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HOMOLOGY MODELLING AND PHYLOGENETIC ANALYSIS OF ALKALINE PHOSPHATASE, ACID PHOSPHATASE AND PHYTASE GENES FROM *ASPERGILLUS FUMIGATUS*

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ABSTRACT

This research work reports the homology modeling and phylogenetic analysis of acid phosphatase, phytase and alkaline phosphatase genes of *A. fumigatus*. Since soil is a reservoir for the isolation of such fungi, soil microbes were screened for phosphatases and a strain of *Aspergillus fumigatus* was isolated. A typical fungal colony was isolated after serial dilution, identified as *A. fumigatus* was used in this study. On molecular identification, the strains identified on the basis of morphological and physiological features were confirmed to belong to *A. fumigatus*. Their ITS1-ITS4 sequences were up to 99% homologous to reference strains of *A. fumigatus* from the GenBank. Acid phosphatase, alkaline phosphatase and phytase cDNAs were synthesized from *Aspergillus fumigatus* RNA by PCR reactions using primers (designed from published NCBI sequences) with reverse transcriptase. This resulted in amplicons corresponding to the predicted gene size of the respective enzymes. They were cloned into bacteria and were subjected to automated DNA sequencing. DNA sequencing after plasmid isolation revealed that the entire nucleotide sequence of the alkaline phosphatase, acid phosphatase and phytase genes from genomic DNA of *A. fumigatus* was 99.9 % similar to NCBI sequences of *Aspergillus fumigatus.* The closest homolog to alkaline phosphatase gene of *A. fumigatus* was *Neosartorya fumigata* and *Neosartorya fumigata.* The closest homolog to acid phosphatase gene of *A. fumigatus* was *Neosartorya fischeri.* The closest homolog to phytase gene *of A. fumigatus* was *Neosartorya fumigata*. Phylogenetic analysis was performed for the deduced amino acid sequences of acid phosphatase, phytase and alkaline phosphatase of *A. fumigatus*. The phylogenetic trees were built using Phylip program option and a bootstrap value of 100 was used. The phylogenetic tree revealed that alkaline phosphatase and phytase formed the branches on the root of acid phosphatase thus inferring that acid phosphatase is the common ancestor for both acid phosphatase and phytase. Thus, based on the phylogram obtained this evolutionary relationship between acid phosphatase, phytase and alkaline phosphatase of *A. fumigatus* could be inferred as a rooted phylogenetic tree.

Keywords: Alkaline phosphatase, Acid phosphatase, Phytase, Homology modelling, Phylogenetic analysis

INTRODUCTION

Aspergillus fumigatus originally is a fungus of the genus *Aspergillus*. It is one of the most common *Aspergillus* species that occur as a saprotroph widespread in nature and plays an essential role in carbon and nitrogen recycling by decaying organic matter such as compost heaps. The fungus also causes diseases in humans suffering from immunodeficiency. This study focuses on the homology modeling and phylogenetic analysis of alkaline phosphatase, acid phosphatase and phytase of *Aspergillus fumigatus* to infer the evolutionary relationship.

EXPERIMENTAL

Total RNA extraction was done from *Aspergillus fumigatus.* Reverse transcription reaction was performed to obtain cDNA using Reverse transcriptase enzyme. Published NCBI gene producing sequences for acid phosphatase, alkaline phosphatase and phytase of *A. fumigatus* were taken for PCR primer design. Primer design was performed using the NCBI Accession no. AF462065 *A. fumigatus* acid phosphatase phoA gene, Accession no. U59804 *A. fumigatus* phytase *phy*A gene and Accession no. XM_744353 *A. fumigatus* Af293 alkaline phosphatase pho8 gene. PCR was performed with gene specific

primers for the three genes. The amplified PCR products of alkaline phosphatase, acid phosphatase and phytase genes of *Aspergillus fumigatus* were confirmed on running on 0.8% agarose gel electrophoresis. The PCR reaction amplified an amplicon size of approximately 1820 bp characteristic of alkaline phosphatase gene, 1320 bp characteristic of acid phosphatase and 1340 bp characteristic of phytase gene which covers the entire gene without introns of the fungal *A. fumigatus* genomic DNA. The purified PCR was ligated in pET-28a vector. This was then subjected to transformation. The positive transformants were subjected to plasmid isolation. After the confirmation of the insert, the plasmid was sequenced by automated DNA sequencing method.

DNA sequencing

Sequencing of the clones was performed with an automatic DNA sequencer (ABI Prism 3100) by using the Big-Dye terminator cycle sequencing kit (PE Applied Biosystems) using the T7 promoter primer and T7 terminator primer.

Homology modeling and Phylogenetic analysis of the genes of acid phosphatase, alkaline phosphatase and phytase of *A. fumigatus*

The conversion of DNA to protein sequences of acid phosphatase, alkaline phosphatase and phytase of *Aspergillus fumigatus* was performed using Expasy translate DNA to protein tool (http://expasy.org/tools/dna.html). The 5'3' Frame 1 deduced amino acid sequences were used for further multiple sequence alignment and phylogenetic studies. The analysis involved FASTA sequences of similar homologs which were found in GenBank using NCBI FASTA tool. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the p-distance method. Evolutionary analyses were conducted in MEGA 6.05[1-6].

RESULTS AND DISCUSSION

DNA sequencing revealed an Open Reading Frame of 1824 base pairs downstream from a potential ribosome-binding site that encoded alkaline phosphatase gene beginning with an ATG (start codon) and ending with TAG (stop codon) was identified. The deduced amino acid sequence of the mature protein comprised of 607 amino acids with a calculated molecular mass of 66.28 KDa. The deduced amino acid sequences from the *pho*8 gene contained the consensus motifs (VTDSAAGAT) at amino acid positions 147-155 and which are conserved among alkaline phosphatases family. An Open Reading Frame of 1344 base pairs downstream from a potential ribosome-binding site that encoded acid phosphatase gene beginning with an ATG (start codon) and ending with TGA (stop codon) was identified. The deduced amino acid sequence of the mature protein comprised of 447 amino acids with a calculated molecular mass of 48.97 KDa. The deduced amino acid sequences from the *pho*A gene contained the consensus motifs (SPVSNVEGVAFNRFFQV) at amino acid positions 41-57 and which are conserved among acid phosphatases family. An Open Reading Frame of 1398 base pairs downstream from a potential ribosome-binding site that encoded phytase gene beginning with an ATG (start codon) and ending with TAG (stop codon) was identified. The deduced amino acid sequence of the mature protein comprised of 465 deduced amino acids with a calculated molecular mass of 50.84 KDa. The deduced amino acid sequences from the *phy*A gene also contained the consensus motif (MYVDFSHDNSMVSIFFA) at amino acid positions 353-369 and which are conserved among Histidine acid phosphatases family. Sequence comparisons showed 99.9 % homology to the reported sequences in the NCBI.

Homology Modeling

Insilico sequence analysis

Homology modeling was performed for the protein sequences of acid phosphatase, alkaline phosphatase and phytase of *Aspergillus fumigatus* using NCBI FASTA**.**

Acid phosphatase

The 5'3' Frame 1 protein sequence of acid phosphatase gene was subjected to NCBI FASTA search. The acid phosphatase producing *pho*A gene of *A. fumigatus* showed 100% identity to *A. fumigatus,*

Neosartorya fumigata, Neosartorya fischeri and 95% and 97% identity to acid phosphatase sequences of *A. clavatus, A. oryzae, A. niger, A. flavus* species confirming that it was the acid phosphatase clone**.** The FASTA search gave a high E value of 2.6E-190.

Alkaline phosphatase

The 5'3' Frame 1 protein sequence of alkaline phosphatase gene was subjected to NCBI FASTA search. The alkaline phosphatase producing *pho*8 gene of *A. fumigatus* showed 100% identity to *A. fumigatus, Neosartorya fumigata, Neosartorya fischeri and* 96% and 97% identity to alkaline phosphatase sequences of *A. fumigatus* and *A. terreus, A. clavatus, A. nidulan* species confirming that it was the alkaline phosphatase clone**.** The FASTA search gave a high E value of 2.6E-106.

Phytase

The 5'3' Frame-1 protein sequence of phytase gene was subjected to NCBI FASTA search. The phytase producing *phy*A gene of *A. fumigatus* showed 100% identity to *A. fumigatus, Neosartorya fumigata, Neosartorya fischeri* and 94% and 96% identity to phytase sequences of *A. fumigatus, A. oryzae and A. flavus* species confirming that it was the phytase clone**.** The FASTA search gave a high E value of 1.5E-213.

Phylogenetic Analysis

Acid phosphatase

The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 9 FASTA sequences of similar homologs which were found in GenBank using NCBI FASTA tool. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA 6.05. All these protein sequences were of fungal origin. A bootstrap value of 500 was used. The acid phosphatase of *A. fumigatus* showed **98**% identity to acid phosphatase sequences of Neosartorya *fumigata, Neosartorya fischeri, A. clavatus and A. niger* species**.** Thus, based on the phylogenetic tree obtained this evolutionary relationship between alkaline phosphatase of various *Aspergillus species* could be inferred as a rooted phylogenetic tree. The closest homolog to acid phosphatase of *A. fumigatus* was *Neosartorya fischeri* (Fig.-1).

Alkaline phosphatase

The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 10 FASTA sequences of similar homologs which were found in GenBank using NCBI FASTA tool. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA 6.05. All these protein sequences were of fungal origin. A bootstrap value of 500 was used. The alkaline phosphatase of *A. fumigatus* showed 98% identity to alkaline phosphatase sequences of *Neosartorya fumigata, Neosartorya fischeri* and *A. clavatus* species**.** Thus, based on the phylogenetic tree obtained this evolutionary relationship between alkaline phosphatase of various *Aspergillus species* could be inferred as a rooted phylogenetic tree. The closest homologs to alkaline phosphatase of *A. fumigatus* were *Neosartorya fumigata and Neosartorya fischeri.* The result of the phylogenetic tree is presented in figure-2.

Phytase

The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 10 FASTA sequences of similar homologs which were found in GenBank using NCBI FASTA tool. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA 6.05. All these protein sequences were of fungal origin. A bootstrap value of 500 was used. The phytase of *A. fumigatus* showed 98% identity to alkaline phosphatase

sequences of *Neosartorya fumigata, Neosartorya fischeri* and *A. clavatus* species. Thus, based on the phylogenetic tree obtained this evolutionary relationship between phytase of various *Aspergillus* species could be inferred as a rooted phylogenetic tree. The closest homologs to phytase of *A. fumigatus* were *Neosartorya fumigata and Neosartorya fischeri.* The result of the phylogenetic tree is presented in figure-3.

The results from FASTA search yielded similar protein sequence results for alkaline phosphatase, acid phosphatase and phytase sequences confirming that the cloning experiments was successful. Alkaline phosphatase of *Aspergillus fumigatus* had 97% homology with *A. terreus.* Acid phosphatase *Aspergillus fumigatus* had 98% homology with *A.clavutus.* Phytase of *Aspergillus fumigatus* had 99% homology with *A. oryzae.* The deduced amino acid sequences were translated insilico for homology modeling and phylogenetic analysis. Evolutionary analyses were conducted using MEGA 6.05 software. The evolutionary history was inferred using the Neighbor-Joining method with a bootstrap value of 500. On phylogenetic analysis, *Neosartorya fumigata* and *Neosartorya fischeri* were closely homologous to *A. fumigatus* alkaline phosphatase. Likewise, the closest homolog of A. fumigatus acid phosphatase was *Neosartorya fischeri* while that of phytase was *Neosartorya fumigata*. The phylogenetic tree also revealed the evolutionary relationship of the three enzymes that acid phosphatase was the common ancestor for alkaline phosphatase and phytase. Phylogenetic analysis was performed for the deduced amino acid sequences of acid phosphatase, phytase and alkaline phosphatase of *Aspergillus fumigatus.* The phylogenetic tree revealed that alkaline phosphatase and phytase formed the branches on the root of acid phosphatase thus inferring that acid phosphatase is the common ancestor for both acid phosphatase and phytase.

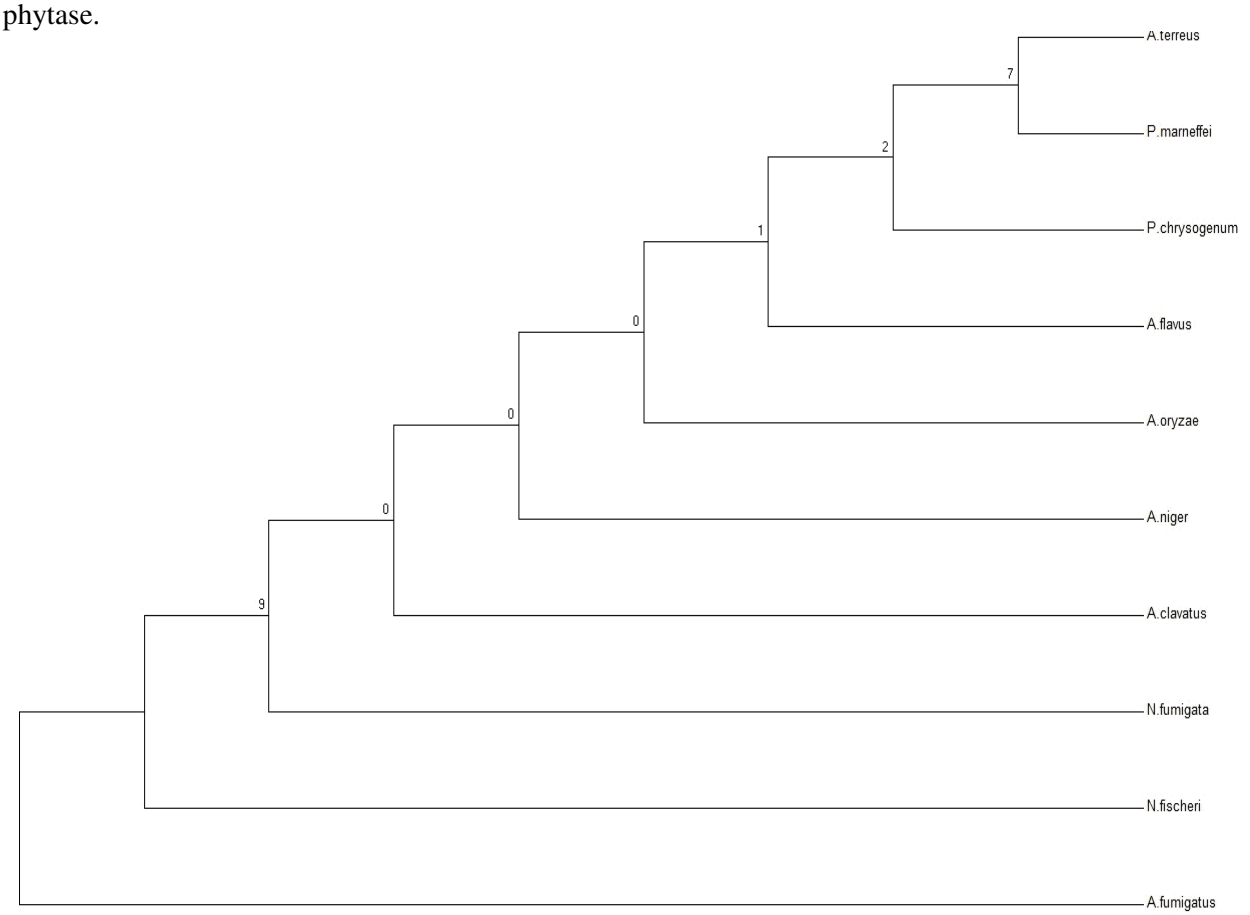

Fig.-1: Neighbor-joining phylogenetic tree based on similar homolog gene sequences showing the position of acid phosphatase gene of *A. fumigatus* and related strains

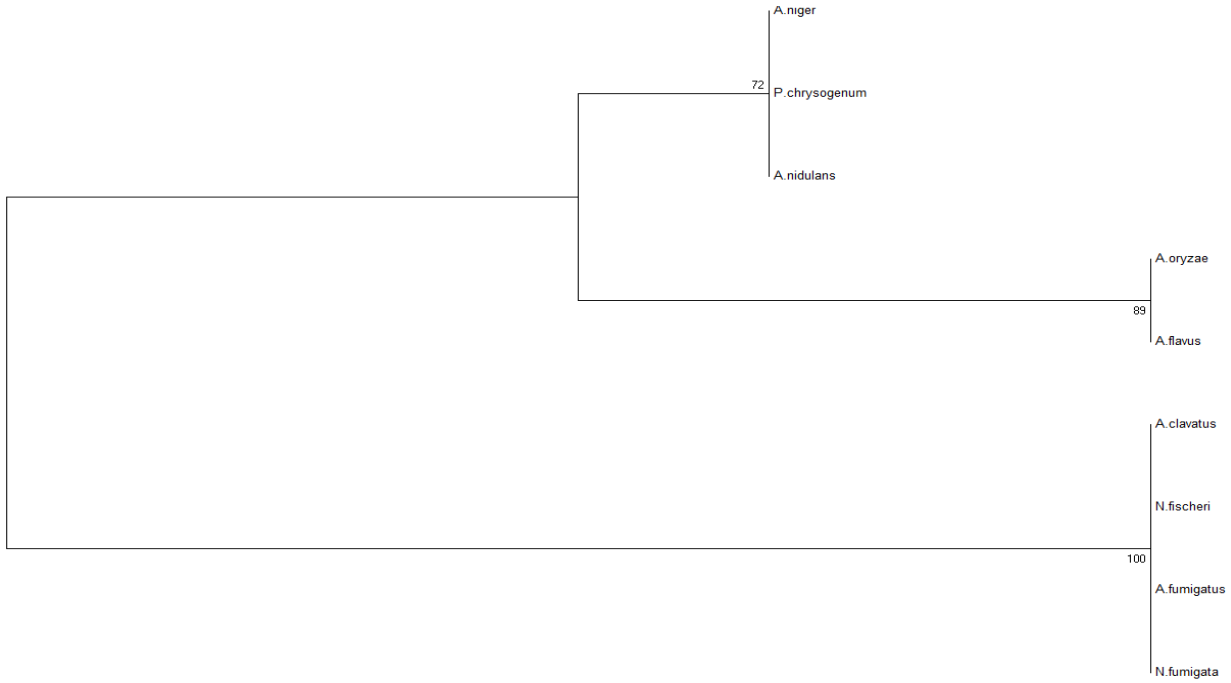

Fig.-2: Neighbor-joining phylogenetic tree based on similar homolog gene sequences showing the position of Alkaline phosphatase gene of *A. fumigatus* and related strains

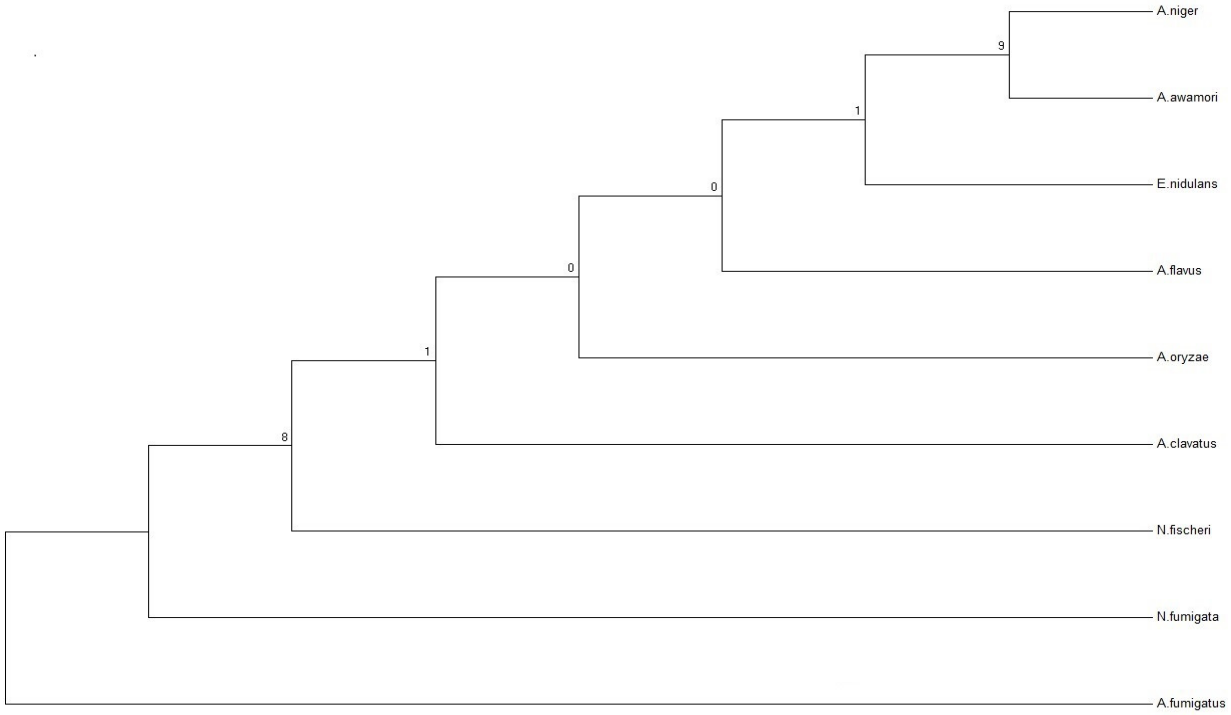

Fig.-3: Neighbor-joining phylogenetic tree based on similar homolog gene sequences showing the position of phytase gene of *A. fumigatus* and related strains

CONCLUSION

Homology modeling and phylogenetic analysis revealed high identify for acid phosphatase *pho*A gene, phytase *phy*A gene and alkaline phosphatase *pho*8 gene with other genes of the same *Aspergillus* species. The phylogenetic tree revealed that alkaline phosphatase and phytase formed the branches on the root of acid phosphatase thus inferring that acid phosphatase is the common ancestor for both acid phosphatase and phytase. Thus, based on the phylogram obtained this evolutionary relationship between acid phosphatase, phytase and alkaline phosphatase of *A. fumigatus* could be inferred as a rooted phylogenetic tree. Rooted phylogenetic tree obtained for the three deduced amino acid sequences thereby indicate the degree of similarity between proteins as an indication of distant amino acid sequence homology.

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