WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.11 (3&4): 235-241. 2013 *www.world-food.net*

Molecular characterization of glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene from *Aspergillus fumigatus*

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Received 6 June 2013, accepted 20 October 2013.

Abstract

First-strand cDNA was reverse transcribed from mRNA of *Aspergillus fumigatus* mycelium culture. The nucleotide sequence of *gapdh* was found to contain an ORF of 1083 bp, capable of coding for a protein of 360 amino acid residues. The signal peptide was predicted to be 19 amino acids in length. The alignment of sequence analysis of this fragment with the previously determined nucleotide sequence led to the definition of the gene (*gapdh* - cDNA, accession no. AB683056). Sequence analysis revealed the presence of a potential site for substrate binding (ASCTTNCV) at position 172-179. Amino acids potentially associated with catalysis were found at amino acid positions 174 (C) and 201 (H). Potential phosphorylation sites were located at positions 13-21, 204-212, 219-227, 272-280 and 339-346. The amino acid residues at positions 7 (D) and 311 (N) corresponded to the putative NAD^+ binding sites. Amino acids at positions 145 (S), 150 (T), 204 (T) and 205 (R) were found to be probable sites for inorganic phosphate binding. Positions 180 and 222 were found to be amino acid residues that putatively related to the binding of the phosphate from the substrate (T and R). The calculated molecular weight of deduced polypeptide is 38.7 kDa, and the estimated isoelectric point (pI) is 7.28. The deduced amino acid sequence revealed the most abundant amino acid was alanine followed by leucine, whereas the rare amino acids are methionine followed by tryptophan.

Key words: *Aspergillus fumigatus*, glyceraldehyde-3-phosphate dehydrogenase gene, signal peptide, putative sites.

Introduction

Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) is a key enzyme of glycolysis and gluconeogenesis [1, 2]. *Gapdh* has been implicated in several non-metabolic processes such as surface antigen [3], membrane transport and fusion, nuclear RNA transport [4-6], transcription activation, initiation of apoptosis [7] and cell surface protein to assist the adhesion of pathogen on host tissue [8, 9].

In filamentous fungi, the *gapdh* gene is generally present as a single copy [3, 10-15], but some species may contain two [11] or three [16] copies of the gene, not all of which are necessarily transcriptionally active [11, 16].

The *gapdh* gene is frequently very highly expressed, with GAPDH protein accounting for up to 5% of the total content of soluble cellular proteins in various eukaryotes [17], e.g. *Aspergillus nidulans* [2, 18], while in yeast, 2-5% of the poly (A^+) RNA is *gapdh* mRNA [19]. This elevated expression raises interesting practical questions about the regulation of this gene. The promoter sequences of native *gapdh*-encoding genes have proven useful for efficient expression of heterologous genes in several yeasts and fungi such as *Pichia pastoris* [20, 21], *Mucor circinelloides* [16], *Lentinus edodes* [22] and *Aspergillus niger* [23].

Aspergillus fumigatus is one of the most ubiquitous of the airborne saprophytic fungi. Humans and animals constantly inhale numerous conidia of this fungus. The conidia are normally eliminated in the immunocompetent host by innate immune mechanisms, and aspergilloma and allergic bronchopulmonary aspergillosis, uncommon clinical syndromes, are the only infections observed in such hosts. Thus, *A. fumigatus* was considered for years to be a weak pathogen. With increases in the number of immunosuppressed patients, however, there has been a dramatic increase in severe and usually fatal invasive aspergillosis, now the most common mold infection worldwide [24].

The aim of this study was to characterize *Aspergillus fumigatus* glyceraldehydes-3-phosphate dehydrogenase gene (*gapdh* – cDNA), also the alignment of cDNA and the deduced amino acid sequence with previously published gene database.

Materials and Methods

Bacterial and fungal strains and plasmid: For standard bacterial cloning, *Escherichia coli* DH5α [25] was grown in Luria–Bertani (LB) medium (Sigma) supplemented with 10 μg ml^{-1} of ampicillin. *Aspergillus fumigatus* was maintained by periodic transfer at 4°C on mineral medium agar plates. The pGEMs-T Easy Vector system I (Promega, Madison) was used in subcloning of *gapdh*-cDNA.

Cultivation of Aspergillus fumigatus: Cultivation was carried out in 250 ml Erlenmeyer flasks each containing 100 ml of mineral broth medium. Inoculum was prepared by harvesting spores from 7-day-old PDA culture of *Aspergillus fumigatus* in sterilized spore buffer (per 100 ml: NaCl, 0.9 g, Tween-80, 1ml). The concentrations of spore suspensions were determined in a hemacytometer and adjusted to $2x10^6$ spores/ml. Each flask was inoculated with 1ml spore suspension. The flasks were incubated for 2 days at 28±2°C

in a shaking incubator (180 rpm). Mycelium was harvested by filtration and kept at -40°C until used.

Isolation of genomic DNA: DNA was isolated by using the mixer mill isolation protocol. The mycelia were ground in liquid N_2 and suspended in a DNA isolation buffer [50 mM Tris-HCl (pH 7.9), 250 mM NaCl, 10 mM EDTA (pH 8.0) and 0.5% sodium dodecyl sulfate (SDS) 0.5%] with a metal ball. The tubes were placed in a mixer mill (4 min at 15 Hz, followed by 20 s at 20 Hz). The tubes were then centrifuged at 1050*g* for 15 min at room temperature (RT), and the supernatant was transferred to a new Eppendorf tube. Then, 10 µl RNAse (10 mg ml^{-1}) was added and incubated for 10 min at 65°C and for 30 min at 37°C, followed by the addition of 600 µl of PCI and centrifugation at 1050*g* for 10 min at RT. The supernatant was transferred to a new Eppendorf tube and *c*. 250 µl chloroform was added and centrifuged at 1050*g* for 10 min at RT. The supernatant was transferred to a new Eppendorf tube and *c*. 600 µl isopropanol was added and incubated for 30 min at RT, followed by centrifugation at 1050*g* for 10 min at 4°C. The pellet was washed by using 70% EtOH (*c*. 100 µl), and centrifuged at 1050*g* for 5 min at 4°C. The pellet was dissolved in 100 µl water and heated at 60°C for 20 min for complete dissolution. A final centrifugation at 1050*g* for 5 min at 4°C was performed. About 90 µl was transferred to a new Eppendorf tube.

Isolation of total RNA and PCR for gapdh: RNA was isolated using RNA isolation solution (Omega Bio-Tek Inc.). The cDNA sequence of *gapdh* was analyzed by reverse-transcribed PCR from total RNA using Superscript (Invitrogen). DNA and cDNA were used for PCR amplification of the *gapdh* gene in 50-µl reaction volumes containing 1µl template, 4 µl dNTPs, 5 µl buffer 3 (containing $MgCl_2$), 2 µl of each primer and 1µl expand high fidelity PCR enzyme (Roche Applied Science). The primers were synthesized at Microsynth Co. (Switzerland). The forward primer was Glyceraldehyde 1F 5´-ATTGAATTCAT GGCTCCCT CCATT AACG-3t and reverse primer was Glyceraldehyde 1R 5´-ATT GAATTC CTAT GCTCCCAGTTCTTGTGAC-3´ to create an *EcoRI* sits (underlined) in the start codon and after the stop codon, respectively.

cDNA cloning and transformation: The PCR product was eluted from the gel using the Micro- EluteTM gel extraction kit (Omega Bio-Tek Inc.) and digested using *EcoR* I restriction enzyme. The pGEM-T easy vector (Promega) was digested using *EcoR* I. The digested PCR product was ligated to the vector using a ligation kit (BioLabs, UK). The cloned gene was transformed inside *E. coli* DH5α cells (Stratagene) following standard procedures [26]. In a precooled 15-ml tube, 200 or 400 µl of *E. coli* cells were transferred, followed by the ligation reaction, and mixed gently. The mixture was incubated for 20–30 min on ice, heat shocked for 90 s at 42°C and cooled for 2 min on ice. The mixture was made up to 1 ml with LB and different volumes of the cells were plated on LB containing ampicillin, and incubated at 37°C overnight.

Miniprep plasmid isolation: The isolated colonies indicating positive transformation were selected and transferred to tubes containing ml LB medium with ampicillin. They were incubated overnight at 37°C and shaken. The cultures were centrifuged in Eppendorf tubes at 2150*g* at 4°C for 5 min, and the pellet was suspended in 300 µl of buffer A (5 mM Tris-HCl, 10 mM EDTA and 400 mg ml^{-1} RNAse A). Then 300 µl of buffer B was added (0.2M NaOH, 1% SDS), mixed by inverting and kept at RT for 5 min, and 300 µl of buffer C was added (2.55 M potassium acetate, pH 4.8) and kept on ice for 5 min. The mixture was centrifuged at 1050*g* for 10 min at 4°C. The supernatant was transferred to a new Eppendorf tube and 600 µl isopropanol was added, incubated at RT for 15–20 min. The mixture was centrifuged at 1050*g* for 10 min at 4°C. The pellet was washed by using 70 µl of 70% ethanol, dried and resuspended in 20 µl water.

Sequencing of the gapdh gene: Nucleotide sequences were determined using the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) on ABI automated sequencers (ABI 3100). The genomic nucleotide sequence for the *gapdh* gene is available on the GenBank database with accession no. AB683056 assigned to the cDNA. Nucleotide and amino-acid sequence similarity searches used the BLAST method [27] from the National Center for Biotechnology Information databases.

Results

First-strand cDNA was reverse transcribed from mRNA of *Aspergillus fumigatus* mycelium culture. An amplification experiment was performed using the designed primer with *EcoRI* as restriction site. The fragment which counted for the *gapdh* cDNA, was cloned using pGEM-T easy vector. The nucleotide sequence of *gapdh* was found to contain an ORF of 1083 bp (Fig. 1), capable of coding for a protein of 360 amino acid residues. A typical translation initiation codon (ATG) and translation termination codon (TAG), the most frequently found codon in filamentous fungi, were identified in the *gapdh* cDNA indicating a full length coding sequence of the gene. Using the programme SIGNALP 3.0 (http://www.cbs.dtu.dk/ services/SignalP/), the signal peptide was predicted to be 19 amino acids in length (Fig. 2). The alignment of sequence analysis of this fragment with the previously determined nucleotide sequence led to the definition of the gene (*gapdh* - cDNA, accession no. AB683056). *A. fumigatus gapdh*-cDNA gene shared significant homology with *gapdh* of *Aspergillus flavus* NRRL3357 (XM002383978) and *Aspergillus oryzae* RIB40 (AP007167) with 74%, while *Neosartorya fischeri* NRRL181 (XM001261902) 94% (Table 1).

Sequence analysis revealed the presence of a potential site for substrate binding (ASCTTNCV) at position 172-179. Amino acids potentially associated with catalysis were found at amino acid positions 174 (C) and 201 (H). Potential phosphorylation sites were located at positions 13-21, 204-212, 219-227, 272-280 and 339-346 (Fig. 3). The amino acid residues at positions 7 (D) and 311 (N) corresponded to the putative NAD^+ binding sites. Amino acids at positions 145 (S), 150 (T), 204 (T) and 205 (R) were found to be probable sites for inorganic phosphate binding. Positions 180 and 222 were found to be amino acid residues that putatively related to the binding of the phosphate from the substrate (T and R) (Fig. 3). The isolated gene codes for a protein of 360 amino acids. The encoded amino acid sequence is reported in Fig. 3. The calculated molecular mass of deduced polypeptide is 38.7 kDa (Table 2), and the estimated isoelectric point (pI) is 7.28.

The multiple alignment of deduced amino acids sequence of *A. fumigatus* GAPDH shared significant homology with GAPDH *A. flavus* NRRL3357 (XM002383978), *A. oryzae* RIB40 (AP007167),

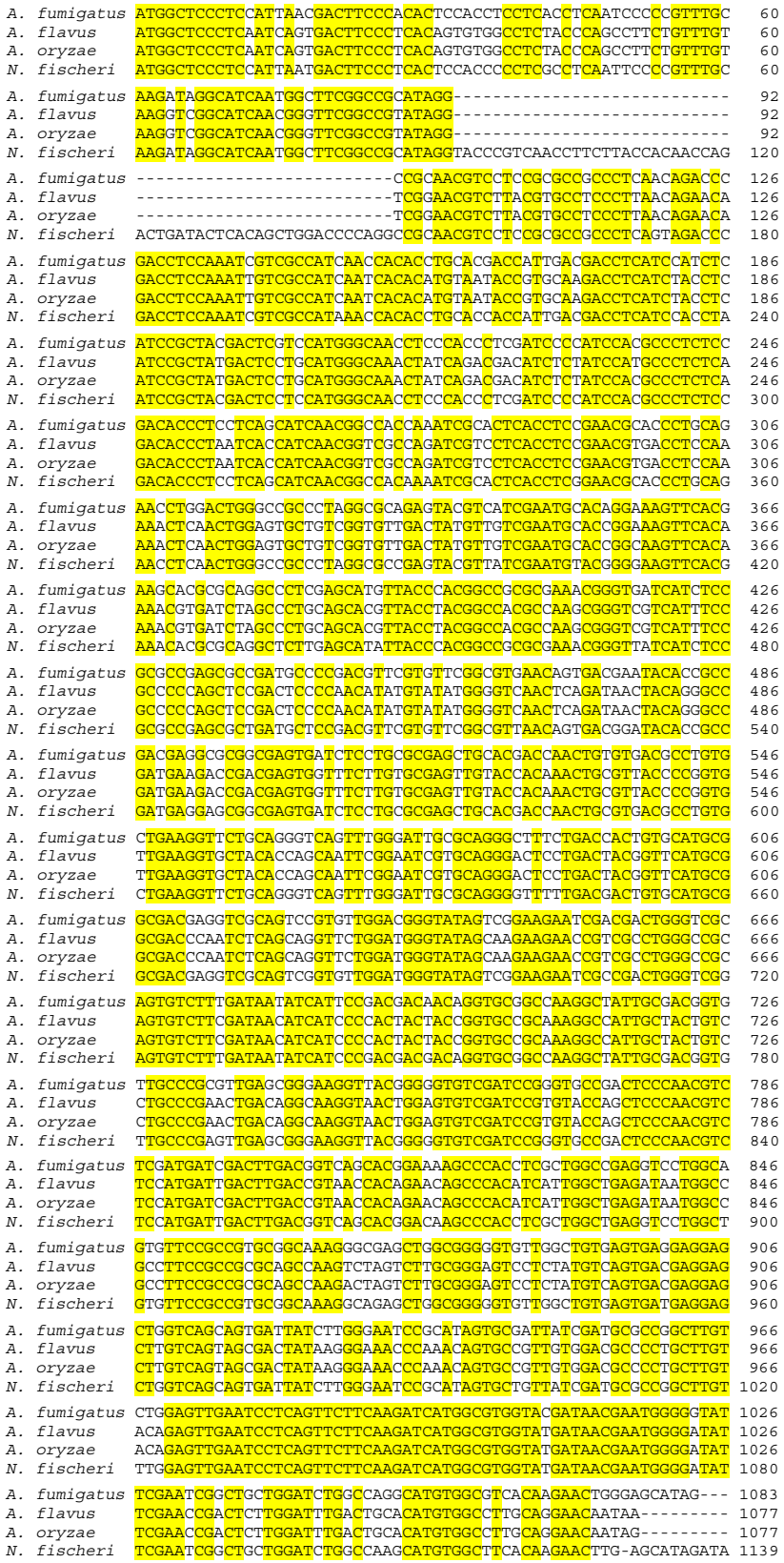


Figure 1. Multiple sequence alignment of nucleotide sequences of *Aspergillus fumigatus* glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh*) sequences (AB683056). The *gapdh* sequences of the *Aspergillus flavus* NRRL3357 (XM002383978), *Aspergillus oryzae* RIB40 (AP007167), *Neosartorya fischeri* NRRL181 (XM001261902) were downloaded from the GenBank with accession numbers and additional resource information. Alignment was done with ClustalW 2.0 software 33.

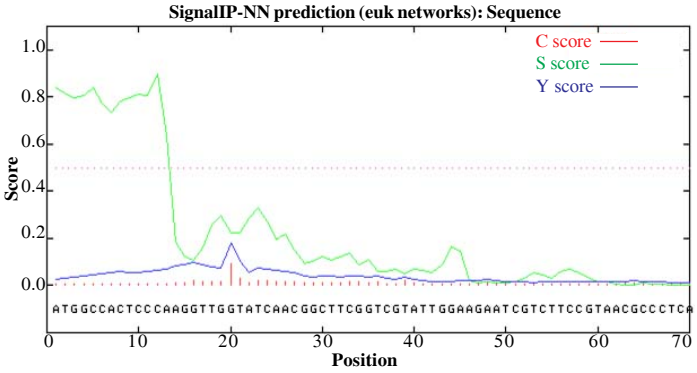
Neosartorya fischeri NRRL181 (XM001261902) shared high homology with GAPDH in Databank (Fig. 4, Table 3). The deduced amino acid sequence revealed the most abundant amino acid was alanine followed by leucine, whereas the rare amino acid was methionine followed by tryptophan (Table 2).

Discussion

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) is one of the key enzymes in glycolysis and gluconeogenesis. In the former pathway, it catalyses the oxidative phosphorylation of glyceraldehyde-3-phosphate into 1,3-biphosphoglycerate in the presence of nicotinamide adenine dinucleotide and inorganic phosphate, and in the latter pathway, it catalyses the reverse reaction [1, 2].

First-strand cDNA was reverse transcribed from mRNA of *Aspergillus fumigatus* mycelium culture. The nucleotide sequence of *gapdh* was found to contain an ORF of 1083 bp, capable of coding for a protein of 360 amino acid residues. The signal peptide was predicted to be 19 amino acids in length. The alignment of sequence analysis of this fragment with the previously determined nucleotide sequence led to the definition of the gene (*gapdh* -cDNA, accession no. AB683056). The calculated molecular weight of deduced polypeptide is 38.7 kDa, and the estimated isoelectric point (pI) is 7.28. The deduced amino acid sequence revealed the most abundant amino acid was alanine followed by leucine, whereas the rare amino acids is methionine followed by tryptophan.

It is well known in a wide variety of organisms that this circumstance favours the synthesis of a specific set of proteins, known as heat shock proteins (HSPs) [28]. Among them, a protein with the apparent molecular mass of 36 kDa was chosen and analyzed. N-terminal amino acid sequences analysis revealed that this protein is GAPDH which has been previously reported as a heat shock protein in *Saccharomyces cerevisiae*[29]. Gene encoding this enzyme has been cloned and characterized in several filamentous fungi such as *Cryphonectria parasitica* [30], *L. edodes* [2], *M. circinelloides* [16], *Phaeosphaeria nodorum* [31], *Pleurotus sajorcaju* [32], *Rhizomucor miehei* [13], *Ganoderma lucidum* [33], *Penicillium marneffei* [34], *Moniliophthora perniciosa* [35] and *Trichoderma virens*[36]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a classic glycolytic enzyme that is active as a tetramer of identical 37kDa subunits catalyzing the oxidative phosphorylation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate by converting NAD+ to NADH. More recently, GAPDH emerged as a multifunctional protein with defined functions in



Measure	Position	Value	Cutoff	Signal peptide
Max. C	20	0.096	0.32	NO
Max. Y	20	0.181	0.33	NO
Max. S	12	0.900	0.87	YES
Mean S	1-19	0.603	0.48	YES
D	1-19	0.392	0.43	NO

Figure 2. Signal P-NN result of glyceraldehyde-3-phosphate dehydrogenase cDNA (*gapdh*). The most likely cleavage site between pos. 19 and 20: TTG-GT, with sequence length = 70.

Table 1. Results of CLUSTAL 2.1 multiple sequence alignments of glyceraldehyde-3-phosphate dehydrogenase cDNA (*gapdh*).

Name	*A. fumigatus*	*A. flavus*	*A. oryzae*	*N. fischeri*
A. flavus	74			
A. oryzae	74	99		
N. fischeri	94	74	74	

Table 2. Amino acid content of the predicted amino acid sequence of glyceraldehyde-3-phosphate dehydrogenase deduced amino acid sequence (GAPDH).

Residue			Number Found	Residue			Number Found
A	Ala	Alanine	39	K	Lys	Lysine	11
R	Arg	Arginine	20	M	Met	Methionine	4
N	Asn	Asparagine	17	F	Phe	Phenylalanine	11
D	Asp	Aspartate	17	P	Pro	Proline	19
Q	Gln	Glutamine	11	S	Ser	Serine	31
E	Glu	Glutamate	15	T	Thr	Threonine	27
G	Gly	Glycine	23	Y	Tyr	Tyrosine	7
H	His	Histidine	11	V	Val	Valine	27
I	Ile	Isoleucine	26	W	Trp	Tryptophan	3
L	Leu	Leucine	34	C	Cys	Cysteine	7

```
ATGGCTCCCTCCATTAACGACTTCCCACACTCCACCTCCTCACCTCAATCCCCCGTTTGC   60
M  A  P  S  I  N  D  F  P  H  S  T  S  S  P  Q  S  P  V  C
AAGATAGGCATCAATGGCTTCGGCCGCATAGGCCGCAACGTCCTCCGCGCCGCCCTCAAC  120
K  I  G  I  N  G  F  G  R  I  G  R  N  V  L  R  A  A  L  N
AGACCCGACCTCCAAATCGTCGCCATCAACCACACCTGCACGACCATTGACGACCTCATC  180
R  P  D  L  Q  I  V  A  I  N  H  T  C  T  T  I  D  D  L  I
CATCTCATCCGCTACGACTCGTCCATGGGCAACCTCCCACCCTCGATCCCCATCCACGCC  240
H  L  I  R  Y  D  S  S  M  G  N  L  P  P  S  I  P  I  H  A
CTCTCCGACACCCTCCTCAGCATCAACGGCCACCAAATCGCACTCACCTCCGAACGCACC  300
L  S  D  T  L  L  S  I  N  G  H  Q  I  A  L  T  S  E  R  T
CTGCAGAACCTGGACTGGGCCGCCCTAGGCGCAGAGTACGTCATCGAATGCACAGGAAAG  360
L  Q  N  L  D  W  A  A  L  G  A  E  Y  V  I  E  C  T  G  K
TTCACGAAGCACGCGCAGGCCCTCGAGCATGTTACCCACGGCCGCGCGAAACGGGTGATC  420
F  T  K  H  A  Q  A  L  E  H  V  T  H  G  R  A  K  R  V  I
ATCTCCGCGCCGAGCGCCGATGCCCCGACGTTCGTGTTCGGCGTGAACAGTGACGAATAC  480
I  S  A  P  S  A  D  A  P  T  F  V  F  G  V  N  S  D  E  Y
ACCGCCGACGAGGCGCGGCGAGTGATCTCCTGCGCGAGCTGCACGACCAACTGTGTGACG  540
T  A  D  E  A  R  R  V  I  S  C (A  S  C  T  T  N  C  V) T
CCTGTGCTGAAGGTTCTGCAGGGTCAGTTTGGGATTGCGCAGGGCTTTCTGACCACTGTG  600
P  V  L  K  V  L  Q  G  Q  F  G  I  A  Q  G  F  L  T  T  V
CATGCGGCGACGAGGTCGCAGTCCGTGTTGGACGGGTATAGTCGGAAGAATCGACGACTG  660
H  A  A  T  R  S  Q  S  V  L  D  G  Y  S  R  K  N  R  R  L
GGTCGCAGTGTCTTTGATAATATCATTCCGACGACAACAGGTGCGGCCAAGGCTATTGCG  720
G  R  S  V  F  D  N  I  I  P  T  T  T  G  A  A  K  A  I  A
ACGGTGTTGCCCGCGTTGAGCGGGAAGGTTACGGGGGTGTCGATCCGGGTGCCGACTCCC  780
T  V  L  P  A  L  S  G  K  V  T  G  V  S  I  R  V  P  T  P
AACGTCTCGATGATCGACTTGACGGTCAGCACGGAAAAGCCCACCTCGCTGGCCGAGGTC  840
N  V  S  M  I  D  L  T  V  S  T  E  K  P  T  S  L  A  E  V
CTGGCAGTGTTCCGCCGTGCGGCAAAGGGCGAGCTGGCGGGGGTGTTGGCTGTGAGTGAG  900
L  A  V  F  R  R  A  A  K  G  E  L  A  G  V  L  A  V  S  E
GAGGAGCTGGTCAGCAGTGATTATCTTGGGAATCCGCATAGTGCGATTATCGATGCGCCG  960
E  E  L  V  S  S  D  Y  L  G  N  P  H  S  A  I  I  D  A  P
GCTTGTCTGGAGTTGAATCCTCAGTTCTTCAAGATCATGGCGTGGTACGATAACGAATGG 1020
A  C  L  E  L  N  P  Q  F  F  K  I  M  A  W  Y  D  N  E  W
GGGTATTCGAATCGGCTGCTGGATCTGGCCAGGCATGTGGCGTCACAAGAACTGGGAGCA 1080
G  Y  S  N  R  L  L  D  L  A  R  H  V  A  S  Q  E  L  G  A
TAG 1083
*
```

Figure 3. Nucleotide and deduced amino acid sequences of *Aspergillus fumigatus* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Nucleotides are numbered from the first nucleotide from 5A˜ end of the sequence. Amino acids are indicated below the nucleotide sequence in single-letter codes. Translation stop codon is indicated by an asterisk. The sites putatively related to inorganic phosphate binding are indicated by boxes around the amino acids. Amino acids potentially associated with catalysis are in shaded boxes. Amino acid residues related to the binding of phosphate from substrate are in bold and underlined. Amino acid putatively related to the NAD+ binding is in bold italic. The substrate-binding site is marked with a bracket. The putative phosphorylation sites are indicated by underlines.

(A)

```
A. fumigatus  MAPSINDFPHSTSSPQSPVCKIGINGFGRIG-----------------RNVLRAALNRP 42
A. flavus     MAPSISDFPHSVASTQPSVCKVGINGFGRIG-----------------RNVLRASLNRT 42
A. oryzae     MAPSISDFPHSVASTQPSVCKVGINGFGRIG-----------------RNVLRASLNRT 42
N. fischeri   MAPSINDFPHSTPSPQFPVCKIGINGFGRIGTRQPSYHNQTDTHSWTPGRNVLRAALSRP 60
              *****.*****..*.* .***:*********                  ******:*.*.

A. fumigatus  DLQIVAINHTCTTIDDLIHLIRYDSSMGNLPPSIPIHALSDTLLSINGHQIALTSERTLQ 102
A. flavus     DLQIVAINHTCNTVQDLIYLIRYDSCMGKLSDDISIHALSDTLITINGRQIVLTSERDLQ 102
A. oryzae     DLQIVAINHTCNTVQDLIYLIRYDSCMGKLSDDISIHALSDTLITINGRQIVLTSERDLQ 102
N. fischeri   DLQIVAINHTCTTIDDLIHLIRYDSSMGNLPPSIPIHALSDTLLSINGHKIALTSERTLQ 120
              ***********.*::***:******.**:*. .*.********::***::*.***** **

A. fumigatus  NLDWAALGAEYVIECTGKFTKHAQALEHVTHGRAKRVIISAPSADAPTFVFGVNSDEYTA 162
A. flavus     KLNWSAVGVDYVVECTGKFTKRDLALQHVTYGHAKRVVISAPSSDSPTYVYGVNSDNYRA 162
A. oryzae     KLNWSAVGVDYVVECTGKFTKRDLALQHVTYGHAKRVVISAPSSDSPTYVYGVNSDNYRA 162
N. fischeri   NLNWAALGAEYVIECTGKFTKHAQALEHITHGRAKRVIISAPSADAPTFVFGVNSDGYTA 180
              :*:*:*:*.:**:********:  **:*:*:*:****:*****:*:**:*:***** * *

A. fumigatus  DEARRVISCASCTTNCVTPVLKVLQGQFGIAQGFLTTVHAATRSQSVLDGYSRKNRRLGR 222
A. flavus     DEDRRVVSCASCTTNCVTPVLKVLHQQFGIVQGLLTTVHAATQSQQVLDGYSKKNRRLGR 222
A. oryzae     DEDRRVVSCASCTTNCVTPVLKVLHQQFGIVQGLLTTVHAATQSQQVLDGYSKKNRRLGR 222
N. fischeri   DEERRVISCASCTTNCVTPVLKVLQGQFGIAQGFLTTVHAATRSQSVLDGYSRKNRRLGR 240
              ** ***:****************: ****.**:********:**.******:*******

A. fumigatus  SVFDNIIPTTTGAAKAIATVLPALSGKVTGVSIRVPTPNVSMIDLTVSTEKPTSLAEVLA 282
A. flavus     SVFDNIIPTTTGAAKAIATVLPELTGKVTGVSIRVPAPNVSMIDLTVTTEQPTSLAEIMA 282
A. oryzae     SVFDNIIPTTTGAAKAIATVLPELTGKVTGVSIRVPAPNVSMIDLTVTTEQPTSLAEIMA 282
N. fischeri   SVFDNIIPTTTGAAKAIATVLPELSGKVTGVSIRVPTPNVSMIDLTVSTDKPTSLAEVLA 300
              ********************** *:***********:**********:*::******::*

A. fumigatus  VFRRAAKGELAGVLAVSEEELVSSDYLGNPHSAIIDAPACLELNPQFFKIMAWYDNEWGY 342
A. flavus     AFRRAAKSSLAGVLYVSDEELVSSDYKGNPNSAVVDAPACTELNPQFFKIMAWYDNEWGY 342
A. oryzae     AFRRAAKTSLAGVLYVSDEELVSSDYKGNPNSAVVDAPACTELNPQFFKIMAWYDNEWGY 342
N. fischeri   VFRRAAKAELAGVLAVSDEELVSSDYLGNPHSAVIDAPACLELNPQFFKIMAWYDNEWGY 360
              .****** .***** **:******** ***:**::***** ******************

A. oryzae     SNRLLDLTAHVALQEQ--- 358
A. flavus     SNRLLDLTAHVALQEQ--- 358
A. fumigatus  SNRLLDLARHVASQELGA- 360
N. fischeri   SNRLLDLAKHVASQELEHR 379
              *******: *** **
```

(B)

```
A. fumigatus  ASCTTNCV
A. flavus     ASCTTNCV
A. oryzae     ASCTTNCV
N. fischeri   ASCTTNCV
```

Figure 4. Multiple sequence alignment of predicted amino acid sequence of *Aspergillus fumigatus* glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (A) The GAPDH sequences of the *Aspergillus flavus* NRRL3357 (XM002383978)**,** *Aspergillus oryzae* RIB40 (AP007167)**,** *Neosartorya fischeri* NRRL181 (XM001261902) were downloaded from from the GenBank with accession numbers and additional resource information. (B) Potential site for substrate binding. Alignment was done with ClustalW 2.0 software (http://www.ebi.ac.uk/Tools/clustalw2/ index.html).

Table 3. Results of CLUSTAL 2.1 multiple sequence alignments of glyceraldehyde-3-phosphate dehydrogenase deduced amino acids sequence (GAPDH).

Name	*A. fumigatus*	*A. flavus*	*A. oryzae*	*N. fischeri*
A. flavus	79			
A. oryzae	79	99		
N. fischeri	95	79	79	

numerous subcellular processes, namely a primary role in apoptosis and in a variety of critical nuclear pathways [6, 37]. The glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene was cloned from the violet root rot fungus, *Helicobasidium mompa*, and characterized. The *H. mompa gpd* gene was found to encode a protein of 335 amino acids and its putative protein [38]. The glyceraldehyde-3-phosphate dehydrogenase gene (GPD) of the sophorolipid producing yeast *Candida bombicola* was isolated using degenerated PCR and genome walking. The obtained 3,740 bp contain the 1,008 bases of the coding sequence. The corresponding protein shows high homology to the other known *GPD* genes [39].

The ATG at 10 bp from the start of the cDNA is likely to encode the initial methionine residue, based on homology to other *gpd* sequences; the sequence surrounding this codon, GCCATCATGTC, is quite consistent with the consensus for translation start sites in *Neurospora crassa* [40]. Most of the 5' untranslated region appears to be missing from the cDNA clone, based on the size of the mRNA (1.8 kb). The molecular mass calculated from the sequence is 36 kDa [41].

Sequence analysis revealed the presence of a potential site for substrate binding (ASCTTNCV) at position 172-179. Amino acids potentially associated with catalysis were found at amino acid positions 174 (C) and 201 (H). Potential phosphorylation sites were located at positions 13-21, 204-212, 219-227, 272-280 and 339-346. The amino acid residues at positions 7 (D) and 311 (N) corresponded to the putative NAD^+ binding sites. Amino acids at positions 145 (S), 150 (T), 204 (T) and 205 (R) were found to be probable sites for inorganic phosphate binding. Positions 180 and 222 were found to be amino acid residues that putatively related to the binding of the phosphate from the substrate (T and R). As reported in *Rhizomucor miehei* (Cys150) and *Cryptococcus neoformans* (Cys150) [13, 42]. In *Eremothecium ashbyi*, His176 hydrogen bonds to Cys149 in the NAD^+ binding site and Lys183 is involved in phosphate binding to NAD [43]. In the NAD^+ binding domain of *E. ashbyi*, the residues Phe35 and Phe100 interact with the adenine ring, Asp33 with the adenosine ribose, Gly10, Arg11 and Ile12 with the NAD^+ phosphate and the Gly98 and Ala121 with the nicotinamide. Thus, the catalytic amino acids in the GPD peptide sequences appear to be conserved throughout evolution in the same position in all the organisms from different phyla [43].

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