Biotechnology: Products derived from Microbes

Questions for Today:
1. What are some major microbial products and how are they made?
2. What is biocatalysis?
3. What is primary vs. secondary metabolism?
4. What is a Fermentor, how is it used?
5. How does one discover/produce new antibiotics?

Overview of microbial products
• Foods
  • Breads, cheeses, yogurt, mushrooms, wine, beer, soy sauce, sake, etc.

• Commodities:
  • Food additives – amino acids, thickening agents, vitamins
  • Solvents – butanol, ethanol
  • Biofuels – ethanol, methane, hydrogen

• Fine chemicals:
  • Pharmaceuticals – antibiotics, antifungals
  • Laboratory and diagnostic reagents – enzymes biochemicals, proteins
Bread, beer
Food additives:  
*Amino acids* – microbes produce the useful L-isomers (optically pure isomers)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Annual production worldwide (metric tons)</th>
<th>Uses</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Glutamate (monosodium glutamate, MSG)</td>
<td>370,000</td>
<td>Various foods</td>
<td>Flavor enhancer; meat tenderizer</td>
</tr>
<tr>
<td>l-Aspartate and alanine</td>
<td>5,000</td>
<td>Fruit juices</td>
<td>“Round off” taste</td>
</tr>
<tr>
<td>Glycine</td>
<td>6,000</td>
<td>Sweetened foods</td>
<td>Improve flavor; starting point for organic syntheses</td>
</tr>
<tr>
<td>l-Cysteine</td>
<td>700</td>
<td>Bread</td>
<td>Improves quality</td>
</tr>
<tr>
<td>l-Tryptophan + l-Histidine</td>
<td>400</td>
<td>Fruit juices</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Aspartame (made from l-phenylalanine + l-aspartic acid)</td>
<td>7,000</td>
<td>Various foods, dried milk.</td>
<td>Low-calorie sweetener.</td>
</tr>
<tr>
<td>l-Lysine</td>
<td>70,000</td>
<td>Blood (Japan), food additives</td>
<td>Nutritive additive</td>
</tr>
<tr>
<td>ox-Methionine</td>
<td>70,000</td>
<td>Soy products, food additives</td>
<td>Nutritive additive</td>
</tr>
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</table>

Problem: a microbe usually produces an amino acid for *growth* – so how do we get the bug to make excess amino acids (e.g. 100 g Glu/L)?

Strain optimization:
- **Regulatory mutants**: select mutants that have lost normal feedback inhibition of biosynthetic pathway; example: lysine production by *Brevibacterium flavum*

- **Other beneficial mutations**: altered cell membrane; example: *Corynebacterium glutamicum* for glutamate production
**Vitamins**

Vitamin B$_{12}$ Propionibacterium, Pseudomonas Riboflavin

**Other food additives**

Citric acid Aspergillus niger
(Increase yield by Fe deficient growth)

Acetic acid Acetobacter
Other commodity products: solvents, fuels, other chemicals

Increase yields by strain selection, metabolic pathway engineering, and process improvement; enzymes may be added by incorporation of genes from other organisms (Lec. 28)

<table>
<thead>
<tr>
<th>Product</th>
<th>Organism</th>
<th>New gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td><em>E. coli</em></td>
<td>Pyruvate decarboxylase, and alcohol dehydrogenase from <em>Zymomonas</em></td>
</tr>
<tr>
<td>Itaconic acid</td>
<td><em>Aspergillus</em></td>
<td>Submerged culture</td>
</tr>
<tr>
<td>lactic acid</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>bovine lactate dehydrogenase</td>
</tr>
<tr>
<td>xylitol</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>xylose reductase from <em>Pichia stipitis</em></td>
</tr>
</tbody>
</table>

Microbial production of industrial enzymes

A $1.5 billion industry

• Glucose isomerase – high fructose corn syrup
• Saccharases – starch breakdown
• Proteases – industrial detergents, food processing; subtilisin from *Bacillus subtilis* largest enzyme market
• Cellulases – fuel production, “stonewashed” denim
• Extremozymes – stable at extreme pH, temp, salt conditions (e.g., *taq* polymerase)
• Amylase, pectinase, rennin, lipase, etc. (Table 30.4)
• New enzymes by directed evolution
Biocatalysis, bioconversion, or biotransformation: microbial conversion of substrates to more useful compounds
Use whole microbial cells as live “bag of enzymes” to perform useful chemical conversions

Alternatively, the cells or the purified enzyme can be “immobilized” (bound to a resin or encapsulated) for use in a “bioreactor”. Substrate flows through and is converted to product.

Primary and secondary metabolism
Some definitions used in industry:

*fermentation* – large-scale growth (usually in vats or tanks) of microorganisms (this differs from the term used to describe bioenergetics!)
*primary metabolites* – substances produced during the primary growth phase of a culture
*secondary metabolites* – are produced near the end of the growth phase or in stationary phase
Secondary metabolites are:
-- Unpredictable -- formation is not consistent among all members of a species
-- Non-essential for growth
-- Highly dependent on growth conditions
-- Often found in families of related chemical structures
Fermentors and process control

- Mixing, aeration, and O₂ monitoring (getting enough dissolved oxygen is a major problem)
- Temperature control
- Sterilization
- Sampling and harvest ports
- pH control

Scale-up: from benchtop to commercial fermentors

- Flask cultures: feasibility testing
  - Laboratory fermentor: 1-10 Liter
  - Pilot plant: ~300-3000 Liter; adjust conditions to be close to what will be encountered in commercial scale; cost not yet a major factor
  - Commercial fermentor: up to 500,000 liters
Growth media used in industrial fermentations

High production of products will not be useful if they are too expensive to grow – need cheap ingredients for formulation of growth media for industrial-scale cultures.

For example: 500,000 liters of growth medium used for laboratory-scale *E. coli* cultures would cost over $200,000!

Solution – use agricultural and food-product by-products and other inexpensive nutrient sources

<table>
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<tr>
<th>Source</th>
<th>Raw material</th>
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<tr>
<td>Carbon/energy</td>
<td>Molasses, whey, agricultural waste (e.g. corncobs)</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Corn-steep liquor, soybean meal, ammonium salts, stick liquor</td>
</tr>
<tr>
<td></td>
<td>(slaughterhouse by-products)</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Crude animal and plant products</td>
</tr>
<tr>
<td>Iron and trace elements</td>
<td>Crude inorganic chemicals, fertilizer-grade phosphates</td>
</tr>
</tbody>
</table>
Other types of mass culture methods

[The goal is to maximize product while minimizing the expense!]

Air lift fermentors: air flow keeps culture mixed

Solid state fermentation: growth without added water (e.g., silage)

Fixed-bed reactor: microbes grow on porous solid surface

Fluidized-bed reactor: microbes grow on surface of inert particles suspended in flowing growth medium
Antibiotics: an example of drug discovery to industrial production

Traditional screening test for antibiotic producers. (Recall that you already learned about zones of inhibition for pure antibiotics measured in a disc agar diffusion assay using a test organism)

Usually a particular antibiotic producer does not make sufficient quantities for industrial production – the microbial strain must be optimized to obtain high-yielding variants

Microbes producing antibiotics usually fall into 3 types: Spore-forming Gram-positive bacteria, Actinomycetes, Fungi
Initial microbial antibiotic producing strain
  ↓
Mutagenesis using chemicals, UV, or radiation along with genetic manipulation (gene amplification, metabolic engineering)
  ↓
Screen for strains producing higher quantities under optimal conditions
  ↓
Repeat the cycle until strain has desired production
  ↓
Chemically modify the purified compound to produce a “semisynthetic” antibiotic
Penicillin fermentation using *Penicillium chrysogenum*

- Production increases as culture enters stationary phase
- Extra feeding with carbon and nitrogen sources maintains production longer for greater yields
- Precise feeding 0.018 g/liter-hour needed for maximal production