Expression analysis of genes related to metabolism and virulence of *Mycobacterium leprae* during infection in human host by microarray

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**Background**

- Leprosy is one of the oldest and notorious, but least understood diseases of man which continues to be a challenge to health worldwide, with about 250,000 new cases being currently detected every year.

- Although we got many successes in controlling the Leprosy, still there is a need for research on early diagnosis, treatment, and prevention.

- Functional analysis of *M. leprae* genome has potential to provide enormous opportunities for the better understand of disease as well as designing the rationale for effective early diagnosis and control strategies.
To identify genes of *M. leprae* those are highly transcribed in human host.
Materials & Methods

i. Designing of gene specific oligo-nucleotides probes of *M. leprae*
   Gene specific oligos (70mer length) by OligoPicker 3.2.1.

ii. Preparation of Partial DNA Chip of *M. leprae* :
   - A panel of 64 targets
     (60 genes specific oligos + 4 controls)
   - Targeted genes known to be associated with virulence in other organisms as well as basic metabolism.
   - Positive control: 16S rRNA gene of *M. leprae*
   - Negative control: β-actin (Human)
   - Labeling controls : Cy3 and Cy5 labelled DNA.

Partial DNA Chip of *M. leprae*
Scalpel biopsies samples from Leprosy cases (untreated or Bacteriological Index >3)  

Biopsies from healthy (Non Leprosy) controls

Processing of specimen and RNA isolation

Purification of M. leprae RNA from host RNA

- Preparation of Cye 3, Cye 5 labeled cDNA
- Hybridization of cDNA to partial DNA Chip

Expression profiling by Microarray analysis

- Identification of significantly expressed genes

Validation of gene expression by Real Time RT-PCR/ In-situ RT PCR

Bioinformatic analysis of selected genes
Results

- Out of 60 selected ORFs, 11 were found to be over-expressed (Signal to noise ratio > 2.49 and visible against background)
- Of these 11 ORFs identified, 6 belong to metabolism and 5 were related to bacterial virulence
- No such signals were detectable in RNA derived from normal human skin.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Gene ID</th>
<th>Gene Name</th>
<th>Function</th>
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<tbody>
<tr>
<td>1</td>
<td>ML1095c</td>
<td>sucA</td>
<td>Metabolism</td>
</tr>
<tr>
<td>2</td>
<td>ML1363</td>
<td>pyrG</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ML0726c</td>
<td>accA3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ML0160</td>
<td>purN</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ML1900</td>
<td>mmaA1</td>
<td>Virulence</td>
</tr>
<tr>
<td>6</td>
<td>ML2230</td>
<td>purB</td>
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</tr>
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<td>7</td>
<td>ML0774</td>
<td>mtrB</td>
<td></td>
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<tr>
<td>8</td>
<td>ML2496c</td>
<td>dnaK</td>
<td></td>
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<td>9</td>
<td>ML0979</td>
<td>pgsA3</td>
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</tr>
<tr>
<td>10</td>
<td>ML2038c</td>
<td>bfrA</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>ML 1358</td>
<td>tlyA</td>
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</tr>
</tbody>
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Continued....
Real Time RT PCR: Relative quantification results show consistent over-expression of all eleven genes in TT, BT, BB, BL, LL as well as reaction cases of leprosy.

**Fig 1.** Relative expression of genes related to metabolism

**Fig 2.** Relative expression of genes related to virulence

*Higher transcript level of tlyA (ML1358) as compared to 16S rRNA and other genes*
**In-situ RT PCR:**

Expression of identified genes were also confirmed at the site of infection by *in-situ* RT-PCR.

Figure: Section from BT/BB case showing multiple positive signals with *in situ* RT-PCR targeting accA (ML0726). Grade +

Figure: Section from LL case showing multiple positive signals with *in situ* RT-PCR targeting PurN(ML160). Grade +++
Relative expression of accA gene in different disease conditions

Patients were from endemic region (Ghatampur, UP, India)

accA was found to be hyper-expressed during the reactions in leprosy patients
Bioinformatic analysis of *accA* gene

Homology structure of accA protein showing epitopes hydrophilic (PEPTIDE-2) and B-Cell epitope (PEPTIDE-1) used for study sero-reactivity in leprosy patients.
Cloning and expression of identified peptides

Physico-chemical properties of peptides selected for cloning and sero-reactivity

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Length</th>
<th>Region</th>
<th>Molecular weight (MW)</th>
</tr>
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<tbody>
<tr>
<td>PEP-1</td>
<td>130</td>
<td>180 to 310</td>
<td>14732.5</td>
</tr>
<tr>
<td>PEP-1</td>
<td>100</td>
<td>450 to 550</td>
<td>10611.9</td>
</tr>
</tbody>
</table>

Transformed *E.coli* cell lysate showing over expression of antigenic region (PEP-1 = 14.7 kD) and antigenic region (PEP-2 = 10.6 kD) in lane 2 and 3 respectively.

SDS-PAGE electrophoresis of cell lysate transformed and transformed non transformed *E.coli* bacterial cell

Lane 1: Non-transformed *E.coli* cell Lysate, Lane 2: Transformed *E.coli* cell lysate; Lane 3: Transformed *E.coli* cell lysate; Lane 4: Molecular weight marker.
Sero-reactivity of accA3 (LM0726) in different types of leprosy cases against selected antigenic regions

Leprosy patients: 40 (10 BL/LL + 10BB/BT + 10 reaction (5RR+5ENL) + 10 healthy)

No significant difference ($P>0.05$) in sero-reactivity was observed between the patient/s of same clinical type with and without reaction.
Conclusions

1. The highly expressed accA appeared as useful molecular marker for monitoring the diseases specially reactions in the leprosy.

2. RT-PCR targeting tlyA appears to be more sensitive then 16S rRNA for detection of viable bacilli, therefore it may be a quantitative viability assessment of *M. leprae*.
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Thanks ...