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Full Length Article

Genetic Variation and Post-Translational Modifications of Cytochrome C Oxidase-1 (*COX1*) in different Strains of *Sordaria fimicola*

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Abstract

Post translational modifications, not only alter the protein structure but also its dynamics due to which there is a definite change in the performance of that protein resulting in remarkable diversity and complexity. The main purpose behind this study was to point out genetic variations in Cytochrome c oxidase-1 at gene level, protein level and modifications of protein in six parental strains and subsequent generations (F3 and F4) of *Sordaria fimicola*. All genetic variations and protein modifications were compared with reference organism (*Neurospora crassa*). Overall, point mutations on 10 different positions were observed in all strains when compared with reference organism, 7 of these were exhibited by the strains isolated from harsh environmental conditions while variations on 3 positions were common between strains of two opposite environments. Various post-translational modification predictor tools were used to predict post-translational modifications. The accuracy of results of PTMs can be enhanced by combining some bioinformatics techniques with mass spectrophotometry, which fascinate the researchers who are working in the field of proteomics.

Keywords: Evolution canyon; Glycosylation; Nucleotide; Polymorphisms; Phosphorylation

Introduction

In eukaryotes, proteins after translation need some chemical alterations to modify their functions. Post translational modifications (PTMs) enhance the role of proteins by linking up with other biochemical functional groups *i.e.,* acetate, phosphate, various lipids and carbohydrates and by altering the chemical composition and structure of proteins such as the formation of disulfide bridges etc. These modifications, not only alter the protein structure but also its dynamics due to which there is a definite change in the performance of that protein (Walsh and Jefferis, 2006; Bhadauria *et al*., 2007; Li *et al*., 2010; Arif *et al*., 2017a). PTMs are of more than 200 types on the basis of functional group attached or type of bond formation (Yu *et al*., 2007). The proteins transitions after their modifications, work as regulatory factor for maintaining many physiological and cellular processes such as differentiation of cells, degradation, signaling and regulation of proteins, gene expression and interaction of proteins with other proteins (Minguez *et al*., 2012).

Recently mass spectrophotometry technique has been designed for the identification, purification and quantification of modified sites on proteins. The accuracy of results of PTMs can be enhanced by combining some techniques like, Affinity-based improvement by combining extraction methods and; multidimensional separation techniques with mass spectrophotometry, Which fascinate the researchers who are working in the field of proteomics (Jensen, 2004; Seo and Lee, 2004; Chandramouli and Qian, 2009).

The present study describes different types of PTMs and their positions on Cytochrome c oxidase-1 (*COX1*) protein of *Sordaria fimicola* (Six parental strains) and *Neurospora crassa*. The above mentioned protein is functionally important in respiratory chain reaction of Ascomycetes (Shoubridge, 2001), as it is responsible for accepting electrons from Cytochrome c and completing oxidation reaction (Hough *et al*., 2014; Soto and Barrientos, 2016). In eukaryotes, *COX* protein is positioned in the inner mitochondrial membrane and has multiple subunits. Subunits 1 and 2 (*COX1, COX2*) are the largest constituent and essential to form a complex enzyme, Cytochrome c oxidase. *COX1* (Cytochrome c oxidase subunit-1) consists of heme a, heme a3 and CuB for the reduction of oxygen (Diaz *et al*., 2006; Shingu-VaZquez *et al*., 2010). It is predicted through Hydropathy plots that *COX1* sequences have minimum 12 a-helical transmembrane regions which have redox centers of the enzyme (Fontanesi *et al*., 2008;

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Khalimonchuk *et al*., 2010; Soto and Barrientos, 2016).

To the best of our understanding, no study on post translational modifications of *COX1* in *S. fimicola* has been reported so far. Therefore, current study was conducted to determine some covalent modifications of *COX1* protein such as phosphorylation, glycosylation, acetylation and methylation in *S. fimicola* and *N. crassa*. Regulation of *COX1* activity by the post-translational modifications could characterize a new field of interest regarding the cell signal process.

Materials and Methods

Culture Retrieving of *S. fimicola*

Six pure culture strains (collected by Prof. Nevo from Evolutionary Canyon) of *S. fimicola* were made available by Molecular Genetics Research Laboratory of Botany Department, Punjab University Lahore. Three of these strains (S1, S2 and S3) were collected from South Facing Slop (SFS) at 60 m, 90 m and 120 m height above sea level respectively and remaining three strains (N_5, N_6 and N_7) were collected from North Facing Slop (NFS) at the same heights. These six parental strains cultures were retrieved on PDA media under aseptic conditions and incubated for 12–14 days at 18°C for subsequent analyses.

DNA Extraction

DNA of six strains of *S. fimicola* was extracted by modified Pietro's method of DNA extraction (Pietro *et al*., 1995). For more detail please see Rana *et al*. (2018).

Cytochrome c Oxidase-1 PCR Amplification and Sequencing

Forward and reverse primers for the amplification of *COX1* gene were designed by using Primer3 software. Touchdown PCR cycling conditions (Korbie and Mattick, 2008) were used for *COX1* amplification. PCR reaction mixture of 15 μL (2 μL DNA sample, 10 μL Master Mix (GeneAll), 1 μL Forward primer, 1 μL Reverse primer and 1 μL Double distilled water) was used. For the verification of amplification of desired PCR product 1% agarose gel electrophoresis was done with 1 Kb DNA ladder (Invitrogen) and PCR amplicons were sequenced and analyzed by sequence analysis software (Chromas). Nucleotide sequence of *COX1* of *N. crassa* was obtained from NCBI under the accession number KY_498478.1.

Prediction of PTMs on *COX1* Protein

For the predictions of glycosylation, phosphorylation, acetylation and methylation on *COX1* following online servers: YinOYang 1.2 (www.cbs.dtu.dk/services/YinOYang/), Netphos 3.1 (www.cbs.dtu.dk/services/NetPhos/), PAIL (bdmpail.biocuckoo.org/) and ModPred (montana.informatics.indiana.edu/ModPred/faq.html) were used respectively. All of these servers provided detail of PTMs positions on *COX1*.

Results

All the nucleotide sequences of *COX1* gene in reference specie and all strains of *Sordaria fimicola* were aligned by clustal omega to calculate polymorphic sites. This alignment showed polymorphisms at 10 different positions (Table 1 and 2). Glycosylation predicted sites by YinOYang 1.2 server (Table 3) showed glycosylation at positions 127, 128 on threonine residue and at positions 299, 322 on serine residue in *N. crassa*. Further, S_1, S_2, S_3, N_5, N_6 and N_7 have same positions of glycosylation *i.e.*, 126 and 127 on threonine residue but no Ser modifications were observed in any strain. One conserved position of glycosylation (127T) was found in *N. crassa* and *S. fimicola*. Graphical representation of glycosylation in *COX1* protein of *N. crassa*, and parental strains of *S. fimicola* are shown in Fig. 1a and b.

NetPhos 3.1 server results (Table 3) showed that there was phosphorylation on *COX1* of *N. crassa*, S_1, S_2, S_3, N_5, N_6 and N_7 on three types of residues that are Ser (Serine), T (Threonine) and Y (Tyrosine), respectively. In total, 59 residues of *N. crassa* and 57 residues of *S. fimicola* exhibited phosphorylation while only 8 phosphorylation sites were found to be conserved in all starins of *S. fimicola* and *N. crassa*. Furthermore, all threonine residues were found to be conserved among all strains of *S. fimicola*, however, variations were observed in case of serine and tyrosine residues *i.e.*, 370 Ser and 318 Y in S1 and S2 strains while 313 Ser and 315 Y in S_3, N_5, N_6 and N_7 strains, respectively (Fig. 2).

Prediction of Acetylation on Internal Lysines (PAIL) server is shown in Table 4. Acetylation sites differ between *N. crassa* and S_1, S_2, S_3, N_5, N_6 and N_7 at 3 positions *i.e.*, 297 K, 310 K and 439 K in *N. crassa* while 308 K, 437 K and 438 K in all strains of *S. fimicola.* One acetylation at lysine residue 440 present in *N. crassa* was found to be absent in *S. fimicola*. All others positions (1, 16, 19, 64 and 68) were conserved positions of acetylation on internal lysine residue in *N. crassa* and *S. fimicola*.

In addition, methylation (Table 4) was conserved; at position 1 on lysine residue methylation was conserved in *N. crassa* and *S. fimicola*. *N. crassa* has 7 methylation sites whereas S_1, S_2, S_3, have methylation on arginine residue at position 112 R only and on lysine residues at positions 1 K, 186 K, 308 K, 406 K. N_5, N_6 and N_7 have methylation on arginine residues at positions 112 R, 347 R and on lysine residues at positions 1 K, 186 K, 308 K, 406 K. All the positions of methylation are same in all strains of *S. fimicola* except at position 347 on arginine residue which is only observed in N_5, N_6 and N_7 strains of *S. fimicola*.

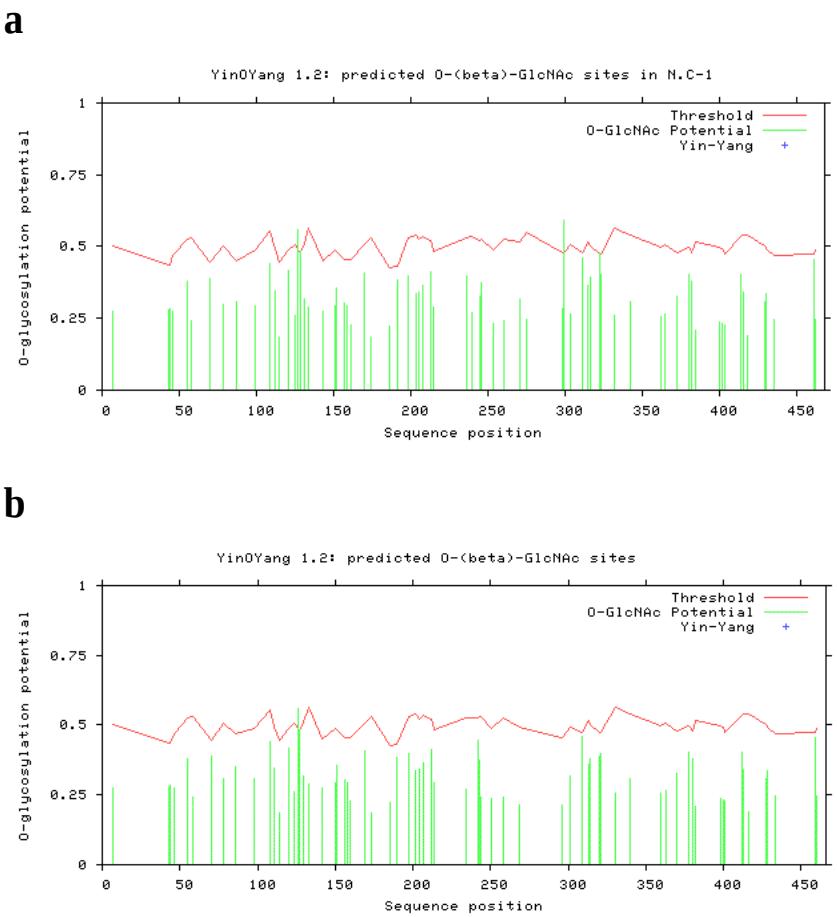

Fig. 1: Graphical Representation of Glycosylation Potential in *COX1* Protein (**a**) In *N. crassa* (**b**) In S_1, S_2, S_3, N_5, N_6 and N_7. Red Zigzag Line Shows the Threshold (0.5), Green Lines Shows the O-Glycosylation Potential. Green Lines Exceeding Threshold Shows the Positions of Glycosylation

Discussion

For broad scale assessment of genetic diversity, nucleotide variation is used (Wright, 2005). In Clark *et al.* (2007) examined the pattern of nucleotide sequence variation in *Arabidopsis thaliana* and reported that essential single nucleotide polymorphisms (SNPs) were more than 1 million and 4% of the genome sequence was found to be divergent from reference genome sequence. *COX1* gene Clustal analysis also revealed 10 variations (Table 1) in nucleotide sequence out of which 7 variations were within specie and these variations were also found in all the SFS (South Facing Slope) strains but not in any strain of the N-slope.

Hebert *et al.* (2003) also had worked on polymorphism of *COX1* gene for the discrimination of closely allied species because *COX1* have high rate of sequence change in most animal groups. Mogadam and Yousefi (2013) inspected the *COX1* gene polymorphism in Malaysia population. They found 13 nucleotide variations within 1548 bp. Nucleotide diversity in *COX1* of *Cooperia oncophora* was not more than 2% between species (Vaulin and Novikov, 2012). *COX1* sequence alignment results have shown 10 variations of protein residues along with conservation of residues in similar groups at five positions and conservation of residues in weakly similar groups at 2 positions. All these molecular analysis results showed the genetic diversity between different strains of *S. fimicola*.

Baer and Millar (2016) reported that DNA has crucial role in evolution due to genetic variability in it and genetic diversity of DNA directly affects the expression of proteins (which form from translation of its genes). More over post translational modifications influence significant variations in mature proteins of same gene leading to evolutionary changes and adaptations as PTMs are actually responsible for the complexity of the proteome (Audagnotto and Peraro, 2017). Tahir *et al.* (2016) found genetic diversity in Cytochrome c oxidase I among the 44 different species of Anopheles mosquitoes.

In eukaryotes, protein glycosylation is vital post translational modification that is of generally three types *i.e.*, *N*-linked, *O*-linked and glycosyl-phosphatidylinositol-anchored. During this research work, we have found *O*-

Table 1: Pairwise sequence alignment of *COX1* gene to determine genetic diversity between different *S. fimicola* strains and *N. crassa*

N.c	CATTGCAGATAATCAATTATACAATGCTATAATAACTGCACATGCGATCTTAATGATTTT	300
S_1	CATTGCAGATAATCAATTATACAATGCTATAATAACTGCACATGCGATCTTAATGATATT	300
S_2	CATTGCAGATAATCAATTATACAATGCTATAATAACTGCACATGCGATCTTAATGATATT	300
S_3	CATTGCAGATAATCAATTATACAATGCTATAATAACTGCACATGCGATCTTAATGATATT	300
N_5	CATTGCAGATAATCAATTATACAATGCTATAATAACTGCACATGCGATCTTAATGATATT	300
N_6	CATTGCAGATAATCAATTATACAATGCTATAATAACTGCACATGCGATCTTAATGATATT	300
N_7	CATTGCAGATAATCAATTATACAATGCTATAATAACTGCACATGCGATCTTAATGATATT	300
	**********	
N.c	TATGCTATTAACAGATAGAAATTTTAATACATCATTCTTTGAAACAGCTGGTGGTGGTGA	780
S_1	TATGGTATTAACAGATAGAAATTTTAATACATCATTCTTTGAAACAGCTGGTGGTGGTGA	780
S_2	TATGGTATTAACAGATAGAAATTTTAATACATCATTCTTTGAAACAGCTGGTGGTGGTGA	780
S_3	TATGGTATTAACAGATAGAAATTTTAATACATCATTCTTTGAAACAGCTGGTGGTGGTGA	780
N_5	TATGGTATTAACAGATAGAAATTTTAATACATCATTCTTTGAAACAGCTGGTGGTGGTGA	780
N_6	TATGGTATTAACAGATAGAAATTTTAATACATCATTCTTTGAAACAGCTGGTGGTGGTGA	780
N_7	TATGGTATTAACAGATAGAAATTTTAATACATCATTCTTTGAAACAGCTGGTGGTGGTGA	780
	***** ****** **********	
N.c	TCCTATTTTATTCCAACATCTTTTCTGATTCTTCGGGCATCCTGAGGTTTACATTTTAAT	840
S_1	CCCTATTTTATTCCAACATCTTTTCTGATTCTTCGGGCATCCTGAGGTTTACATTTTAAT	840
S_2	CCCTATTTTATTCCAACATCTTTTCTGATTCTTCGGGCATCCTGAGGTTTACATTTTAAT	840
S_3	CCCTATTTTATTCCAACATCTTTTCTGATTCTTCGGGCATCCTGAGGTTTACATTTTAAT	840
N_5	CCCTATTTTATTCCAACATCTTTTCTGATTCTTCGGGCATCCTGAGGTTTACATTTTAAT	840
N_6	CCCTATTTTATTCCAACATCTTTTCTGATTCTTCGGGCATCCTGAGGTTTACATTTTAAT	840
N_7	CCCTATTTTATTCCAACATCTTTTCTGATTCTTCGGGCATCCTGAGGTTTACATTTTAAT	840
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N.c	AAGTCATCATATGTATACAGTTGGTTTAGACGTGGATACAAGAGCGTATTTCACAGCAGC	1020
S_1	AAGTCACCACATGTGTACAGTTGGGTTAGACGTGGATACAAGAGCGTATTTCACAGCAGC	1020
S_2	AAGTCACCACATGTGTACAGTTGGGTTAGACGTGGATACAAGAGCGTATTTCACAGCAGC	1020
S_3	AAGTCACCACATGTGTACAGTTGGTTTAGACGTGGATACAAGAGCGTATTTCACAGCAGC	1020
N_5	AAGTCATCACATGTATACAGTTGGTTTAGACGTGGATACAAGAGCGTATTTCACAGCAGC	1020
N_6	AAGTCATCACATGTATACAGTTGGTTTAGACGTGGATACAAGAGCGTATTTCACAGCAGC	1020
N_7	AAGTCATCACATGTATACAGTTGGTTTAGACGTGGATACAAGAGCGTATTTCACAGCAGC	1020
	******* ** ***** ****** ***** ******** **********	
N.c	TACATTAATTATTGCAGTTCCTACAGGAATTAAAATATTCTCATGATTAGCTACATGTTA	1080
S_1	AACATTAATTATTGCAGTTCCTACTGGAATTAAAATATTCTCATGATTAGCTACATGTTA	1080
S_2	AACATTAATTATTGCAGTTCCTACTGGAATTAAAATATTCTCATGATTAGCTACATGTTA	1080
S_3	TACATTAATTATTGCAGTTCCTACTGGAATTAAAATATTCTCATGATTAGCTACATGTTA	1080
N_5	TACATTAATTATTGCAGTTCCTACGGGAATTAAAATATTCTCATGATTAGCTACATGTTA	1080
N_6	TACATTAATTATTGCAGTTCCTACGGGAATTAAAATATTCTCATGATTAGCTACATGTTA	1080
N_7	TACATTAATTATTGCAGTTCCTACGGGAATTAAAATATTCTCATGATTAGCTACATGTTA	1080
	********** ******* **********	
N.c	TGGAGGTTCTATTAGATTAACTCCTTCTATGTTATTTGCTTTAGGTTTTGTATTTATGTT	1140
S_1	TGGAGGTTGTATTAGATTAACTCCTTCTATGTTATTTGCTTTAGGTATTGTATTTATGTT	1140
S_2	TGGAGGTTGTATTAGATTAACTCCTTCTATGTTATTTGCTTTAGGTATTGTATTTATGTT	1140
S_3	TGGAGGTTGTATTAGATTAACTCCTTCTATGTTATTTGCTTTAGGTTTTGTATTTATGTT	1140
N_5	TGGAGGTTCTATTAGATTAACTCCTTCTATGTTATTTGCTTTAGGTTTTGTATTTATGTT	1140
N_6	TGGAGGTTCTATTAGATTAACTCCTTCTATGTTATTTGCTTTAGGTTTTGTATTTATGTT	1140
N_7	TGGAGGTTCTATTAGATTAACTCCTTCTATGTTATTTGCTTTAGGTTTTGTATTTATGTT	1140
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Highlighted region shows mutation on that point

linked glycosylation of *COX1* protein in *S. fimicola* and *N. crassa* because these *O*-linked modifications occurred only on serine or threonine residues and not on tyrosine residues and these modifications are also called mucin-type *O*-linked glycosylation.

In glycosylation, the hydroxyl groups of serine and threonine residues (O-glycosylation) get transformed (Bektasa *et al*., 2011; Jamil *et al*., 2018) but present results show glycosylation only on threonine residue in *COX1* of *S. fimicola*. *COX1* have two glycosylation modification positions on threonine (T) residues and two on serine (S) residues in *N. crassa* whereas all strains of *S. fimicola* have glycosylation only on two threonine (T) residues. In conclusion one position was found to be conserved in *N. crassa* and *S. fimicola* (Table 3).

Phosphorylation modification of proteins have gained much consideration in regulatory process of living organisms in which protein kinases and their corresponding phosphotases have central role (Knorre *et al*., 2009) and most of the protein kinases studied along with protein phosphorylation belongs to eukaryotes (Trost and Kusalik, 2011). Present results have four major protein kinases involved in phosphorylation of Cytochrome c oxidase where PKC, unsp, PKA, cdc2 have their contribution in phosphorylation respectively. In Liko *et al*. (2016) reported 8 phosphorylated sites (only on serine and threonine residues) in bovine Cytochrome c oxidase with no phosphorylation on tyrosine residue while in contrast

Table 2: Pairwise amino acid sequence alignment of *COX1* protein of *S. fimicola* strains and *N. crassa*

N.c	TWNSIFCFNKNGVKRTWGAVHCR*SIIQCYNNCTCDLNDFLYGYASINRWIW*FLVTIIS	112
S_1	TWNSIFCFNKNGVKRTWGAVHCR*SIIQ*YNNCTCDLNDFLYGYASINRWIW*FLVTIIS	111
S_2	TWNSIFCFNKNGVKRTWGAVHCR*SIIQ*YNNCTCDLNDFLYGYASINRWIW*FLVTIIS	111
S_3	TWNSIFCFNKNGVKRTWGAVHCR*SIIQ*YNNCTCDLNDFLYGYASINRWIW*FLVTIIS	111
N_5	TWNSIFCFNKNGVKRTWGAVHCR*SIIQ*YNNCTCDLNDFLYGYASINRWIW*FLVTIIS	111
N_6	TWNSIFCFNKNGVKRTWGAVHCR*SIIQ*YNNCTCDLNDFLYGYASINRWIW*FLVTIIS	111
N_7	TWNSIFCFNKNGVKRTWGAVHCR*SIIQ*YNNCTCDLNDFLYGYASINRWIW*FLVTIIS	111
	********** **********	
N.c	YAINR*KF*YIIL*NSWWW*SYFIPTSFLILRAS*GLHFNYTWFWYNKYNNISLF**ISI	276
S_1	YGFN**KF*YIIL*NSWWW*PYFISTSFLILRAS*GLHFNYTWFWYNKYNNISLF**ERI	274
S_2	YGFN**KF*YIIL*NSWWW*PYFISTSFLILRAS*GLHFNYTWFWYNKYNNISLF**ERI	274
S_3	YGFN**KF*YIIL*NSWWW*PYFISTSFLILRAS*GLHFNYTWFWYNKYNNISLF**ERI	274
N_5	YGFN**KF*YIIL*NSWWW*PYFISTSFLILRAS*GLHFNYTWFWYNKYNNISLF**ERI	274
N_6	YGFN**KF*YIIL*NSWWW*PYFISTSFLILRAS*GLHFNYTWFWYNKYNNISLF**ERI	274
N_7	YGFN**KF*YIIL*NSWWW*PYFISTSFLILRAS*GLHFNYTWFWYNKYNNISLF**ERI	274
	.. ********** **** ********** *	
N.c	RLYWYGLRHDVYWNIRIYCLKSPYVYSWFRRGYKSVFHSSYINYCSSYRN*NILMISYML	335
S_1	RLYWYGLRHDVYWNIRIYCLKSPHVYSWVRRGYKSVFHSSNINYCSSYWN*NILMISYML	333
S_2	RLYWYGLRHDVYWNIRIYCLKSPHVYSWVRRGYKSVFHSSNINYCSSYWN*NILMISYML	333
S_3	RLYWYGLRHDVYWNIRIYCLKSPHVYSWFRRGYKSVFHSSYINYCSSYWN*NILMISYML	333
N_5	RLYWYGLRHDVYWNIRIYCLKSPHVYSWFRRGYKSVFHSSYINYCSSYGN*NILMISYML	333
N_6	RLYWYGLRHDVYWNIRIYCLKSPHVYSWFRRGYKSVFHSSYINYCSSYGN*NILMISYML	333
N_7	RLYWYGLRHDVYWNIRIYCLKSPHVYSWFRRGYKSVFHSSYINYCSSYGN*NILMISYML	333
	********** :****.********** ******* **********	
N.c	WRFY*INSFYVICFRFCIYVHNWGIKWSCFSECIFRYSIPRYLLRSCSFSLCIKYGCCIC	394
S_1	WRLY*INSFYVICFRYCIYVHNWGIKWSCFSECIFRYSITRYLLRSCSFSLCIKYGCCIC	392
S_2	WRLY*INSFYVICFRYCIYVHNWGIKWSCFSECIFRYSITRYLLRSCSFSLCIKYGCCIC	392
S_3	WRLY*INSFYVICFRFCIYVHNWGIKWSCFSECIFRYSIQRYLLRSCSFSLCIKYGCCIC	392
N_5	WRFY*INSFYVICFRFCIYVHNWGIKWSCFSECIFRYSIPRYLLRSCSFSLCIKYGCCIC	392
N_6	WRFY*INSFYVICFRFCIYVHNWGIKWSCFSECIFRYSIPRYLLRSCSFSLCIKYGCCIC	392
N_7	WRFY*INSFYVICFRFCIYVHNWGIKWSCFSECIFRYSIPRYLLRSCSFSLCIKYGCCIC	392
	:********:********** **********	

Highlighted region shows mutation on that point

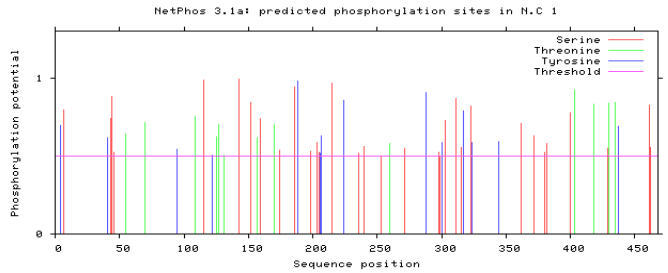
Table 3: Prediction of glycosylation and phosphorylation on serine, threonine and tyrosine residues in *N. crassa* and in different strains of *S. fimicola*

		COX1		Kinases involved			
Organism	**Residue**	**Glycosylation**	**Phosphorylation**	**unsp**	**PKA**	**PKC**	**cdc2**
N. crassa	Serine	299, 322	7, 43, 44, 46, 115, 143, 152, 159, 174, 186, 198, 203, 205, 215, 236, 240, 253, 271, 298, 299, 303, 311, 315, 323, 362, 372, 380, 382, 400, 429, 461, 462 Total= 32 sites	43,115,143, 159, 186, 215, 311, 323	43,44,115, 143, 203, 240, 253, 271, 315, 362, 372, 382, 400, 429, 461,	7,115,152,159,186,23 6, 240, 298, 303, 311, 323, 462,	44,46,152, 198, 205, 298, 299, 380,
	Threonine	127, 128	55, 70, 109, 125, 127, 131, 157, 170, 260, 403, 418, 430, 435 Total= 13 sites	157	Nil	55, 70, 109, 125, 127, 131, 157, 170, 260, 403, 418, 430, 435,	170, 430,
	Tyrosine	**Nil**	4, 41, 95, 122, 188, 206, 207, 224, 288, 300, 317, 324, 344, 437 Total= 14 sites	4, 41, 188, 207, 224, 288, 300, 317, 324, 437	Nil	Nil	Nil
S_1, S_2, S_3 Strains	Serine	**Nil**	7, 43, 44, 46, 114, 142, 151, 158, 173, 185, 197, 202, 204, 214, 242, 244, 251, 269, 296, 309, 321, 330, 360, 370, 378, 380, 398, 427, 459, 460 Total= 30 sites	43, 114, 142, 158, 185, 214, 296, 309	43, 44, 114, 142, 202, 242, 251, 269, 330, 360, 370, 380, 398, 427, 459	7, 114, 151, 158, 185, 309, 321, 460	44, 46, 151, 197, 204, 244, 378
	Threonine	126, 127	55, 70, 108, 124, 126, 130, 156, 169, 258, 401, 416, 428, 433 Total= 13 sites	156	Nil	55, 70, 108, 124, 126, 130, 156, 169, 258, 401, 416, 428, 433,	169, 428,
	Tyrosine	-	4, 41, 94, 121, 187, 205, 206, 223, 229, 286, 318, 322, 342, 435 Total= 14 sites	4, 41, 206, 223, 229, 286, 318, 322, 435	187	-	-
N_5, N_6, N_7	Serine	-	7, 43, 44, 46, 114, 142, 151, 158, 173, 185, 197, 202, 204, 214, 242, 244, 251, 269, 296, 309, 313, 321, 330, 360, 378, 380, 398, 427, 459, 460 Total= 30 sites	43, 114, 142, 158, 185, 214, 296, 309	43, 44, 114, 142, 202, 242, 251, 269, 313, 330, 360, 370, 380, 398, 427, 459	7, 114, 151, 158, 185, 309, 321, 460	44, 46, 151, 197, 204, 244, 378
	Threonine	126, 127	55, 70, 108, 124, 126, 130, 156, 169, 258, 401, 416, 428, 433 Total= 13 sites	156	-	55, 70, 108, 124, 126, 130, 156, 169, 258, 401, 416, 428, 433,	169, 428,
	Tyrosine	-	4, 41, 94, 121, 187, 205, 206, 223, 229, 286, 315, 322, 342, 435 Total= 14 sites	4, 41, 206, 223, 229, 286, 315, 322, 435	187	-	-

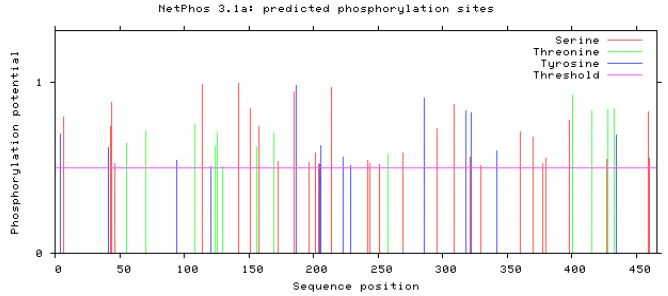
Highlighted area = conserved regions

present research results showed that tyrosine residue of *COX1* have phosphorylation modification in both *N. crassa* and *S. fimicola*. Trost and Kusalik (2011) confirmed that computational prediction of potential sites of phosphorylation is more significant than time taking experimental procedures. Liko *et al*. (2016) have

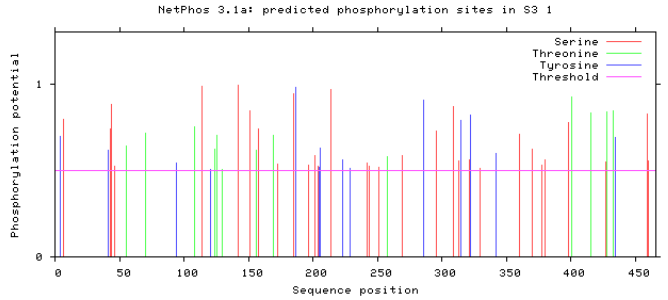
a



b



c



d

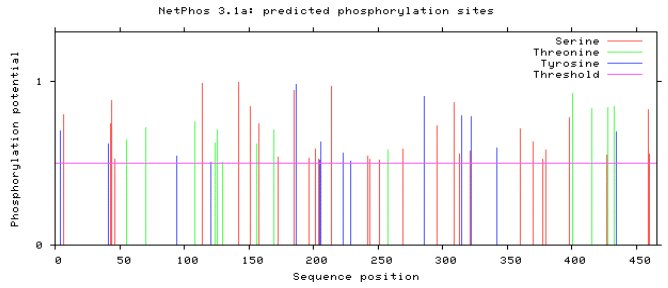


Fig. 2: Graphical Representation of Phosphorylation Potential in *COX1* Protein (**a**) In *N. crassa* (**b**) In S_1 and S_2 (c) In S_3 (d) In N_5, N_6 and N_7. Red Lines= Serine, Green Lines= Threonine, Blue Lines= Tyrosine and Pink Line= Threshold. Lines Exceeding Pink Line (Threshold= 0.5) Shows the Positions of Phosphorylation in *COX1* and Red, Green, Blue Color of Lines Shows the Residue Present at that Position

reported 14 acetylation sites in *COX* of bovine. Rieder and Bosshard (1980) compared attachment sites on Cytochrome c for *COX* and *CyC1* where they also found 6 positions of acetylated lysine residues. Vassilev *et al*. (1995) had also reported acetylation at 324 on internal lysine of Cytochrome c oxidase subunit 1 for formation of myristic acid.These computational analysis results are quite satisfactory due their improved prediction accuracy and these predictions of methylation on covalently modified lysine and arginine residues are important information for

Table 4: Comparison of *COX1* Acetylation Sites, Methylation Sites on Lysine (K) and Arginine (R) Residues in *N. crassa* and *S. fimicola*

		COX1	
Organism	Residue	Acetylation Sites	Methylation Sites
N. crassa	K	1, 16, 19, 64, 68, 297, 310, 439, 440 Total = 9sites	1, 187, 229, 310, 408 Total = 5sites
	R	-	113, 349 Total = 2sites
S_1, S_2, S_3	K	1, 16, 19, 64, 68, 308, 437, 438 Total = 8sites	1, 186, 308, 406 Total = 4sites
	R	-	112 Total = 1site
N_5, N_6, N_7	K	1, 16, 19, 64, 68, 308, 437, 438 Total = 8sites	1, 186, 308, 406 Total = 4sites
	R	-	112, 347 Total = 2sites

future experimental research (Deng *et al*., 2017). Various workers have applied the same protein modification predictor tools to determine the post translational modifications of mating type and frequency clock proteins (Arif *et al*., 2017b); Histone 3 and 4 proteins (Jamil *et al*., 2018); Manganese oxide super dismutase (MnSOD) protein (Rana *et al*., 2018) in different strains of *S. fimicola* as used in present study.

Hoffmann and Hercus (2000) also mentioned environmental stress as an evolutionary force which starts with variation in genetic makeup. It is concluded from above results that genetic variations help to overcome the harsh conditions confronted by an organism (Arif *et al*., 2017a). These genetic variations within same species due to any force (point mutation) initially takes place in the nucleotide sequence of genes leading to translation of these genes with diverse amino acid sequence of the same protein in same species. Finally, the protein modifications are also effected by genetic variations in basic unit of genome due to which dynamics and functions of protein are enhanced.

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