Current Situation and Prospect of the Japanese Bio-Industry

2013/7/2
Japan Bioindustry Association
Director, Strategic Planning Department
Eikoh SHIMIZU
Biotechnology and Bio-industry

* By elucidation of life and its function, biotechnology is to protect and enrich the human health, foods and environment.

* Bio-industry is expected to be bigger to realize the human dreams.

Past
(To live)

Experience=Risk evaluation

Medicine, Health
• Pharmaceutical plant

Agriculture, Foods
• Experience + Breeding
  Rice, Vegetables, animal
• Fermented foods
  Cheese, Wine, Miso
• Seasoning

Present
(Population Increasing/Aging)

Science=Risk evaluation
Modern biotechnology
• Genetically modified tech.
• Fermentation

Medicine, Health
• New drug, Biopharmaceutical

Agriculture, Foods
• GM crops
• AA, NA fermentation
⇒ Food & Feed additives

Environment & Energy

Future
+(Environ. Resource)

Science=Risk evaluation
• Gene=elucidation of life
• Food production with harmonizing to environment

Medicine, Health
• Regenerative, personalized

Agriculture, Foods
• High efficiency, productivity
• Health foods

Environment & Energy
The Era of Big World Competition in Biotechnology

**Europe** (Support of innovation)
- The 7th Frame work plan (FP7)(2007-2013)

**England** : Frame work of science & innovation investment 2004～2014
- Science & research foundation plan

**Korea**
- The second basic plan of science and technology
- (577 Initiative) (2008-2012)
- New growth vision (2009)

**Japan**
- The 4th Science and Technology Basic Plan (2012～2017)
- Comprehensive Science and Technology Innovation Strategy (2013)
- Japan Revitalization Strategy (2013)

**China**
- National medium-to-long term technology development plan (2006-2020年)
- The 15th 5 years plan (’11~’15)
- Bioindustry : ‘20 30〜45Trillion ¥
- Biofuel : ’20 11mt

**India**
- The 12th 5 years plan (2012-2017)
- India Vision 2020
- Science and Technology, Innovation plan

**Singapore**
- Economic revitalization committee
- Biopolice
- Singapore medicine campaign

**USA**
- Competitiveness Initiative (2006)
- The America COMPETES Act (2007)
- A Strategy for American Innovation (2011)
- The Green New Deal (2009)

**Brazil**
- Bioethanol 28mkℓ (2010)
- 51mkℓ (2021, Estimation)

The global launch by an open innovation
The Summary of Development of Bio-industry (Japan)

**Traditional biotechnology**
- Agriculture, Food
  - Food Culture (Japan)
    - Fermented foods
      - Miso, Soy soup
  - Seasoning
    - UMAMI: MSG (1908)

**Modern biotechnology & life science**
- Penicillin (1929)
- DNA-dowbl helix (1953)
- GM technology (1973)
- Human insulin (1976)
- Gene analysis
- Human genome
- GM crop (1996~)
- iPS (2007)

**Extract. & Purif.**
- (1909~1950’)
- Screening of microbe
- Breeding: metabolic cont.
- Ferment.: pure & large
- Separation & Purification
- Quality control

**Ferment. (1960~)**
- AA, NA
- Antibiotics

**Japan: Research ⇒ Production ⇒ Industry ⇒ Technology Transfer**

(Ref.)
- Medicine: 6.8 Trillion J¥
- Agriculture/Foods: 47.7 Trillion J¥
  - Agriculture: 11.1 Trillion J¥
  - Food: 36.6 Trillion J¥
## Food Additives

<table>
<thead>
<tr>
<th>Food Additives</th>
<th>Object and Effect (Usage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosodium Glutamate</td>
<td>Seasoning</td>
</tr>
<tr>
<td>5'-Inosinic Acid (Nucleotide) 5'-Guanylic Acid (Nucleotide)</td>
<td>Seasoning</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>Acidifier, Preservative, pH adjuster</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>Preservative, Acidifiers</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>Acidifiers</td>
</tr>
<tr>
<td>Aspartame</td>
<td>Sweetener</td>
</tr>
<tr>
<td>Amino Acids (Ile, Thr, Val, His...)</td>
<td>Seasoning, Nutrient enrichment</td>
</tr>
<tr>
<td>Enzymes (Amylase, Glucose Isomerase...)</td>
<td>Enzyme</td>
</tr>
</tbody>
</table>

## Feed Additives

<table>
<thead>
<tr>
<th>Feed Additives</th>
<th>Object and Effect (Usage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acids (Lys, Thr, Trp...)</td>
<td>Supply of nutrient ingredients and other effective ingredients of feed</td>
</tr>
<tr>
<td>Vitamin (Ascorbic Acid...)</td>
<td>Supply of nutrient ingredients and other effective ingredients of feed</td>
</tr>
<tr>
<td>Enzymes (Amylase, Protease...)</td>
<td>Promote efficient use of feed nutrient ingredients</td>
</tr>
</tbody>
</table>

### Reference

- **MHLW** Food Designated additives (2013/5/15) 434
- **MAFF** Existing food additives (2011/5/6) 365
- **MAFF** Feed additives (2012/7/1) 157
Biosynthesis of Organic Acids and Amino Acids

Key Pathways:
- Glucose metabolism to pyruvate
- Pyruvate metabolism to acetyl-CoA
- Glucose metabolism to oxaloacetate
- Acetyl-CoA metabolism to acetyl-CoA
- Glucose metabolism to 3-phosphoglycerate
- 3-phosphoglycerate metabolism to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate
- Glyceraldehyde-3-phosphate metabolism to fructose-1,6-bisphosphate
- Fructose-1,6-bisphosphate metabolism to glyceraldehyde-3-phosphate
- Glyceraldehyde-3-phosphate metabolism to pyruvate
- Pyruvate metabolism to acetyl-CoA
- Acetyl-CoA metabolism to oxaloacetate
- Oxaloacetate metabolism to fumarate
- Fumarate metabolism to succinate
- Succinate metabolism to α-ketoglutarate
- α-Ketoglutarate metabolism to glutamate
- Glutamate metabolism to ornithine
- Ornithine metabolism to arginine
- Arginine metabolism to histidine

Specific Pathways:
- Glucose metabolism to fructose-1,6-bisphosphate
- Fructose-1,6-bisphosphate metabolism to glyceraldehyde-3-phosphate
- Glyceraldehyde-3-phosphate metabolism to pyruvate
- Pyruvate metabolism to acetyl-CoA
- Acetyl-CoA metabolism to oxaloacetate
- Oxaloacetate metabolism to fumarate
- Fumarate metabolism to succinate
- Succinate metabolism to α-ketoglutarate
- α-Ketoglutarate metabolism to glutamate
- Glutamate metabolism to ornithine
- Ornithine metabolism to arginine
- Arginine metabolism to histidine
# Industrial Amino acids Production Process

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Ferment.</th>
<th>Enzyme</th>
<th>Chem. syn.</th>
<th>Extraction</th>
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<tr>
<td>L-Glu</td>
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<td></td>
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<tr>
<td>L-Lys</td>
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<tr>
<td>L-Thr</td>
<td>○</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<tr>
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<tr>
<td>L-Tyr</td>
<td>○</td>
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</tbody>
</table>
Breeding Technology of Amino Acid Producer

1. Control of metabolic flows:
   - Auxotrophic mutants (deletion of key enzymes) \(\Rightarrow\) Lys, Thr etc
   - Analog-resistant mutants \(\Rightarrow\) Lys, Thr etc
     (Desensitization of feedback inhibition of key enzymes)
   - Activation of AA-exporter \(\Rightarrow\) Glu etc

2. Conversion by microbial enzymes (Bioreactor):
   \(\Rightarrow\) Asp, Cys etc

3. Genetically modified microbes:
   - Amplification of modified genes of key enzymes
   - Amplification of specific AA transporter genes etc
Discovery and Industrialization of Umami-Seasoning from three major Japanese Traditional Soup (“Dashi”)

Konbu (Kelp)

Shiitake (Lentinula edodes)

Katsuobushi (dried, fermented, and smoked shipjack tuna)

Glutamic acid

(Prof. Ikeda, 1908)

Guanosine-5’-monophosphate (5’-GMP)

(Dr. Kuninaka, 1960)

Inosine-5’-monophosphate (5’-IMP)

(Dr. Kodama, 1913)
MSG (Mono-sodium Glutamate)

① Seasoning : UMAMI taste
② Discovery of MSG as the essence of UMAMI taste by Prof. IKEDA

(1909～1950’s) Extraction

Wheat protein
Soy bean protein → Glutamic acid

(1960～) Fermentation

Glucose (Starch)
Sucrose, Molasses → Glutamic acid

(Corynebacterium glutamicum)
(= "Micrococcus glutamicus" “Brevibacterium lactofermentum” etc.)

* Japanese researchers in Kyowa Hakko Kogyo Co. first isolated this excellent microbe in 1956

=C. glutamicum
Industrial production of 5’-IMP and 5’-GMP

**Conventional process**

**Phosphorylation of nucleosides by chemical method**

Glucose (Bacillus subtilis) fermentation → chemical reaction

**Direct fermentation of 5’-IMP from sugar**

Glucose (Corynebacterium ammoniagenes etc) fermentation → (5’-IMP)

**New process**

**C5’-regioselective phosphorylation using pyrophosphate (PPi)**

Glucose (Bacillus subtilis) fermentation → phosphotransferase from enteric bacteria

(Base)
1. hypoxanthine ⇒ 5’-IMP
2. guanine ⇒ 5’-GMP

High productivity
High selectivity

Low productivity
Low selectivity
Citric Acid

① Acidifier
   (Preservative, pH adjuster)
② In citrus fruits: oranges, lemons・・・

③ Production

Static culture
Starch, sucrose
(Solid or Liquid)

Fermentation by
Aspergillus niger

Submerged culture
Glucose, Sucrose
(Liquid, aeration)

Citric acid

Citric Acid

Citric Acid
Aspartame

① Non-saccharide sweetener, a sugar substitute
  * 200 times sweeter than sucrose
  * Dipeptide ⇒ Hydorolysis
② Discovery: James. M. Schlatler in 1965
③ USA-FDA approval in 1998
   EU Authority approval in 2002, 2006
④ Production
   (Current process)
   Fumaric acid \(\xrightarrow{\text{NH}_3}\) Asp
   (Asparate ammonialyase of \(E.\ coli\))
   Glucose \(\xrightarrow{\text{Fermentation by } C.\ glutamicum}\) Phe
   (chemical reaction)
   Aspartame
   (Asp-Phe methyle ester)
   (New process & future)
   Glucose \(\xrightarrow{\text{Fermentation by new microbe}}\) Phe
   (enzymatic reaction)
   Aspartame
   Under investigation

Aspartame
Lysine and Threonine Fermentation

Breeding of Lys & Thr producer by analogue resistance and auxotrophic mutation in *C. glutamicum*

**Wild strain**
(AA prod. is strictly controlled)

**Lys producer**

**Thr producer**

Sugar → Aspartate → Aspartyl-P → Aspartylsemialdehyde → Homoserine → Lysine → Threonine

Sugar → Aspartate → Aspartyl-P → Aspartylsemialdehyde → HD deletion → Homoserine → Lysine → Threonine

Sugar → Aspartate → Aspartyl-P → Aspartylsemialdehyde → HD mut. → Homoserine → Lysine → Threonine

AK = aspartokinase
HD = homoserine dehydrogenase
Application of New Technology to Lys Producer

Conventional technology

- Based on conventional mutation in *C. glutamicum*

New (Future) technology

- Rational construction of producer in *E. coli*

Glucose (sucrose) → Aspartate → Aspartyl-P → Aspartylsemialdehyde → Homoserine → Lysine

AK mutation (analog resist) - HD deletion (auxotroph)

Targets to be aimed at;
- Optimiz. of metabolic pathway
  - Substrate uptake↑
  - AA discharge↑
  - AA degradation↓
- Osmotolerant
- High yield & high speed ferment.
- Usage of novel substrate such as biomass, methanol, CO₂ etc
- Low O₂ demand etc
Modern Biotech Products* Market in Japan

Products* by modern biotechnology (Gene engineering, Cell fusion, Cell culture)

Market (Trillion J ¥) vs. Growth rate (%)

- Medicine / Health care
- Agriculture / Food
- Environment / Energy
- Instrument / Information / Services
- Chemicals / Others

Market except the Import (GM crop)

Total Market

(Source: Nikkei Bio File 2013)
Modern Biotech Products* Market in Japan (2012)

34.1 US billion dollar

Pharmaceuticals 44.7%

Bio-pharmaceuticals 32.5%

Instruments /Reagent 6.0%

Agriculture/ Food 29.2%

Chemicals 9.1%

Diagnosis 7.2%

Pharmaceutical Ingredients 3.5%

Vaccine 8.7%

Services 1.6%

Others 1.2%

Bioinformatics 1.2%

Notice: 80.82 Japanese yen/ dollar (2012 average)

Source: Nikkei Bio File 2013
Trend of Bio-Ventures in Japan

Source: Japan Bioindustry Association
Business Field of Bio-Venture in Japan (2012)

Source: Japan Bioindustry Association
New Industry of Health Foods/Functional Foods

- Healthy
- Half-Healthy (“Tokuho”*)
- Illness - Disease

Market of Foods: >100 Trillion ¥

Health Foods/Functional Foods Market: 20 Trillion ¥ ↔ 2 Trillion ¥

Biotech-driven Health Promotion

Functional analysis of foods focusing on health benefits and development of “New Foods”

Creation of Functional Food-Related Industry

*Foods for specified health uses (FOSHU) = “TOKUHO”
[1105 items approved (2013/May)]

Examples of health claims:
- Digestive effects (e.g., Lactic acid bacteria)
- Blood-Pressure Control (e.g., Lacto-tripeptide)

Market of Medicines: 8 Trillion ¥
Thank you!