.: In silico Characterization of Cellulases from Genus Bacillus

7.	Glu (E)	3.8	9.2	8.5	8.2	3.1	3.9
8.	Gly (G)	9.0	7.2	8.1	6.4	8.6	8.2
9.	His (H)	1.4	1.6	3.2	2.0	1.4	1.0
10.	Ile (I)	6.4	5.9	6.2	6.6	4.6	4.0
11.	Leu (L)	7.0	6.9	6.6	7.8	6.6	5.5
12.	Lys (K)	9.0	4.2	8.5	7.6	6.6	4.7
13.	Met (M)	1.4	1.9	1.9	3.1	1.4	1.3
14.	Phe (F)	3.0	3.4	4.9	4.7	3.3	2.8
15.	Pro (P)	3.4	6.1	4.1	3.7	6.7	6.1
16.	Ser (S)	7.4	5.5	3.4	4.7	7.1	8.0
17.	Thr (T)	6.8	5.2	4.7	4.3	8.8	8.3
18.	Trp (W)	2.8	2.5	3.0	1.6	2.0	1.9
19.	Tyr (Y)	4.0	3.1	7.2	6.6	3.8	4.7
20.	Val (V)	5.0	7.6	6.2	4.5	6.0	8.3

Table 4: Hydrophobic score and position of cellulases using Protscale tool.

S. No.	Organism	Accession number	Position		Score	
			Min	Max	Min	Max
1.	Bacillus subtilis	P10475	143	13	-3.044	2.811
2.	Bacillus akibai	Po6564	794,795	1718	-3.044	3.489
3.	Bacillus thuringiensis	M1QQC9	57	169	-3.100	1.878
4.	Bacillus pumilus	B2ZHC9	68	320	-2.889	1.922
5.	Paenibacillus polymyxa	E3EEC5	366	21	-2.800	2.044
6.	Paenibacillus macerans	AoAo9oY895	857	16,17	-2.400	1.889

Table 5: Prediction of secondary structure of xylose reductases using Expasy's GOR IV and SOPMA tool.

S. No.	Organism	Accession	GOR IV analysis		SOPMA Prediction			
		number	<mark>α-helix</mark> (Hh) (%)	Extended strand (Ee) (%)	Random coils (%)	<mark>α-helix</mark> (Hh) (%)	Extended strand (Ee) (%)	Random coils (%)
1.	Bacillus subtilis	P10475	18.24	28.66	53.11	22.85	26.45	36.47
2.	Bacillus akibai	Po6564	28.38	17.25	54.37	27.88	23.75	38.25
3.	Bacillus thuringiensis	M1QQC9	29.64	23.88	46.48	35.18	22.39	30.06
4.	Bacillus pumilus	B2ZHC9	39.34	18.24	42.42	46.52	15.16	28.28
5.	Paenibacillus polymyxa	E3EEC5	19.80	20.36	59.83	22.32	22.04	44.91
6.	Paenibacillus polymyxa	AoAo9oY895	19.69	23.51	56.81	25.08	21.82	41.96

teletocolescore sartu	WITHING I DEA	tr[M100C9]M100C9_BACTU	
** 2774C0 2774C0 24701		tr B22HC9 B22HC9 BACPU	KYFACNDIKLDIEEODEOILKEGIVDPVA
ta ESEEKS STEEKS DALES	CURTEEN/TONE CARDON/CERTINE DETAILINGSOOD OF EAH PARE ANY ONE	tr EBEECS EBEECS PAEPS	REYTOKGLINGTTYFYWSATNAKGTSKIDSATVSAEPKGTPTD
tr ESCECS ESCECS_PACES	STUTSDAY INANUMARY THE PRACTICE STOCKED IN EXCLOSED	tr ABA090Y895 ABA090Y895 PAEMA	TVYTDTW/INGTTYYYVISA/MGAQVSPEST/V/SATPM/EPAPOTPAGLKAT-ASNAEVK
CF MONESCE 335 MENAPORE 335 FROM	when a second seco	sp P18475 GUN2 BACSU	LKYLDSKTISK
sp P06564 GUN_BACA3		sp P86564 GUN_BACA3	TEFLNEIWITSH
		relations and activ	NVVDTRVERVDNDTVCEEUCRENCTI ADDADCENCEVDTV
OF INDOGCO INDOGCO BACIN	***************************************	** 8774C0 8774C0 84C00	TOWNORMER BOARD BOARD OF STORE THE
TP B2DRC9 B22HC9_BACPU		tr backs backs backs	T211/2001/CA
tr EBEECS EBEECS_PAEPS	PITAEFIVYDLPGROCHALASNGELPLTQAGLETYKKDYIDKIASIFANPKYKDIRIVAI	UP (EDECS) ESEELS_PREPS	
tr ABAR90Y895 ABAR90Y895_PAEMA	PITASPYTYDMPGROCHALASNGELPLTQDALERYKKEYTDVTAOTPSNPKYKDTRTVTV	TE ADADOCTOSS ADADOCTOSS_FAERA	LOWARKURED WAR
sp P18475 GUN2_BACSU		sp P20475 GUN2_UACSU	WWWLSDIGE
sp P86564 GUN_BACA3		sp P06564 GUN_BACA3	ANASLITNKNEVSGAFTPFELGKSNATSLDPGPDQVMVPEE
tr M1QQC9 M0QQC9_BACTU	***************************************	e-importainmoora avettu	PROVETVENDAETSODGELOGI MILY NEVGETUNA/TENG
tr B22HC9 B22HC9_BACPU		ch landfes landfes avenu	HIGH CLOWER DOOL OF THE MONTH OF THE
tr E3EEC5 E3EEC5_PAEPS	IEPOSLPNLVTNLDTPQCGQAKSTGIYEAGVKYTHNKLNEIPWVYKV/DIGHSGALGADN	TF BEERS BEERS BALFU	MERLELSQUARIDATELAISLINLTURINA -PUPILEIRE
tr ABA898Y895 ABA898Y895 PAEMA	IEPOSLPNLVTNLSTPACAAANSSNIYRDGITYALDQLHDIPW/YKYLDIGHSGALGHDS	TP ESCECS ESCELS PREPS	
sp P18475 GUN2 BACSU		TF ABARSET895 ABARSET895_FAEMA	NWCSGESSDSNA-YSAAPYELPICADLWAQYRNGDSSA
sp P06564 GUN_BACA3	ULLISLEPTALAAEGNTREDNEK	sp P26475 GUN2_BACSU sp P86564 GUN_BACA3	LSASGTFVRENTLGTKDSTKDIPETPSKDKPTQENGISVQVRAGDGSM
tr M100C9 M100C9_BACTU		Income Income autors	annut service by the part interest on the
tr B2ZHC9 B2ZHC9 BACPU		culuational watera meria	GOEDY11-DEETWWYR-107TEAPLAYWARAIEEGDALAGYMAGYIDLLSAL
tr EBEECS EBEECS PAEPS	NRAATVSLFTTIFKOTSKELASVOGFTTNTANTSPLTEPNLPOPNLNIGGOPDKSSKPVE	TP B22HC9 B22HC9_BACPU	GARDLETACKETHEDAK+TDA+++CKGHTEGAK+++ENAKERADDARALINPOCTDET2C2
tr ABABSBY895 ABAB9BY895 FAEMA	NLOPAWNI, YTSYVOGTKIGEFSSVOGFVTNTANTTPLEEPNL PNANLOUNGOPURAAKYVE	TF EREEG_PALPS	······AWAAKPERN-LICKGTTPVILSELICIX/YETKDESQELQSAVDwAQVGND
50 P18475 GIN2 RACSU		tr ABA898Y895 ABA898Y895 PAEMA	••••••TDNQTRPQFN•IX0WGSTAVKLSDVKLRYYFTKE650AM••QAWDWAQV655
sp P06564 GUN_BACA3	VKRPSEAGALQUQEVDGQMTLVDQHGEKIQLRGMSTHGLQ	sp [P10475] GUN2_BACSU sp [P06564] GUN_BAC43	 NENQTRPQLQ-TXNVGNTTVDLKDVTARVVYKAKINKSQNEOQDVAQTGC5
++ latorplanoorg partu	KADARCHARTCOMPERCITOR THATTACTURE PLANEY	shiresseriess"erec	
the particle partice partic		tr M100C9 M100C9_BACTU	N-GYK
to balling balling been		tr B22HC9 B22HC9_BACPU	150*5
UP ESCEUS ESCEUS_PWEPS	WAY DESCRIPTION AND A STORE AN	tr EJEECS EJEECS PAEPS	WALRTIKONVIEIGESAMGTLAAG
TL NEWBOLLEND WEWBALLAND	WYTFUEAUPTAALTSGYVAXGB/SUUSPLIUTSWEaBOPW/PIGAS	tr ABA898Y895 ABA898Y895 PAEMA	NWWTFTDSVMEVGFTSGAGSLAPG
sp[P104/5]00N2_BACSU	WYGEYYWKDSLKMLKDDWGLTVPKAAPYTADGGYDDW-S	sp P18475 GUN2 BACSU	WTHKPVTLHOPKOGAD-T
sp P86564 GUN_BACA3	WEPETUNDNAYKALANDNESNPTRLAYFYGENDROSNPELI	sp 986564 GUN_BACA3	KLTPDVIV-DEPTTVSTAAIPQGPSANA/NP/RAIXVEPTNFVPLEDKFKAELTITSADS
tr M100C9 M100C9_BACTU	KEDVRLMAEMGLESYR-FSISMARILPTGD-GKVMEKGIEFYMMLIDECLKYGIVPF	an lan open langeren anothi	
tr B22HC9 B22HC9_BACPU	EEDIALFAEMGFKVFR-LSISWARIFPTGLEDKPMEEGLAFYDM/FDEC/KYGIEPL	ra landide a landide a particip	KUTO TENDED TELEVISION CONTRACTOR AND
tr E3EEC5 E3EEC5_PAEPS	GIDINSYNNSGRUDIRSHROWIDISSGAGHETPPQT	TE BEERS BEERS BALFU	NATULITY/ACCORDING LOWTANDS
tr ABA898Y895 ABA898Y895 PAEMA	GSDINT/VDSGRIDKILHROWOWAGAGIG/PPOA	TP EREECS EREECS_PARPS	AGLEDTÖTKUMPORPADIZEDU.ZEDU.ZEDU.LELZUSENKALFEHIDKTARDTEL-
50 P18475 GUN2 BACSU	KNKVKEAVEAAKELGEVVETDMHELNDGNPNONK-EKAKEFFKEMSSLYGNTPN	tr ABA898Y895 ABA898Y895_PAEMA	GQTGDIQDRAAKSSWTNFDETDDYSYNATQTSFANNEKVTLYQNGELWWGIEPGV
sp P86564 GUN_BACA3	KSRVIKGIDLAIENDMYVIVDMMMAPGDPRDPVYAGAEDFFRDIAALYPNNPH	sp P10475 GUN2_BACSU sp P06564 GUN_BACA3	ASTIGNIQURUHNOOVSWIAQSGDYSFFKSN-TEKTTKKITLYDQGKLINGTEPN- PSLEATAW-AENNKINNITUFVGTEGADVIYLDNIKVIGTEVEIPV/HD
triM00009 M00009 BACTU	VTLYHOLFLELE	41	· · · · · · · · · · · · · · · · · · ·
1# 877WF9 877WF9 84FPU	VTIKSWENDTTI T	tr MODOC9 MODOC9 BACTU	
to EDEEVE EDEEVE DAEPE	THE MARK DOMENTS OF THE PROPERTY AND THE DESIGNATION OF THE DESIGN OF TH	TE 877409 877409 84081	
- AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	IDAL MARTING CONTRACT CONTRACT TO A DEAD COLLEGE	TR ESCENE ESCENE PAEPE	
LF Adverter and Adverters Frank	TORE WITH PRODUCT IS A DOME OF THE PRODUCT OF THE P	te annoyens annoyens name	
30 P104/5 GUN2_BACSU	VITE DATE PRODUCTION AND A DATE A	co papererora ascou	
spiPeoso4/GUN_BACAS	TITELAWEPSSWW00401PWEE00AWA	spirzowrojounz_awcou	NATION OF COMPANY AND
tr M100C9 M100C9 BACTU	ÉTVMFCGLEYLKGA-HPPG10NDVPK	shirecoordonaTowney	FRAMEWORK ON THE PROPERTY OF T
THE ROOM OF ROOM OF REAL PROVIDENCE	FIRMMENCE V	an Income Income Party	
+# EBEEFE EBEEFE DAEDS	ADAL VIEWORTUDCCCC, UND	culuntificaluntificaTeerin	***************************************
an ananovene innanovene name	OCTUDENT ALTO SHOT TO THE ADDITION OF THE ADDI	TP 822HC9 822HC9_BACPU	
UF AGAESCI 355 AGAESCI 555 PACHA	UPPELIQUETRALPLSOREDEDITPTRPDTLTAATTURISAESAKSTRAMAATSAEGP	tr EBEECS EBEECS PAEPS	
301610e12100e12100e1210	EAT2ATE	TY ABAPPEYRPS ABAPPEYRPS PAEMA	
sp[P86564[GUN_BACA3	PTVERURDSGMDDKLITIVGSPWSQRPCLAAD	en PIAS25 GIN2 BACSU	
telationes anone parti-	VENITAVEVA	(n 206564 (20 86/13	DELOCATION VICENTIATEDEVI DOVELTECHNITH VEDERTVOALDAD TYTTNED
tr B2ZHC9 B22HC9_BACPU	ANQASH QFLASAL TVKAAKEI IPHAQIGO/UNQIEAY	** Internet Internet Barrie	
tr EBEECS EBEECS PAEPS		+= 2374C0 2374C0 24C0	
tr ABA898Y895 ABA898Y895 PAEMS	FAV1ASWARGLTYTDASIA SOTTYYYYYSSIAACAGESUDGANOVTVTTSYGARGUDTUDLA	C DEERS BEERS BEERS	
se P10475 GIN2 R4/SU		TF ESCES ESEES_PARPS	***************************************
sa Passed QIN BACA3	APTION THE TOULAST	TE I NONOVETERS NUMBORY 895_PREMA	
shir on callona "barres	Treasure and treasure (D)	sp[#18475[GUN2_BACSU	
tr M100C9 M100C9 BACTU	SVDOQKENTRAANHANEYETYNYYDPILKGEYPSYW	sp[P86564]GUN_BACA3	ELEEPNQVVGLVHVEVKINVROITNIQOOTLLRNHHOIFADVESDFAGRVFVDWVRFEGA
tr B2ZHC9 B2ZHC9 BACPU	AKTTKPEDQLQAWKSN-QLWFYPDVQARGEYPTYMV	telenooralistoora avenu	
tr EBEECS EBEECS PAEPS	PS6LXATVGNAQVTLTWDASTIGADSYTVKRATSEAGPFTAWAPLVTA	** 837WD 877WC 84CM	
tr ABAB98Y895 ABAB98Y895 PAFMA	PAKVTAKAGDEONKLINKNAVGGAEGYTVKRAVDAAGPEEDIAETVIIS	to starts bacht a berry	
50 P18475 QIN2 R4(SI	EDKANY AL SKSAPTPYTEVETSDASCHCOVELDYKREU	U ESCOS ESCOS PARPS	
IN POSSA GIN BACAT	DETDISCEROMAKATRY	LT MONOSCIESS AGARSTESS FREMA	***************************************
the second and markets	The second	sp1+28475 0082_84C50	***************************************

Figure 1: Multiple Sequence Alignment result of translated Cellulases Contig showing differences in 5' and 3' UTR.



Figure 2: Kyte and Doolittle Plots for cellulases from different microorganisms (A: *Bacillus subtilis*P10475; B: *Bacillus ak-ibai*P06564; C: *Bacillus thuringiensis* M1QQC9; D: *Bacillus pumilus*B2ZHC9; E: *Paenibacillus polymyxa*E3EEC5; F: *Paenibacillus macerans*A0A090Y895).



Figure 3: Phylogenetic tree constructed by Mega 6.0 software (Molecular Evolutionary Genetics Analyses) with Neighbor Joining method showing evolutionary relationship among xylose reductase sequences from different origin. Bootstrap values are depicted at the nodes with Bar value of 0.2.