ANALYSIS OF *MYCOBACTERIUM LEPRAE* GENOME: IN SILICO SEARCHING FOR DRUG TARGETS

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Abstract. The author performed a database search to find the recorded complete genes with complete sequences of *Mycobacterium leprae* and studied their homology to human genomes by BLAST method. From a total of 35 genes, the potential candidates for further target-based drug development were identified.

INTRODUCTION

Leprosy is a chronic tropical infectious disease which attacks the skin, peripheral nerves and mucous membranes. This disease, also known as Hansen's disease because the causative bacillus, *Mycobacterium leprae*, was discovered by GA Hansen in 1873 (Luis Fernandez *et al*, 2004). The high complexity and variability of clinical presentation, together with epidemiology and drug accessibility, results in various treatment regimens being currently used. This makes eradication difficult and contributes to the existence of many different treatments following different recommendations on leprosy (Luis Fernandez *et al*, 2004). Current treatments share a common basis made up of several combined drugs, particularly rifampicin, dapsone, and clofazimine (Luis Fernandez *et al*, 2004). Antimicrobials most recently added to the therapy of leprosy include fluoroquinolones, tetracyclines, and macrolides (Luis Fernandez *et al*, 2004). Supervised multi-drug therapy (MDT) for fixed durations is highly effective for all forms of the disease (Britton and Lockwood, 2004). The widespread implementation of MDT has been associated with a fall in the prevalence of the leprosy but as yet no reduction in the case-detection rate globally (Britton and Lockwood, 2004).

An important problem in the control of leprosy is drug resistance (Mistry and Antia, 1993; Williams and Gillis, 2004). Some recent studies indicate that the problem of drug resistance in leprosy is very concerning. With an increasing number of new cases with low bacterial loads, the conventional *in vivo* and *in vitro* methods for determination of drug resistance may not help (Gupta and Katoch, 1999). Newer molecular approaches, including the polymerase chain reaction (PCR), may be more useful and it will be important to undertake studies to develop such tools (Gupta and Katoch, 1999). The use of advanced molecular biology technology to discover new drugs to treat resistant organisms is needed.

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MATERIALS AND METHODS

Searching for the *M. leprae* gene set

The author used the database of the National Center for Biotechnology (Wheeler *et al*, 2003) to search for the gene sequences of *M. leprae*. The gene set with complete sequences was investigated for further homology identification.

Homology identification

In order to identify the target genes with high selectivity, the *M. leprae* gene sequences were compared to those of human genes. Basic Local Alignment Search Tool (BLAST) (McGinnis and Madden, 2004) was used as the main tool in searching for the homology. The genes that lacked significant homology to the human genome were identified as potential candidates for further target-based drug development.

RESULTS

According to the database search, 308 nucleotide sequences (as of September 2004) have been derived. A gene set consisting of 35 complete gene nucleotide sequences was derived for further homology study (Table 1). Of these genes, all were found to lack significant homologues to the human genome and were identified as potential candidates for further target-based drug development (Table 1).

DISCUSSION

Leprosy remains an important health problem worldwide (Britton and Lockwood, 2004). At the beginning of 2004, the number of leprosy patients under treatment in the world was around 460,000.

Table 1
Genes of *Mycobacterium leprae* with complete sequences in the Database (http://www.ncbi.nlm.nih.gov/).

Accession numbers	Description	Homology to human genome
X77655	*M. leprae* ASPS gene	No
X55022	*M. leprae* 16S ribosomal RNA	No
AB019194	*M. leprae* rpoT gene, complete cds, strain:Kyoto-2	No
AB019193	*M. leprae* rpoT gene, complete cds, isolate:Thai 53	No
U82117	*M. leprae* 34 kDa antigen precursor gene	No
U82112	*M. leprae* tRNA-Ala gene, complete sequence	No
Z68206	*M. leprae* DNA gyrase subunit A gene	No
X77128	*M. leprae* Ag84 (CIE) gene	No
X76501	*M. leprae* M43L gene	No
X73822	*M. leprae* recA gene	No
X68431	*M. leprae* sra gene for serine-rich antigen	No
Z21949	*M. leprae* of MPT51 gene	No
Z21950	*M. leprae* of 85A protein gene	No
Z21951	*M. leprae* of 85C protein gene	No
X65546	*M. leprae* pra gene for proline rich antigene	No
X51328	*M. leprae* mla12A gene	No
X53487	*M. leprae* LSR2 gene	No
X63470	*M. leprae* gene for biotin carboxyl carrier protein	No
X60934	*M. leprae* gene for 85-B antigen	No
X58888	*M. leprae* gene for 16S ribosomal RNA	No
X53999	*M. leprae* gene for 16S rRNA	No
Z46257	*M. leprae* aceA gene for isocitrate lyase	No
L10660	*M. leprae* putative 46 kDa membrane protein gene	No
M90649	*M. leprae* 85-C antigen gene, complete cds	No
M90648	*M. leprae* 85-A antigen gene, complete cds	No
X90946	*M. leprae* L45 gene	No
X80124	*M. leprae* rpsL gene	No
Z26766	*M. leprae* (her1) gene for acyltransferase (putative)	No
M67510	*M. leprae* antigen 15 kDa gene, complete cds	No
M22587	*M. leprae* 18 kDa antigen gene, complete cds	No
M23232	*M. leprae* 28-kDa protein gene, complete cds	No
D43841	*M. leprae* gene for antigen 85 complex A, complete cds	No
D50488	*M. leprae* gene for MPT51 like protein, complete cds	No
AF117618	*M. leprae* dihydropteroate synthase gene	No
AB022517	*M. leprae* ML-LBP21 gene for 21-kDa laminin-2-binding protein, complete cds	No

About 515,000 new cases were detected during 2003 (WHO, Leprosy Elimination Group, 2004). Among them, 43% were multibacillary cases, 12% were children, and 3% were diagnosed with severe disabilities (WHO, Leprosy Elimination Group, 2004). Meima *et al* (2004) said that there was no general decline in case detection to date, and several countries still have high case detection rates. Since 1940, treatment using dapsone has been used to suppress leprosy (WHO, Leprosy Elimination Group, 2004). Seldom can leprosy be completely eradicated from a patient's skin and tissues; modest expectations for newer and better drug combinations led to MDT for the control of leprosy (Noordeen, 2000, WHO, Leprosy Elimination Group, 2004).

Previously, the assessment of chemotherapy efficacy in leprosy was difficult, since the only reliable method for determining whether *M. leprae* was viable depended on its growth on mouse foot pads (Jamil *et al*, 1993). A new method based on PCR has been developed (Jamil *et al*, 1993). The PCR technique was not useful for follow-up of treatment, but for detection of drug resistance (Mistry and Antia, 1993; Williams and Gillis, 2004). The *folP1*, *rpoB*, and *gyrA* genes were the main groups of *M. leprae* genes identified for their correspondence to drug resistance. Maeda *et al* (2001) analyzed 88 isolates of *M. leprae* from leprosy patients in Japan, Haiti, Indonesia, Pakistan, and the Philippines and found that 14.8% showed representative mutations in more than two genes, suggesting the emergence of multi-drug resistant *M. leprae*. Finding new drugs to treat multi-drug resistant *M. leprae* is therefore a useful leprosy research (Pattyn, 1986; Gupta and Katoch, 1999).

Since it is generally believed that the genomes of bacteria contain both genes with and without homologues to the human host. Drugs that specifically target genes with high homology to the host can lead to unwanted toxicity, therefore, finding new anti-leprosy drugs should based on genome homology. Here, the author performed a database search to find the recorded genes with complete sequences of *M. leprae* using the similar method noted by Thammarongtham and Palittapongarnpim (2002), and studied the homology to human genomes by the BLAST method. According to this study, there are 35 identified target genes. These genes can be the target for future "magic bullet" anti-leprosy drugs.

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