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Hypothetical Protein from *Aspergillus niger* contains Chromate Reductase Motifs

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Abstract
Chromate reductase is an enzyme that converts toxic and carcinogenic Cr^{6+} to non-carcinogenic and less soluble Cr^{3+}. A hypothetical protein (ref.XP-001388504.1) from *Aspergillus niger* has been identified as chromate reductase using bioinformatics techniques. The techniques involved Clustal W alignment and Basic Local Alignment Search Tool (BLAST). Using Clustal W alignment the motifs VTPEYN and LKNAID motifs were identified to be common among the different chromate reductase homologues aligned. The same motifs reappeared when the hypothetical protein was aligned with other chromate reductase homologues. The BLAST search was able to also identify VTPEYN and LKNAID motifs on the A. *niger* genome indicating the likelihood of the hypothetical protein being chromate reductase. The homologue was recovered as putative chromate reductase gene encoded by an open reading frame of 2227 bp nucleotide sequence. These findings clearly demonstrate the hypothetical protein is likely to be chromate reductase.
Key words: *Aspergillus niger*, gene, motifs, chromate reductase

INTRODUCTION

Chromate (Hexavalent chromium ion)is a widespread environmental contaminant that is toxic, mutagenic and carcinogenic. It is produced as a by – product of many industrial processes which include: leather tanning, chrome plating, stainless steel welding, pigment production and nuclear weapons generation. The ability of some microorganisms to remove toxic hexavalent chromium has been attributed to the presence of a group of enzymes that have raised enormous interest in hexavalent chromium bioremediation called chromate reductases (Garcia-Arellano *et al.,* 2004). They are a group of enzymes that catalyze the reduction of toxic and carcinogenic Cr^{6+} to the less soluble and less toxic Cr^{3+} (Garcia-Arellano *et.al.,* 2004) and have been found in both aerobic and anaerobic microorganisms (Cheung and Gu, 2007). The enzyme has been partially purified from *Pseudomonas putida* PRS 2000 (Ishibashi *et. al.,* 1990). Suzuki *et al.,* (1992) reported a 38 fold purification of soluble chromate reductase from *Pseudomonas ambigua* G – 1 and Park *et al.,* (2000) was able to attain a 600 fold purification of a soluble chromate reductase from *Pseudomona putida* MK1. More recently, characterization of chromate reductase from *Thermusscotodutus* SA-O1 was reported by Oppermanetal., (2008). Although *Aspergillus niger* has also been implicated in the reduction of hexavalent chromium (Gouda 2000), as at now there is no report on identification of chromate reductase gene in any of the *Aspergillus* species although Sallau*et al.* (2014) purified and characterized chromate reductase from A. *niger.*

However, this claim has not been substantiated through molecular biology approaches, as such whether the enzyme activity reported was due to chromate reductase or other oxidoreductases with chromate reducing capabilities are questions that have remained unanswered. This paper therefore attempts to answer this question.

MATERIALS AND METHODS

Alignment of Chromate Reductase Homologues: Alignment of chromate reductase homologues from different bacterial species as well as strains of oxidoreductase homologues from *Aspergillus* species were conducted using Clustal W, part of the MacVector 7.0 software. Identification of A.*niger* hypothetical protein as chromate reductase was also done using the National Institute of Health (NIH) website (www.nlm.nih/gov).

The bacterial chromate reductase homologues used were : *Bordetella bronchiseptica, Pseudomonas putuda, Vibroharveyi, Bacilluss subtilis, Bordetella avium, Bacillus coagulans, Alkaphilusoremlandi, three strains of E.coli, Commonastestosteroni, Coryneabacteria diphteriaea, Bordetella peratusis, Bacillus thuringiensis, Delfitiaacidovorans, Mycobacterium bovis, Mycobacterium tuberculosis, Lactobacillus sakaei, Delfitiaspp, Oceanobacillus Ihiensis HTE, Symbiobacterium thermophillum, Stigmatella aurentia, Pedobacteriaspp, Oceanicola batiensis, Bordetella perapertusis ,Oceanobacillus isrealiensis*and *Rolatomia metallidurans*. The chromate reductase homologues were alsoobtained from the gene bank through www.nlm.nih/gov.

Blast Search: Basic local Alignment Search Tool (BLAST) was used; specifically tblastn was used, involving the hypothetical protein homologue (Query) and the Aspergillus (Subject).

RESULTS

Figure 1 presents a section of the Clustal W alignment output of the aforementioned different bacterial chromate reductase homologues (61 – 90) indicating the VTPEYN motif. Figure 2 is a continuation of Figure 1 (92-120) indicating the KNAID motif. From the Figure it is clear that except the *Bacillus thuringiensis* chromate reductase homologue which did not have the VTPEYN and KNAID motifs, all other homologues had them further asserting that the motif seems to be common among the various chromate reductase enzymes. In Figure 3, the alignment included the hypothetical protein (XP_001883504.1) mined from the NIH website (Gene bank). There is no doubt that the protein aligned with the other homologues clearly indicating the presence of the motifs in the hypothetical protein. Figure 4 indicates a FASTA sequence of the hypothetical protein which is what was used in the BLAST search (tblastn). Figure 5 is showing a section of the blast hits (61-120) obtained after the BLAST search. The VTPEYN and the LKNAID sequences are clearly indicated on the hits. In Figure 6, a presentation of the open reading frame (ORF)/nucleotide sequence of the hypothetical protein is presented. It is a 2227 kb nucleotide sequence.

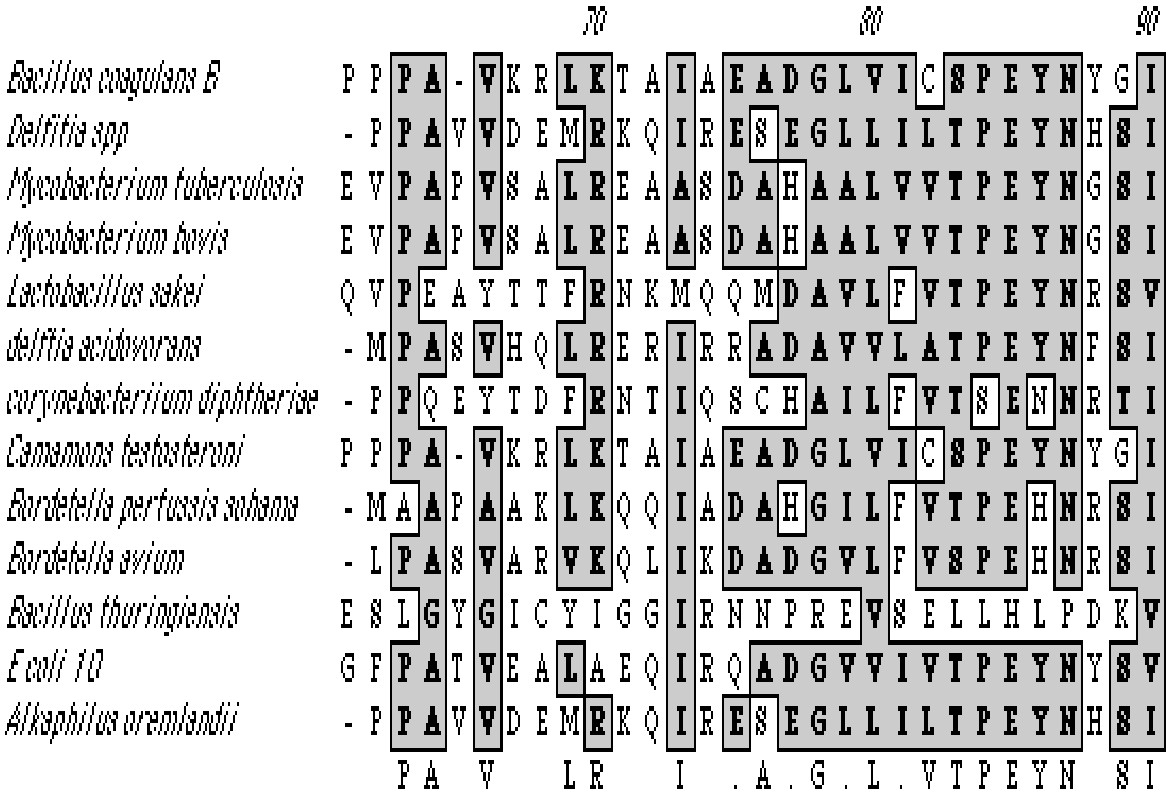

Figure 1. Section of the Alignments of The Chromate Reductase Homologues Indicating The ***VTPEYN*** Motif. The (Motifs Can be Seen on The Last Line of The Alignment)

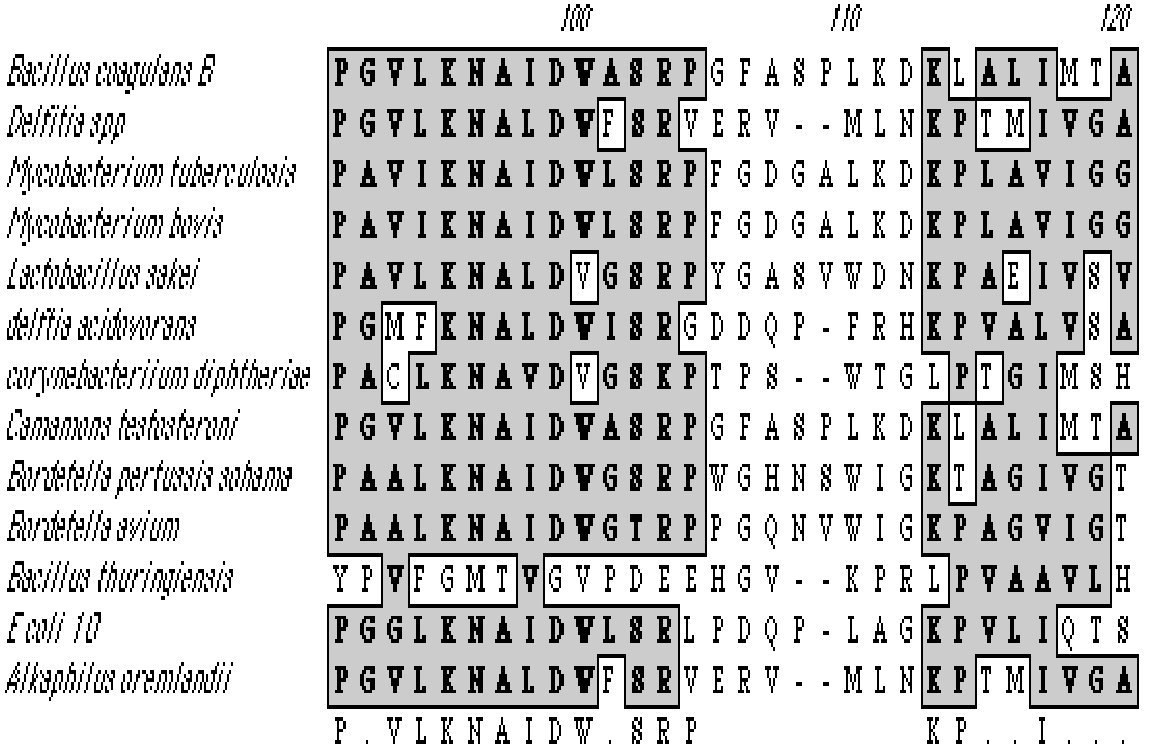

Figure 2. Section of the Alignments of The Bacterial Chromate Reductase Homologues Indicating The ***LKNAID*** Motif. The (Motifs Can be Seen on The Last Line of The Alignment)

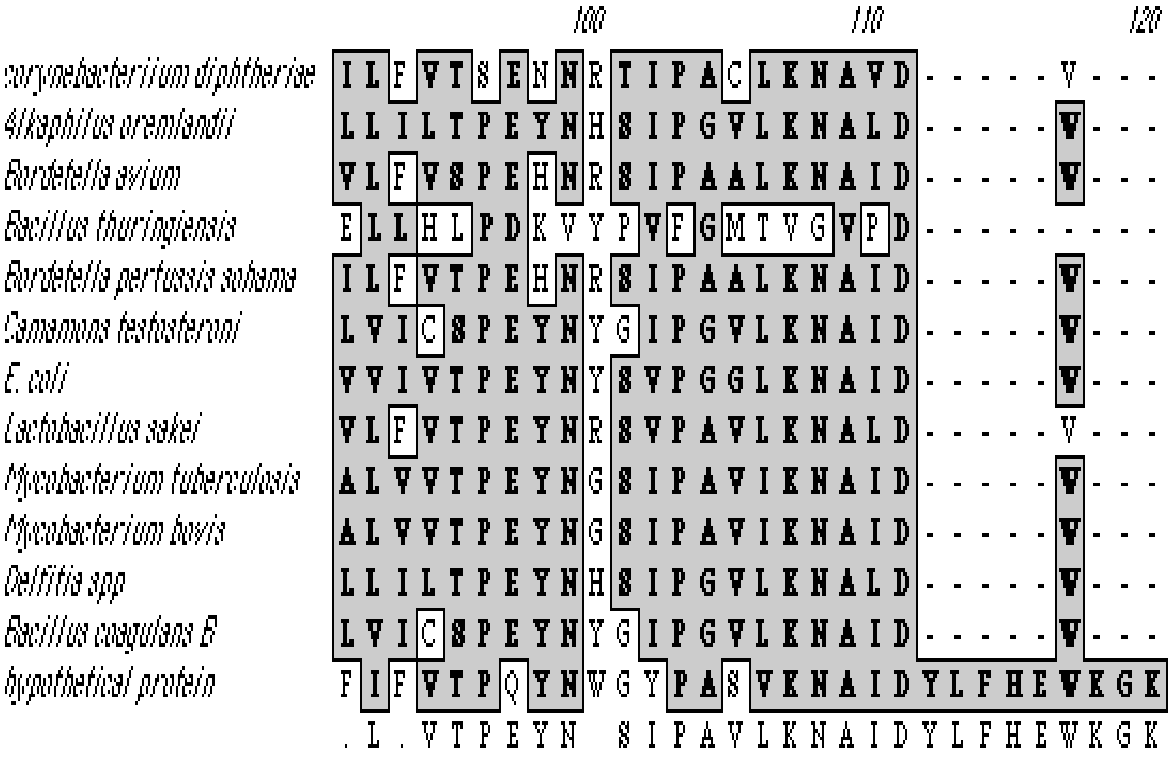

Figure 3. Section of the Alignments of TheBacterial Chromate Reductase Homologues Including Hypothetical Protein Indicating The ***LKNAID*** and ***VTPEYN*** Motifs. The (Motifs Can be Seen on The Last Line of The Alignment)

MPPSIGLIICSQRTPRAGPQIATFIHNTIRESYPPETATITTIDLAKWNLPLYNESGMPS

FINSADEYEHEHTKAWSREISRHEAFIFVTPQYNWGYPASVKNAIDYLFHEWKGKAA LVV

SYGGHGGGKAAAQLRQVLQGVRMRPLERMVELRFPEIEEVKRAAKGEDLGLNVTG YPEVE

IEVPRKKILSQVHDTNRIDAGTRHAGTLHSQGNLMSAAYDSLFVMRMPGCIISYYPT GLF

FKPAYNHRSTHCIPLLYYHSSLMVHFISLFYTLVGLLPAAALPSPTHHSLETITCDICIL

GGGSSGTYSAIQLKDAGKHVVVIEPNNRLGGHAATLYLPDGNYVDYGVEGVFNNEL SRNY

FLRLGVDWKPLLPLNKRTDFVDFSTGERVNPAAGILQALVSTFLYRSSIKHWTFLTRG VY

DLPDPVPEELLRPFREFIEEHSLEAALQLVFLFSQNVGNLLDMPTLYAIQNFGVPHVD AL

IHGYITPKNGMYELYRQAREILGSGVLFQTNVIATNRSDSGVEVTVQHANGTRQLIK
ARN

LLITFPPLIKKLQGFDLDETETELFQKWFWRTYYVAVVNNTGIPDKLWVTNTDPTNG
LGY

LPRMPFDFLLQYMGAPGYSTSKLVGDMNFTEEDARDLLAADFARMKAKGTYPIHDP
QIVV

FGDSSPETLMVSPEDIRNGFYRKLVIYPISARYNYSVKLNDTKICLHTGSDFEVALKN
LK
LWPFFLSRKSRLFVNHSPDTTDNRIYESVLRPLIQATI

Figure 4. FASTA sequence of XP 001388504.1(The Hypothetical Protein Identified As Chromate Reductase)

Query 64
SADEYEHEHTKAWSREISRHEAFIF**VTPQYNWGYPASVKNAID**YLFHEWKGKAAL
VVSYG 123
S DEY HE TKAWSREI+ H FIF**VTPQYNWGYPASVKNAID**YLFHEWKGK
A++VSYG
Sbjct 181
SIDEYTHETTKAWSREIASHAGFIF**VTPQYNWGYPASVKNAID**YLFHEWKGKPAMI
VSYG 360

Figure 5. Section of The Blast Hits Obtained After Blast Search (The Motifs *VTPQYN* and *KNAID* are Indicated in Bold)

ATGCCTCCCTCTATCGGCCTCATAATCTGCAGCCAACGCACTCCACGCGCAGGCCC
TCAAATCGCCACCT
ATGGAACCTTCCACTCTACAATGAGTCGGGGATGCCATCGTTCATCAACTCAGCGG
ACGAGTACGAGCAC
GAGCACACAAAGGCCTGGTCACGGGAGATATCGCGCCACGAAGCGTTTATTTTCG
TCACACCGCAGTATA
ACTGGGGGTATCCCGCAAGCGTGAAGAATGCGATTGATTACTTGTTCCATGAGTGG
AAGGGAAAGGCGGC
GTTGGTGGTGAGCTATGGGGGGCATGGGGGCGGGAAGGCGGCGGCGCAATTGAG
GCAGGTGTTGCAGGGG
GTGAGGATGAGGCCATTGGAGAGGATGGTCGAGCTGAGGTTTCCGGAGATCGAG
GAAGTTAAGAGGGCCG
CTAAGGGGGAGGATCTGGGACTGAATGTCACAGGTTACCCCGAGGTTGAGATAGA
AGTCCCACGAAAGAA
GATATTGTCACAAGTTCATGATACTAACAGGATAGATGCAGGAACCAGGCACGCTG
GCACACTCCACTCC
CAAGGAAATCTGATGAGCGCAGCATATGATTCCCTGTTTGTCATGAGGATGCCGGG
ATGTATCATCTCAT
ACTATCCAACTGGGCTATTCTTCAAACCGGCGTATAATCACAGAAGCACTCACTGT
ATACCATTATTATA
TTATCATTCGTCTCTGATGGTCCACTTTATAAGCTTATTCTATACCCTCGTAGGCTTA
CTACCAGCGGCT

TCATTCACAATACTATTCGTGAATCCTACCCCCCCGAAACAGCCACAATTACGACC
ATTGACCTAGCAAA
GCCCTTCCCTCACCAACCCACCACTCTCTCGAGACAATAACCTGCGACATTTGCAT
CCTCGGAGGTGGAA
GCTCAGGGACGTACAGCGCCATCCAACTCAAAGATGCAGGGAAGCACGTGGTAGT
AATAGAGCCCAACAA
CAGGCTAGGAGGGCACGCAGCGACATTATACCTGCCAGATGGCAACTACGTCGAC
TACGGCGTCGAAGGC
GTCTTCAACAACGAGCTCTCCCGCAACTATTTCCTCCGACTCGGAGTAGACTGGA
AGCCACTACTCCCAC
TGAACAAACGAACCGACTTTGTCGACTTCTCGACAGGTGAACGCGTGAACCCCG
CAGCAGGAATCTTGCA
AGCCCTCGTATCAACATTTCTTTACCGCTCATCCATAAAGCACTGGACGTTTCTCAC
AAGAGGGGTTTAC
GACCTCCCCGACCCAGTACCAGAAGAACTTCTCCGCCCATTCCGTGAATTCATCGA
GGAACATTCCCTCG
AAGCTGCTCTGCAGTTGGTATTTCTCTTTTCTCAGAATGTAGGAAATCTGCTCGATA
TGCCCACCCTCTA
TGCGATCCAGAATTTCGGGGTCCCGCATGTTGACGCTCTCATTCATGGATACATCA
CACCGAAAAACGGC
ATGTACGAGCTTTACAGACAAGCGAGGGAAATACTCGGCTCGGGTGTGCTGTTCC
AGACAAACGTGATCG
CAACAAATAGGTCCGATTCCGGCGTGGAGGTAACAGTTCAGCATGCGAACGGTAC
TCGTCAGCTCATTAA
AGCCAGAAACCTCCTCATTACCTTCCCTCCGCTCATAAAAAAGCTCCAAGGCTTCG
ATTTGGATGAAACA
GAAACGGAACTATTCCAGAAATGGTTCTGGAGGACATACTACGTTGCCGTGGTGA
ATAATACCGGTATCC
CGGACAAGCTGTGGGTCACCAATACAGACCCGACTAATGGACTGGGGTATTTACC
TCGCATGCCATTTGA
TTTCCTGCTGCAGTATATGGGAGCACCAGGGTATTCAACCAGCAAGTTAGTAGGCG
ATATGAACTTCACC
GAGGAAGATGCAAGAGACCTTCTAGCGGCGGATTTTGCGCGGATGAAGGCAAAG
GGGACATACCCGATTC
ATGATCCTCAAATCGTGGTGTTTGGGGATAGTTCGCCGGAGACTCTGATGGTGTCG
CCGGAGGATATTCG
TAACGGATTTTATCGCAAGTTGGTTATTTATCCTATTTCAGCAAGATACAATTACTCA
GTGAAACTGAAC
GATACGAAGATATGTTTGCATACTGGTAGTGATTTTGAAGTGGCCTTGAAGAACCT
TAAATTGTGGCCAT
TTTTCTTGAGCAGGAAGTCTAGGCTTTTCGTAAACCATTCTCCAGATACAACTGAT
AATCGCATATATGA
GTCGGTCTTGAGACCATTAATCCAAGCCACTATTTGA

Figure 6. Open Reading Frame (ORF) of 2227 kb Nucleotide/Coding Sequence of Hypothetical Protein.

DISCUSSION

Chromate reductase has been identified in various prokaryotic organisms including *Pseudomonas spp* and E.*coli* (Ackerly *et al*, 2004; Bae *et al*, 2005) and more recently from *Aspergillus*, an eukaryote (Sallau *et al*, 2014).This is the first report on the identification of chromate reductase gene in a eukaryotic organism. However, several proteins have been attributed to the activity of chromate reductase in some microorganisms (Kwak *et al*, 2003). The clustal W alignment outputs presented in Figures 1,2 and 3 all revealed the presence of the motifs VTPEYN and LKNAID as sequences common to the homologues. This is an indication of a particular property that is common to the chromate reductases. Ackerly *et al*, (2004) reported LFVTPEYNXXXXXXLKNAIDXXS as a signature sequence of NADH dehydrogenase family to which chromate reductase belongs. The motif has been speculated to be involved in FMN binding since residence of similar identity bind the cofactor in *Vibrio fisheri* reductase (Tanner *et al*, 1996). In addition, most of the amino acids that are highly common within chromate reductase homologues are also present in the chromate reductase homologues aligned including the hypothetical protein suggesting that the hypothetical protein could be chromate reductase. The blast search results (Blast hits) also confirmed the presence of the motifs notable in the chromate reductase homologues in the A*spergillus* genome further buttressing that hypothetical protein obtained from *Aspergillus niger* genome is likely to be chromate reductase. Although the complete coding sequence of the hypothetical protein (chromate reductase) was 2.2kb and is larger than that of *Pseudomonas putida* chromate reductase gene (1.5kb) whose protein has been cloned and expressed, the bigger size of that of the A. *niger* could be attributed to the organism's level of organization. Being a eukaryotic organism which is essentially associated with higher genome size, complex genome organization, this could account for the gene size been larger than that of its prokaryotic counterpart. Although a close look at one of the motifs from the blast hits indicated VTPQYN instead of VTPEYN as indicated in the alignments and the molecular signature for all oxidoreductases, the two amino acids are very much alike structurally E (Glutamic acid) and Q (Glutamine), but differ functionally having acidic and amido groups respectively. The substitution of E with Q will not impact significantly on the secondary structure of the protein since most –CO groups present in polypeptides especially the mid-chain are connected to –NH groups in the formation of α-helical structure and beta conformations (Berg *et al*., 2002), and incidentally both amino acids ie glutamic acid and glutamine have the –CO groups. By implication, both amino acids can favourably form similar secondary conformations. An interesting feature in the hypothetical protein primary structure is the presence of Glu (E) which Sallau *et al,*(2014) were able to demonstrate its presence in the soluble integral protein, chromate reductase they isolated. This further confirms the hypothetical protein to be chromate reductase.

CONCLUSION

Conclusively, it is clear that the hypothetical protein (XP-001388504.1) mined from *Aspergillus niger* proteome is chromate reductase, going by its molecular signature and the chromate reductase motifs present in it which also corroborated with those of other chromate reductases as aligned.

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