antibiotics from microbial sources
1. antibiotics producers: *Streptomyces* and related actinomycetes
2. antibiotic biosynthesis
3. Search for novel antibiotics
How are secondary metabolites made?

Application of genomic technologies requires knowledge of genes. Knowledge of genes requires understanding function.
gene clusters

all genes required for biosynthesis, regulation and resistance

size: 10-120 kb
oligomerization reactions

primary metabolites

class I reactions

monomers
- amino acids
- carboxylic acids
- sugars
- isopentenyl diphosphate (IPP)

class III reactions
A

- β-alanina
- acido α-amino adipico
- ornitina
- 3,5-diidrossi-fenilglicina
- 4-idrossi-fenilglicina

B

- prefenato
- 4-p-idrosssi-fenilglicina

Reazioni chimiche:
- DPH
- HmaS
- Hmo
- HpgT
PEP + E4P → shikimic acid → AHBA → amino acidi aromatici

amino acidi
rifamicina
main classes of microbial secondary metabolites

peptides
polyketides
terpenes
oligosaccharides
Oligopeptides

ribosomal origin

lantibiotics (e.g. nisin), microcins

non ribosomal origin

β-lactams, glycopeptides, cyclosporin, bacitracin, etc.
made by nonribosomal peptide synthetases (NRPS)
a lantibiotic: nisin
oligopeptides of ribosomal origin

synthesis of a prepropeptide

post-translational modifications + cleavage
Microcin B17, M, 1157
(E. coli)

ramoplanina, M, 2554
(Actinoplanes sp.)

vancomicina, M, 1592
(Amycolatopsis orientalis)
NRPS clusters

**gramicidin**
Phe-Pro-Val-Orn-Leu

**cephalosporin**
Aaa-Cys-Val

**surfactin**
Glu-Leu-Leu-Val-Asn-Leu-Leu

**cyclosporin**
Ala-Leu-Leu-Val-Bmt-Abu-Sar-Leu-Val-Leu-Ala
an NRPS module

Minimal module

Condensation    Adenylation    Thiolation
oligopeptide synthesis

M1 → M2 → M3

aa1 → aa2 → aa3

3 ATP → 3 AMP

SH → S → SH

M1 → M2 → M3

aa1 → aa2 → aa3
additional domains
bacA (15.8 kb) → bacB (7.8 kb) → bacC (19.1 kb)

BacA

BacB

BacC

Bacitracina A
Examples

bacA: Ala Leu Leu Val Bmt Abu Gly Leu Val Leu Ala

bacB: Ile Cys Leu Glu Ile Lys Orn Ile Phe His Asp Asn

bacC: Ile Cys Leu Glu Ile Lys Orn Ile Phe His Asp Asn

Adenylation A domain
Thiolation T domain
Condensation C domain
Epimerization E domain
N-methylation M domain
Heterocylization Cy domain
Thioesterase Te domain

Examples

bacA

bacB

bacC
main classes of microbial secondary metabolites

peptides
polyketides
terpenes
oligosaccharides
examples of polyketides
Avermectina

Tetraciclina

Oleandomicina

Actinorodina
<table>
<thead>
<tr>
<th>Type of PKS</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I PKS</td>
<td>Multifunctional proteins</td>
</tr>
<tr>
<td></td>
<td>Iterative (e.g. in fungi)</td>
</tr>
<tr>
<td></td>
<td>Modular in bacteria</td>
</tr>
<tr>
<td>Type II PKS</td>
<td>Multienzyme complexes</td>
</tr>
<tr>
<td></td>
<td>Found in bacteria</td>
</tr>
<tr>
<td></td>
<td>Make mostly aromatic polyketides</td>
</tr>
<tr>
<td></td>
<td>(e.g. tetracycline, daunomycin)</td>
</tr>
<tr>
<td>Type III PKS</td>
<td>Single enzyme, uses directly CoA derivatives</td>
</tr>
<tr>
<td></td>
<td>Typical of plants</td>
</tr>
<tr>
<td></td>
<td>Present also in bacteria</td>
</tr>
<tr>
<td></td>
<td>(e.g. dihydroxyphenylglycine)</td>
</tr>
</tbody>
</table>
identifying the polyketide core

erythromycin
polyketides and fatty acids
new cycle
CoAS

ACP

AT

new cycle

KS

ACP

KR

DH

ER
modulo minimo

A

CoAS \rightarrow \text{AT} \\text{ACP} \rightarrow \text{AT} \\text{ACP} \\
\text{KS} \\text{AT} \\text{ACP}

B

\text{CoAS} \rightarrow \text{AT} \\text{ACP} \rightarrow \text{AT} \\text{ACP} \rightarrow \text{AT} \\text{ACP}

C

\text{KS} \\text{AT} \\text{ACP} \rightarrow \text{KS} \\text{AT} \\text{ACP} \rightarrow \text{KS} \\text{AT} \\text{ACP} \rightarrow \text{KS} \\text{AT} \\text{ACP} \\
\text{ER} \\text{DH} \\text{KR} \\text{ACP} \\
\text{KS} \\text{AT} \\text{ACP} \\
\text{KS} \\text{AT} \\text{ACP} \\
\text{KS} \\text{AT} \\text{ACP}
modular PKS
1 PropionylCoA + 6 MethylmalonylCoA

\[ \text{eryA} \rightarrow \text{6-Deoxyerythronolide B} \]

\[ \text{eryF} \rightarrow \text{Erythronolide B} \]

\[ \text{eryB} \rightarrow \text{eryC} \]

\[ \text{eryG} \rightarrow \text{Erythromycin A} \]

\[ \text{eryK} \rightarrow \text{Erythromycin C} \]

\[ \text{Erythromycin D} \]
manipulation of a modular PKS
<table>
<thead>
<tr>
<th>Feature</th>
<th>PS</th>
<th>PKS</th>
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</thead>
<tbody>
<tr>
<td>Unit</td>
<td>amino acid</td>
<td>Carboxylic acid</td>
</tr>
<tr>
<td></td>
<td>( \text{C} \text{N} \text{O} )</td>
<td>( \text{C} \text{C} )</td>
</tr>
<tr>
<td>Bond formation</td>
<td>( \text{C} \text{AMP} \text{O} )</td>
<td>( \text{C} \text{S-CoA} \text{O} )</td>
</tr>
<tr>
<td>Activation</td>
<td>(N-methylation)</td>
<td>none, KR, DH, ER</td>
</tr>
<tr>
<td>Processing</td>
<td>Pantetheine</td>
<td>Pantetheine</td>
</tr>
<tr>
<td>Prosthetic group</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Racemization</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Modularity</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Size of module</td>
<td>1000-1800 aa</td>
<td>1000-2000 aa</td>
</tr>
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2. antibiotic biosynthesis
3. Search for novel antibiotics
Combinatorial biosynthesis: “the way”

- Isolation of the biosynthesis gene cluster
- Characterization of genes and assignment of functions to the gene products
- Understanding natural product biosynthesis
- Engineering biosynthesis pathways

Producer host
Nonproducer host

Novel compounds
Indolocarbazoles

Lechevalieria aerocolonigenes

Streptomyces longisporoflavus

Rebeccamycin
DNA topoisomerase I inhibitor

Staurosporine
Protein kinase inhibitor
over 20,000 microbial metabolites have been described

most of the metabolites are produced by either

- filamentous fungi
- filamentous actinomycetes

does this reflect superior biosynthetic ability by these strains?

are there other microbes capable of producing secondary metabolites?
Increasing the odds in NP research

- Increase the number of strains
- Employ novel assays
- Utilize novel approaches to strains
  - novel strains
  - metagenomic libraries
  - genome mining
Opportunities in NP research

• Only a small fraction of the bacterial world has been cultivated

• Bacteria have had millions of years of evolution to experiment with chemistry to fight competing organisms

biodiversity is a necessary requirement to access chemical diversity
novel strains from new habitats

- strains have been screened mostly from terrestrial sources, while marine habitats have been largely neglected
- marine strains have substantially diverged from terrestrial ones and are likely to produce different compounds
abyssomicin

- searched for compounds active against *B. subtilis* in MM whose activity could be reverted by pABA addition
- screened a limited number of extracts from marine or rare strains isolated from a marine
- identified abyssomicin from the marine actinomycete *Verrucosispora sp.*

novel strains from soil

- strains phylogenetically related to known antibiotic producers are likely to possess the same genetic potential for antibiotic production

- strains not represented in 16S rRNA databases are unlikely to have been intensively screened in the past

search for previously uncultured actinomycetes
novel actinomycete taxa

S. thermoviolaceus

“Beta”

T. alba

N. dassonvillei

“Alpha”

M. rosea

N. salmonae

“Delta”

S. albida

A. herbida

D. roseum

A. auranticolor

A. erythreum

“Gamma”

B. aggregatus

Frankia sp. CN10

A. alba

C. diphteriae

Streptomycesaceae

Nocardiopsaceae

Streptosporangiaceae

Thermomonosporaceae

Micromonosporaceae

Micrococcineae

Frankineae

Pseudonocardineae

Corynebacterineae

“Catenulisporineae”
Ktedobacter racemifer
A new division of filamentous, spore-forming, gram-positive bacteria

(Cavaletti et al., 2006)