Complete nucleotide sequence of pLD-TEX-KL, a 66-kb plasmid of *Legionella dumoffii* TEX-KL strain

Plasmid, 2007

Tian Qin a,*, Hideki Hirakawa b, Ken-ichiro Iida a, Kenshiro Oshima c, Masahira Hattori c, d, Kosuke Tashiro e, Satoru Kuhara e, Shin-ichi Yoshida a

a Department of Bacteriology, Faculty of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
b Graduate School of Systems Life Sciences, Kyushu University, Hakoizaki, Higashi-ku, Fukuoka 812-8581, Japan
c Kitasato Institute for Life Science, Kitasato University, 1-15-1, Kitasato, Sagamihara, Kanagawa 228-8555, Japan
d Department of Computational Biology, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8561, Japan
e Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University, Hakoizaki, Higashi-ku, Fukuoka 812-8581, Japan
Legionella sp.

- Gram-negative bacteria coccobacilli
- *Legionella* spp. are ubiquitous.
- 0.3–0.9 μm in width and 2–20 μm in length
- Proteins rather than carbohydrates are used as an energy source.
- Legionellae are obligate aerobes, and grow at temperatures ranging from 20° to 42° C.
- Clinically important *Legionella* species grow best at 35° C in humidified air.
Legionella sp.

- found in natural aquatic environments (streams, rivers, ponds, lakes and thermal pools) in moist soil and in mud, also in the canopy of the rain forest.
- able to survive in moist environments for long periods of time and can withstand temperatures of 0-68°C and a pH range of 5.0-8.5.
- They can survive chlorination and thus enter water supply systems and proliferate in thermal habitats, including air-conditioning cooling towers, hot water systems, shower heads, taps, whirlpool spas and respiratory ventilators which are found in biofilms on the surfaces of these systems.
Among the Legionella species that cause human pneumonia, Legionella dumoffii is the fourth most common causative agent.

Capable of growing within alveolar macrophages and epithelial cells after being accidentally transmitted to humans.

Discovery of a plasmid in L. dumoffii TEX-KL strain, which we named pLD-TEX-KL.

Methodology

*L. dumoffii* TEX-KL strain was cultured then isolate the plasmid

shear the plasmid and construct the shotgun library

Ligase into pTS1 vector and transform into *E.coli* DH5α

sequencing

Assemble with Phred-Phrap-Consed program (error rate < $10^{-4}$ bases)

Sequence analysis
Sequence analysis

- Identify the open reading frames (ORFs) by
  - Genome Gambler
  - CRITICA
  - GeneHacker
  - Glimmer2.0

- Protein functional annotation by
  - BLASTP searches against the non-redundant protein database

- Functional classification of ORFs by
  - Clusters of Orthologous Groups of Proteins (COGs)

Comparative analysis

- L. pneumophila strains Lens (pLPL)  
  - about 59 kb

- L. pneumophila strains Paris (pLPP)  
  - about 130 kb

Sequences were obtained from NCBI (National Center for Biotechnology Information)

Results & Discussion
pLD-TEX-KL – General features

- Circular plasmid of 66,512 bp
- 38.9% GC content
- 88.5% putative coding sequence
- 57 putative ORFs were identified
- Average ORF length of 343.3 bp

pLD-TEX-KL

- a plasmid discovered in *L. dumoffii* TEX-KL strain
- harbors two functional regions
  - transfer (*tra*) region
  - *hel* (heavy-metal transporter) region
- Has DNA polymerase III epsilon subunit gene

The ORFs in pLD-TEX-KL and their annotations

<table>
<thead>
<tr>
<th>98</th>
<th>205</th>
<th>Hypothetical protein</th>
<th>Bacteriophage QBSV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>09</td>
<td>247</td>
<td>TraT</td>
<td>Legionella pneumophila. Lens</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>TraA</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>11</td>
<td>98</td>
<td>TraL</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>12</td>
<td>189</td>
<td>TraE</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>13</td>
<td>239</td>
<td>TraK</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>14</td>
<td>457</td>
<td>TraB</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>15</td>
<td>865</td>
<td>TraC</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>16</td>
<td>111</td>
<td>TrbI</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>17</td>
<td>211</td>
<td>TraW</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>18</td>
<td>329</td>
<td>TraU</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>19</td>
<td>140</td>
<td>TrbC</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>20</td>
<td>562</td>
<td>TraN</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>21</td>
<td>249</td>
<td>TraF</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>22</td>
<td>169</td>
<td>Hypothetical protein plp10027</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>23</td>
<td>454</td>
<td>TraH</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>24</td>
<td>949</td>
<td>TraG</td>
<td>Salmonella typhi plasmid pED208</td>
</tr>
<tr>
<td>42</td>
<td>1041</td>
<td>HelA</td>
<td>Legionella pneumophila. Lens</td>
</tr>
<tr>
<td>43</td>
<td>416</td>
<td>HelB</td>
<td>Legionella pneumophila. Lens</td>
</tr>
<tr>
<td>44</td>
<td>414</td>
<td>HelC</td>
<td>Legionella pneumophila. Lens</td>
</tr>
<tr>
<td>49</td>
<td>306</td>
<td>DNA polymerase III subunit epsilon</td>
<td>Brucella suis 1330</td>
</tr>
</tbody>
</table>

Linear comparison between pLPL, pLD-TEX-KL, and pLPP

Genome atlas of pLD-TEX-KL
BLAST atlas of pLD-TEX-KL
zoom of pLD-TEX-KL BLAST atlas
DNA Polymerase III E- subunit

Conclusion

- Very much similar to other *Legionella* plasmids.
- Three distinct regions—transfer region, hel region, and DNA polymerase III epsilon subunit.

Tian et al., 2007. Complete nucleotide sequence of pLD-TEX-KL, a 66-kb plasmid of *Legionella dumoffii* TEX-KL strain. PLASMID 58, 261-268
THANK YOU