Expression of antibiotic resistance genes under natural conditions

Dennis Versluis
Antibiotics resistance is natural

• Antibiotic resistance (AR) determinants have been found in various biological niches
  – Soil
  – Marine sediments
  – Permafrost
  – Mexican caves
Aims

• To investigate which antibiotic resistance genes are expressed in different microbial communities

• To study how such expression would relate to the circumstances in that particular biological niche
  – Presence of notorious antibiotic-producing bacteria
  – Expression of antibiotic biosynthesis genes
Approach

• Metatranscriptome analysis (gene-centric) of antibiotic resistance genes
## Available data

<table>
<thead>
<tr>
<th>Origin</th>
<th>Mean read length</th>
<th>Total no. of reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human microbiota</td>
<td>101</td>
<td>650,000,000</td>
</tr>
<tr>
<td>Pig microbiota</td>
<td>101</td>
<td>350,000,000</td>
</tr>
<tr>
<td>Mouse microbiota</td>
<td>101</td>
<td>500,000,000</td>
</tr>
<tr>
<td>USA coastal water (plankton)*</td>
<td>210</td>
<td>1,200,000</td>
</tr>
<tr>
<td>Forest soil**</td>
<td>385</td>
<td>1,200,000</td>
</tr>
</tbody>
</table>

* Gifford et al, 2011
**Stewart et al, 2011
Workflow

Metatranscriptomic data

Read pre-processing

Evaluation of the expression of AR genes

Evaluation of the expression of antibiotic biosynthesis genes

Community composition analysis
Pre-processing

• Removal of rRNA reads
• Read trimming (ppred score cut-off: 21)
• Removal of duplicate reads
AR expression analysis

Two dedicated AR databases exist:

- Antibiotic resistance database (ARDB)
- Resistance determinants database (RED-DB)
## AR expression analysis

There are 2 dedicated AR databases:

<table>
<thead>
<tr>
<th></th>
<th>ARDB</th>
<th>RED-DB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Latest update</strong></td>
<td>2009</td>
<td>Last month</td>
</tr>
<tr>
<td><strong>Update procedure</strong></td>
<td>Automatically generated</td>
<td>Automatically/Manually updated</td>
</tr>
<tr>
<td><strong>Basis for classification</strong></td>
<td>Resistance class and type</td>
<td>Resistance phenotype</td>
</tr>
</tbody>
</table>
Alignment cut-off settings

- E-value $\leq 1.0\times 10^{-07}$
- Alignment length $\geq 40$
- % Nucleotide identity $\geq 90$
- No. of reads $\geq 3$
AR expression analysis

USA coastal water

No. of reads

RED-DB:

No. of reads

β-lactamase resistance genes

ARDB:

Class A β-lactamases

No. of reads
AR expression in the forest soil
Secondary metabolite biosynthesis

antiSMASH2 released
Dear antiSMASH users, the antiSMASH team is proud to present you with antiSMASH2, the new version of antiSMASH. This version offers improved HTML output, more secondary metabolites detected, more details on NRPS, PKS and Lantibiotics clusters and improvements of this version. Happy new year 2013.

- all
- polyketides (type I)
- polyketides (type IV)
- lantibiotics
- aminoglycosides / aminocyclitols
- ectoines
- nucleosides
- oligosaccharides
- thiopeptides
- polyketides (type II)
- nonribosomal peptides
- bacteriocins
- aminocoumarins
- butyrolactones
- phosphoglycolipids
- furans
- phenazines
- polyketides (type III)
- terpenes
- beta-lactams
- siderophores
- indoles
- melanins
- homoserine lactones
- others
Conclusions

• Antibiotic resistance genes are expressed under natural conditions
• Expression can either be the result of prior anthropogenic pressure or niche-specific roles of the antibiotics
Future work

• Community composition analysis
• Evaluation of the expression of antibiotic synthesis gene clusters
Acknowledgements

• Mark van Passel
• Hauke Smidt