# In Silico Characterization of Fatty Acid Synthase of Mycobacterium tuberculosis H37Rv

C Kumar, C Anuradha, K Rao, K Venkateswara Swamy

## Abstract

A study was carried out to characterize fatty acid synthase (FAS) protein of Mycobacterium tuberculosis H37Rv by in silico methods using various bioinformatics tools. Functional characterization of FAS protein was done by submitting the sequence to InterProscan which revealed the presence of Acetyl transferase, beta-keto-acyl synthase and MaoC dehydratase domains. Based on the presence of domains the FAS protein of M. tuberculosis was split into 3 subunits and correspondingly the amino acid sequence of each subunit were used to generate tertiary structure using online tool at 3D PSSM server. For the first subunit of FAS protein the 3DPSSM suggest that this protein belongs to Acyl transferase fold and malonyl-coenzyme acyl carrier protein family. MaoC dehydratase structure predicted by 3DPSSM server assigned lyase fold followed by assigning enoyl-coA hydratase superfamily and hydratase protein to MaoC dehydratase sequence.

## INTRODUCTION

It is well known fact that Tuberculosis (TB) is a leading infectious disease responsible for death and represents more than a quarter of the world's preventable deaths (Cole et al, 1998). Mycobacterial cell wall contains unique lipids such as mycolic acids, very long chain fatty acids and multimethyl-branched fatty acids (Kikuchi et al., 1992). According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2002), there are two discrete enzyme systems in the biosynthesis of fatty acids in mycobacteria, namely fatty acid synthase (FAS) I and II (Cole, et al., 1998, Kolattukudy, et al., 1997, Kanehisa et al., 2002). The FAS I system consists of a single polypeptide with multiple catalytic activities that creates short precursors for elongation by other fatty acid systems (Cole, et al, 1998). In fatty acid elongation system the FAS-II is involved in the biosynthesis of mycolic acids, which are major and specific long-chain fatty acids of the cell envelope of M. tuberculosis and other mycobacterium (Ducasse-Cabanot, et al., 2004). The protein MabA, also named FabG1, has been shown recently to be part of FAS-II and to catalyse the NADPH-specific reduction of long chain beta-ketoacyl derivatives whose activity corresponds to the second step of an FAS-II elongation round (Ducasse-Cabanot, et al., 2004). The FAS II system includes a host of enzymes involved in the elongation of substrates bound to an acyl-carrier protein to produce mycolic acids (Cole, et al, 1998). Further it has been

demonstrated that multifunctional fatty acid synthase (FAS) is having the unique capability of catalyzing both de novo synthesis and chain elongation of fatty acids (Kikuchi, et. al., 1992). Mycolic acids consist of long-chain alpha-alkyl beta-hydroxy fatty acids that are produced by successive rounds of elongation catalyzed by a type II fatty acid synthase. It is a well known fact that FAS is the primary enzyme responsible for the synthesis of fatty acids in many organisms . Genes such as AcpM (Acyl carrier protein), kasA (beta-keto acyl synthase), kasB (beta-keto acyl synthase), accD6 (Acetyl-CoA carboxylase), fbpc (trehalose dimycolyl transferase), fadE24 (Fatty acyl-CoA dehydrogenase), ahpc (Alkyl hydroperoxide reductase), efpA (Efflux protein) are responsible for fatty acid synthase (Anonymous). All of the reactions of fatty acid synthesis are carried out by the multiple enzymatic activities of FAS which are beta-keto-ACP synthase (condensing enzyme), beta -keto-ACP reductase, 3-OH acyl-ACP dehydratase and enoyl-ACP reductase (Michael, 2003). Acyl carrier protein synthase (acpS) catalyzes the formation of holo-ACP, which mediates the transfer of acyl fatty-acid intermediates during the biosynthesis of fatty acids and lipids (Chopra, et al., 2002).

From these studies it has been inferred that there is not much information available on characterization of FAS protein sequence of M. tuberculosis that is involved in mycolic acid synthesis using bioinformatics tools. Hence the focus of

present work is to characterize FAS protein (NP_217040) of M. tuberculosis H37Rv by using online and off-line computational tools.

## METHODOLOGY

### SEQUENCE ANALYSIS OF FAS

M. tuberculosis H37Rv strain was selected as the candidate organism for the present study whose complete genome sequence (gi No. 57116681) is available at http://www.ncbi.nlm.nih.gov. The protein sequence of FAS (NP_217040) was downloaded from NCBI (http://www.ncbi.nlm.nih.gov). FAS protein sequence was searched for similarity search using blast-P against PDB database at http://www.ncbi.nlm.nih.gov. Since the BLAST algorithm detects local as well as global alignments, regions of similarity embedded in otherwise unrelated proteins can be detected (Altschul, 1990). The derived homologues sequences of FAS protein were aligned using ClustalW (Chenna, 2003). ClustalW calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. The clustal alignment file of the selected sequences was used for the basic parameter for further creating the phylogenetic tree with FAS protein as query sequence.

### FUNCTIONAL CHARACTERIZATION OF FAS

Functional characterization of FAS protein sequence was done by submitting the amino acid sequence of FAS protein to Prosite (http://au.expasy.org/prosite/) and InterProscan (http://www.ebi.ac.uk/InterProScan/). InterProscan is a searchable database providing information on sequence function as well as annotation and further, these sequences are grouped based on protein signatures (Apweiler, et al., 2001). Prosite is a database of protein families and domains (Falquet et al., 2002). The output of Prosite and interProscan was recorded in terms of the length of amino residues of FAS protein with specific functional domain. Further, the results that were obtained from both Prosite as well as InterProscan were compared for better interpretation.

### SECONDARY AND 3D STRUCTURE OF FAS COMPLEX

The secondary structure of FAS protein was obtained from Jpred (http://www.compbio.dundee.ac.uk/~www-jpred/) by submitting the sequence which predicts secondary structure using a neural network called Jnet (Cuff and Barton, 2000). The secondary structure prediction is the definition of each residue into either alpha helix, beta sheet or random coil secondary structures. Analysis of IinterProscan results

suggest that FAS protein as comprising of 3 different subunits such as B_ketoacyl synthase, Acyl transferase, MaoC-like dehydratase. Hence to generate 3D structures of functional domains of FAS protein, the amino acid sequence of each subunit of FAS protein (as suggested by InterProscan results) was submitted to 3DPSSM server (http://www.sbg.bio.ic.ac.uk/~3dpssm/), which is a automatic fold recognition server for predicting the 3D structure (Lawrence Kelley, et al (2000). Thus generated 3D structures of B_ketoacyl synthase, Acyl transferase, MaoC-like dehydratase were visualized by RASMOL (Sayle and Milner, 1995).

## RESULTS

The amino acid sequence of FAS protein was retrieved from NCBI and blast-P program was used to find out the sequences that shared structure and sequence similarity against PDB database (Table 1). The result suggest that protein sequence with PDB ID of IJ3N A, which belongs to 3-Oxoacyl- (Acyl-Carrier Protein) Synthase II of T. thermophilus was having the highest degree of similarity to FAS protein as query sequence, indicated through E-value.

### Figure 1

Table 1: Summary of blast-P () of FAS protein sequence when searched against PDB database

| Subject ID | % identity | Mis match | Query start | Query end | Subject start | Subject end | E value | Bit score |
|---|---|---|---|---|---|---|---|---|
| gi|29726335|pdb|1J3N|A | 32.4 | 155 | 2686 | 2948 | 124 | 373 | 4.8e-15 | 81.65 |
| gi|4557950|pdb|1KAS| | 29.53 | 156 | 2696 | 2946 | 140 | 373 | 3.7e-11 | 68.55 |
| gi|13096639|pdb|1E5M|A | 26.86 | 157 | 2707 | 2947 | 154 | 378 | 2.0e-09 | 63.16 |
| gi|6573501|pdb|1DD8|A | 24.51 | 224 | 2607 | 2950 | 42 | 370 | 3.0e-09 | 62.39 |
| gi|10120827|pdb|1FJ4|A | 24.51 | 224 | 2607 | 2950 | 42 | 370 | 3.1e-09 | 62.39 |
| gi|30750044|pdb|1OX0|A | 23.98 | 162 | 2692 | 2936 | 156 | 379 | 8.6e-09 | 60.85 |
| gi|14278633|pdb|1F91|A | 25.88 | 137 | 2727 | 2950 | 170 | 370 | 8.8e-06 | 50.83 |
| gi|14278623|pdb|1EK4|A | 25.65 | 135 | 2727 | 2950 | 182 | 382 | 9.1e-06 | 50.83 |
| gi|1431717|pdb|1MLA| | 37.37 | 49 | 1432 | 1530 | 86 | 171 | 0.005 | 41.59 |
| gi|28948376|pdb|1IO6|A | 30.91 | 67 | 1226 | 1330 | 29 | 134 | 0.016 | 40.05 |
| gi|21730255|pdb|1GX3|A | 32.20 | 28 | 941 | 999 | 136 | 182 | 5.7 | 31.57 |

Further, FAS protein was subjected to ClustalW for multiple sequence alignment and phylogram analysis. The result suggests that FAS protein of M. tuberculosis was very much similar to M. bovis than other mycobacterium (Figure 1).

### Figure 2

Figure 1: Phylogram analysis of query (FAS protein of ) through ClustalW.
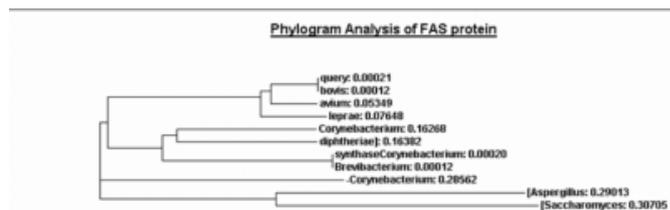


The function of FAS protein of M. tuberculosis was analyzed by submitting the amino acid sequence to Prosite

and InterProscan servers. Prosite analysis suggested the functionality of FAS protein with domains identified for characteristic functionality (Table 2 A). Based on InterProscan results, the important domains such Acyl transferase, Beta-keto acyl synthase and MaoC dehydratase were assigned to FAS protein amino acid sequence correspondingly (Table 2 B).

Table 2 A : Functional characterization of FAS protein of M. tuberculosis H37Rv at Prosite (http://au.expasy.org/prosite/)

Prosite tool

(i) RGD Cell attachment sequence Sequence length: 2804 – 2806 Function : The sequence Arg-Gly-Asp, found in fibronectin, is crucial for its interaction with its cell surface receptor, an integrin. What has been called the 'RGD' tripeptide is also found in the sequences of a number of other proteins, where it has been shown to play a role in cell adhesion. These proteins are: some forms of collagens, fibrinogen, vitronectin, von Willebrand factor (VWF), snake disintegrins, and slime mold discoidins.

(ii) ATP_GTP_A ATP/GTP-binding site motif A (P-loop) Sequence length: 2080 - 2087 Function : From sequence comparisons and crystallographic data analysis it has been shown that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha helix. This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence or the 'P-loop'.

There are numerous ATP- or GTP-binding proteins in which the P-loop is found. We list below a number of protein families for which the relevance of the presence of such motif has been noted:

- ATP synthase alpha and beta subunits, Myosin heavy chains, Kinesis heavy chains and kinesin-like proteins, Dynamins and dynamic-like protein, Guanylate kinase, Thymidine kinase, Thymidylate kinase, Shikimate kinase, Nitrogenase iron protein family, ATP-binding proteins involved in 'active transport' (ABC transporters), DNA and RNA helicases, GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF- 2, etc.), Ras family of GTP-binding proteins (Ras, Rho, Rab, Rally, Ypt1, SEC4, etc.), Nuclear protein ran, ADP-ribosylation factors family, Bacterial dnaA protein, Bacterial recA protein, Bacterial recF protein,

Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.), DNA mismatch repair proteins mutS family, Bacterial type II secretion system protein E.

(iii) LEUCINE_ZIPPER Leucine zipper pattern Sequence length: 400 - 421 Function : A structure, referred to as the 'leucine zipper', has been proposed to explain how some eukaryotic gene regulatory proteins work. The leucine zipper consist of a periodic repetition of leucine residues at every seventh position over a distance covering eight helical turns. The segments containing , these periodic arrays of leucine residues seem to exist in an alpha-helical conformation. The leucine side chains extending from one alpha-helix interact with those from a similar alpha helix of a second polypeptide, facilitating dimerization; the structure formed by cooperation of these two regions forms a coiled coil. The leucine zipper pattern is present in many gene regulatory proteins, such as:

CCATT-box and enhancer binding protein (C/EBP), cAMP response element (CRE) binding proteins (CREB, CRE-BP1, ATFs), Jun/AP1 family of transcription factors, yeast general control protein GCN4, fos oncogene, and the fos-related proteins fra-1 and fos B, C-myc, L-myc and N-myc oncogenes, octamer-binding transcription factor 2 (Oct-2/OTF-2).

(iv) B_ ketoacyl synthase Sequence length: 2711 - 2727 Function

Beta-ketoacyl-ACP synthase (KAS) is the enzyme that catalyzes the condensation of malonyl-ACP with the growing fatty acid chain.

It is found as a component of the following enzymatic systems:

Fatty acid synthetase (FAS), The multifunctional 6-methysalicylic acid synthase (MSAS) from Penicillium patulum, Polyketide antibiotic synthase enzyme systems, Rhizobium nodulation protein nodE,

(v) ALA_RICH Alanine-rich region Sequence length: 2331 – 2414  Function : Many proteins contain compositionally biased sequence regions, which are also called low-complexity regions. Typically, such regions are highly enriched in one or a few amino acids. We have included profiles specific for each of the 20 amino acids so as to search for regions that are significantly enriched in a particular amino acid. The behavior of these profiles is controlled by two parameters, the match and mismatch

scores. These parameters were chosen such that the "target frequencies" of the corresponding amino acids computed according to the Karlin-Altschul theory approximate 35% for the residue.

**Figure 3**

Table 2 B : Functional domain identification of FAS protein of H37Rv Proscan. ().

| Domain No. | Function of Domain | Position in the FAS protein |
|---|---|---|
| 1 | Acyl transferase region | 1333 - 1625 |
| 2 | MaoC-like dehydratase | 1197 - 1321 |
| 3 | B_ketoacyl synthase | 2571 - 2808<br>2816 - 2992<br>2711 - 2727 |

Jpred program that was used to predict secondary structures in M. tuberculosis suggest that FAS proteins were composed more of helices than beta sheets (Fig 2).

**Figure 4**

Figure 2: Secondary structure prediction of FAS protein of by Jpred) tool.
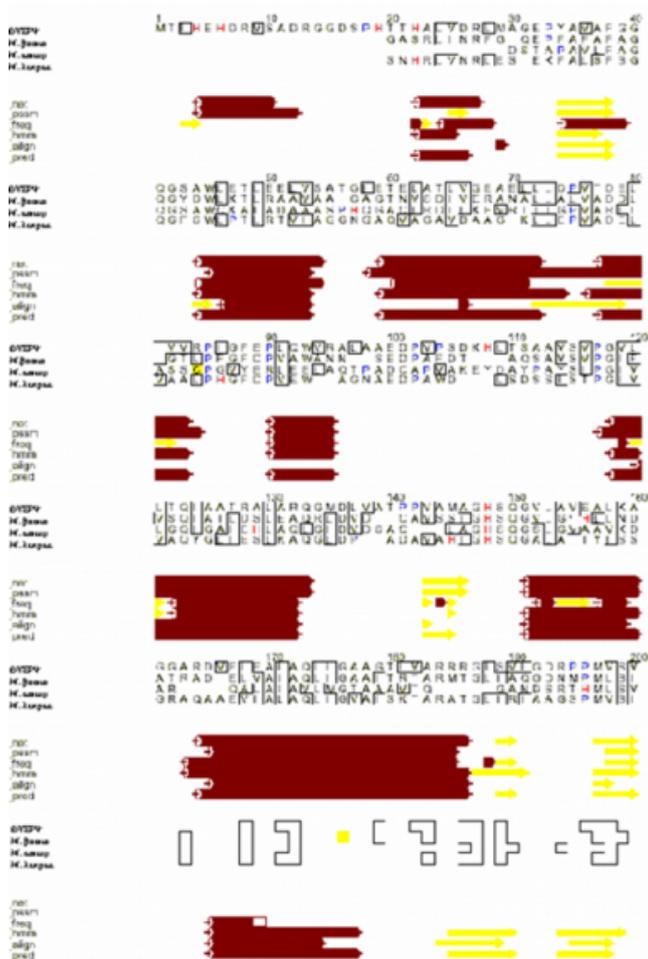


Based on the presence of domains in FAS protein of M.

tuberculosis, the amino acid sequence of FAS protein was split into 3 subunits. The amino acid sequence of each subunit of FAS protein was submitted to 3D PSSM server for 3D structure prediction individually. In addition to predicting the structure of protein the 3DPSSM was also able to classify the proteins based on SCOP database. The results of three dimensional structure for Acyl transferase domain of FAS protein of M. tuberculosis that was predicted by 3DPSSM (Figure 3 A), suggest that this protein belonged to Acyl transferase fold and malonyl-coenzyme acyl carrier protein family (No. of Helices-13; No. of strands-11; No of turns-25).
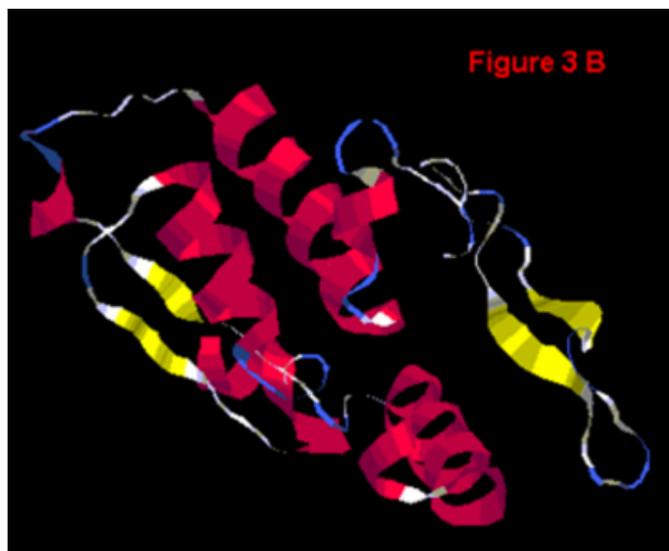
**Figure 5**

Figure 3 A: 3D structure of Acyl transferase as predicted by 3DPSSM server () and visualized through Rasmol. Violet colour: helix; Yellow colour: sheets; other colour: turns. Fold: acyltransferase; Family: malonyl-coenzyme acyl carrier protein. No.of Helices-13; No.of strands-11; No of turns-25



In case of beta keto acyl synthase, 3D PSSM was able to predict that these proteins belonged to alpha and beta class of proteins, (Figure 3 B) fold, family and superfamily were related to thiolase like, thiolase related and thiolase like respectively and the protein name was assigned as beta-keto-acyl ACP synthase II (No. of helices-8; No. of strands-4; No. of turns-12).

## Figure 6

Figure 3 B: 3D structure of beta keto acyl synthase as predicted by 3DPSSM server () and visualized through Rasmol. Violet colour: helix region; Yellow colour: sheets; other colour: turns. Class-Alpha and beta proteins (a/b); Fold-Thiolase-like; Family-Thiolase-related; Super family Â–Thiolase-like; Protein: Beta-ketoacyl-ACP synthase II; No. of helices-8; No. of strands-4; No. of turns-12



The structure of MaoC dehydratase (Figure 3 C) that was predicted by 3DPSSM server was able to assign lyase fold followed by assigning enoyl-coA hydratase superfamily and hydratase protein to MaoC dehydratase sequence (No. of helices-4; No. of strands-9; No. of turns-10).

## Figure 7

Figure 3 C: 3D structure of MaoC dehydratase as predicted by 3DPSSM server () and visualized through Rasmol. Violet colour: helix region; Yellow colour: sheets; other colour: turns. Fold-: lyase; Super Family: enoyl-coa hydratase; Protein-hydratase. No. of helices-4; No. of strands-9; No. of turns-10
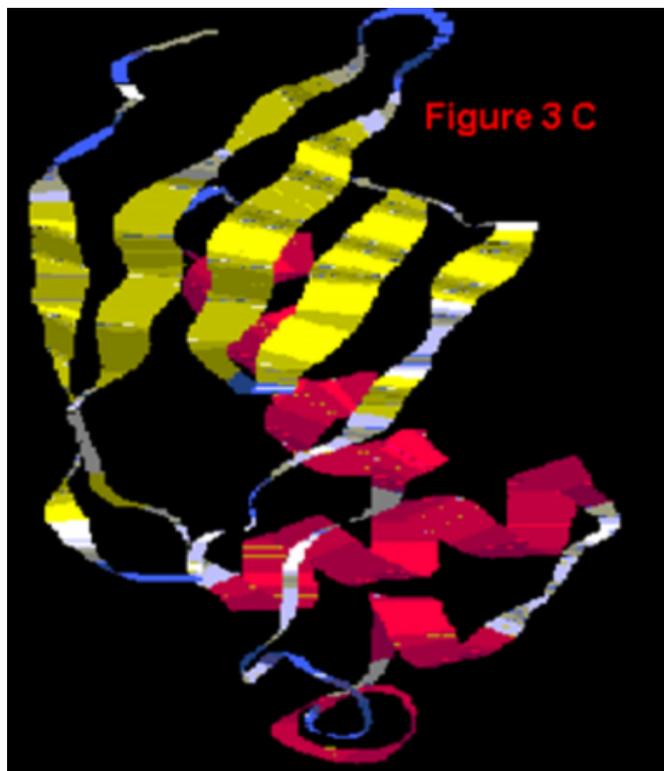


## DISCUSSION

TB, which is killing more people than any other infectious disease, was declared as global emergency by the World Health Organization (Kremer and Besra, 2002). About 32 % or 1.86 billion of the world populations are infected with TB. Every year approximately 8 million people develop active TB and almost 2 million of these people die from this disease (Dye et. al., 1999). A survey in 72 countries suggested that the multidrug-resistant (MDR) TB problem is more widespread than previously thought and likely is worsening. If not prevented and controlled, MDR TB likely will become more widespread in other areas of the world, including developed countries.

Combining sequence information with 3D structure gives invaluable insights for the development of effective rational strategies for experiments such as site directed mutagenesis, studies of disease related mutations, or the structure based design of specific inhibitors. Techniques for experimental structure solution have made great progress in recent years. However, experimental structure determination is still a

time-consuming process without guaranteed success. No experimental structural information is available for the vast majority of protein sequences hence theoretical methods for proteins structure prediction aiming to bridge this structure knowledge gap have gained much interest in recent years. Knowledge based approaches, combined with the current explosion in sequence and structure data, may move us to a new prospective paradigm in which it may be possible to discover a suitable drug against a given target long before any application is known. Combined with advances in single-nucleotide polymorphism detection, this may take possible individualized medicines in which each patient gets a drug designed against his or her particular form of the target (Pfost, 2000). As we move toward a situation where drug discovery projects are bathed in structural and sequence information, it is the role of the structural bioinformatician to integrate this wealth of data accelerating drug discovery. New drugs are urgently needed to reduce the potential impact of the emergence of multidrug-resistant strains of the causative agent M. tuberculosis. The front-line antibiotic isoniazid (INH), and several other drugs, target the biosynthesis of mycolic acids and especially the Fatty Acid Synthase-II (FAS-II) elongation system. This biosynthetic pathway is essential and specific for mycobacteria and still represents a valuable system for the search of new anti-tuberculous agents. Hence in the present study we have concentrated to characterize the FAS protein of M. tuberculosis from sequence to function and structure at molecular level using bioinformatics tools. Design of inhibitors and probability of generation small molecule inhibitors of FAS protein of M. tuberculosis will open gates to formulate new drugs for MDR strains M. tuberculosis which is under progress.

## CORRESPONDENCE TO

Chitta Suresh Kumar, Ph.D

Associate Professor

Center for Bioinformatics

Department of Biochemistry

Sri Krishnadevaraya University

ANANTAPUR-515 003, A.P.

India.

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## Author Information

**Chitta Suresh Kumar, Ph.D.**
Associate Professor, Center for Bioinformatics, Department of Biochemistry, Sri Krishnadevaraya University

**C. M. Anuradha, M. Phil**
Research Scholar, Department of Biochemistry, Sri Krishnadevaraya University

**K. Venkata Rao, M.Sc.**
Research Scholar, Department of Microbiology, Sri Krishnadevaraya University

**K. Venkateswara Swamy, M.Sc.**
Research Scholar, Department of Biochemistry, Sri Krishnadevaraya University