Bioresources, genome sequencing project and service in BCRC

Sung-Yuan Hsieh, Lina Huang, Chien-Chi Chen, Shin-Ying Lee,
Wen-Shen Chu, Gwo-Fang Yuan
Contents

• Introduction of BCRC
  – Background and history
  – Bioresource Holding and distribution

• Genome project
  – Barcode project, MALDI-TOF and Database
  – Genome Project on Monascus pilosus
  – Whole genome sequencing

• Conclusions
1982
Establishment of CCRC
(Culture Collection Research Center) with the support of MOEA
(Ministry of Economic Affairs)

1984
Member of WFCC (the World Federation for Culture Collections)

1994
Act a National Authority for the Deposit of Patent-related Bioresource with the support of the IPO (Intellectual Property Office)

1996
Being a Core facility for Cell Bank with the support of NHRI (National Health Research Institute)

2000
ISO 9001:2000 Certificated

2001
Microbial genome project with the support of MOEA

2002
Rechristened as BCRC (Bioresource Collection and Research Center)

2004
A draft genome sequence of Monascus pilosus BCRC38072

2007
Outstanding Project Manager Award for the Projects (2004–2007) from MOEA (Ministry of Economic Affairs)

2008
ISO 9001:2008 Certificated

2009
Joined GBRCN and became the first GBRCN Cooperating Partner.

2010
TSCB (Taiwan Stem Cell Bank) is funded from MOEA to distribute the hESC lines to researchers under strict supervision by a Stem Cell Steering Committee

2011
Joining GBRCN and became the first GBRCN Cooperating Partner.

2012
Getting TAF-accreditation to be as a reference material producer based on ISO guide 34

2013
Joined WDCM/GCM (global catalogues of microorganism) through uploading dataset

2014
Being the only depository of microorganism for bio-pesitcides with the supporting of COA(Council of Agriculture)

2015
ISO Guide 34 RMP

2016
Being a member of ACM

2017
Host the ACM 14

2018
R&D Service Excellence Award of MOEA

2018
Accreditation of New version of ISO certification

 landedmark of BCRC

Landmark of BCRC
Bioresource Preservation in BCRC

Cell Resources
(Total ~ 14,088 cells)
- Cell Bank
- Stem Cell Bank
- Taiwanese Genetic Cell Bank

Microbial Resources
(Total ~ 31,911 strains)
- Bacteria
- Actinomycetes
- Archaea
- Host
- Fungi
- Mushrooms
- Yeasts
- Algae

Gene Resources
(Total ~ 1,205,569 clones)
- Vectors
- Genomic DNAs
- Clones/Plasmids
- Genetic Libraries

Patent Deposit
(Total ~ 2,348 strains)
- Microbes, Cells, Plasmids

(until Sep. 22, 2018)
Microbes Preservation

- Total of 31,911 microbial strains were preserved, about 50% of microbes isolated from various unique environments in Taiwan.
- The most abundance collections are fungi, showing the fugal researches in Taiwan is pretty active.

<table>
<thead>
<tr>
<th>Microbial strains isolated from Taiwan</th>
<th>~50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycetes</td>
<td>17%</td>
</tr>
<tr>
<td>Archaea</td>
<td>53%</td>
</tr>
<tr>
<td>Bacteria</td>
<td>40%</td>
</tr>
<tr>
<td>Filamentous Fungi</td>
<td>59%</td>
</tr>
<tr>
<td>Mushrooms</td>
<td>59%</td>
</tr>
<tr>
<td>Yeasts</td>
<td>56%</td>
</tr>
<tr>
<td>Algae</td>
<td>70%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbial Strains Isolated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycetes</td>
<td>1,525</td>
</tr>
<tr>
<td>Archaea</td>
<td>70</td>
</tr>
<tr>
<td>Bacteria</td>
<td>10,827</td>
</tr>
<tr>
<td>Filamentous Fungi</td>
<td>10,930</td>
</tr>
<tr>
<td>Mushrooms</td>
<td>2,755</td>
</tr>
<tr>
<td>Yeasts</td>
<td>5,101</td>
</tr>
<tr>
<td>Algae</td>
<td>191</td>
</tr>
<tr>
<td>Phages</td>
<td>204</td>
</tr>
<tr>
<td>Hosts</td>
<td>308</td>
</tr>
<tr>
<td>Archaea</td>
<td>70</td>
</tr>
</tbody>
</table>

(about 50% of microbes isolated from various unique environments in Taiwan)

(known until Sep. 22, 2018)
Diversity and New Species

- BCRC has collected 31,911 microbial strains belongs to 1,961 genus, 5,253 species. Among them, some strains are new species and some are isolated from various unique environments in Taiwan.

<table>
<thead>
<tr>
<th>Collections</th>
<th>Type</th>
<th>Domestic</th>
<th>Type &amp; Domestic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus</td>
<td>1,961</td>
<td>777</td>
<td>1,159</td>
</tr>
<tr>
<td>Species</td>
<td>5,253</td>
<td>3,096</td>
<td>2,202</td>
</tr>
<tr>
<td>Strains</td>
<td>31,911</td>
<td>4,053</td>
<td>15,578*</td>
</tr>
</tbody>
</table>

*Some strains haven’t been identified yet.

(until Sep. 22, 2018)
• In average, about 5,000 bioresources were distributed to the users around the world per year from 2008 to 2017.

• Total amount of 120,667 strains were applied to research activities in the field of pharmaceutical, life science, biotech and agriculture and so on during the past 30 years (from 1984 to 2017)
Systematic Management

- Backup storage and information bank at remote site

Freezing Dry Stock bank (4°C)

Liquid Nitrogen Tank Bank (-196°C)

Ultra-low Temp Freezer Bank (-80°C)

Copy backup Bank in Chia-Yi

新竹 HSIN CHU (Since 1982)

嘉義 CHIA YI (Since 2012)

Synchronize management in both sites
Barcoding and Authentication

• Since 2010, a barcoding project started for strain authentication.
• And an in-house DNA barcoding database was generated from 17 genes with 14,258 sequences of 10,292 BCRC strains.

(unti{l Sep. 22, 2018})
Microbial Barcoding DB

http://mcoddb.bcrc.firdi.org.tw/

• This database contained major of food microbes, agriculture and industrial microbes in BCRC. Contained more than 10,000 strains, including 1,707 fungal species and 1,161 bacteria species in this database.

• The web service of MCodDB mainly offers a DNA comparison tool using two alignment algorithms, FASTA3 and Smith-Waterman, against data source from BCRC in-house database and Genbank.
MALDI-TOF MS (Biotyper) with in-house Database for Microbial Identification

- Project strat from 2014, the main spectrum profiles (MSP) of more than 1,000 important strains mainly used in food, and bio-industry were accomplished in the database under the optimized culture and sample treating conditions by using Bruker Biotyper

Ex. Main Spectrum Profile (MSP) of Enzyme Producing Bacillus species

Discrimination at strain level by PCA analysis of MALDI-TOF MSP
# Genome Project on *Monascus pilosus* (紅麴)

*Monascus* was important fermented fungi, and have being used historically, in East Asia, particularly in China and Japan.

- No reference genome available in 2001

<table>
<thead>
<tr>
<th><strong>Monascus</strong> database</th>
<th>polyketide gene clusters research</th>
<th><strong>Monascus</strong> pigment research</th>
<th>2nd metabolic chemicals research</th>
</tr>
</thead>
<tbody>
<tr>
<td>gene function annotation</td>
<td>gene finding &amp; alternative splicing</td>
<td>ORF finding</td>
<td>EST contig to cDNA sequence</td>
</tr>
<tr>
<td>genome assembly to genome sequence draft</td>
<td>genome assembly to genome sequence draft</td>
<td>EST sequencing</td>
<td>EST sequencing</td>
</tr>
<tr>
<td>Whole genome sequencing</td>
<td>Whole genome sequencing</td>
<td>EST sequencing</td>
<td>EST sequencing</td>
</tr>
<tr>
<td>WGS shotgun/BAC libraries construction</td>
<td>WGS shotgun/BAC libraries construction</td>
<td>Expression cDNA libraries construction</td>
<td>Expression cDNA libraries construction</td>
</tr>
<tr>
<td>strain identification &amp; karyotyping</td>
<td>strain identification &amp; karyotyping</td>
<td>strain identification &amp; karyotyping</td>
<td>strain identification &amp; karyotyping</td>
</tr>
</tbody>
</table>

- ~ 50 scientific papers and posters have been published
- Patents in USA and other countries
- Create *Monascus* industry application forum (紅麴產業聯盟)
- Many *Monascus* technical transfer cases (技轉及工服)
From Genome to Function and Metabolite

Selected Publication & Patent

Polyketide biosynthesis characterization


Metabolites discovery

Genomic Applications in BCRC

- **Species identification**
  - ANI (Average Nucleotide Identity)

- **Strain typing**
  - MLST marker selection
  - genomeMLST

- **Phylogenomic analysis**
  - Pan-genome analysis

- **Probiotic property evaluation**
  - Safety assessment by WGS
  - Probiotic related genes
  - Genome stability
Genomic Applications in BCRC

Species identification
- ANI (Average Nucleotide Identity)

Strain typing
- MLST marker selection
- genomeMLST

Phylogenomic analysis
- Pan-genome analysis

Probiotic property evaluation
- Safety assessment by WGS
- Probiotic related genes
- Genome stability
Species identification - *Micrococcus luteus* clades

**DDH**

- **M. luteus**
  - Schleifer et. al., 1972
- **M. yunnanensis**
  - Zhao et al., 2009
- **M. aloeverae**
  - Prakash et. al., 2014

**16S rRNA gene**

Similarity: >99%
# Whole genome-based comparisons

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Micrococcus luteus</td>
<td>BCRC 11034&lt;sup&gt;T&lt;/sup&gt;</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Micrococcus yunnanensis</td>
<td>BCRC 80243&lt;sup&gt;T&lt;/sup&gt;</td>
<td>97.0&lt;sup&gt;a&lt;/sup&gt; / 78.5&lt;sup&gt;b&lt;/sup&gt; / 65.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3 Micrococcus aloeverae</td>
<td>BCRC 80870&lt;sup&gt;T&lt;/sup&gt;</td>
<td>97.3 / 78.5 / 53.3</td>
<td>98.01 / 85.0 / 41.3</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> ANI values; <sup>b</sup> dDDH values, <sup>c</sup> DDH values (Zhao et al., 2009; Prakash et al., 2014).

*Micrococcus yunnanensis* and *M. aloeverae* were synonyme of *Micrococcus luteus*
Genomic Applications in BCRC

Species identification
- ANI (Average Nucleotide Identity)

Strain typing
- MLST marker selection
  - genomeMLST

Phylogenomic analysis
- Pan-genome analysis

Probiotic property evaluation
- Safety assessment by WGS
  - Probiotic related genes
  - Genome stability
Multilocus Sequence Typing (MLST) Technology for molecular strain typing

**Probiotics**

*Bifidobacterium*

→ Discrimination of 37 strains in 8 *Bifidobacterium* species by MLST (using 5 gene loci)

**Medicinal Fungi**

*Cordyceps* spp.

→ Discrimination of 36 strains in *Cordyceps* and related species by MLST

New species

*Cordyceps militaris* (北蟲草)
*Cordyceps cicadae* (金蠅草)
*Ophiocordyceps sobolifera* (蛹蟲草)
*Ophiocordyceps sinensis* (冬蟲夏草)
*Metacordyceps sinensis* (孢囊蟲草)
Genomic Applications in BCRC

Species identification
- ANI (Average Nucleotide Identity)

Strain typing
- MLST marker selection
  - Genome MLST

Phylogenomic analysis
- Pan-genome analysis

Probiotic property evaluation
- Safety assessment by WGS
  - Probiotic related genes
  - Genome stability
Whole genome-based comparisons using ANI and dDDH (%)

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Accession no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 <em>Lactobacillus chiayiensis</em> sp. nov.</td>
<td>NCYUAS&lt;sup&gt;T&lt;/sup&gt;</td>
<td>MSSM00000000</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 <em>Lactobacillus chiayiensis</em> sp. nov.</td>
<td>BCRC 18859</td>
<td>NOXN00000000</td>
<td></td>
<td></td>
<td>98.6&lt;sup&gt;a&lt;/sup&gt; / 87.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3 <em>Lactobacillus casei</em></td>
<td>ATCC 393&lt;sup&gt;T&lt;/sup&gt;</td>
<td>GCA_000829055.1</td>
<td>88.5 / 36.1</td>
<td>88.4 / 35.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 ‘<em>Lactobacillus zeae</em>’</td>
<td>DSM 20178&lt;sup&gt;T&lt;/sup&gt;</td>
<td>GCA_001433745.1</td>
<td>88.3 / 35.6</td>
<td>88.2 / 35.3</td>
<td>94.6 / 57.3</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>5 <em>Lactobacillus paracasei</em> subsp. paracasei</td>
<td>JCM 8130&lt;sup&gt;T&lt;/sup&gt;</td>
<td>GCA_000829035.1</td>
<td>77.5 / 22.8</td>
<td>77.2 / 21.8</td>
<td>78.0 / 24.9</td>
<td>77.8 / 24.8</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>6 <em>Lactobacillus paracasei</em> subsp. tolerans</td>
<td>DSM 20258&lt;sup&gt;T&lt;/sup&gt;</td>
<td>NZ_AYYJ00000000</td>
<td>77.4 / 22.3</td>
<td>77.4 / 22.0</td>
<td>78.3 / 24.7</td>
<td>78.0 / 24.1</td>
<td>98.3 / 84.9</td>
<td>100</td>
</tr>
<tr>
<td>7 <em>Lactobacillus rhamnosus</em></td>
<td>JCM 1136&lt;sup&gt;T&lt;/sup&gt;</td>
<td>GCA_000615245.1</td>
<td>79.0 / 23.2</td>
<td>78.9 / 22.9</td>
<td>79.2 / 23.6</td>
<td>79.2 / 23.5</td>
<td>77.0 / 22.8</td>
<td>77.3 / 22.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> ANI values; <sup>b</sup> isDDH values.

ANI and dDDH values with cut-off threshold 95–96% and 70% for delineation of prokaryotic species.
Whole genome-based a split network tree

<table>
<thead>
<tr>
<th>Organisms</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Lactobacillus casei ATCC 393^T</td>
<td>100</td>
<td>42</td>
<td>44</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>70</td>
</tr>
<tr>
<td>2 Lactobacillus chiyiensis BCRC 18859</td>
<td>45</td>
<td>100</td>
<td>90</td>
<td>8</td>
<td>7</td>
<td>11</td>
<td>48</td>
</tr>
<tr>
<td>3 Lactobacillus chiyiensis BCRC 81062^T (NCYUAST^T)</td>
<td>44</td>
<td>84</td>
<td>100</td>
<td>9</td>
<td>7</td>
<td>11</td>
<td>47</td>
</tr>
<tr>
<td>4 Lactobacillus paracasei subsp. paracasei JCM 8130^T</td>
<td>12</td>
<td>7</td>
<td>9</td>
<td>100</td>
<td>68</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>5 Lactobacillus paracasei subsp. tolerans DSM 20258^T</td>
<td>11</td>
<td>8</td>
<td>8</td>
<td>85</td>
<td>100</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>6 Lactobacillus rhamnosus JCM 1136^T</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>7 'Lactobacillus zeae' DSM 20178</td>
<td>65</td>
<td>41</td>
<td>43</td>
<td>9</td>
<td>8</td>
<td>11</td>
<td>100</td>
</tr>
</tbody>
</table>

By Gegenees and Splits Tree softwares
New species from cow manure of Taiwan

Polyphasic characterization of a novel species in the *Lactobacillus casei* group from cow manure of Taiwan: Description of *L. chiayiensis* sp. nov.☆

Chien-Hsun Huang\textsuperscript{a}, Jong-Shian Liou\textsuperscript{a}, Ai-Yun Lee\textsuperscript{a}, Min Tseng\textsuperscript{a}, Mika Miyashita\textsuperscript{b}, Lina Huang\textsuperscript{a}, Koichi Watanabe\textsuperscript{a,c,*}

\textsuperscript{a} Bioresource Collection and Research Center, Food Industry Research and Development Institute, 331 Shih-Pin Rd., Hsinchu 30062, Taiwan, ROC
\textsuperscript{b} Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), 2-5-8, Kazusakamata, Kisarazu, Chiba 292-0818, Japan
\textsuperscript{c} Department of Animal Science and Technology, College of Bioresources and Agriculture, National Taiwan University, No. 50, Ln. 155, Sec. 3, Keelung Rd., Taipei 10673, Taiwan, ROC
Genomic Applications in BCRC

- Species identification
  - ANI (Average Nucleotide Identity)

- Strain typing
  - MLST marker selection
    - genomeMLST

- Phylogenomic analysis
  - Pan-genome analysis

- Probiotic property evaluation
  - Safety assessment by WGS
    - Probiotic related genes
    - Genome stability
Functional Food in Taiwan

• In Taiwan, the total market value of functional food reached 129 billion NT dollars (4.3 billion US dollars) in 2017. The value of probiotic products of LAB was nearly 50 billion.

The current status of evidence shows that probiotic effects of LAB are strain specific. Providing the strain typing data of the functional strains used in the probiotic product for health claimed is essential on the product registration upon the TFDA regulation.
Genome-based Safety Assessment

**Genome comparison technology**

1. Microbial genomic DNA
2. NGS de novo Assemble
3. NGS reference
4. Blast against the DB of virulence

<table>
<thead>
<tr>
<th>Category of Genes</th>
<th>Recorders*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein toxin</td>
<td>8632</td>
</tr>
<tr>
<td>Virulence protein</td>
<td>8726</td>
</tr>
<tr>
<td>Pathogenicity island</td>
<td>5895</td>
</tr>
<tr>
<td>Antibiotic resistance</td>
<td>5642</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>681</td>
</tr>
</tbody>
</table>

*The DB accumulated 64,711 genes from various strains

Analysis by genome comparison of pathogen and probiotic strains
Proposing novel species
• Potential microbes
Conclusions

• BCRC is continuously improving the technologies in microbial identification, isolation and cultivation as well as maintaining the functions as a BRC.
• BCRC makes efforts on various exploitation of bioresource, providing not just bioresource but also related services and techniques for bio-industries.
Thanks for your kind attention