MYCOBACTERIUM LEPRAE GENOME REVISITED USING THE LATEST BIOINFORMATICS TOOLS

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Abstract

M.Leprae genome was analysed and compared to M.tuberculosis genome to seek explanations for the unique features of M.Leprae. The protein implicated in M.leprae's nerve predilection is shared by many mycobacteria. No human nerve protein homologues were found. 18KDa cytoplasmic antigen of M.Leprae showed little similarity to other mycobacterial proteins.

Introduction

Leprosy is a chronic infectious disease caused by mycobacterium leprae. Mycobacterium leprae closely resembles mycobacterium tuberculosis though biochemical differences do exist between the two.

M. Leprae, one of the earliest human pathogens to be identified is still considered unique. The physiological basis for many of these unique behaviour is still not understood completely.

The important distinguishing features of M.Leprae are the following.

1. Very long incubation period which ranges from 2-7 years.
2. Predilection for schwann cells of the nerves.
3. Inability to grow in the conventional culture media.
4. Only few organisms are susceptible to infection with m.leprae apart from humans.
5. Intracellular location of the bacilli
6. Can initiate a wide range of host immunological responses.

This article is an attempt to seek explanation to some of the above factors by analysing the m.leprae genome.

The complete genome of M.leprae TN strain contains 3,268,203 base pairs with a GC content of 57.8%.1 The other details are listed in Table 1.

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NERVE DESTRUCTION IN LEPROSY

Leprosy is primarily a disease of the nerves, and the skin manifestations are mostly secondary to the nerve involvement. Morbidity in leprosy is mainly due to the nerve destruction.[2]

Nerve involvement in Leprosy has been the focus of many research activities. However the cause of nerve predilection still remains a mystery. The affinity of M.Leprae for schwann cells is hypothesised to be due to an alpha 2 laminin binding protein. This protein was later found to be a 21 KDa histone like protein (HLP) coded by ML-LBP21 gene.[3] The EMBL Accession number for this gene is AB022517.

I conducted a BLAST (Basic Local Alignment Search Tool)4 search using the above gene and found that homologues are found in most of the atypical mycobacteria including M.Tuberculosis. It is still not understood how a shared gene can account for this unique feature.

Autoantibodies against neural antigens have also been demonstrated in leprosy.[5] I conducted blast search of many neural membrane antigens, but did not find any similarity with mycobacterial antigens. Hence it is possible that these antibodies are part of non-specific humoral immunity hyperactivity in lepromatous leprosy. Another possibility mentioned is that schwann cells process and present antigens of M.Leprae to Th cells which may subsequently damage and lyse infected schwann cells.[6]

DOWNSIZING OF GENOME AND ITS IMPLICATIONS

One of the most interesting thing which has happened to M.leprae genome over the years is its downsizing compared to other mycobacteria.[7] This is evident from the data presented in Table 1. M. Leprae seems to have lost a significant part of its non-coding repeats compared to M.Tuberculosis. In the process it is likely that M.Leprae lost few of its genes associated with metabolic pathways, making it more fastidious than M.Tuberculosis.8 Hence a detailed comparative genomic study of M.Leprae and M.Tuberculosis might provide insight into the unique features of M.Leprae like its long generation time and inability to grow in conventional culture media.

COMPARISON OF M.LEPRAE AND M.TUBERCULOSIS GENOME

M.Leprae genome was simultaneously compared with M.Tuberculosis and draft human genome using NCBI's (National Centre for Biotechnology Information) Taxplot (Fig. 1). Taxplot is the graphical representation of com-

parison of whole genome to that of two other reference organisms. All coding areas are translated to corresponding protein and blast is performed with every protein of the reference organism. The highest score for each organism is plotted on a graph. If a protein lies on the midline it shows equal similarity to corresponding protein of each organism. If it lies on X or Y axis it is only to that particular organism's protein. If it lies on either side of midline, it is similar to one organism more than the other. The points towards the origin of the graph represent proteins, which show minimal similarity to the reference organisms.

One of the interesting features observed from the TaxPlot was that prolyl t RNA synthetase of M.Leprae (gil15827816) resembled human glutamyl prolyl tRNA (gil16158948) than any protein of M.Tuberculosis. (Figure. 2) This unusual resemblance to human protein can be explained by 'horizontal gene transfer'[9] sometimes observed between the host and the intracellular organisms. Though the clinical relevance of this transfer is not known at present, further research may bring to light more clinically relevant gene transfers.[10]

The bottom area of the graph (Figure. 3) represents proteins in M.Leprae with minimal resemblance to M.tuberculosis or human proteins. One relevant protein found in this region is 18 KDa cytoplasmic antigen. The closest M.Tuberculosis protein is a 19KDa protein with a blast similarity score of only 88 (E=0.22). Hence 18 KDa antigen is the most unique of M.Leprae proteins and can be used as a marker for the organism to differentiate it from other mycobacteria. However recent studies have demonstrated cross reactivity for this antigen too.11

Bioinformatics is still in the nascent stage. Further research in this field might lead to the identification of missing genes in M.Leprae, replacement of which can lead to faster cultivation of bacteria for research purposes. Identification of specific genes of M.Leprae can also help in epidemiological studies to differentiate relapse and reinfection and to develop specific skin tests for leprosy

ACKNOWLEDGEMENT

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REFERENCES

1. Cole ST, Honore N, Eiglmeier K. Preliminary analysis of the genome sequence of mycobacterium leprae. Lepr Rev., 2000; S162-S164.

2. Pereira JH. Mycobacteria in nerve trunks of long term treated patients. Lepr Rev. Jun 1991; 62(2):134-42

3. Shimoji Y. A 21 KDa Surface protein of Mycobacterium leprae binds peripheral nerve laminin 2 and mediates schwann cell invasion. Proc Natl Acad Sci USA. Aug 1999; 96(17):9857-62

4. Altschul SF, Gish W, Miller W et al. Basic Local Alignment Search Tool. J Mol Biol. 1990; 215: 403-10

5. Corsico B. Identification of myelin basic protein in circulating immune complexes associated with lepromatous leprosy. Clin Immunol Immunopathol. Apr 1999; 71(1): 38-43

6. Spierings E. Mycobacterium leprae specific, HLA Class II restricted killing of human schwann cells by CD4+ Th1 cells: a novel immunopathogenic mechanism of nerve damage in leprosy. J Immunol. May 2001; 166(10): 5883-5888

7. Cole ST. Massive gene decay in the leprosy bacillus. Nature. Feb 2001; 409(6823): 1007-11

8. Eiglmeier K. The decaying genome of Mycobacterium Leprae. Lepr Rev. Dec 2001; 72(4): 387-98

9. Lawrence JG. Reconciling the many faces of lateral gene transfer. Trends Microbiol. Jan 2002; 10(1): 1-4

10. Webb T. Researchers explore role of gene transfer in tumor growth. J Natl Cancer Inst. Mar 2002; 94(6): 413-4

11. Vikerfors T. Serological response in leprosy and tuberculosis patients to the 18 KDa antigen of Mycobacterium Leprae and antigen 85B of mycobacterium bovis BCG. Int J Lepr Other Mycobact Dis. Dec 1993; 61(4): 571-80

Table. 1: Comparison of M.Leprae genome with M.Tuberculosis genome.

Feature	M.leprae	M.Tuberculosis
Base Pairs	3,268,203	4,411,529
Accession Numbers	AL450380 (EMBL) NC_002677 (GenBank)	AL123456 (EMBL) NC_000962 (GenBank)
GC Content	57%	65%
Number Of Genes	2,500	3,918

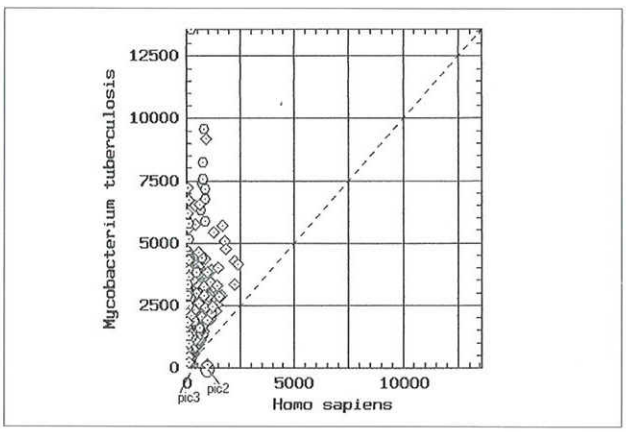

Figure 1. Complete TaxPlot View.

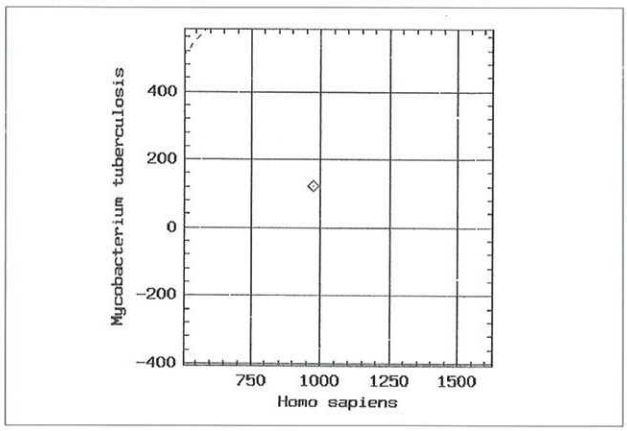

Figure 2. Magnified view of the area marked pic2 in Figure 1, to show the 'horizontal gene shift'.

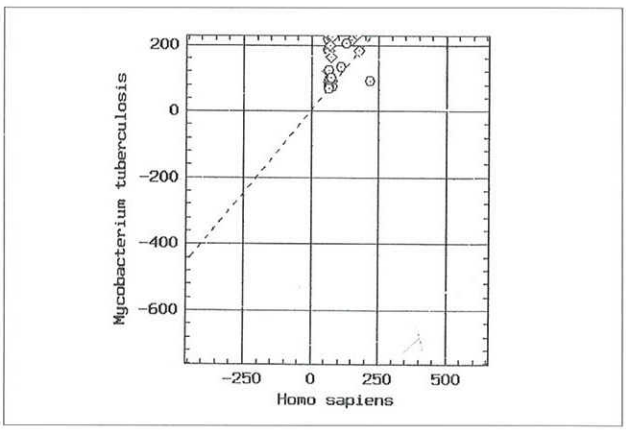

Figure 3. Magnified view of the area marked pic3 in Figure 1, to show the proteins with least similarity to compared organisms.