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Sequence analyses of genes coding key enzymes responsible for degradation of phenolic compounds by *Aspergillus fumigatus* strain AL3

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ABSTRACT

Two genes coding proteins with phenol hydroxylase and catechol 1,2-dioxygenase enzyme activities which take part in the degradation pathway of phenolic compounds in Antarctic fungal strain *A. fumigatus* AL 3 have been identified. The primers applied in the PCR analyses for both genes were designed on the basis of the identical genes sequences of *A. fumigatus* strain Af293. For the detection of phenol hydroxylase and catechol 1,2-dioxygenase genes were created and used two sets each consisted of 3 pairs of primers. The obtained oligonucleotide DNA fragments were sequenced by Sanger method. The overlapped three fragments for each one of the investigated genes were combined. The sequence obtained for phenol hydroxylase gene (1398 bp without introns) was organized into 3 exons and 2 introns. The length of the partial catechol 1,2-dioxygenase gene count without introns was 642 bp. It included 4 exons and 3 introns. The basic logical alignment search confirmed that the phenol hydroxylase gene had 99% nucleotide identity with the corresponding gene of *A. fumigatus* Af293. The same percent of identity of the catechol 1,2-dioxygenase genes of *A. fumigatus* Af293 and *A. fumigatus* AL 3 was established. The translation of the obtained DNA sequences revealed a protein with phenol hydroxylase activity consisting of 465 amino acids as well as a protein with catechol 1,2–dioxigenase activity consisting of 213 amino acids. The proximity of the studied proteins with closely related enzymes with similar functions were demonstrated on the created distant tree's cladograms.

Key words: *Aspergillus fumigatus*, DNA-sequence, phenol hydroxylase, catechol 1,2-dioxygenase

Introduction

Eukaryotic organisms from kingdom of Fungi are already well-known for their ability to remove and to detoxify numerous industrial waste compounds. These abilities are maintained in the greatest extent of their diverse enzyme profiles (Atagana, 1999; Mendonça, 2004; Santos & Linardi, 2004; Leatham, 1983; Aleksieva, 2002; Yemendzhiev, 2008). The phenol degradation by fungi proceeds via *ortho*-cleavage pathway (Varga & Neujahr, 1970). In this route the benzene ring of phenol is initially hydroxylated by phenol hydroxylase to form catechol. Catechol is oxidized by catechol 1,2-dioxygenase to cis-cis muconic acid.

There are many reports for different species of genus *Aspergillus* which are able to digest aromatic hydrocarbons such as phenol and phenolic derivatives (Santos & Linardi, 2003; Atagana, 2004; Basha, 2010; Stoilova 2006; Ghanem, 2009; Sharma & Gupta, 2012; Hanafi, 2013).

In our previous study we have reported that strains of *Aspergillus fumigatus*, including *A. fumigatus* strain AL 3 are capable to assimilate completely 0.5 g/l phenol as a sole carbon source within less than two weeks (Gerginova, 2013a). In other experiments we have established that *A.*

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fumigatus AL 3 degraded as well, two phenol derivatives – catechol and *o*-cresol. The values of phenol hydroxylase and catechol-1,2-dioxygenase enzyme activity were determined spectrophotometrically (Gerginova, 2013b)

With the progress in molecular techniques increases the knowledge of microbial communities involved in the degradation of toxic environmental pollutants (El-Sayed *et al.*, 2003). PCR techniques and analysis of DNA sequences are used successfully in the study of genes involved in the catabolism of the phenol and its derivatives (Okuta *et al*., 1998; Kahng & Oh, 2005). More than 300 catabolic genes involved in the catabolism of aromatic compounds by cultivable microorganisms are cloned and characterized. These approaches give new perspectives on the processes of bioremediation (Futamata *et al.*, 2001; Jorgensen, 2008; Ai *et al.*, 2008).

The genes coding proteins with phenol hydroxylase and catechol 1,2-dioxygenase enzyme activities were identified and sequenized in many microorganisms, including fungi, which are capable to degrade aromatic compounds (Nierman *et al*. 2005, ; Nurk et *al*., 1991).

Two main tools are used in the analysis of new sequences. The first one is a search of similar sequences in the internet data base with BLAST (Basic Local Alignment Search Tool) analysis (Altschul *et al*., 1990; Casey, 2005). BLAST is one of the widely used computing programs used in the bioinformatics. It is an algorithm for comparing of primary biological sequence information, as for example amino acids sequences of different nucleotides from the investigated DNA or proteins. The BLAST searching gives the opportunities for comparing the researched sequence with a database of sequences and for identification of those which resemble the wanted sequence above a certain threshold (Chao & Zhang 2008; Boratyn et *al*., 2013). In that way the sequences most similar to the studied one are checked.

Clustal W platform can be used when the similarity between two specific sequences should be investigated. This is a program for selection and alignment of divergent nucleic acids or protein sequences with the significant degree of matching (Chenna *et al*., 2003; Wallace *et al*., 2005).

In the current study we have identified the presence of genes coding proteins with phenol hydroxylase and catechol 1,2-dioxygenase activities in the cells of *A. fumigatus* AL 3 strain. The sequences obtained for both genes were studies by the BLAST analysis. The translation of the obtained DNA sequences revealed proteins with closely related enzymes with similar functions in fungi.

Materials and Methods

Microorganisms and cultivation conditions

Earlier, a multitude of filamentous fungi had been isolated from soil samples collected on Livingston Island, South Shetland Archipelago, Antarctica (Kostadinova *et al*., 2009). Taxonomic identification of the isolates was performed based on morpho-dimensional parameters following the available identification keys for the different genera (Tosi *et al*., 2002).

In this study we used one of these isolated Antarctic strains - *Aspergillus fumigatus* AL3 (NCBI Acc. N KT781127).

Universal beer agar BA (Fluka) contained per liter deionized water: 12g/L agar, 16.1g/L dextrose, 0.31g/L dipotassium phosphate, 0.006g/L ferrous sulfate, 0.12g/L magnesium sulfate was used to store the strain.

Yeast extract peptone dextrose YEPD, complete medium for yeast growth contained per liter deionized water: 10 g yeast extract (Difco), 20 g peptone (Difco), 10 g Dextrose (glucose) was used for the strain cultivation.

The media were autoclaved for 20 min at 110 °C. The broths and solid media were with same content. In the solid media the quantity of agar-agar was 1.5%.

The main parameters of cultivation were: temperature – 23°C, aeration on laboratory Basic orbital shaker (IKA KS 130, Sigma) at 400 rpm in Erlenmeyer flasks with suitable volume of nutrient media.

Genomic DNA isolation

The obtained biomass of the strain *A. fumigatus* AL3 was filtered through a Büchner funnel, washed twice with distilled water and then centrifuged at 1500 x g for 15 min. The A. *fumigatus* AL3 cells were disrupted by the three cycle's freeze-thaw procedure including liquid nitrogen treating and heating to 90° C (Manasiev et al., 2008). The pellet was put in a laboratory mortar. The cell destruction continues mechanically by grind with quartz sand and 2 ml 1xTE buffer (10 mM Tris/HCl, pH 8.0, 1 mM EDTA) for about 5 min. The final crushed biomass was centrifuged at 1500 *g* for 15 min. The following extraction of genomic DNA was performed by using standard phenol-chloroform method (Maniatis *et al*., 1982). The probe was dried over night at 4°C and dissolved in 30 μl ТЕ-буфер. The DNA was purified via GFX columns (GE Health Care, Little Chalfont,

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Buckinghamshire, UK). The quantity and quality of purified DNA was measured by Jenway 6305 UV-VIS spectrophotometer with the absorbance at wave lengths of 280 nm. The above prepared DNA was used for the PCR.

DNA amplification and electrophoretical analysis

PCR was done using 'puReTaq Ready-To-Go PCR Beads' (Amersham Biosciences). The genes for the two of enzymes were amplified by applying the programs and the primers indicated in Table 1. The primers for all sets of reactions were designed on the basis of the identical genes' sequences of *A. fumigatus* strain Af293. The amplification reactions were performed in a 25 μl volume containing 10 pmol of each primer. The PCR in the current study was carried out on a T100 Thermal cycler (Bio-Rad Laboratories, Inc.).

The genomic DNA from the cell-free extract and PCR samples was detected by agarose gel electrophoresis accomplished in 1xTBE buffer (Maniatis et al., 1982) with 0.8 and 2% agarose gel respectively for genomic DNA and for the amplified genes. The gel was run at 80V for 1 hour. The DNA molecules in the gel were visualized after submersion in a solution of Ethidium Bromid.

DNA sequencing

The sequencing of amplified fragments was performed on an ABI Prism 310 Genetic Analyzer by using BigDye Terminator Kit version 3.1. The row data from the genetic analyzer were edited by Sequence scanner Version 1.0 software (Applied Biosystems). The authenticity of the obtained data was verified with program Sequence Scanner V1.0 (Applied Biosystems, Foster City, CA, USA). The DNA sequences were turned in FASTA format which is suitable for BLAST comparison with database of NCBI (Altschul *et al*., 1990). The comparative analysis of the both sequences were accomplished by using ClustalW 2 program (Manasiev *et al*., 2008).

Results

Nucleotide and the amino acid sequences of the gene for phenol hydroxylase

Two genes coding proteins with phenol hydroxylase and catechol 1,2-dioxygenase enzyme activities which take part in the degradation pathway of phenolic compounds in Antarctic fungal strain *A. fumigatus* AL 3 have been identified. The primers applied in the PCR analyses for both genes were designed on the basis of the identical genes sequences of *A. fumigatus* strain Af293 (Table 1.).

For the detection of phenol hydroxylase and catechol 1,2-dioxygenase genes were created and used two sets each consisted of 3 pairs of primers. The obtained oligonucleotide DNA fragments were sequenced by Sanger method. The overlapped fragments for each one of the investigated genes were combined.

Table 1. *The programs and primers used in PCR reactions.*

Gene	Fragments	Primers	PCR conditions
Phenol hydroxylase	1	PPHF1varf acactgcatctcgggaatacagaa PPHF3r gtagattgtccaccagtcgc	Initial step, 95°C, 5 min; 34 cycles amplification, denaturing at 95°C, 30 s; annealing at 60°C, 30 s; extension at 72°C, 1 min, and final step - 72°C, 5 min
	2	pPHF1f atgatgggtcttgaggtctcaagg PPHF3r gtagattgtccaccagtcgc	
	3	PPHF3f gcgactggtggacaatctac PPHF6r tcagcgcgtgaagatgggatg	
Catechol 1,2-dioxygenase	1	AFKF2 ttgtctgtgacgtgatcggg AFKR2 tgccacacctccaccgtcgc	Initial step, 95°C, 5 min; 34 cycles amplification, denaturing at 95°C, 30 s; annealing at 55°C, 30 s; extension at 72°C, 1 min, and final step - 72°C, 1 min
	2	AFKF3 tcatgcacggccgggtgatc AFKR3 gggtgtcggtccatgagctc	
	3	AFKF3 tcatgcacggccgggtgatc AFKR4 ctacgcctggtccgccacca	

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      M  M  G  L  E  V  S  R  M  N  C  F  T  L  H  L  G  N  T  E  A  I  L  I  D
   1  ATGATGGGTCTTGAGGTCTCAAGGATGAATTGTTTTACACTGCATCTCGGGAATACAGAAGCAATTCTGATCGAC   75

      A  I  Q  L  R  G  G  P  R  I  E  R  G  V  V  P  V  A  M  E  L  E  E  G  V
  76  GCCATCCAACTACGGGGCGGGCCCCGGATTGAACGAGGAGTCGTGCCAGTGGCGATGGAACTGGAGGAGGGCGTA  150

      A  D  D  P  D  A  Y  P  L  K  I  Q  L  R  H  Q  K  L  E  H  L  T  A  W  R
 151  GCGGACGACCCCGACGCATATCCTCTGAAGATACAGCTGCGCCACCAGAAACTGGAGCATCTGACTGCGTGGCGG  225

      T  N  A  H  S  E  R  G  G  I  D  A  A  I  H  S  G  R  D  G  E  R  D  T  E
 226  ACCAACGCTCACAGTGAGAGAGGAGGCATTGACGCGGCCATCCATTCCGGACGCGACGGGGAGCGTGACACCGAG  300

      P  V  L  T  G  E  E  G  S  L  K  T  I  R  A  K  Y  V  I  G  S  D  G  A  H
 301  CCCGTCCTGACCGGGGAGGAGGGCAGCCTCAAGACCATCCGCGCAAAATATGTCATTGGCAGCGATGGCGCCCAC  375

      S  W  V  R  R  W  L  G  F  E  M  E  G  D  S  T  N  A  V  W  G  V  V  D  A
 376  AGCTGGGTGCGTCGCTGGCTGGGCTTTGAGATGGAGGGGGATTCCACCAATGCAGTGTGGGGCGTGGTGGATGCC  450

      I  L  D  S  D  F  P  D  F  R  R  H  C  T  I  L  S  Q  H  G  T  I  L  S  V
 451  ATTCTCGACTCAGATTTTCCCGACTTTCGCCGACACTGCACAATTCTCTCGCAGCATGGGACCATCTTGAGCGTG  525

      P  R  E  N  G  M  T  R  L  Y  V  Q  L  P  D  S  M  K  D  I  C  L  T  D  A
 526  CCTCGAGAAAACGGCATGACCCGCCTTTACGTCCAGCTACCCGATTCGATGAAAGATATCTGCCTCACCGACGCA  600

      A  Q  V  V  K  I  M  A  V  A  R  R  S  L  F  P  Y  T  L  E  Y  S  Y  C  D
 601  GCTCAGGTGGTGAAGATCATGGCTGTCGCTCGCAGGAGTTTGTTTCCTTATACTCTGGAATATTCCTACTGCGAC  675

      W  W  T  I  Y  R  V  G  R  R  V  A  N  H  F  T  Y  K  Q  R  V  F  L  G  G
 676  TGGTGGACAATCTACCGGGTGGGTAGGAGAGTTGCCAACCATTTCACCTACAAGCAGCGCGTCTTCCTCGGTGGG  750

      D  A  V  H  T  H  T  P  K  G  G  Q  G  M  N  V  S  M  Q  D  A  Y  N  L  G
 751  GACGCTGTACACACACATACCCCCAAGGGGGGTCAGGGGATGAATGTCTCCATGCAGGATGCATACAACCTGGGA  825

      W  K  L  G  G  V  L  R  G  Q  L  R  P  S  V  L  A  T  Y  E  S  E  R  R  P
 826  TGGAAGCTGGGTGGTGTACTGCGTGGCCAACTGCGTCCGTCCGTACTGGCGACGTACGAATCCGAACGTCGACCC  900

      V  A  Q  D  L  I  K  L  D  T  S  M  G  R  V  L  A  G  E  T  M  S  E  T  P
 901  GTGGCCCAGGACCTGATCAAGCTAGATACGAGTATGGGCCGCGTGCTGGCGGGCGAGACCATGTCCGAGACTCCC  975

      E  V  L  Q  V  Y  E  Q  L  R  N  Y  G  S  G  A  N  I  C  Y  S  P  N  I  L
 976  GAGGTGCTTCAGGTTTATGAGCAGCTGCGCAACTACGGCAGCGGCGCCAACATCTGCTACTCGCCAAACATCCTT 1050

       V  A  S  P  Q  Q  S  Q  Q  H  L  A  A  H  L  R  L  G  M  R  F  P  S  H  P
1051  GTGGCGAGTCCCCAGCAGTCCCAGCAACACCTGGCCGCGCATCTGAGGCTGGGAATGCGGTTCCCCAGCCATCCG 1125

      V  V  N  L  A  S  A  I  T  M  E  S  Q  S  L  L  P  S  N  G  S  W  R  L  W
1126  GTGGTCAACCTTGCCAGCGCGATCACCATGGAGAGCCAGAGTCTACTGCCGAGTAATGGCTCGTGGCGGCTCTGG 1200

      V  F  A  G  N  V  V  A  C  P  A  Q  L  K  R  V  N  S  L  G  E  K  L  C  A
1201  GTCTTTGCCGGCAATGTTGTCGCTTGTCCAGCGCAGTTGAAGCGGGTGAACAGCCTCGGGGAGAAGCTGTGCGCT 1275

      L  T  A  R  L  A  A  L  Q  M  L  S  T  P  F  L  E  I  L  L  L  Y  K  G  R
1276  CTGACAGCCCGTCTTGCTGCGCTCCAGATGCTCTCCACGCCGTTTCTCGAGATACTGCTCCTCTACAAAGGTCGA 1350

      V  E  E  M  E  V  S  D  F  H  P  I  F  T  R  *
1351  GTGGAGGAGATGGAAGTCAGTGATTTCCATCCCATCTTCACGCGCTGA 1398
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Figure 1. *The nucleotide sequence of the sense strand and a corresponding amino acid sequence of phenol hydroxylase gene of A. fumigatus AL 3 (NCBI Acc. No. KT781126).*

The performed nucleotide sequence analysis of a gene for phenol hydroxylase of *A. fumigatus* AL 3 (NCBI Acc. N KT781126) resulted in a fragment with a length of 1513 bp which was organized into 3 exons and 2 introns. The first intron started from 241 to 267 bp (27 bp) and the second one occupied position from 501 bp to 588 bp (88 bp). The length of the coding DNA sequence (cds) is 1398 bp. (Figure 1). The translation of the obtained DNA sequences revealed a

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single open reading frame consisting of 465 amino acids (Figure 1). The BLAST analysis confirmed that the phenol hydroxylase gene of *A. fumigatus* AL 3 has 99% identity with the nucleotide as well as amino acid sequences reported for the corresponding gene of *A. fumigatus* Af293 (Nierman et al., 2005). Comparing to the identical genes sequences of *A. fumigatus* strain Af293 and *A. fumigatus* AL 3 there are a difference of 9 single-nucleotide substitutions.

Nucleotide and the amino acid sequences of the gene for catechol 1,2-dioxygenase

The overall length of the received nucleotide sequence of the gene for the catechol 1,2-dioxygenase *of A. fumigatus AL 3* It was defined as consisting of 843 base pairs (NCBI Acc. N KT781125). It was composed of 4 exons and 3 introns. The position of the introns is as follows: the first is located between 21 and 102 bp (74 bp), the second is covering the region between 464 and 534 (71 bp), and the third is between 604 and 657 bp (54 bp). The established length of the partial coding DNA sequence of the gene counted 642 bp (Figure 2). The corresponding protein sequencion is consisting of 213 amino acids. Since it is a partial sequence the initial ATG codon isn't indicated. (Figure2). There was again 99% identity of nucleotide and protein sequences of the catechol 1,2-dioxygenase genes of *A. fumigatus* Af293 and *A. fumigatus* AL 3. The received in our experiments DNA sequence has only 2 single-nucleotide substitutions which distinguish it from that established in *A. fumigatus* Af293.

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V  C  D  V  I  G  L  E  S  L  V  D  E  I  T  F  K  L  A  D  E  A  A  D  A
   1 GTCTGTGACGTGATCGGGCTCGAGTCGCTTGTCGACGAAATCACCTTCAAGCTTGCTGATGAGGCCGCCGACGCA   75

   P  T  A  T  A  I  L  G  P  F  F  R  A  D  T  P  Y  R  N  N  G  D  N  I  V
  76 CCCACCGCGACCGCCATCCTCGGCCCCTTCTTCCGCGCTGATACCCCCTACCGCAACAACGGCGACAATATCGTC  150

   K  D  V  P  D  G  E  M  V  F  M  H  G  R  V  I  D  F  Q  T  K  K  P  L  V
 151 AAGGACGTGCCGGACGGCGAGATGGTGTTCATGCACGGCCGGGTGATCGATTTCCAGACCAAGAAGCCGCTCGTT  225

   G  A  T  V  E  V  W  Q  A  S  T  N  G  L  Y  E  Q  Q  D  P  N  Q  E  E  F
 226 GGAGCGACGGTGGAG GTGTGGCAGGCGTCCACGAACGGGCTGTATGAGCAGCAGGATCCGAACCAGGAGGAGTTT 300

   N  L  R  G  K  F  K  T  D  A  D  G  R  Y  Y  F  Y  C  L  R  P  T  P  Y  P
 301 AACTTGCGGGGGAAGTTCAAGACGGATGCTGACGGGCGGTACTACTTTTACTGCCTGCGCCCGACGCCGTATCCT  375

   V  P  N  D  G  P  A  G  K  L  L  E  L  M  D  R  H  P  F  R  P  A  H  I  H
 376 GTTCCGAATGATGGTCCCGCCGGGAAGTTACTCGAGCTCATGGACCGACACCCCTTCCGCCCTGCCCATATCCAC  450

   I  I  A  T  H  D  G  H  K  P  L  T  T  Q  I  F  D  R  Q  D  K  Y  L  T  N
 451 ATCATTGCTACGCACGATGGCCACAAGCCCCTCACCACGCAGATCTTCGACCGCCAGGACAAGTACCTAACAAAC  525

   D  S  V  F  A  V  K  D  S  L  I  V  D  F  V  P  R  K  D  D  P  Q  A  G  L
 526 GACTCAGTATTTGCCGTCAAGGACTCGCTGATCGTGGACTTTGTGCCGCGCAAGGATGACCCGCAGGCTGGACTT  600

   E  L  E  Y  D  V  K  L  V  A  D  Q  A  *
 601 GAGCTTGAATACGACGTCAAGCTGGTGGCGGACCAGGCGTAG 642
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Figure 2. *The nucleotide sequence of the sense strand and a corresponding amino acid sequence of catechol 1,2-dioxygenase gene of A. fumigatus AL 3 (NCBI Acc. No. KT781125).*

Discussion

The DNA-molecular analyses carried out showed the presence of nucleotide sequences typical for genes coding key enzymes for the catabolism of aromatic compounds. The sequencing of both genes aimed better realizing the genetic characteristics of enzymes.

In the comparison made of the obtained amino acid sequences with those of other members of the flavin adenine dinucleotide (FAD) dependent adhesive monooxygenases (FMO) can be seen that the similarity range between 36 and 45%. This result confirms the observation that despite the similar folding typical of flavin-containing aromatic hydroxylase they can vary greatly in terms of their amino acid sequence (Figure 3).

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Figure 3. *Alignment analysis of the second FAD-binding region of flavin-containing proteins with monooxygenase activity (The marked amino acids show conserved "GD" motif sequence):*
1. Aspergillus fumigatus AL 3 (this study); 2. Phanerochaete chrysosporium (Nakamura et al., 2012.); 3. Polaromonas naphthalenivorans (Park et al., 2007); 4. Pseudomonas sp. (Nurk et al., 1991); 5. Alcaligenes eutrophus (Perkins et al., 1990); 6. Trichosporon cutaneum ATCC 46490 (L04488.1); 7. A. fumigatus AL8 (Gerginova et al., 2013); 8. A. fumigatus Af293 (XM_743491.1); 9. Neosartorya fischeri NRRL 181 (XP_001265717.1).

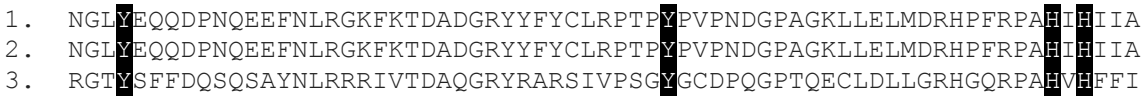

Figure 4. *Alignment analysis of the iron-binding side in proteins with catechol 1,2-dioxygenase activities from 1. Aspergillus fumigatus AL 3 (this study), 2. Aspergillus fumigatus Af293 (XM_744333), 3. Pseudomonas arvilla C-1 Isozyme αα (Nakai et al., 1996). The four marked amino acids form the iron-binding side.*

A similar comparison was done for catechol dioxygenase in several representatives of *Aspergillus*. Again it can be seen the existence of common conserved motifs in parallel with significant differences in the arrangement of the amino acids in the rest of the molecule (Figure 4).

The proximity of the studied proteins with closely related enzymes with similar functions were demonstrated on the created distant tree's cladograms.

Based on the amino acid sequences obtained for the enzymes with phenol hydroxylase activity in the strains *A. fumigatus* AL3, *A. fumigatus* (AL3, AL8, AL9, AL15), and related sequences in the database of the NCBI was created cladogram reflecting the significant sequence identity of this enzyme within the species *A. fumigatus*. This group of enzymes with similar amino acid sequences includes a phenol hydroxylase from *A. glaucus* AL1, and phenol 2-monooxygenase described for strain *Fuzarium oxysporum* f. sp. (Figure 5).

By the same approach was created cladogram reflecting the sequence identity of the enzyme catechol 1,2-dioxygenase obtained in strains *A. fumigatus* AL3, *A. fumigatus* (AL3, AL8, AL9, AL15), and related sequences in the database of the NCBI. The demonstrated group of enzymes includes as well some functionally related aminoacid sequences of *A. glaucus* AL1, and similar enzymes, described in other strains belonging mainly to the genus *Aspergillus* (Figure 6).

As a conclusion from the carried out analyses and comparisons it can be claimed that in the present study were identified catabolic genes encoding enzyme proteins with a key role in the degradation of highly toxic aromatic compounds such as phenol and phenol derivatives.

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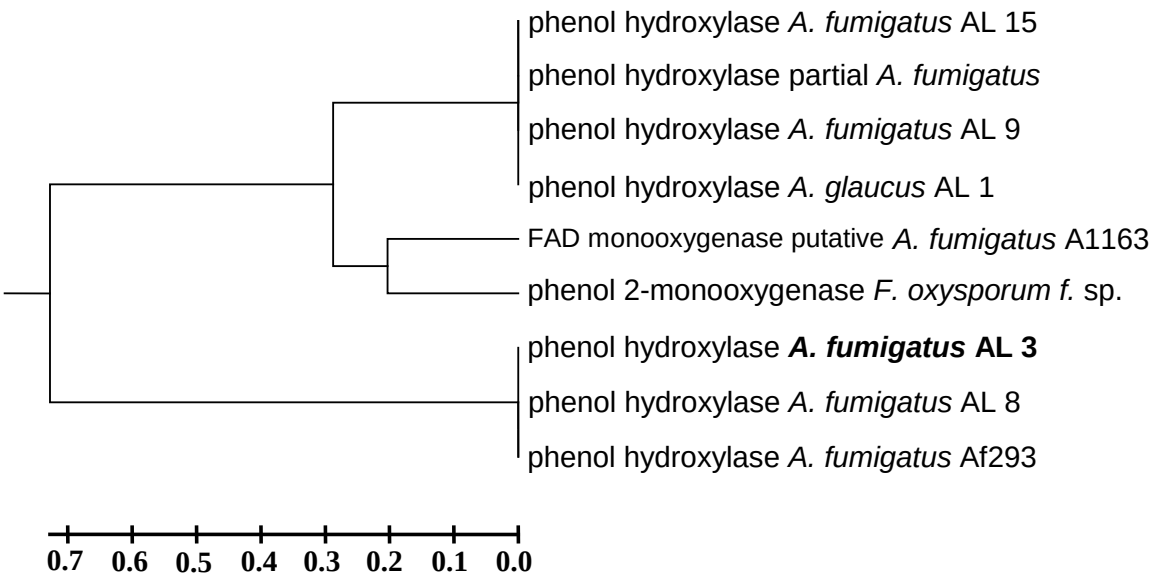

Figure 5. *Cladogram created on the base of amino acid sequences, deduced for phenol hydroxylases by different strains of Aspergillus, as well as published in NCBI database related sequences.*

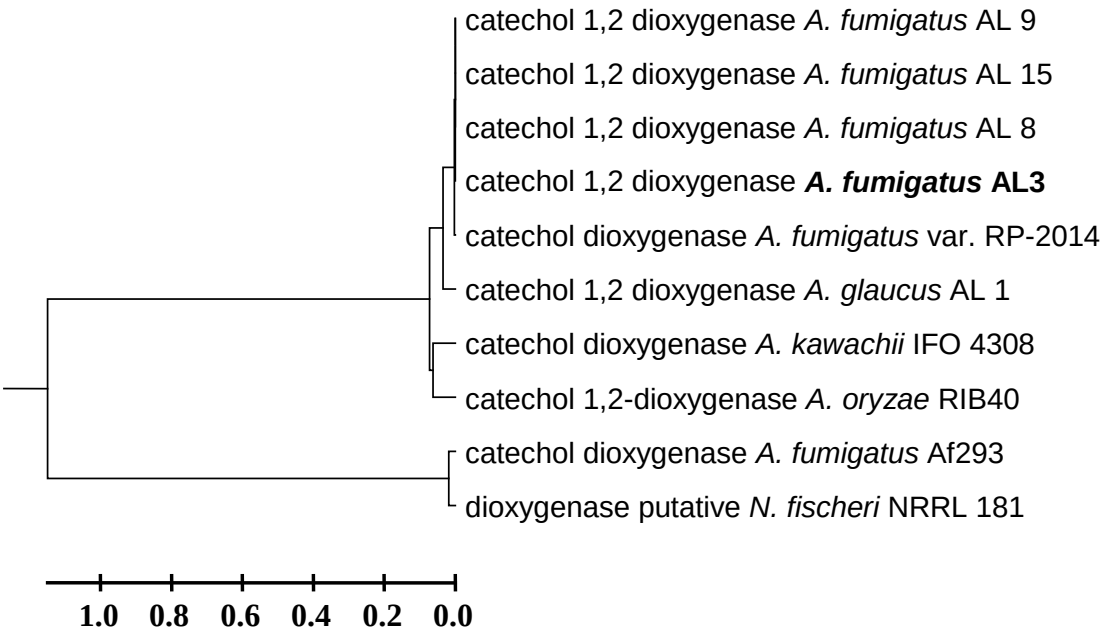

Figure 6. *Cladogram created on the base of amino acid sequences, deduced for catechol 1,2-dioxygenase by different strains of Aspergillus, as well as published in NCBI database related sequences.*

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