

In Silico metabolic pathway analysis of pathogenic microbial genomes

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Abstract: Studies involving pathogenic microbes and their genetic organization contributes to enrichment of knowledge on control of infectious human diseases. Sequencing of genomes of various pathogenic micro organisms and humans requires to be analysed so as to understand the pathogen- host interactions and function and functions of related genes and proteins. Present study details some efforts undertaken application bioinformatics software tools and comparative metabolomics in understanding the genetic organization in pathogenic microbes.

Keywords: Pathogens, microorganisms, genome annotation, Shikomat pathway, chorismate synthase, Helico bacter pyroli..

According to estimates of the World Health Organization, infectious diseases caused nearly 20 million deaths in 2007 around the world, accounting for 26% of the total global mortality. Integration and analysis of various data related to pathogens and pathogen-host interactions (PHIs) will yield a better understanding and means for control of infectious diseases induced by such pathogens.

Sequencing of model organisms and the available genomic information is a wonderful source for elucidating the gene, protein functions and most importantly enriches our knowledge about the disease mechanism and pathogen - host inter actions. Pathogen genome information thus obtained forms the basis for study of pathogen related genes & proteins and synthesized toxins, defense compounds and computer design of potential drugs & vaccines. Sequencing of Genomes of Humans, rat, Drosophila, E. coli, Yeast and rice plant are providing huge genetic data that can be analyzed and potentially translated in improvement of research efficiency in public health. Several genome data resources, such as the National Center for Biotechnology Information (NCBI), European Bioinformatics Institute (EBI) and Swiss Institute of Bioinformatics (SIB), are available to the public n the world wide web. However, data obtained from these sources is often raw in form and are not annotated (ie. analyzed) completely to elucidate the biological functions of targeted genes and proteins. Various bioinformatics analysis tools such as BLAST (for sequence comparison & detection of orthologous genes), COGS (for detection of orthologs in other species), PFAM (for detection of functional domains) OMIM (Online inheritance of Man: a database of human disease information), MOE For detection of ligand-Rceptor interaction & pathogen active binding sites) and PDB (a database of protein structure information) can be readily utilized in day to day research and any information about genes or diseases can be searched using even simple key words.



Fig.1:Front view of Ensembl, Human Genome annotation tool.

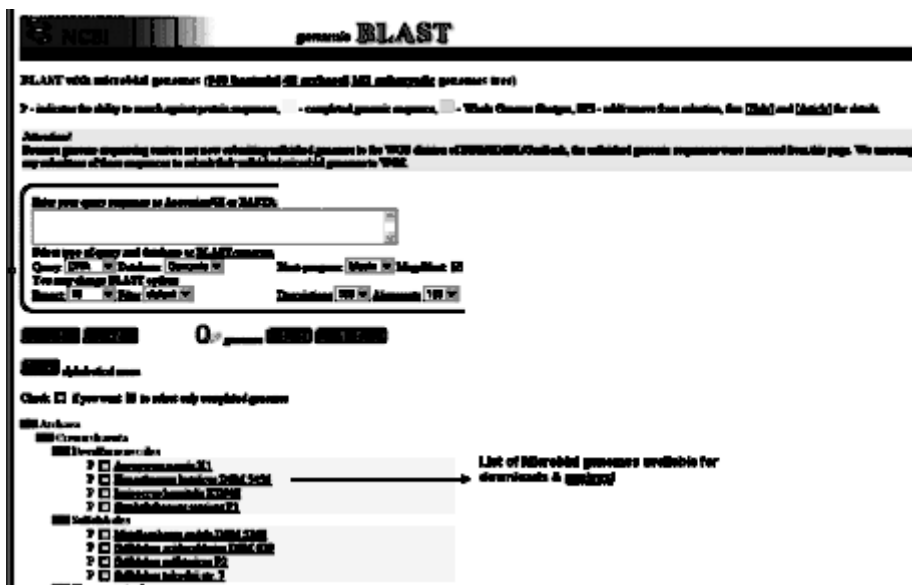


Fig.2. Genome BLAST page front view and the arrow in red points to downloadable microbial genome data.

Application of Comparative Genomics to better understand organization of Pathogen genomes:
 Annotation of Pathogenic Genomes:

Case study- Pathogenic genome Viewer, Marla and Singh, 2007 (1).

Pathogenic genome Viewer is a Java based unique microbial genome display & annotation tool developed by Bioinformatics centre, G.B.Pant University of Agric. & Tech., Pantnagar (1). The Java based tool takes in downloaded genomic data of any data format and readily builds chromosomal maps depicting positions of genes (from both sense & anti sense strands) and enables translation and downloading of corresponding protein sequences. The tool enables users to select the particular area on the chromosome where the suspected gene is located, magnify the region and annotate to know its functions using various bioinformatics tools. Similarly PHIDAS (2) is another Bioinformatics tool that enables Pathogen- Host data analysis and integration.



Fig. 3. Pathogenic Genome Display tool (Marla et al, 2007)

Comparative Metabolonomics and elucidation of disease information:

A living cell comprises a large number of compounds and metabolites such as amino acids, nucleic acids, carbohydrates, lipids and other biomolecules. Synthesis of these metabolites that support the functioning of a living cell is carried through hundreds of biochemical reactions occurring parallelly in a cell. The biochemical pathway charts of different microorganisms, plants and animals are displayed and available online. Kyoto Encyclopedia (KEGG:3), EcoCYc (4), BioCYc (5), MetaCYc (6) for microorganisms & AraCYc & RiceCYc for plants (7) and are the major sources for obtaining information on metabolic pathway reactions. These biochemical databases combine the molecular information from the genome databases with the simulation of metabolic networks. These are powerful interactive tools with applications in understanding diseases, drug development. There are various integrative tools that offer wonderful opportunities to analyze the metabolism of pathogenic microbes to understand the processes of pathogenesis in relation with human hosts.

Case study- Analysis of shikimate pathway in pathogenic microbes:

Comparative Structure analysis of Chorismate synthase from shikimate pathway in *Helicobacter pylori* and *Prevotella rumnicola*, from Type-B, gastritis (Marla et al, 2006 ,2).

It has been reported that *Helicobacter pylori* is responsible for causing several water born diseases in humans including type-B gastritis, peptic-ulcer and lymphoid-tissue (Maltoma: Blaser, 1992). However we report here in the present study the activity of chorismate synthase and involvement of microorganism *Prevotella rumnicola* in Gastritis disease pathogenesis. *Prevotella rumnicola* also *Prevotella rumnicola* is a gram-positive obligate anaerobic bacterium chiefly inhabiting in the rumen of live stock is also involved in the infection of bowel and gastroenteritis in humans (Blaser, 1992; Nicolich et al., 1992). The structure of chorismate synthase enzyme was predicted and compared with its counter part in *Helicobacter pylori*.

Recent appearance of antibiotic-resistant *H. pylori* strains poses a threat to disease therapeutics. In the pathogenesis of gastric mucosa associated with lymphoid-tissue, the long chain of flavodoxin of *H. pylori* functions as an electron acceptor to the Peruvate-oxidoreductase (POR) enzyme in chorismate metabolism (Hughes et al. 1995; Kaihovaara et al. 1998). It was also discovered that sera from patients with gastric MALToma contained antibodies against a 19 KD protein flavodoxin (Chang et al, 1999 ; Sheish et al. 2000). *H. pylori* encodes flavodoxins- acidic redox proteins belonging to Flavin Mononucleotide (FMN) containing proteins involved in a variety of electron transfer reactions (Tomb et al. 1997 ; Alm et al. 1999). Chorismate synthase enzyme from the shikimate pathway has an absolute requirement for reduced flavin mononucleotide for the synthesis of aromatic amino acids, though the former is not consumed in the reaction (Kitzing et al. 2004). Chorismate is used as a substrate for other pathways that lead to synthesis of folates, ubiquinones, naphthoquinones and amino acids like phenylalanine, tryptophan, tyrosine, Vitamin K, P and amino benzoic acid (PABA). PABA is also converted in the reaction to folates. The shikimate pathway and flavodoxins are present in both eukaryotes and prokaryotes but not in mammals (Osborne et al. 1991; Romero et al. 1996). The shikimate pathway is also present in apicomplexan parasites like *Toxoplasma gondii*, *Plasmodium falciparum*, *Cryptosporidium parvum*, *Clostridium difficile* (Roberts et al. 2002). The discovery of potential inhibitors of the shikimate pathway may aid in design of a spectrum of antimicrobial agents that are effective agents in control of bacterial, fungal pathogens and apicomplexan parasites. The *fldA* gene in genome of *H. pylori* encodes the small acidic redox protein flavodoxins (Tomb et al. 1997). Genes encoding various enzymes in shikimate pathway including chorismate synthase in fungi and plants have already been reported. But in microorganism to date only a few reports are present (Davies et al,

1994). Recently, we found evidence for the presence of chorismate synthesis in Thermodesulfobacteriales (gi: 70907682), Holoferax volcani (gi: 68146591), Dichelobacter nodosus (gi:68146596),Thrmodesulfobacterium commune. (gi: 70907682) and Salinibacter rubber (gi: 68146594). These results are recently submitted to EMBL database. The presence of the chorismate synthase genes in above organisms has been verified in wet lab studies using PCR amplification.

Prevotella rumnicola is a gram-positive obligate anaerobic bacterium chiefly inhabiting the rumen of live stock is also involved in the infection of bowel and gastroenteritis in humans (Blaser, 1992; Nicolich et al., 1992). The genome of P. rumnicola is not yet been fully sequenced (TIGR, 2008) but is being annotated. The bioinformatics analysis of this enzyme provides tools to develop drugs as potential antibacterial drugs.

We present here our work on comparative genomic analysis and discovery of genes and modeling of chorismate synthase enzyme structure from P. rumnicola based on the crystal structure of chorismate synthase from H. pyroli and other micro organisms. Results obtained from chorismate sequence analysis and detection of conserved residues gave leads to probe structure inference. The detected active binding sites of the enzyme were further validated by Ramachandran plot (PROCHECK). Phylogenetic tree was constructed and evolutionary relationships were used to support the observed homology among the analyzed microorganisms. The microorganisms analysed are:Helicobacter pyroli, Prevotella rumnicola, Thermodesulfobacteriales, Holoferax volcani, Dichelobacter nodosus, Salinibacter rubber and Thrmodesulfobacterium commune.

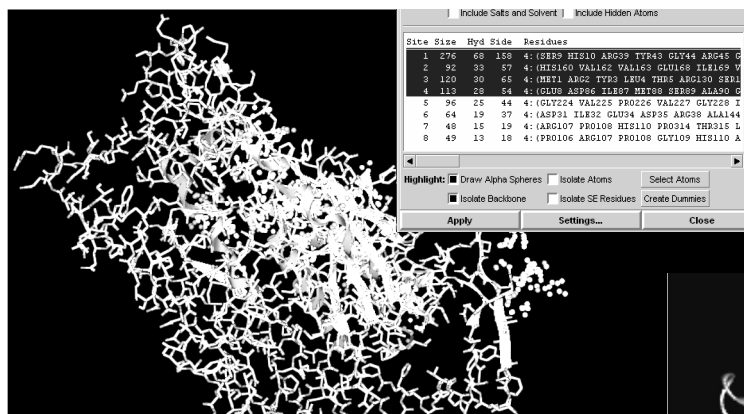


Fig. 4. Location of four active site binding FMN in chorismate synthase in P. rumnicola (MOE V.11.0). Various energy parameters associated with potential binding sites are also shown in the MOE tool output displayed.

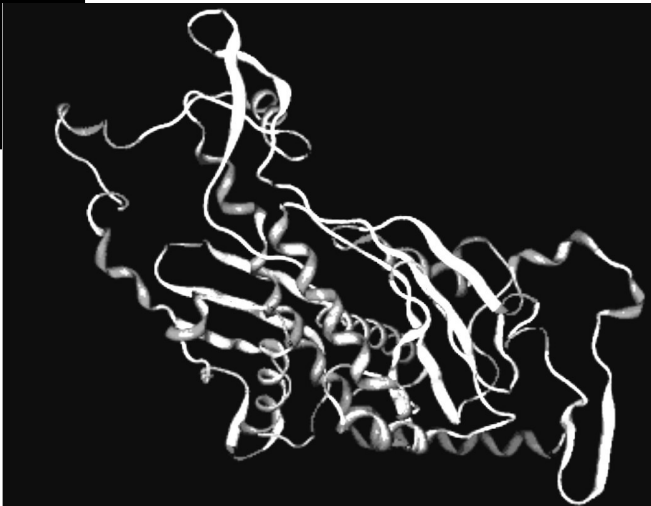
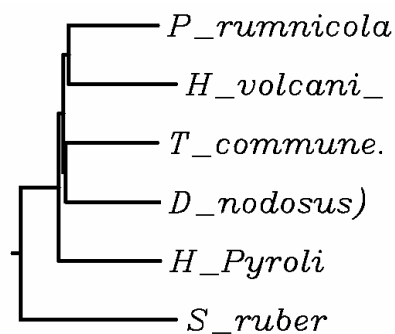


Fig.6. Phylogenetic tree displaying evolutionary relationships among various microorganisms for chorismate synthase activity from shikimate pathway (Tree View Tool from CLUSTALW).



PHIDAS (<http://genomebiology.com/2007/8/7/R150#B1#B1>)

MetaCyc (<http://biocyc.org/metacyc/>)

EcoCyc (<http://www.ecocyc.org/>)

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