Laboratory to industry

Laboratorial information to biotech industrialisation

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Laboratory to industry

Biosciences → Biotech → Bioindustry

Strain collection → Modification → Fermentation → Purification → Pilot production

Software-People
Hardware-Facilities
Integration of research groups in IM

- Biomanufacture
- Sustainable Agriculture
- Health-care
- Environmental engineering

Laboratory to industry
Collection of genetic information

- Strain library (10,000 species, 100,000 strains)
  - Cultivable microbes

- Gene library (>1,500,000 clones)
  - Genomes
  - Functional genes

- Metabolite library (>100,000 deposits)
  - Extracts
  - Purified and identified

- Cell library (3,000 strains)
  - mABs hybridoma
Functional layout of TDTC

1. **菌种改造**
   - Strain Improvement
   - Screening, Mutagenesis, Metabolic engineering

2. **发酵优化**
   - Fermentation Optimization
   - Understanding the pathway how a product produced and applying the information to produce it

3. **分离提取**
   - Extraction & Separation
   - Environmentally friendly method to purify a target component

4. **工艺开发**
   - Process Development
   - Integrate optimised whole-process of production, equipment and facility layout, continuous process design to establish entire production package
Platform 1: Microbial information collection

- Identify the key factor in metabolism by Genomics and Transcriptomics
- Modify the related genes and operons to control metabolism
**Technology for strain modification**

- Screening for optimal strains
- Mutagenesis (physical and Chemical)
- Metabolic pathway control
- Hybridisation
- Protoplast
- Metabolic engineering

**Targeting at:**

- Increase desired products
- Block by-passes
- Construct novel pathways
- Whole-cell catalysts
- High-efficient microbial agents
- Enzyme manufactory
- IP protection
Fermentation process design - Data Collection

Laboratory experiment

Pilot production

Industrialisation
Advantage in Fermentation-Facility platform

- **5L lab fermentors**: Single and multi-factor experiment, response surface models
- **10L-100L amplifying fermentor**: Small scale production mimicking (Lab), amplification key factor determination
- **0.1M³-1M³ - 10M³ Pilot facilities**: Pilot production for real production mimicking, Facility and process design for manufactory


Advantage in Fermentation-Flexible strategies

Facility understanding in-depth: stirring, ventilation, mass and energy transfer

Metabolism-directed fermentation design and operation

Universal process for enzyme expression in constructed strains

Understanding balance between various factors in manufacturing: e.g. high efficiency and energy saving
Advantage in Fermentation - Smooth upstream to downstream

- Seamless integration of upstream metabolic engineering and middle fermentation control
- Optimisation of fermentation to ease downstream purification
Extraction and purification

Platform of extraction and purification - More than purer

Laboratory scheme

Pilot proving
Extraction and purification

Advantage in Purification- Full range facilities

- Supernatant separation: centrifuge, all types of membrane filtration, MF, UF, ROF
- High pressure homogenization to release component within cells
- Hydrophilic compound extraction and purification pipeline: UF, Ion-exchange etc
- Lipophilic compound extraction and purification pipeline: counter current, reverse phase chromatography, vacuum concentration
- Crystallization
- All types of dryers
- Non-standard devises designed
Advantage in Purification - Various strategies

- Combination of better processes on basis of understanding: physical, chemical, fermentation, metabolites

- Chain of thought attaching importance to various requirement

- Key point extraction and detail solution: team work

- Principle to balance quality and costs

- Professional analytical capability directing to target products
Advantage in Purification- Up & Down Connection

- Feedback to fermentation for optimisation
- Provide a proper form of product for downstream drying process
Cover the gap between laboratory and industrial production
- One-stop technology package

- Professional R&D scientists and engineers to integrate entire technology processes
- Various IP positions from strain construction, fermentation, purification and devise design
- Broad technology connections with CAS, universities for scientific solutions, and comprehensive collaboration with devise suppliers for technology solutions
- One-stop universal pilot platform for technology prove, optimisation, up & down integration
- Typical package of technology: dicarboxylic acids, N-acetylneuraminic acid, pullulan polysaccharide
What-we-do: I. strain construction

- Strengthen some anabolitic pathways to increase target products
- Block bypass to reduce by products or catabolitic pathways
- Whole-cell catalyst
- Enzyme expression and bioconversion platform
**Metabolic pathway modification of dicarboxylic acid production strain**

Data collection and processes → Information applying

- Genome
- Transcriptome
- Target
- Modification
- Producing strains

**β-oxidation** (degradation) → **ω-oxidation** (synthesis) → Co-factors balance

**β-oxidation**

- Long-chain fatty acid
- Long-chain dicarboxylic acid
- Acetyl-CoA
- Acyl-CoA
- Acyl carnitine
- Fatty acyl-CoA
- Fatty acyl carnitine
- Fatty acid synthesis

**ω-oxidation**

- Fatty alcohol oxidation
- Fatty aldehyde oxidation
- Fatty acid oxidation
- TCA cycle
- Oxidative phosphorylation

**Co-factors balance**

- NADH
- NADPH
- ATP
- NAD+
Metabolic pathway modification of dicarboxylic acid production strain

Remove genes related to β-oxidation

Strengthen promoters or increase copies to enhance ω-oxidation
Molecular modification for the pullulan production strain

A constructed novel strain with minimum melanin production

1. Bypass blockade - reduce melanin pigments in the product

2. IP position protection with secret gene sequences
Whole cell catalyst to produce sialic acid

- Brain component
- Infant formula
- Health-care

Sialic acid

E. Coli cells

N-GluNac  Isomerase  AGE  N-ManNac  N-NeuAc

cyanobacteria

Completely novel metabolic pathway design whole cell catalyse strategy
Production of terpine compound using microbial fermentation method

Industrial strategies

Chemical synthesis

Plant extraction

Microbial cell factory

Production of terpine compound using microbes will be the best strategy
Starch, glucose

Understanding and information collection of biosynthetic pathways

Precursors
IPP, DMAPP

Introduction of heterogenous metabolic pathway

Balance material energy redox

Microbial cell factory
Terpine production by recombinant microbes

Key factor determination
- HTS platform
- Metabolistic network

Gene screening substitution
- Microbial gene resources
- Plant gene resources

Cell factory
- Starting strains
- Adding up
- Target 1 → Target 2 → Target 3 → Target 4

before  after
Gene screen → Metabolic pathway → Fermentation by controlled metabolism → Purification → Purity 98%
Recombinant E. coli to produce hyaluronic acid
**Energy metabolism:**

- **ATP:** 6.5
- **NH$_3$:** 1
- **NAD$:** 4
- **Glucose:** 2.5
- **Conversion:** 10%

**Optimization undertaken:**

1. HA will damage cell wall
2. Mass transfer problem
3. Stirring sensitive of HA
4. degradation of HA by enzyme secreted by host
Laboratory equipments
Laboratory equipment II
Pilot platform for Fermentation

2m³ Feeding system

10m³ Fermentors

1m³ - 10m³ culture transferable fermentors

10m³ fermentors
Pilot platform for purification

Ceramic membrane

UF

Disc separator

Frame filter press and Diatomite filter

High pressure squeezer frame filter
Pilot platform for purification

Extraction system

Rectification and recycle system

Lyophiliser

Oil refinery system
Drying systems

- Belt Vacuum dryer
- Thin film evaporator
- Rake vacuum dryer
- Fluid bed dryer
- Spray dryer
- Spin flash dryer
Microbes for ensilage

- Short of herbage for livestock in autumn and winter
- Ensile herbage by *Bacillus sp.* and *Lactobacillus sp.* will significantly increase the body weights by 8% in winter.
- Mapping of enzymes at the molecular level, which are related to degrade herb cellulose, starch, to use various nitrogen source, to release lactic acid
Projects

Application

Bio-based material
- Synthetic chem
- Direct use
- pullulan, trehalose, amino acids

Proteins
- Enzyme preparations
- Other
- for trehalose, stevia glucosides
- EGF, IGF

Microbe agent
- Probiotics
- Lactobacilli, bacilli, yeast
- Environmental
- water treatment

Health-care
- Neutraceuticals
- DHA, carotenoids, sialic acid
- Cosmetics
- β-arbutin, pullulan,
Looking forward to collaborations