Assessing the permissiveness of complex bacterial communities towards conjugal plasmids – Development of a novel method

Klümper, Uli; Riber, Leise; Sannazzaro, Analia; Dechesne, Arnaud; Musovic, Sanin; Hansen, Lars H.; Sørensen, Søren J.; Smets, Barth F.

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Assessing the permissiveness of a bacterial community towards conjugal plasmids

Uli Klümper¹, Leise Riber³, Analia Sannazzaro³, Arnaud Dechesne³, Sanin Musovic¹, Lars H. Hansen¹, Søren J. Sørensen¹, Barth F. Smets³
¹Technical University of Denmark, DTU Miljø, Kgs. Lyngby
²University of Copenhagen, Section of Microbiology, Copenhagen

Introduction
A crucial parameter governing horizontal gene transfer (HGT) in complex bacterial communities is community permissiveness. The permissive fraction of a microbial community is defined as that fraction able to receive a given plasmid¹.

Permissiveness in complex, natural communities has not been extensively studied at the quantitative or at the phylogenetic level because no suitable methods have been available.

Materials and Methods

Plasmids
<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Inc-group</th>
<th>Phenotype</th>
<th>host range</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP4::Plac::gfp</td>
<td>IncP-1</td>
<td>resistance</td>
<td>broad</td>
</tr>
<tr>
<td>pIP02T::Plac::gfp</td>
<td>IncQ-mobilizer</td>
<td>cryptic</td>
<td>broad</td>
</tr>
<tr>
<td>pKJK5::Plac::gfp</td>
<td>IncP-1</td>
<td>catabolic</td>
<td>broad</td>
</tr>
</tbody>
</table>

3 gfp-repressing red fluorescent donor strains:
- Pseudomonas putida KT2440::lacIq-pLpp-mCherry
- Pseudomonas putida KT2440::lacIq-pLpp-DsRed
- Kluyvera spp. KT2440::lacIq-pLpp-mCherry
- Escherichia coli KT2440::lacIq-pLpp-mCherry

1 recipient community:

<table>
<thead>
<tr>
<th>Soil origin</th>
<th>Soil type</th>
<th>Soil treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRUCIAL, Taastrup, Denmark</td>
<td>agricultural</td>
<td>untreated</td>
</tr>
</tbody>
</table>

Plating of transconjugal pool

Filtering of colonies based on mCherry, gfp and Green Fluorescent Activated Cell Sorting (FACS) of transconjugants

Gate 1: size
- Before 1st sort
- After 1st sort
- After 2nd sort

Gate 2: Green fluorescence

Gate 3: No red fluorescence

Before 1st sort
- Gate 1
- Gate 2
- Gate 3

After 1st sort
- Gate 1
- Gate 2
- Gate 3

After 2nd sort
- Gate 1
- Gate 2
- Gate 3

Sorting of cells from a filter mating of the soil community with the mCherry tagged donor Pseudomonas putida KT2440 carrying the gfp-tagged conjugative plasmid pIP02T:
- After the sorting, the isolation of pure transconjugants from the original community was achieved.
- 20,000 transconjugants could be isolated from the filter mating within less than 12 hours.

Conclusions
- High-throughput isolation of transconjugants from a complex microbial community is possible.
- The number of isolated transconjugants is sufficient for subsequent pyrosequencing.
- The new method allows one to assess the permissiveness of a soil microbial community simultaneously at the quantitative and at the taxonomic levels.

References:
1 Musovic et al., 2010, AEM 76(14):4813
2 Burmølle et al., 2003, Microb Ecol 45:226–236

Email: ulik@env.dtu.dk
Research supported by:
CREAM, a VKR Center of Excellence