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Full Length Article

Genetic Diversity and Post-Translational Modification Analysis of L-Asparaginase in Six Strains of *Sordaria fimicola*

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Abstract

Filamentous fungi are a preferable source of production of L-asparaginase as compare to other organisms. In the present study L-asparaginase gene NUC05624, which encode its production was amplified and sequenced to assess the genetic diversity and post-translational modifications (PTMs). A comparison was made among six different strains of filamentous fungus *Sordaria fimicola* with reference species *Neurospora crassa*. During this study, we calculated single nucleotide polymorphism (SNP) in the exonic region of the L-asparaginase gene in six parental strains (S1, S2, S3, N5, N6 and N7) of *S. fimicola.* SNP(s) on seven different nucleotides i.e. G(158)C; T(256)A; A(715)T; T(936)A; G(1026)C; C(1137)G; T(1301)A with 50% of percentage prevalence was observed in S1, S2, S3 strains of *S. fimicola*, isolated from the S-slope of EC. Genetic polymorphism on position T(559)G and A(1665)C with 50% of percentage prevalence was calculated in N5, N6 and N7 strains only not in any strain of S-slope. PTMs were predicted by using various online bioinformatics tools both in *S. fimicola* and reference species. The molecular and post-translational modification data in this study revealed that environmental stresses affected specific genes by SNP, which may result in genomic diversity among the organisms and their proteins. © 2020 Friends Science Publishers

Keywords: Amplification; Evolution canyon; SNPs, mutations; 3D structure; Variations

Introduction

The importance of L-asparaginase enzyme has been increasing tremendously during recent years due to its therapeutic potential and role in food processing. L-asparaginase is an important enzyme, which is not produced in humans but is present in plants, animals and microorganism. L-asparaginase enzyme (L-asparagine amido hydrolase E.C. 3.5.1.1) is very popular due to its anti-carcinogenic potential and its role in food industry. L-asparaginase catalyzes the L-asparagine into aspartic acid and ammonia (Verma *et al.* 2007). Taeymans *et al.* (2005) studied its clinical role in cancer therapies and discovered its antitumor properties. In food processing industry, it produces Acrylamide free food, which is otherwise formed in baked and fried foods containing carbohydrates.

The eukaryotic microorganisms like yeast and few genera of filamentous fungi such as *Aspergillus*, *Penicillium* and *Fusarium* are commonly reported in scientific literature to produce a substantial amount of extracellular L-asparaginase with less adverse effects (Sarquis *et al*. 2004; Isaac and Abu-Tahon 2016; Bedaiwy *et al.* 2016). Fungal sources are reported to be the second largest L-asparaginase producer expected to overtake bacterial L-asparaginase as it is cost effective and ecofriendly in nature (Sarquis *et al.* 2004; Elzainy and Ali 2006; Ferrara *et al*. 2006; Prakasham *et al.* 2007).

Genes encoding L-asparaginase enzymes have been amplified in different filamentous fungi like *Aspergillus niger*, *A. oryzae* and *Trichoderma reesei* (Bhamare *et al.* 2018). After transcription its protein goes through some modifications, i.e., cleavage or attachment of some functional group through covalent bond on specific amino acid residues. Following their synthesis, the post-translational modifications (PTMs) increase the functional diversity of the proteins with the attachment of small chemical molecules with amino acid residues compare to non-PTM proteins (Arif *et al*. 2017; Raveendran *et al.* 2018). Glycosylation has vital role in folding of proteins, secretion and enzymatic properties (Banerjee *et al*. 2007).

Sumoylation is a unique kind of PTMs, which brings chemical alterations in the protein. This modification involves the covalent attachment of small ubiquitin like modifier polypeptide to the lysine residue. It is involved in the regulation of cellular processes, regulates the transcription and has therapeutic potential. In this regard, these chemical alterations are different from other PTMs (Yang and Chiang 2013).

In this study, for the first-time different strains of *S. fimicola* were evaluated for the presence of L-asparaginase gene while *Neurospora crassa* was used as reference fungus. Gene producing L-asparaginase NUC05624 was amplified and studied to observe the polymorphism and PTMs in various strains of *S. fimicola,* which were previously collected from Evolution Canyon (EC). EC is a microscale divergent environment. All organisms must face different environmental stress in the form of elevated temperature, drought, and high UV rays in their life. Theses environmental factors lead towards the mutations and molecular diversity (Nevo 2011).

In the current study, an attempt was made to study the genetic variation and polymorphism in coprophilous fungus *S. fimicola.* These strains were collected from the south facing slope (SFS) of EC which is xeric, has high UV rays, high temperature and from the north facing slope (NFS) of EC which is milder and greener (Arif *et al*. 2017). It was tempting to study the impact of environmental stress on genetic variation and evolvability. For this purpose, gene NUC05624 was amplified in all the six strains of *S. fimicola* and gene sequence was compared with reference species of *N. crassa.*

Materials and Methods

Experimental organism

Six different strains (S1, S2, S3, N5, N6 and N7) of *S. fimicola* were used in the present study to amplify L-asparaginase gene NUC05624 and to predict PTMs. For this purpose, all the strains were taken from stock already available at Molecular Genetics Research Laboratory, Department of Botany University of the Punjab, Lahore. The strains were originally collected from two entirely different environments. S1, S2, and S3 were isolated from the dung samples collected from south facing slope (SFS) of Evolution Canyon (EC) which is xeric in nature while N5, N6 and N7 belongs to mild North facing slope (NFS) which is mesic in nature. Sub culturing was done under sterilized conditions and was stored at 20°C in potato dextrose agar media (PDA).

Genomic DNA extraction

Genomic DNA of all the strains of *S. fimicola* was extracted by using QIAamp DNA kit (Qiagen, Germany) by following the company's instructions. The extracted DNA was subjected to 1% agarose gel electrophoresis; ethidium bromide was used as a dye. To check the presence or absence of DNA, gel was visualized in GEL Doc system (Syngene, Germany). Extracted genomic DNA was subjected to PCR for gene amplification.

Primer designing

For the amplification of NCU05624 gene different pairs of primers (forward and reverse) were designed manually and by using primer 3plus software available at https://primer3plus.com. The primers were; NUCF1 (5′-TGGAATACAAGCCCCAACCC-3′); NUCR1 (5′-GACATCAGGCTCCCCATCTC-3′); NUCF2 (5′-GGCGTTGGAAAGGGAGAAGA-3′); NUCR2 (5′-GATCATCGGCGCTCTTCTGA-3′); NUCF3 (5′-AAGGCGA AGGTGGCATC ATC-3′) and NUCR3 (5′-TTTGCGAATGTGTTACCGGC-3′) of Bioron, Germany were used in this study.

Polymerase chain reaction (PCR) amplification of g-DNA

Amplification of L-asparaginase gene NUC05624 was made by using touch down PCR cycling conditions. Reaction mixture of 20 µL was made by using 10 µL of master mix, containing 2.5 µL of DNA, 5.5 µL of distilled water, 1 µL of forward primer and 1 µL of reverse primer. PCR optimized conditions were 50°C for 2 min then 60 °C for 35 min, followed by 50 cycles of 95°C for 10 s, 60°C for 30 s using a Light Cycler® 480 DNA real-time PCR system (Roche Applied Science, Mannheim, Germany). The reaction was observed in real time by SYBR® Green fluorescence. Amplified products were subjected to 1% agarose gel electrophoresis followed by ethidium bromide staining.

Sequence and data analysis

After PCR amplifications, the PCR products were sequenced to analyze the nucleotide sequences of L-asparaginase genes of different strains of *S. fimicola*, they were also confirmed by BLAST database search method provided by NCBI (http://www.ncbi.nlm. nih.gov). Meanwhile, the translation tool provided by the ExPASy server available at (http://web.expasy.org/translate/) was used to translate the nucleotide sequence in order to obtain the amino acid sequences of L-asparaginase. Clustal-O and Jalview programs were also utilized for multiple sequence alignment to locate the single nucleotide polymorphism (SNP) between strains in comparison to reference organism of *N. crassa*. Amino acid sequence of NUC05624 gene of *N. crassa* was retrieved from Uniprot available at https://www.uniprot.org.

To predict protein PTMs, various bioinformatics tools were used in this study. YinOYang 1.2 server

(http://www.cbs.dtu.dk/services/YinOYang/), NetPhos 3.1 server (http://www.cbs.dtu.dk/services/NetPhos/), PAIL (http://pail.biocuckoo.org/), were used for prediction of acetylation, phosphorylation and glycosylation.

Results

Calculation of single nucleotide polymorphism (SNP)

During this study, we calculated SNP in the exonic region of the *L-asparaginase* gene in six parental strains (S1, S2, S3, N5, N6 and N7) of *S. fimicola.* These parental strains were collected from the South slope (S-slope) and the North slope (N-slope) of EC. SNP(s) on seven different nucleotides i.e. G(158)C; T(256)A; A(715)T; T(936)A; G(1026)C; C(1137)G; T(1301)A with 50% of percentage prevalence was observed in S1, S2, S3 strains of *S. fimicola* which were isolated from the S-slope of EC. Genetic polymorphisms' on position T(559)G and A(1665)C with 50% of percentage prevalence was calculated in N5, N6 and N7 strains only not in any strain of S-slope. We also observed G(677)C and A(1150)T base substitutions in S2 and S3 strains while T(707)A; and C(1246)G changes are present only in S3 strain whereas genetic variation on position G(784)C with 33.33% of percentage prevalence was found only in N6 and N7 strains of *S. fimicola* (Table 1).

Analysis of 3D structures

The 3D structure of asparaginase protein of S3 strain, N6 strains and reference strain of *N. crassa* were generated by using Phyr2 (Protein Homology/analogy Recognition Engine V 2.0) online server tool available at http://www.sbg.bio.ic.ac.uk/~phyre2. We found visual variations in the orientation of alpha and beta helix in all the strains due to the presence of polymorphic sites in protein (Fig. 1). The motifs shown helical form are α-helix, motifs in arrow form are β-sheets and it also indicates the loop regions for the attachment of ligand.

Prediction of phosphorylation

Total of 67 phosphorylation sites were predicted in *N. crassa* and NFS strains of *S. fimicola,* out of which 37 were on Ser, 21 on Thr and 09 on tyrosine while in SFS total 63 sites (36 on Ser, 18 on Thr and 09 on tyrosine) were predicted by Netphos 2.1 server (Table 2). Furthermore, phosphorylation modifications on Ser 11, Ser 103, Ser 269, Thr134, Y13 and Y 273 were highly conserved in all the strains of *S. fimicola* and reference organism *N. crassa* with a threshold of 0.9. We found that total 14 different kinds of kinases were involved in the phosphorylation of these amino acids in all the strains and *N. crassa* (Table 2).

Prediction of glycosylation

Glycosylation modifications on Ser/Thr and YinOYang sites (the sites where glycosylation and phosphorylation interplay with each other) were predicted by using online predictor tool YinOYang 2.1 with a threshold of 0.5. A total of 23 glycosylation modifications in *N. crassa* were calculated out of which 18 modification sites were predicted on Ser residue while 08 sites were present on Thr residue (Fig. 2). Among these 23 sites of modifications 11 sites (S43, S96, S100, S103, S105, S265, S330, S375 S387, S399 and S411) had the potential to interplay between phosphorylation and glycosylation. Among these YinOYang sites, serine modification on S43, S103, S265, S330 and S399 had a threshold greater than 0.9. Therefore, these sites had the highest potential or chance of modifications than the other sites. In case of N6 strain of *S. fimicola* we found that glycosylation with highest number of modifications on 16 serine sites were predicted while in case of S3 strain only 13 sites on serine glycosylation modifications were predicted.

Prediction of acetylation

PAIL Server predicted acetylation modification on Lysine (K) residue in all the strains of *S. fimicola* and *N. crassa*.

Discussion

In this study, the sequences for L-asparaginase enzyme from the *N. crassa* was used as a template for designing suitable primers for finding the equivalent genes from the genome of the *S. fimicola*. This approach led to the amplification of the coding sequences of L-asparaginase (1506 bp) in S1, S2 and S3 strain while (1502 bp) in N5, N6 and N7 strain of *S. fimicola* when compared with the reference species of *N. crassa*. By a similar approach, Safary *et al.* (2019) amplified the same gene as amplified in the current study in different strains of *Bacillus* sp. The DNA sequence analysis of these genes showed a high degree of identity 97% to the same gene from *N. crassa* with nine base substitution i.e. G(158)C; T(256)A; T(559)G; G(677)C; T(707)A; A(715)T; G(784)C; T(936)A and G(1026)C or SNPs (Tables 1) leading to three silence and six residue changes in the protein sequence (S84 to T, L185 to V, G224 to A, none, G260 to R, D310 to E), respectively (Fig. 2). From the primary sequence alignment of asparaginase (Fig. 2), it was clear that the identified L-asparaginases from North facing slope strains i.e. N5, N6 and N7 had more conserved amino acids compared to the L-asparaginases from south facing slope strain i.e. S1, S2 and S3 strains of *S. fimicola* represented in the dark pink columns. These variations in proteins were also affected the dimensions of alpha and beta helix in 3D structures of proteins (Fig. 1).

The reasons of these variations could be environmental stress in EC. Environmental stress usually leading to high genetic variability and molecular diversity (Nevo 2011). Genomics, proteomics, phonemics, genetic polymorphism both at DNA and protein level has also been

Table 1: Polymorphisms detection in the exonic region of L-asparaginase genes amplified in the different strains of the *S. fimicola* in comparison with the L-asparaginase gene of *N. crassa*

SNPs in exon of the L-asparaginase gene	S1	S2	S3	N5	N6	N7	Percentage prevalence (%)
G(158)C	+	+	+	-	-	-	50
T(256)A	+	+	+	-	-	-	50
T(559)G	-	-	-	+	+	+	50
G(677)C	-	+	+	-	-	-	33.33
T(707)A	-	-	+	-	-	-	16.66
A(715)T	+	+	+	-	-	-	50
G(784)C	-	-	-	-	+	+	33.33
T(936)A	+	+	+	-	-	-	50
G(1026)C	+	+	+	-	-	-	50

Table 2: Prediction of phosphorylation in *N. crassa* and *S. fimicola*

Organism	Serine	Threonine	Tyrosine	Kinases
N. crassa	11,19, 46,80, 81,99, 103, 105,106,107,108, 112, 132, 153, 266, 269, 273, 299, 334, 340, 346, 367, 378, 379, 391, 398, 403, 415, 416, 419, 430, 436, 463, 471, 506, 521, 542	66,84,94,95,97, 122, 164, 198, 238, 271, 300, 304, 314, 327, 372, 383, 384, 385, 393, 407, 424	13,75, 235, 273, 345, 414, 494, 508, 528	Unsp, cdk5, CKI, EGFR, INSR, PKC, p38MAPK, cdc2, PKA, DNAPK, GSK3, CKII, CaM-II ATM
Total	37	21	09	14
SFS strains of *S. fimicola*	11, 16, 43, 78, 96, 100, 102, 103, 104, 105, 109,129, 150, 262, 269,267, 269, 295, 330,336, 342, 363, 374, 375, 381, 387, 394, 399, 411, 412, 426, 459, 467, 502, 517, 538	63, 91, 92, 94, 119, 161, 195, 235, 296, 300, 314, 323, 368, 379, 380. 389. 403, 420,	13, 72, 75, 232, 273, 341, 490, 504, 524	Unsp, cdk5, CKI, EGFR, INSR, PKC, p38MAPK, PKA, DNAPK, GSK3, cdc2, ATM, CKII, CKI
Total	36	18	09	14
NFS strains of *S. fimicola*	11, 17, 44, 78, 79 97, 101, 103, 104, 105, 106, 110, 130, 151, 264, 269, 271, 297, 332,338, 344, 365, 376, 377, 389, 396, 401, 413, 414, 417, 428, 434, 461, 469, 504, 519, 540	64, 82, 92, 93, 95, 120, 162, 196, 236, 269, 298, 302, 314, 325, 370, 381, 382, 383, 391, 405, 422	13, 73, 75, 233, 273, 343, 492, 506, 526	Unsp, cdk5, CKI, EGFR, INSR, PKC, p38MAPK, PKA, DNAPK, GSK3, cdc2, ATM, CKII, CKI
Total	37	21	09	14

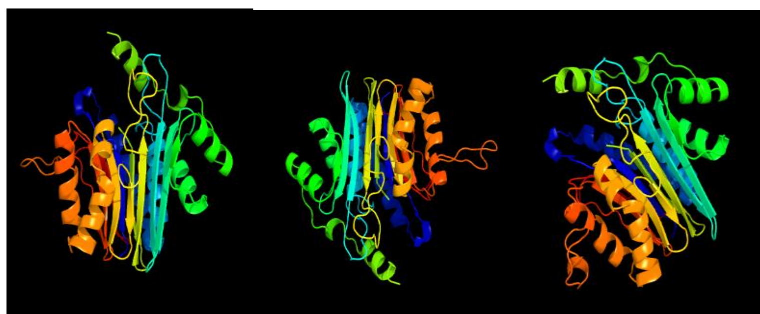

Fig. 1: 3D structure of asparaginase protein in *N. crassa* (Left), SFS (Center) and NFS (Right) of *S. fimicola*
Image coloured by rainbow N → C terminus
N. crassa= Model dimensions (Å): **X**: 50.357 **Y**: 50.599 **Z**: 48.611
SFS= Model dimensions (Å): **X**: 51.538 **Y**: 54.648 **Z**: 48.611
NFS= Model dimensions (Å): **X**: 50.357 **Y**: 46.395 **Z**: 48.631

revealed by the "EC" model (Nevo 2006–2007; 2009). Our current findings are in agreement with Arif *et al*. (2017) who found more polymorphisms in the strains that were isolated from the stressed environmental conditions when studied for the genotyping of short sequence repeats. Saleem *et al*. (2001) also gave similar results and favored the hypothesis that high temperature, higher solar radiations and xeric conditions compel an organism to bring changes even at molecular level.

Recently a new temperature resistant *Bacillus* sp. (SL-1) was extracted from brackish pond of Iran (Safary *et al.* 2013) was explored as the basic source of fresh L-asparaginases (Safary *et al*. 2016). Similarly, we also found *S. fimicola* as an efficient source of L-asparaginase among the ascomycetes. Prior studies have also revealed that polymorphisms in nucleotide region of L-asparaginase gene in different bacteria and fungi cause the overexpression of these proteins and affect the solubility of L-asparaginases enzyme in different strains of *E. coli* (Sudhir *et al*. 2014; Sindhu and Manonmani 2018; Saeed *et al*. 2018).

The PTMs on different residues like serine, threonine, and tyrosine and lysine play an important role in

Table 3: Prediction of Acetylation on Lysine residue in *N. crassa* and different strains of *S. fimicola*

N. crassa			SFS strains S1, S2, S3		NFS strains N5, N6, N7	
Peptide	Position	Score	Position	Score	Position	Score
RNSPYIKSGRERV	15	1.51	15	1.51	15	1.51
SPSSSSKLPSALE	106	2.47	106	2.47	106	2.47
VSRGQAKRGVGVT	155	0.53	155	0.53	155	0.53
PILLAKKVLEHGK	175	1.01	175	1.01	175	1.01
KVLEHGKDDLLGR	181	1.36	181	1.36	181	1.36
DLLGRGKKLDNNT	189	1.07	189	1.07	189	1.07
LLGRGKKLDNNTG	190	1.05	190	1.05	190	1.05
HGPTAEKLARQYG	218	2.09	218	2.09	218	2.09
RALEREKREQQDL	250	1.54	249	1.54	249	1.54
DPTSPHKNGSRNP	334	1.04	333	1.04	333	1.04
VLSDALKRLIADC	355	1.26	354	1.26	354	1.26
SVVALTKVAGPSG	422	0.38	421	0.38	421	0.38
PSGELQKSADDRW	432	0.54	431	0.54	431	0.54
YARIFRKDQDISS	497	0.69	496	0.69	496	0.69
SVCLPKKREILFG	547	0.64	546	0.64	546	0.64
FFRAEAKHPR***	562	2.56	561	2.56	561	2.56

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N5     PHPITNHTRNSPYIKS*GRERVDNQQVIHQET*AATMEYKPQPSSPTRVQVQPRLIIHGG     58
N6     PHPITNHTRNSPYIKS*GRERVDNQQVIHQET*AATMEYKPQPSSPTRVQVQPRLIIHGG     58
N7     PHPITNHTRNSPYIKS*GRERVDNQQVIHQET*AATMEYKPQPSSPTRVQVQPRLIIHGG     58
S3     PHPITNHTRNSPYIKS*GRERVDNQQVIHQET*AATMEYKPQPSSPTRVQVQPRLIIHGG     58
S2     PHPITNHTRNSPYIKS*GRERVDNQQVIHQET*AATMEYKPQPSSPTRVQVQPRLIIHGG     58
Ref    PHPITNHTRNSPYIKS*GRERVDNQQVIHQET*AATMEYKPQPSSPTRVQVQPRLIIHGG     58
S1     PHPITNHTRNSPYIKS*GRERVDNQQVIHQET*AATMEYKPQPSSPTRVQVQPRLIIHGG     58
       ************************************************************

N5     AGNITPASLGPERYVAYRSSLLTIVSKAHTYLTTPTQSNYNSPSSSSKLPSALEAATYAV     118
N6     AGNITPASLGPERYVAYRSSLLTIVSKAHTYLTTPTQSNYNSPSSSSKLPSALEAATYAV     118
N7     AGNITPASLGPERYVAYRSSLLTIVSKAHTYLTTPTQSNYNSPSSSSKLPSALEAATYAV     118
S3     AGNITPASLGPERYVAYRSSLLTIVTKAHTYLTTPTQSNYNSPSSSSKLPSALEAATYAV     118
S2     AGNITPASLGPERYVAYRSSLLTIVTKAHTYLTTPTQSNYNSPSSSSKLPSALEAATYAV     118
Ref    AGNITPASLGPERYVAYRSSLLTIVSKAHTYLTTPTQSNYNSPSSSSKLPSALEAATYAV     118
S1     AGNITPASLGPERYVAYRSSLLTIVTKAHTYLTTPTQSNYNSPSSSSKLPSALEAATYAV     118
       *************************:**********************************

N5     TLLEDNPLFNSGHGAVFTRDGINELEASVMVSRGQAKRGVGVTGLRHVRNPILLAKKVLE     178
N6     TLLEDNPLFNSGHGAVFTRDGINELEASVMVSRGQAKRGVGVTGLRHVRNPILLAKKVLE     178
N7     TLLEDNPLFNSGHGAVFTRDGINELEASVMVSRGQAKRGVGVTGLRHVRNPILLAKKVLE     178
S3     TLLEDNPLFNSGHGAVFTRDGINELEASVMVSRGQAKRGVGVTGLRHVRNPILLAKKVLE     178
S2     TLLEDNPLFNSGHGAVFTRDGINELEASVMVSRGQAKRGVGVTGLRHVRNPILLAKKVLE     178
Ref    TLLEDNPLFNSGHGAVFTRDGINELEASVMVSRGQAKRGVGVTGLRHVRNPILLAKKVLE     178
S1     TLLEDNPLFNSGHGAVFTRDGINELEASVMVSRGQAKRGVGVTGLRHVRNPILLAKKVLE     178
       ************************************************************

N5     HGKDDLVGRGKKLDNNTGDGEPDVPSAQGHTLIHGPTAEKLARQYGLEMVDPSYFFTQKR     238
N6     HGKDDLVGRGKKLDNNTGDGEPDVPSAQGHTLIHGPTAEKLARQYGLEMVDPSYFFTQKR     238
N7     HGKDDLVGRGKKLDNNTGDGEPDVPSAQGHTLIHGPTAEKLARQYGLEMVDPSYFFTQKR     238
S3     HGKDDLLGRGKKLDNNTGDGEPDVPSAQGHTLIHGPTAEKLARQYALEMVDPSYFYTQ*R     237
S2     HGKDDLLGRGKKLDNNTGDGEPDVPSAQGHTLIHGPTAEKLARQYALEMVDPSYFFTQ*R     237
Ref    HGKDDLLGRGKKLDNNTGDGEPDVPSAQGHTLIHGPTAEKLARQYGLEMVDPSYFFTQKR     238
S1     HGKDDLLGRGKKLDNNTGDGEPDVPSAQGHTLIHGPTAEKLARQYGLEMVDPSYFFTQ*R     237
       ******:**************************************.*********:** *

N5     WDEHVRALEREKREQQDLLGAGTASGVSATWSKDEYLPQGTVGAVALDVEGVICVATSTG     298
N6     WDEHVRALEREKREQQDLLGARTASGVSATWSKDEYLPQGTVGAVALDVEGVICVATSTG     298
N7     WDEHVRALEREKREQQDLLGARTASGVSATWSKDEYLPQGTVGAVALDVEGVICVATSTG     298
S3     WDEHVRALEREKREQQDLLGARTASGVSATWSKDEYLPQGTVGAVALDVEGVICVATSTG     297
S2     WDEHVRALEREKREQQDLLGARTASGVSATWSKDEYLPQGTVGAVALDVEGVICVATSTG     297
Ref    WDEHVRALEREKREQQDLLGAGTASGVSATWSKDEYLPQGTVGAVALDVEGVICVATSTG     298
S1     WDEHVRALEREKREQQDLLGARTASGVSATWSKDEYLPQGTVGAVALDVEGVICVATSTG     297
       ********************* **************************************

N5     GMTNKLTGRIGDTPVVGAGFWAEEWTEEGDPTSPHKNGSRNPLYSPGPAVVLSDALKRLI     358
N6     GMTNKLTGRIGDTPVVGAGFWAEEWTEEGDPTSPHKNGSRNPLYSPGPAVVLSDALKRLI     358
N7     GMTNKLTGRIGDTPVVGAGFWAEEWTEEGDPTSPHKNGSRNPLYSPGPAVVLSDALKRLI     358
S3     GMTNKLTGRIGETPVVGAGFWAEEWTEEGDPTSPHKNGSRNPLYSPGPAVVLSDALKRLI     357
S2     GMTNKLTGRIGETPVVGAGFWAEEWTEEGDPTSPHKNGSRNPLYSPGPAVVLSDALKRLI     357
Ref    GMTNKLTGRIGETPVVGAGFWAEEWTEEGDPTSPHKNGSRNPLYSPGPAVVLSDALKRLI     358
S1     GMTNKLTGRIGETPVVGAGFWAEEWTEEGDPTSPHKNGSRNPLYSPGPAVVLSDALKRLI     357
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Fig. 2: Pairwise alignment of asparaginase protein in six strains of *S. fimicola* and *N. crassa*. Alphabets in red indicating the posttranslational modification sites on Ser/Thr/Y and Lysine residue for potential acetylation, glycosylation and phosphorylation. Alphabets in Pink indicating polymorphic regions

phosphorylation, glycosylation and acetylation of certain proteins. We in this study tried to predict how these residues get modified and affect the cellular activities of asparaginase enzyme in *S. fimicola* and *N. crassa*. Filamentous fungi retain protein serine/threonine and tyrosine kinases, which have the ability to phosphorylate numerous substrates.

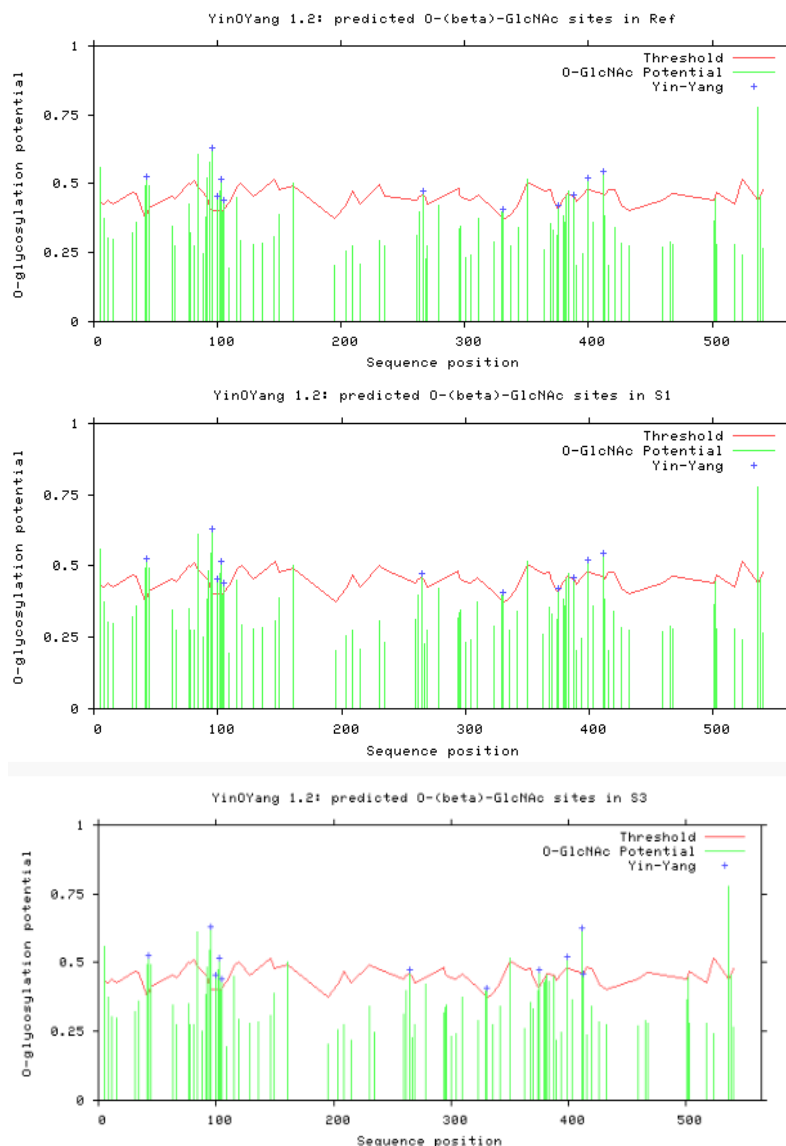

Fig. 3: Graphical representation of Prediction of Potential Glycosylation and YinOYang sites in *N. crassa* (A), SFS strains (B) and NFS strains (C) of *S. fimicola* at 0.5 thresholds

We currently, assumed that the similarity might spread further, and fungal kinases may also go through common phosphorylation and activation, which is at present reflected as a trademark of fungal kinase networks. In order to test this assumption, we tried to predict the ability of all members of diverse classes of serine/threonine and tyrosine kinases present in the two model fungi *S. fimicola* and *N. crassa* to phosphorylate each other *in silico*. The current data suggested that PKC, PKA, Unsp and CDK5 are important kinases, which are involved in the phosphorylation of Ser/Thr/Y residues among *S. fimicola* and *N. crassa*. Arif *et al.* (2019) reported protein kinases (PKC, Unsp, PKA, cdc2) involved in phosphorylation of the COX1 protein of *S. fimicola*.

To the best of our knowledge, no study has been found on the post-translational modifications of L-asparaginase in fungi. This study is first time reporting the PTMs of L-asparaginase in different strains of *S. fimicola.* There is one study related to the N-glycosylation of asparaginase in humans, which reported six N-glycosylation sites by the NetNGlyc 1.0 server (Dantas *et al.* 2019). In the current study, 23 O-glycosylation sites have been observed in *N. crassa*, 16 serine sites in N6, and 13 serine sites in the S3 strain of *S. fimicola*.

Glycosylation is one of the important PTMs, which shows interplay with phosphorylation (Pang *et al.* 2007; Jamil *et al.* 2018). This interplay has been observed during this study and we found 11 YinOYang sites in *N. crassa*, but no such site has been observed in any strain of *S. fimicola* (Fig. 3).

We have reported a total of 67 phosphorylation sites in *N. crassa* and NFS strains and 63 sites in SFS strains. Six sites (Ser11, Ser103, Ser269, Thr314, Y13, and Y273) are conserved in *N. crassa* and all studied strains (Table 2). One of the recent studies reported phosphorylation at serine, threonine and tyrosine residues of histone H3/H4 proteins by NetPhos 3.1 server and predicted acetylation at three lysine residues of *S. fimicola* by PAIL server (Jamil *et al.* 2018). Likewise, we investigated 16 conserved acetylation sites with three sites having a threshold level of more than 2.0 (Table 3), which indicated that these sites are more likely to be acetylated and might have important roles.

Conclusion

L-asparaginase gene is first time amplified in *S. fimicola* and is investigated for polymorphism and post-translational modifications analysis. It is concluded that environmental stress has influence in generating polymorphism in the exonic regions of genes and this effects the post-translational modifications of proteins. This is evidenced by the presence of different PTM sites in each strain and some conserved PTM sites for L-asparaginase of *S. fimicola*.

References

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*. *BioMed Res Intl* 2017; Article 1268623

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

Banerjee S, P Vishwanath, J Cui, DJ Kelleher, R Gilmore, PW Robbins, J Samuelson (2007). The evolution of N-glycan-dependent endoplasmic reticulum quality control factors for glycoprotein folding and degradation. *Proc Natl Acad Sci USA* 104:11676–11681

Bedaiwy MY, OA Awadalla, AM Abou-Zeid, HT Hamada (2016). Optimal conditions for production of L-asparaginase from *Aspergillus tamarii*. *Egypt Exp Biol Bot* 12:229–237

Bhamare HM, HP Jadhav, RZ Sayyed (2018). Statistical optimization for enhanced production of extracellular laccase from *Aspergillus sp.* HB_RZ4 isolated from bark scrapping. *Environ Sustain* 1:159–166

Dantas RC, LF Caetano, ALS Torres, MS Alves, ETMF Silva, LPR Teixeira, DC Teixeira, R de Azevedo Moreira, MHG, Fonseca, SG Neto, LT Martins (2019). Expression of a recombinant bacterial L-asparaginase in human cells. *BMC Res Notes* 12; Article 794

Elzainy TA, TH Ali (2006). Detection of the antitumor glutminase-asparaginase in the filamentous fungi. *Appl Sci* 6:1389–1395

Ferrara MA, NMB Severino, JJ Mansure, AS Martin, EMM Oliveira, AC Siani, NP Jr, FAG Torres, EPS Bon (2006). Asparaginase production by a recombinant *Pichia pastoris* strain harboring *Saccharomyces cerevisiae* ASP3 gene. *Enz Microb Technol* 39:1457–1463

Isaac GS, MA Abu-Tahon (2016). Production of extracellular anti-leukemic enzyme L-asparaginase from *Fusarium solani* AUMC 8615 grown under solid-state fermentation conditions: Purification and characterization of the free and immobilized enzyme. *EJBO* 56:799–816

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

Nevo E (2011). *Selection overrules gene flow at ''Evolution Canyon'' Israel,* Vol. 5, pp: 67–89. In: Urban K (ed). Advances in genetic research. Nova Science Publishers New York, USA

Nevo E (2006). ''Evolution Canyon' a microcosm of life's evolution focusing on adaptation and speciation. *Isr J Ecol Evol* 52:485–506

Nevo E (2007). Mosaic evolution of subterranean mammals: tinkering, regression, progression, and global convergence. *In: Subterranean Rodents: News from Underground,* pp: 375–388. Beagall S, H Burda, CE Schleich (eds). Springer-Verlag Berlin, Germany

Nevo E (2009). Evolution in action across life at ''Evolution Canyon'', Israel. *Trends Evol Biol* 1; Article e3

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

Pang CNI, A Hayen, MR Wilkins (2007). Surface accessibility of protein post-translational modifications. *J Proteome Res* 6:1833–1845

Prakasham RS, CS Rao, RS Rao, GS Lakshmi, PN Sarma (2007). L-asparaginase production by isolated *Staphylococcus* sp.–6A: design of experiment considering interaction effect for process parameter optimization. *Appl Microbiol* 102:1382–1391

Raveendran S, B Parameswaran, S Beevi Ummalyma, A Abraham, A Kuruvilla Mathew, A Madhavan, S Rebello, A Pandey (2018). Applications of microbial enzymes in food industry. *Food Technol. Biotechnol* 56:16–30

Saeed H, H Ali, H Soudan, A Embaby, A El-Sharkawy, A Farag (2018). Molecular cloning, structural modeling and production of recombinant *Aspergillus terreus,* L. asparaginase in *Escherichia coli Intl Biol Macromol* 106:1041–1051

Safary A, R Moniri, SM Mirhashemi, H Nikzad, MA Khiavi (2013). Phylogenetic and biochemical characterization of a new halo-thermotolerant, biofilm-forming *Bacillus* from Saline Lake of Iran. *Polish Microbiol* 62:419–425

Safary A, R Moniri, M Hamzeh-Mivehroud, S Dastmalchi (2016). Identification and molecular characterization of genes coding pharmaceutically important enzymes from halo-thermo tolerant *Bacillus*. *Adv Pharm Bull* 6:551–561

Safary A, R Moniri, M Hamzeh-Mivehroud, S Dastmalchi (2019). Highly efficient novel recombinant L-asparaginase with no glutaminase activity from a new halo-thermotolerant *Bacillus* strain. *Bioimpacts* 9:15–23

Sarquis MIM, EMM Oliviera, AS Santos, GL da-Costa (2004). Production of L-asparaginase by filamentous fungi. *Mem Inst Oswaldo Cruz* 99:489–492

Sindhu R, H Manonmani (2018). Expression and characterization of recombinant l-asparaginase from *Pseudomonas fluorescens*. *Protein Expr Purif* 143:83–91

Sudhir AP, BR Dave, AS Prajapati, K Panchal, D Patel, R Subramanian (2014). Characterization of a recombinant glutaminase-free L-asparaginase (ansa3) enzyme with high catalytic activity from bacillus *licheniformis. Appl Biochem Biotechnol* 174:2504–2515

Taeymans D, A Anderson, P Ashby, I Blank, P Gonde, P Van Eijck, V Faivre, SP Lalljie, H Lingnert, M Lindblom, R Matissek, D Muller, RH Stadler, A Studer, D Silvani, D Tallmadge, G Thompson, T Whitmore, J Wood, D Zyzak (2005). Acrylamide: update on selected research activities conducted by the European food and drink industry. *JAOAC Intl* 88:234–241

Verma N, K Kumar, G Kaur, S Anand (2007). L-asparaginase: a promising chemotherapeutic agent. *Crit Rev Biotechnol* 27:45–62

Yang XJ, CM Chiang (2013). Sumoylation in gene regulation, human disease, and therapeutic action. *F1000prime Rep* 5; Article 45