Primary and secondary metabolites

Source:
1. Fermentation Microbiology and Biotechnology By EMT Mansi et al
2. Industrial Microbiology : An Introduction By MJ Waites
3. Industrial Microbiology By HS Patel
4. Food and Industrial Microbiology By Raveendra Reddy
Introduction

• Applications of microbes for industrial production of primary and secondary metabolites - Industrial Microbiology.

• Metabolism in microorganisms involves two pathways:

  **Primary metabolic pathways** (PMPs, produced during the growth phase of the organism) produce too few end products, while

  **secondary metabolic pathways** (SMPs, produced during the stationary phase) produce a variety of products.

• There are some similarities between the pathways that produce primary and secondary metabolites:
  ❑ The product of one reaction is the substrate for the next and
  ❑ The first reaction in each case is the rate-limiting step.
  ❑ Also the regulation of secondary metabolic pathways is interrelated in complex ways to primary metabolic regulation.
Production of primary and secondary metabolites.

(a) A primary metabolite, such as ethanol from yeast, has a production curve that lags only slightly behind the line showing cell growth.

(b) A secondary metabolite, such as penicillin from mold, begins to be produced only after the logarithmic growth phase of the cell (trophophase) is completed. The main production of the secondary metabolite occurs during the stationary phase of cell growth (idiophase).
Industrial fermentation based on the end-product application, can be categorized into four types:

1. **Biomass:** The end-product is viable cellular material eg, single cell protein, baker’s yeast, probiotic cultures.

2. **Extracellular metabolites:** Chemical compound intermediates of microbial biochemical pathways are produced and can be divided two groups:
   a. **Primary metabolites** *(produced during the growth phase of the organism)*, eg, ethanol, citric acid, glutamic acid, lysine, vitamins and polysaccharides.
   b. **Secondary metabolites** *(produced during the stationary phase)*, eg, penicillin, cyclosporin A, gibberellin, and lovastatin.

3. **Enzymes and other proteins (intracellular components):** A key component of this process is lysis of cells at the end of fermentation. Proteins are typical end products and need to be purified and crystallized.

4. **Substrate transformations:** Raw material is biologically transformed into a finished product. Generally used for steroid transformations, food fermentations and sewage treatment.
Primary metabolites

Involved in **growth, development and reproduction**. Hence, **essential for survival and existence of the organism and reproduction.**

- Formed at the same time as new cells.
- Production curve follows the growth curve.
- Formed in **trophophase** during exponential growth as normal end products of primary metabolism.
- Also called **central metabolites** as these maintain normal physiological processes.
- Cells maintain optimum concentration of all macromolecules (**proteins, DNA, RNA** etc.).
- Produced in **adequate amount to sustain cell growth** for example vitamins, amino acids, nucleosides etc.
- **Overproduction can be genetically manipulated.** Auxotrophic (auxo, “increase,” and trophos, “food”) mutants having a block in steps of a biosynthetic pathway for the formation of primary metabolite.
- **Growth rate slows down due to limited supply of any other nutrient.** Metabolism does not stop but product formation stops.

Industrially important for example ethanol, acetone, lactic acid, CO₂.

Common food supplements, L-glutamate and L-lysine, are produced and purified via the mass production *Corynebacterium glutamicum*.

Citric acid, commonly used in pharmaceutical and cosmetic industries is produced by *Aspergillus niger*. 
Over production of primary metabolite.

- To maximize production manipulation of feedback inhibition pathways is performed.
- Another approach is to use auxotrophic mutant with defective metabolite production.
Secondary metabolites

• Secondary metabolites are not produced until the microbe has largely completed its logarithmic growth phase and entered the stationary phase of the growth cycle. Period of production is called idiophase and metabolites as idiolites.

• In the idiophase, cells do not divide but are metabolically active.

• Idiolites are organic compounds produced only after considerable number of cells and a primary metabolite have accumulated (end or near the stationary phase of growth). Rather it can be said that these are produced under sub-optimal concentrations of O$_2$, deviations of pH or when primary nutrient source is depleted.

• Though idiolites are a characteristic feature of fungal, yeast, actinomycetes and bacterial growth but are not produced by a few strains of E. coli.

• In some strains secondary metabolite are produced by further conversion of a primary metabolite.

• Not necessary for growth, development, and reproduction like primary metabolites. Their production is influenced by environmental factors.

• Secondary metabolites are synthesized for a finite period by cells that are no longer undergoing balanced growth.

• A single microbial type can produce very different metabolites.
• Their production is regulated by complex biochemical pathways and some strains can produce a variety of idiolites. For example a strain of *Streptomyces* can produce a variety of 35 anthracyclines.

• Overproduction of secondary metabolites can be achieved by manipulating larger number of genes (gene cassettes).

Typical examples include antibiotics, toxins and pigments to name a few.

During the trophophase, the cell mass increases logarithmically but as the resources become limiting, growth rate drops and production stops.
<table>
<thead>
<tr>
<th>Primary metabolites</th>
<th>Secondary metabolites</th>
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</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>Antibiotics</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Pigments</td>
</tr>
<tr>
<td>Nucleic acid</td>
<td>Toxins</td>
</tr>
<tr>
<td>Polysachchrides</td>
<td>Alkaloids</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Steroids</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Polymeric substances eg gums, rubber</td>
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</tbody>
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Industrial Production of Microbial Metabolites

Basic steps are:

1. Screening, selection, maintenance of source microorganism for the production of target metabolite (primary or secondary).
2. Optimization and standardization of growth medium and conditions (w.r.t. choice of fermenter vessel, aeration, temperature, agitation, pH, etc.) for large-scale (fermentation) protocol.

*Preparation of the microorganism and the raw materials required for the microorganism to grow and produce the desired product is called upstream processing.*

3. Sterilization of the medium, fermenter and ancillary equipment.
4. Active, pure culture in sufficient quantities is used to inoculate growth medium in fermenter.
5. The growth of the organism in the production fermenter under optimum conditions for product formation.

*Growth of the target microorganism in a large bioreactor (usually >100 litres) with the consequent production (biotransformation) of a desired compound is the phase of fermentation and transformation.*

6. The extraction of the product and its purification.
7. Disposal of effluents produced by the process.

*Purification of the desired compound from either the cell medium or the cell mass is called downstream processing.*
Scheme for industrial production of metabolites