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A CARBOHYDRATE HYDROLYSIS ENZYMES ENCODING GENES IN *NEUROSPORA CRASSA*

Ravi Gedela
Department of Bio-Science, Rajiv Gandhi University of Knowledge Technologies (RGUKT) IIIT, Nuzvid-521201, Andhra Pradesh, India.
e-mail id: ravibio531@rgukt.in

ABSTRACT

Neurospora crassa, NCU05882.7 (423aa) and NCU09774.7 (303 aa) (NCU, Neurospora crassa unit) genes encoding a Cellulase, which hydrolysis the Cellulose. In addition to that, reporting here other 35 Carbohydrate hydrolysis enzymes encoding genes in *N.crassa*. A metagenomic analysis for multiple sequences alignment and Phylogenetics analysis, the evaluated result showed high sequence similarity and 99% homology to the other class of fungi; in the bacterial species showed extremely very less sequence similarities and 100 % homology. Where as in inter species between fungi and bacteria, the results showed extremely less sequence similarities and 97 % homology. The studies on physiochemical properties of Cellulase using GeneDoc, the evaluated results showed Cellulase was an amphoteric (polor), aromatic, aliphatic and highly repeated amino acids of glycine and proline. These metagenomic studies could help to straightforward isolation of Cellulase enzymes from NCU05882.7 (Chromosome/Linkage Group-VII), NCU09774.7 (Chromosome Linkage Group- II) and other 35 Carbohydrate hydrolysis enzymes encoding genes in *N.crassa*.

Key Words: Enzyme, Cellulase, Cellulose, Neurospora crassa, Carbohydrates

Academic Discipline And Sub-Disciplines

Fungal Biology

SUBJECT CLASSIFICATION

Carbohydrate Hydrolysis Enzymes

TYPE (METHOD/APPROACH)

Regular Article

Cellulose is the most abundant renewable biological and a low-cost energy resource in the environment (Lynd et al., 2008; Zhang, 2009). The production of bio-based products and bioenergy from less costly renewable lignocelluloses materials would bring benefits to the local economy, environment, and national energy security (Bayer, 1998a, 2004 and 2007; Zhang, 2008). Cellulases is the enzymes that hydrolyze β-1, 4 linkages in cellulose chains (Bayer, 1998a, 2004 and 2007; Lynd et al., 2002, Tian, 2009). They are produced by using fungi, bacteria, protozoans, plants, and animals (Bayer, 1998a, 2004 and 2007; Zhang, 2008; Tian, 2009).

Cellulases and hemicellulases in filamentous fungi

Filamentous fungi have the capacity to secrete large amounts of lignocellulosic degrading enzymes, and this ability has been exploited by industry to produce cellulases in quantities exceeding 100 g/L of culture (Bayer, 1998a, 2004 and 2007; Pauly and Keegstra , 2010; Cherry and Fidantsef , 2003). The most commonly used organism for the production of cellulases in an industrial setting is Trichoderma reesei (Hypocrea jecorina)(Le Crom, 2009). The related filamentous fungus, *Neurospora crassa* also has an innate ability to secrete lignocellulose-degrading enzymes. While Bruce Eberhart first examined this characteristic in the late 1970s, almost nothing further was reported until published a systems analysis of *N. crassa* grown on Miscanthus in 2009 (Tian, 2009). While *N. crassa* might not be known as an industrial workhorse like H. jecorina, it has the unique advantage of being a NIH model organism, most commonly known for its role proving the "one gene, one enzyme" hypothesis by Edward Beadle and George Tatum (Beadle and Tatum, 1941). Given the high conservation of the lignocellulose degrading machinery in filamentous fungi, have begun to develop *N. crassa* as a model to understand the global change such an organism requires to go from energy generation using a simple sugar to a much more complex and recalcitrant molecule such as cellulose (Bayer, 2004 and 2007; Znameroski, 2012).

Cellulases and their roles in cellulose hydrolysis

Cellulolytic microorganisms have evolved two strategies for utilizing their cellulases: discrete noncomplexed cellulases and complexed cellulases (Lynd et al., 2002; Bayer, 2004 and 2007). The catalytic modules of cellulose have been classified into numerous families based on their amino acid sequences and crystal structures (Henrissat, 1991). In general, most of aerobic cellulolytic microorganisms degrade cellulose by secreting a set of individual cellulases, which catalyses either N-terminus or C-terminus of linker peptide catalytic module (Bayer, 1998a and 2004; Lynd et al., 2002; Tian, 2009). In nature, complete cellulose hydrolysis is mediated by a combination of three main types of cellulases: (1) endoglucanases (EC3.2.1.4), (2) exoglucanases, including cellobiohydralyses (EC 3.2.1.91), and (3) β-glucosidase (EC 3.2.1.21). To hydrolyze and metabolize insoluble cellulose, the micro-organisms must secrete the cellulases (except β-glucosidase) that are either free or cell-surface-bound (Lynd et al., 2002; Bayer, 1998a, 2004 and 2007; Himmel et al., 1999; Zaldivar et al., 2001).

Cellulose are increasingly being used for a large variety of industrial purposes-in the textile industry, pulp and paper industry, food as well additive in detergents and improving digestibility of animal feeds. Now Cellulases accounts for a significant share of the worlds industrial enzyme markets (Bayer et al., 2004 and 2007; Himmel et al., 1999; Zaldivar et al., 2001; Tian, 2009; Zhang, 2008, 2009).

On this basis of ground, I reporting here total 37 Carbohydrate hydrolysis enzymes encoding genes in *N. crassa* (Table 1, 2).

MATERIALS & METHODS

Cellulase Enzymes encoding Genes info at NCBI:

N. crassa Cellulase gene info obtained from NCBI, and I found NCU05882.7 (Cellulase), NCU09774.7 (cellulose) and NCU06971.7 (Xylan degradation regulator-1) genes encoding Carbohydrate hydrolysis enzymes in *N.crassa*; BLAST (Altschul et al., 1990) analysis was performed using BLAST tools available in NCBI (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi).

Sequence analysis

The protein sequences were aligned with Clustal X 1.83 (Thompson et al., 1997) and transferred to GeneDoc for visualization (Nicholas et al., 1997). The phylogenetic trees were constructed from these alignments by using the maximum likelihood method (Rzhetsky and Nei, 1992), with 500 bootstrap value replications as a test of phylogeny (Felsenstein, 1985) and the software MEGA5.05 (Tamura et al., 2007). The BLAST (Altschul et al., 1990) analysis was performed using software tools available from NCBI (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi), the Conserved Domain Database (CCD; Marchler-Bauer and Bryant, 2004; Marchler-Bauer et al. 2009) was used to identify conserved domains in the protein.

RESULTS & DISCUSSION

The metagenomic studies for multiple sequences and Phylogenetics tree analysis, the results showed high sequence similarity and 100 % homology to other class of fungi (Figure2.1, 2.2), where as in the bacterial species results showed very less sequence similarity and 100 % homology to other class of bacteria (Figure2.3, 2.4). The comparisons of Cellulase protein sequences between inter species of fungal and bacterial, the evaluated results showed extremely less sequence similarities and 97 % homology (Figure3.1, 3.2). Cellulase gene encoding protein Cellulose in *N.crassa*, using smart BLAST analysis, on consideration of Max.score 874, 700, identity 100%, 100%, e-value 0.0 to 8.00E-154. (http://www.ncbi.nlm.nih.gov/gene) (Table 3). The evaluated results showed NCU05882.7 (Cellulase), NCU09774.7 (Cellulase) and NCU06971.7 (Xylan degradation regulator-1) genes encoding Carbohydrate hydrolysis enzymes in *N.crassa* respectively. These results straightforward assisted to recognizing of 37 Carbohydrate hydrolysis enzymes encoding genes in *N.crassa* (Table1,2),Broadinstitute(http://www.broadinstitute.org/annotation/genome/neurospora/) and NCBI genomes (Figure1.1). The studies on Cellulase's physiochemical properties using GeneDoc (color index: light green color indicates amphoteric (polor), dark green color: Grey color: aromatic and aliphatic, red color: glycine and proline, blue color: negative and positive, black color: hydrophobic charged amino acids) (Figure1.2), the evaluated results showed Cellulase was an amphoteric (polor), aromatic and aliphatic nature as well highly repeated of glycine and proline amino acids residues (Figure1.2).

The Cellulase genes encoding size of Cellulose proteins 423aa, we placed in the genome databases of *N.crassa* (http://www.broadinstitute.org/annotation/genome/neurospora/); the results showed 37 genes encoding of carbohydrate hydrolysis enzymes in *N.crassa* (Table1, 2). In nature, complete Cellulose hydrolysis is mediated by a combination of three main types of Cellulases endoglucanases, Exoglucanases and β-Glucosidase (Lynd et al., 2002; Bayer, 1998a, 2004, Bayer et al., 2007; Himmel et al., 1999; Zaldivar et al., 2001).

The evolutionary studies perspective conserve domain of Cellulase, showed 100 % homology and high sequence similarity other class of fungi (Fig2.1, 2.2), where as in bacteria less sequences similaritie; it indicates conserve domain of Cellulase widespread and predominant in the other class of fungi (Fig2.1, 2.2). These metagenomic studies reveal 37 Carbohydrate hydrolysis enzymes encoding genes in *N. crassa* (Table 1, 2).

CONCLUSION

Cellulase encoding cellulose protein (423aa) of physiochemical properties was an amphoteric (polor), and highly repeated of glycine and proline amino acids residues (Figure1.2). The evaluated results suggested that 37 genes encoding Carbohydrates hydrolysis enzymes in *N.crassa*. These results could help to molecular level studies and straightforward isolation, characterization, and functional analysis of Carbohydrate hydrolysis enzymes in *N.crassa* and other class of fungi as well.

The most useful thing is that *Cellulase* enzyme, straightforwardly we can isolate from *N.crassa* as well grow vastly in Bio-processing units, could be supplied waivers less for industrial purposes-in the textile, pulp, and food (Bayer et al., 2007; Himmel et al., 1999; Zaldivar et al., 2001; Tian, 2009; Zhang, 2008, 2009).

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S. No	Name/Gene ID	Encoding enzyme	No. of Amino Acids	Location of chromosome	Organism
1	NCU05882.7	Cellulase	423 aa	Chromosome VII (Supercontig 7: 4155062-4158498 +)	*N.Crassa*
2	NCU09774.7	Cellulase	303 aa	Chromosome II (Supercontig 2: 4271243-4272151 +)	*N.Crassa*
3	NCU06971.7	Xylan degradation regulator-1	945 aa	Chromosome IV (Supercontig 4: 4706112-4709560 -)	*N.Crassa*

Conflict of interest:

The authors declare that, I have no conflict of interest.

Tables:

Table: 1 Putative Cellulase encoding genes in *N.crassa*

Note: Date obtain from http://www.broadinstitute.org/annotation/genome/neurospora/FeatureSearch.html

Table: 2 Putative Cellulase encoding genes in N.crassa

S.No /Locus	Gene Name	Location	Length	Gene Symbol	Annotations	Alleles	Relevence	Matching Fields
4]NCU07340.7	cellobiohydrolase-1	N. crassa OR74A (NC12): Supercontig 4: 4311049-4314145 -	3097	cbh-1	CA-32961, CA-32962, CA-32963		0.75	blastx, citation, hmmer, product, terms
5]NCU05104.7	glycosylhydrolase family 7-4	N. crassa OR74A (NC12): Supercontig 6: 3732709-3734273 +	1565	gh7-4	CA-39969, CA-39970, CA-39971		0.69	blastx
6]NCU09416.7	cellulose-binding GDSL lipase /acylhydrolase	N. crassa OR74A (NC12): Supercontig 7: 1189102-1190441 -	1340		CA-34963		0.45	blastx, desc, hmmer, product
7]NCU06599.7	cellulose-binding protein	N. crassa OR74A (NC12): Supercontig 4: 1762371-1763523 -	1153				0.36	desc, product
8]NCU02344.7	fungal cellulose binding domain-containing protein	N. crassa OR74A (NC12): Supercontig 7: 2842202-2844057 -	1856		CA-38504, CA-38505	KO1	0.34	blastx, citation, desc, product, terms

9]NCU05751.7	cellulose-binding protein	N. crassa OR74A (NC12): Supercontig 7: 3708740-3709661 -	922		CA-35799, CA-35800	KO1	0.32	desc, product
10]NCU08785.7	fungal cellulose binding domain-containing protein	N. crassa OR74A (NC12): Supercontig 3: 5130163-5131035 -	873		CA-39940, CA-39941		0.3	blastx, desc, product
11]NCU08042.7	cellulose degradation regulator-2	N. crassa OR74A (NC12): Supercontig 4: 4001190-4004643 +	3454	clr-2	CA-34131	KO1	0.26	desc, terms
12]NCU07705.7	cellulose degradation regulator-1	N. crassa OR74A (NC12): Supercontig 4: 2149157-2154036 -	4880	clr-1	CA-35351, CA-35352	KO1	0.23	desc, terms
13]NCU02240.7	glycosylhydrolase family 61-1	N. crassa OR74A (NC12): Supercontig 7: 3218962-3221181 -	2220	gh61-1	CA-38584, CA-38585, CA-38586	KO1	0.16	blastx, citation, hmmer, terms
14]NCU02916.7	glycosylhydrolase family 61-3	N. crassa OR74A (NC12): Supercontig 1: 8701416-8703127 +	1712	gh61-3	CA-33161, CA-33162, CA-33163		0.16	blastx, citation, hmmer, terms
15]NCU07760.7	glycosylhydrolase family 61-2	N. crassa OR74A (NC12): Supercontig 5: 394274-396048 +	1775	gh61-2	CA-39532, CA-39533, CA-39534		0.16	blastx, citation, hmmer, terms
16]NCU00836.7	glycosylhydrolase family 61-7	N. crassa OR74A (NC12): Supercontig 1: 7005196-7006890 +	1695	gh61-7	CA-36947, CA-36948, CA-36949		0.16	blastx, citation, hmmer, terms
17]NCU08760.7	glycosylhydrolase family 61-5	N. crassa OR74A (NC12): Supercontig 3: 5242495-5244087 +	1593	gh61-5	CA-38472, CA-38473, CA-38474		0.15	citation, hmmer, terms
18]NCU01867.7	glycosylhydrolase family 61-10	N. crassa OR74A (NC12): Supercontig 1: 2206690-2207912 -	1223	gh61-10	CA-33636, CA-33637, CA-33638		0.14	blastx, hmmer, terms
19]NCU09680.7	glycosylhydrolase family 6-2	N. crassa OR74A (NC12): Supercontig 5: 858215-859731 -	1517	gh6-2	CA-33934, CA-33935, CA-33936		0.12	blastx, citation, hmmer, terms
20]NCU01050.7	glycosylhydrolase family 61-4	N. crassa OR74A (NC12): Supercontig 5:	773	gh61-4	CA-36510, CA-36511, CA-36512		0.11	blastx, citation, terms

		2769232-2770004 +					
21]NCU05121.7	glycosylhydrolase family 45-1	N. crassa OR74A (NC12): Supercontig 6: 3681542-3682601 -	1060	gh45-1	CA-37463, CA-37464, CA-37465	0.11	hmmer, terms
22]NCU07898.7	endoglucanase IV	N. crassa OR74A (NC12): Supercontig 4: 1412641-1413821 -	1181		CA-33980, CA-33981	0.1	blastx, citation, terms
23]NCU05955.7	Cel74a	N. crassa OR74A (NC12): Supercontig 6: 3326807-3330420 +	3614		CA-39275, CA-39276	0.1	hmmer
24]NCU03328.7	glycosylhydrolase family 61-6	N. crassa OR74A (NC12): Supercontig 2: 1091218-1092875 +	1658	gh61-6	CA-33155, CA-33156, CA-33157	0.09	blastx, citation, terms
25]NCU04952.7	glycosyl hydrolase family 3-4	N. crassa OR74A (NC12): Supercontig 4: 846341-849048 -	2708	gh3-4	CA-37496, CA-37497, CA-37498	0.09	blastx, citation, terms
26)NCU00206.7	cellobiose dehydrogenase-1	N. crassa OR74A (NC12): Supercontig 3: 3004156-3008108 -	3953	cdh-1	CA-37974, CA-37975	0.08	citation, hmmer
27]NCU05057.7	glycosylhydrolase family 7-1	N. crassa OR74A (NC12): Supercontig 6: 3894456-3895844 +	1389	gh7-1	CA-39629, CA-39630, CA-39631	0.07	citation, terms
						0.07	blastx
					CA-42905	0.07	hmmer
28]NCU04997.7	glycosylhydrolase family 10-3	N. crassa OR74A (NC12): Supercontig 6: 4144717-4146414 +	1698	gh10-3	CA-32361, CA-32362	0.07	hmmer
29]NCU04500.7	glycosylhydrolase family 18-1	N. crassa OR74A (NC12): Supercontig 4: 3162545-3164692 -	2148	gh18-1	CA-37043, CA-37044	0.07	hmmer
30]NCU05159.7	acetylxylan esterase	N. crassa OR74A (NC12): Supercontig 6: 3537954-3540330 +	2377		CA-38014, CA-38015	0.07	hmmer
31]NCU07225.7	glycosylhydrolase 11-2	N. crassa OR74A (NC12): Supercontig 5: 6198659-	1322	gh11-2	CA-33247, CA-33248, CA-33249	0.07	hmmer

		6199980 -						
32]NCU08114.7	cellodextrin transport-2	N. crassa OR74A (NC12): Supercontig 1: 318873-321079 -	2207	cdt-2	CA-33513, CA-33514		0.06	terms
33]NCU04854.7	glycosylhydrolase family 7-2	N. crassa OR74A (NC12): Supercontig 4: 477626-480111 -	2486	gh7-2	CA-36413, CA-36414		0.05	terms
34]NCU07190.7	glycosylhydrolase family 6-3	N. crassa OR74A (NC12): Supercontig 5: 6293016-6295011 -	1996	gh6-3	CA-39007, CA-39008, CA-39009		0.05	citation, terms
35]NCU06358.7	high affinity glucose transporter RGT2	N. crassa OR74A (NC12): Supercontig 4: 2795914-2798939 -	3026			CA-32964		0.25
36]NCU01813.7	high affinity glucose transporter	N. crassa OR74A (NC12): Supercontig 2: 2341940-2344977 +	3038			CA-38103		0.25
37]NCU00762.7	glycosylhydrolase family 5-1	N. crassa OR74A (NC12): Supercontig 1: 7271711-7273785 -	2075	gh5-1		CA-35893, CA-35894, CA-35895		0.65

Note: Date obtain from http://www.broadinstitute.org/annotation/genome/neurospora/FeatureSearchResults.html?sp=*15

Table: 3 Putative Cellulase encoding genes in other class of fungi

Cellulase in other class of Fungi	Encoding enzyme	Max Score	Identity	E-value	Accessin No
Neurospora crassa	Cellulase	874	100%	0	XP_959873.1
Sporothrix schenckii	Cellulase	568	100%	0	KJR83574.1
Sporothrix brasiliensis	Cellulase	561	92%	0	KIH93867.1
Gaeumannomyces graminis var	Cellulase	481	99%	2.00E-163	XP_009218474.1
Magnaporthiopsis poae	Cellulase	479	100%	1.00E-162	KLU90982.1
Aspergillus fumigatus	Cellulase	460	91%	4.00E-156	KMK62928.1

Neosartorya fischeri	Cellulase	456	91%	2.00E-154	XP_001259223.1
Aspergillus nomius	Cellulase	450	91%	3.00E-152	KNG88126.1
Aspergillus oryzae	Cellulase	447	91%	3.00E-151	XP_001727126.2
Aspergillus flavus	Cellulase	437	91%	8.00E-147	KOC12502.1
Aspergillus kawachii	Cellulase	432	90%	5.00E-145	GAA92545.1
Aspergillus niger	Cellulase	427	90%	2.00E-143	XP_001390007.2
Magnaporthe oryzae	Cellulase	422	91%	2.00E-140	XP_003719440.1

Note: Date obtain from http://blast.ncbi.nlm.nih.gov/Blast.cgi

Figure

Fig:1.1 Carbohydrates hydrolysis by enzymes encoding gene information in *N.crassa* (http://www.ncbi.nlm.nih.gov/gene)

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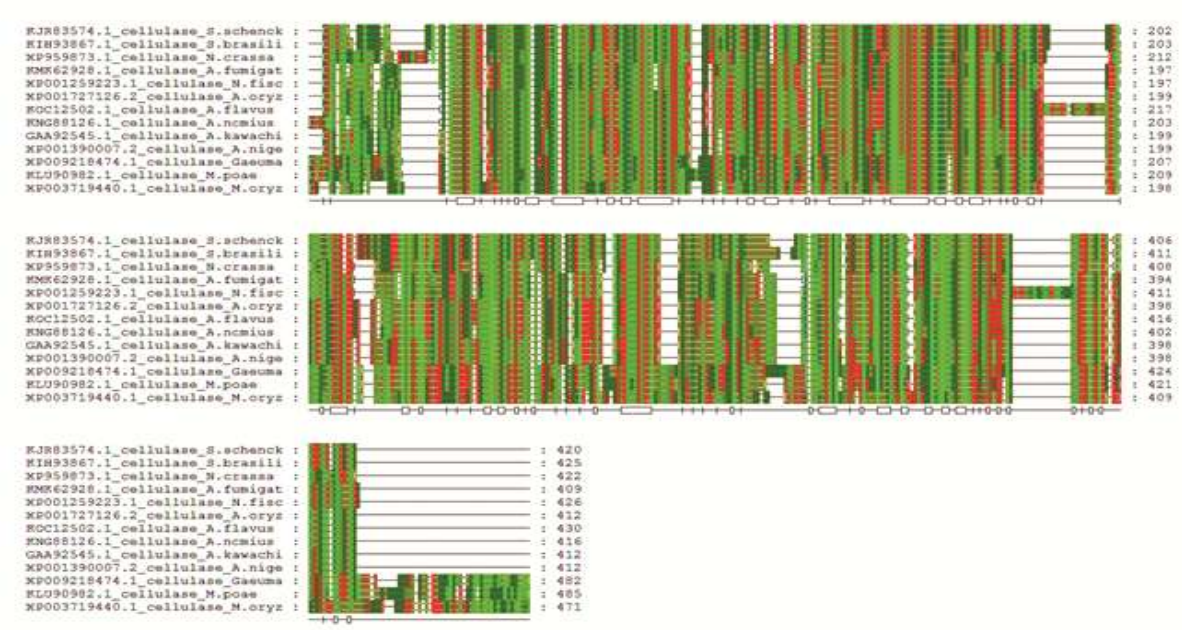

Fig: 1.2 Physico-chemical parameter of cellulase protein in *N.Crassa*, analyzed by using Genedoc: light green color indicates amphoteric (polor), dark green color: Grey color: aromatic and aliphatic, red color: glysin and prolin, blue color: negative and positive , black color : hydrophobic charged amino acids.

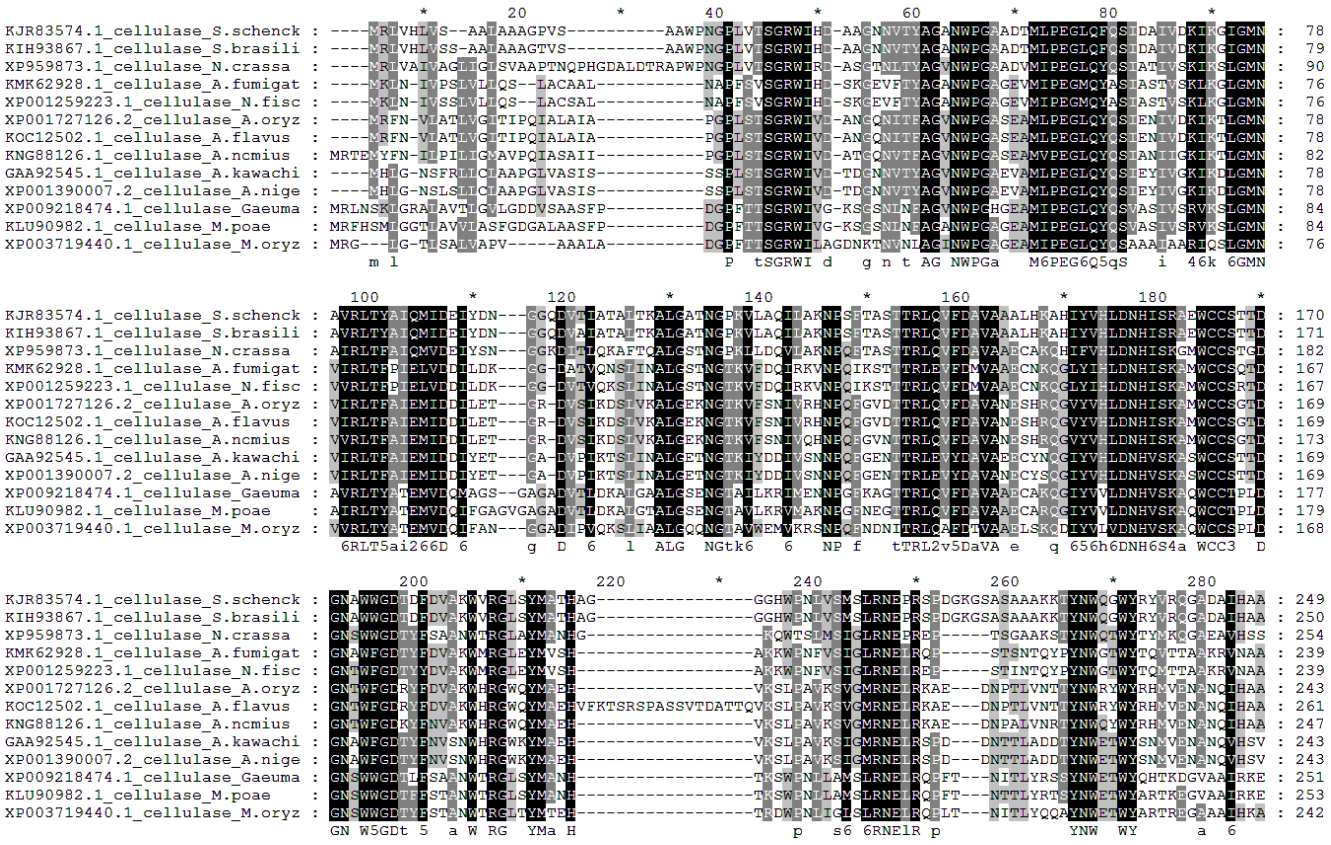

Fig: 2.1 Multiple sequence analysis on Cellulase encoding protein in *N. Crassa*, using GeneDoc software (Black color indicates: 100% similarities, grey color indicates: 80% similarities)

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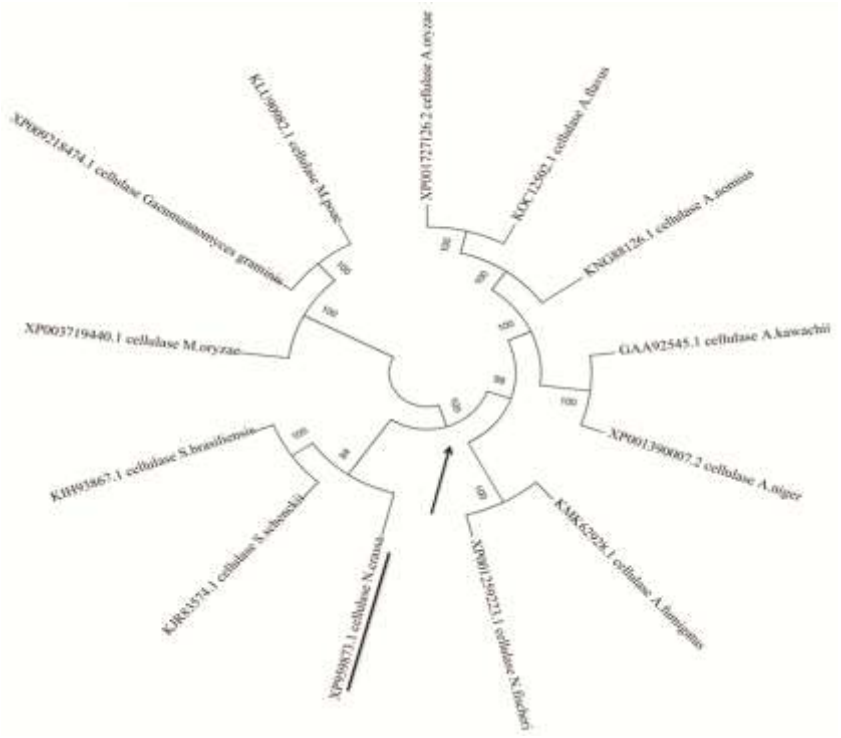

Fig: 2.2 Phylogenetics tree analyses in *N.crassa*, using the maximum likelihood method, 500 Bootstrap replications (bootstrap values are indicated in the point at nodes) as test of phylogeny, and the software MEGA5.05

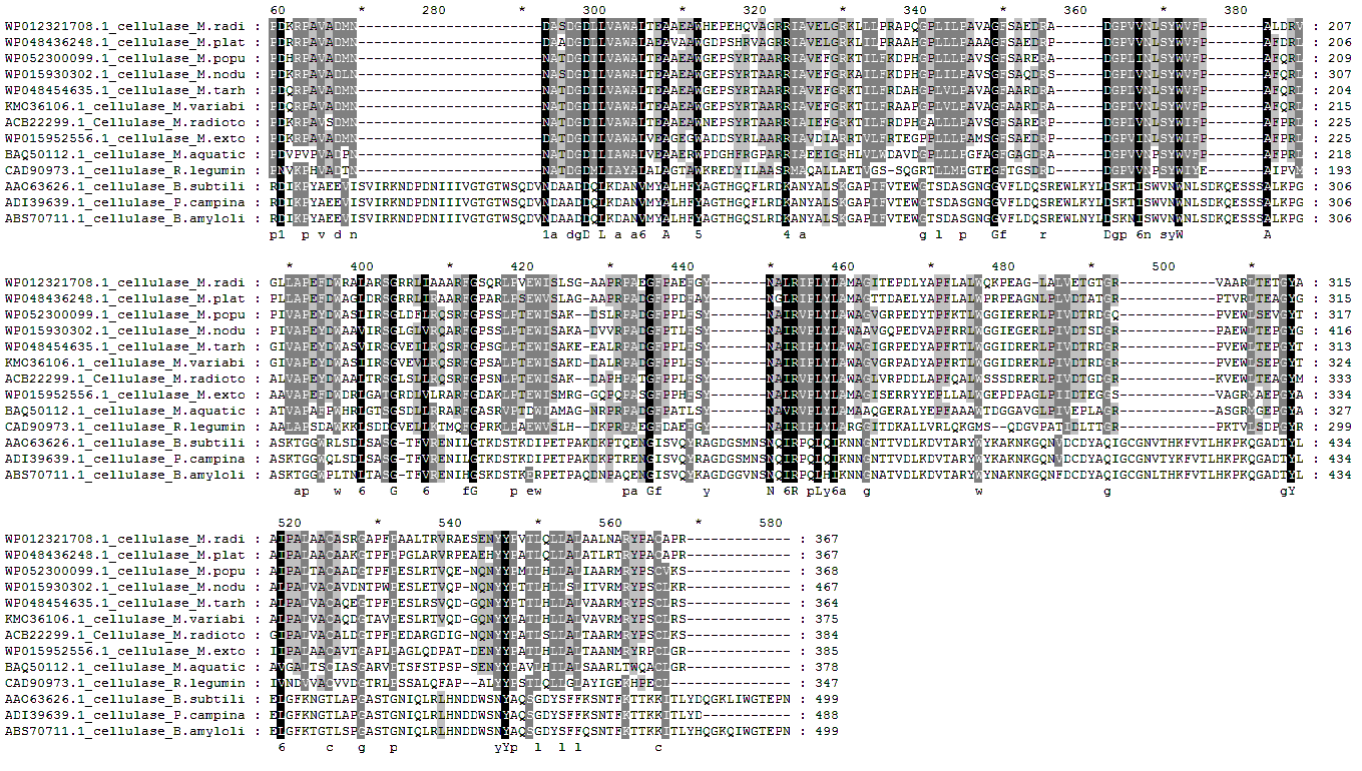

Fig: 2.3 Multiple sequence analysis on Cellulase encoding protein in *Bacillus subtilis*, using GeneDoc software (Black color indicates: 100% similarities, grey color indicates: 80% similarities)

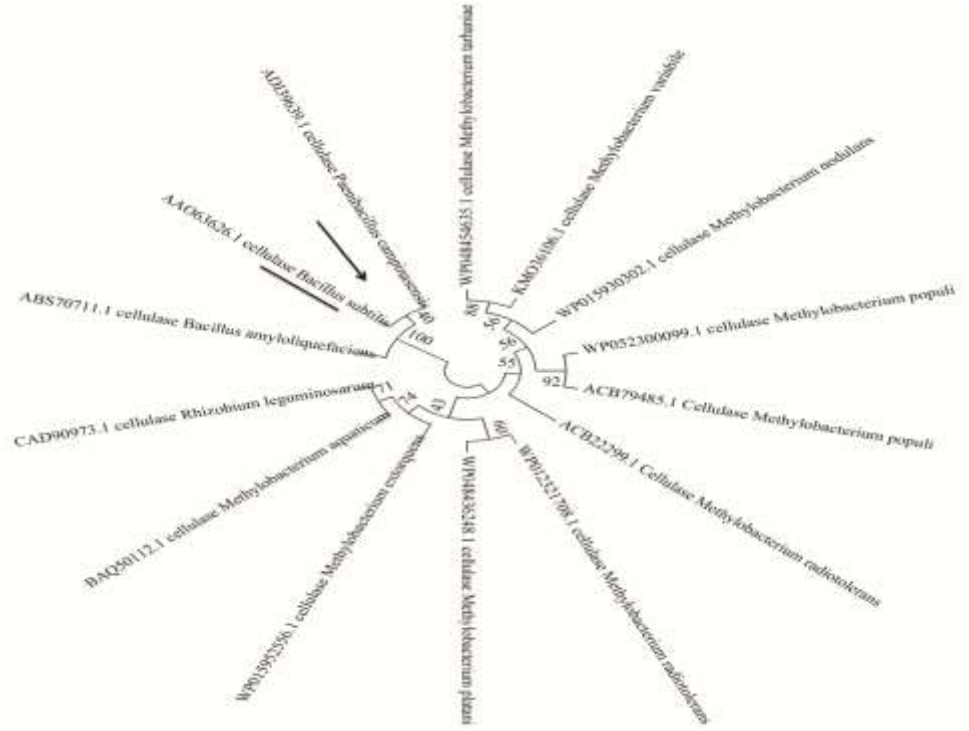

Fig: 2.4 Phylogenetics tree analyses in *Bacillus subtilis*, using the maximum likelihood method, 500 Bootstrap replications (bootstrap values are indicated in the point at nodes) as test of phylogeny, and the software MEGA5.05

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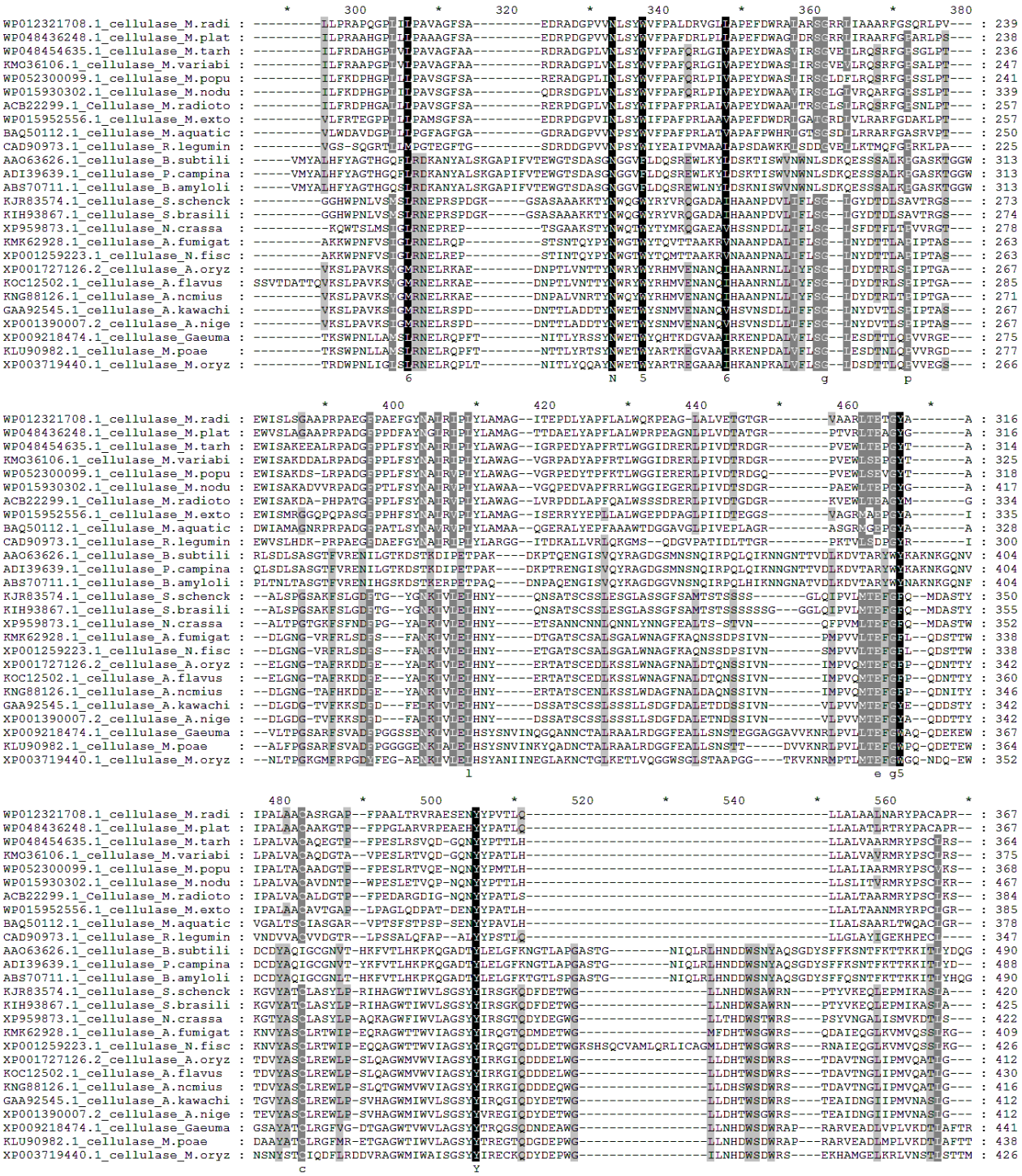

Fig: 3.1 Multiple sequence analysis between interspecies of Bacteria and Fungi, on Cellulase encoding protein Cellulose, using GeneDoc software (Black color indicates: 100% similarities, grey color indicates: 80% similarities)

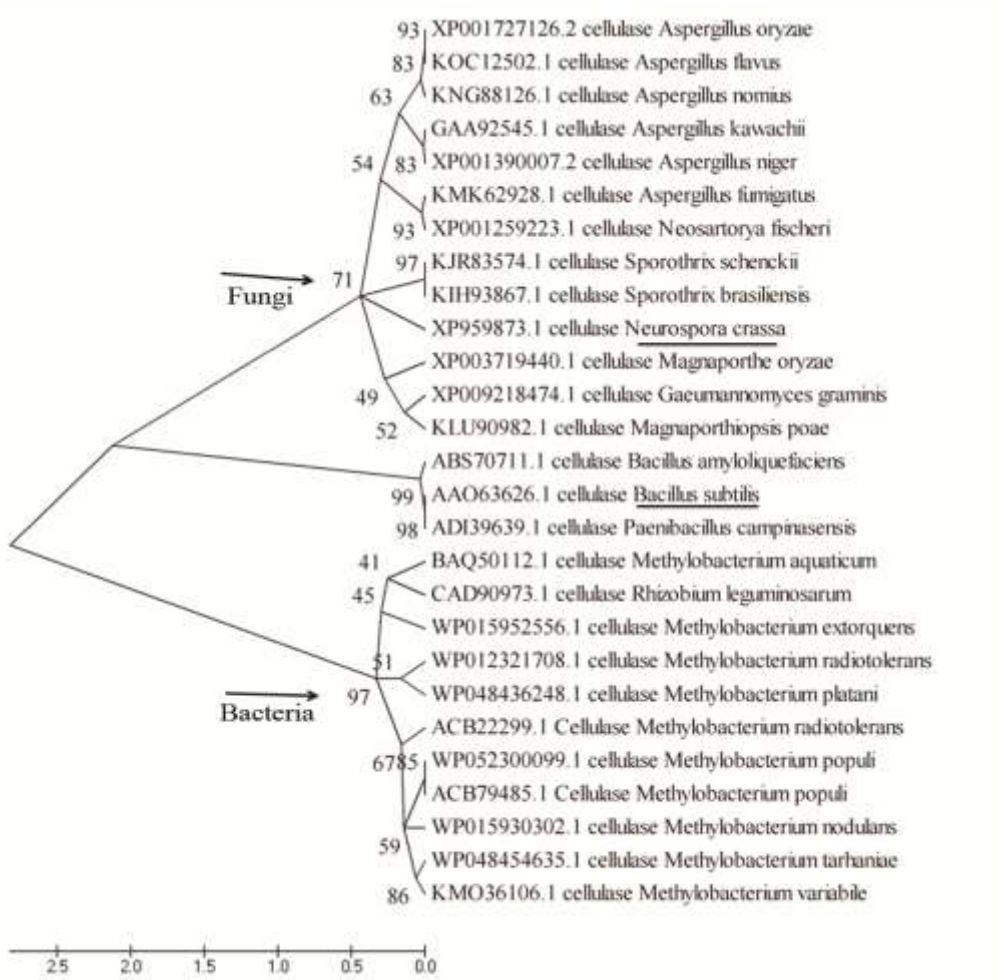

Fig: 3.2 Phylogenetics tree analyses between Bacteria and Fungi, using the maximum likelihood method, 500 Bootstrap replications (bootstrap values are indicated in the point at nodes) as test of phylogeny, and the software MEGA5.05

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