Genome mining for drug discovery: Activation of silent biosynthetic gene clusters

Project acronym: GenoDrug
Project no: EIB.10.023
GenoDrug Final Report

Structure:

1. Background
2. Project aim and approach
3. Project partners
4. Progress and cooperation
5. Achievement of milestones and objectives
6. Problems encountered; proposed changes
GenoDrug Final Report

Structure:

1. Background
2. Project aim and approach
3. Project partners
4. Progress and cooperations
5. Achievement of milestones and objectives
6. Problems encountered; proposed changes
Streptomyces strains contain numerous secondary metabolite biosynthetic gene clusters, much more than the number of products isolated from one strain.
GenoDrug Final Report

Structure:

1. Background
2. Project aim and approach
3. Project partners
4. Progress and cooperations
5. Achievement of milestones and objectives
6. Problems encountered; proposed changes
GenoDrug Project: Aim

Aim: Exploit the unused potential

A new technology for drug discovery:
Activation of previously silent biosynthetic gene clusters of microbial genomes → Novel bioactive compounds
GenoDrug Project: Aim and Approach

Aim

A new technology for drug discovery: Activation of previously silent biosynthetic gene clusters of microbial genomes → Novel bioactive compounds

General approach

1) Bioinformatic genome analysis of actinomycete strains ⇒ new gene clusters.
2) Development of strategies for the activation and expression of silent biosynthetic gene clusters using:
   a) global and pathway-specific regulators;
   b) introduction of artificial promoters;
   c) heterologous expression of gene clusters in genetically engineered host strains.
3) Production of new compounds from the engineered strains and testing for bioactivities, especially antibiotic and anticancer activities.
GenoDrug Final Report

Structure:

1. Background
2. Project aim and approach
3. Project partners
4. Progress and cooperations
5. Achievement of milestones and objectives
6. Problems encountered; proposed changes
## GenoDrug Partners

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Country</th>
<th>Research Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Wohlleben</td>
<td>Microbiology Tübingen University</td>
<td>Germany</td>
<td>Bioinformatics, Systems biology, Genetic engineering</td>
</tr>
<tr>
<td>Prof. Zakrzewska-Czerwińska</td>
<td>Biotechnology University of Wrocław</td>
<td>Poland</td>
<td>Gene regulation studies, Upregulation of expression</td>
</tr>
<tr>
<td>Prof. Heide</td>
<td>Pharmaceutical Institute Tübingen University</td>
<td>Germany</td>
<td>Gene cluster assembly, Heterologous expression, Combinatorial biosynthesis</td>
</tr>
<tr>
<td>Prof. Méndez</td>
<td>Functional Biology University of Oviedo</td>
<td>Spain</td>
<td>Precursor supply, Genetic engineering, Mutasynthesis</td>
</tr>
<tr>
<td>Dr. Ylihonko</td>
<td>Galilaeus Oy (SME) Kaarina</td>
<td>Finland</td>
<td>Strain optimization, Fermentation, Compound generation</td>
</tr>
<tr>
<td>Dr. Moris</td>
<td>EntreChem SL (SME) Oviedo</td>
<td>Spain</td>
<td>Structure elucidation, Biological testing, Toxicity testing</td>
</tr>
</tbody>
</table>
GenoDrug Final Report

Structure:
1. Background
2. Project aim and approach
3. Project partners
4. Progress and cooperations
5. Achievement of milestones and objectives
6. Problems encountered; proposed changes
1. *Streptomyces argillaceus* ATCC 12956
2. *Streptomyces albus* J1074
3. *Streptomyces collinus* Tü 365
4. *Amycolatopsis balhimycina* DSM 5908
5. *Catenulispora acidiphila* DSM 44928
6. *Amycolatopsis japonicum*
7. *Streptomyces tendae* Tü1028
Development of New Bioinformatic Tools: antiSMASH

- **anti**biotics and **S**econdary **M**etabolites **A**nalysis **S**HELL

  - **A**im:
    - Integration of all available prediction methods for secondary metabolite biosynthesis genes into user friendly pipeline

  [Link: http://antismash.secondarymetabolites.org/]

  - M. Medema / E. Takano / R. Breitling
  - K. Blin / T. Weber / W. Wohlleben
  - P. Cimermancic / M. Fischbach

  - Tilmann Weber

_Blin et al., Nucleic Acids Res., 2013_
antiSMASH – Input Form

- DNA of Eukaryotic origin

Gene cluster types to search:
- all
- polyketides (type I)
- polyketides (type II)
- polyketides (type III)
- nonribosomal peptides
- terpenes
- lantibiotics
- bacteriocins
- beta-lactams
- aminoglycosides / aminocyclitols
- aminocoumarins
- siderophores
- ectoines
- butyrolactones
- indoles
- nucleosides
- phosphoglycolipids
- melanins
- others

- smCOG analysis for functional prediction and phylogenetic analysis of genes

- Gene Cluster Blast Comparative Analysis

- Whole-genome BLAST results in EMBL output

- Whole genome PFAM results in EMBL output

Senden
<table>
<thead>
<tr>
<th>Cluster</th>
<th>Type</th>
<th>From</th>
<th>To</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>Ectoine</td>
<td>397859</td>
<td>408248</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>Other</td>
<td>1879080</td>
<td>1922856</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>Nrps</td>
<td>2449130</td>
<td>2519199</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>T1pks</td>
<td>2505679</td>
<td>2610944</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>Other</td>
<td>2978921</td>
<td>3022823</td>
</tr>
<tr>
<td>Cluster 6</td>
<td>T4pks</td>
<td>3188097</td>
<td>3238424</td>
</tr>
<tr>
<td>Cluster 7</td>
<td>T1pks</td>
<td>3233337</td>
<td>3277536</td>
</tr>
<tr>
<td>Cluster 8</td>
<td>Lantipeptide</td>
<td>3305024</td>
<td>3328143</td>
</tr>
<tr>
<td>Cluster 9</td>
<td>Nrps</td>
<td>3387615</td>
<td>3459171</td>
</tr>
<tr>
<td>Cluster 10</td>
<td>Terpene</td>
<td>3925707</td>
<td>3946837</td>
</tr>
<tr>
<td>Cluster 11</td>
<td>Other</td>
<td>3948382</td>
<td>3992446</td>
</tr>
<tr>
<td>Cluster 12</td>
<td>Nrps</td>
<td>4069149</td>
<td>4120964</td>
</tr>
<tr>
<td>Cluster 13</td>
<td>Nrps</td>
<td>4111374</td>
<td>4163603</td>
</tr>
<tr>
<td>Cluster 14</td>
<td>Nrps</td>
<td>4379325</td>
<td>4441530</td>
</tr>
<tr>
<td>Cluster 15</td>
<td>Nrps</td>
<td>4530446</td>
<td>4587520</td>
</tr>
<tr>
<td>Cluster 16</td>
<td>T1pks</td>
<td>4713730</td>
<td>4750149</td>
</tr>
<tr>
<td>Cluster 17</td>
<td>Nrps-T1pks</td>
<td>4838508</td>
<td>4932058</td>
</tr>
<tr>
<td>Cluster 18</td>
<td>T1pks-T4pks</td>
<td>5121459</td>
<td>5172192</td>
</tr>
<tr>
<td>Cluster 19</td>
<td>Terpene</td>
<td>5278355</td>
<td>5299473</td>
</tr>
<tr>
<td>Cluster 20</td>
<td>Bacteriocin</td>
<td>5496314</td>
<td>5507129</td>
</tr>
<tr>
<td>Cluster 21</td>
<td>Terpene</td>
<td>5831519</td>
<td>5852448</td>
</tr>
<tr>
<td>Cluster 22</td>
<td>T1pks</td>
<td>6415623</td>
<td>6461442</td>
</tr>
<tr>
<td>Cluster 23</td>
<td>T3pks-nrps</td>
<td>6800521</td>
<td>6897743</td>
</tr>
<tr>
<td>Cluster 24</td>
<td>T1pks</td>
<td>6902710</td>
<td>6949003</td>
</tr>
<tr>
<td>Cluster 25</td>
<td>Amglyccycl</td>
<td>7571659</td>
<td>7618166</td>
</tr>
<tr>
<td>Cluster 26</td>
<td>Terpene</td>
<td>8232022</td>
<td>8254163</td>
</tr>
<tr>
<td>Cluster 27</td>
<td>Other</td>
<td>8286964</td>
<td>8327650</td>
</tr>
</tbody>
</table>
antiSMASH Results
PKS / NRPS Domain Organisation

PKS/NRPS domain annotation

SCO3230 (nrps)

SCO3231 (glycopeptide nrps)

SCO3232 (nrps)

Domain AMPbinding3 (SCO3230)
Location: 2785-3184 AA
Predicted substrate: trp
-NRPSPredictor code: trp
-NRPSPredictor SVM: trp
-Minowa HMM: trp
NCBI BlastP on this domain

Monomers prediction: ser thr trp
asp asp hpg asp gly asn nrp trp trp
SCO3230:
  -NRPSPredictor code prediction, A1: ser
  -NRPSPredictor SVM prediction, A1: ser
  -Minowa prediction, A1: ser
  Prediction details
  -NRPSPredictor code prediction, A2: thr
  -NRPSPredictor SVM prediction, A2: thr
  -Minowa prediction, A2: thr
  Prediction details
  -NRPSPredictor code prediction, A3: trp
  -NRPSPredictor SVM prediction, A3: trp
  -Minowa prediction, A3: trp
  Prediction details
Products of *Amycolatopsis* Strains

*Amycolatopsis mediterranei*
- Rifampicin

*Amycolatopsis orientalis*
- Vancomycin

*Amycolatopsis japonicum*

*Amycolatopsis balhimycinina*
- Balhimycin
Identification of a Glycopeptide Biosynthetic Gene Cluster in *A. japonicum*

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Type</th>
<th>From</th>
<th>To</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>Ectoine</td>
<td>397859</td>
<td>408248</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>Other</td>
<td>1879080</td>
<td>1922856</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>NrpS</td>
<td>2449130</td>
<td>2519199</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>T1pks</td>
<td>2505679</td>
<td>2619944</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>Other</td>
<td>2978921</td>
<td>3022823</td>
</tr>
<tr>
<td>Cluster 6</td>
<td>T4pks</td>
<td>3188097</td>
<td>3238424</td>
</tr>
<tr>
<td>Cluster 7</td>
<td>T1pks</td>
<td>3233337</td>
<td>3277536</td>
</tr>
<tr>
<td>Cluster 8</td>
<td>Lantipeptide</td>
<td>3305024</td>
<td>3328143</td>
</tr>
<tr>
<td>Cluster 9</td>
<td>NrpS</td>
<td>3387615</td>
<td>3459171</td>
</tr>
<tr>
<td>Cluster 10</td>
<td>Terpene</td>
<td>3925707</td>
<td>3994683</td>
</tr>
<tr>
<td>Cluster 11</td>
<td>Other</td>
<td>3948382</td>
<td>3992446</td>
</tr>
<tr>
<td>Cluster 12</td>
<td>NrpS</td>
<td>4069149</td>
<td>4120964</td>
</tr>
<tr>
<td>Cluster 13</td>
<td>NrpS</td>
<td>4111374</td>
<td>4163603</td>
</tr>
<tr>
<td>Cluster 14</td>
<td>NrpS</td>
<td>4379325</td>
<td>4441530</td>
</tr>
<tr>
<td>Cluster 15</td>
<td>NrpS</td>
<td>4530446</td>
<td>4587520</td>
</tr>
<tr>
<td>Cluster 16</td>
<td>T1pks</td>
<td>4713730</td>
<td>4750149</td>
</tr>
<tr>
<td>Cluster 17</td>
<td>NrpS-T1pks</td>
<td>4838508</td>
<td>4932058</td>
</tr>
<tr>
<td>Cluster 18</td>
<td>T1pks-T4pks</td>
<td>5121459</td>
<td>5172192</td>
</tr>
<tr>
<td>Cluster 19</td>
<td>Terpene</td>
<td>5278355</td>
<td>5299473</td>
</tr>
<tr>
<td>Cluster 20</td>
<td>Bacteriocin</td>
<td>5496314</td>
<td>5507129</td>
</tr>
<tr>
<td>Cluster 21</td>
<td>Terpene</td>
<td>5831519</td>
<td>5852448</td>
</tr>
<tr>
<td>Cluster 22</td>
<td>T1pks</td>
<td>6415623</td>
<td>6461442</td>
</tr>
<tr>
<td>Cluster 23</td>
<td>T3pks-NrpS</td>
<td>6800521</td>
<td>6897743</td>
</tr>
<tr>
<td>Cluster 24</td>
<td>T1pks</td>
<td>6902710</td>
<td>6949003</td>
</tr>
<tr>
<td>Cluster 25</td>
<td>Amsglycycl</td>
<td>7571659</td>
<td>7618166</td>
</tr>
<tr>
<td>Cluster 26</td>
<td>Terpene</td>
<td>8232022</td>
<td>8254163</td>
</tr>
<tr>
<td>Cluster 27</td>
<td>Other</td>
<td>8286964</td>
<td>8327650</td>
</tr>
</tbody>
</table>

Blin et al., Nucleic Acids Res., 2013
"Cluster 23" Encodes the Biosynthesis of a Ristomycin-like Glycopeptide

NRPS:
- 7 x aromatic amino acids
- 3 x Hpg
- 2 x β-Ht
- 2 x Dpg

Tailoring enzymes:
- 4 x P450 monooxygenases
- 0 x acetyltransferase
- 6 x glycosyltransferase
- 2 x methyltransferase

Chemical structures showing arabinose, glucose, rhamnose, mannose, and ristosamine linked by various bonds and functional groups.
Cluster 23 encodes the biosynthesis of a ristomycin-like glycopeptide

---

NRPS:
- 7 x aromatic amino acids
  - 3 x Hpg
  - 2 x β-Ht
  - 2 x Dpg

Tailoring enzymes:
- 4 x P450 monooxygenases
- 0 x acetyltransferase
- 6 x glycosyltransferase
- 2 x methyltransferase

---

- Cluster 23 is the first example of a type III glycopeptide gene cluster
- Ristomycin is a component of a diagnostic kit to screen for von Willebrand disease (vWD) and Bernard-Soulier syndrome (BSS)
- Market price: 100 mg: 560 €
‟Cluster 23“ Encodes the Biosynthesis of a Ristomycin-like Glycopeptide

NRPS:
- 7 x aromatic amino acids
  - 3 x Hpg
  - 2 x β-Ht
  - 2 x Dpg

Tailoring enzymes:
- 4 x P450 monooxygenases
- 0 x acetyltransferase
- 6 x glycosyltransferase
- 2 x methyltransferase

But the wild type does not produce a detectable amount of ristomycin
Heterologous Expression of the Regulator Gene *bbr* in *Amycolatopsis japonicum*

*Amycolatopsis japonicum* WT → Below detection limit

Ristomycin A standard

*Amycolatopsis japonicum* *bbr*+ produces 50 mg/l of a ristomycin-like glycopeptide in shaking flasks and 200 mg/l in the fermenter

Under negotiation with company XYZ for commercialisation
**Amycolatopsis japonicum**, a Talented Producer of Bioactive Compounds

*Amycolatopsis japonicum* produces S,S-EDDS which has similar chelating properties as EDTA, but which is biodegradable.

EDDS = Ethylene-diamine-$N,N'$-disuccinic acid

**Problem:** Synthesis is strongly Zn-repressed and biosynthesis was unknown.
EDDS-Production in *A. japonicum* Is Repressed in the Presence of Zinc

- Identification of the Zn-dependent regulator
- Identification of the EDDS biosynthetic gene cluster

In the presence of Zn: Transcription is blocked

In the absence of Zn: Transcription is possible
An *A. japonicum* Δzur Mutant Produces EDDS in High Yield in Complex Media

In the presence of Zn: Transcription is blocked

In the absence of Zur: Transcription is possible

- Identification of the Zn-dependent regulator
- Identification of the EDDS biosynthetic gene cluster
- Elimination of the Zn-repression
- EDDS-production in complex media in the presence of Zn

---

**Solution**

In the presence of Zn:

Transcription is blocked

In the absence of Zur:

Transcription is possible

---

**Graph:**

- ZnSO₄ concentrations (0, 6, 50, 500, 1000, 2500, 5000 µM)
- % activity
- DM and CM categories

---

**Patent Application:** P 53563 DE
Heterologous Expression of Cosmids from *Streptomyces collinus* in *Streptomyces albus*

Cluster 1, Cluster 4, Cluster 7, Cluster 10, Cluster 16, Cluster 18, Cluster 19, Cluster 24

lantibiotic, carotenoid, t1pks-nrps, t3pks, t2pks, t1pks-nrps, nrps, t1pks

„Cluster 4“ induces visible morphological changes in *S. albus*

Rückert et al., J. Biotechnol., 2014
Heterologous expression of “Cluster 4” resulted in the production of four carotenoids.
Heterologous Expression of Cosmids from *Streptomyces collinus* in *Streptomyces albus*

<table>
<thead>
<tr>
<th>Cluster 1</th>
<th>Cluster 4</th>
<th>Cluster 7</th>
<th>Cluster 10</th>
<th>Cluster 16</th>
<th>Cluster 18</th>
<th>Cluster 19</th>
<th>Cluster 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>lantibiotic</td>
<td>carotenoid</td>
<td>t1pks-nrps</td>
<td>t3pks</td>
<td>t2pks</td>
<td>t1pks-nrps</td>
<td>nrps</td>
<td>t1pks</td>
</tr>
</tbody>
</table>

„Cluster 1“ is predicted to encode a new lantibiotic

Rückert *et al.*, J. Biotechnol., 2014
Heterologous Expression of „Cluster 1“ in *S. coelicolor* M1146

„Cluster 1“ lantibiotic: Streptocollin

![Diagram showing gene expression and lantibiotic structure](image)

<table>
<thead>
<tr>
<th>Genus</th>
<th>Gene</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. collinus</em> Tü365</td>
<td>stcL</td>
<td>MENHDIELLLAHLHALPETDPFGVDGAPFAA</td>
</tr>
<tr>
<td></td>
<td>stcA</td>
<td>TC</td>
</tr>
<tr>
<td></td>
<td>stcT</td>
<td>VC GLL T</td>
</tr>
<tr>
<td></td>
<td>stcH</td>
<td>TVCIGISCA</td>
</tr>
<tr>
<td><em>S. venezuelae</em></td>
<td>venL</td>
<td>MENQTLELLAHLHALPETDPPEVGDGAFAAN</td>
</tr>
<tr>
<td></td>
<td>venA</td>
<td>TC</td>
</tr>
<tr>
<td></td>
<td>venT</td>
<td>VC GLL T</td>
</tr>
<tr>
<td></td>
<td>venH</td>
<td>VC VGI SC</td>
</tr>
</tbody>
</table>

Characterisation of the first isolated lantipeptide belonging to the type IV class

Iftime *et al.*, in preparation
Selection of Bioinformatically Identified Gene Clusters from *S. albus* and *S. argillaceus*

Cluster 9 (NRPS)

Cluster 26 (NRPS-PKS)

Cluster 1701 (PKS)

Cluster 909 (lanthipeptide)

Cluster 25 (lanthipeptide)

Cluster 1705 (other)
Activation of Silent Gene Clusters from *S. albus*

**Cluster 9 (NRPS)**

- NRPS
- 311, 312, 313, 314, 315, 316
- *S. albus::pO1313*  
- *S. albus wt*
- promoter insertion

**Cluster 26 (NRPS-PKS)**

- Overexpression of regulator
- 5706, 5707, 5708, 5709, 5710, 5711, 5712, 5717
- REG
- promoter insertion
- *S. albus::pO15712*  
- *S. albus::pO15713*  

**Novel compounds**

- 6-epi-alteramide A (1)
- 6-epi-alteramide B (2)
Activation of the Silent Gene Cluster 1701 from S. argillaceus

Cluster 1701 (PKS)  

S. argillaceus ΔPKS  

S. argillaceus 01SARP::aac(3)IV  

promoter insertion  

Inactivation biosynthesis gene  

S. argillaceus 01tetR::aac(3)IV  

Inactivation repressor  

S. argillaceus WT  

S. argillaceus 01tetR::aac(3)IV  

S. argillaceus ΔPKS
Bioassay of Novel Compounds Encoded by Cluster 1701 of S. argillaceus

Nigrifactin (P3)

Bioassays vs *M. luteus*

Argimycin I (P1) + Argimycin II (P2)

No activity observed

Argimycin IV (P4)

No activity observed

Argimycin V (P5)

Argimycin VI (P6)
Proposed Biosynthesis Pathway for Argimycins

Argimycin I

Argimycin II

Argimycin VI

Argimycin V

Argimycin IV

Nigrifactin
Increasing the Intracellular Pool of Glucose-1-phosphate and Malonyl-CoA in *S. argillaceus* to Increase Production of Argimycins

Zabala et al., Metab. Engineer., 2013
Evaluation of Argimycin 2 (Cytotoxicity Study)

Argimycin 2 showed no cytotoxic activity against A459 lung cancer cells

Strategies to Activate Other Silent Gene Clusters from *S. argillaceus*

**Cluster 909 (lanthipeptide)**

- *S. argillaceus* ΔM909
- Inactivation of biosynthesis gene
- *S. argillaceus::erm909*
- Promoter insertion
- *S. albus* cos12D6
- Heterologous expression

**Cluster 25 (lanthipeptide)**

- *S. argillaceus* Δ25lanB
- Inactivation of biosynthesis gene
- *S. argillaceus::erm25E*
- *S. argillaceus::erm25D*

**Cluster 1705 (other)**

- *S. argillaceus pEM4T1705SARP1*
- *S. argillaceus pEM4T1705SARP9*
- Overexpression of activators
- *S. argillaceus Δ1705-0rf19-20*
- Deletion of biosynthesis genes
Bioinformatic Identification of a Gene Cluster for an Aminocoumarin Antibiotic in *Catenulispora acidiphila*

Caci2717: CloL-like
Caci2718: CloK-like
**Caci2719: hypothetical protein**
Caci2720: CloJ-like
Caci2721: CloY-like
Caci2722: CloI-like
Caci2723: CloH-like
Caci2724: FADH-dependent halogenase (similar to *clohal*)

Aminocoumarin moiety + amide bond + halogenase
Different profiles of expression of genes encoding an activator and a repressor during culture growth

Expression of cluster genes is correlated with the expression of the activator gene
Activation of Heterologous Expression by Overexpression of the *luxR* Regulatory Gene

caci2725, a *luxR* family regulatory gene belonging to the predicted cacibiocin cluster was cloned into pUWL201 under control of the *ermE* promoter
Production of 60 mg/L cacibiocin A in *S. coelicolor* M1152(caciJZ08)

Production of 4.6 mg/L cacibiocin B in *Catenulispora acidiphila*

Different optimal media for *C. acidiphila* and the heterologous host
Fermentation of the Engineered Aminocoumarin Producer Strain

100 liter fermentation of *S. coelicolor* M1152 10E2int pXL11 at Galilaeus Oy

≈ 120 mL extract cacibiocin A and B (new natural products)
Structure Elucidation of Cacibiocin A and B
New Industrially Relevant Products/Processes from GenoDrug

Argimycins/ Nigrifactin: New antibiotics

Cacibiocins: New aminocoumarin

Carotenoid: New isorenieratene (?) derivative

EDDS: Biodegradable chelator: High yield production in complex media

Ristomycin: Production strain for a high value component of a diagnostic kit

Streptocollin: First type IV lantibiotic

+ many silent gene clusters and products thereof, awaiting further characterisation

Patent application Commercialisation
GenoDrug Final Report

Structure:
1. Background
2. Project aim and approach
3. Project partners
4. Progress and cooperations
5. Achievement of milestones and objectives
6. Problems encountered; proposed changes
### Achievement of Milestones

<table>
<thead>
<tr>
<th>Project</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioinformatic identification of clusters</td>
<td>6</td>
</tr>
<tr>
<td>Bioinformatic identification of regulators</td>
<td>6</td>
</tr>
<tr>
<td>Transcription profiles of gene clusters</td>
<td>9</td>
</tr>
<tr>
<td>Metabolite profiles under different growth conditions</td>
<td>9</td>
</tr>
<tr>
<td>Techniques for overexpression/deletion of regulatory genes</td>
<td>15</td>
</tr>
<tr>
<td>Gene clusters with introduced artificial promoters constructed</td>
<td>15</td>
</tr>
<tr>
<td>Heterologous expression constructs generated</td>
<td>15</td>
</tr>
<tr>
<td>Engineering strategies for increasing precursor supply established</td>
<td>18</td>
</tr>
<tr>
<td>Expression of previously silent clusters</td>
<td>18</td>
</tr>
<tr>
<td>Chemical identification of novel compounds</td>
<td>21</td>
</tr>
<tr>
<td>New compounds from different chemical classes by fermentation</td>
<td>24</td>
</tr>
<tr>
<td>Structure elucidation of new compounds</td>
<td>27</td>
</tr>
<tr>
<td>Pharmacological testing of new compounds initiated</td>
<td>27</td>
</tr>
</tbody>
</table>
## Achievement of Deliverables

<table>
<thead>
<tr>
<th>Deliverables</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>D 1  Report of Co-ordinator at kick-off seminar</td>
<td>1</td>
</tr>
<tr>
<td>D 2  DNA sequences biosynthetic gene clusters of antibiotics</td>
<td>6</td>
</tr>
<tr>
<td>D 3  Improved bioinformatic technologies</td>
<td>12</td>
</tr>
<tr>
<td>D 4  Report on transcription profiles of gene clusters of interest</td>
<td>12</td>
</tr>
<tr>
<td>D 5  Strains containing heterologous gene clusters</td>
<td>18</td>
</tr>
<tr>
<td>D 6  Gene clusters with introduced artificial promoters</td>
<td>18</td>
</tr>
<tr>
<td>D 7  Mid-term progress report</td>
<td>18</td>
</tr>
<tr>
<td>D 8  Strains manipulated with respect to regulatory genes</td>
<td>24</td>
</tr>
<tr>
<td>D 9  Strains manipulated with respect to precursor supply</td>
<td>24</td>
</tr>
<tr>
<td>D 10 Strains expressing new bioactive compounds</td>
<td>24</td>
</tr>
<tr>
<td>D 11 Successful genetic strategies for the activation of gene clusters</td>
<td>24</td>
</tr>
<tr>
<td>D 12 Technologies for metabolic engineering to increase production</td>
<td>24</td>
</tr>
<tr>
<td>D 13 Technologies for upscaling</td>
<td>24</td>
</tr>
<tr>
<td>D 14 New bioactive compounds for drug discovery programs</td>
<td>24</td>
</tr>
<tr>
<td>D 15 Chemical structures of new bioactive compounds</td>
<td>30</td>
</tr>
<tr>
<td>D 16 Report on bioactivities and toxicities</td>
<td>36</td>
</tr>
<tr>
<td>D 17 Process patents</td>
<td>36</td>
</tr>
<tr>
<td>D 18 Substance patents</td>
<td>36</td>
</tr>
<tr>
<td>D 19 Scientific publications</td>
<td>36</td>
</tr>
<tr>
<td>D 20 Final progress report</td>
<td>36</td>
</tr>
</tbody>
</table>
GenoDrug Final Report

Structure:
1. Background
2. Project aim and approach
3. Project partners
4. Progress and cooperations
5. Achievement of milestones and objectives
6. Problems encountered; changes

no major problems or changes
EIB.10.023 GenoDrug
Genome mining for drug discovery: Activation of silent biosynthetic gene clusters

Thank you!