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Full Length Article

Genetic Diversity Analysis and *In Silico* Investigation of Post-Translational Modifications of Carboxypeptidase A1 (CpA1) in *Sordaria fimicola*

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Abstract

Post-translational modifications (PTMs) regulate different complex mechanisms of cell and affect cell growth, stress, evolution of living organisms and adaptations due to environment. The purpose of the present research is to investigate the genetic diversity and PTMs of protease (Carboxypeptidase A1) n *Sordaria fimicola*. They perform a variety of functions ranging from housekeeping: *e.g.*, protein maturation, signal peptide cleavage, signal transduction, intracellular protein turnover, immune response, apoptosis, and reproduction. *S. fimicola* is a microscopic filamentous fungus, has been preferably used in this study because of its easy growing pattern on Potato Dextrose Agar (PDA) and a short life cycle of 7 to 12 days. The genomic DNA of six of the strains *S. fimicola* was used to amplify the carboxypeptidases A1 gene (*CpA1*), the product size was 940 bp. The multiple sequence alignment of the nucleotide sequences of six strains of *S. fimicola* with *Neurospora crassa* (as a reference strain) was studied. The numbers of polymorphic sites in six strains of *S. fimicola* with respect to *N. crassa* were six. Posttranslational modifications were depicted by using bioinformatics tools *i.e.*, YinOYang1.2, NetPhos 3.1 and NetNES 1.1 Server to calculate O-glycosylation, phosphorylation sites, and nuclear export signals respectively. The study has predicted 19 phosphorylation sites on serine residues for protease Carboxypeptidase A1 in S1 strains of *S. fimicola* while 15 phosphorylation sites on serine in N7 strain and 17 serine phosphorylation modifications were predicted in *N. Crassa.* The results of this research will be helpful for further *in vitro* investigations of this industrially important enzyme under study. © 2021 Friends Science Publishers

Keywords: Acetylation; Coprophilous fungus; Glycosylation; Phosphorylation; Protease; Strains

Introduction

Proteases account for 60% of total commercial enzyme market and the sources of proteases are microbes, fungi, animals, and plants (Boominadhan *et al.* 2009; Muszewska*et al.* 2017). Microbial proteases are widely utilised in several industries such as brewing, detergent, leather, dairy, and food-processing factory (Arber 2000; Wood *et al.* 2011) The first aim of the current research is to explore genetic variations of protease Carboxypeptidase A1 (*CpA1*) of different strains of *S. fimicola* collected from the north-facing slope (NFS), south-facing slope (SFS) of "Evolution Canyon".

S. fimicola is a microscopic coprophilous fungus belongs to the class Sordariomycetes and is closely related to *Neurospora* and *Podospora*. It is found all over the world and produces black perithecia containing asci of eight dark ascospores in a linear arrangement. Due to having short life cycle of usually 7–12 days and easily grown in culture, *S. fimicola* is considered as a model organism for genetics study (Arif *et al*. 2019).

The natural selection of living organisms is competed for the adverse environmental conditions by genetic variations. Thus, evolution depends upon these variations because these are the causes of the evolutionary potential of organisms (Arif *et al*. 2017). Mutations are generated by these genetic variations and finally create frontier diversity of biomolecules like proteins by several PTMs. Living organisms preferred them to compete for the environmental stresses such as temperature, light, wind, water *etc*. The advancement of molecular biology quickly examines the exploration of the genetic biodiversity of different species. Bioinformatics and proteomics tools are predicting the details of modified sites of the molecules, which are used for joining and disjoining of functional groups (Marquez *et al.* 2018). This information of joining and disjoining of functional groups to the molecules is essential for the details of post-translational modifications network in the cell of living organisms (Shen 2013). Yu *et al.* (2007) reported that Ascomycota possesses more than two hundred types of

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PTMs. Marquez *et al.* (2018) and Jimenez-Morales *et al.* (2013) said that PTMs like acetylation, methylation, glycosylation, phosphorylation, S-nitrosylation and Ubiquitination commonly occur in eukaryotes (Chandramouli and Qian 2009).

This research aims to investigate the various PTMs of protease CpA1 using bioinformatics tools, which is a hot topic now a day because of their role in the understanding of different biological processes at the cellular level and designing of drugs against many diseases especially cancer (Chou 2019). This study has also been reported some particular protein kinases that are included in the phosphorylation of protease. Protein kinase C (PKC) accomplishes the role of controlling many proteins by adding the phosphate on the –OH (hydroxyl) groups of serine and threonine. This enzyme is activated in the increased concentration of diacylglycerol or calcium ions in the presence of signals such as an increase in the level of calcium ions or diacylglycerol (Khoury *et al.* 2011). The other protein kinase, CK2 (Casein kinase 2) is well known to occur in a physiological complex of tetramer (Sibanda *et al*. 2010). The DNA-Pkc is a threonine and serine protein kinase that consists of a single polypeptide chain made of 4128 amino acids (Turnham and Scott 2016).

To the best of our information, no scientific study was done on PTMs of protease CpA1 protein such as glycosylation, phosphorylation, and acetylation in *N. crassa* and *S. fimicola*. Regulation of protease Carboxypeptidase A1 protein by PTMs will characterize a new path of interest regarding the cell processes and cellular signalling and help in establishing the platform to produce proteases on small as well as on a large scale.

Materials and Methods

Collection of samples and extraction of genomic DNA

The samples of six parental strains obtained from the north-facing slope (NFS) and south-facing slope (SFS) of "Evolution Canyon", Israel, which possesses diverse environmental conditions. (These strains were received from Genetics department of Imperial College London). The sub-culturing of total six strains was done on PDA (potato dextrose agar) that is a nutrient medium for the growth of filamentous fungi followed by incubation at 20∘C in an incubator. We obtained mature fungal growth in 8 days and then these sub-cultures were subjected for DNA extraction. DNA extraction was performed by adopting the method described by Pietro *et al*. (1995) and the DNA concentration, as well as quality, was assessed by calculating the absorbance at OD260/OD280.

Amplification and sequencing

Forward and reverse primers were designed through Primer 3 software for the amplification of CpA1 genes of all strains. The sequences of the forward primer were ATCTTTCCTCACCGCC, and reverse primer was GTACTCGGCGACCATGGTAG. The PCR reaction volume was 15 μL, which contained 10 μL PCR master mix (Gene All), 1 μL forward primer, 1 μL reverse primer and 3 μL ddH_2O. Then amplification was carried out by one round of amplification consists of initial denaturation at 95°C for 15 min followed by 30 cycles of denaturation at 95°C for 20 s, annealing at 50°C for 40 sec and extension at 72°C for 1 min, with a final extension step of 72°C for 5 min. The amplification of PCR results was confirmed by running the product on gel electrophoreses on 0.8% agarose gel. The required bands were eluted and were sent for sequencing to Macrogen Korea.

Prediction tools used for post-translational modifications

PTMs were investigated with the help of these bioinformatics tools like YinOYang1.2, NetPhos 3.1 and NetNES 1.1 Servers. YinOYang 1.2 server is utilised to calculate glycosylation. NetPhos 3.1 server is used for phosphorylation sites prediction on residues of serine, threonine and tyrosine, whereas NetNES 1.1 server is utilised for nuclear export signals (NES). Online tool 'EMBOSS Transeq' was used to obtain the sequences of amino acid of amplified genes while the amino acid sequences of reference strain were retrieved from Uniprot.

Homology modelling and model validation

Phyre2server (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index) is a reliable 3D structure prediction tool, which was used to build 3D models of protease with 100% confidence prediction. Afterward, the RAMPAGE tool was used to validate the 3D models available at http://mordred.bioc.cam.ac.uk/~rapper/rampage.php.

Tertiary structure refinement

Galaxy Refine (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) tool was used to check the refinement of 3D structures of the template-based modelled protein. A unique web server uses a side chain algorithm with packaging and structural relaxation by molecular dynamics simulation. This tool increases the overall local and global quality of 3D structures. The tertiary structures of S1, N7 and *N. crassa* were subjected to the Galaxy Refine webserver to refine and enhance the quality of 3D models on mild and aggressive relaxation algorithm.

Results

The genomic DNA of all strains was used to amplify the protease *CpA1* gene and product size of 940 bp was obtained. Polymorphism study was carried out by aligning the nucleotide sequences of six strains of *S. fimicola* with a

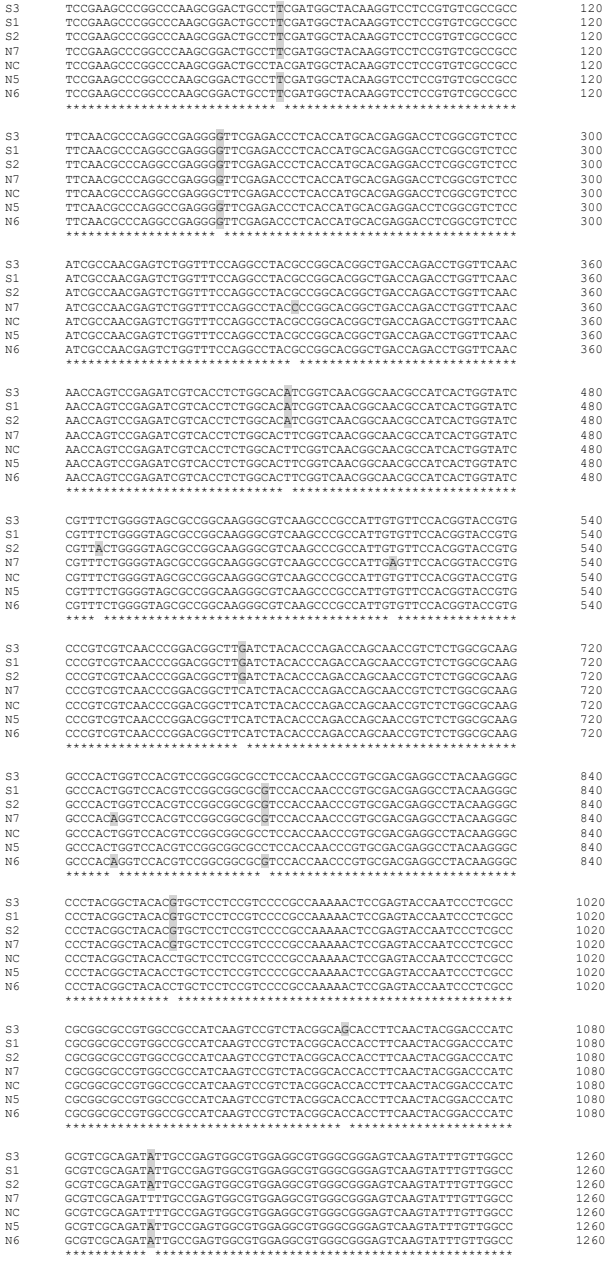

Fig. 1: Multiple sequence alignment of nucleotides sequence of protease *CpA*1 regions of six *S. fimicola* strains with reference sequence of *N. crassa.* The gaps or spaces are showing polymorphic sites and symbols (*) are showing similar or non-polymorphic sites

reference organism *N. crassa* in clustal omega online tool. The numbers of polymorphic sites in the strains of *S. fimicola* compared to the *N. crassa* are 12 (Fig. 1). After sequencing, the sequences were subjected to blast tool at NCBI to check homologous sequences to those found for *S. fimicola*. BLAST used the *S. fimicola* sequence as a query sequence to find out the homologous region in *N. crassa.* The alignment of the amino acid sequences of six strains of

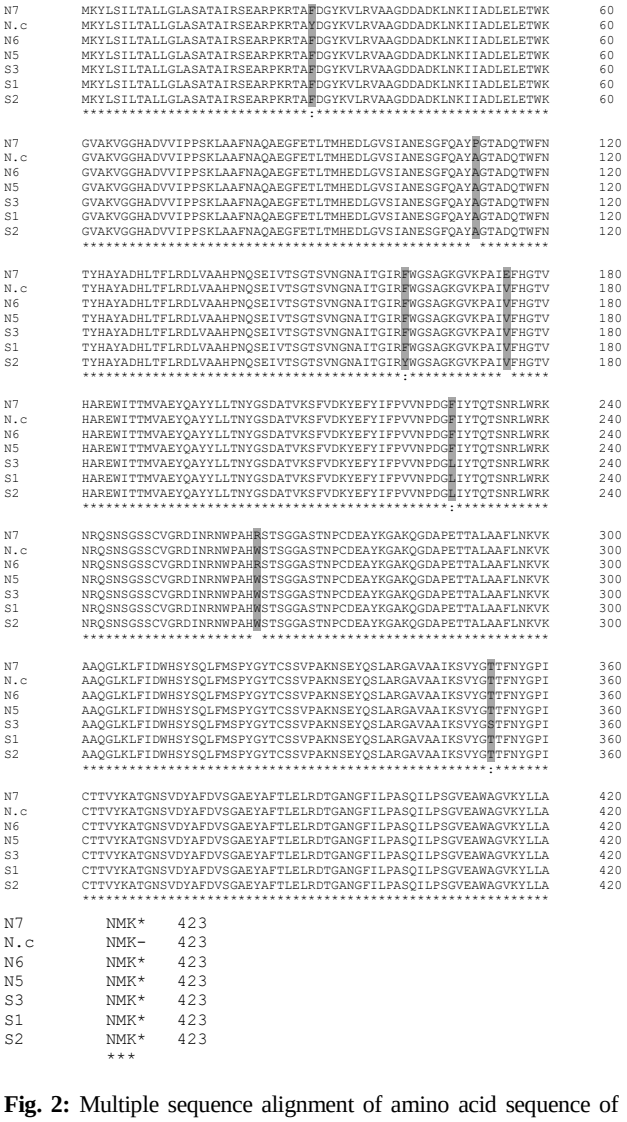

Fig. 2: Multiple sequence alignment of amino acid sequence of six strains of *S. fimicola* with reference sequence of *N. crassa.* The gaps are showing polymorphic sites and symbol (:) is showing the conservation among the species of strongly similar properties

S. fimicola with the reference sequence of *N. crassa* showed seven polymorphic sites, and four sites were found to be highly conserved among the species of strongly similar properties. The gaps indicate polymorphic sites and symbols (:) present the conservation among the species of significant features of similarities. The asterisks (*) at the end of the amino acid sequence indicate the presence of stop codons (Fig. 2).

O-glycosylation and YinOYang – predicted sites

YinOYang and O-glycosylation sites at Serine, Threonine residues for CpA1 of *N. crassa* and *S. fimicola* were attained by YinOYang 1.2 (Table 1). In *N. crassa*, glycosylation was found on eight Serine residues, and five Threonine residues. SFS strains had nine serine, and four Threonine

Table 1: Table is showing predicted O-glycosylation sites at Serine (S), Threonine (T) and Tyrosine (Y) residues as well as acetylation on Lysine (K) residues for carboxypeptidase A1 of *N. crassa* and *S. fimicola.* Glycosylation sites with asterisks are YinOYang sites, where interplay of phosphorylation and glycosylation is taking place.

Organism	Amino Acid Residues	Glycosylation Positions	Acetylation on Lysine (K)
N. crassa	S	200*, 202, 220, 222*, 283, 284, 290,335 Total=08	33, 57, 77, 91, 108, 202, 240, 310, 313, 331, 337, 363, 379, 447, 454 Total=14
	T	103,221,245, 281,348 Total=05	
SFS Strains	S	174*,184*, 186, 204, 206*, 267, 268, 274, 319 Total=09	2, 26, 34, 46, 60, 77, 171, 209, 279, 282, 300, 306, 332, 348, 416, 423 Total=16
	T	87, 205, 265, 332 Total=04	
NFS Strains	S	184*, 186, 204, 206*, 267, 268, 274, 319 Total=08	2, 26, 34, 46, 60, 77, 171, 209, 279, 282, 300, 306, 332, 348, 416, 423 Total=16
	T	53, 87, 205*, 229, 265, 332 Total=06	

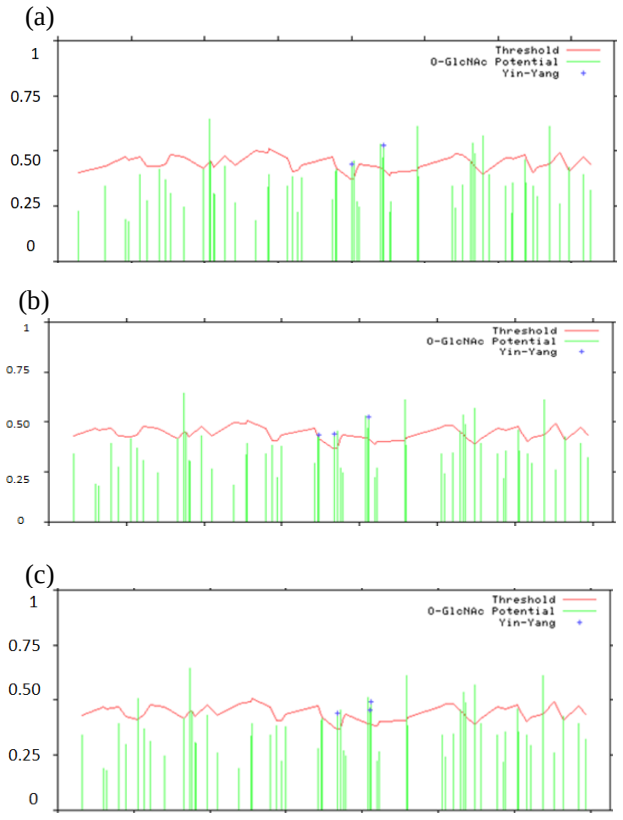

Fig. 3: Graphs are showing glycosylation potential of each O-GlcNAc (O-linked acetyl glucosamine) modified sites (**a**) *N. crassa* (**b**) SFS strains and (**c**) NFS strains of *S. fimicola.* Vertical lines in green color are showing O-GlcNAc potential, red horizontal line is showing threshold level (0.5) and blue plus (+) signs are representing YinOYang sites

glycosylation modifications; while NFS strains have eight serine glycosylation modifications and six threonine modifications. The residues with asterisks are YinOYang sites where the interchange of phosphorylation and glycosylation is taking place. The potential of all glycosylation sites is shown in Fig. 3.

Prediction of acetylation, phosphorylation and nuclear export signals (NES)

Predicted sites of acetylation of internal lysine residues for Protease CpA1of *S. fimicola* and *N. crassa* are shown in Table 1. We found 14 acetylation sites in N. crassa and 16 in each of SFS and NFS strains. All possible phosphorylation sites of *N. crassa* and *S. fimicola* are given in Table 2. The sites (S-407, T-58, T-231, T-325, T-353, and T-363) of *S. fimicola* are different from *N. crassa* due to the genetic variation after PTMs. The sites (S-403, T- 266, T-279, Y-58, Y-230 and Y-231) of *N. crassa* are different from *S. fimicola* due to genetic variations. Nuclear export signals on residue 56-L (Lucien) and 93-M (Methionine) in *N. crassa* and *S. fimicola* have been predicted as shown in Fig. 4.

Molecular modelling and structure validation

All of the three proteins of *N. crassa*, *Sordaria fimicola* N7, and *Sordaria fimicola* S1 were modelled using the Phyre2 structure prediction server. The template used to model the N7, S1, and *N. crassa* proteins were the human CpA1 (PDB ID: 5OM9). Based on this template, all amino acid residues of input protein sequences were modelled as one domain. The overall uGDT (un-normalized global distance test) of *S. fimicola* N7 was 317 (74), *S. fimicola* S1 was 316(74), and *N. crassa* protein was 319 (75), presenting the same residues number in the alignment. A total of 432 amino acid residues were modelled as a single domain with an 8% disorder. Each model has its own dimensions (Å) with small differences; (a) X: 50.735, Y: 50.990, Z: 60.736 (b) X: 50.716, Y: 55.215, Z: 60.736 (c) X: 50.728, Y: 51.564, Z: 60.73 (Fig. 5).

Secondary structure information revealed the presence of 35% helix, 15% Beta sheet, and 49% coiled structure. The P-value of the 3D model suggests the relative quality of the predicted model, lesser the P-value, excellent the quality of the model. The P-value got for the predicted N7 model was 2.40e−14, 3.35e−14 for S1 and 4.56e−14 for N. *crassa* expressing the excellent quality of the model. Galaxy Refine

Table 2: Phosphorylation predicted sites with their protein kinases for Carboxypeptidase A1 protein of *N. crassa* and different strains of *S. fimicola.* Numbers in third column are showing the phosphorylation positions on serine, threonine and tyrosine residues of Carboxypeptidase A1. The numbers in the others columns (last six) are showing the positions, where the specific protein kinase involved in the phosphorylation of its respective residue *i.e*., serine, threonine, and tyrosine

Organisms	Residues	Phosphorylation Sites	Protein Kinases					
			CDC2	CK2	UNSP	PKC	PKA	DNAPK
N. crassa	Serine (S)	21,143,210,234,244,246, 248,249,313,315,320,327 328,371,379,402,403 Total= 17	246,248 249,315 320,327 407	379	210,231 244,249 320,407	21,143 266,327 328,353	371	313,402
	Threonine (T)	17,28,90,92,117,150,158 179,188,200,207,233,265 266,279 Total= 15	150,158 325,363	90,92 289	28,92,117 188,207 253,266	158,179 200,207 233,266 363	--	--
	Tyrosine (Y)	33,58,215,230,231,275,289 322,324,336,365,383 Total=12	--	--	33,58,215 230,278,322 324,336,365 383	--	--	231
S. fimicola (SFS)	Serine (S)	21,143,210,244,246,248,249 266,270,313,315,320,322,327 328,371,379,402,407 Total= 19	246,248 449,315 320,327 407	379	210,244,249 266,270,320 322,402,407	21,145 266,327 328	371	313,402
	Threonine (T)	17,28,58,90,92,117,150,158,179 188,200,207,231,233,234,265,325 363 Total:=19	17,150 158,325 363	90,92	58,92,117 188,207,235 363,234	158,179 200,207 233,253	--	--
	Tyrosine (Y)	33,215,278,289,322,324,326,365 383 Total= 9	--	--	--	--	--	--
S. fimicola (NSF)	Serine (S)	21,143,210,244,246,248,249,266 270,313,315,320,327,328,353,371 379,402,407 Total= 19	246,248 249,315 320,327 407	379	210,244,249 266,270,320 407	21,143 265,327 328,353	379	313,402
	Threonine (T)	17,28,58,90,92,117,150,158,179, 188,200,207,231,233,234,265,289 325,354,363 Total: 20	17,150 158,325 363	90,92 289	58,93,117, 188,207,233 234,363	28	--	231
	Tyrosine (Y)	33,215,278,327,324,336,365,383 Total=9	--	--	33,215,278 312,324,336 365,383	--	--	--

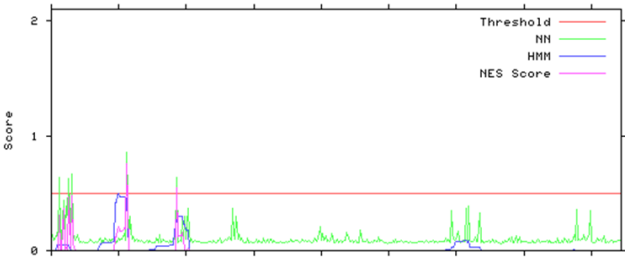

Fig. 4: Graphical representation of leucine rich nuclear export signals (NES) potential for of *N. crassa* and six *S. fimicola* strains. Green peaks are showing NN signals, blue peaks are showing HMM signals, purple peaks are showing NES signals and red horizontal line is presenting threshold level, which is 0.5 and above
Key: X-axis showing sequence position; Y-axis showing O-Glycosylation potential

showed that the number of residues increased in the favoured region. After the refinement of N7 model, 95.5% residues were present in the favoured region, 3.6% residues in the allowed region and only 1% of residues were in the outlier region. S1 model refinement results presented 96.2% residues in the favoured region, 3.1% residues in the allowed region and only 0.7% of residues were in the outlier region. Likewise, *the N. crassa* protein model has 97.1% residues in the favoured region, while 2.4% residues in the allowed region and only 0.5% residues were present in the outlier region (Fig. 6). These refinement results show the reliability of the Phyre2 3D model prediction tool and the validity of prediction.

Discussion

The protease CpA1is first time reported in *S. fimicola*. Genetic variations were studied in the protease CpA1 gene of *S. fimicola*. Our study has sharply linked the genetic diversity of *CpA1* with PTMs of protease CpA1 in *S. fimicola*. We had observed more polymorphic sites in the SFS strains than in the NFS strains. Other co-workers have also been found more polymorphism in the SFS strains as compared to the NFS strains of *S. fimicola* in their studies (Saleem *et al.* 2001; Ishfaq *et al.* 2014; Arif *et al*. 2017; Bukhari *et al*. 2020; Mobeen *et al.* 2020). Due to the harsh and xeric environmental conditions at SFS slope, the strains of this slope bear more polymorphism than the strains of NFS slope (having mild conditions) of "Evolution Canyon".

Walsh *et al.* (2005) said that PTMs are referred to as biochemical processes that take place after its synthesis. This study has predicted four types of PTMs; phosphorylation, O-glycosylation, acetylation and nuclear export signals (NES). The phosphorylation process takes

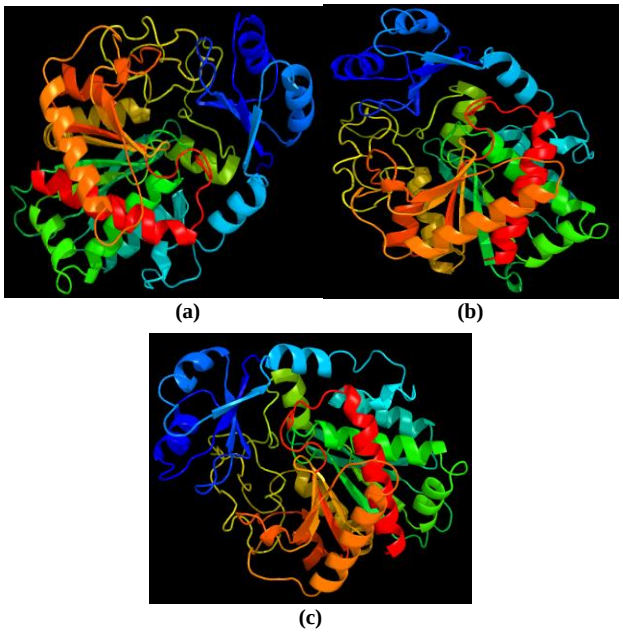

Fig. 5: 3D structure of (**a**) *N. crassa* (**b**) S1 and (**c**) N7 strains with 100% confidence prediction by Phyre2. Arrows in the structure are showing β–sheets, coiled ribbons are α–helix and sticks are coils. Each model has its own dimensions (Å) with small differences; (**a**) X: 50.735, Y: 50.990, Z: 60.736 (**b**) X: 50.716, Y: 55.215, Z: 60.736 (**c**) X: 50.728, Y: 51.564, Z: 60.73

place at specific residues of serine and threonine. It has effects on structural and signalling of the cell, whereas the percentage of phosphorylation at residues of Tyrosine is only 1%, which is linked in the cell signalling (Ishfaq *et al.* 2017). We predicted 19 phosphorylation sites on serine residues for CpA1 in SFS strains of *S. fimicola*. In comparison, 15 phosphorylation sites on serine in NFS strains and 17 serine phosphorylation modifications were predicted in *N. crassa* (Table 2).

The present study has found phosphorylation on Ser-143; Ser-248 in *N. crassa* and as well as in *S. fimicola* (NFS & SFS strains), so these are considered to be highly conserved in them. In most of the eukaryotes from fungi to mammals, Ser-248 and Thr-233 were found to play a conserved task in controlling the development of cells (Horn *et al*. 2009). Huang *et al*. (2012) have been found phosphorylation on these sites experimentally.

During the present investigation, we have found that serine and threonine phosphatases are actively involved in the phosphorylation of protease CpA1 of *S. fimicola* and *N. crassa*. CDC2, CK2, UNSP, PKC, PKA, DNA-PK are found to be highly engaged in phosphorylation of CpA1 of *N. crassa* and *S. fimicola* (Table 2). The role of protein kinases is very vital in phosphorylation. Their function is to transfer a phosphate group from adenosine triphosphate to the protein substrate and changed it into phosphorylated. PKC and PKA kinases in fungi perform many essential features like regulation of cell, growth, synthesis of protein,

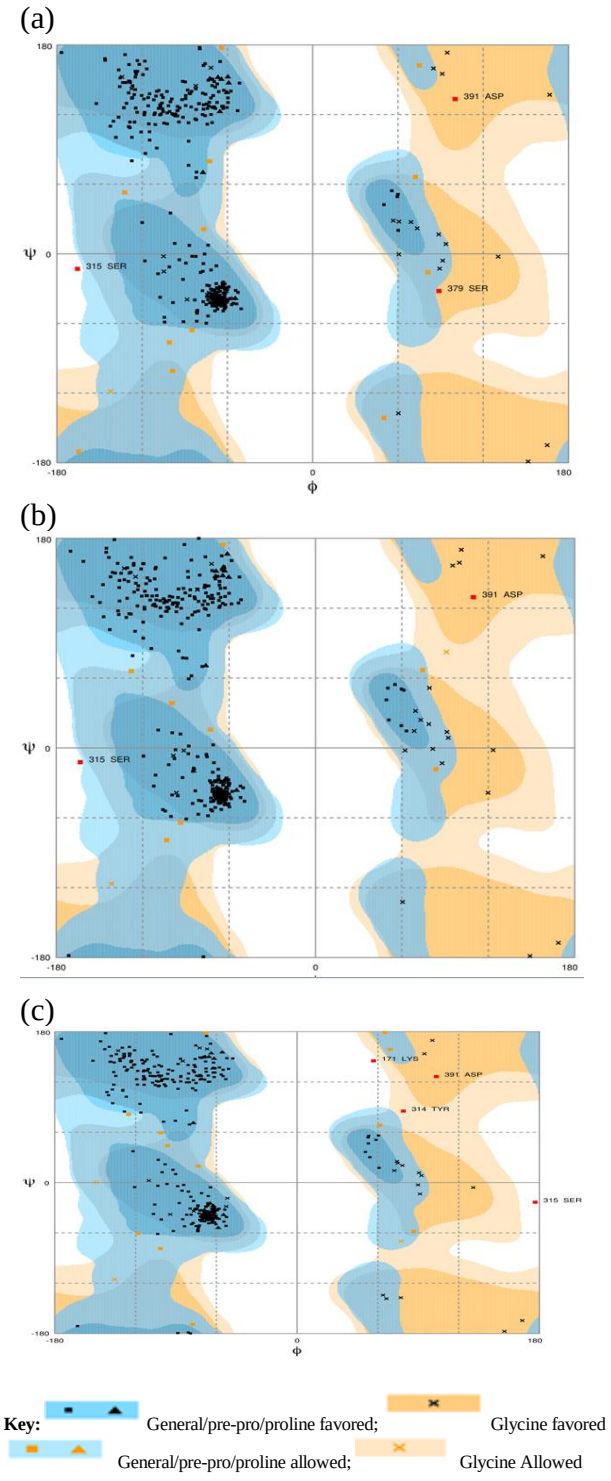

Fig. 6: These graphs are showing the refinement results of 3D models of (**a**) *N. crassa* (**b**) S1 (**c**) and N7 strains of *S. fimicola* for protease CpA1 using Galaxy Refine server to check the validity and reliability of 3D models

and maintain cell integrity (Albataineh *et al.* 2014). A current BLAST search has also shown the occurrence of homologs for numerous significant kinases (PKA, Cek1-

MAPK, PKC,) and enzymes like phosphatases are expected to play roles in pathogenicity (Leach and Brown 2012). Protein kinases (PKC, CDC2, UNSP, and PKA) involved in phosphorylation of COX1 (Cytochrome c oxidase) reported by (Arif *et al.* 2019).

O-glycosylation is another alterable type of modification, which is responsible for immunity, survival, signalling and transcription (Zhang *et al.* 2011). We have found 13 and 14 O-glycosylation sites for proteases CpA1in SFS and NFS strains, respectively. Some differences in the O-GlcNAc (O-linked acetyl glucosamine) modified sites among strains are found *i.e*., NFS strains have two novel sites (T-53 and T-229) that are absent in SFS strains, likewise S-174* is not present in the NFS strains. These differences are the reflections of polymorphism. Some sites (T-205*, S-174*, S-184*, T-205*, S-206*) have shown interplay between glycosylation and phosphorylation (Table 1). Jamil *et al.* (2018) have been reported interplay between O-glycosylation and phosphorylation at six serine and threonine residues for Histone H3 of *S. fimicola* using YinOYang server-a reliable tool for the prediction of protein O-glycosylation.

The acetylation is a process of transfer of an acetyl group (CH_3CO) to other molecules. The acetylation is a modification that affects the function of a protein by changing its properties such as solubility, hydrophobicity, and properties of the surface. All these changes can affect protein conformation and interactions with substrates, cofactors, and other macromolecules (Christensen *et al.* 2019). We have observed 14 acetylation modifications in *N. crassa* and 16 similar acetylation modifications on internal lysine (K) in all strains of *S. fimicola* (Table 1). Carabetta *et al*. (2016) had reported the acetylation a K-240 in *Bacillus subtilis*, which reduces cell length, width, and peptidoglycan thickness. This study has also been reported K-240 modification, which might perform the same functions as have been reported in *B. subtilis* by Carabetta *et al.* (2016).

Nuclear export signals are exceedingly essential elements for the biomolecules because these signals regulate the subcellular localization of these molecules. These signals are responsible for the export of proteins and transcriptional factors from the nucleus to the cytoplasm (Fischer *et al.* 1995). Nuclear export signals (NES) on residue 56-L (Leucine) and 93-M (Methionine) in *N. crassa* and *S. fimicola* have been predicted are shown in Fig. 4. The existence of NES in protease CpA1 in *N. crassa* and all strains of *S. fimicola* evidenced that these nuclear export signals have their role in the regulation of this protein.

Conclusion

The SFS strains of have more tendency of genetic variation than NFS strains due to the stressful conditions of south-facing slope. These variations on the CpA1 region might be helpful in the survival under stressful conditions by producing diverse protein motifs through various post-translational modifications. Although the molecular basis of these genetic variations has been investigated in this study, but the functional study of each polymorphic site is required to device the specific functions related to these sites.

Author Contributions

Uzma Naureen perform the major experiments Rabia Arif and Muhammad Saleem Plan the research work Faiza Akram and Memuna Ghafoor Shahid help in manuscript write up.

References

Albataineh MT, A Lazzell, JL Lopez-Ribot, D Kadosh (2014). Ppg1, a PP2A-type protein phosphatase, controls filament extension and virulence in *Candida albicans*. *Eukaryot Cell* 13:1538–1547

Arber W (2000). Genetic variation: Molecular mechanisms and impact on microbial evolution. *FEMS Microbiol Rev* 24:1–7

Arif R, SH Bukhari, M Ishfaq, MG Shahid, SF Lee, M Saleem (2019). Genetic variation and post-translational modifications of cytochrome c oxidase-1 (COX1) in different strains of *Sordaria fimicola*. *Intl J Agric Biol* 21:1055–1062

Arif R, F Akram, T Jamil, H Mukhtar, SF Lee, M Saleem (2017). Genetic variation and its reflection on posttranslational modifications in frequency clock and mating type a-1 proteins in *Sordaria fimicola*. *Biol Med Res Intl* 2017; Article 1268623

Boominadhan U, R Rajakumar, PKV Sivakumaar, MM Joe (2009). Optimization of protease enzyme production using *Bacillus* spp. isolated from different wastes. *Bot Res Intl* 2:83–87

Bukhari SH, I Mobeen, U Naureen, F Akram, R Arif, MG Shahid, M Saleem (2020). Analysis of genetic polymorphisms and posttranslational modifications of cytochrome C-1 in *Sordaria fimicola*. *Intl J Agric Biol* 23:675–680

Carabetta VJ, TM Greco, AW Tanner, IM Cristea, D Dubnau (2016). Temporal regulation of the *Bacillus subtilis* acetylome and evidence for a role of Mre B acetylation in cell wall growth. *mSystems* 1; Article e00005-16

Chandramouli K, P Qian (2009). Proteomics: Challenges, techniques and possibilities to overcome biological sample complexity. *Hum Genom Proteom* 2009:1–22

Chou KC (2019). Progresses in predicting post-translational modification. *Intl J Pept Res Ther* 26:873-888

Christensen DG, X Xie, N Basisty, J Byrnes, SM Sweeney, B Schilling, A. Wolf (2019). Post-translational protein acetylation: An elegant mechanism for bacteria to dynamically regulate metabolic functions. *Front Microbiol* 10; Article 1604

Fischer U, J Huber, WC Boelens, IW Mattaj, R Luhrmann (1995). *Cell* 82:475–483

Horn DL, EJD Neofytos, JA Anaissie, WJ Fishman, AJ Steinbach, KA Olyaei, MA Marr, CH Pfaller, KM Chan, KM Webster (2009). Epidemiology and outcomes of candidemia in 2019 patients: Data from the prospective antifungal therapy alliance registry. *Clin Infect Dis* 48:1695–1703

Huang OW, X Ma, J Yin, J Flinders, T Maurer, N Kayagaki, Q Phung, I Bosanac, D Arnott, VM Dixit, SG Hymowitz (2012). Phosphorylation-dependent activity of the deubiquitinase DUBA. *Nat Struct Mol Biol* 19:171–175

Ishfaq M, N Mahmood, IA Nasir, M Saleem (2017). Biochemical and molecular analysis of superoxide dismutase in *Sordaria fimicola* and *Aspergillus niger* collected from different environments. *Pol J Environ Stud* 26:115–125

Ishfaq M, N Mahmood, IA Nasir, M Saleem (2014). Molecular and biochemical screening of local *Aspergillus niger* strains efficient in catalase and laccase enzyme production. *Intl J Agric Biol* 16:177–182

Jamil T, N Sami, R Arif, Q Rashid, M Saleem (2018). H3/H4 Histone genes variations and its effect on posttranslational modifications in various strains of *Sordaria fimicola. Intl J Agric Biol* 20:1021–1026

Jimenez-Morales D, L Adamian, D Shi, J Liang (2013). Lysine carboxylation: Unveiling a spontaneous post-translational modification. *Acta Crystallogr D Biol Crystallogr* 70:48–57

Khoury GA, RC Baliban, CA Floudas (2011). Proteome-wide post-translational modification statistics: Frequency analysis and curation of the swiss-prot database. *Sci Rep* 1; Article 90

Leach MD, AJ Brown (2012). Posttranslational modifications of proteins in the pathobiology of medically relevant fungi. *Eukaryot Cell* 11:98–108

Marquez J, SR Lee, N Kim, J Han (2018). Post-translational modifications of cardiac mitochondrial proteins in cardiovascular disease not lost in translation. *Kor Circ J* 46:1–12

Mobeen I, R Arif, A Rasheed, F Akram, MG Shahid, M Saleem (2020). Genetic and post-translational modification analysis of translational associated protein RKM4 in *Sordaria fimicola*. *Intl J Agric Biol* 23:935–942

MuszewskaA, MM Stepniewska-Dziubinska, K Steczkiewicz, J Pawlowska, A Dziedzic, K Ginalski (2017). Fungal lifestyle reflected in serine protease repertoire. *Sci Rep* 7; Article 9147

Pietro S, TM Fulton, J Chunwongesm, SD Tanksley (1995). Extraction of high-quality DNA for genome sequencing. *Mol Biol Rep* 13:207

Saleem M, BC Lamb, E Nevo (2001). Inherited differences in crossing over and gene conversion frequencies between wild strains of *Sordaria fimicola* from "Evolution Canyon". *Genetics* 159:1573–1593

Shen B (2013). *Bioinformatics for Diagnosis, Prognosis and Treatment of Complex Diseases*, Vol. 4. Springer Science + Business Media, Berlin, Germany

Sibanda BL, DY Chirgadze, TLBlundell (2010). Crystal structure of DNA-PKcs reveals a large open-ring cradle comprised of HEAT repeats. *Nature* 463:118–121

Turnham RE, JD Scott (2016). Protein kinase A catalytic subunit isoform PRKACA; History, function and physiology. *Gene* 577:101–108

Walsh CT, S Garneau-Tsodikova, GJ Gatto (2005). Protein posttranslational modifications: The chemistry of proteome diversifications. *Angew Chem Intl* 44:7342–7372

Wood JR, JM Wilmshurst, TH Worthy, A Cooper (2011). *Sporormiella* as a proxy for non-mammalian herbivores in island ecosystems. *Quat Sci Rev* 30:915–920

Yu LR, HJ Issaq, TD Veenstra (2007). Phosphoproteomics for the discovery of kinases as cancer biomarkers and drug targets. *Proteomics* 1:1042–1057

Zhang S, K Roche, HP Nasheuer, NF Lowndes (2011). Modification of histones by sugar β-N-Acetylglucosamine (GlcNAc) occurs on multiple residues, including histone H3 Serine 10, and is cell cycle-regulated. *J Biol Chem* 286:37483–37495