EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 3
506-518
Year: 2016

COMPUTATIONAL ANALYSIS OF T7SS ASSOCIATED GENES IN MYCOBACTERIUM

Taimoor Khan[1], Shazia Mannan[1], Shaeeq Ur Rehman[2] and Shahid Raza[2*]

[1]COMSATS Institute of Information Technology, Sahiwal, Pakistan.
[2]University of South Asia, Lahore, Pakisan.

***Author for Correspondence: Shahid Raza**
University of South Asia, Lahore, Pakisan.

Article Received on 19/01/2016 **Article Revised on 09/02/2016** **Article Accepted on 29/02/2016**

ABSTRACT
Mycobacteria are a genus of Actinobacteria and belong to the family of *Mycobacteriacae*. They are immobile, obligate aerobic, acid-fast Gram-positive bacilli with high genomic G+C content (59-66%). Basically, Mycobacteria are a type of germ and exist in many different forms. The most common one causes tuberculosis. Another one causes leprosy. Still others cause infections that are called atypical mycobacterial infections. The study is focused on analysis, identification and annotation of T7SS associated genes and proteins, along with phylogenetic analysis and their comparison at genome level by using bioinformatics tools and software's. Genome wide annotation will help for studying and analysing future genes at large scale.

KEYWORDS: Actinobacteria, tuberculosis, phylogenetic analysis.

INTRODUCTION

Now days a lot of research work has been conducted on bacteria. As the advancement in sequencing technologies moves on a dramatic increase has also been recorded in bacterial genomes. Due to this advancement the sequencing technologies have become more effective and efficient. A large amount of data was also collected by using these techniques. These techniques and software tools that have been specially designed for the analysis and assembly enable us to handle the specific characteristics of new generation sequencing data that could be supportive for the comparison of bacterial species acquired from different environment.

The bacterium which I studied is Mycobacterium. Mycobacterium is a genus of Actinobacteria, given its own family, the Mycobacteriaceae. The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis (Mycobacterium tuberculosis) and leprosy (Mycobacterium leprae). Mycobacteria are aerobic and nonmotile bacteria (except for the species *Mycobacterium marinum*, which has been shown to be motile within macrophages) that are characteristically acid-alcohol-fast. Mycobacteria do not contain endospores or capsules and are usually considered Gram-positive. *Mycobacterium marinum* and perhaps *M. bovis* have been shown to sporulate; however, this has been contested by further research. While mycobacteria do not seem to fit the Gram-positive category from an empirical standpoint (i.e., in general, they do not retain the crystal violet stain well), they are classified as an acid-fast Gram-positive bacterium due to their lack of an outer cell membrane. All *Mycobacterium* species share a characteristic cell wall, thicker than in many other bacteria, which is hydrophobic, waxy, and rich in mycolic acids/mycolates. The cell wall consists of the hydrophobic mycolate layer and a peptidoglycan layer held together by a polysaccharide, arabinogalactan. The cell wall makes a substantial contribution to the hardiness of this genus. The biosynthetic pathways of cell wall components are potential targets for new drugs for tuberculosis.

(Stanley SA, Raghavan S, Hwang WW, Cox JS 2003).

Many *Mycobacterium* species adapt readily to growth on very simple substrates, using ammonia or amino acids as nitrogen sources and glycerol as a carbon source in the presence of mineral salts. Optimum growth temperatures vary widely according to the species and range from 25°C to over 50°C.

Mycobacteria are widespread organisms, typically living in water (including tap water treated with chlorine) and food sources. Some, however, including the tuberculosis and the leprosy organisms, appear to be obligate parasites and are not found as free-living members of the genus.

The physiological ecology of environmental mycobacteria refers to the identification of physiological characteristics of environmental mycobacteria that are determinants of their ecology and hence epidemiology. These important characteristics of the slow-growing

environmental mycobacteria are presented in Table 2. Slow growth of mycobacteria is due to the possession of either one (slow growers) or two (rapid growers, except M. chelonae and M. abscessus, which have only one) (92) 16S rRNA cistrons (E. coli has seven operons), impermeability of the lipid-rich cell wall, and the synthetic energy cost of the long-chain mycolic acids (e.g., C60 to C90). While the possession of a single rRNA cistron constrains mycobacteria to their characteristic slow growth, it also grants them greater ease of accumulating a resistance mutation for ribosomal-targeting antibiotics. The lower metabolic rate of slow growth also imparts more time for adaptation in stressful environments.

There are a variety of situations where human and mycobacterial geographic and environmental distributions can overlap and lead to exposure of humans as well as impacting mycobacterial ecology. A major overlap occurs with water. Humans are exposed to mycobacteria in water through drinking, swimming, and bathing. Aerosols generated during these activities can also lead to human exposure. Cervical lymphadenitis in children is hypothesized to be a result of mycobacteria in drinking water and possibly from soil which contaminates dirty objects placed in the mouth. The presence of environmental mycobacteria in water coupled with their disinfectant resistance leads to the presence of environmental mycobacteria in hot tubs, solutions used in medical treatment, e.g., gentian violet, and water-oil emulsions used to cool metalworking tools. Dusts can be rich sources of environmental mycobacteria, especially dust rich in peat. Foods and cigarettes may also be sources of mycobacterial infection.

M. Tuberculosis esx-1 is required for virulence in mice, macrophages growth, and suppression of macrophage inflammatory and immune responses, including arrest of phagosome maturation and the reduced expression of il-12 and tnf-α [1–6]. The homologous *m. Marinum* esx-1 is required for virulence in zebrafish, growth in macrophages, cytolysis and cytoxicity, and cell-to-cell spread, in addition to esat-6 and cfp-10 secretion. In zebrafish embryo infections, *m. Marinum* esx-1 is required for macrophage aggregation and granuloma formation. In *m. Smegmatis*, esx-1, in addition to being required for secretion of esat-6 and cfp-10, modulates conjugal dna transfer. In contrast, most strains of *m. Ulcerans*, which is genetically closely related to *m. Marinum* and *m. Tuberculosis*, but persists in extracellular locations during mammalian infection, lack most of the esx-1 components as well as orthologs of the genes extending from *rv3879c* thru *rv3883c*. Although the esx-1 secretion machinery (rv3870, rv3871, and rv3877) is required for the arrest of phagosome maturation by *m. Tuberculosis* during an infection of macrophages, the known esx-1 substrates are dispensable. The multiple phenotypes and host responses dictated by the esx-1 secretory apparatus suggest that there may be additional substrates, components, and regulatory molecules yet to be identified.

(Guinn KM, Hickey MJ, Mathur SK, Zakel KL, Grotzke JE, et al. 2004)

ESX 1/Type 7 secretion system

Recent evidence shows that mycobacteria have developed novel and specialized secretion systems for the transport of extracellular proteins across their hydrophobic, and highly impermeable, cell wall. Strikingly, mycobacterial genomes encode up to five of these transport systems. Two of these systems, ESX-1 and ESX-5, are involved in virulence - they both affect the cell-to-cell migration of pathogenic mycobacteria. Here, we discuss this novel secretion pathway and consider variants that are present in various Gram-positive bacteria.

Given the unique composition of this secretion system, and its general importance, we propose that, in line with the accepted nomenclature, it should be called type VII secretion.

It is now well established that pathogenesis of *Mycobcterium tuberculosis* depends on the secretion of key virulence factors, such as the 6 kD early secreted antigenic target ESAT-6 (EsxA) and its protein partner, the 10 kD culture filtrate protein CFP10 (EsxB) via the ESX-1 secretion system. ESX-1 represents the prototype system of the recently named type VII secretion systems that also exist in a range of other mycobacteria and actinobacteria. Indeed, three of the most well known attenuated strains in the history of tuberculosis research, *Mycobacterium bovis* BCG (BCG), *Mycobacterium microti*, and *M. tuberculosis* H37Ra (“a “stands for avirulent) are impaired for ESAT-6 and CFP10 secretion due to different genomic lesions or defects.

M. tuberculosis contains a total of five ESX systems that show similarity in gene content and gene order. While the genes conserved in at least four of the five systems were recently named as ESX conserved components (Ecc), these systems also contain genes coding for proteins defined as ESX-1 secretion-associated proteins (Esp). Research on type VII secretion systems has recently become a large and competitive research topic that is tightly linked to studies of host-pathogen interactions of pathogenic mycobacteria. Insights into this matter are of utmost importance for the improvement of current prevention strategies, diagnostics and therapy of tuberculosis as well as for a better understanding of the virulence mechanisms employed.

Abbreviations

BMDM, bone marrow–derived macrophage; CFP-10, culture filtrate protein 10; EspB, ESX-1 substrate protein B; ESAT-6, early secretory antigenic target 6; ESX-1, ESAT-6 system 1; extRD1, extended region of

difference 1; FAP, fibronectin attachment protein; OD, optical density; RD1, region of difference 1.

Aims and Objectives

- To check the presence of T7SS associated genes in bacterial genomes of mycobacterium whose genome has been done.
- To identify known T7SS regulatory proteins.
- Perform the phylogenetic analysis to check out the diversity among the species.
- Draw the phylogenetic tree of selected species to check out the association or link among them.

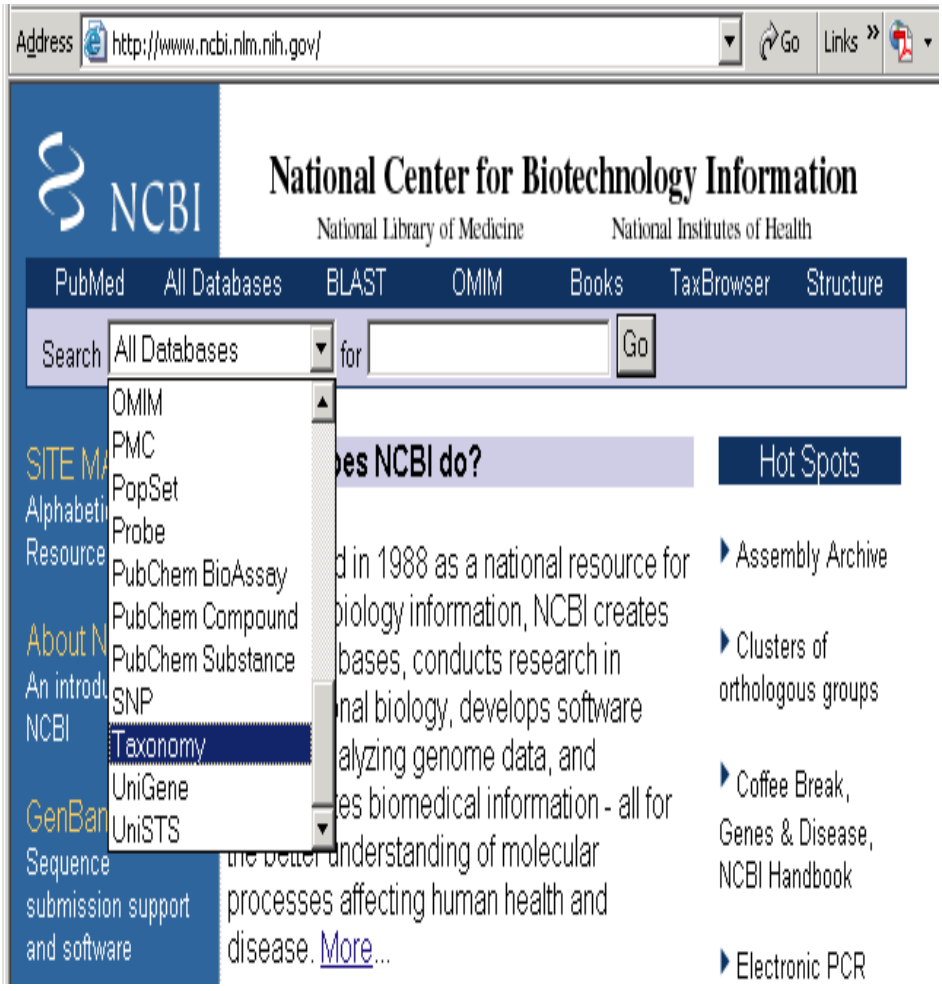
MATERIALS AND METHODS

Biological data is one of the most important things for in silico processing. As per the objectives of my project I need to perform the genome wide analysis of Type 7 secretion system in all species of mycobacterium and for this purpose I need to carry the in silico processing of biological data obtained from different sources. Now I am going to elaborate the sources and the steps being followed to collect the genomic data.

Retrieval of genomic data

NCBI

Collection of genomic data is one of the first and most important step in the project. The whole work depends upon this. The National Centre for Biotechnology Information (NCBI) is one of the most major databank for biological data which is a part of United States National Library of Medicine (NLM) and is a branch of National Institutes of Health. The NCBI has had responsibility for making available the GenBank DNA sequence database since 1992. Gen Bank coordinates with individual laboratories and other sequence databases such as those of the European Molecular Biology Laboratory (EMBL) and the DNA Data Bank of Japan (DDBJ).



PATRIC

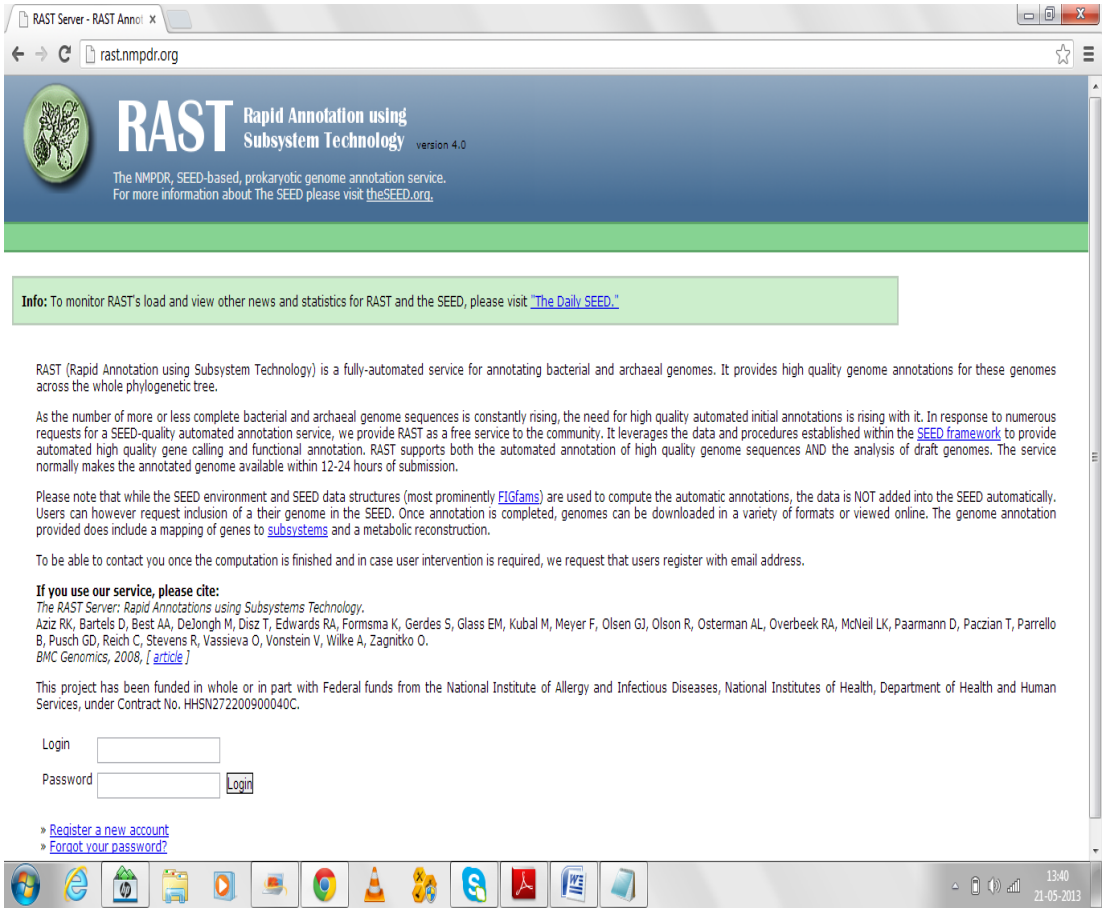
Pathosystems Resource Integration Centre abbreviated as **PATRIC** is the Bioinformatics Resource Centre covering Bacteria. **PATRIC** is basically an information system designed to support the biomedical research community`s work on bacterial infectious diseases. As I have to collect the genomic data about Mycobacterium and for that purpose I used the **PathoSystems Resource Integration Center (PATRIC).**

The steps to be followed while using the **PATRIC** to gather the data are as follows:

- First of all go to **PATRIC**.
- There will appear two options on the start page of **PATRIC**. One is of bacteria and the other one is of viruses. Select bacteria from there.
- Then on main page of **PATRIC** there is an option of organism. Choose your desired organism from the list.
- As my requisite organism is Mycobacterium so I selected it.
- The click on the No. of genomes which in the case of Mycobacterium are 195.
- The go to each genome one by one and select the feature table option and download it.
- After downloading the feature tables of the Mycobacterium having completed genome projects which are 195 according to the **PathoSystems Resource Integration Center (PATRIC)** till the date. Among these 195 species 54 has complete genome sequences and 141 are Whole genome sequences (WGS)**.** I organized the tables according to my project and deleted the undesired columns of information from the the excel files.
- After that I find T7SS components in all the tables.

RAST

RAST is a fully automated service for annotating bacterial and archaeal genomes. The service identifies protein-encoding, rRNA and tRNA genes, assigns functions to the genes and predicts which subsystems are represented in the genome, uses this information to reconstruct the metabolic network and makes the output easily downloadable for the user. In addition, the annotated genome can be browsed in an environment that supports comparative analysis with the annotated genomes maintained in the SEED environment.



The steps to be followed while using RAST for the annotation of genome is as under:

- Create an account on RAST.
- Then after the account is created upload your job i.e. upload your sequence.
- Then upload your query sequence.
- Within 12 to 24 hours it will give you the annotated data.
- As the annotation procedure is very much time consuming so we just used RAST to know how annotation procedure is carried out.
- For saving our time we take annotated data from PATRIC.

Comparative analysis of Secretary Pathway system

Kyoto encyclopedia of genes and genomes (KEGG) database is used to investigate the secretion system and pathways for T7SS in *Mycobacterium*. The KEGG, the Kyoto Encyclopedia of Genes and Genomes, was initiated by the Japanese human genome programme in 1995. According to the developers they consider KEGG to be a "computer representation" of the biological system. The KEGG database can be utilized for modeling and simulation, browsing and retrieval of data. It is a part of the systems biology approach.

We then analyzed all the genome sequences of mycobacterium available at Kyoto encyclopedia of genes and genomes (KEGG) database and crosschecked all the species to investigate whether there is presence of any other secretory pathway or not. It is also used to check that which proteins of T7SS are involved in secretory pathway of Mycobacterium.

Blast N by using BIOEDT

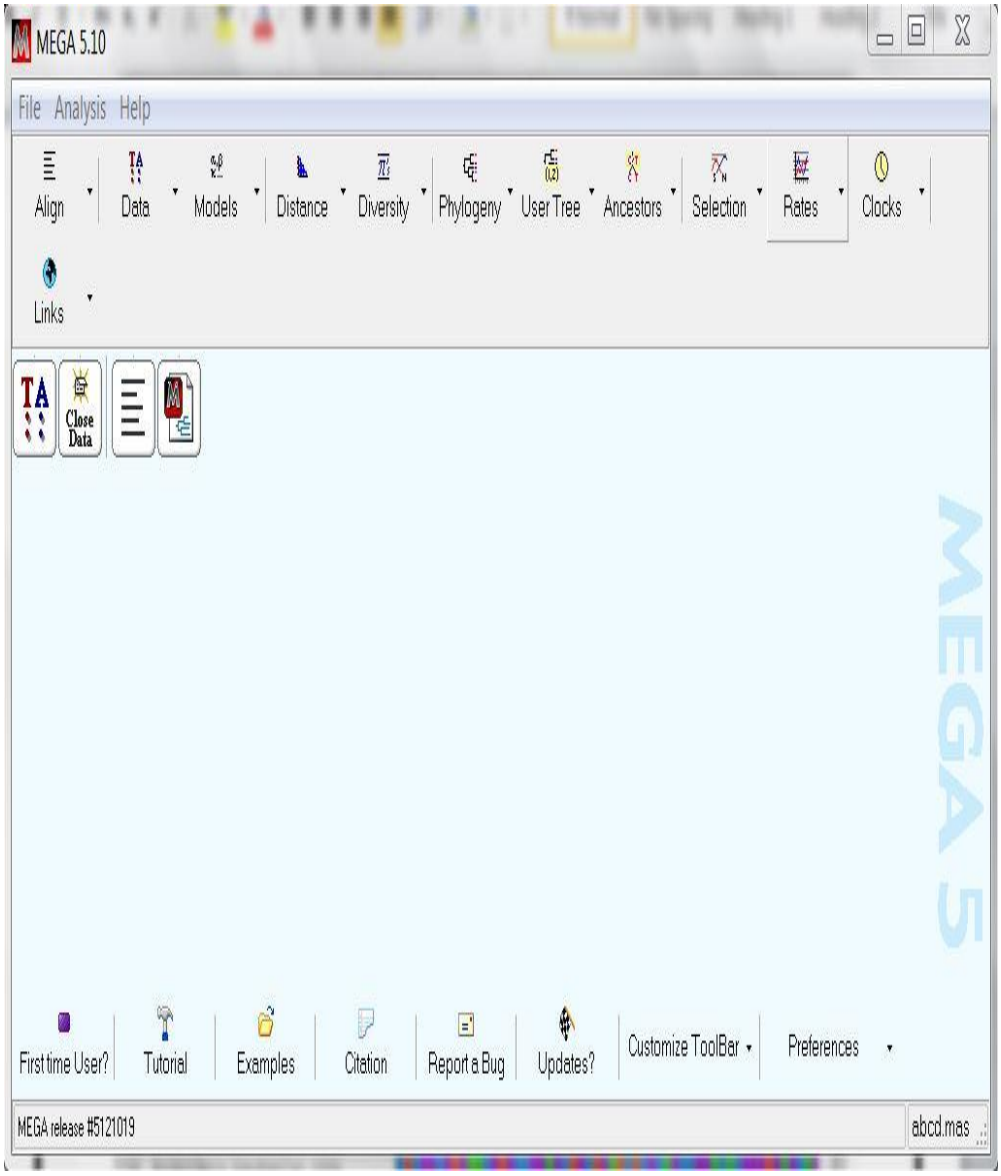
- Download the 16s rRNA sequences of mycobacterium from ncbi.
- After downloading the seuences I aligned them by using BIOEDIT tool.
- When the alignment of sequences is completes I saved it in fasta format.
- Then I performed local blast with BIOEDIT.
- Several modes of hand alignment.
- Automated ClustalW alignment.
- Automated Blast searches (local and WWW).

Phylogenetic study of T7SS components and 16 SrRNA

Phylogenetic analysis is conducted using the biological software Molecular Evolutionary Genetic Analysis which is abbreviated as (MEGA) and I used the 5th version of this software based on the approach described by Bingle et al. (2008). In case of T7SS loci amino acids sequences were aligned using ClustalW (Thompson et al., 1994). Similarly, to prove the ancestry of the T7SS and to understand their evolutionary pattern, 16S rRNA sequences of some selected species corresponding to species used by Bingle et al. (2008) were downloaded from RNA database **NCBI**. After that I constructed 16S phylogenetic tree of the selected species of

Mycobacterium using MEGA version 5. (Thompson et al., 1994). The steps followed for phylogenetic study were as under:

- First of all I downloaded the 16s rRNA sequences of sum some of the selected species of mycobacterium from NCBI **(NATIONAL CENTER FOR BIOINFORMATICS).**
- After that I pasted all the sequences in a text file on notepad.
- Then I aligned the sequences by using ClustalW (Thompson et al., 1994) with default parameters.
- Pairwise alignment and multiple sequence alignment were carried out.
- After the alignment is being done phylogenetic tree is being constructed.



- The snapshot shown above shows the sequences with multiple sequence alignment and pairwise alignment by using MEGA version 5 by Clustal W.
- It is the final sequence alignment after removing gaps and spaces within the sequences.

RESULTS AND DISCUSSION

Identification of SS genes clusters in Campylobacter

Representatives of the secretion system genes and core components recently defined by Boyer and coworkers were used as baits to identify SS loci by sequential BLASTN, BLASTP and TBLASTX searches using all publicly available sequences from *195 Mycobacterium* genome sequencing projects (completed or in progress). The data analyzed included both chromosome and plasmid sequences. Overall, in our results we determined that type 7 secretion system was widely distributed in Campylobacter as shown in Table 1. Not any other type of secretion system was found in Mycobacterium. Research on type VII secretion systems has recently become a large and competitive research topic that is tightly linked to studies of host-pathogen interactions of pathogenic mycobacteria. Insights into this matter are of utmost importance for the improvement of current prevention strategies, diagnostics and therapy of tuberculosis as well as for a better understanding of the virulence mechanisms employed.

Table NO. 1 Table above shows the presence of T7SS components in different species of *Mycobacterium.*

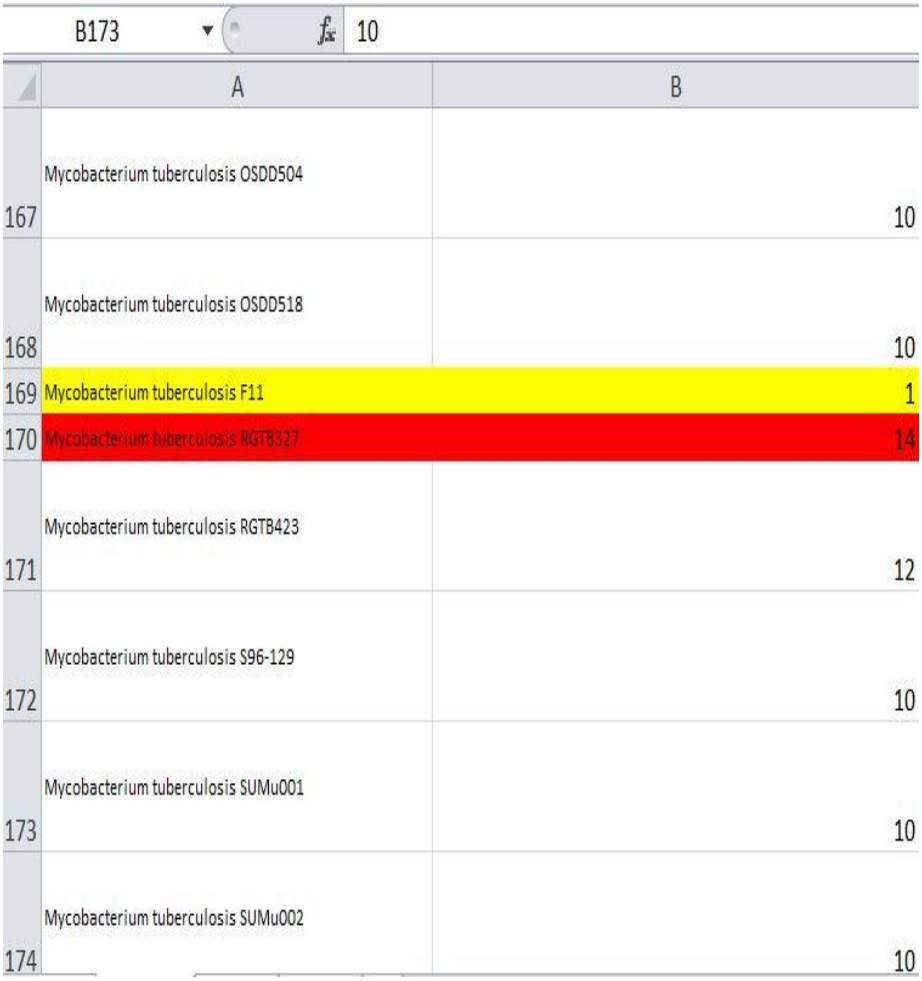
Genome Name	T7SS
Mycobacterium abscessus	3
Mycobacterium abscessus 3A-0119-R	3
Mycobacterium abscessus 3A-0122-R	3
Mycobacterium abscessus 3A-0122-S	3
Mycobacterium abscessus 3A-0731	3
Mycobacterium abscessus 3A-0810-R	3

Mycobacterium abscessus 3A-0930-R	3
Mycobacterium abscessus 3A-0930-S	3
Mycobacterium abscessus 47J26	3
Mycobacterium abscessus 4S-0116-R	2
Mycobacterium abscessus 4S-0116-S	2
Mycobacterium abscessus 4S-0206	2
Mycobacterium abscessus 4S-0303	2
Mycobacterium abscessus 4S-0726-RA	2
Mycobacterium abscessus 4S-0726-RB	2
Mycobacterium abscessus 5S-0304	5
Mycobacterium abscessus 5S-0421	5
Mycobacterium abscessus 5S-0422	4
Mycobacterium abscessus 5S-0708	4
Mycobacterium abscessus 5S-0817	5
Mycobacterium abscessus 5S-0921	4
Mycobacterium abscessus 5S-1212	4
Mycobacterium abscessus 5S-1215	5
Mycobacterium abscessus 6G-0125-R	3
Mycobacterium abscessus 6G-0125-S	3
Mycobacterium abscessus 6G-0212	3
Mycobacterium abscessus 6G-0728-R	3
Mycobacterium abscessus 6G-0728-S	3
Mycobacterium abscessus 6G-1108	3
Mycobacterium abscessus M115	3
Mycobacterium abscessus M139	2
Mycobacterium abscessus M148	5
Mycobacterium abscessus M152	2
Mycobacterium abscessus M154	2
Mycobacterium abscessus M156	3
Mycobacterium abscessus M159	3
Mycobacterium abscessus M172	3
Mycobacterium abscessus M24	3
Mycobacterium abscessus M93	3
Mycobacterium abscessus M94	3
Mycobacterium abscessus subsp. bolletii BD	3
Mycobacterium africanum GM041182	11
Mycobacterium avium	
Mycobacterium avium 104	10
Mycobacterium avium subsp. avium ATCC 25291	10
Mycobacterium avium subsp. avium DT 78	9
Mycobacterium avium subsp. avium Env 77	9
Mycobacterium avium subsp. paratuberculosis 1281	9
Mycobacterium avium subsp. paratuberculosis 4B	10
Mycobacterium avium subsp. paratuberculosis ATCC 19698	9
Mycobacterium avium subsp. paratuberculosis CLIJ361	9
Mycobacterium avium subsp. paratuberculosis CLIJ623	9
Mycobacterium avium subsp. paratuberculosis CLIJ644	10
Mycobacterium avium subsp. paratuberculosis DT 3	1
Mycobacterium avium subsp. paratuberculosis Env 210	10
Mycobacterium avium subsp. paratuberculosis JQ5	12
Mycobacterium avium subsp. paratuberculosis JQ6	10
Mycobacterium avium subsp. paratuberculosis JTC 1285	9
Mycobacterium avium subsp. paratuberculosis K-10	10
Mycobacterium avium subsp. paratuberculosis Pt139	9
Mycobacterium avium subsp. paratuberculosis Pt144	10
Mycobacterium avium subsp. paratuberculosis Pt145	10
Mycobacterium avium subsp. paratuberculosis Pt146	9
Mycobacterium avium subsp. paratuberculosis Pt154	9

Mycobacterium avium subsp. paratuberculosis Pt155	9
Mycobacterium avium subsp. paratuberculosis Pt164	9
Mycobacterium avium subsp. paratuberculosis S397	10
Mycobacterium avium subsp. paratuberculosis S5	10
Mycobacterium bovis AF2122/97	10
Mycobacterium bovis BCG str. ATCC 35733	10
Mycobacterium bovis BCG str. ATCC 35740	10
Mycobacterium bovis BCG str. ATCC 35743	10
Mycobacterium bovis BCG str. China	10
Mycobacterium bovis BCG str. Mexico	10
Mycobacterium bovis BCG str. Pasteur 1173P2	10
Mycobacterium bovis BCG str. Tokyo 172	10
Mycobacterium canettii CIPT 140010059	10
Mycobacterium canettii CIPT 140060008	10
Mycobacterium canettii CIPT 140070008	10
Mycobacterium canettii CIPT 140070010	10
Mycobacterium canettii CIPT 140070017	10
Mycobacterium chubuense NBB4	1
Mycobacterium colombiense CECT 3035	10
Mycobacterium fortuitum subsp. fortuitum DSM 46621	10
Mycobacterium gilvum PYR-GCK	4
Mycobacterium gilvum Spyr1	4
Mycobacterium hassiacum DSM 44199	3
Mycobacterium indicus pranii MTCC 9506	10
Mycobacterium intracellulare ATCC 13950	10
Mycobacterium intracellulare M.i.198	11
Mycobacterium intracellulare MOTT-02	10
Mycobacterium intracellulare MOTT-64	10
Mycobacterium kansasii ATCC 12478	10
Mycobacterium leprae Br4923	7
Mycobacterium leprae TN	7
Mycobacterium liflandii 128FXT	11
Mycobacterium mageritense JR2009	3
Mycobacterium marinum M	9
Mycobacterium massiliense 1S-151-0930	3
Mycobacterium massiliense 1S-152-0914	3
Mycobacterium massiliense 1S-153-0915	3
Mycobacterium massiliense 1S-154-0310	3
Mycobacterium massiliense 2B-0107	4
Mycobacterium massiliense 2B-0307	3
Mycobacterium massiliense 2B-0626	3
Mycobacterium massiliense 2B-0912-R	3
Mycobacterium massiliense 2B-0912-S	3
Mycobacterium massiliense 2B-1231	3
Mycobacterium massiliense CCUG 48898 = JCM 15300	3
Mycobacterium massiliense M18	4
Mycobacterium massiliense str. GO 06	3
Mycobacterium parascrofulaceum ATCC BAA-614	4
Mycobacterium phlei RIVM601174	3
Mycobacterium rhodesiae JS60	5
Mycobacterium rhodesiae NBB3	3
Mycobacterium smegmatis JS623	1
Mycobacterium smegmatis str. MC2 155	4
Mycobacterium sp. JDM601	9
Mycobacterium sp. JLS	4
Mycobacterium sp. KMS	4
Mycobacterium sp. MCS	4
Mycobacterium sp. MOTT36Y	10

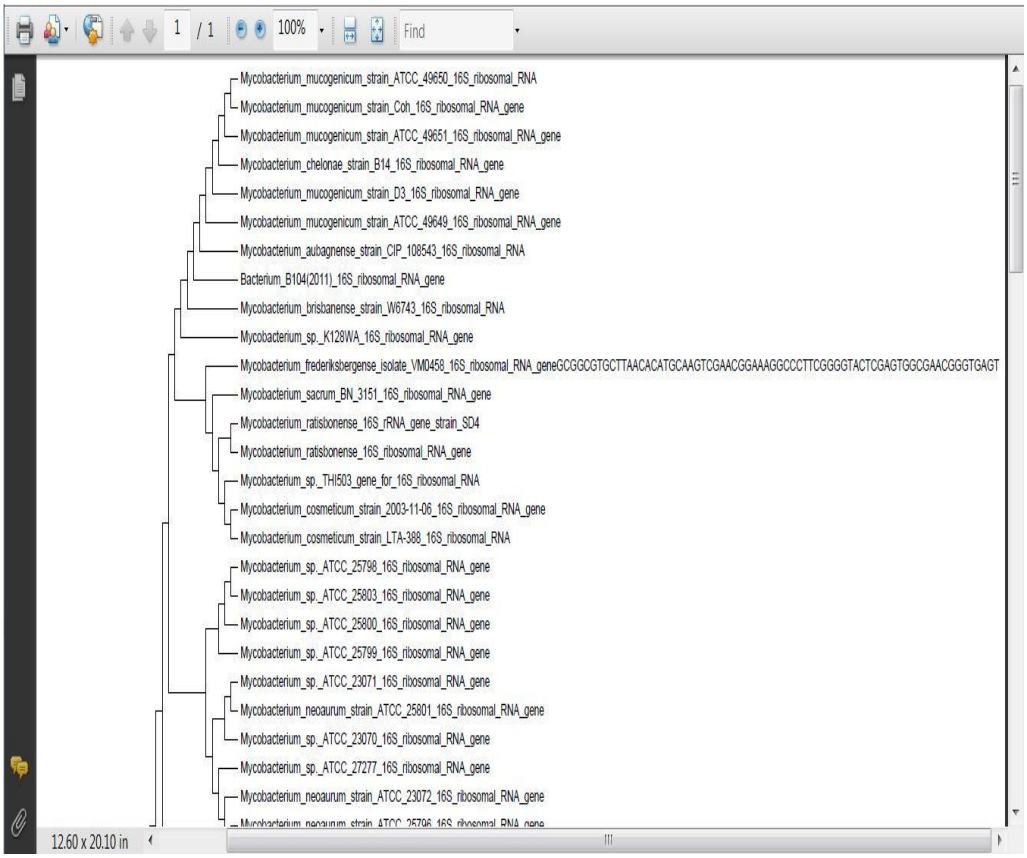
Mycobacterium sp. VKM Ac-1815D	3
Mycobacterium thermoresistibile ATCC 19527	4
Mycobacterium tuberculosis	11
Mycobacterium tuberculosis '98-R604 INH-RIF-EM'	11
Mycobacterium tuberculosis 02_1987	1
Mycobacterium tuberculosis 210	10
Mycobacterium tuberculosis 94_M4241A	12
Mycobacterium tuberculosis BTB05-552	10
Mycobacterium tuberculosis BTB05-559	10
Mycobacterium tuberculosis C	10
Mycobacterium tuberculosis CCDC5079	11
Mycobacterium tuberculosis CCDC5180	10
Mycobacterium tuberculosis CDC1551	11
Mycobacterium tuberculosis CDC1551A	11
Mycobacterium tuberculosis CPHL_A	10
Mycobacterium tuberculosis CTRI-2	10
Mycobacterium tuberculosis CTRI-4	10
Mycobacterium tuberculosis EAS054	12
Mycobacterium tuberculosis GM 1503	11
Mycobacterium tuberculosis H37Ra	10
Mycobacterium tuberculosis H37Ra [WGS]	12
Mycobacterium tuberculosis H37Rv	11
Mycobacterium tuberculosis H37Rv (Broad)	11
Mycobacterium tuberculosis H37RvAE	10
Mycobacterium tuberculosis H37RvCO	11
Mycobacterium tuberculosis H37RvHA	10
Mycobacterium tuberculosis H37RvJO	11
Mycobacterium tuberculosis H37RvLP	10
Mycobacterium tuberculosis H37RvMA	10
Mycobacterium tuberculosis HN878	10
Mycobacterium tuberculosis K85	
Mycobacterium tuberculosis KZN 1435	10
Mycobacterium tuberculosis KZN 4207	10
Mycobacterium tuberculosis KZN 4207 (Broad)	10
Mycobacterium tuberculosis KZN 605	10
Mycobacterium tuberculosis KZN R506	10
Mycobacterium tuberculosis KZN V2475	10
Mycobacterium tuberculosis NA-A0008	12
Mycobacterium tuberculosis NA-A0009	11
Mycobacterium tuberculosis NCGM2209	10
Mycobacterium tuberculosis OSDD071	10
Mycobacterium tuberculosis OSDD504	10
Mycobacterium tuberculosis OSDD518	10
Mycobacterium tuberculosis F11	1
Mycobacterium tuberculosis RGTB327	14
Mycobacterium tuberculosis RGTB423	12
Mycobacterium tuberculosis S96-129	10
Mycobacterium tuberculosis SUMu001	10
Mycobacterium tuberculosis SUMu002	10
Mycobacterium tuberculosis SUMu003	10
Mycobacterium tuberculosis SUMu004	10
Mycobacterium tuberculosis SUMu005	11
Mycobacterium tuberculosis SUMu006	10
Mycobacterium tuberculosis SUMu007	10
Mycobacterium tuberculosis SUMu008	10
Mycobacterium tuberculosis SUMu009	11
Mycobacterium tuberculosis SUMu010	9
Mycobacterium tuberculosis SUMu011	11

Mycobacterium tuberculosis SUMu012	11
Mycobacterium tuberculosis T17	10
Mycobacterium tuberculosis T46	10
Mycobacterium tuberculosis T85	10
Mycobacterium tuberculosis T92	10
Mycobacterium tuberculosis UM 1072388579	10
Mycobacterium tuberculosis UT205	11
Mycobacterium tuberculosis W-148	10
Mycobacterium tuberculosis X122	10
Mycobacterium tuberculosis str. Erdman = ATCC 35801	11
Mycobacterium tuberculosis str. Haarlem	11
Mycobacterium tusciae JS617	3
Mycobacterium ulcerans Agy99	0
Mycobacterium vaccae ATCC 25954	5
Mycobacterium vanbaalenii PYR-1	4
Mycobacterium xenopi RIVM700367	10



The snapshot above shows the two highlighted species of *Mycobacterium*. The one which is highlighted yellow named *Mycobacterium Tuberculosis F11* has the least number of T7SS components present in it. Therefore it causes less pathogenicity and virulence whereas on the other hand the 2nd specie of *Mycobacterium* highlighted in red colour named *Mycobacterium Tuberculosis RGTB327* with the highest number of T7SS components present in it.

Phylogenetic tree



The snapshot shown above shows the phylogenetic tree which shows the association and closeness among species on the basis of evolutionary relationships among the species and also on the basis of secretory pathway similarities or differences between the species.

- First of all I downloaded the feature tables from **Patho Systems Resource Integration Center (Patric).**
- These feature tables contains the genomic data of all the species of mycobacterium. After downloading the feature tables I have arranged all the tables according to my requirements and deleted the undesired information from these tables.
- Then I found all the T7SS present in all species of Mycobacterium.
- The species in which there is more T7SS genes present in cluster form cause more virulence and pathogenicity as compared to those who have less number of T7SS present in them shows less virulence and pathogenicity.
- The tree above shown in results show the phylogenetic analysis.
- The tree is formed on the basis of clusters of secretory components present in them.
- The species which are closely related on the basis of secretory genes are clustered together in the tree.
- They have same number of secretory genes present in them and those which are situated far in the phylogenetic tree have different number of secretory protiens in them.
- The secretory proteins present in bacteria helps them in survival within the environment also helps in adaptation within different environment.
- The secretion system also assists in adherence, virulence and pathogenicity.
- As we know that the secretion system functions mainly in pathogenicity and virulence.
- After knowing the genes encoding T7SS we can alter them in laboratory and can manipulate the information within these genes and we can use this information in pharmaceutical and in drug designing for the benefit of mankind.

REFERENCES

1. Stanley SA, Raghavan S, Hwang WW, Cox JS (2003).
2. Pallen MJ: The ESAT-6/WXG100 superfamily—and a new Gram-positive secretion system? Trends Microbiol, 2002; 10: 209-212.
3. Abdallah A, Gey van Pittius N, Champion P, Cox J, Luirink J, Vandenbroucke-Grauls C, Appelmelk B, Bitter W: Type VII secretion–mycobacteria show the way. Nat Rev Microbiol, 2007; 5: 883-891.Very complete and comprehensive overview of the ESX-related features in a large range of actinobacteria.
4. Gey van Pittius NC, Sampson SL, Lee H, Kim Y, van Helden PD, Warren RM: Evolution and expansion of the Mycobacterium tuberculosis PE and PPE multigene families and their association with the duplication of the ESAT-6 (esx) gene cluster regions. BMC Evol Biol, 2006; 6: 95.This

paper nicely shows evidence that PE and PPE proteins have coevolved and have preceded the PE-PGRS and PPE-MPTR proteins.

5. Stanley SA, Raghavan S, Hwang WW, Cox JS Acute infection and macrophage subversion by Mycobacterium tuberculosis require a specialized secretion system. Proc Natl Acad Sci U S A, 2003; 100: 13001–13006.
6. Hsu T, Hingley-Wilson SM, Chen B, Chen M, Dai AZ, et al. The primary mechanism of attenuation of bacillus Calmette-Guerin is a loss of secreted lytic function required for invasion of lung interstitial tissue. Proc Natl Acad Sci U S A, 2003; 100: 12420–12425.
7. Guinn KM, Hickey MJ, Mathur SK, Zakel KL, Grotzke JE, et al. Individual RD1-region genes are required for export of ESAT-6/CFP-10 and for virulence of Mycobacterium tuberculosis. Mol Microbiol, 2004; 51: 359–370.
8. Brodin P, Rosenkrands I, Andersen P, Cole ST, Brosch R ESAT-6 proteins: Protective antigens and virulence factors? Trends Microbiol, 2004; 12: 500–508.