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In-Silico ANALYSIS OF THE GENE AND PROTEIN SEQUENCES OF THE ENZYME MUSHROOM TYROSINASE (POLYPHENOL OXIDASE, PPO)

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ABSTRACT: Tyrosinase (E.C. 1.14.18.1) is a multifunctional copper-containing enzyme which catalyses the biosynthesis of melanin (a huge polyphenolic bio-macromolecule) in human, plants and animals. The irregularity in the expressions of this enzyme causes severe clinical problems in humann beings like hyperpigmentation and depigmentation, as well as vitiligo and albinism-type severe dermatological problems. In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms. This enzyme and its receptors (TRP1 and TRP2) are involved in the skin melanoma. The gene and protein sequences of this enzyme and their primary, secondary, tertiary and quaternary structures have been analyzed through several bioinformatic approaches.

Keywords: Tyrosinase, Polyphenol oxidase, copper, melanin, hyperpigmentation, depigmentation, melanoma, TRP1, TRP2, bioinformatics.

Abbreviations used: DDBJ (DNA database of Japan), DMSO (Dimethyl sulfoxide), EBI (European Bioinformatics Institute), EMBL (European Molecular Biology Laboratory), EST (expressed sequence tag), ExPASy (Expert Protein Analysis System), GRAVY (grand average of hydropathicity), GSS (genome survey sequence), HTGS (high-throughput genomic sequences), ORF (open reading frame), PCR (polymerase chain reaction), PDB (protein databank), PPO (Polyphenol oxidase), SIB (Swiss Institute for Bioinformatics), STS (sequence tagged sites), TRP1 (Tyrosinase related protein 1), TRP2 (Tyrosinase related protein 2),

INTRODUCTION: Over the past 30 years the enzyme tyrosinase (Polyphenol oxidase, PPO, EC (1.14.18.1) has received considerable attention as an indispensable tool in the performance of studies on a wide range of topics. Since the first biochemical investigations were carried out in 1895 on the mushroom *Russula nigricans*, the cut flesh of which turned red and then black on exposure to air (Bourquelot and Bertrand, 1895), a number of studies have been made to find the factor mainly responsible for the color change. This factor was later identified as enzyme tyrosinase, the active site of which contains a binuclear copper cluster (Schoot-Uiterkamp and Mason, 1973; Nishioka, 1978). In higher plants and fungi, tyrosinases occur in various isoforms such as immature, mature latent (Sanchez-Ferrer, et al., 1989; ibid, 1990) and active forms; however, the bio-chemical descriptions regarding the kinetic characterization and relationship

between these isoforms is yet to be established. The biosynthetic pathway for melanin formation, operating in insects, animals, and plants, has largely been elucidated by Raper (in 1928), Mason (in 1948), and Lerner et al. (in 1949).

Contradictory results were reported regarding the role of tyrosinase in cancer as some papers suggest a tumor-suppressing effect of mushroom tyrosinase, whereas others predict a possible role in mutagenicity. Vogel et al. (1977) reported that a stable phenol, χ- L - glutaminyl- 4- hydroxybenzene (GHB), is oxidized by tyrosinase to a quinone and another oxidation product, which together suppress mitochondrial energy production and synthesis of nucleic acids and proteins. Incubation of cultured murine L1210 leukemia and B-16 melanoma cells with purified quinone was found to block tumor growth in the mice, but when these cells were incubated in the presence of GHB, tumor suppression was observed only in B-16 melanoma cells and not in L1210 leukemia cells due to the absence of the enzyme tyrosinase, indicating that the cytotoxic effect of GHB is dependent on the presence of tyrosinase. The antitumor effect of L-glutamic acid and χ-(p-hydroxyanilide), on B-16 melanoma was studied *in vivo*. In the presence of mushroom tyrosinase it inhibited the DNA polymerase activity while its 3,4-dihydroxy derivative inhibited the thymine production. On the other hand the 2,5-dihydroxy derivative inhibited the uracil and leucin incorporation into nucleic acid and proteins of melanoma cells (Wick et al. 1980). Papaparaskeva-Petrides et al. (1993) found that tyrosinase is responsible for enhancing the mutagenicity of mushroom extract through the production of phenolic and quinoid compounds. Moreover, this muta genic response was inhibited by catalase, superoxide dismutase, glutathione, and solvent dimethyl sulfoxide (DMSO), which indicated the role of phenolic and quinoid compounds in the generation of reactive oxygen species (ROS). A similar increase in mutagenicity was also observed with the extract of baked mushroom (Walton et al. 1998). Aromatic hydrazines apparently play an important role in the carcinogenicity of mushroom (Toth, 1988 and ibid, 1995), and a number of studies have been performed to establish the relationship between hydrazines and mushroom tyrosinase.The main hydrazine candidate for mediating the carcinogenicity of the mushroom is agaritine [*N*-(χ-L-(+)-glutamyl) -4- (hydroxymethyl) phenyl-hydrazine]. Walton et al. (Walton et al. 1997) studied the mutagenicity of putative agaritine metabolites in the presence of mushroom tyrosinase and found that among all the metabolites, tyrosinase can effectively enhance the mutagenicity of *N*-acetyl-4-(hydroxy-methyl) phenyl-hydrazine. Recently, the same group reported that the whole mushroom homogenate readily metabolizes agaritine, whereas the mushroom tyrosinase has the potential to metabolize both agaritine and *N*-acetyl-4-(hydroxy-methyl)-phenyl-hydrazine, in the latter case forming some genotoxic metabolites (Walton et al. 2001). Agaritine is activated by the loss of the χ-glutamyl

group, catalyzed by χ-glutamyl transpeptidase, to release the free hydrazine [4-(hydroxymethyl) phenylhydra-zine], which is further oxidized to generate the 4-(hydroxymethyl) benzene diazonium ion. It is interesting to note that the mutagenicity of agaritine is much lower than that of its metabolite, 4-(hydroxymethyl)-benzene diazo-nium ion (Walton et al. 1997). The contribution of this pathway in the mutagenicity of ethanolic mushroom extracts (Papaparaskeva, et al. 1991) or the metabolism and/ or carcinogenicity of hydrazines in animals remain to be elucidated.

Experimental:

Analysis of gene sequences and homology buildup: The nucleotide sequences were obtained from the public domain of Entrez-Nucleotide (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide) and then translated into respective amino-acid sequences with the help of ORF (open reading frame). After translation the nucleotide sequences was analyzed in BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul et al., 1997). For the blast analysis, "nr" (non-redundant) parameter has been taken for consideration. In this parameter, sequences will be searched in GenBank, EMBL (European Molecular Biology Laboratory), DDBJ (DNA database of Japan), PDB (protein data bank) sequence databases, but not the EST (expressed sequence tag), STS (sequence tagged sites), GSS (genome survey sequence) or HTGS (high throughput genomic sequences) sequences. This may remove most of the repetitions.

Differential analysis of protein sequence of the genome: Physico-chemical parameters of a protein sequence (amino-acid and atomic compositions, *pI*, extinction coefficient, etc.) were determined by ProtParam tool (http://us.expasy.org/tools/protparam.html), which allowed the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered sequence. The computed parameters include the molecular weight, theoretical *pI*, amino acid composition, atomic composition,extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

PCR Primer design to clone the genes: Here we are trying to design primers for the PCR of the same genes. This is done with the help of algorithm based program Primer 3 developed by Massachusetts Institute of technology, USA. The sequences were uploaded through the site http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi and the out put (possible primer for the PCR of the respective genome) came from http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www_results.cgi

RESULTS AND DISCUSSION:

This paper describes the gene sequence of enzyme tyrosinase (polyphenol oxidase, PPO), which contains 1916 base-pairs. Its translation into corresponding amino acid sequence has also been analyzed and discussed through bio-informatic approaches. This enzyme is

responsible for the biosynthesis of melanin in humanbeing, animals and plants. In this genome, seq. no 1401-164 is 100% homologous with the 20732-20756 no seq. of *Mus musculus* chromosome 17; also similar homology were found with *Homo sapiens* chromosome 5 seq. no. 9567-9546, seq. no. 169714-169693, DNA seq. of chromosome 20 (pseudo gene of beta-1, 6- N- acetylglucosaminyl transferase, GCNT1, RNA polymerase III, 60s ribosomal protein L21, RPL21, a 40s ribosomal protein S19, RPS19; a gene for novel protein similar to retinoblastoma binding protein, RBBP9), chromosome 16 seq. no 91416-91396, chromosome 15 96567-96587, DNA seq. on chromosome 9 p21.1-22.3 of seq. no. 95554-95534.

The mRNA sequence of tyrosinase (Ebbelaar, et al., 1995), PPO1 gene:

```
   1 ccggcacgag cttgtttctt cagagtttcc atccgctctg tctccgcact ctcttgacca
  61 ttccactctt ttttttcttt tgatttagat gtctcatctg ctcgtttctc ctcttggagg
 121 aggcgttcaa cctcgtcttg aaataaataa ttttgtaaag aatgaccgtc aattctctct
 181 ttacgttcaa gctctcgacc ggatgtacgc cacccctcag aatgaaactg cgtcctactt
 241 tcaagtagct ggagtgcatg gatacccact catccctttc gatgatgcag tcggtccaac
 301 cgagttcagt ccttttgacc aatggactgg gtattgcact cacggctcaa ctctttttcc
 361 aacttggcat cgtccttatg ttttgattct cgaacaaatt ttgagtggac acgctcaaca
 421 aatcgccgat acttacactg tcaataaatc cgagtggaaa aaggcggcaa ccgaattccg
 481 tcatccgtat tgggattggg catctaatag cgttcctcct ccggaagtca tctccctacc
 541 caaagtcact atcacgactc cgaatggcca aaagacgagc gtcgccaacc cactgatgag
 601 gtatactttc aactctgtca acgacggcgg tttctatggg ccgtataatc agtgggatac
 661 tactttgaga caacccgact cgacgggtgt gaacgcaaag gataacgtta ataggcttaa
 721 aagtgttttg aaaaatgctc aagccagtct tacacgggct acttacgaca tgttcaaccg
 781 cgtcacgact tggcctcatt tcagcagcca tactcctgcg tctggaggaa gtaccagtaa
 841 tagtatcgag gcaattcatg acaatatcca tgtcctcgtc ggtggtaacg gccacatgag
 901 tgatccttct gtcgcccccc ttgatcctat cttcttcttg catcatgcga acgttgatcg
 961 actgattgct ttatggtcgg ctattcgtta cgatgtgtgg acttccccgg gcgacgctca
1021 atttggtaca tatactttga gatataagca gagtgttgac gagtcgaccg accttgctcc
1081 gtggtggaag actcaaaatg aatactggaa atccaatgaa ctgaggagca ccgagtcgtt
1141 gggatacact taccccgagt ttgttggttt ggatatgtac aacaaagacg cggtaaacaa
1201 gaccatttcc cgaaaggtag cacagcttta tggaccacaa agaggagggc aaaggtcgct
1261 cgtagaggat ttatcaaact cccatgctcg tcgtagtcaa cgccctgcga agcgctcccg
1321 ccttggtcaa ctcttgaaag ggttattctc ggattggtct gctcaaatca aattcaaccg
1381 ccatgaagtc ggccagagct tctcggtttg tcttttcctg ggcaatgttc ctgaagaccc
1441 gagggagtgg ttggttagcc ccaacttggt tggcgctcgt catgcgttcg tccgttcggt
1501 caagaccgac catgtagccg aggaaatagg tttcattccg attaaccagt ggattgccga
1561 gcacacgggt ttaccttcgt ttgcagtaga ccttgtaaaa ccactcttgg cacaaggttt
1621 acagtggcgc gtgctcttgg cggatggaac ccctgctgag ctcgattcac tggaagtgac
1681 tatattggag gtcccatccg agctgaccga cgatgagcct aatccccgct ccaggccgcc
1741 caggtaccac aaggatatta cacacggaaa gcgtggtggt tgccgcgagg cttgataggt
1801 gttattcatt ggacattgga cttgttgcta gaagtatata gataaagtta gcgtacatgg
1861 tttaattgat ttaccttgtt tgagaaaaaa aaaaaaataa tgntannaaa aaaaaa
```

Accession no: X85113, **Total no of bp:** 1916, **Base count:** 477a, 472c, 442g, 522t and 3 others

Translation of the mRNA into protein sequence:

mshllvsplgggvqprleinnfvkndrqfslyvqaldrmyatpqnetasyfqvagvhgyplipfddavgptefspfdqwtgycthgstlf
ptwhrpyvlileqilsghaqqiadtytvnksewkkaatefrhpywdwasnsvpppevislpkvtittpngqktsvanplmrytfnsvnd
ggfygpynqwdttlrqpdstgvnakdnvnrlksvlknaqasltratydmfnrvttwphfsshtpasggstsnsieaihdnihvlvggngh
msdpsvapfdpifflhhanvdrlialwsairydvwtspgdaqfgtytlrykqsvdestdlapwwktqneywksnelrsteslgytypefv
gldmynkdavnktisrkvaqlygpqrggqrslvedlsnsharrsqrpakrsrlgqllkglfsdwsaqikfnrhevgqsfsvclflgnvped
prewlvspnlvgarhafvrsvktdhvaeeigfipinqwiaehtglpsfavdlvkpllaqglqwrvlladgtpaeldslevtilevpseltdde
pnprsrppryhkdithgkrggcrea

BLASTn alignment analysis: 100% homologous with *Mus musculus* chromosome 17 clone RP24-413E14, complete sequence [score 50.1 bit and E value is 0.014]

```
Query: 140   gaaataaataattttgtaaagaatg 164
              |||||||||||||||||||||||||
Sbjct: 20732 gaaataaataattttgtaaagaatg 20756
```

96% homologous with *Octopus dofleini* hemocyanin gene, partial cds [score 48.1 bit and E value is 0.054]

```
Query: 930  tcttcttcttgcatcatgcgaacgttga 957
             |||||||||||||||| ||||||||||
Sbjct: 1950 tcttcttcttgcatcattcgaacgttga 1977
```

96% homologous with *Octopus dofleini* hemocyanin G-type subunit (Odhcy) mRNA, partial cds [score 48.1 bit and E value is 0.054]

```
Query: 930 tcttcttcttgcatcatgcgaacgttga 957
           |||||||||||||||| ||||||||||
Sbjct: 611 tcttcttcttgcatcattcgaacgttga 638
```

100% homologous with *Homo sapiens* chromosome 5 clone RP11-116O11, complete sequence [score 44.1 bit and E value is 0.84]

```
Query: 1092 ctcaaaatgaatactggaaatc 1113
             |||||||||||||||||||||
Sbjct: 9567 ctcaaaatgaatactggaaatc 9546
```

100% homologous with *Homo sapiens* chromosome 5 clone CTD-2074D8, complete sequence [score 44.1 bit and E value is 0.84]

```
Query: 1092   ctcaaaatgaatactggaaatc 1113
               |||||||||||||||||||||
Sbjct: 169714 ctcaaaatgaatactggaaatc 169693
```

100% homologous with Human DNA sequence from clone RP11-189K21 on chromosome 20. Contains the 5' end of a novel gene, a novel gene, a β-1,6-*N*-acetylglucosaminyl transferase (GCNT1) pseudogene, the gene for RNA

polymerase III subunit RPC39, a 60S ribosomal protein L21 (RPL21) pseudogene, a gene for a novel protein similar to retinoblastoma binding protein (RBBP9), a 40S ribosomal protein S19 (RPS19) pseudogene and the 5' end of the SEC23B gene for Sec23 (*S. cerevisae*) homolog B. Contains ESTs, STSs, GSSs and CpG islands, co> [score 44.1 bit and E value is 0.84]

```
Query: 142   aataaataattttgtaaagaat 163
              |||||||||||||||||||||
Sbjct: 52037 aataaataattttgtaaagaat 52058
```

100% homologous with *Homo sapiens* chromosome 16 clone RP11-351A20, complete sequence [score 42.1 bit and E value is 3.3]

```
Query: 1082  tggtggaagactcaaaatgaa 1102
              ||||||||||||||||||||
Sbjct: 91416 tggtggaagactcaaaatgaa 91396
```

96% homologous with *Mus musculus* chromosome 10 clone RP23-240G3, complete sequence [score 42.1 bit and E value is 3.3]

```
Query: 143   ataaataattttgtaaagaatgacc 167
              ||||||| ||||||||||||||||
Sbjct: 99160 ataaatattttgtaaagaatgacc 99184
```

100% homologous with *Homo sapiens* chromosome 15, clone RP11-41I15, complete sequence [score 42.1 bit and E value is 3.3]

```
Query: 141   aaataaataattttgtaaaga 161
              |||||||||||||||||||||
Sbjct: 23501 aaataaataattttgtaaaga 23481
```

100% homologous with *Homo sapiens* chromosome 15, clone RP11-616M17, complete sequence [score 42.1 bit and E value is 3.3]

```
Query: 141   aaataaataattttgtaaaga 161
              |||||||||||||||||||||
Sbjct: 96567 aaataaataattttgtaaaga 96587
```

100% homologous with Mouse DNA sequence from clone RP23-88O15 on chromosome 2, complete sequence [score 42.1 bit and E value is 3.3]

```
Query: 143    ataaataattttgtaaagaat 163
               |||||||||||||||||||||
Sbjct: 220530 ataaataattttgtaaagaat 220550
```

100% homologous with Human DNA sequence from clone RP11-330J23 on chromosome 9p21.1-22.3, complete sequence [score 42.1 bit and E value is 3.3]

```
Query: 144
taaataattttgtaaagaatg 164
|||||||||||||||||||||

Sbjct: 95554
taaataattttgtaaagaatg 95534
```

Differential analysis of protein sequence of the mRNA: Physico-chemical parameters (amino acid and atomic compositions, *pI*, extinction coefficient, etc.) of the protein sequence of the enzyme was performed with ProtParam tool (http://us.expasy.org/tools/protparam.html). The computed parameters include the molecular weight, theoretical *pI*, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

Number of amino acids: 568, **Molecular weight:** 63897.6, **Theoretical *pI*:** 6.58
Amino acid composition:

Ala (A)	37	6.5%	His (H)	20	3.5%
Ile (I)	21	3.7%	Leu (L)	48	8.5%
Lys (K)	22	3.9%	Met (M)	6	1.1%
Phe (F)	24	4.2%	Pro (P)	40	7.0%
Ser (S)	47	8.3%	Thr (T)	38	6.7%
Trp (W)	16	2.8%	Tyr (Y)	21	3.7%
Val (V)	41	7.2%	Asx (B)	0	0.0%
Glx (Z)	0	0.0%	Xaa (X)	0	0.0%
Arg (R)	32	5.6%	Asn (N)	30	5.3%
Asp (D)	32	5.6%	Cys (C)	3	0.5%
Gln (Q)	26	4.6%	Glu (E)	26	4.6%
Gly (G)	38	6.7%			

Total number of negatively charged residues (Asp + Glu): 58
Total number of positively charged residues (Arg + Lys): 54
Atomic composition:
C 2871, H 4362, N 798, O 847, S 9
Formula: $C_{2871}H_{4362}N_{798}O_{847}S_9$
Total number of atoms: 8887

Extinction coefficients: Conditions: 6.0 M guanidium hydrochloride, 0.02 M phosphate buffer, pH 6.5. Extinction coefficients are in Units/M/cm. The first table lists values computed assuming all Cys residues appear as half cystines, whereas the second table assumes that none do table lists values computed assuming all Cys residues appear as half cystines, whereas the second table assumes that none do.

276 nm	278 nm	279 nm	280 nm	282 nm
Ext. coefficient				
116995	119127	118925	118040	114920
FAbs 0.1% (=1 g/l)				
1.831	1.864	1.861	1.847	1.799

276 nm	278 nm	279 nm	280 nm	282 nm
Ext. coefficient				
116850	119000	118805	117920	114800
Abs 0.1% (=1 g/l)				
1.829	1.862	1.859	1.845	1.797

Estimated half-life: The *N*-terminal of the sequence considered is M (Met). The estimated half-life is 30 hours (mammalian reticulocytes, *in vitro*), >20 hours (yeast, *in vivo*), >10 hours (*Escherichia coli*, *in vivo*).

Instability index: The instability index (II) is computed to be 42.67. This classifies the protein as unstable. **Aliphatic index:** 74.82

Grand average of hydropathicity (GRAVY): -0.486

Active sites: From the analysis of the PDB structure, it was found that the active sites of the enzyme are [http://www.biochem.ucl.ac.uk/bsm/pdbsum/1bt1]:–

For chain A:Cu1-HIS A 88 HIS A 109 HIS A 118;Cu3-HIS A 240 HIS A 244 HIS A 274

For chain B: Cu2-HIS B 88 HIS B 109 HIS B 118; Cu4-HIS B 240 HIS B 244 HIS B 274

PCR Primer design to clone the genes: The output from the program Primer 3 showed the following primer to run the PCR experiments with this mRNA (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.results.cgi)(main primer) -OLIGO start len tm gc% any http://www-genome.wi.mit.edu/cgi-bin/primer/ primer3 www results help.cgi-PRIMER THREE 3, seq

LEFT PRIMER	1187	20	60.00	50.00	4.00	2.00
gacgcggtaaacaagaccat						
RIGHT PRIMER	1430	20	60.05	45.00	4.00	2.00
gaacattgcccaggaaaaga						
HYB OLIGO	1371	20	59.94	45.00	4.00	2.00
aattcaaccgccatgaagtc						

SEQUENCE SIZE: 1916, INCLUDED REGION SIZE: 1916

Some other primers are also suggested, they are:-

start len tm gc% any http://www-genome.wi.mit.edu/cgi-bin/primer/ primer3_www_results_help.cgi - PRIMER_THREE 3' seq

LEFT PRIMER	1189	20	60.00	45.00	4.00	0.00
cgcggtaaacaagaccattt						
RIGHT PRIMER	1430	20	60.05	45.00	4.00	2.00
gaacattgcccaggaaaaga						
HYB OLIGO	1371	20	59.94	45.00	4.00	2.00
aattcaaccgccatgaagtc						

PRODUCT SIZE: 242, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 2.00

LEFT PRIMER	1118	20	59.99	60.00	4.00	3.00
gaactgaggagcaccgagtc						
RIGHT PRIMER	1292	20	60.08	50.00	4.00	2.00
gacgagcatgggagtttgat						
HYB OLIGO	1187	20	60.00	50.00	4.00	2.00
gacgcggtaaacaagaccat						

PRODUCT SIZE: 175, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 1.00

LEFT PRIMER	1187	20	60.00	50.00	4.00	2.00
gacgcggtaaacaagaccat						
RIGHT PRIMER	1425	20	60.09	45.00	4.00	0.00
ttgcccaggaaaagacaaac						

HYB OLIGO 1371 20 59.94 45.00 4.00 2.00
aattcaaccgccatgaagtc
PRODUCT SIZE: 239, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 0.00
LEFT PRIMER 1131 20 59.99 55.00 5.00 1.00
ccgagtcgttgggatacact
RIGHT PRIMER 1292 20 60.08 50.00 4.00 2.00
gacgagcatgggagtttgat
HYB OLIGO 1187 20 60.00 50.00 4.00 2.00
gacgcggtaaacaagaccat
PRODUCT SIZE: 162, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 3.00

Geometry of the Enzyme from PDB:

Table 1: The dihedral angles of the PDB structure (chain A and B).

Chain 1BT1:A							
Angle	**total #**	**average**	**Stddev**	**min**	**at**	**max**	**at**
Phi	286	273.99	39.315	-41.53	PHE 114	2.85	GLU 256
Phi (PRO)	27	-64.71	16.746	-97.40	PRO 136	-3.84	PRO 175
Phi (GLY)	21	200.64	95.584	-51.95	GLY 130	63.67	GLY 94
Phi helix	106	-65.40	11.834	-124.54	CYS 92	-41.53	PHE 114
Psi	313	221.75	111.041	-0.85	TYR 91	0.37	ASN 303
Psi (GLY)	21	175.88	137.887	-7.25	GLY 95	5.32	GLY 259
Psi helix	106	-39.54	12.566	-82.99	PHE 114	-0.85	TYR 91
Omega	334	180.70	9.954	-0.57	VAL 13	175.31	SER 275
Chi1 g(-)	45	61.51	17.766	3.47	VAL 177	104.11	TYR 262
CHI1 trans	85	185.22	16.377	126.96	VAL 318	229.19	GLU 7
Chi1 g(+)	122	-67.57	14.714	-113.02	GLU 256	-32.42	ASP 295

Chain 1BT1:B							
Angle	**total #**	**average**	**Stddev**	**min**	**at**	**max**	**at**
Phi	286	273.43	40.105	-42.68	ALA 16	4.49	GLU 256
Phi (PRO)	27	-64.11	15.977	-83.98	PRO 136	-3.49	PRO 175
Phi (GLY)	21	200.86	95.193	-52.56	GLY 321	65.60	GLY 94
Phi helix	107	-66.17	11.524	-123.71	CYS 92	-46.30	PHE 81
Psi	313	227.51	108.976	-0.05	ASN 158	0.52	PRO 136
Psi (GLY)	21	161.29	138.132	-7.46	GLY 225	2.57	GLY 259
Psi helix	107	-38.47	12.700	-77.62	PHE 114	-1.01	GLN 286
Omega	334	180.57	9.963	-0.30	VAL 13	175.61	ILE 243
Chi1 g(-)	43	61.74	17.024	1.69	VAL 177	104.01	TYR 262
CHI1 trans	86	185.69	16.987	125.94	VAL 318	231.65	LYS 131
Chi1 g(+)	123	-67.81	14.671	-112.09	GLU 256	-22.81	SER 137

Table 2: The common bond angles of the PDB structure (chain A & B).

Chain 1BT1:A							
Angle	**Total #**	**average**	**stddev**	**min**	**At**	**max**	**at**
N-CA-C	288	110.81	3.895	99.55	ILE 3	127.06	GLU 256
N-CA-C (P)	27	112.55	3.913	105.01	PRO 29	125.95	PRO 175
N-CA-C (G)	21	114.11	4.073	107.66	GLY 233	120.75	GLY 259
N-CA-CB	207	110.30	1.517	105.28	ASN 144	115.40	TRP 145
N-CA-CB (A)	30	110.07	0.666	108.97	ALA 66	111.59	ALA 214
N-CA-CB (P)	27	102.32	0.732	100.79	PRO 50	103.71	PRO 239
N-CA-CB (I,T,V)	51	110.84	2.241	103.38	VAL 318	115.05	THR 297
CA-C-O	315	120.52	0.917	115.97	ALA 288	123.46	ASN 93
CA-C-O (G)	21	120.26	1.163	117.77	GLY 134	121.69	GLY 248
CA-C-N	286	116.60	1.288	111.87	ASN 255	122.18	GLU 256
CA-C-N (P)	27	116.99	1.266	114.02	PRO 228	119.99	PRO 175
CA-C-N (G)	21	116.92	1.486	115.09	GLY 130	120.59	GLY 134
CB-CA-C	234	110.14	1.858	105.51	PHE 306	116.52	ASP 257
CB-CA-C (A)	30	110.25	0.922	108.77	ALA 59	113.57	ALA 304
CB-CA-C (I,T,V)	51	110.39	1.927	105.01	ILE 133	113.92	VAL 316
O-C-N	227	122.81	0.972	120.65	ASN 80	125.52	GLN 107
O-C-N (P)	27	122.38	0.979	120.28	PRO 242	124.86	PRO 228
C-N-CA	286	121.60	1.768	109.74	ASP 257	126.45	SER 229
C-N-CA (P)	27	119.88	4.663	106.34	PRO 239	130.36	PRO 175
C-N-CA (G)	21	120.55	1.359	118.70	GLY 149	124.32	GLY 139
Chain 1BT1:B							
angle	**total #**	**average**	**stddev**	**min**	**at**	**Max**	**at**
N-CA-C	288	110.78	3.968	100.74	ILE 3	129.61	GLU 256
N-CA-C (P)	27	112.45	3.833	104.16	PRO 29	124.27	PRO 175
N-CA-C (G)	21	114.01	3.729	108.06	GLY 233	119.82	GLY 265
N-CA-CB	207	110.36	1.504	104.54	ASN 144	114.89	GLU 256
N-CA-CB (A)	30	110.25	0.578	109.21	ALA 334	111.50	ALA 90
N-CA-CB (P)	27	102.58	0.584	101.12	PRO 228	103.72	PRO 102
N-CA-CB (I,T,V)	51	110.84	2.081	102.85	VAL 318	116.04	VAL 190
CA-C-O	315	120.56	0.973	116.19	GLU 256	123.84	TRP 143
CA-C-O (G)	21	120.22	1.028	118.20	GLY 330	122.47	GLY 219

CA-C-N	286	116.62	1.313	111.81	GLN 107	122.15	GLU 256
CA-C-N (P)	27	116.92	1.417	113.85	PRO 154	120.00	PRO 175
CA-C-N (G)	21	117.02	1.020	115.34	GLY 21	118.48	GLY 94
CB-CA-C	234	110.19	1.843	105.42	PHE 306	117.00	ASP 257
CB-CA-C (A)	30	110.36	0.953	108.69	ALA 232	113.38	ALA 304
CB-CA-C (I,T,V)	51	110.48	1.826	106.57	THR 237	116.03	VAL 316
O-C-N	227	122.72	0.858	120.64	ASN 147	125.17	GLN 107
O-C-N (P)	27	122.56	0.752	121.31	PRO 175	124.59	PRO 228
C-N-CA	286	121.50	1.705	109.77	ASP 257	127.88	VAL 318
C-N-CA (P)	27	119.56	5.267	105.13	PRO 176	131.75	PRO 175
C-N-CA (G)	21	120.38	1.008	118.25	GLY 95	121.82	GLY 313

Table 3: The bond lengths of the PDB structure (chain A and B).

Chain 1BT1:A							
bond	**Total #**	**average**	**stddev**	**min**	**at**	**max**	**at**
C-N	307	1.33	0.007	1.31	THR 161	1.35	LEU 153
C-N (PRO)	27	1.34	0.007	1.32	PRO 228	1.35	PRO 116
C-O	336	1.23	0.010	1.20	ILE 133	1.27	ALA 288
CA-C	315	1.52	0.017	1.46	GLU 256	1.56	THR 197
CA-C (GLY)	21	1.52	0.012	1.49	GLY 150	1.53	GLY 248
CA-CB	234	1.52	0.012	1.49	PRO 175	1.57	ASN 260
CA-CB (ALA)	30	1.51	0.023	1.47	ALA 304	1.55	ALA 90
CA-CB (I,T,V)	51	1.54	0.021	1.50	ILE 128	1.60	VAL 177
N-CA	288	1.46	0.012	1.43	VAL 208	1.51	ARG 294
N-CA (GLY)	21	1.45	0.007	1.43	GLY 225	1.46	GLY 259
N-CA (PRO)	27	1.46	0.010	1.44	PRO 230	1.49	PRO 239
Chain 1BT1:B							
Bond	**total #**	**average**	**stddev**	**min**	**at**	**max**	**at**
C-N	307	1.33	0.007	1.30	TRP 143	1.35	PHE 306
C-N (PRO)	27	1.34	0.009	1.32	PRO 339	1.36	PRO 154
C-O	336	1.23	0.011	1.20	VAL 108	1.27	LEU 341
CA-C	315	1.52	0.017	1.46	GLU 256	1.56	SER 275
CA-C (GLY)	21	1.51	0.011	1.49	GLY 225	1.54	GLY 219
CA-CB	234	1.53	0.012	1.49	PRO 175	1.57	LEU 287
CA-CB (ALA)	30	1.51	0.023	1.46	ALA 1	1.55	ALA 186
CA-CB (I,T,V)	51	1.55	0.022	1.50	VAL 23	1.60	VAL 318
N-CA	288	1.46	0.012	1.42	SER 162	1.52	ARG 294
N-CA (GLY)	21	1.45	0.007	1.44	GLY 95	1.46	GLY 130
N-CA (PRO)	27	1.46	0.008	1.45	PRO 29	1.48	PRO 19

CONCLUSION: The gene sequence of tyrosinase contains 1916 base-pair. Its translation into corresponding amino acid sequence has been analyzed and discussed approaches. These studies will be helpful to develop new drugs against different disease conditions related to tyrosinase.

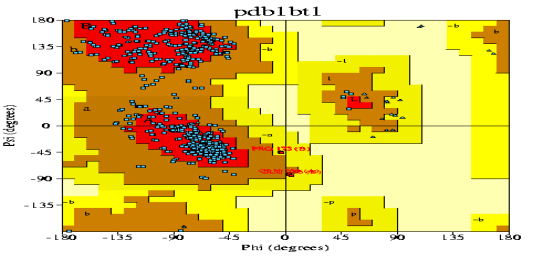

Figure-1: Ramachandran plot for the PDB structure of 1BT1

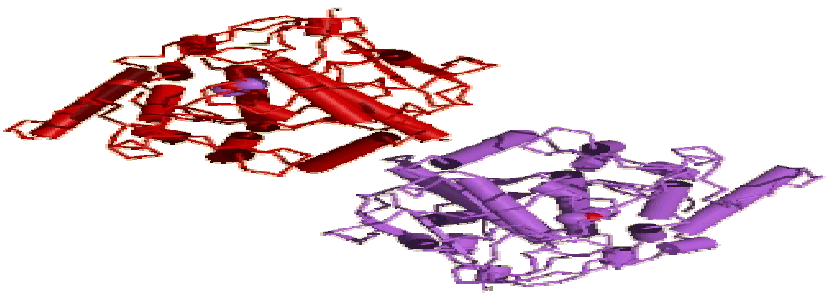

Figure-2: Three-dimensional cartoon structure of tyrosinase enzyme, in red color A chain (up) and in purple color B chain (down http://www.biochem.ucl.ac.uk /bsm/pdbsum/1bt1/tracel_r.html].

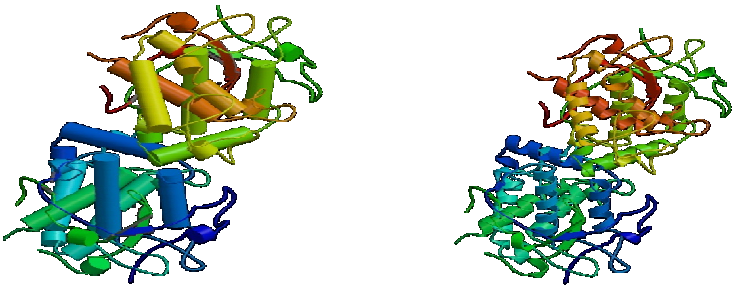

Figure-3: Protein data bank (PDB, 1BT1) structure of tyrosinase enzyme in cylinder (left) and ribbon (right) forms [http://www.rcsb.org/pdb/.

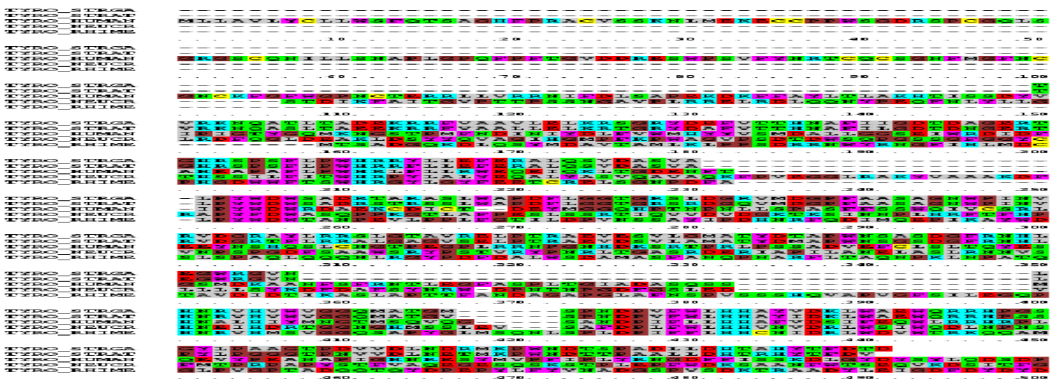

Figure 4: Comparisons of sequences of tyrosinase enzyme from different sources.

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