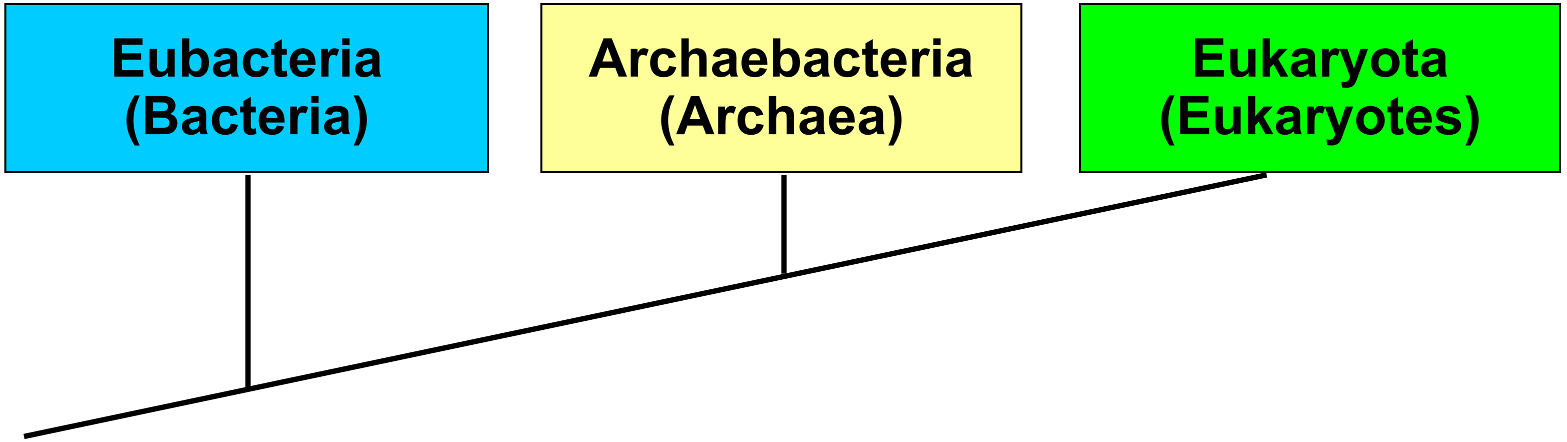
Microbial Biotechnology

What are microbes?

- Microbes are small single-celled organisms
- Either free-living or in colonies
- They can belong to any of the three domains

Three Domains



Eubacteria

- Gram-negative and gram-positive prokaryotes
- Either autotrophs or heterotrophs
- Can be aerobic or anaerobic
- Mesophiles
- Examples:

  E. coli

  Lactobacillus

  Agrobacterium

  Staphylococcus

Archea

- Ancient domain, but only recently identified
- Through DNA analysis they were determined to differ significantly from eubacteria
- Found predominantly in extreme environments (Extremophiles)

  Thermophiles 50- 110°C

  Psychrophiles 0- 20°C

  Alkaliphiles pH>9

  Halophiles 3- 20% salt

  Methanogens use H_2 + CO_2 to produce CH_4

Eukaryotes

Predominately yeasts/molds, protists, algae

Sac shaped cells that form sexual spores

Examples:

Sacchromyces

Penicillium

Aspergillus

Pichia

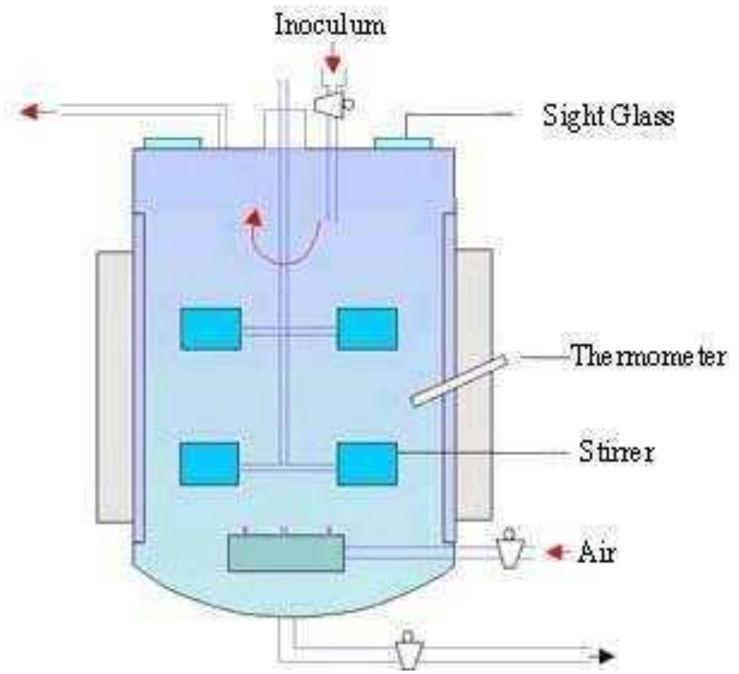
Commercial Uses of Microbes

- Products
- Bioconversion/Biocatalysis
- Agriculture
- Bioremediation
- Oil/Mineral Recovery

Fermentation is a process for the production of useful products through mass culture of single-cells

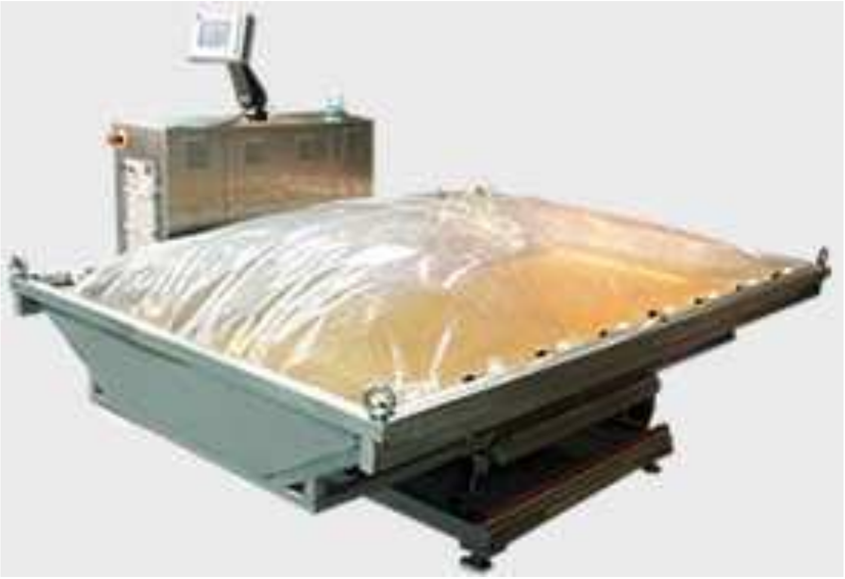
The end products or the various intermediate products (metabolites) are siphoned off & purified for commercial use

Fermenter or Bioreactor



stirred tank reactor

15 000L Fermenter



1000L Disposable Bag

http://www.wavebiotech.com/products/wave_bioreactor/system500/index.html
http://www.pharmaceutical-technology.com/projects/lonza/lonza1.html

Types of Products Produced in Microbes

- Amino Acids
- Vitamins
- Food Additives
- Enzymes
- Recombinant Protein Drugs
- Antibiotics
- Fuels
- Plastics

Examples of bacterially-expressed proteins:

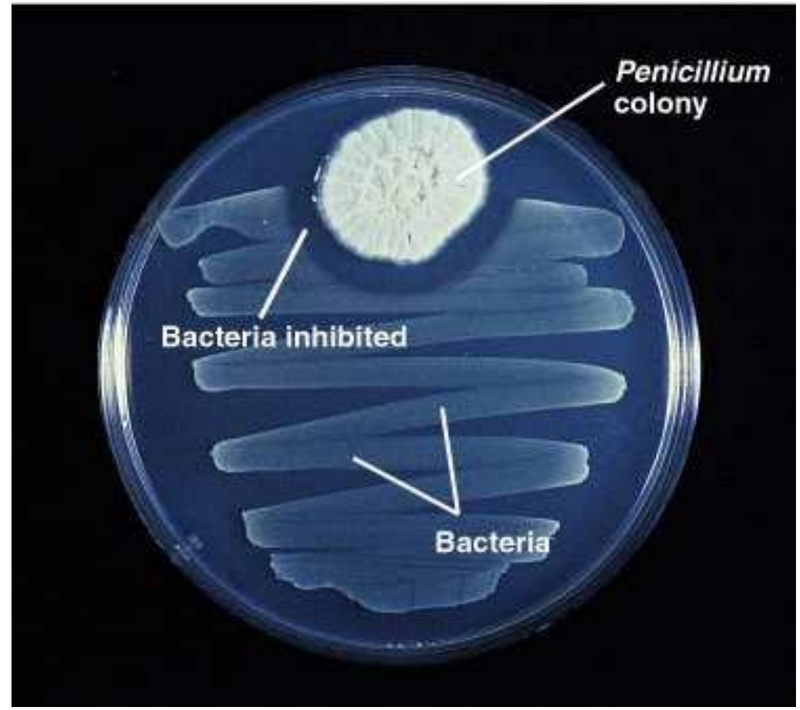
Enzyme: chymosin - the enzyme used to curdle milk products

Hormone: bST - bovine somatotropin; used to increase milk production

1928: Alexander Fleming discovered the first antibiotic.

He observed that *Penicillium* fungus made an antibiotic, penicillin, that killed *S. aureus*.

1940s: Penicillin was tested clinically and mass produced.



Original *Penicillium* moulds produced less than 10 units of penicillin per ml of fermentation broth (1943)

By 1955 *Penicillium* strains produced 8000 units/ml

Mutation with UV, mustard gas, and X-Ray, strain selection / culture improvement

Is this GMO?

How Are Microbes Modified?

- Artifical Selection
- Recombiant DNA
- Metabolic Engineering

Recombinant DNA Microbes

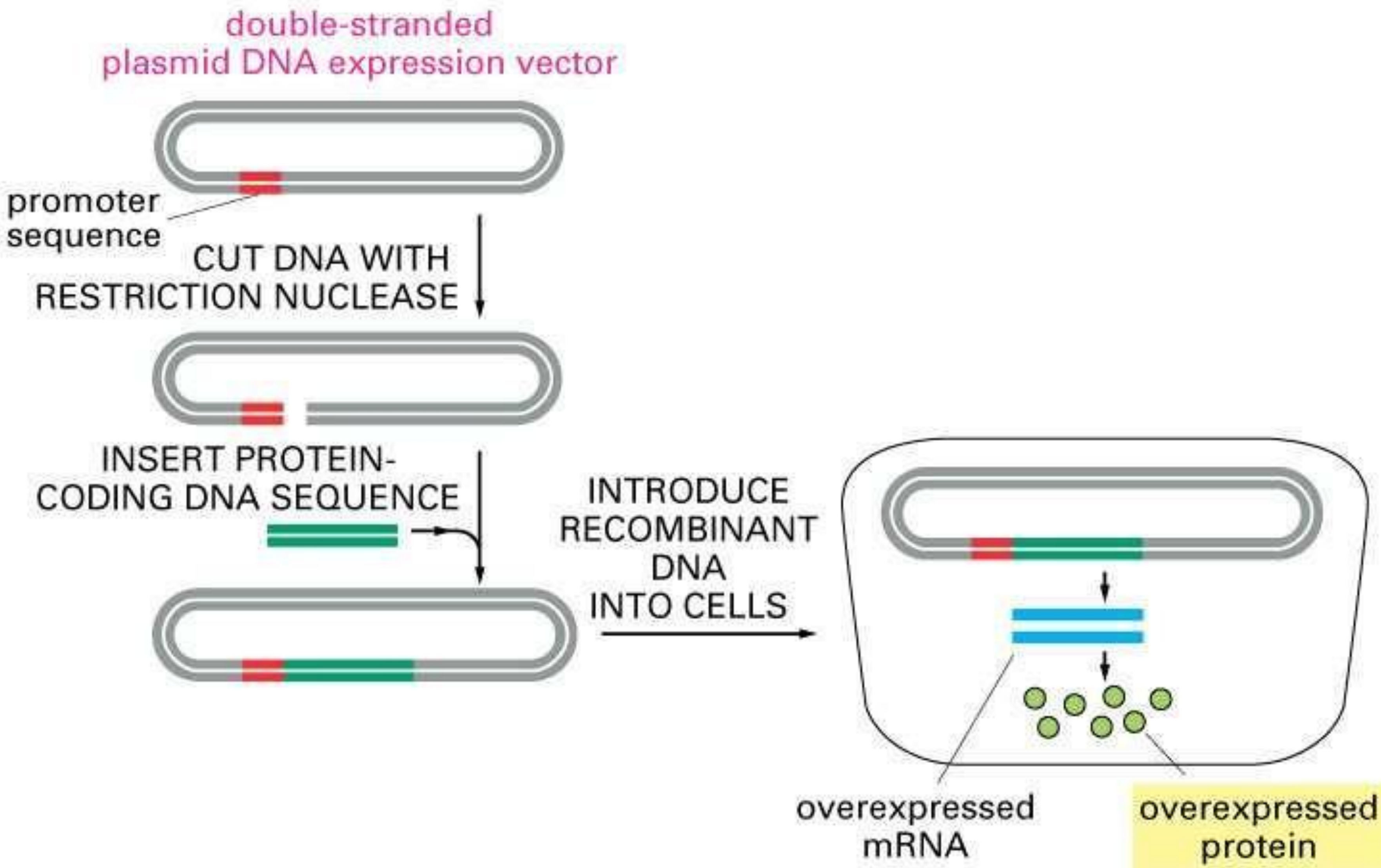
Transgenic microbes are created when cDNAs for the protein product are cloned into expression vectors

Human genes inserted into *E. coli*

Genes from extremophiles are moved to mesophiles

Due to the ease in culturing of mesophiles

Mesophiles also have 5 to 10x higher growth rates



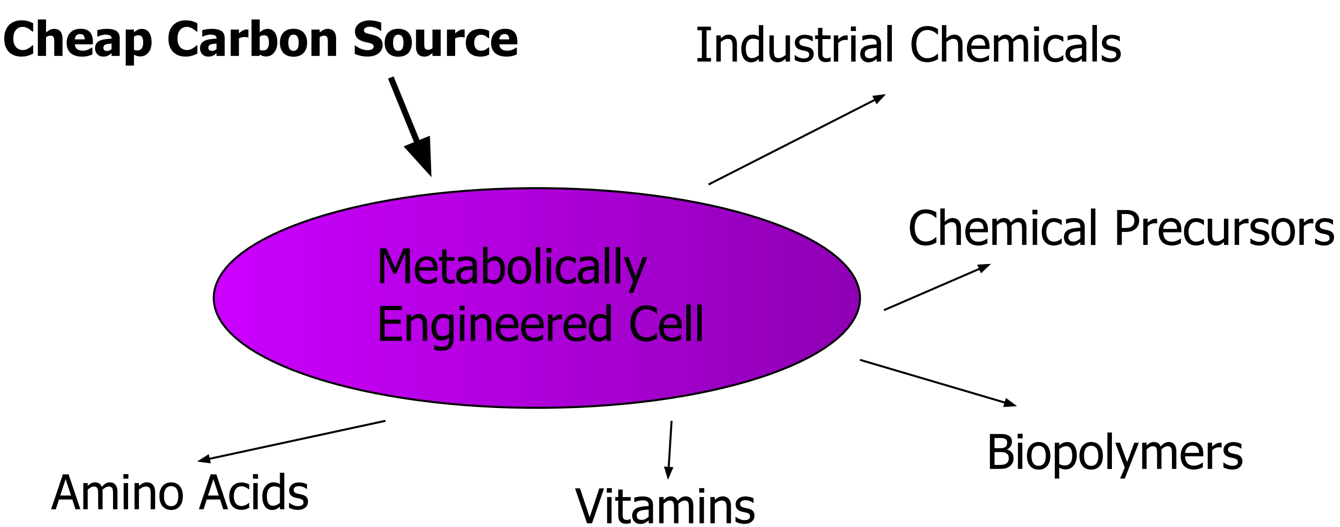
Enzyme/Drug

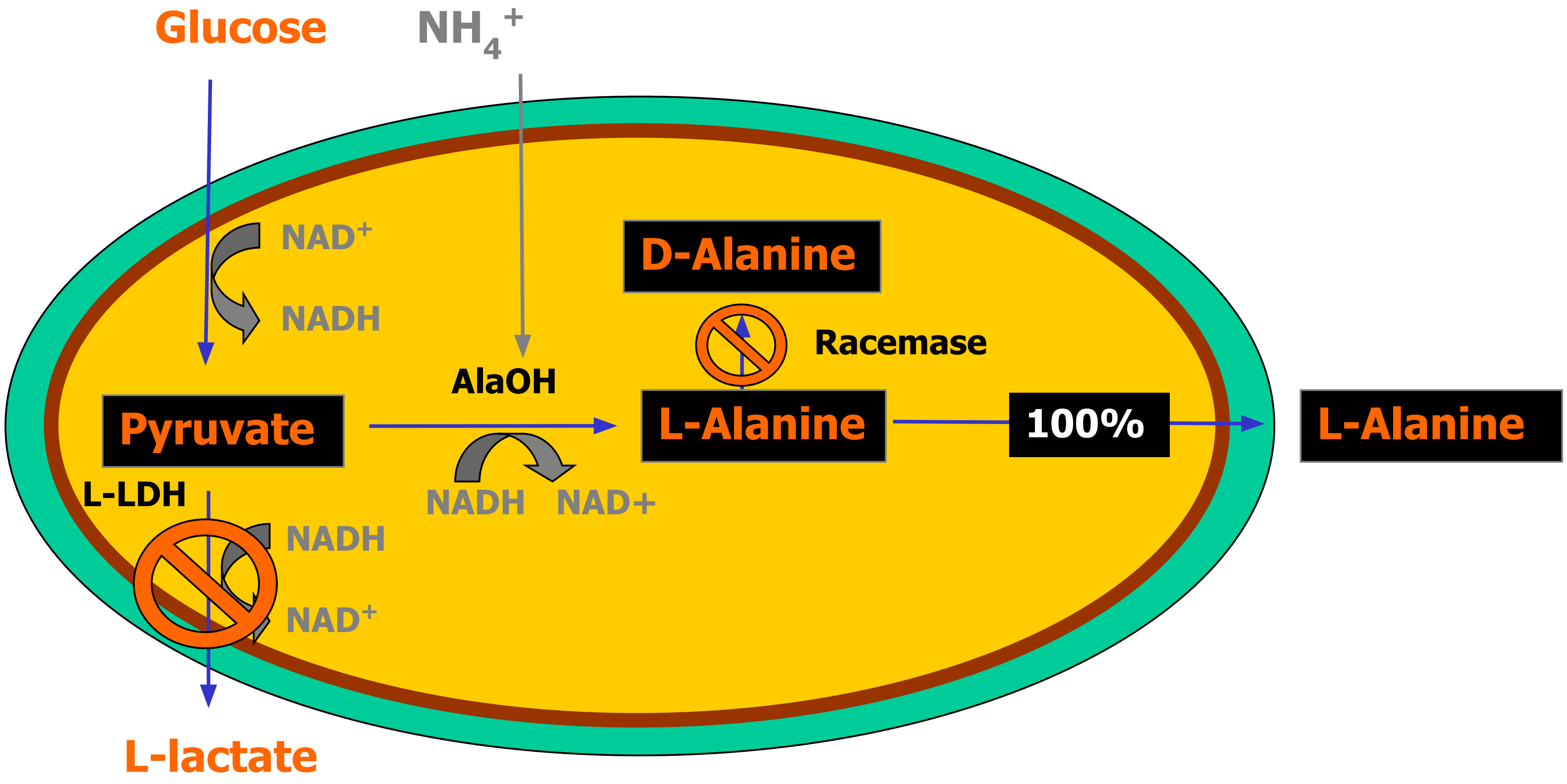
Figure 10-32 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Metabolic Engineering, manipulation of pathways within an organism to optimize the production of a compound

Done by turning off particular genes, either through mutation or deletion

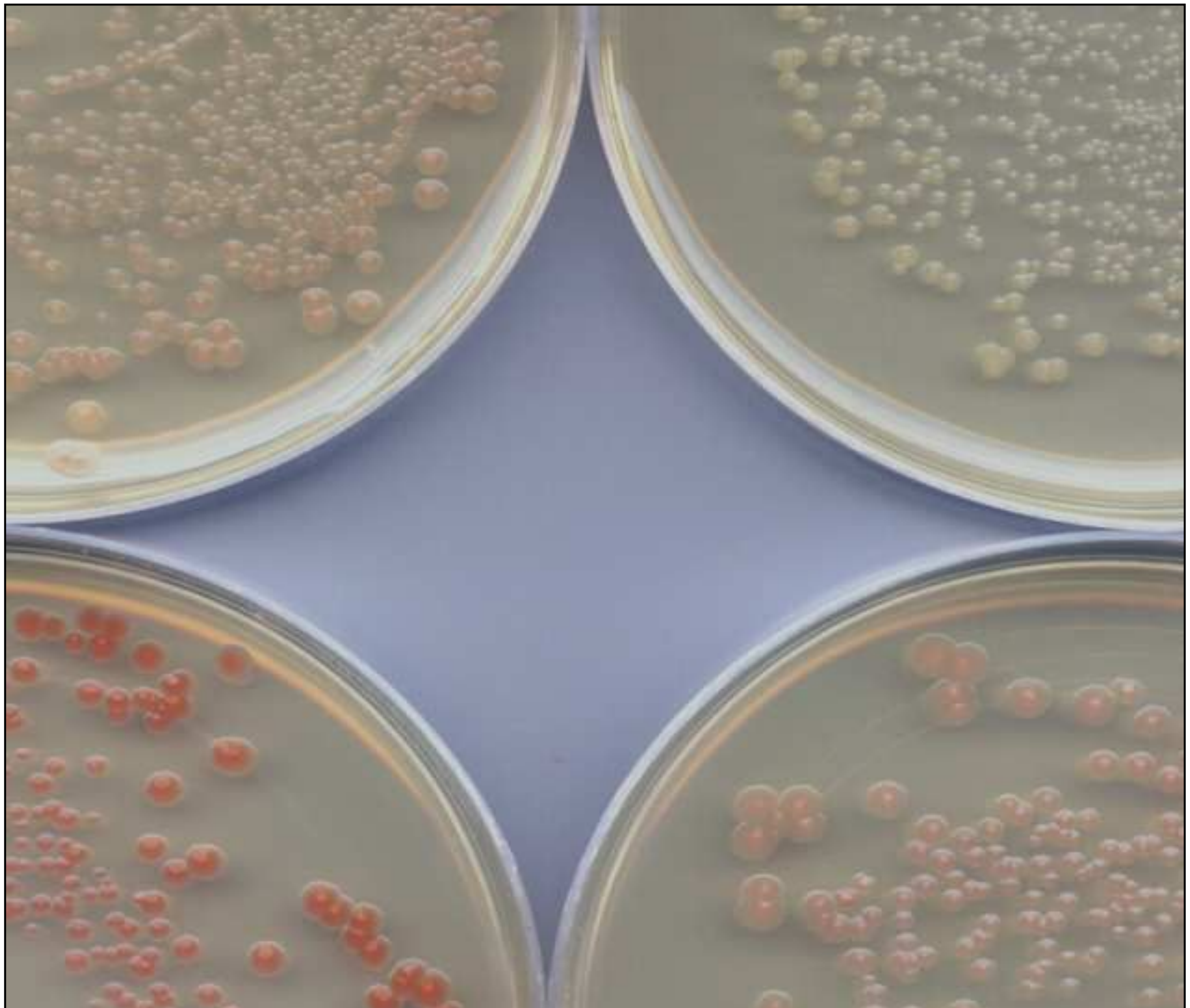
Products are also gained by altering the microbe's environment





The microbe is forced to produce alanine at higher than normal amounts

Carotenoid production in E.coli cells



Fermentation Products

Enzymes

Enzymes

Enzymes, the most common product produced by microbes

Overall value of industrial enzymes is about $2.0 billion[1]

They are found in many household items that you would never think to have a biotechnology component

[1] 2004 data

Enzyme Name	*GE Organism*	*Use (examples)*
α-acetolactate decarboxylase	bacteria	Removes bitter substances from beer
α -amylase	bacteria	Converts starch to simple sugar
Catalase	fungi	Reduces food deterioration
Chymosin	bacteria or fungi	Clots casein to make cheese
β-glucanase	bacteria	Improves beer filtration
Glucose isomerase	bacteria	Converts glucose to fructose
Glucose oxidase	fungi	Reduces food deterioration
Lipase	fungi	Oil and fat modification
Maltogenic amylase	bacteria	Slows staling of breads
Pectinesterase	fungi	Improves fruit juice clarity
Protease	bacteria	Improves bread dough structure
xylanase (hemicellulase)	bacteria or fungi	Enhances rising of bread dough

http://www.geo-pie.cornell.edu/crops/enzymes.html

Detergent Enzymes

Detergents are the largest application of industrial enzymes

Traditionally these are lipolases, proteases & amylases

A recent innovation is the addition of mannanase

This enzyme aids in removing stains containing guar gum

These enzymes are engineered to improve stability in the presence of detergent, alkaline pH, and cold water

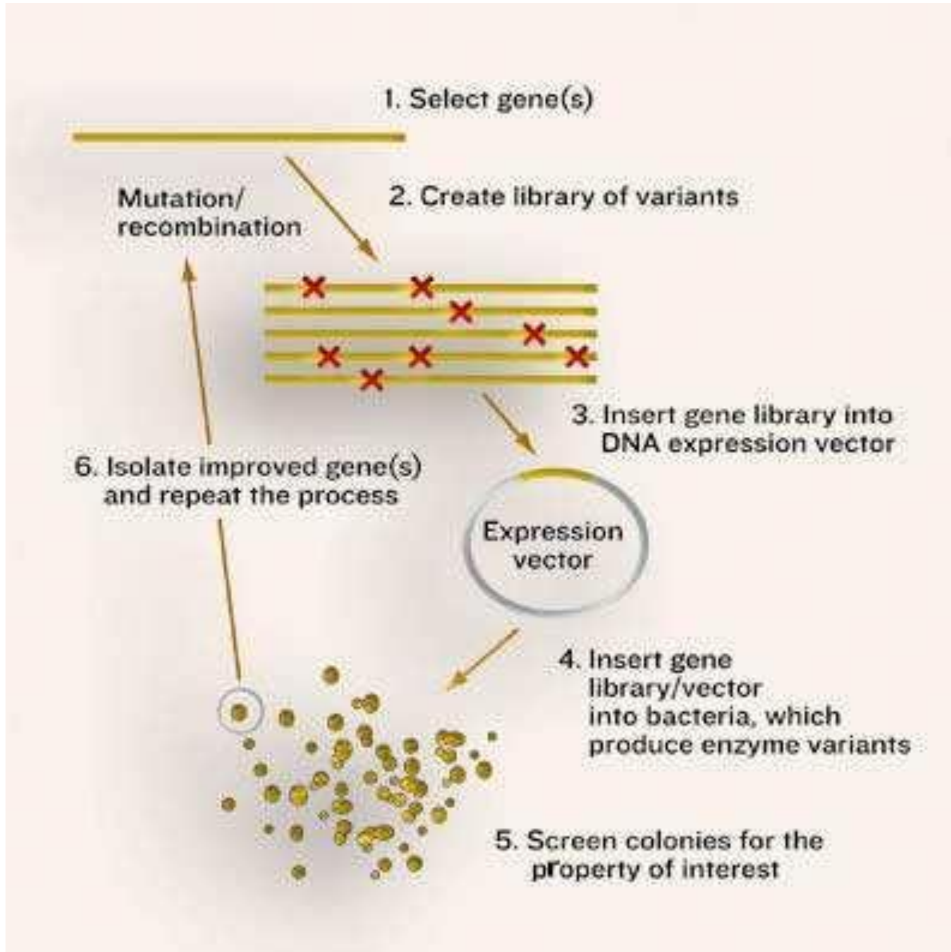
Subtilisin, a protease used in laundry detergents

The recombinant protein was engineered to remain active in the presence of bleach

Bleach caused the oxidation of one amino acid (methionine) and the enzyme lost 90% of its activity

By replacing this amino acid with alanine, the engineered enzyme was no longer sensitive to oxidation

Directed evolution is the most recent tool utilized in the creation of new and better enzymes (& other proteins)



http://www.rsc.org/chemistryworld/Issues/2004/July/rational.asp

Subtilisin normally functions in aqueous solution

Mutations were introduced randomly throughout the structure of the enzyme

Only 0.1–1% of the mutations were beneficial, but…

Activity in 60% dimethylformamide was improved 256-fold

doi:10.1016/S1367-5931(02)00396-4

Enzymes for Feed

Enzymes are used in animal feed to breakdown cellulose (cellulase)

New use of enzymes (phytases) which breakdown phytic acid

This allows better utilization of plant phosphorus stores

Allowing bone-meal to be removed from feeds

The latest generation of phytases are from fungus and have been engineered to survive high temperatures used during food processing

65% of poultry and 10% of swine feeds contain enzymes

Where do the genes for these enzymes come from?

Nature is still an important source (Gene Prospecting)

~<1% of the microbes have been grown in pure cultures

But what if you cannot find the enzyme you want?

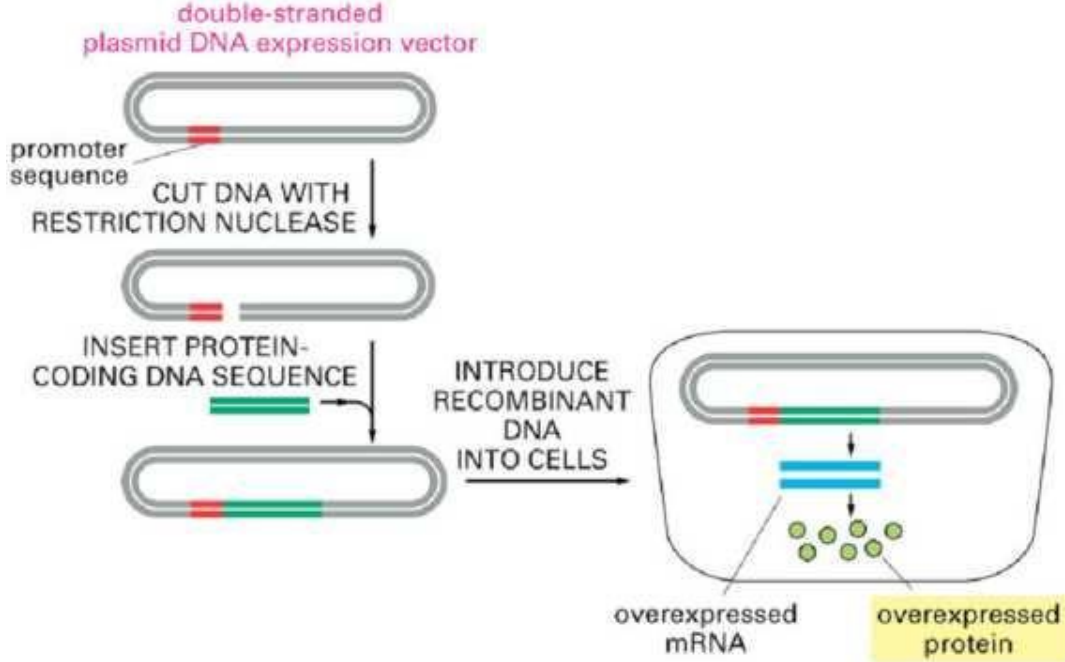
You engineer it…

In the 1980's rational protein engineering was introduced as a way of optimizing enzymes

Recombinant Drugs

Besides antibiotics which are derived from microorganisms

Protein medicines are produced by inserting human genes into microbes



1982, FDA approves the first recombinant protein drug, human insulin produced by *E. coli* developed by Genentech

Today there are >75 recombinant protein drugs approved by the FDA with 100s more being studied

Currently the global market for recombinant protein drugs is $47.4 billion[1]

(2006)

Product	Microbe	Purpose
Insulin	*E. coli*	Diabetes treatment
Interleukin-2	*E. coli*	Cancer/immune system stimulant
EGF	*E. coli*	wound healing
Interferons	*E. coli*/yeast	Cancer/virus treatments
Prourokinase	*E.coli*/yeast	Anticoagulant/heart attacks
CSF	*E. coli*/yeast	Immune stimulant
Taxol	*E. coli*	ovarian cancer

Other Products From Microbes

Fuels, Plastics, Medications

Ethanol Production

Produced via anaerobic fermentation by yeast

Corn starch is hydrolyzed to glucose monomers

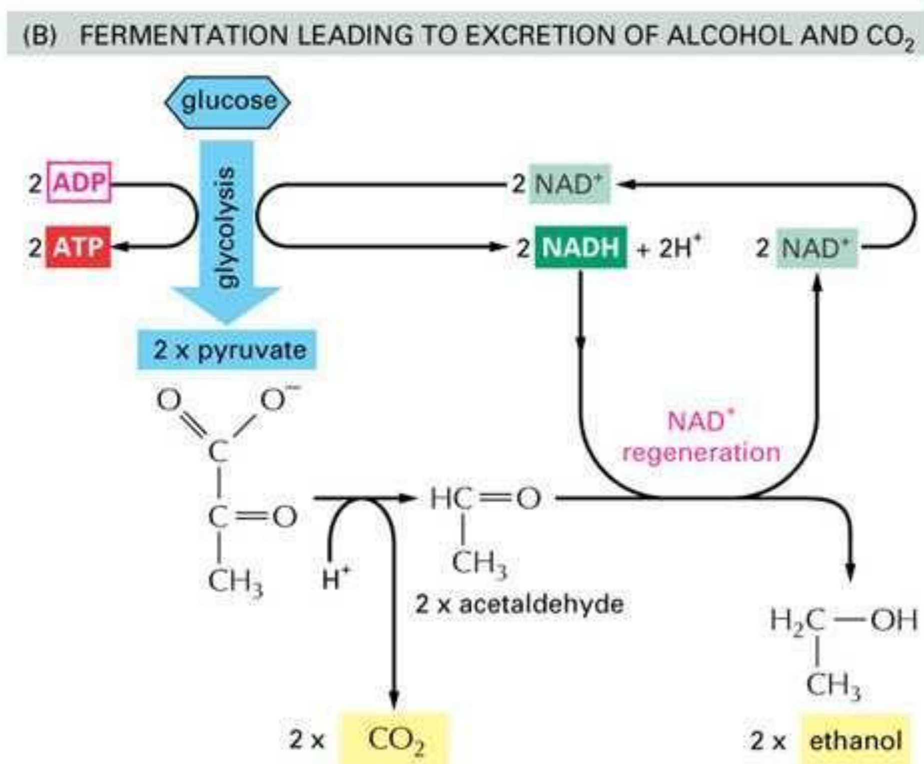


Figure 13-4 part 2 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Problem with Corn Ethanol

Ethanol contains 76000BTU/gal

Takes ~98000BTU/gal to produce from corn sugar

Gasoline contains 112000BTU/gal

Costs 22000BTU/gal to extract and refine

A BTU (British thermal unit) is defined as the amount of heat required to raise the temperature of one pound of water by one degree Fahrenheit

2004 3.4 billion gallons of ethanol were produced

U.S. consumes 140 billion gallons of gasoline/yr

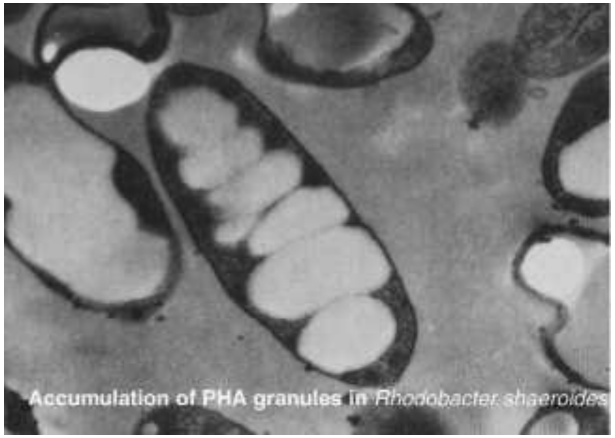
Plastics

Polyhydroxyalkanoate (PHA) is a polymer made by some microbes as a way of storing carbon

Up to 80% of the microbe's biomass is plastic

PHA is sold to make shampoo bottles in Germany, and disposable razors in Japan

The microbe *Pseudomonas putida* converts styrene to PHA



http://www2.unil.ch/ibpv/WWWPoirier/images/Fig1.jpg

http://www2.unil.ch/ibpv/WWWPoirier/images/Fig2.jpg

Bioconversion

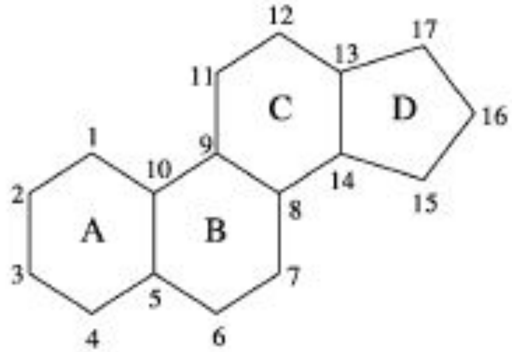
Utilization of microbes to modify a compound

Useful when multi-step chemical synthesis is expensive or inefficient

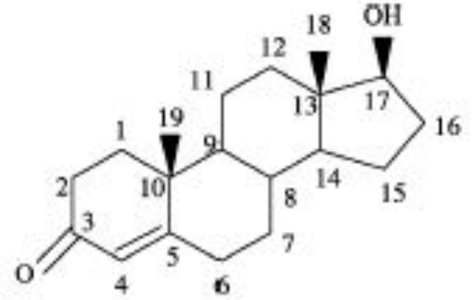
Often microbial conversion is combined with traditional chemistry to reduce the steps necessary

The most common use of bioconversion is in the synthesis of steroids such as hormones & corticosteroids

starting product

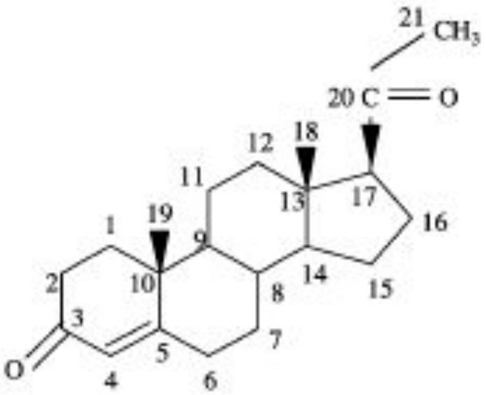


Cyclopentanoperhydrophenantrene (sterane)

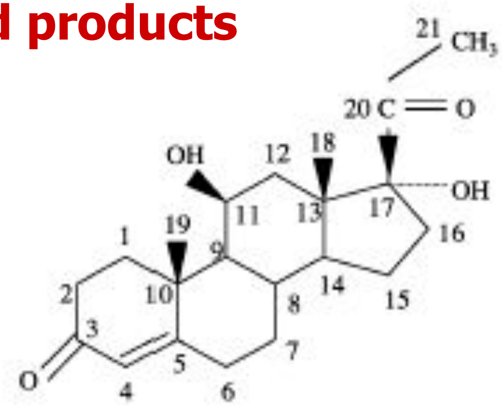


Testosterone

End products



Progesterone



Prednisolone

Microbes and Agriculture

Frost Damage

Frost damages many crops such as citrus trees & strawberries

When fruit freeze the ice crystals form

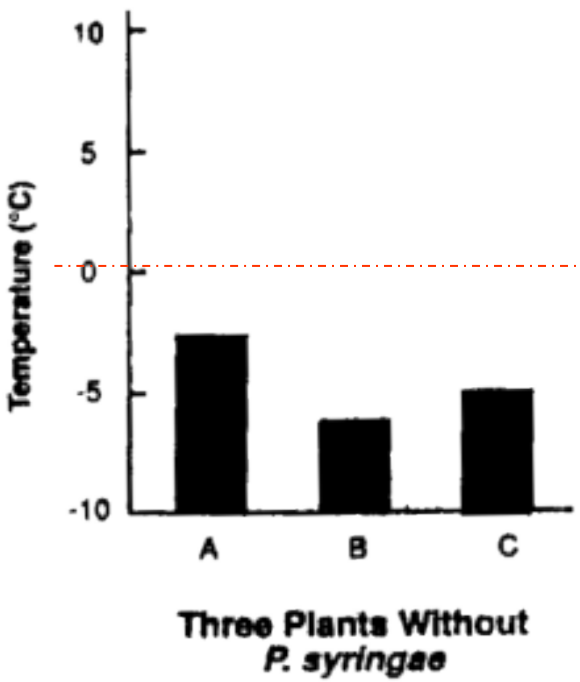
As the plants thaws they are effectively turned to mush

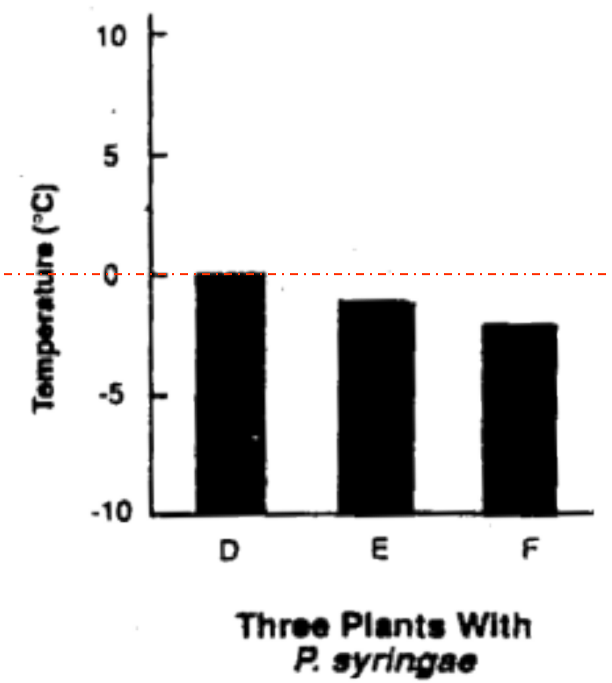
Frost damage to an orange leaf and fruit

Some ice crystal nucleation is due to bacterial activity

Pseudomonas syringae promotes the development of ice at 0 to 2°C

If the bacteria are not present ice does not form until between –6 and –8°C





A strain of *P. syringae* called “ice minus” was developed

Plants were to be sprayed with the ice minus strain

This inhibits colonization by the “ice plus” (wild) strain

The EPA declared the new strain to be a pesticide

This made the review process lengthy and burdensome

The company thought it too expensive to pursue

However the “ice plus” strain has found a purpose…

Microbial Pesticides

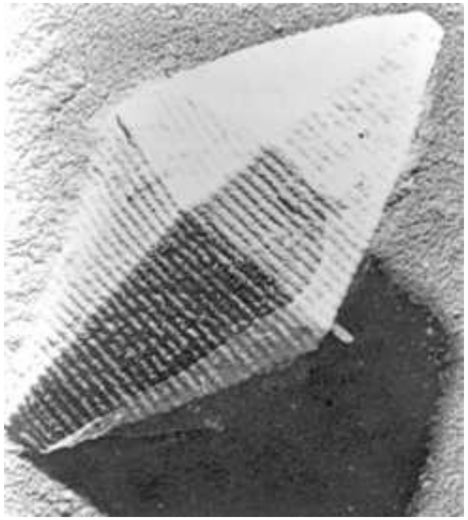
Bacillus thuringiensis (Bt) is an aerobic spore-forming bacterium

During sporulation produces insecticidal crystal protein (ICP), a toxin (Cry)

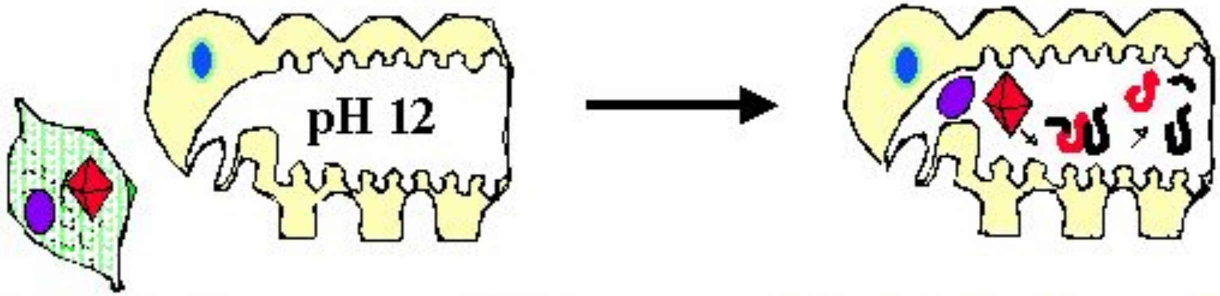
The toxin brakes down quickly in the environment

They have no toxicity to humans & there is no withholding period on produce sprayed with Bt

Cry toxins vary in their toxicity and specificity

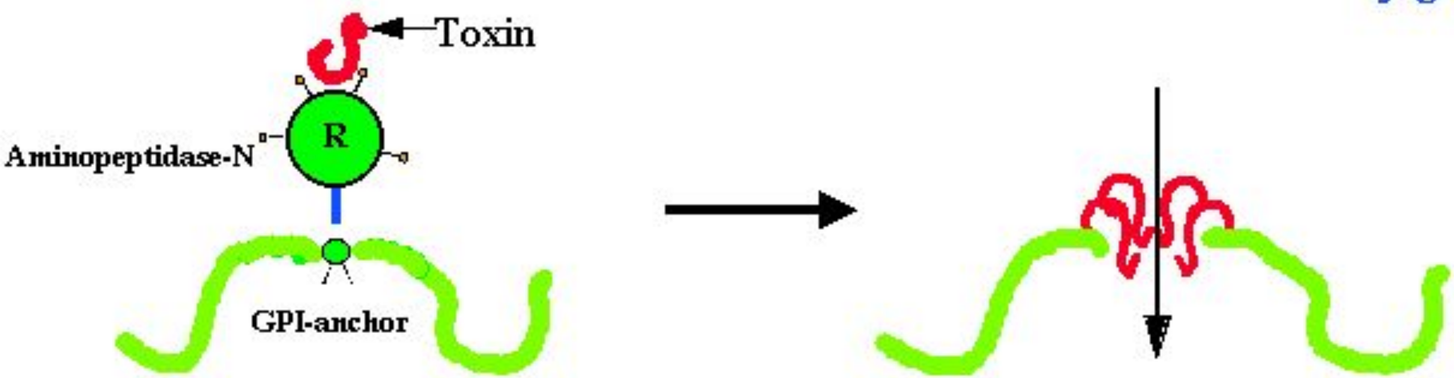


MECHANISM OF TOXIN ACTION



1 Crystal and spore eaten by insect

2 Crystal dissolves and protoxin is processe
to smaller 'active' form by gut enzymes



3 Activated toxin binds to receptor (R)
in the midgut epithelium

4 Toxin inserts into the membrane
making it permeable to ions and small
molecules so that the cell bursts

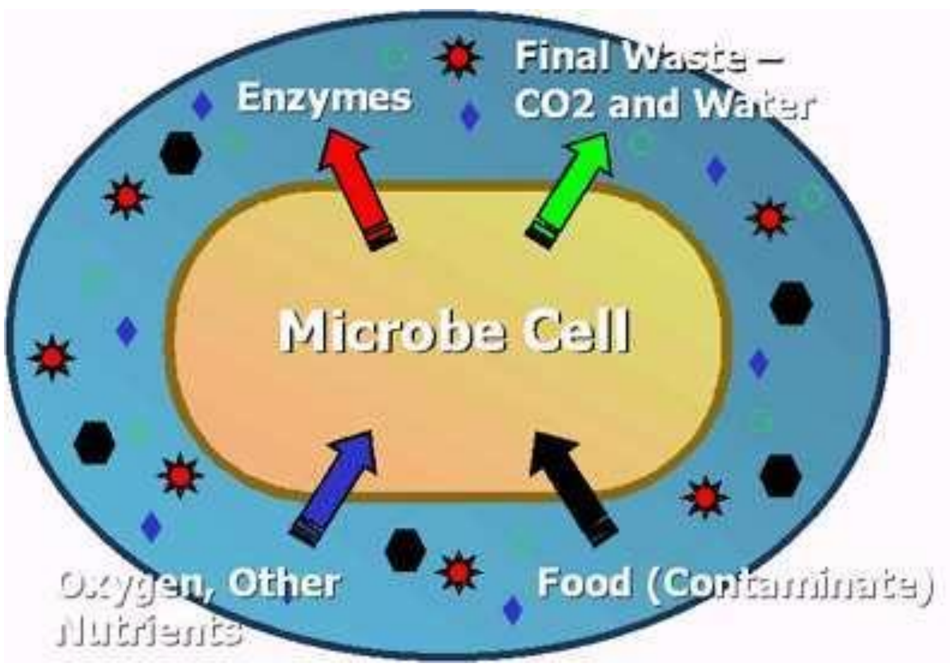
http://www.bioc.cam.ac.uk/~dje1/


Thuricide
Bacillus Thuringiensis (BT)
Concentrate
KEEP OUT OF REACH OF CHILDREN
CAUTION
Net Contents 8 FL. OZ. (.23 liter)
Thuricide
Bacillus Thuringiensis (BT)
Concentrate
KEEP OUT OF REACH OF CHILDREN
CAUTION
Net Contents 16 FL. OZ. (1 PT.) (473 ML.)
TRUSTED SINCE 1926
BONIDE
Controls moth larvae (caterpillars), certain leaf eating worms and Gypsy Moths.

Bioremediation

Bioremediation is reclaiming or cleaning of contaminated sites using microbes or other organisms

This entails the removal, degradation, or sequestering of pollutants &/or toxic wastes



http://www.cleanearthltd.com/en/contamination_cleanup/index.php

Bacteria are isolated based on their efficiency at digesting & converting the waste

The bacteria are tested for performance and safety

Bacteria are placed back in the waste environment in high concentrations

The bacteria grow & in the process digest & convert the waste into CO_2 and H_20

What can be cleaned up using bioremediation?

- Oil spills
- Waste water
- Plastics
- Chemicals (PCBs)
- Toxic Metals

Oil/Wastewater Cleanup

Bioremediation

Bacteria degrade organic matter in sewage.

Bacteria degrade or detoxify pollutants such as oil and mercury

Microbes that digest hydrocarbons found throughout the environment

These naturally occurring microbes are utilized during a spill to clean shore lines

Fertilizer is added to supply the nutrients phosphorus and nitrogen

This was approach was used after the Exxon Valdez

Stimulated the natural rate of biodegradation by 2 to 5x

There have yet to be any other instances of this being used on a large-scale

Exxon Valdez off the Coast of Alaska

http://blogs.abcnews.com/photos/uncategorized/exxon_valdez.jpg

Smaller scale cleanup is feasible

For 3 months nutrients and microbes were sprayed on this field

After 11 months the site was deemed clean

Before

After

6000yards^3 petroleum conc. Before 4000ppm After 100ppm

Wastewater

Treatment of domestic sewage or industrial waste

Utilizes aeration to oxygenate allowing aerobic microbes to digest solid waste

Before After

Plastic Degradation

140 million tons of plastics are produced each year

Traditional plastics are very stable and do not degrade

Some plastics have been shown to be biodegradable

Strains of bacteria have been isolated that breakdown:

- Polyurethane
- Polyvinyl alcohol
- Nylon-66

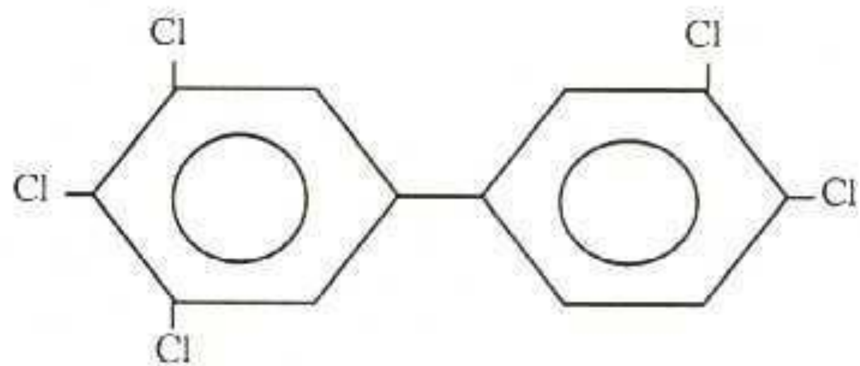
The degradation pathways are currently under study

Chemicals

Polychlorinated biphenyls (PCBs)

PCBs have low water solubility, good insulating properties, high boiling points and resistance to chemicals

The largest uses for PCBs was in capacitors, transformers, & as plasticizers



(b) 3,3',4,4',5-Pentachlorobiphenyl (IUPAC #126)
(A true coplanar PCB)

1977, Monsanto (main producer) stops all PCB production

Millions of lbs of PCBs are still in place around the world

The stability properties that made PCBs so useful have allowed them to persist in the environment

Most people in industrialized countries have PCBs in their tissue

Microbes that dehalogenate PCBs have been isolated

This process is referred to as haloremiration

Involves the replacement of the Cl with an –OH

This process is multi-step with four enzymes required

These enzymes are now the target of protein engineering to optimize their performance

Heavy Metal Clean up

Uranium processing has left contaminated groundwater sites across the United States and the world

Traditional “pump-and-treat” methods take decades and expose workers to toxic levels of uranium

Geobacter to convert soluble uranium to insoluble uraninite

Uraninite stays put instead of mixing with water used for drinking or irrigation

The microbes are encouraged to multiply by injecting acetate

In ~50 days, 70% of the uranium is converted into uraninite

Biomining

Microbe assisted mining has gone on for millennia

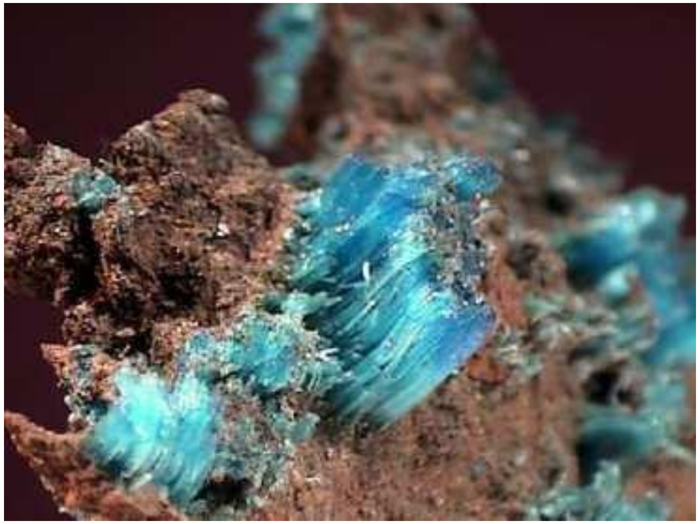
Early copper miners used microbes to leach copper from ore without even knowing it

Low-grade ore and mine tailings are exploited biologically

Sulfides of metals like zinc, copper, nickel, cobalt, iron, tungsten, lead are insoluble in water

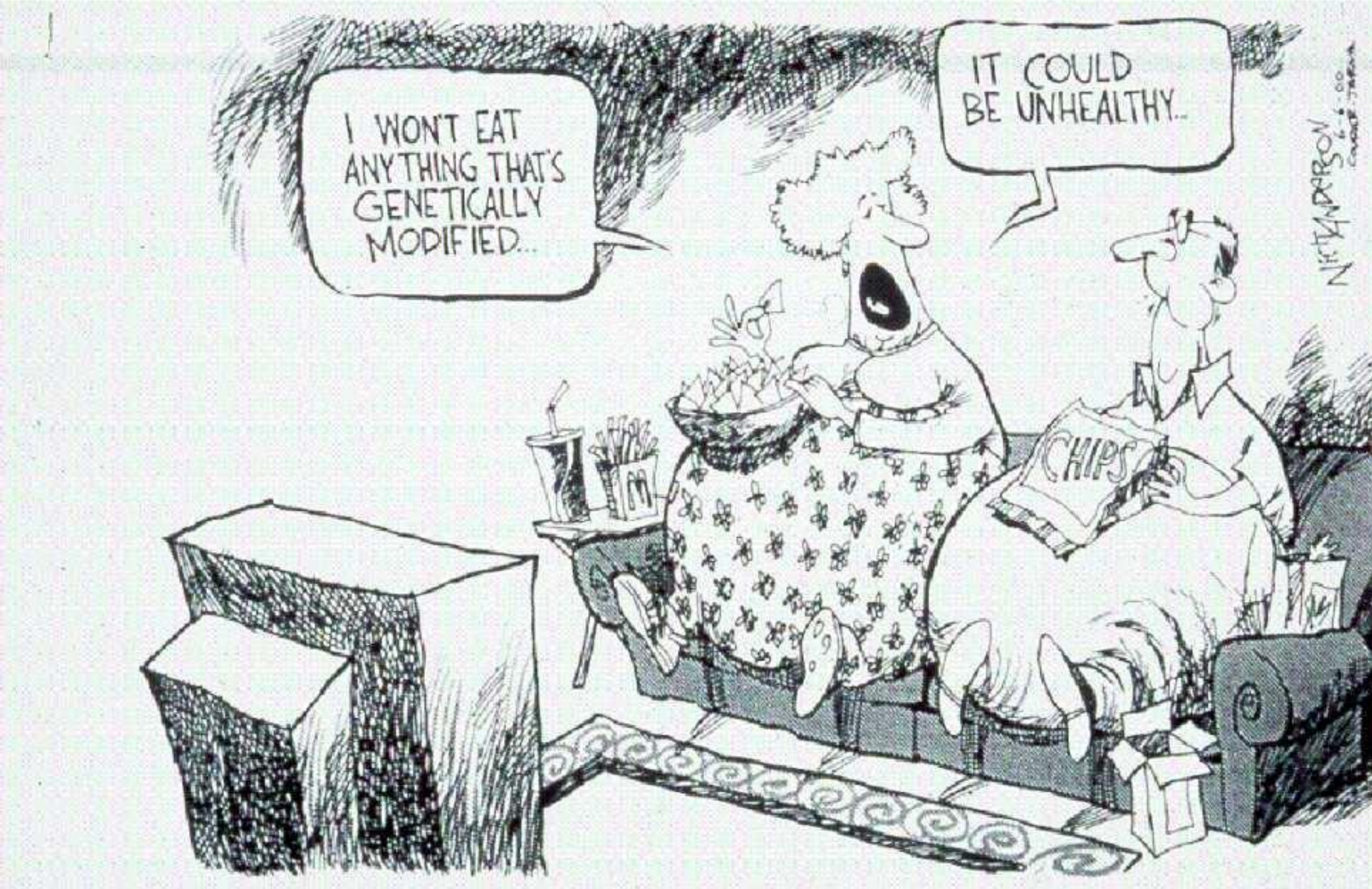
These sulfides are converted to sulfate which are soluble

The sulfates leach out of the ore and are then extracted



Cu_2S not soluble $CuSO_4$ is soluble

Commercial Bioleaching Tanks



Rex Babin is on vacation

Anderson / Courier Journa