Infection, Genetics and Evolution 12 (2012) 853–856

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Potential of *Mycobacterium vanbaalenii* as a model organism to study drug transporters of *Mycobacterium tuberculosis*, *Mycobacterium marinum* and *Mycobacterium ulcerans*: Homology analysis of *M. tuberculosis* drug transporters among mycobacterial species

Anuj Kumar Gupta [1], V.M. Katoch *, D.S. Chauhan, Mallika Lavania [2]

Department of Microbiology & Molecular Biology, National JALMA Institute for Leprosy & Other Mycobacterial Diseases (NJILOMD), Tajganj, Agra 282001, India

ARTICLE INFO

Article history:
Available online 23 November 2011

Keywords:
Mycobacterium vanbaalenii
Model organism
Drug efflux pumps
Mycobacterium tuberculosis
Mycobacteria

ABSTRACT

Drug efflux pumps have been one of the important mechanisms of drug resistance in *Mycobacterium tuberculosis*. There is a prerequisite to study the behavior and mechanisms of these drug efflux pumps in detail for being considered in future anti-TB drug designing. The need of a rapid grower non-pathogenic mycobacterium with significant genomic homology for such type of studies is often being felt. During microarray and Real-Time PCR analysis of drug efflux pump genes of *M. tuberculosis*, we found 10 genes to be over-expressed during stress induced by common anti-TB drugs. In the present study homology analysis of these genes was done in order to know its phylogenetic relationship among other bacteria /mycobacteria. It was found that amino acid sequences of 7 out of 10 genes were significantly (>40%) identical to a non-pathogenic rapid grower environmental mycobacterium, *M. vanbaalenii*. The protein sequences of *M. vanbaalenii* share important sequence motifs with *M. tuberculosis* useful for drug efflux mechanism based study across species. Like *M. smegmatis*, it can be used as a model organism to study drug efflux pumps of *M. tuberculosis* and also other pathogenic mycobacteria such as *M. ulcerans* and *M. marinum*.

1. Introduction

Drug transporters are important contributing factors of drug resistance in a number of clinically important microorganisms. Basically they are active so that noxious substance could be transported out of the organism/cell, allowing survival (Webber and Piddock, 2003). All the bacterial genomes studied so far have been found to contain several different efflux pumps, which indicate their ancestral origin. It has been estimated that ~5–10% of all bacterial genes are involved in transport and a large proportion of these encode efflux pumps (Lomovskaya and Watkins, 2001).

The highest numbers of predicted drug efflux systems have been observed to be present in the soil or environmental bacteria: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Deinococcus radiodurans*, as well as *Escherichia coli* and *Saccharomyces cerevisiae*. This justifies the origin of drug transporters as excretion systems for secondary metabolites or as a mechanism of defense against environmental toxins and also antibiotics. However, other organisms such as intracellular *Mycobacterium tuberculosis* and *Rickettsia prowazekii* also have relatively large numbers of predicted drug efflux systems, thus serving other physiological role. The abundance of drug efflux systems in these different organisms does not necessarily clarify the natural physiological roles of these transporters, which would be varying in different conditions (Paulsen et al., 2003).

The genome of *M. tuberculosis* contains around 20 genes, which have been annotated as probable drug efflux pumps (Welcome Trust's Sanger Institute, ftp://ftp.sanger.ac.uk/pub/pathogens/Mycobacterium/tuberculosis/functional_classes – see functional class III.A.6). Only some of them have been experimentally demonstrated to extrude a variety of substances including active antibiotics (DeRossi et al., 2006, da Silva et al., 2011). Several studies dealt with efflux pump identification involved cloning and expressing a gene into a non-pathogenic host and looking whether it contributes to a phenotypic difference in the host cell. The primary problem in studying structural and functional properties of efflux pumps in *M. tuberculosis* lies behind its highly pathogenic and slow growing

* Corresponding author. Present address: Secretary, Department of Health Research, Ministry of Health and Family Welfare, Govt. of India, and Director General, Indian Council of Medical Research, Ansari Nagar, New Delhi 110029, India (Ex-Director, NJILOMD, Agra). Tel.: +91 11 26588204; fax: +91 11 26588662.
E-mail address: vishwamohan_katoch@yahoo.co.in (V.M. Katoch).
[1] Present address: Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India.
[2] Present address: Stanley Browne Lab, TLM Community Hospital, Nand Nagari, New Delhi 110093, India.

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doi:10.1016/j.meegid.2011.10.028

nature, which requires more skilled handling and relatively lengthy experiment duration. None of the non-pathogenic mycobacterial species has been found to possess a significant identity among efflux pump protein sequences useful in analyzing structural and functional properties of efflux pumps which can be almost similar to that of *M .tuberculosis*. The need to have a non-pathogenic rapid growing model organism is often being felt for research aimed at tuberculosis (El-Etr et al., 2001). This would be even more desirable to have models/screens that could be common to *M. tuberculosis* and important slow growing pathogenic non-tuberculous mycobacterial species such as *Mycobacterium avium*.

2. Materials and methods

In our microarray and Real-Time PCR based studies, ten efflux pumps have been identified to contribute in drug resistance in mycobacteria (Gupta et al., 2010a, 2010b) (Indian Patent Application No. 2071/DEL/2007). During our attempts to study phylogenetic relationship among protein sequences of different drug transporters of a number of bacterial species, we performed bioinformatic analysis of their protein sequences. Phylogenetic analysis of these efflux pump proteins was done in order to investigate their intergeneric as well as intrageneric relationships among clinically important bacteria as well as mycobacteria. The protein sequences of these drug transporters were obtained from tuberculosis database (TubercuList web server; http://genolist.pasteur.fr/TubercuList/). Firstly, the amino acid sequence homology was searched among all organisms and among mycobacterial species separately using BLASTp and Position Specific Iterated BLAST (PSI-BLAST). The sequences with close homologous relation with these efflux proteins were selected from BLAST analysis. These sequences were subjected to multiple sequence alignment using ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and were used to draw phylogenetic trees by using online programme Phylodendron (http://iubio.bio.indiana.edu/treeapp/treeprintform.html) as well as by NCBI web server (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Protein structural motifs were searched and aligned among these sequences using MEME programme (Multiple Em for Motif Elicitation; version 4.0.0; http://meme.sdsc.edu/meme4/cgi-bin/meme.cgi).

3. Results and discussion

While orthologous genes in *M. smegmatis* showed significant homology with > 40% amino acid identity (Petsko and Ringe, 2004) with five of the ten *M. tuberculosis* drug transporters (Rv2688, Rv2994, Rv1819c, Rv2846 and Rv2477), efflux pump genes of another non-pathogenic fast growing environmental species *M. vanbaalenii* with biodegradation capabilities (Khan et al., 2002) showed significant homology to seven sequences, i.e. Rv2688, Rv1819c, Rv3065, Rv2938, Rv2994, Rv2846 and Rv2477 (Table 1). Other three genes (Rv2459, Rv3728 and Rv2209) showed relatively limited homology (<40% amino acid identity) with *M. vanbaalenii* sequences, although they have little differences (30-39% identity) (Table 2).

The phylogenetic analysis of mycobacterial efflux pumps revealed existence of their neighbour sequences with similar functions in other bacteria and reflected dynamic and interesting scenarios of evolutionary importance. It is seen from the phylogenetic trees drawn within highly similar sequences that efflux

Table 1
Drug efflux pumps of *M. tuberculosis* showing >40% amino acid identity with sequences of other mycobacteria.

M. tuberculosis efflux pumps	Species in which homology found	Description of homologous sequence	% Identity
Rv2688	*M. bovis AF2122/97*	Probable antibiotic-transport ABC transporter	99
	M. vanbaalenii PYR-1	ABC transporter-related protein	80
	M. marinum M	Antibiotic-transport ABC transporter	80
	M. smegmatis MC2 155	ABC transporter, ATP-binding protein	80
Rv1819c	*M. bovis BCG*	Putative drugs-transport transmembrane ABC protein	99
	M. ulcerans Agy99	drug-transport transmembrane transporter	87
	M. marinum M	ATP-binding protein ABC transporter	88
	M. avium	Hypothetical protein MAP1531c & sbmA	76
	M. vanbaalenii PYR-1	ABC transporter domain protein	60
Rv3065	*M. avium 104*	SMR family protein	82
	M. leprae TN	Probable multidrug resistance protein ML1776	78
	M. marinum M	Multidrug-transport integral membrane protein Mmr	74
	M. ulcerans Agy99	Multidrug-transport integral membrane protein Mmr	72
	M. vanbaalenii PYR-1	Small multidrug resistance protein	72
Rv2938	*M. leprae TN*	Probable antibiotic resistance membrane protein ML2350	78
	M. ulcerans Agy99	Daunorubicin-transport integral membrane ABC protein DrrC	72
	M. marinum M	Daunorubicin-transport integral membrane ABC transporter	72
	M. gilvum PYR-GCK	ABC drug efflux pump, inner membrane subunit, DrrB family	48
	M. vanbaalenii PYR-1	ABC drug efflux pump, inner membrane subunit, DrrB family	47
Rv2994	*M. marinum M*	Conserved integral membrane transport protein	81
	M. avium 104	Sugar transporter family protein	74
	M. leprae TN	Hypothetical protein MLCB637.27c	61
	M. smegmatis MC2 155	Sugar transporter family protein	59
	M. vanbaalenii PYR-1	Major facilitator superfamily MFS_1	56
Rv2846	*M. bovis AF2122/97*	Possible integral membrane efflux protein efpA	99
	M. marinum M	Integral membrane efflux protein EfpA	90
	M. ulcerans Agy99	Integral membrane efflux protein EfpA	89
	M. avium	Transporter, major facilitator family protein EfpA_2	88
	M. vanbaalenii PYR-1	Major facilitator superfamily transporter	76
Rv2477	*M. bovis AF2122/97*	Putative ABC transporter ATP-binding protein	99
	M. avium	Putative ABC transporter ATP-binding protein	94
	M. marinum M	ATP-binding component of an ABC transporter	93
	M. ucerans Agy99	Putative ABC transporter ATP-binding protein	93
	M. vanbaalenii PYR-1	Putative ABC transporter ATP-binding protein	89