5. Industrial Microbiology

the roles of bacteria yeast in food and alcohol production

Learning Outcomes

- Fermentation
- Food/beverage production with microbes
- Industrial microbiology
- Bioprospecting

Microbes as food

- Edible fungi :
  - Mushrooms : fungal fruiting bodies
    - *Agarcius bisporu:*button and portobellos
  - Quorn : *Fusarium venetum*
- Edible algae: Seaweed
  - Red algae *Porphyra*
    - Nori (sushi)
  - Brown algae *Macrocystis*
    - Alginate
- Edible bacteria - Nucleic acid often too concentrated for food - Cyanobacterium Spirulina: - Single-celled protein

Fermentation

- Fermentation is the completion of catabolism without the electron transport system and a terminal electron acceptor.
  - The hydrogens from NADH + H+ are transferred back onto the products of pyruvate, forming partly oxidized fermentation products.
- Most fermentations do not generate ATP beyond that produced by substrate-level phosphorylation.
  - Microbes compensate for the low efficiency of fermentation by consuming large quantities of substrate and excreting large quantities of products

Energetic and Redox Considerations

- Two mechanisms for catabolism of organic compounds
- Respiration
  - Exogenous electron acceptors are present to accept electrons generated from the oxidation of electron donors
- Fermentation
  - Fermented substance is initially the electron donor and later becomes the electron acceptor
  - Relatively little energy yield

Mechanisms

- In fermentation, ATP is synthesised by substrate-level phosphorylation
    - Energy-rich phosphate bonds from phosphorylated organic intermediates transferred directly to ADP
- Organic molecules within the cell act as electron acceptors
- Therefore, redox balance is achieved by production and secretion of fermentation products

Microbial Fermentations

- Oxidation of NADH produced by glycolysis
    - Necessary to restore Redox balance
- Pyruvate or derivative used as endogenous electron acceptor
    - This results in waste products which are no longer used by the cell
- Oxygen not needed
- Oxidative phosphorylation does not occur
    - ATP formed by substrate-level phosphorylation

Carbohydrate Catabolism

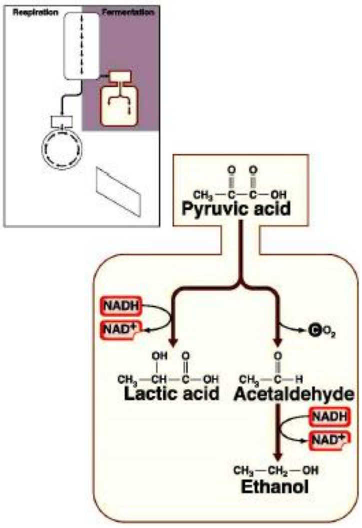
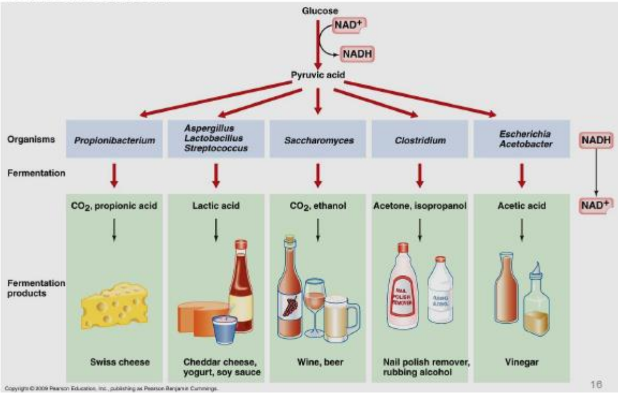


Table 5.4 Comparison of Aerobic Respiration, Anaerobic Respiration, and Fermentation

	Aerobic Respiration	Anaerobic Respiration	Fermentation
Oxygen required	Yes	No	No
Type of phosphorylation	Substrate-level and oxidative	Substrate-level and oxidative	Substrate-level
Final electron (hydrogen) acceptor	Oxygen	NO_3^-, SO_4^{2-}, CO_3^{2-}, or externally acquired organic molecules	Cellular organic molecules
Potential molecules of ATP produced	38 in prokaryotes, 36 in eukaryotes	2–36	2

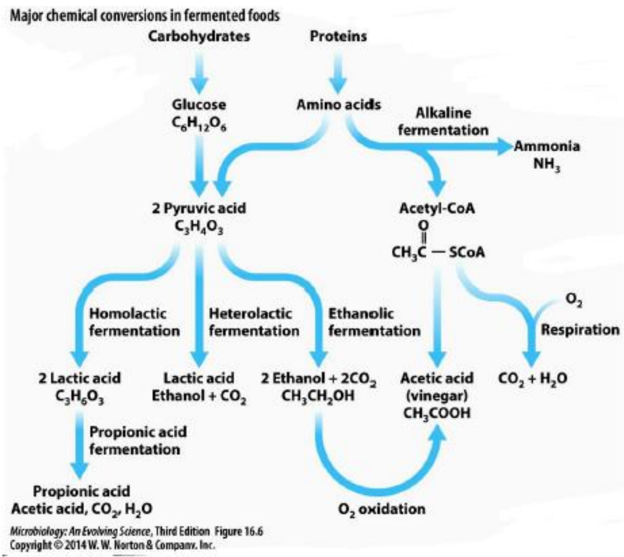
Fermentation Pathways

- **<u>Homolactic fermentation :</u>** Produces two molecules of lactic acid
- **<u>Ethanolic fermentation</u>** : Produces two molecules of ethanol and two CO2
- **<u>Heterolactic fermentation</u>** : Produces one molecule of lactic acid, one ethanol, and one CO2
- **<u>Mixed-acid fermentation</u>** : Produces acetate, formate, lactate, and succinate, as well as ethanol, H2, and CO2
- 

Applications of Fermentation

Overview of fermented foods

- Virtually all human cultures have developed varieties of fermented foods, which are food products modified biochemically by microbial growth.
- The purposes of food fermentation include:
  - To preserve food: By limiting microbial growth
  - To improve digestibility: By breaking down fibers
  - To add nutrients (such as vitamins) and flavor molecules (such as esters and sulfur compounds
- Traditional fermented foods usually depend on indigenous flora (found naturally in the food) or starter cultures (from a previous fermentation).
- Major classes of fermentation reactions include:
  - Homolactic acid fermentation
  - Propionic acid fermentation
  - Heterolactic acid fermentation
  - Ethanolic fermentation
  - Alkaline fermentation



Acidic Fermentation of Dairy Products

- Milk fermentation begins by lactic acid fermentation with Lactobacillus and Streptococcus.
- This is followed by rennet proteolysis (by chymosin and pepsin), rendering casein insoluble.
- The cleaved peptides coagulate to form a semisolid curd.
  - Separated from the liquid portion called whey

Cheese

- Involves a standard series of steps:
  - Milk is filtered, centrifuged, and subjected to flash pasteurization.
  - Fermenting microbes are added as a starter culture.
  - The solid curd is then cut, or cheddared.
  - Curd is then heat-treated.
  - The pressed curd is shaped into a mold.
  - The cheese is the ripened (or aged).
- In all fermented foods, microbial metabolism generates by-products that confer characteristic aroma and flavour.

Fermented Vegetables

Acidic vegetable fermentation

- Soybeans
  - Microbes remove harmful elements.
  - Mold: Rhizopus – Tempeh
  - Mold: Aspergillus – Miso, soy sauce
- Cabbage, cucumbers, olives –
  - Pickling: fermentation in brine (high salt) –
  - Bacterium Leuconostoc: sauerkraut, pickles, kimchi

Alkaline Vegetable Fermentation

- Soybeans
  - Bacteria: Bacillus – Natto
- Eggs
  - Bacteria: Bacillus – Pidan
  - "Thousand-year eggs"
- Locust beans
  - Bacteria: Bacillus – Dawadawa

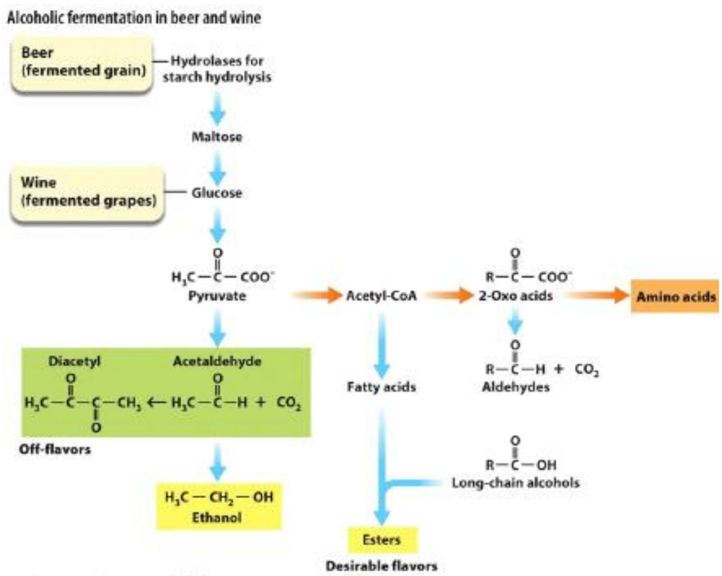
Ethanolic Fermentation

Bread

- Saccharomyces cerevisiae: Baker's yeast
- Pyruvate → Ethanol + CO_2
  - CO_2 causes bread to rise.
  - Easier to chew and digest
  - More gluten → more rise
  - Ethanol is removed by baking
- A prolonged fermentation with extensive microbial activity; occurs in the dough for an Ethiopian bread called injera
- Made from teff, a grain with no glutten
- Makes an edible "tablecloth"
- Fermentation dominated by the yeast Candida

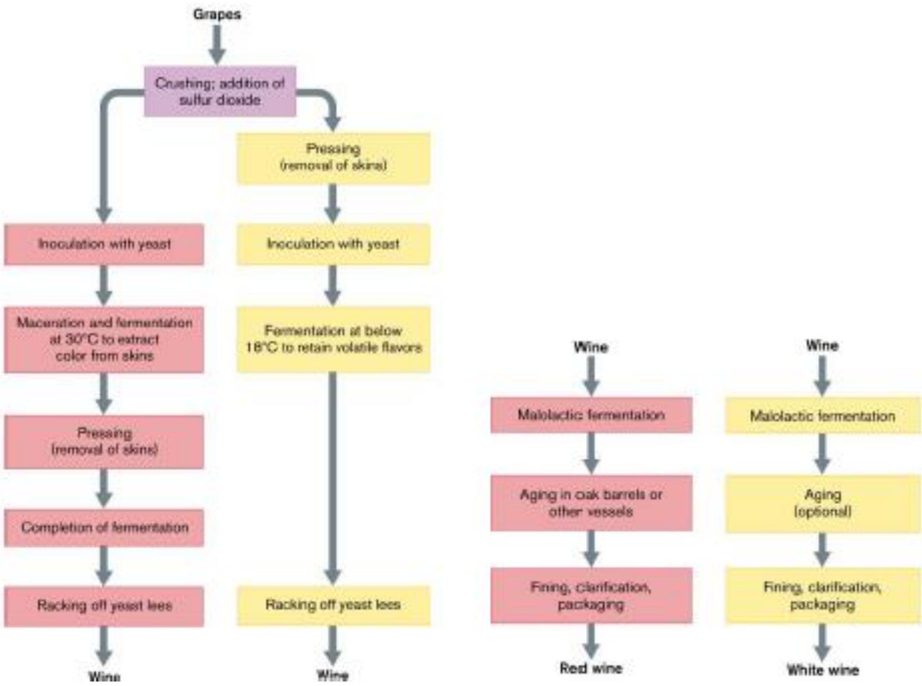
Beer

- Saccharomyces cerevisiae: Brewer's yeast
- Beer derives from alcoholic fermentation of grain.
    - Barley grains are germinated, allowing enzymes to break down the starch to maltose for yeast fermentation.
    - Secondary products, such as long-chain alcohols and esters, generate the special flavours of beer.



Microbiology: An Evolving Science, Third Edition Figure 16.23

Wine



- Saccharomyces cerevisiae: Brewer's yeast
- Wine derives from alcoholic fermentation of fruit, usually grapes.
    - The grapes are crushed to release juices.
        - For white wine (and not red wine) the skin is removed.
- The inoculated yeast ferments glucose to alcohol.
- Both kinds of wine usually undergo malolactic fermentation by *Oenococcus oeni* bacteria

Lactic Acid Fermentation

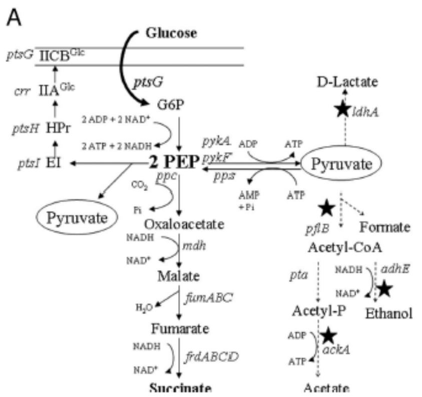
Homolactic fermenters – fermented milk products

- Cultured milks
    - Mesophilic fermentation → buttermilk, sour cream
        - Lactobacillus & L. lactis for acid and flavour
    - Thermophilic fermentation (45°C) → yoghurt
        - Streptococcus thermophilus – acid
        - Lactobacillus bulgaricus – flavour
    - Increased acid content also extends the life of the milk

Lactic and mixed-acid Fermentations

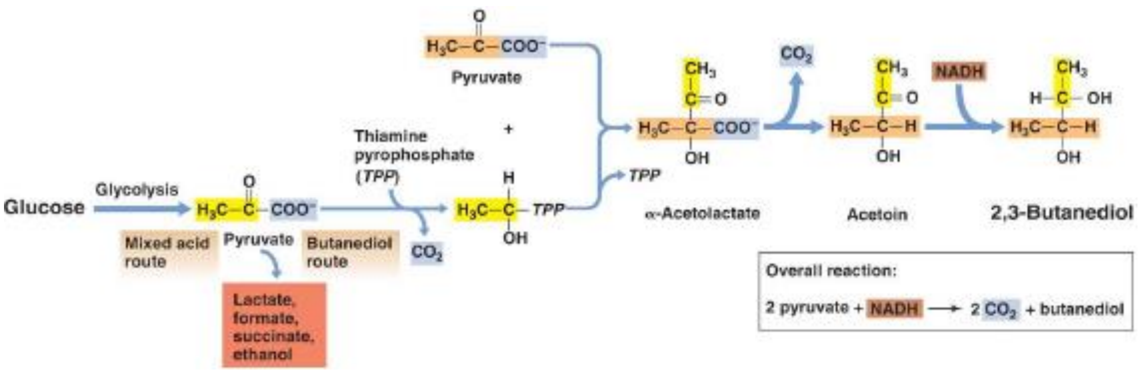
- Mixed-Acid Fermentations
    - Generate acids
        - Acetic, lactic, and succinic
- Sometimes also generate neutral products
    - e.g., butanediol
- Characteristic of enteric [intestinal] bacteria and is an important diagnostic tool

Mixed acid pathway for glucose fermentation in native *E.coli*

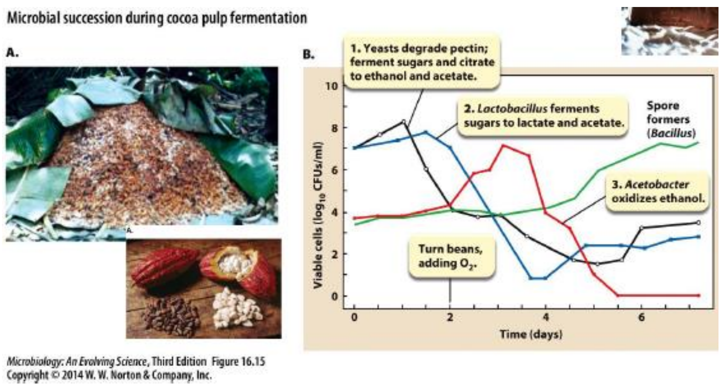


Butanediol Production in Mixed Acid Fermentations

- Butanediol is not a product of *E. coli* mixed acid fermentation



Fermentation of cocoa beans



Common Bacterial Fermentations

Table 21.2 Common bacterial fermentations and some of the organisms carrying them out

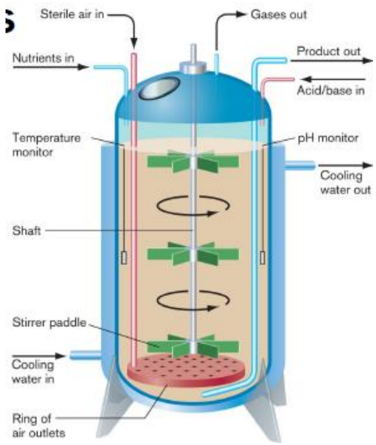
Type	Reaction	Organisms
Alcoholic	Hexose → 2 Ethanol + 2 CO_2	Yeast, *Zymomonas*
Homolactic	Hexose → 2 $Lactate^-$ + 2 H^+	*Streptococcus*, some *Lactobacillus*
Heterolactic	Hexose → $Lactate^-$ + Ethanol + CO_2 + H^+	*Leuconostoc*, some *Lactobacillus*
Propionic acid	3 $Lactate^-$ → 2 $Propionate^-$ + $Acetate^-$ + CO_2 + H_2O	*Propionibacterium*, *Clostridium propionicum*
Mixed acid[a]	Hexose → Ethanol + 2,3-Butanediol + $Succinate^{2-}$ + $Lactate^-$ + $Acetate^-$ + $Formate^-$ + H_2 + CO_2	Enteric bacteria[b] *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*
Butyric acid[b]	Hexose → $Butyrate^-$ + 2 H_2 + 2 CO_2 + H^+	*Clostridium butyricum*
Butanol[b]	2 Hexose → Butanol + Acetone + 5 CO_2 + 4 H_2	*Clostridium acetobutylicum*
Caproate/Butyrate	6 Ethanol + 3 $Acetate^-$ → 3 $Butyrate^-$ + $Caproate^-$ + 2 H_2 + 4 H_2O + H^+	*Clostridium kluyveri*
Homoacetogenic	Fructose → 3 $Acetate^-$ + 3 H^+	*Clostridium aceticum*

[a]Not all organisms produce all products. In particular, butanediol production is limited to only certain enteric bacteria. Reaction not balanced.
[b]Stoichiometry shows major products. Other products include some acetate and a small amount of ethanol (butanol fermention only).

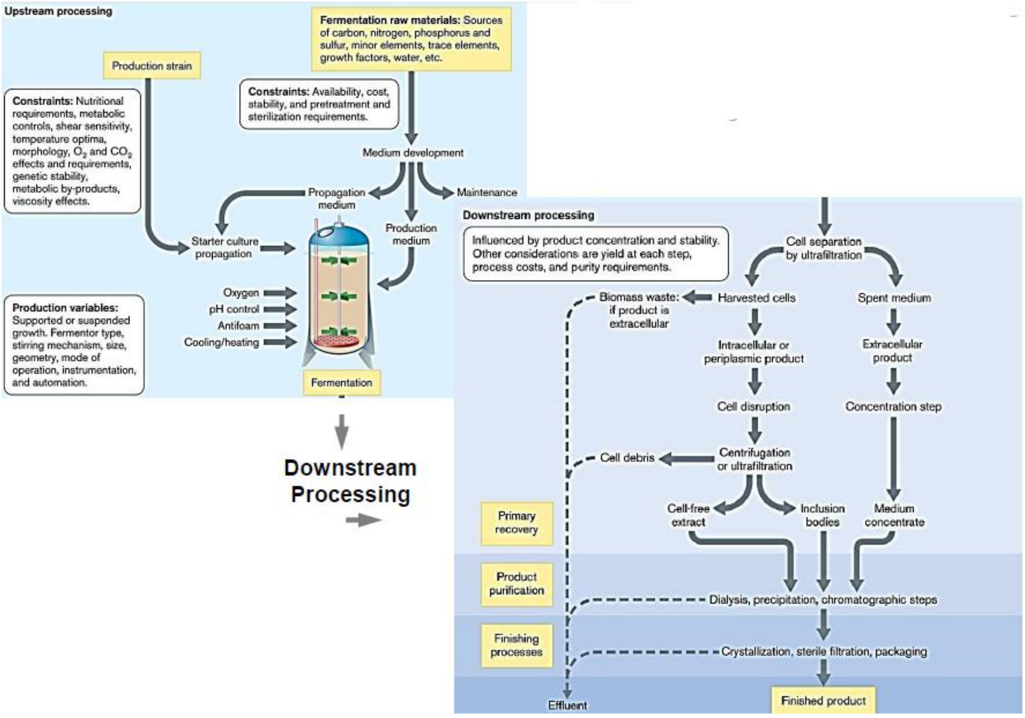
Industrial Microbiology

- Industrial microbiology is the commercial exploitation of microbes.
- It includes food production and preservation.
- Also the production of:
  - Vaccines and clinical devices
  - Industrial solvents and pharmaceuticals
  - Genetically modified plants and animals

Fermentation Systems

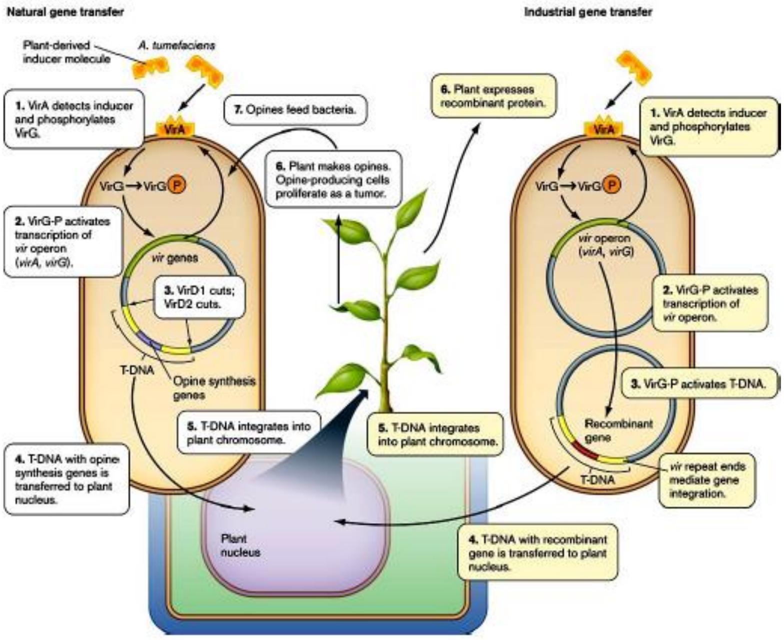


- Industrial fermenters provide environment for maximum microbial production.
  - pH, temperature, and oxygenation are controlled.
- Upstream processing
  - Culturing of the industrial microbe to produce large quantities of the product
  - Downstream processingHarvesting of the culture and purification of the product



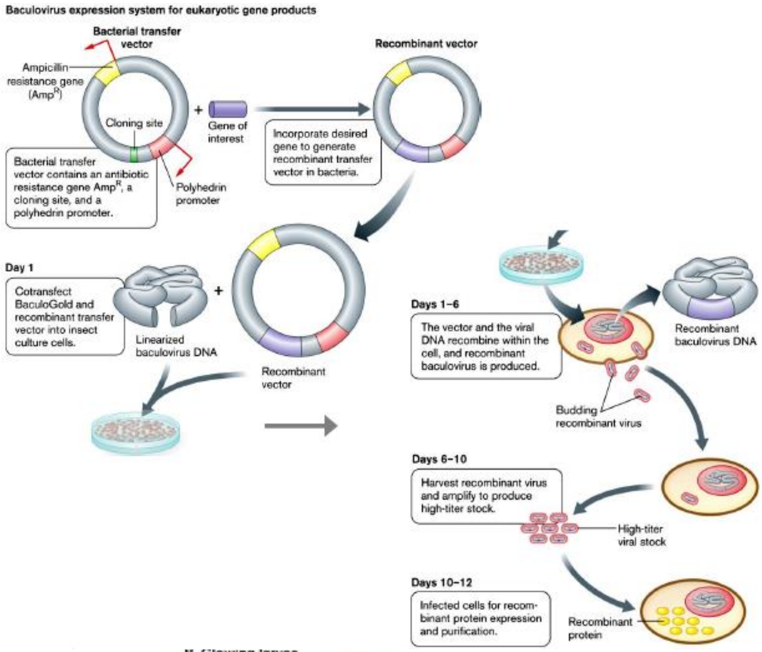
Microbial production in plants

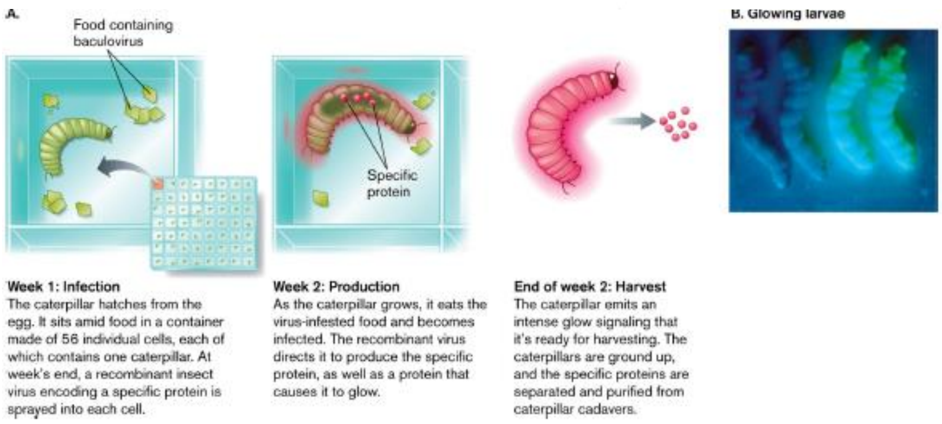
- Agrobacterium tumefaciens
- Can be used to clone genes in plant genomes
- A recombinant strain has the Ti plasmid divided into two separate plasmids:
  - One with the vir operon that transfers DNA
  - Other with the T-DNA with most of its genes substituted by the desired recombinant gene
- Thus allowing genomic integration without tumor induction or opine production



Microbial Production in Animals

- Baculovirus
- Can be used to clone genes in insect genomes
- Desired gene (e.g., antibody) is spliced into a bacterial transfer vector.
- Insect culture cells are co-transfected with recombinant vector and a fluorescent marker protein.
- The two DNAs recombine within the cell, and a recombinant baculovirus is produced.
- Can be used to infect caterpillars (in their food)





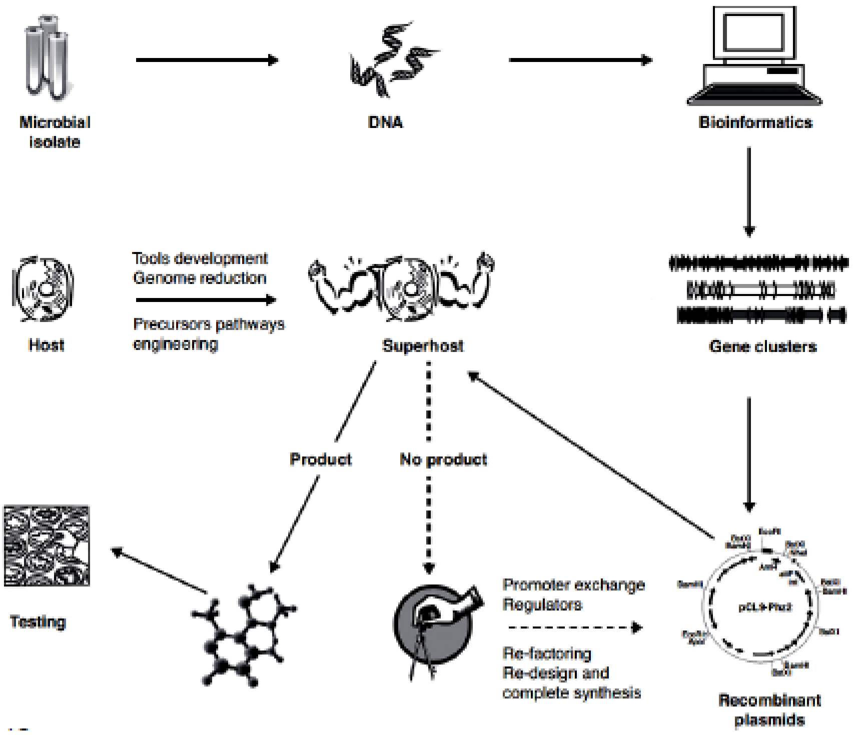
Bio prospecting

- Bio-prospecting is the search for organisms with potential commercial applications.
- An important aspect is "mining the genome."
- The cloned genes are transferred into an industrial strain, which must have the following attributes:
    - Genetic stability and manipulation
    - Inexpensive growth requirements
    - Safety
    - High level of protein expression
    - Ready harvesting of product

Bioprospecting to date

- Observation of organisms in extreme environments has yielded enzymes used in processes as diverse as washing detergents and PCR:
  - Washing detergent – cold active lipases
  - PCR – Thermally stable DNA polymerase
- There has been a strong drive to identify naturally occurring biomolecules that can be used in medicine:
  - Penicillin; other antibiotics from actinomycetes
  - Plant-derived anticancer drugs
- Production of medicines had moved towards high content screening of synthetic chemical
- GlaxoSmithKline spent six years trying to use synthetic chemistry to generate new antibiotics. This failed due to lack of chemical diversity in the library of synthetic chemicals
- Exploring the diversity of genes present in bio-systems and ecological niches still provides the greatest sources of new molecules
  - Abyssomicin C is a new antibiotic from marine bacteria. It is the first natural product to act as a pABA biosynthesis inhibitor

Genome based bioprospecting



Readings

•Chapter 13 Energetics and Catabolism Section 13.1 – p 476-7 – Swiss Cheese Production All Sections – p 483-486 - Fermentation

•Chapter 16 Food and Industrial Microbiology Pages 584-600 – Food and Microbes Pages 609-615 – Industrial Microbiology Pages 605-609 – Food Preservation (extra information)