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Ph.D., Analytical Chemistry
UO-Moss, University of Oregon
PhD, Organic Chemistry, University of Arkansas

Jim Tang works at Leucine (Laboratories) in Portland, OR, currently as a chemical development manager. He has been with Leucine for 10 years, working on developing new chemical banking projects. Before that, he was a research chemist at diverse Moss Building Campbell, a performing arts center, and chemistry.

He earned his bachelor’s degree from the University of Oregon, his Ph.D. in organic chemistry from the University of West Virginia, with postdoctoral experiences at the University of Iowa. He has interests in chemical, biology, and environmental science, and enjoys reading about new developments in chemistry.

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Biosynthetic Breakthroughs: Paving the Way for Future Drug Development

CHRISTINA SMOLKE, PhD
CEO and Co-founder, Antheia, Inc., and Adjunct Professor, Bioengineering, Stanford University

YI TANG, PhD
Parsons Family Professor, Department of Chemical and Biomolecular Engineering, Department of Chemistry and Biochemistry, UCLA

CATHERINE GOODMAN, PhD
Senior Associate Publisher, American Chemical Society

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Genome Mining of Fungal Natural Products

Yi Tang
Department of Chemistry and Biochemistry
Department of Chemical and Biomolecular Engineering
University of California, Los Angeles, USA
NP Biosynthetic Gene Clusters (BGCs)

Central dogma in biology and biosynthesis

DNA → RNA → Protein → NP

Biosynthetic genes are clustered

Genome

Balk and Tang, Natural Product Biosynthesis – Chemical Logic and Enzymatic Machinery 2017, RSC Press

Major Natural Products Families and BGCs

- Major NP families are assembled by “core, polymerizing” enzymes, and decorated by “tailoring” enzymes.

polyketides

nonribosomal peptides

terpenes

The anchoring core enzymes serve as the starting point for genome-driven NP mining.
Genome Mining of Natural Products


Pathway activation
Constitutively overexpress silent transcriptional factors

Oxaleimides from Penicillium oxalicum

IC_{50}: 0.32 μM (HeLa)

Epigenetic changes
Modify chromatin to make and P_{min} more accessible
Inactivate HDAC in C. arbuscula

Activated >75% of NP pathways, isolated 10 new compounds

Heterologous expression

Filamentous Fungi Baker’s yeast

Harvey, Science Advances 2018

Yee, JACS 2020

Genomics Guided Natural Product Discovery
How to mine new NPs from genomes?

~97% of Fungal biosynthetic gene clusters are uncharacterized

<table>
<thead>
<tr>
<th>Type of pathway</th>
<th>Characterized</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyketides</td>
<td>127</td>
<td>4984</td>
</tr>
<tr>
<td>Nonribosomal peptides</td>
<td>81</td>
<td>2983</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>44</td>
<td>550</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>25</td>
<td>336</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>277 (3.1%)</strong></td>
<td><strong>8853</strong></td>
</tr>
</tbody>
</table>

How to search through genomes for gene clusters of interest?
Can we search gene clusters based on desired biological activity and structural novelty?

Natural Products (NPs) and BGCs

Types of BGC

- Unknown
- Known

PKS, NRPS, Terpene cyclase, Etc.

Natural products (metabolites)

1. Known BGC, Known Compound
2. Unknown BGC, Known Compound
3. Known BGC, Unknown Compound
4. Unknown unknowns, biosynthetic dark matter
Natural Products (NPs) and BGCs

Types of BGC

PKS, NRPS, Terpene cyclase, Etc.

Natural products (metabolites)

Unknown BGC, Known Compound

Gene clusters

Natural products (metabolites)

Known BGC, Unknown Compound

Biosynthetic dark matter


Unknown BGC, Known Compound

From NP Biosynthesis to Biocatalysis

Biosynthesis

Science 2009

JACS 2009

ACIE 2013

Melonyl-CoA → Dihydromonocolin L (DML) → Monocolin J (MJ) → Lovastatin

Semisynthesis by biocatalysis


9 rounds of evolution

H₂O, NH₄OH

25°C, pH 9

98%, 75 g/L

Semisynthesis by biocatalysis


Used in commercial API manufacturing

LovG

LovB

LovC

LovD

LovF

LovE

Ro et al, JACS 2011

Lovastatin

Simvastatin

A U.S. EPA Program

2012

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Natural Products (NPs) and BGCs

Types of BGC

PKS, NRPS, Terpene cyclase, Etc.

Unknown BGC, Known Compound

Known BGC, Unknown Compound

known

unknown

unknowns

biosynthetic dark matter

Unknown BCG-
Known Compounds

fluopsin

Bo Li and coworkers, Science 2021

altemicidin

Ikuro Abe and coworkers, Nature 2022

guanitoxin

Bradley Moore and coworkers, JACS 2022
Natural Products (NPs) and BGCs

Biosynthetic gene clusters (BGCs) were predicted by AntiSMASH 5.0
Output for a biocontrol fungus *Trichoderma afroharzianum* t-22

<table>
<thead>
<tr>
<th>Compound family</th>
<th># of BGC</th>
<th>Reported NPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyketides</td>
<td>16</td>
<td>harzianolide, pachybasin azaphilone</td>
</tr>
<tr>
<td>Nonribosomal Peptides</td>
<td>22</td>
<td>peptaibols, gliotoxin</td>
</tr>
<tr>
<td>Polyketide-peptide hybrids</td>
<td>8</td>
<td>trichosetin, harzianic acid,</td>
</tr>
<tr>
<td>Terpenes</td>
<td>11</td>
<td>abscisic acid*</td>
</tr>
<tr>
<td>RiPPs</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>9</td>
</tr>
</tbody>
</table>

Most predicted BGCs are cryptic and have no associated NPs
How to mine new NPs from genomes?

~97% of Fungal biosynthetic gene clusters are uncharacterized

<table>
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How to search through genomes for gene clusters of interest?

*Can we search gene clusters based on desired biological activity and structural novelty?*

What Makes a Known-Unknown BGC Novel?

- **Novel clusters lead to novel natural products**
  - A cluster that offers minimal clue to the structure of NP
  - For fungi, >30 kB of biosynthetic enzymes
  - Abundance of tailoring enzymes (redox enzymes transferases, PLP-dependent, pericyclases, etc)
  - Hypothetical proteins (including DUFs)
  - Atypical core enzyme domain arrangements
  - Combinations of core enzymes in a single cluster
  - Etc.
Example of KU Mining from Fungi

Penicillium oxalicum from Baja

*OE* wild type

Sato et al., *JACS*, 2017

The cluster is entirely conserved in *Aspergillus oryzae*, *Aspergillus turcosus*, etc.

Yee et al., *JACS*, 2020
Genome Mining for Desired Activity

<table>
<thead>
<tr>
<th>New Compound?</th>
<th>Bioactivity?</th>
<th>Target?</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>oxaleimide J</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

Mining Guided by Self-Resistance Enzyme

Self-resistance enzyme
- provides the essential resistance needed to neutralize the effects of the natural product on the producing host.
- is frequently a mutated version of a housekeeping enzyme that is insensitive to the natural product and performs the same function.
- The encoding gene is colocalized in the natural product biosynthetic gene cluster.

Provides a predictive window to the function of the NP encoded by the gene cluster
Step 1: Biosynthetic cluster Identification

Step 2: NP Production and Target Validation

Step 3: Biological Activity Demonstration

Target: Branched Chain Amino Acid Biosynthesis

The BCAA pathway is present in bacteria, fungi and plants, but absent in animals and humans.

Attractive as targets for antimicrobials (esp anti TB) and herbicide development.
BCAA as herbicide targets

Acetolactate synthase (ALS)
Acetohydroxy acid isomeroreductase (KARI)
Dihydroxy acid dehydratase (DHAD)

1. Targeted for development of herbicide by major ag chemical companies with no success.
2. No crystal structure available.
3. No natural product inhibitor of DHAD is known.

A potential DHAD inhibitor BGC

A conserved fungal terpene BGC contains DHAD as second copy (60% identity) in addition to the housekeeping DHAD.
Heterologous Expression of BGC in Yeast

**Saccharomyces cerevisiae**

- **Kinetic parameters**
  - *Aspergillus terreus*
    - DHAD (housekeeping)
    - \( k_{\text{cat}} = 3.0 \text{ s}^{-1} \)
    - \( K_m > 20 \text{ mM} \)
    - IC\text{}_{50} = 0.31 \text{ mM} (sensitive)
  - **Arabidopsis thaliana**
    - DHAD (target)
    - \( k_{\text{cat}} = 1.2 \text{ s}^{-1} \)
    - \( K_m = 5.7 \text{ mM} \)
    - IC\text{}_{50} = 0.50 \text{ mM} (competitive) sensitive
  - **Aspergillus terreus**
    - AstD (resistance?)
    - \( k_{\text{cat}} = 0.05 \text{ s}^{-1} \)
    - \( K_m = 5.4 \text{ mM} \)
    - IC\text{}_{50} > 8 \text{ mM} (solubility limit) insensitive

---

**Mechanism of Inhibition**

- **DHAD (dihydroxyacid dehydratase)**
  - **DHAD**
    - dihydroxyacetone
    - dihydroxyacetone phosphate
  - **DHAD**
    - ketoacid
    - ketoacid phosphate

---


---

Yan, et al., Nature 2018
Herbicidal Activities of AA

Growth inhibition of plant on agar plate

Yan, et al. Nature 2018

Hexagon Bio
Menlo Park, CA

Genomics
- Strain collecting
- Strain dereplication
- Genome sequencing
- Genome assembly & annotation

Data Science
- Genome assembly & annotation
- Gene cluster mining
- Target prediction & gene cluster scoring
- LCMS data processing and analysis

Synthetic biology
- Manual gene cluster curation
- Cluster activation and expression
- Cluster product analysis and characterization

Drug Discovery
- Assay development
- Bioactivity screening of cluster products
- Medicinal chemistry and lead optimization
Natural Products (NPs) and BGCs

Types of BGC

- Known BGC, Known Compound
- Unknown BGC, Known Compound
- Known BGC, Unknown Compound
- Unknown BGC, Unknown Compound

Unknowns

- PKS, NRPS, Terpene cyclase, Etc.

Unknowns

- Biosynthetic dark matter

Unknown

- New methods to generate molecular scaffold (C-X bond formation)

Unknown

- Abundance of modification enzymes (redox enzymes, transferases, PLP-dependent enzymes, pericyclases, etc)

Natural products (metabolites)

Search for the Unknown/Unknown

What makes a cluster UU?

- No predicted core enzymes (PKS, NRPS, TS, Prenyltransferase) → new methods to generate molecular scaffold (C-X bond formation)
- Abundance of modification enzymes (redox enzymes, transferases, PLP-dependent enzymes, pericyclases, etc)
- Hypothetical protein (HP)
- DUFs (proteins with domains of unknown function)
- Etc.
Example of Unknown-Unknown BGC Mining

<table>
<thead>
<tr>
<th>Gene</th>
<th>Predicted Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>NIS synthetase</td>
</tr>
<tr>
<td>B</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>H</td>
<td>ABC bile acid transporter</td>
</tr>
<tr>
<td>A</td>
<td>Hypothetical protein</td>
</tr>
<tr>
<td>D</td>
<td>PLP-dependent transferase</td>
</tr>
<tr>
<td>C</td>
<td>FAD monooxygenase</td>
</tr>
<tr>
<td>F</td>
<td>O-methyltransferase</td>
</tr>
<tr>
<td>G</td>
<td>Hypothetical protein</td>
</tr>
</tbody>
</table>

Heterologous recon. of ank cluster

New compound, but..

NK13650 C

NK13650 A
Biosynthesis of NK13650

Aspergillus thermomutatus (ank)

NIS P450 ABC HP PLP FMO MT AGE

E B H A D C F G

AnkA??

Building molecular complexity without core “polymerization” enzyme

AnkB C D E F G

NK13650 C

AnkA is the core enzyme?

AnkA is the core enzyme in this pathway!

Verified function in yeast

A Cyclo-Arg-Tyr synthase

AnkA

Verified function

citrate ligase

cytochrome P450

hypothetical protein

PLP-dependent C-O forming

FAD monoxygenase

O-methyltransferase

ATP grasp enzyme

AnkA is the core enzyme in this pathway!

Initial assignment

A hypothetical protein

518 aa protein

No predicted function

No characterized sequence homolog

No structural homolog

No sequence resemblance to NRPS or bacterial CDPS.

Yee, et al, Nature Chemical Biology 2023
Genome Mining of AnkA-like Enzymes

Over 100 homologs of AnkA detected from JGI/NCBI blast search

Fungal AnkA homologs (CDRPSs) generate rare and new-to-nature Cyclo-Arg-Xaa dididpeptides.
Using CDRPS to find UU Natural Products

Aspergillus versicolor (ava)

Aspergillus nidulans A1145

DY225

New compound identified from UU genome mining

<table>
<thead>
<tr>
<th>gene</th>
<th>predicted function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>cRW synthase (verified)</td>
</tr>
<tr>
<td>B</td>
<td>FAD monooxygenase</td>
</tr>
<tr>
<td>C</td>
<td>kynurenine formamidase (KFA)</td>
</tr>
<tr>
<td>D</td>
<td>hypothetical protein</td>
</tr>
<tr>
<td>E</td>
<td>transporter</td>
</tr>
</tbody>
</table>

DY225 may not represent the final NP of the cluster. Surrounding enzyme (including P450s) are currently being tested for function.
Conclusions

unknown BGC, known Compound  |  unknown unknowns
unknown                      |  biosynthetic dark matter
Gene clusters

known BGC, known Compound  |  known BGC, unknown Compound
New Enzymes                  |  New Structures
New Biosynthetic Logic       |  Targeted Bioactivities

Natural products (metabolites)

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