Comparative Study of Tryptophan Synthase Beta Chain between *Pyrococcus furiosus* (Archaea) and *Salmonella typhimurium* (Proteobacteria)

Satpal Singh Bisht, Santosh Kumar Behera, and Amrita Panda

***Abstract*—The present investigation deals with comparative study of beta chain of the enzyme tryptophan synthase from *Salmonella typhimurium and Pyrococcus furiosus.* The study yielded few significant results that the sequence of beta 2 subunit (PF1706) from *Pfu* is closely related to the beta chain of *Salmonella typhimurium* in comparison to the beta 1. The phylogenetics of these two strains indicates that the beta 2 subunit plays a vital role in tryptophan synthesis in *Pyrococcus furiosus*. In the present investigation *Salmonella typhimurium* was used as a reference organism for comparative genomics of tryptophan synthase beta chain amongst a group of organisms 13 archaea and 41 proteobacteria respectively. The study reveals that most of the sequences of archaea are distantly related and was observed that there are certain sequences like pfu PF1706 which are closer to *Stm* that is a proteobacteria.**

***Index Terms*—Chorismate, Pyridoxal phosphate, Hyperthermophile, shikimate pathway**

I. Introduction

In the *Shikimic acid pathway* Chorismate is a key intermediate in the biosynthesis of the aromatic amino acids (*phenylalanine, tyrosine and tryptophan*) [1, 2]. The synthesis of these amino acids is not fully understood. Recent studies on these pathways identified a number of alternative cross-regulated biosynthetic routes with unique evolutionary origins [3]. Chorismate is the substrate for a number of enzymes involved in the biosynthesis of aromatic compounds making it a key branching point for secondary metabolites of the shikimate pathway [4]. There is a large group of plant secondary metabolites mainly originated from shikimic acid pathway [5]. Due to this, chorismate is often considered a limiting factor in the formation of tryptophan, with much of the compound being utilized for a variety of other metabolites. Plants of Anacardiaceae family specifically Syrian and *Chinese sumac* fruits were found to have eighteen amino acids including eight essential amino acids (leucine, isoleucine, lysine, methionine, threonine, phenylalanine, valine and tryptophan) and ten non-essential amino acids [6].

Tryptophan is an essential amino acid to human [7] as the human cells do not synthesize tryptophan therefore it needs to be supplied from out side. There are reports on fish proteins having higher (70-85 mg/g protein) essential amino acid index [8] and [9]. Shellfish have a balanced distribution of all essential amino acids required by an adult per day and have been reported from this fish protein [10]. Tryptophan is synthesized in bacteria, *E.coli,* by a five-step biochemical pathway from chorismate. There are five enzymes that are involved in the biosynthesis of tryptophan and there are five genes in the tryptophan operon in *E.coli* encoding polypeptide chains, which organize themselves to make these five enzymes. [11].

Tryptophan synthase catalyzes the last step of the Tryptophan biosynthetic pathway that involves conversion of Indole-3-glycerol phosphate and Serine to Tryptophan and water. It is a bifunctional, bienzyme complex having two α chains and two β chains that forms the $\alpha_2\beta_2$ tetrameric enzyme complex. The α subunit is an αβ barrel protein composed of a central core of eight parallel β strands with eight parallel α helices packed around the periphery of the barrel. In most of the organisms α chain is encoded by the gene trpA and β chain is encoded by the gene trpB. Structure and function of the monovalent cation site tryptophan synthase from *Salmonella typhimurium* plays an essential role in catalysis and in the regulation of substrate channeling studies [12]. Initially the 3D structure of $\alpha_2\beta_2$ complex of tryptophan synthase from *Salmonella typhimurium (Stm)* has been determined by X–ray crystallography at 2.5 resolutions. The four polypeptides are arranged linearly in αββα order forming a complex 150A˚ long. The length of α subunit is 268 amino acid residues, where as the length of the β subunit is 397 amino acid residues. The total length of the αβ dimer is 665 amino acid residues. The α subunits are smaller than β subunits.

The active site of each α subunit is located near the interface with the subunit, where as the active site of the β subunit is deeply buried in the center of the β subunit [13]. The two active sites of neighboring α and β subunits are separated by a distance of about 25-30 A˚. The β subunit has two domains - N domain and C domain. Part of the N-domain of each β subunit interacts with part of the C domain of the complementary β subunit. The crystal structure of the *Tryptophan synthase* β_2 subunit (Pfb2) from a hyperthermophile, *Pyrococcus furiosus (Pfu),* was determined by X-ray crystallographic analysis at 2.2 A˚ resolution on the similar aspects [14]. The crystal structure of β_2 subunit from *Tryptophan synthase of Pyrococcus furiosus* represented a dimer having two β subunits those are tightly associated in a broad space.

II. Material and Methods

The present investigation was carried out from 10th

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October 2010 to 15th January 2011. The amino acid sequences of both beta 1 and beta 2 subunits of *Tryptophan synthase* were taken from KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database. The phylogenetic analysis was done by taking all the three sequences (beta 1, beta 2 of *Pyrococcus furiosus* and beta subunit of *Salmonella typhimurium)* obtained from PDB (Protein data bank) The amino acid sequence of beta 2 subunit (PF1706) from *P.fu* was obtained from KEGG. Pair wise alignment was done with the amino acid sequence of beta subunit of *S.tm* using BLAST 2 (Basic Local Alignment Search Tool) programme with parameters Matrix: Blosum62, Gap open penalty: 11, Gap extension penalty: 1 [15]. The sequence homology between *Pfu* beta 2 and *Stm* beta was 58 %. The alignment indicates that 6 residues in the N-terminal domain and 3 residues from C-terminal domain were deleted in *Pfu* beta 2 subunit. Pro366 of Stm and Ile63 of *Pfu* beta were inserted in each protein.

Score=470bits (1209), Expect (E value) =e-131, Identities = 230/391 (58%),
Positives=301/391(76%), Gaps=5/391(1%).

Multiple sequence alignment was performed by loading amino acid sequence of Tryptophan synthase beta chain from 41 proteobacteria and 13 archaea in ClustalX programme. This was performed using the default values to find out the conserved residues that take active part in Tryptophan synthase beta chain. The amino acid sequence of *Stm* was considered as the standard sequence as its crystal structure was known earlier then the crystal structure of Pfu beta 2.

III. Results

It was observed from the phylogenetic analysis (Fig.1) that sequence of beta,2 subunit (PF1706) from *Pfu* is closely related to beta chain of *Salmonella typhimurium*. The result of phyllogenetic analysis states that the beta 2 subunit plays a vital role in tryptophan synthesis of *Pyrococcus furiosus,* from the sequence analysis the amino acid composition of both monomers of Pfu β_2 and Stm β was found. The Pfuβ_2 consists of 388 aa residues, but Stm β has 397. The content (%) of hydrophobic residues for Pfu beta was similar to as of for the Stm beta though the number of hydrophobic residues of Pfu beta was slightly lowered the number of hydrophilic residues increased from 110 to 121 in Pfu beta 2, compared to Stm beta chain.

The number of neutral residues of Pfu beta 2 was largely reduced from 73(in Stm beta) to 57 residues. It was also observed from the analysis that there are considerable amount of conservations seen in the amino acid residues (80 conserved positions, which are represented in the form of *,•, & :) here the star (*)represents completely conserved positions, the dot (•) represents mostly conserved residues and the colon (:) represents acceptable substitutions [16]. It was also observed from the results that there are 32 completely conserved positions and 25 acceptable substitutions and 23 mostly conserved. Apart from these 55R, 378G, 380G were also completely conserved except in the organisms i.e. *Pyrobaculum aerophilum* PAE0257 (pai) and *Buchnera aphidicola* (Buc) respectively.

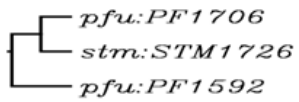

Fig. 1. Phylogenetic relationship between beta 1, beta 2 subunit of *Pfu* and beta subunit of *S.tm*.

The cladograms were constructed, to find the evolutionary relationships among these 13 archaeas and 41 proteobacterias with respect to amino acid sequence of beta chain of tryptophan synthase. It was found that most of the amino acid sequences which belong to archaeal group are located on one side of the tree where as the amino acid sequences that belong to proteobacterial group are located on the other side. This depicts the close relation ship within a particular group of organisms. It was found that some of the amino acid sequences of archaeal group like Pfu PF1706, pab PAB2048, hpy HP1278, mja MJ1037, mth MT1659, afu AF1600, were located towards the side where all the sequences of proteobacteria are presented. This depicted the closeness among the archaeal group and proteobacterial group with respect to the amino acid sequence of tryptophan synthase beta subunit. (Fig. 2).

The phylogenetic tree shows the archaeal organisms are placed at single branch of the tree and it was also found that the sequences of these organisms are found to be close with the sequence of the cjeCj0348 belonging to proteobacteria, which was found to be distantly related with the *Stm* in comparison to other. Therefore it can be concluded that most of the sequences of archaea are distantly related. It was also reported there are certain sequences like pfu PF1706 which was found to be closer with *Stm* which is a proteobacteria. Hence from this it was found that sequences of archaea are similar to sequences of proteobacteria (Fig. 3).

IV. Discussion

Present investigation has been correlated with the many studies made by various scientists, [17-28]. The studies supports the findings of the present study specially the sequence and structure analyzed against the standards taken (Pfu beta 2 subunit with Stm beta subunit of tryptophan synthase) for sequence analysis and structure prediction. The comparative sequence analysis of beta chain was performed for proteobacteria and archaea with reference to the structure and sequence analysis of beta subunit of *Tryptophan synthase* from *Salmonella typhimurium* and *Pyrococcus furiosus*, and the conserved residues were found.The residues Gly232, Gly233, Gly234, Ser 235 and Asn 236 (between strand 7 and helix 9 in stm beta subunit) that corresponds to Gly227, Gly228, Gly229, Ser230 and Ala232 (residues between strand 7 and helix 10 in pfu beta 2 subunit) are conserved in all archaeal and proteobacterial group. This is where the phosphate group of the co-enzyme is highly ligated through hydrogen bonds with the peptide backbone atoms of these residues. The residue His 86 (before N terminal helix3in Stm) that corresponds to residue His 81 (before N terminal end of helix3 in Pfu) was found conserved in all the organisms**,** as the negative charges on phosphate may be neutralized by the imidazole of His 86 and the positive end of

a dipole from helix 9. The residue Lys87 (at the N-termini of helix3 in stm) that corresponds to Lys82 (at the N-termini of helix3 in Pfu beta 2 subunit) was found conserved in both the groups. These are the residues where the PLP forms a covalent bond with ε-amino group of Lys87 of beta subunit. The residues 303Gly, 304Leu (between strand 8 and helix 10 in Stm) residues 298Gly, 299Leu (residues between strand 8 and helix 11 in Pfu) are conserved in all the organisms as these residues fold in a complicated manner and apparently lack any well defined secondary structural elements and these residues are involved in the formation of wall of the tunnel.

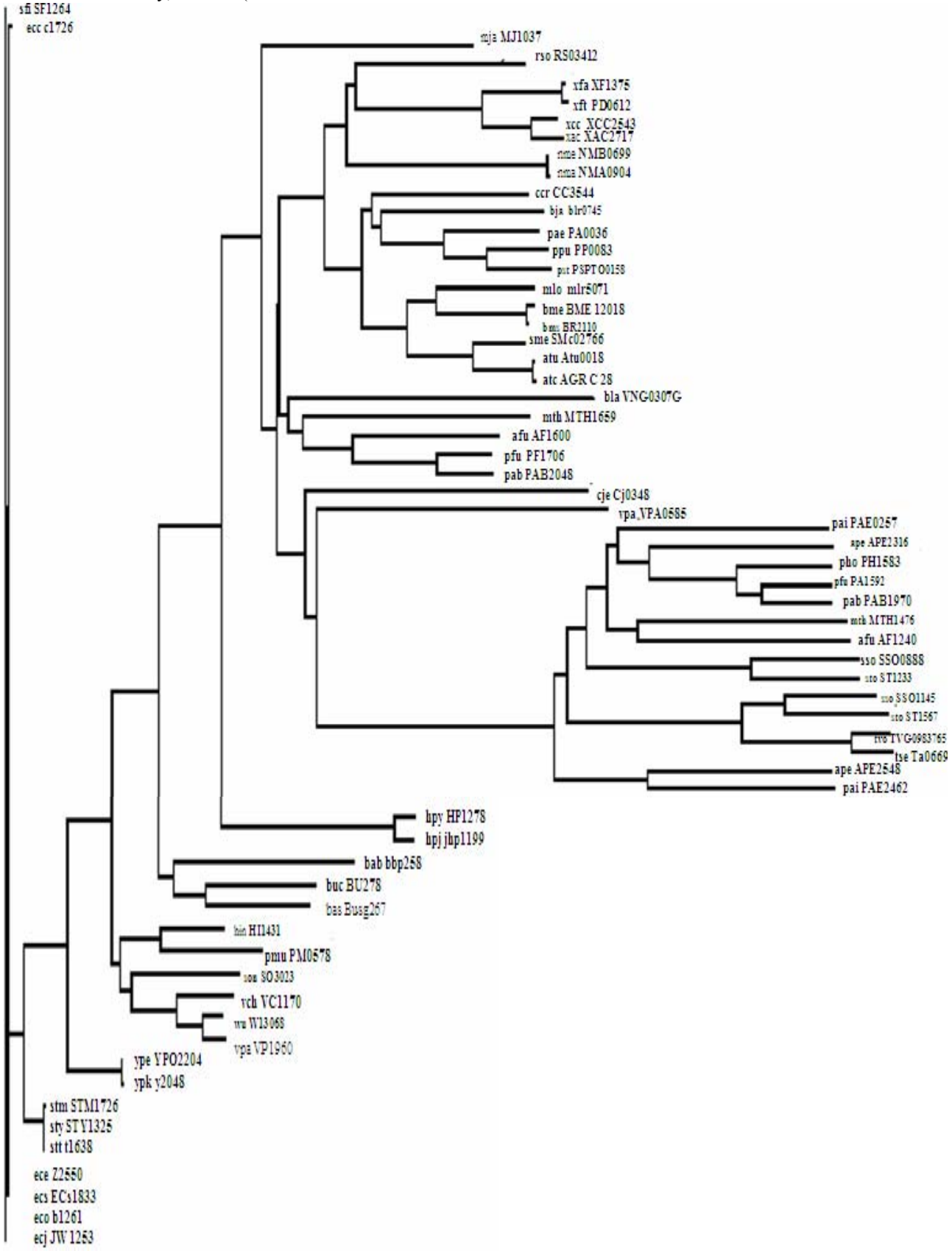

Fig. 2.Phylogenetic tree view of amino acid sequences of beta subunit from 13 archaea and 41 proteobacterias.

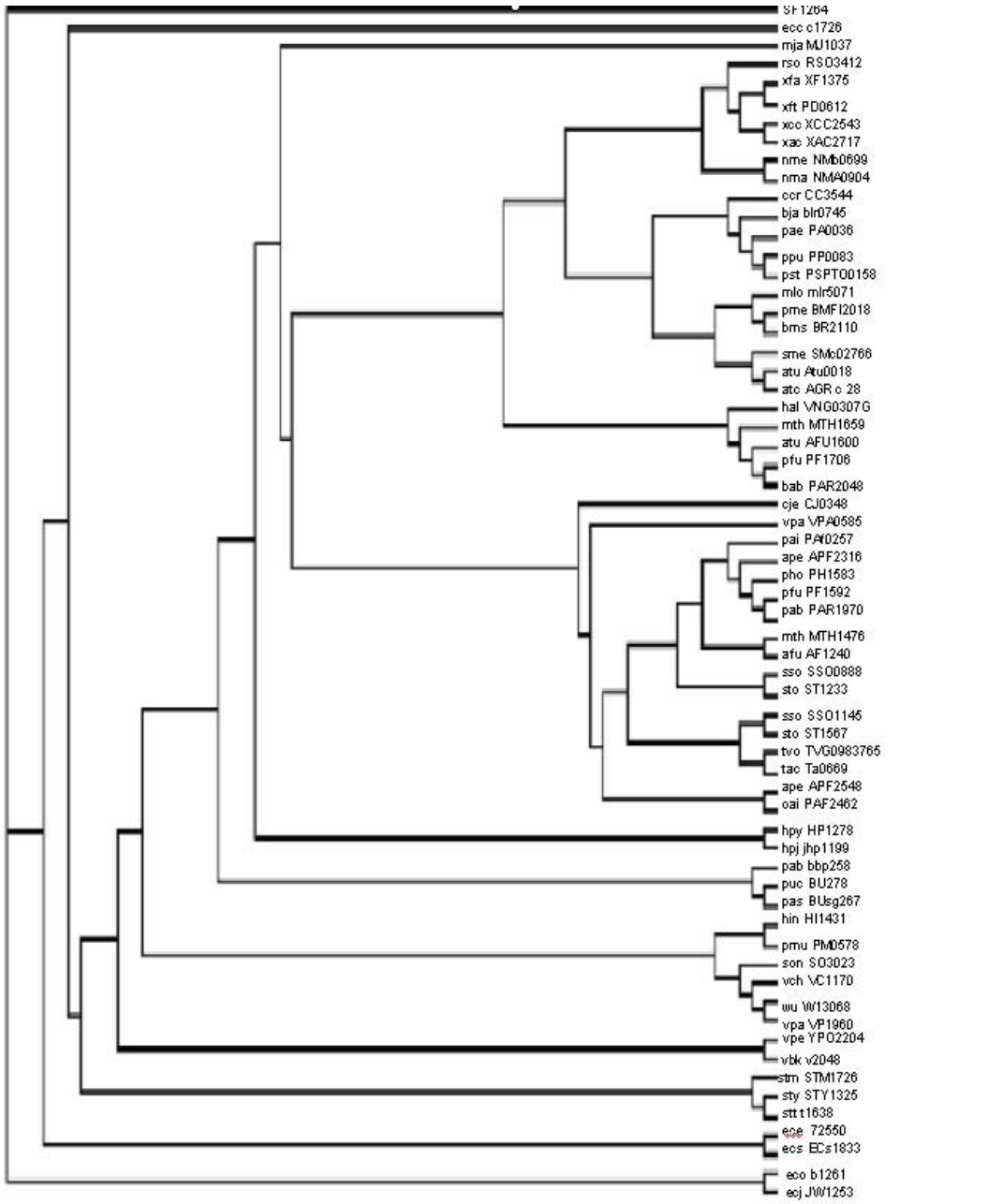

Fig. 3. Rectangular cladogram of amino acid sequences of beta subunit from 13 archaeas and 41 proteobacterias.

The residues 186Y, 189G (between helix 6 and strand 6 in Stm) that corresponds to 181Y, 184G (residues between strand 6 and helix 8 in Pfu beta 2 subunit) are conserved as they are involved in formation of wall of the tunnel. The residues 86His, 87Lys, 89Asn, 109Glu, 110Thr, 111Gly, 112Ala, 113Gly, 116Gly (residues from helix 3,strand 3in Stm) that corresponds to residues 81His, 82Lys, 84Asn, 104Glu, 105Thr, 106Gly, 107Ala, 108Gly, 109Gln, 111Gly, 117Ala (residues from helix 3,strand 3, and helix 4 in Pfu beta 2 subunit) are found to be conserved in both the groups, as these residues are structurally similar with the residues 204Phe, 209Gly, 229Ala, 232Gly, 233Gly, 234Gly, 235Ser, 236Asn, 259Gly (residues including helix 8 to strand 8 along with strand 7,helix 9 in Stm) that corresponds to 206Glu, 210Gln, 227Gly, 228Gly, 229gly, 230Ser, 231Asn (residues including helix 9 to helix 10 in Pfu beta 2 subunit) . As the above residues are useful for its catalytic and substrate binding functions, these residues have important role in tryptophan synthase in all organisms of both the groups. Hence these residues are found conserved in all organisms. The comparative study of tryptophan synthase in both the groups depicted the common functionality in both the groups although there was difference in sequence lengths. The phylogenetic trees depicted the closeness among the sequences between two groups. Although there was a difference in sequence length and residue position, the comparative study indicates that the functional important of tryptophan synthase is highly conserved in both the groups. The superimposition of Pfu beta 2 subunit with Stm beta subunit of tryptophan synthase depicted the structural similarity.

V. Conclusion

The correlation of structural analysis of the tryptophan synthase with theoretical sequence analysis helps in understanding the structure of tryptophan synthase in different organisms. This correlation helps in comparing the sequence and structure of tryptophan synthase in unrelated

groups of organisms. The conserved residues obtained from multiple sequence alignment studies are found to be significantly conserved in both the groups of organisms. The pair-wise alignment had shown a high bit score with low e-value as well as low bit score with high e-value in both the groups. Thus e-value is inversely proportional to the score. This indicates the significant similarity and dissimilarity among the sequences in both the groups. The comparative study of tryptophan synthase in both the groups depicted the common functionality in both the groups although there was difference in sequence length. The phylogenetic trees depicted the relatedness among the sequences between two groups. Though there was a difference in sequence length and residue position, the comparative study indicates that the functional importance of the enzyme tryptophan synthase is highly conserved in both the groups and the superimposition of Pfu beta 2 subunit with Stm beta subunit of tryptophan synthase shows the structural similarity.

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