



PROTEIN AND DRUG DESIGNING OF NOCARDIA BY USING IN SILICO APPROACH

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ABSTRACT

Nocardia asteroides, is a saprophytic, filamentous aerobic, Gram-positive and mycolic acid cell wall bacteria that is responsible for Nocardiosis, which is a chronic bacterial disease of humans and many other animals originating in the respiratory tract and disseminated by way of blood to other organs. It is caused by either introduction of the species into the skin or by inhalation of the species from its habitat. In the present study, we have identified a set of potential drug targets in the Nocardiosis-causing bacterium, *Nocardia asteroides*, by performing in-silico subtractive genomic approach. The bacterial enzymes which bear no significant similarity to human proteins were identified using a BLASTp search. Presence of homologs in the prokaryotic database of essential genes suggests that an enzyme could be essential for survival of the pathogen, and hence was taken as a criterion for short listing the potential drug targets in *Nocardia asteroides*. After that, proteins were searched for their metabolic pathway using KEGG database.

Keywords: *Nocardia asteroides*, saprophytic, filamentous aerobic Protein and Drug Designing

INTRODUCTION

Nocardia asteroides live on dead decaying organic matter in the soil, water, dust and on vegetation. Nocardiosis is a chronic bacterial disease of humans and many other animals originating in the respiratory tract and disseminated by way of blood to other organs. It is caused by either introduction of the species into the skin or by inhalation of the species from its habitat. *Nocardia asteroides* accounts for 86% of systemic nocardiosis in humans and 98% of cerebral nocardial abscesses and 2% of all cerebral abscesses [1, 3, 6]. The majority of infections occur in patients with weakened cell-mediated immunity. Patients commonly also include those who have received bone-marrow transplantations and are on immunosuppressive therapy, those with HIV/AIDS, and those with malignancies [1, 7]. The disease usually begins with malaise, loss of weight, fever and night sweats. Nocardiosis is found worldwide in persons of all ages. *Nocardia* species are generally

resistant to penicillin and require six months to one year of antibiotic treatment. Early treatment of nocardiosis with sulphonamide drugs before the disease has spread to the brain has reduced the mortality rate from nocardiosis. Species of *Nocardia* genus are Gram positive. *Nocardia asteroides* are found worldwide and they are saprophytes, they live on dead decaying organic matter in the soil, water, dust and on vegetation. They are important in the soil because they break down dead and decaying organic matter into simple substances that can be taken up and recycled by plants. *Nocardia asteroides* appear as filaments and their colonies usually appear yellowish in a slant culture media as seen from the Images below. The current study makes use of the subtractive genomics approach, database of essential gene (DEG), differential pathway analysis and sub-cellular localization prediction to analyse the complete proteome of *Nocardia asteroides* to search for potential drug and vaccine targets.

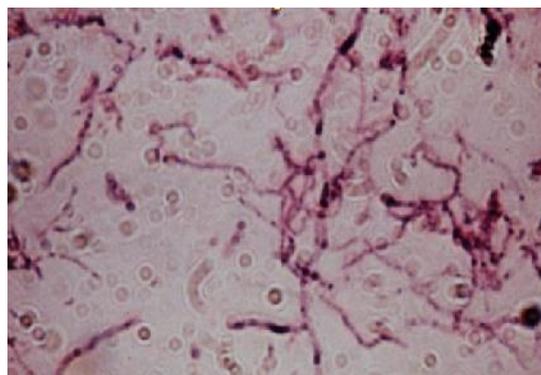


Figure 1: *Nocardia asteroides*



Figure 2: *Nocardia asteroides* colony

Nocardia asteroides

The complete genome structure of *Nocardia asteroides* is not available to the public. The total genome size of *Nocardia asteroides* is 7.5 Mb with a large linear plasmid of 220 kb. It is known though that *Nocardia asteroides* drug pattern VI strain is 99.5 % genomically identical to *Nocardia cyriacigeorgica* in HSP gene sequence [4]. There are only two differences in a 373-bp region; the amino acid sequences are identical in both of the above *Nocardia* species. *Nocardia farcinica* is the *Nocardia* species with a complete genome that is available to the general public. It has one circular chromosome and two circular plasmids. Its circular chromosome consists 6,021,225 bp and circular plasmids, pNf1 and pNF2 consists of 184, 027bp and 87,093 bp respectively [2, 4]. *Nocardia asteroides* species are filamentous aerobic, Gram-positive and mycolic acid cell wall bacteria. *Nocardia* colonies vary from white, to tan, orange and red in colour they have a lipid bilayer cell membrane with associated proteins [1, 7].

Their cell envelopes consist of a Peptidoglycan cell wall. All mycolic acids comprising the cell wall of *Nocardia asteroides* are saturated mycolic acids. As of today, researches have not indicated association of Pili nor flagella on *Nocardia asteroides*. *Nocardia asteroides* experience an optimal growth at body temperature.

Since *Nocardia* is a soil bacterium, it uses different compounds as its source for carbon and energy. The primary source energy for *Nocardia asteroides* is acetate and gluconate while its source of carbon and nitrogen is sucrose and acetamide. During nitrogen assimilation glutamate and glutamine is formed. *Nocardia asteroides* uses the GS/GOGAT pathway to form glutamine which contains the enzymes glutamine synthetase and glutamate synthetase which catalyzes glutamate from α -ketogluterate and ammonia. The genus *Nocardia* is a ubiquitous group of environmental bacteria that usually manifest as an opportunistic infection in immunocompromised hosts. *Nocardia* can be

found in soil, decomposing vegetation, and other organic matter, as well as in fresh and salt water. Both *Nocardia* and *Rhodococcus* are members of the family Nocardiaceae, which belongs to a suborder of aerobic actinomycetes that also includes *Mycobacterium*, *Corynebacterium*, *Gordona*, and *Tsukamurella*.

The taxonomy of *Nocardia* has been challenging because numerous revisions have been made with the identification of more than 50 species. What was originally referred to as *Nocardia asteroides* was later found to be a group of bacteria with a heterozygous pattern of antimicrobial drug susceptibilities [1]. Subsequently named *N asteroides* complex, this group of bacteria is responsible for most clinical human nocardial infections. *N asteroides* complex was later separated and reorganized into different species on the basis of drug susceptibility patterns: *Nocardia abscessus*, *Nocardia brevicatena-paucivorans* complex, *Nocardia nova* complex (which includes *N nova*, *Nocardia veterana*, *Nocardia africana*, *Nocardia kruczakiae*), *Nocardia transvalensis* complex, *Nocardia farcinica*, and *N asteroides* [2]. *Nocardia cyriacigeorgica* was recently differentiated from *N asteroides* and is becoming a more frequently identified clinically relevant pathogen.

Nocardia is a gram-positive bacterium that grows aerobically. Unlike other gram-positive bacteria, *Nocardia* appears as a filamentous

bacterium with hyphaelike branching on direct microscopy. *Nocardia* exhibits varying degrees of acid-fastness, depending on the mycolic acid composition in the cell wall and type of stain used [2]. The modified Kinyoun acid-fast stain uses 1% sulfuric acid as a decolorizer (instead of the more potent hydrochloric acid used in the decoloration step in the Ziehl-Neelsen staining procedure), which enhances the ability of *Nocardia* to retain the colored fuchsin. Unlike mycobacteria, *Nocardia* has a beaded acid-fast appearance on microscope. *Nocardia* can resemble *Actinomyces* species on Gram stain; however, *Actinomyces* species are not acid-fast and grow under anaerobic conditions. *Nocardia* can resemble *Actinomyces* species on Gram stain; however, *Actinomyces* species are not acid-fast and grow under anaerobic conditions.

Nocardiosis

Nocardia usually is an opportunistic pathogen, with the majority of infections occurring in patients with immunosuppressive conditions. Up to one-third of patients with nocardiosis, however, are immunocompetent [5]. Irrespective of a patient's immunologic status, the isolation of *Nocardia* from the respiratory tract or other body source should not be regarded as a contaminant or commensal organism. Patients with depressed cell-mediated immunity especially are at high risk for infection, including those with lymphoma, other selected malignancies, human

immunodeficiency virus infection, and solid-organ or hematopoietic stem cell transplant and those receiving long-term treatment with steroids or other medications that suppress cell-mediated immunity.

Patients with allogeneic hematopoietic stem cell transplants are at much higher risk for nocardiosis than those with autologous hematopoietic stem cell transplants. The development of graft-vs-host disease and subsequent additional immunosuppressive treatments may account for much of the increased risk in allogeneic hematopoietic stem cell transplant patients. Among these patients, nocardiosis can develop at varying time periods, which range from 2 to 3 months to 1 to 2 years after stem cell infusion. Among solid organ transplant recipients, *Nocardia* infection has a frequency of 0.6% to 3% and has been well described in kidney, heart, and liver recipients. A recent review of 5126 solid organ transplant recipients, however, found lung transplant recipients to have the highest incidence of *Nocardia* infection, followed by recipients of heart, small bowel, kidney, and liver transplants. Although the use of cyclosporine has been associated with the development of nocardiosis, combination therapy with cyclosporine and prednisone in some patient groups may pose less risk than azathioprine and prednisone or high-dose prednisone alone. Solid tissue cancers with associated chemotherapy also represent

another novel category for *Nocardia* disease development. Comorbidities and concurrent infections, including diabetes, cytomegalovirus infection, and alcoholism, contribute as well. Chronic obstructive pulmonary disease has a common association with pulmonary nocardiosis, but usually in the setting of concurrent corticosteroid use.

Pulmonary nocardiosis is the most common clinical presentation of infection because inhalation is the primary route of bacterial exposure. The onset of symptoms may be subacute to more chronic and can include productive or non-productive cough, shortness of breath, chest pain, haemoptysis, fever, night sweats, weight loss, and progressive fatigue. The chest radiograph can be variable, displaying focal or multifocal disease with nodular and/or consolidation infiltrate as well as cavitory lesions. Pleural effusions can develop in up to one-third of patients. It can be very difficult clinically and radiographically to differentiate *Nocardia* from filamentous fungal (eg, aspergillosis, mucormycosis) or mycobacterial disease (**Table 1**). Occasionally, *Nocardia* may be identified from the respiratory tract in a person without apparent pulmonary infection. *Nocardia* isolation without apparent pulmonary infection can be encountered in patients with underlying structural lung disease, such as bronchiectasis and cystic fibrosis, and should be interpreted cautiously. The identification of *Nocardia*

from an immunocompromised patient should never be ignored, especially if any abnormal clinical or radiologic pulmonary findings are present.

Extrapulmonary nocardiosis is relatively common and can occur through hematogenous dissemination or a contiguous spread of necrotizing pneumonitis into the pleura, pericardium, mediastinum, and vena cava. Abscess formation is characteristic of extrapulmonary nocardiosis and can resemble a pyogenic bacterial process or evolve into a chronic granulomatous or mixed progressive inflammatory mass. The central nervous system (CNS) is the most common extrapulmonary location for nocardiosis (up to 44% in one series). Patients may have 1 or more brain abscesses and present with headache, nausea, vomiting, seizures, or alteration in consciousness. Neurologic symptoms typically develop gradually, although an acute presentation with rapid progression may occur occasionally. Cerebral nocardiosis commonly accompanies pulmonary disease, but isolated CNS disease may occur. In immune-competent patients, cerebral nocardiosis is less common and may resemble a brain tumor or vascular infarct. Central nervous system imaging should be considered for patients with any adverse neurologic symptoms, severe pulmonary nocardiosis, or significant immunosuppression.

Primary cutaneous and soft tissue nocardiosis can result from traumatic injury to the skin that involves contamination with soil. Unlike other forms of nocardiosis, primary cutaneous disease usually develops in immunocompetent hosts. After skin inoculation, a superficial abscess or localized cellulitis can develop. Cutaneous nocardiosis can resemble soft tissue infections produced by *Staphylococcus aureus* or streptococci; however, this form of nocardial disease is usually more indolent. The infection can spread to the regional lymph nodes and produce a single or linear chain of nodular lesions. Lymphocutaneous nocardiosis is often called *sporotrichoid nocardiosis*, given the similar presentation of sporotrichosis. In more advanced disease, a mycetoma can develop with sinus tract development. *Nocardia brasiliensis* is the most common *Nocardia* species in cutaneous disease (especially progressive and lymphocutaneous disease), although *N asteroides* and *Nocardia otitidiscaviarum* have also occasionally been isolated.

Nocardia bacteremia is less often encountered. In one review of *Nocardia* bacteremia, 64% patients had concurrent pulmonary nocardiosis, 28% had concurrent cutaneous disease, and 19% had concurrent CNS disease. *Nocardia* bacteremia associated with central venous catheter infections has been reported. Polymicrobial bloodstream infections with *Nocardia* and gram-negative bacilli have also

been identified. Hematogenously disseminated nocardiosis has led to infection in the eyes (keratitis), heart valves, liver, spleen, adrenal glands, thyroid gland, and organ tissues.

MATERIAL AND METHODS

Databases and Techniques used

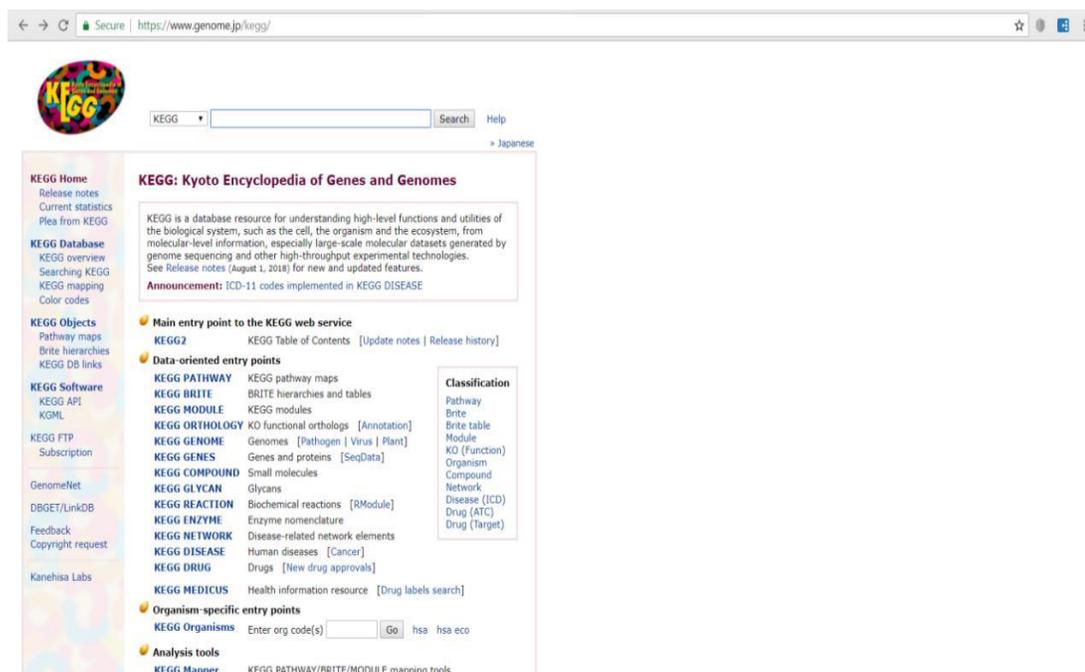
KEGG (Kyoto Encyclopedia of Gene and Genome)

KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome

sequencing and other high-throughput experimental technologies.

PDB (Protein Data Bank)

The main function of this database is to organize 3-D structural data of large biological molecules including proteins and nucleic acids of all the organisms including bacteria, yeast, plants, flies, other animals and humans. The three-dimensional structures of the biological macromolecules data available with PDB is determined by experimental methods such as X-ray crystallography, Nuclear magnetic resonance (NMR) spectroscopy, electron microscopy.



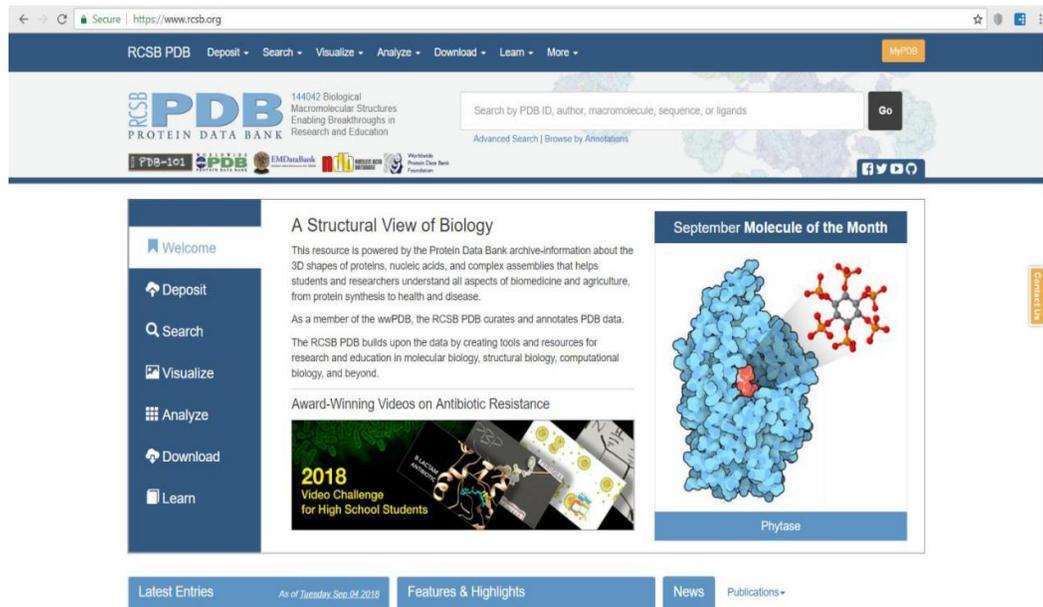


Figure 1

DEG (Database of Essential Genes)

Essential genes are those indispensable for the survival of an organism, and therefore are considered a foundation of life. DEG hosts records of currently available essential genomic elements, such as protein-coding

genes and non-coding RNAs, among bacteria, archaea and eukaryotes. Essential genes in a bacterium constitute a minimal genome, forming a set of functional modules, which play key roles in the emerging field, synthetic biology.

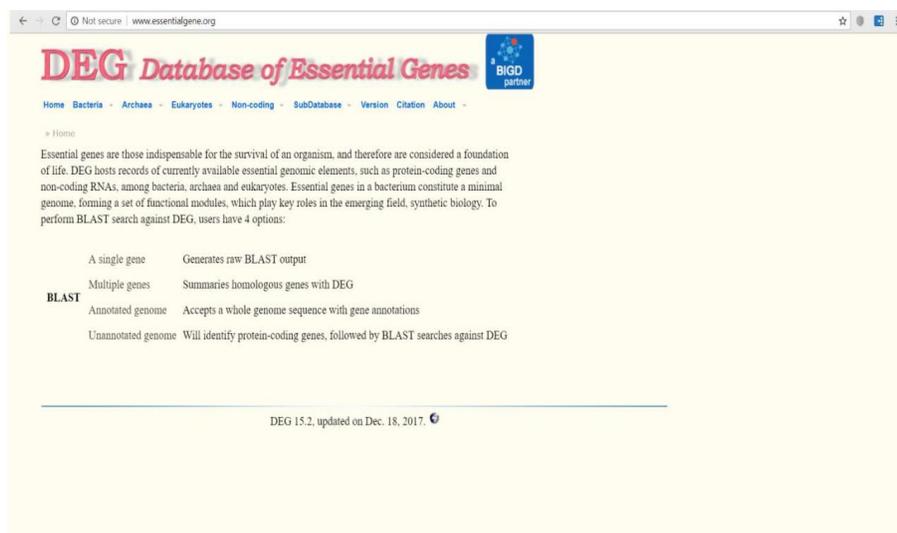


Figure 2

BLAST (Basic Local Search Alignment Tool)

The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence

databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

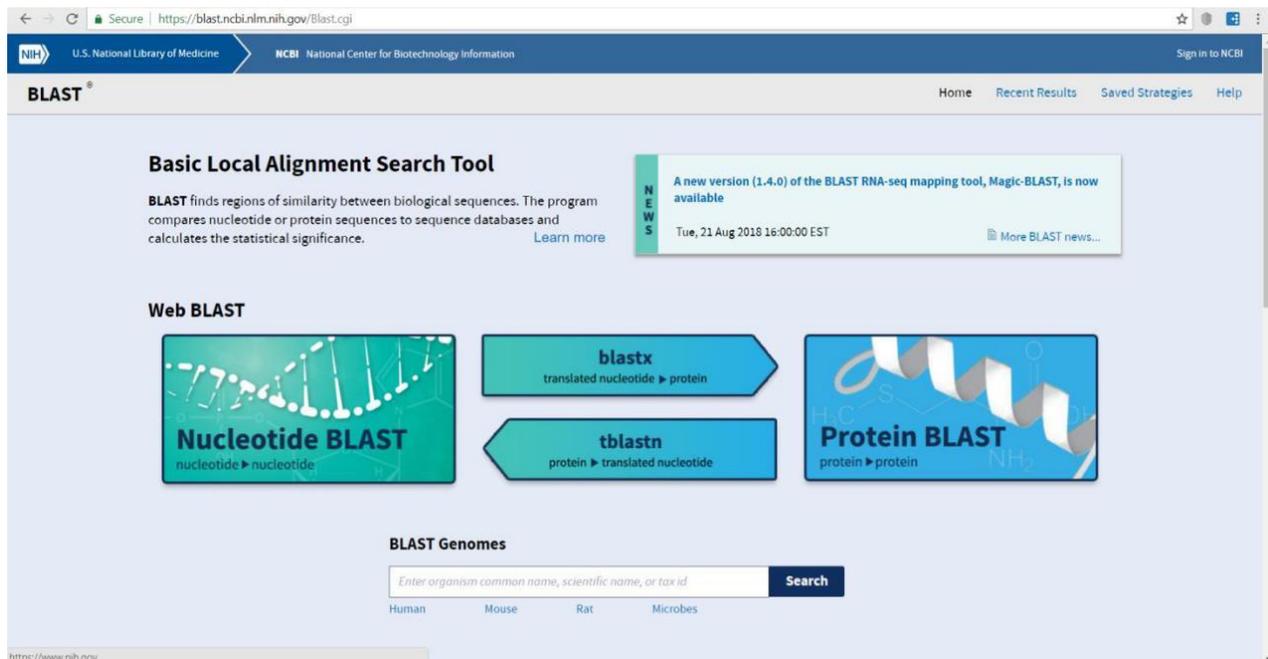


Figure 3

CYTOSCAPE

Cytoscape is an open source software platform for *visualizing* molecular interaction networks and biological pathways and *integrating* these networks with annotations, gene expression profiles and other state data. Although *Cytoscape* was originally designed for biological research, now it is a general platform for complex network analysis and visualization. *Cytoscape core* distribution provides a basic set of features for data

integration, analysis and visualization. Additional features are available as *Apps* (formerly called *Plugins*). *Apps* are available for network and molecular profiling analyses, new layouts, additional file format support, scripting, and connection with databases. They may be developed by anyone using the *Cytoscape* open API based on *Java™* technology and *App* community development is encouraged.



Figure 4

STRING

The STRING database contains information from numerous sources, including experimental data, computational prediction methods and public text collections. It is freely accessible and it is regularly updated. The resource also serves to highlight functional enrichments in user-provided lists of proteins,

using a number of functional classification systems such as [GO](#), [Pfam](#) and [KEGG](#). The latest version 10.5 contains information on about 9.6 million proteins from more than 2000 organisms. STRING has been developed by a consortium of academic institutions including [CPR](#), [EMBL](#), [KU](#), [SIB](#), [TUD](#) and [UZH](#).

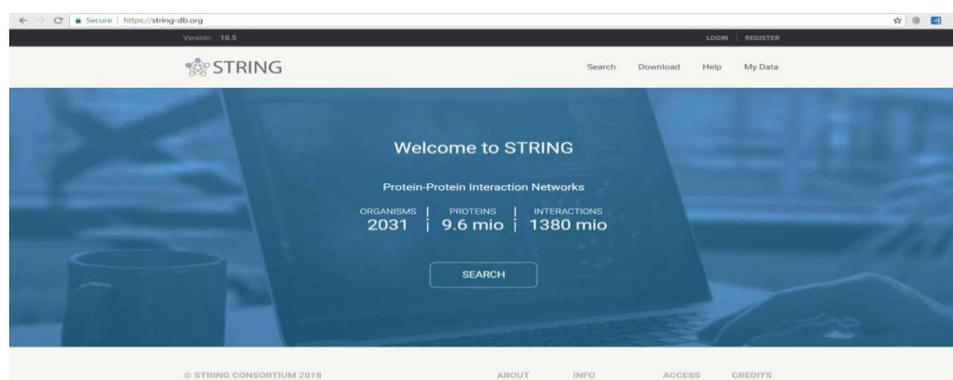


Figure 5

1. Retrieval of proteomes of pathogen and host

The complete proteome of *Nocardia asteroides* NBRC 15531 was retrieved from uniprot (Bairoch and Apweiler, 2000) and

complete Homo sapiens proteome was downloaded from NCBI (Maglott *et al.*, 2007).

UniProtKB results

Quote terms: "nocardia asteroides"

Entry	Entry name	Protein names	Gene names	Organism	Length
U5EMJ3	U5EMJ3_NOCAS	Bifunctional NAD(P)H-hydrate repair...	nnrD nnrE, NCAST_35_00550	Nocardia asteroides NBRC 15531	479
U5E9G8	U5E9G8_NOCAS	Bifunctional protein GImU	gImU NCAST_20_05800	Nocardia asteroides NBRC 15531	496
U5E5C1	U5E5C1_NOCAS	Pyrimidine/purine nucleoside phosph...	ppnP NCAST_01_00060	Nocardia asteroides NBRC 15531	103
Q9EZQ7	BLAC_NOCAS	Beta-lactamase AST-1	bla ast1	Nocardia asteroides	310
U5ECJ0	U5ECJ0_NOCAS	Riboflavin biosynthesis protein Rib...	ribA ribBA, NCAST_21_00930	Nocardia asteroides NBRC 15531	414
U5EDF3	U5EDF3_NOCAS	UDP-N-acetylmuramoyl-L-alanyl-D-glu...	murE NCAST_18_00610	Nocardia asteroides NBRC 15531	530
U5EFP6	U5EFP6_NOCAS	Ribose-phosphate pyrophosphokinase	prs NCAST_20_05790	Nocardia asteroides NBRC 15531	326
U5EEA2	U5EEA2_NOCAS	Aspartate-semialdehyde dehydrogenas...	asd NCAST_25_01750	Nocardia asteroides NBRC 15531	344

Figure 6: *Nocardia asteroides* proteome from UniProtKb

NCBI search results for 'homo sapiens'

Search results: Items: 1 to 20 of 717613

1.	MK2 non-allergic IgE heavy chain IGHV2-5	125 aa protein (primates)	1 sequence, including AB0229114.2
2.	double-stranded RNA-specific adenosine deaminase isoform e	1235 aa protein (primates)	1 sequence, including NP_001351974.1
3.	double-stranded RNA-specific adenosine deaminase isoform c	1181 aa protein (primates)	1 sequence, including NP_056656.3
4.	double-stranded RNA-specific adenosine deaminase isoform b	1200 aa protein (primates)	1 sequence, including NP_056655.3
5.	leucine-rich repeat-containing protein 53 isoform 2	1215 aa protein (primates)	1 sequence, including NP_001351595.1
6.	HLA class II histocompatibility antigen gamma chain isoform d	211 aa protein (primates)	

Figure 7: Human proteome results from NCBI

2. Non-homology analysis

The corresponding protein sequences of common and unique pathways were obtained from UniProt database (www.uniprot.org). They were subjected to BLASTP (Altschul *et al.*, 1997) analysis against the non-redundant database with the e-value inclusion threshold set to 0.005 against human proteome. Proteins

which did not have hit below the e-value were considered as non-homologous protein. The criterion for selection e-value was based on the previous study by Anishetty *et al.* (2005), Butt *et al.* (2012 a, b), and Damteet *et al.* (2013).

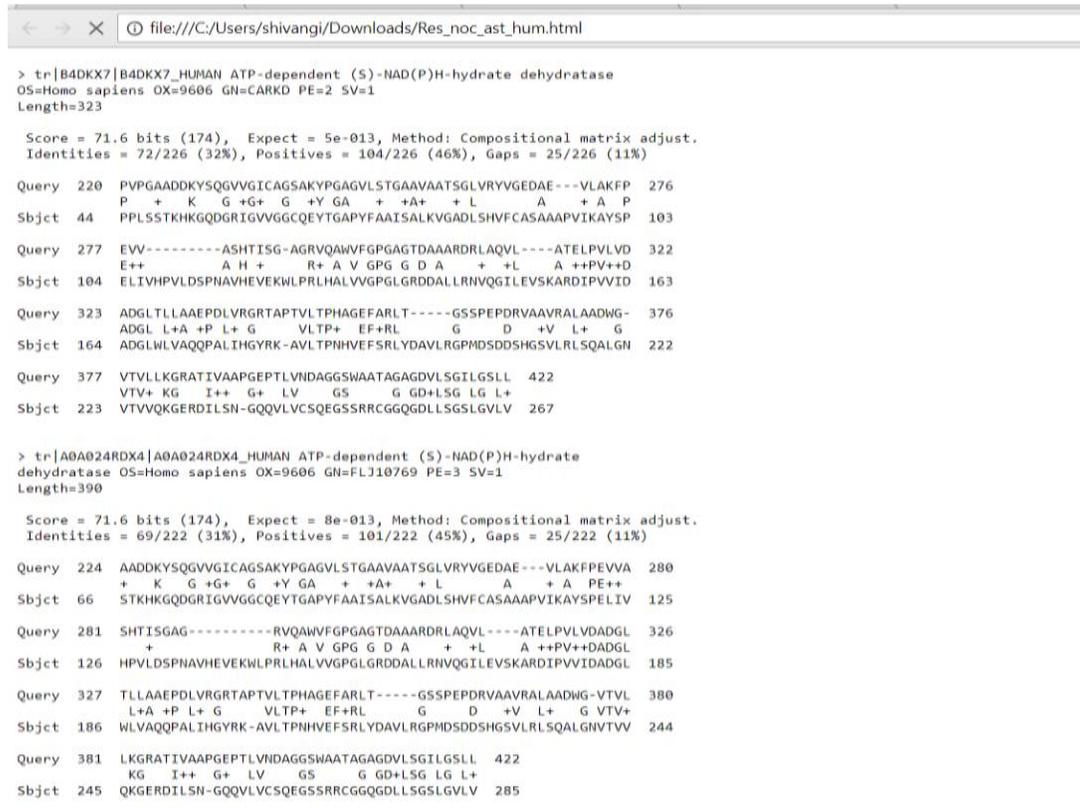
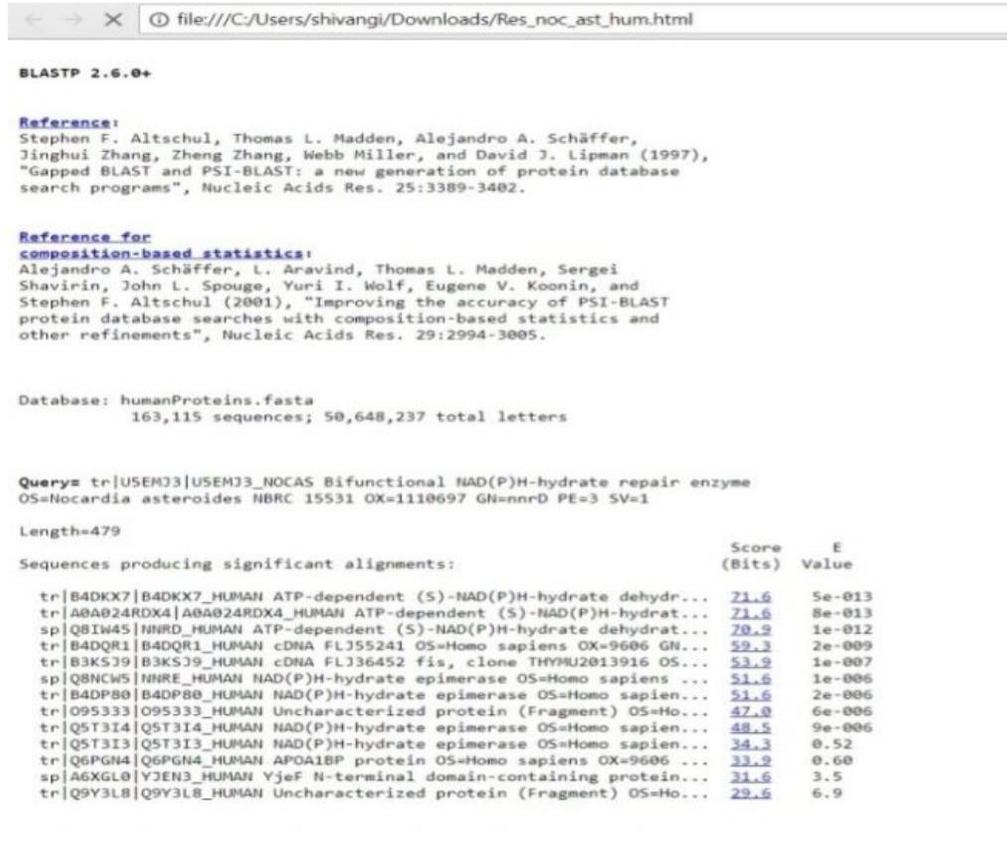


Figure 8: BLAST results

3. Essentiality analysis

Bacteria essential protein sequences were retrieved from the database of essential genes (DEG) (Zhang *et al.*, 2004) (<http://tubic.tju.edu.cn/deg/>)

4. Prediction of subcellular localization

Sub cellular localization of proteins could be used to obtain information about their potential functions. Sub cellular localization of the drug targets were carried out by PSORTb and the results obtained were further validated with CELLO v2.5. PSORTb-PSORTb version 3.0 with improved recall, higher proteome-scale prediction coverage,

and new refined localization subcategories. It is the first SCL predictor specifically geared for all prokaryotes, including archaea and bacteria with atypical membrane/cell wall topologies. It features an improved standalone program, with a new batch results delivery system complementing its web interface. We evaluated the most accurate SCL predictors using 5-fold cross validation plus we performed an independent proteomics analysis, showing that PSORTb 3.0 is the most accurate but can benefit from being complemented by Proteome Analyst predictions.

The screenshot shows the PSORTb web interface. At the top, there is a navigation bar with the PSORTb logo and links for Updates, Documentation, Resources, and Contact. Below this is a section titled "Submit a Sequence to PSORTb version 3.0.2". The text explains that PSORTb v3.0.2 is the most precise bacterial localization prediction tool available, based on a study from 2010. It lists improvements over v2.0.4 and provides a link to the version 2 maintenance page. The main content area contains instructions for submitting sequences in FASTA format, either by copying and pasting or uploading a file. It also mentions that the web display mode is limited to approximately 100 proteins and offers email delivery for larger analyses. A "See also" section lists various resources like updates, precomputed genome results, and user guides. At the bottom, there is a form with several dropdown menus and a text input field for email address, followed by a large text area for pasting FASTA sequences.

Submit a Sequence to PSORTb version 3.0.2

Based on a study last performed in 2010, PSORTb v3.0.2 is the most precise bacterial localization prediction tool available. PSORTb v3.0.2 has a number of [improvements](#) over PSORTb v2.0.4. Version 2 of PSORTb is maintained [here](#).

You can currently submit one or more Gram-positive or Gram-negative bacterial sequences or archaeal sequences in FASTA format (2). Copy and paste your FASTA-formatted sequences into the textbox below or select a file containing your sequences to upload from your computer. Web display mode is limited to the analysis of approximately 100 proteins. For larger analyses, either enter your email address in the form below (results of up to 5000 per submission returned by email) or for even larger analyses we can help you or you can download the standalone version.

See also:

- [Updates](#)
- [Precomputed genome results](#)
- [Limitations of PSORTb v.3.0](#)
- [PSORTb User's Guide](#)
- [Docker PSORTb web service](#) (what is docker?)
- [Download standalone PSORTb](#)
- [Docker standalone PSORTb](#) (what is docker?)

Choose an organism type (2): Required

Choose Gram stain (2): Required

Advanced Gram stain options (2): Required

Output format (2):

Show results (2):

Email address:

Copy and paste your FASTA sequences below

Figure 9: PSORTb

CELLO V.2.5

It is a publicly available, web-based system for screening various properties of a targeted protein and its subcellular localization. Herein, we describe how this platform is used to obtain a brief or detailed gene ontology (GO)-type categories, including subcellular localization(s), for the queried proteins by combining the CELLO localization-predicting and BLAST homology-searching approaches. Given a query protein sequence, CELLO2GO uses BLAST to search for homologous sequences that are GO annotated in an in-house database derived from the UniProt KnowledgeBase database. At the same time, CELLO attempts predict at least one subcellular localization on the basis of the species in which the protein is found. When

homologs for the query sequence have been identified, the number of terms found for each of their GO categories, i.e., cellular compartment, molecular function, and biological process, are summed and presented as pie charts representing possible functional annotations for the queried protein. Although the experimental subcellular localization of a protein may not be known, and thus not annotated, CELLO can confidentially suggest a subcellular localization. CELLO2GO should be a useful tool for research involving complex subcellular systems because it combines CELLO and BLAST into one platform and its output is easily manipulated such that the user-specific questions may be readily addressed.

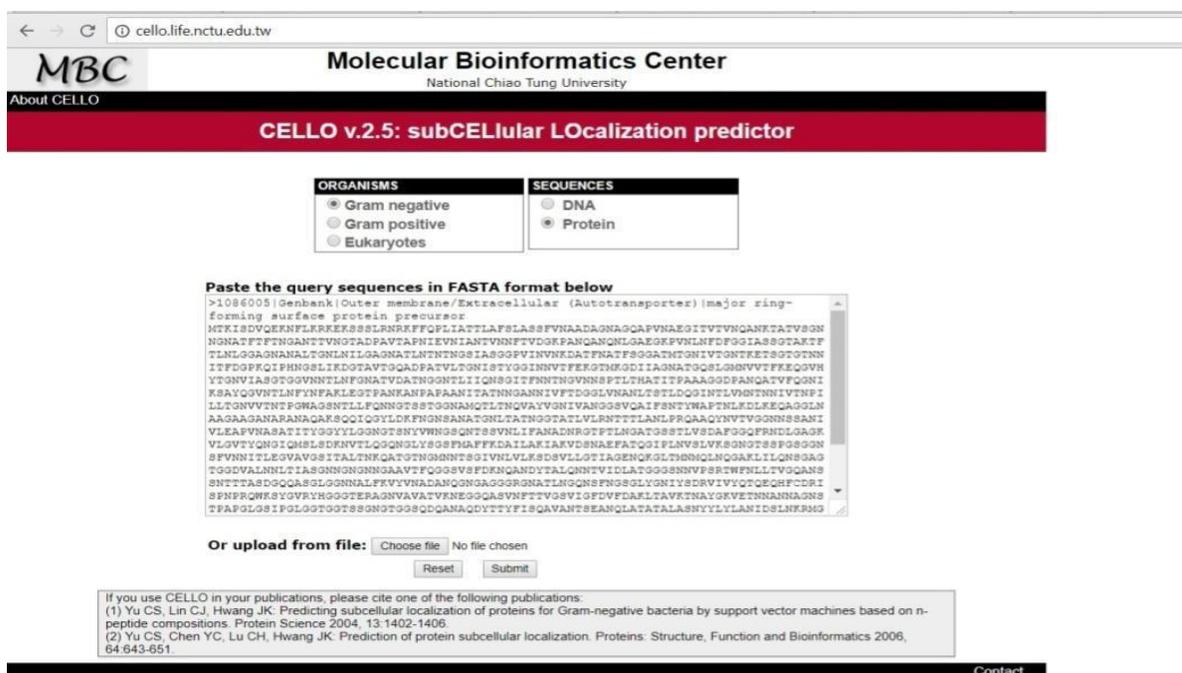


Figure 10: CELLO v2.5

5 Evaluating druggability of the targets.

The Drug Bank (<http://www.drugbank.ca>) database is a distinctive bioinformatics and cheminformatics resource that combines detailed drug data (chemical, pharmacological and pharmaceutical) with comprehensive drug target information (sequence, structure and pathway). The database contains 6796 drug entries, including 1437 FDA-approved small

molecule drugs, 134 FDA-approved biotech (protein/peptide) drugs, 83 nutraceuticals, and 5174 experimental drugs. Additionally, 4285 non-redundant protein (drug target/enzyme/transporter/carrier) sequences are linked to these drug entries. Drug ability of the predicted 37 drug targets were further checked using Drug Bank.

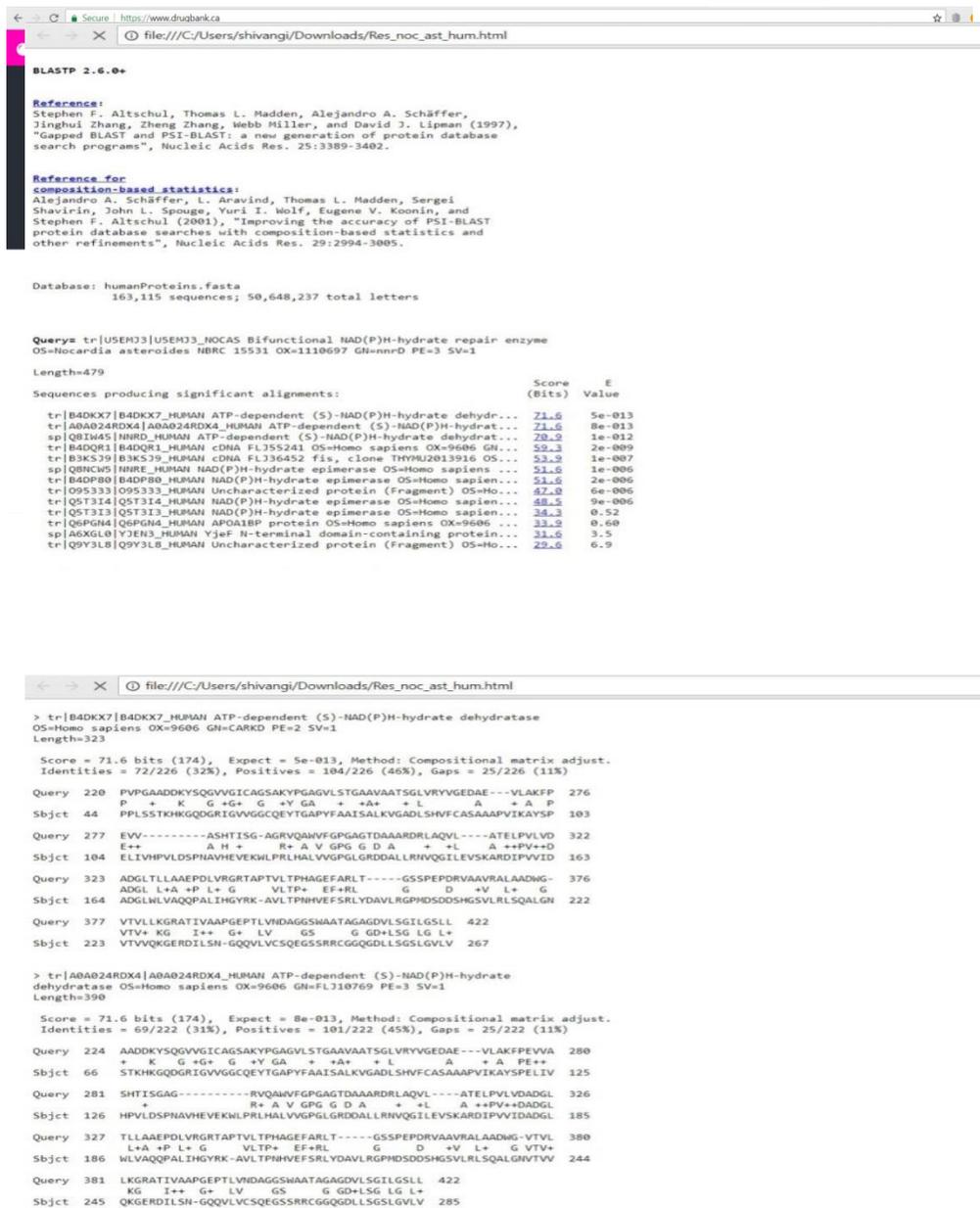


Figure 11: Drug Bank

RESULT AND DISCUSSION

1. Retrieval of proteomes of pathogen and host:

The complete proteome of *Nocardia asteroides* \NBRC 15531 was downloaded

from Uniprot. *Nocardia asteroides* NBRC 15531 have 6,540 proteins. Human proteome was also downloaded from Uniprot.

Entry	Entry name	Protein names	Gene names	Organism	Length
U5EM13	U5EM13_NOCAS	Bifunctional NAD(P)H-hydrate repair...	nnrD nnrE, NCAST_35_00550	Nocardia asteroides NBRC 15531	479
U5E9G8	U5E9G8_NOCAS	Bifunctional protein GlmU	glmU NCAST_20_05800	Nocardia asteroides NBRC 15531	496
U5E5C1	U5E5C1_NOCAS	Pyrimidine/purine nucleoside phosph...	ppnP NCAST_01_00060	Nocardia asteroides NBRC 15531	103
Q9EZQ7	BLAC_NOCAS	Beta-lactamase AST-1	bla ast1	Nocardia asteroides	310
U5ECJ0	U5ECJ0_NOCAS	Riboflavin biosynthesis protein Rib...	riBA ribBA, NCAST_21_00930	Nocardia asteroides NBRC 15531	414
U5EDF3	U5EDF3_NOCAS	UDP-N-acetylmuramoyl-L-alanyl-D-glu...	murE NCAST_18_00610	Nocardia asteroides NBRC 15531	530
U5EFP6	U5EFP6_NOCAS	Ribose-phosphate pyrophosphokinase	prs NCAST_20_05790	Nocardia asteroides NBRC 15531	326
U5EEA2	U5EEA2_NOCAS	Aspartate-semialdehyde dehydrogenas...	asd NCAST_25_01750	Nocardia asteroides NBRC 15531	344

Figure 12: Screenshot Of the proteins of *Nocardia asteroides* NBRC 15531

2. Non-Homology Analysis

Further, the proteins of *Nocardia asteroides* that are homologous to the human proteome could not be considered as the taken directly as potent drug targets since they are showing similarity with human proteins. Therefore, out of 6,543 proteins, 2,290 proteins were identified as non-homologues to the human proteome. Stand-alone BLAST software (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/LATEST/blast-2.2.21-ia32-win32.exe) was used for the BlastP analysis.

3. Essentiality analysis

BlastP analysis was performed for the non-homologous protein sequences of *Nocardia asteroides* against database of essential

proteins retrieved from DEG with E-value cutoff score of 10⁻¹⁰ and 30% identity. A minimum bit-score cut-off of 100 was used to screen out proteins that appeared to represent essential genes. The protein sequences obtained are non-homologous essential proteins of *Nocardia asteroides*. Total, 1200 proteins were identified as essential proteins. These proteins as non-homologous and essential could be a potent drug targets in pathogen.

Table 3: Metabolic pathways present in *Nocardia asteroides* but not in the host *Homo sapiens*.

A	Metabolic Pathways	KEGG pathway ID
1	C5-Branched dibasic	bps00660
2	acid metabolism	bps00710
3	Carbon fixation in	bps00680
4	photosynthetic	bps00300
5	Organisms	bps00460
6	Methane metabolism	bps00473
7	Lysine biosynthesis	bps00540
8	Cyanoamino acid	bps00550
9	metabolism	bps00909
10	D-Alanine metabolism	bps00906
11	Various types of N-	bps00906
12	glycan biosynthesis	bps00903
13	Lipopolysaccharide	bps00281
14	biosynthesis	bps00523
15	Peptidoglycan	bps01053
16	biosynthesis	bps00311
17	Sesquiterpenoid and	bps00332
18	triterpenoid biosynthesis	bps00261
19	Carotenoid biosynthesis	bps00521
20	Limonene and pinene	bps00525
21	degradation	bps00401
22	Geraniol degradation	bps00405
23	Polyketide sugar unit	bps00362
24	biosynthesis	bps00627
25	Biosynthesis of	bps00364
26	siderophore group no	bps00625
27	ribosomal peptides	bps00361
28	Penicillin and	bps00623
29	cephalosporin	bps00622
30	biosynthesis	bps00643
31	Carbapenem	bps00791
32	biosynthesis	bps00930
33	Monobactam	bps00621
34	biosynthesis	bps00626

4. Prediction of subcellular localization

Computational prediction of subcellular localization provides a quick and inexpensive means for gaining insight into protein function, verifying experimental results, annotating newly sequenced bacterial

genomes, and detecting potential cell surface/secreted drug targets. Among the 1200 drug targets, 900 were cytoplasmic, 250 were membrane, and 50 was extracellular from PSORTb. Similar results were also

observed for 37 drug targets using CELLO v2.5.

KEGG pathway analysis

Comparative metabolic pathway analysis of *Homo sapiens* and *Burkholderia pseudomallei* shows that there are 34 pathways unique to the pathogen are listed in Table respectively.

5. Evaluating drug ability of the targets

Ten common potential drug targets were found to be highly similar to the target proteins in Drug Bank. Further, the common drug targets were explored for presence of 3D structures and were explored for the structure-based drug designing (SBDD), to propose novel inhibitor molecules against bacteria.

CONCLUSION

Nocardia asteroides live on dead decaying organic matter in the soil, water, dust and on vegetation. Nocardiosis is a chronic bacterial disease of humans and many other animals originating in the respiratory tract and disseminated by way of blood to other organs. It is caused by either introduction of the species into the skin or by inhalation of the species from its habitat. The identification of *Nocardia* from an immunocompromised patient should never be ignored, especially if any abnormal clinical or radiologic pulmonary findings are present. To develop a vaccine against these diseases we have to know about its metabolic rate and chemical process by which it regulates. *Nocardia asteroides* species are filamentous aerobic,

Gram-positive and mycolic acid cell wall bacteria. *Nocardia* colonies vary from white, to tan, orange and red in colour they have a lipid bilayer cell membrane with associated proteins. Their cell envelopes consist of a Peptidoglycan cell wall. All mycolic acids comprising the cell wall of *Nocardia asteroides* are saturated mycolic acids. As of today, researches have not indicated association of Pili nor flagella on *Nocardia asteroides*. *Nocardia asteroides* experience an optimal growth at body temperature.

Nocardiosis can occur in apparently healthy population but further detailed immunologic evaluation particularly considering interleukin12-gamma interferon pathway deficiency or other immunologic systems may help in diagnosis of these patients' underlying diseases in the future. Occasionally isolation of *Nocardia* is due to colonization, but clinical decision making to receive treatment is according to apparent *Nocardia* related disease and existence of immune deficiency and comorbidity. However, pulmonary colonization in healthy people without disease is not rare. Drug of choice for *Nocardia* is Trimethoprim-sulfamethoxazole (TMP-SMX), but other regimens like Amikacin, Imipenem, Minocycline, Linezolid and Cephalosporins are alternatives. Occurrence of *Nocardia* in patients on TMP-SMX prophylaxis may happen rarely, which means

that this drug is not fully protective agent against Nocardiosis.

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