Diversity and Evolution of Secondary Metabolite Genes in the Marine Actinomycete *Salinispora*

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Natural Products as Drugs

antibiotics

anticancer

antiparasitic

immunosuppressants
Ecological Functions

Fundamental units with which microbes sense and respond to their environment

Moriah Sandy; Alison Butler; Chem. Rev. 2009, 109, 4580-4595.
Classical drug discovery

Strain isolation → fermentation → extraction

structure elucidation ← chromatography ← bioassays
Genome Mining

- Biosynthesis
- Regulators
- Transporters
- Resistance

Targeted Genome Mining

Gene Clusters

Heterologous Expression

Knock in WT

in vitro Reconstitution

Purified Enzymes

Comparative Metabolomics and/or Bioactivity-Guided Fractionation

Bioactive Small Molecule Discovery

cchem.berkeley.edu
Huge amount of data
Big gene clusters, can exceed 100 kb
How to assess novelty
How to predict Bioactivity
Nonribosomal Peptide Synthetases and Polyketide Synthases

Directly connect genes to molecules
Salinispora

• Actinomycetes from marine sediments

Jensen et al., Environ Microbiol. 2005,
Bill Fenical, Brad Moore, Paul Jensen
Salinispora Secondary Metabolites

100 Genomes

cultured strains:  S. arenicola  S. tropica  S. pacifica  culture independent:  Pacific Gyre

locations:
- San Diego
- Sea of Cortez
- Canary Basin
- Madeira Islands
- US Virgin Islands
- Dominica
- Palmyra Atoll
- Guam
- Hawaii
- Red Sea
- South China Sea
- Papau New Guniea
- Fiji
- Mexico
- Caribbean
- Costa Rica
- Red Sea
Diversity and Evolution of Secondary Metabolites

1. How much diversity is out there?
2. How pathways evolve to yield new chemical diversity?
3. Diversity and distribution of biosynthetic pathways?
4. Can we learn lessons about effective sampling strategies?
NaPDoS - The Natural Product Domain Seeker

Screening for NRPS and PKS genes

### 124 NRPS/PKS Pathways

<table>
<thead>
<tr>
<th>species</th>
<th>analyzed genomes</th>
<th>avg genome size</th>
<th>avg contig number</th>
<th>avg total pathways /genome</th>
<th># of distinct pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. arenicola</td>
<td>37</td>
<td>5.7</td>
<td>74</td>
<td>17.5</td>
<td>47</td>
</tr>
<tr>
<td>S. pacifica</td>
<td>31</td>
<td>5.4</td>
<td>76</td>
<td>14.1</td>
<td>88</td>
</tr>
<tr>
<td>S. tropica</td>
<td>7</td>
<td>5.4</td>
<td>90</td>
<td>13.6</td>
<td>19</td>
</tr>
</tbody>
</table>

Ziemert et al, PNAS 2014
Pathway swapping

S. pacifica CNT609
S. pacifica CNS103
S. pacifica CNR942

sporolide cluster
cyanosporaside cluster
PKS32 cluster

spoE 52% 67% 53% 78%
cyaE 32% 54% 54%
Functional Pathway Swapping

S. pacifica  S. arenicola  S. tropica

Ziemert and Crüsemann et al., in prep.
Pathway Evolution

Pathway swapping

- S. pacifica CNT09
- S. pacifica CNS103
- S. pacifica CNR942

...spo cluster...
...cya cluster...
...PKS32 cluster...

Pathway duplication

Mechanisms

Gene gain and gene loss
Simplified species tree

S. arenicola

S. tropica

S. pacifica
genus specific
34 of 45 pathways (78%) occur at branch tips
Summary

75 Salinispora Genomes

- Evolve by gene gain and gene loss, duplication and divergence, horizontal gene transfer of whole units and pathway swapping
- Pathways are mainly located in certain hot spots in the genome
- Strong correlations between species phylogeny and pathways
- Constant sampling of pathways
- Informs collection strategies: target new phylotypes and new locations
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Functional Pathway Swapping

- Desferrioxamine is the more ancient pathway
- NRPS16 is acquired via horizontal gene transfer
- Acquisition of NRPS16 leads to the deletion of the des pathway
- Shows enormous plasticity of secondary metabolite gene clusters

Questions:
What drives evolution of siderophores in marine actinomycetes?
Does NRPS16/Salinichelin provide a better adaptation to the environment?
Cyanosporasides Evolution

Lane et al., 2013
Pathway Distribution

More than half of the pathways were found in only 1 or 2 strains

These pathways were recently acquired

Indicates a high rate of “pathway sampling”
Heterologous Expression

Bonet et al. 2015, J Nat Prod. 2015 Mar 27;78(3):539-42
Genome mining
Select strain/culture

Extract DNA

Sequence

De-replicate

Predict novelty

C&Ks - Database

Bioinformatics

Identify

Bioassay

Culture
Connecting Genes to Molecules

Peptido- and Glycogenomics

Pattern based Genome Mining

Heterologous Expression

Kersten, Ziemert et al., 2013 PNAS

Duncan, Ziemert et al. 2015, Chemistry & Biology

Bonet, Ziemert et al. 2015, J Nat Prod.