HC70A Winter 2004
professor Bob Goldberg

Lecture #5 21st Century Applications of Genetic Engineering

Themes/Concepts

1. Variety of Genetic Engineering Applications
2. Genetic Engineering of bacteria
3. Release of Genetically Engineered bacteria to Environment – A Case Study

Stop 2/5/04 (~1hr)

4. Genetic Engineering Fungi/Yeast
5. Genetic Engineering "Pharm" animals
6. merger of animal Genetic Engineering + cloning
7. Engineering other animals / Regulatory Issues
8. Genetic Engineering Plants

Stop 2/7/04 (~2 hrs)

9. Why GMO Controversy?

Regulating GMOs

"Animal Biotechnology - Science-Based Concerns"
National Research Council
National Academies Press, 2002

"Environmental Effects of Transgenic Plants:
Scope & Adequacy of Regulation"
National Research Council
National Academies Press, 2002

Genetic Engineering & Recombinant DNA ARE USED IN A VARIETY OF Applications

Similar CLASSES of Applications CAN BE Engineered in Several organisms

(1) TRANSGENIC Animals

(4) DNA, RNA, Oligonucleotides

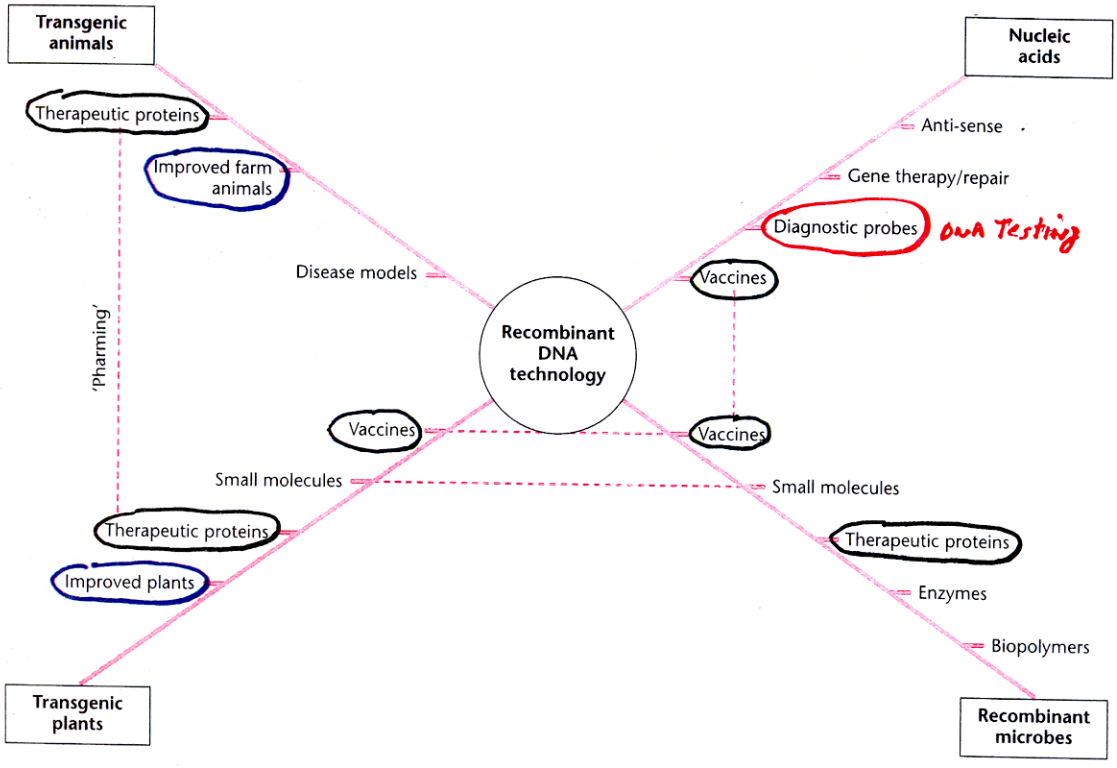


Fig. 14.1 The different ways that recombinant DNA technology has been exploited.

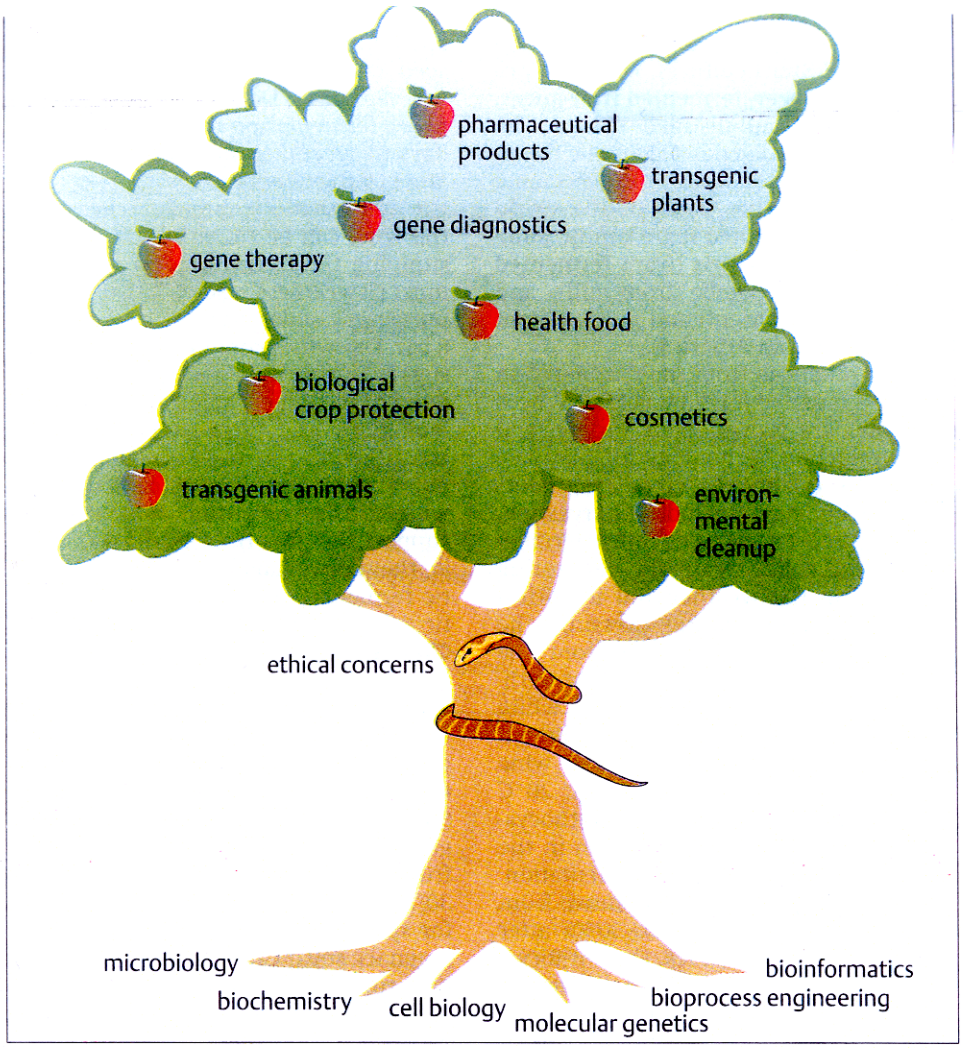
TRANSGENIC PLANTS

(2)

TRANSGENIC Microbes – Bacteria & Fungi

(3)

GENETIC ENGINEERING HAS "COME OF AGE"



Market data of some bioproducts (~ 2000, estimates)

	~ volume	~ value	price/kg
beer	130000000 t	330 billion €	2.50 €/kg
ethanol	19000000 t	5 billion €	0.25 €/kg
glutamic acid	800000 t	800 million €	1.00 €/kg
citric acid	700000 t	700 million €	1.00 €/kg
detergent protease	100000 t	300 million €	3.00 €/kg
aspartame	10000 t	50 million €	5.00 €/kg
cephalosporins	5000 t	2,5 billion €	500.00 €/kg
tetracyclines	5000 t	250 million €	50.00 €/kg
insulin	8 t	1 billion €	125.00 €/kg
erythropoietin	10 kg	4 billion €	500 million€/kg

AND IS NOW A HUGE WORLD-WIDE BUSINESS

VALUE OF DNA TECHNOLOGY IN USA

TABLE 6.2
Ten-Year Sales Forecast of the Value of DNA Technology Products in the United States.

SECTOR	BASE YEAR 1996	FORECAST YEARS 2001	2006	AVERAGE ANNUAL GROWTH RATE (%) 1996-2006
Human therapeutics	7,555[a]	13,935	25,545	13
Human diagnostics	1,760	2,705	4,050	9
Agriculture	285	740	1,740	20
Nonmedical diagnostics	225	330	465	8
Totals	10,100	18,400	32,400	12

[a] Millions of 1996 dollars. Source: Consulting Resources Corp.

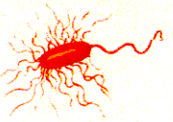
~$30 BILLION!

1976 $0
2004 $30 BILLION (NOT including VALUATION of Biotech Companies) $190 BILLION

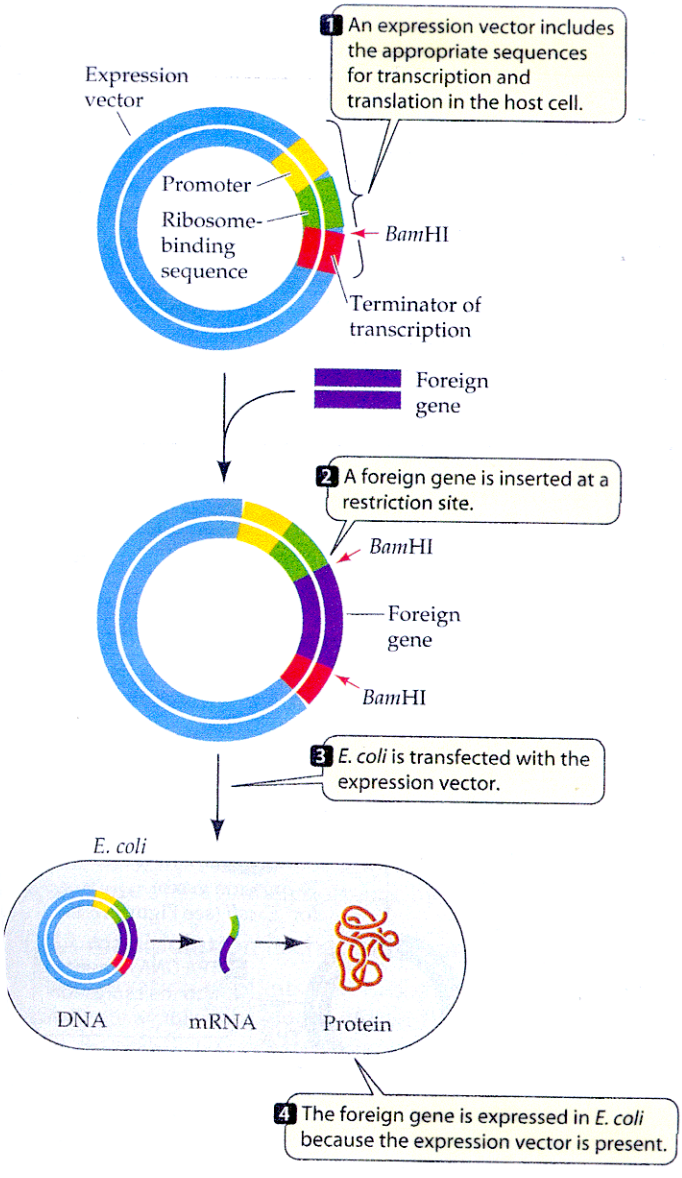
AN INDUSTRY is BORN!

GENETIC ENGINEERING BACTERIAL CELLS

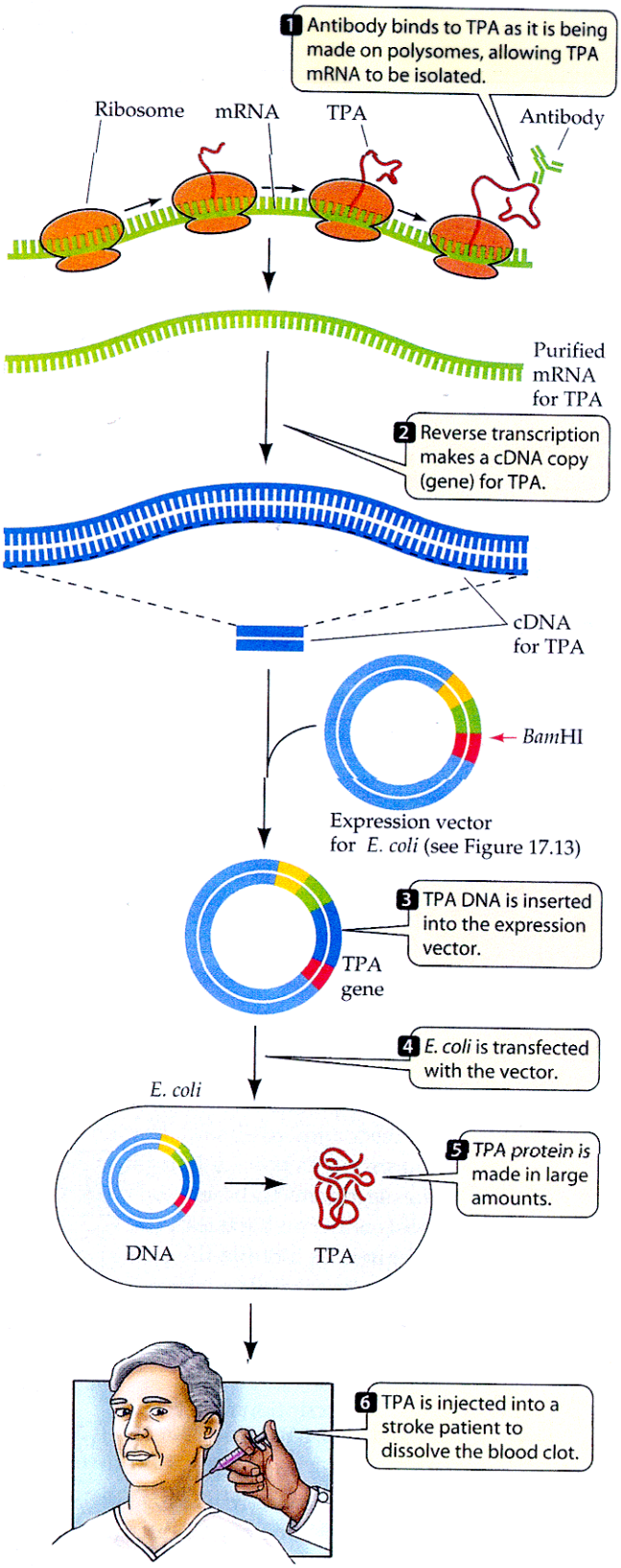
Table 34.1 Bacteria

Major Group	Typical Examples		Key Characteristics
			ARCHAEBACTERIA
Archaebacteria	Methanogens, thermophiles, halophiles		Bacteria that are not members of the kingdom Eubacteria. Mostly anaerobic with unusual cell walls. Some produce methane. Others reduce sulfur.
			EUBACTERIA
Actinomycetes	*Streptomyces*, *Actinomyces*		Gram-positive bacteria. Form branching filaments and produce spores; often mistaken for fungi. Produce many commonly used antibiotics, including streptomycin and tetracycline. One of the most common types of soil bacteria; also common in dental plaque.
Chemoautotrophs	Sulfur bacteria, *Nitrobacter*, *Nitrosomonas*		Bacteria able to obtain their energy from inorganic chemicals. Most extract chemical energy from reduced gases such as H_2S (hydrogen sulfide), NH_3 (ammonia), and CH_4 (methane). Play a key role in the nitrogen cycle.
Cyanobacteria	*Anabaena*, *Nostoc*		A form of photosynthetic bacteria common in both marine and freshwater environments. Deeply pigmented; often responsible for "blooms" in polluted waters.
Enterobacteria	*Escherichia coli*, *Salmonella*, *Vibrio*		Gram-negative, rod-shaped bacteria. Do not form spores; usually aerobic heterotrophs; cause many important diseases, including bubonic plague and cholera.
Gliding and budding bacteria	Myxobacteria, *Chondromyces*		Gram-negative bacteria. Exhibit gliding motility by secreting slimy polysaccharides over which masses of cells glide; some groups form upright multicellular structures carrying spores called fruiting bodies.
Pseudomonads	*Pseudomonas*		Gram-negative heterotrophic rods with polar flagella. Very common form of soil bacteria; also contain many important plant pathogens.
Rickettsias and Chlamydias	*Rickettsia*, *Chlamydia*		Small, gram-negative intracellular parasites. *Rickettsia* life cycle involves both mammals and arthropods such as fleas and ticks; *Rickettsia* are responsible for many fatal human diseases, including typhus (*Rickettsia prowazekii*) and Rocky Mountain spotted fever. Chlamydial infections are one of the most common sexually transmitted diseases.
Spirochaetes	*Treponema*		Long, coil-shaped cells. Common in aquatic environments; a parasitic form is responsible for the disease syphilis.

Expression Vectors are used to make recombinant proteins in bacterial cells



What switches? Terminators? Codon useage (for synthetic genes)?



synthesizing t PA in BACterial cells

17.14 Tissue Plasminogen Activator: From Protein to Gene to Pharmaceutical

TPA is a naturally occurring human protein that prevents blood from clotting. Its isolation and use as a pharmaceutical agent for treating patients suffering from blood clotting in the brain or heart—in other words, strokes and heart attacks—was made possible by recombinant DNA technology.

BACTERIAL FACTORIES FOR DRUGS

INSULIN

GROWTH HORMONE

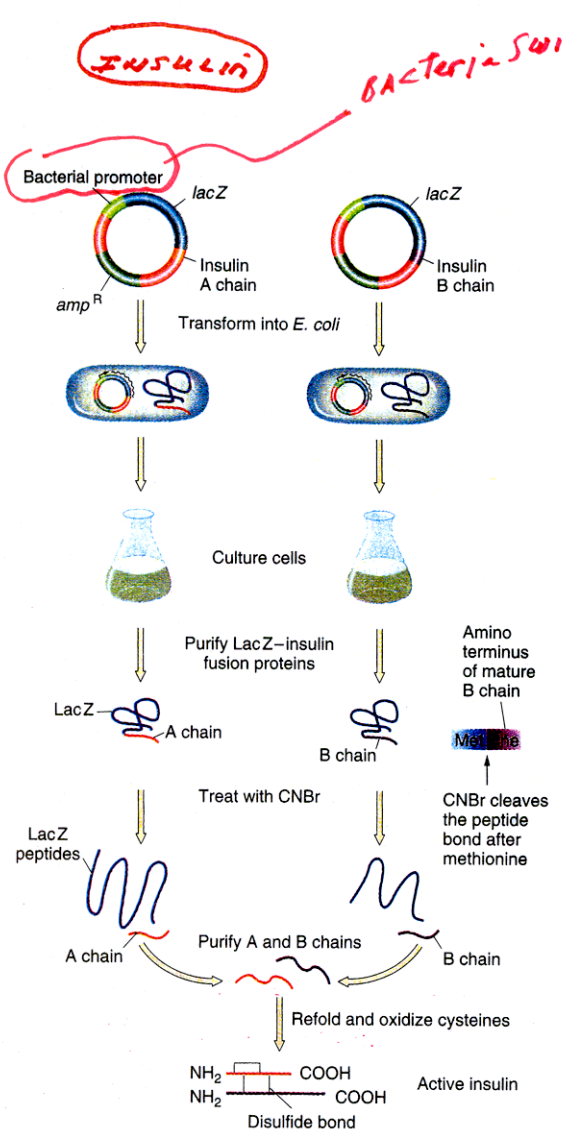


Figure 13-6 Expression of human insulin in *E. coli*. The two chains of insulin are made separately as fusion proteins with β-galactosidase. They are processed chemically and then mixed, and active insulin forms. (Copyright © 1992 by J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright © Scientific American Books.)

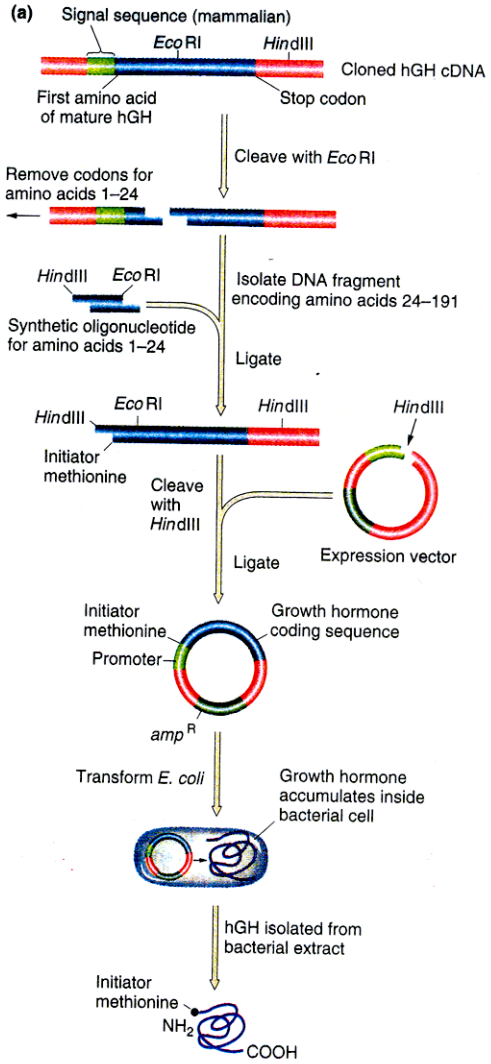


Figure 13-7 Expression of human growth hormone (hGH) in *E. coli*. (a) The human signal sequence is removed, enabling the protein to be produced in bacterial cells. The product contains an extra bacterial methionine. (b) A bacterial signal sequence that targets the protein for secretion to the outside can be added. In this method, the product has no extra methionine. (Copyright © 1992 by J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright © Scientific American Books.)

(7) MANY other Protein Classes — Enzymes for food Processing, etc.

RECOMBINANT PROTEINS MADE IN BACTERIA TO TREAT HUMAN DISEASES

Table 10.1 Some human proteins that have been produced by recombinant DNA technology for treating various disorders

Protein	Disorder(s)
α_1-Antitrypsin	Emphysema
Adrenocorticotropic hormone	Rheumatic diseases
B-cell growth factors	Immune disorders
Bactericidal/permeability-increasing protein	Infections
Brain-derived neurotrophic factor	Amyotrophic lateral sclerosis (Lou Gehrig's disease)
Calcitonin	Osteomalacia
Colony-stimulating factors	Cancer
Chorionic gonadatropin	Female infertility
Endorphins and enkephalins	Pain
Epidermal growth factor	Burns
Erythropoietin	Anemia, kidney disorders
Factor VIII	Hemophilia
Factor IX	Hemophilia
Growth hormone	Growth defects
Growth hormone-releasing factor	Growth defects
Hemoglobin	Anemia
Insulin	Diabetes
Insulin-like growth factor	Diabetes, renal failure
Interferons (α, β, γ)	Viral diseases, cancer, multiple sclerosis
Interleukins	Cancer, immune disorders
Interleukin-1 receptor	Asthma, rheumatoid arthritis
Lymphotoxin	Cancer
Macrophage-activating factor	Cancer
Nerve growth factor	Nerve damage
Platelet-derived growth factor	Atherosclerosis
Relaxin	Birthing
Serum albumin	Insufficient plasma proteins
Somatomedin C	Growth defects
Thyroid-stimulating hormone	Thyroid cancer
Tissue plasminogen activator	Blood clots
Tumor necrosis factor	Cancer
Urogastrone	Ulcers
Urokinase	Blood clots

MANY Recombinant Proteins Have Been Approved As Drugs

2002 List

Table 10.1 Some recombinant proteins that have been approved for human use in either the United States or the European Union

Compound	Company	Disorder
Factor VIII	Baxter Healthcare, Genetics Institute, Centeon, Bayer	Hemophilia A
Factor VIIa	Novo Nordisk	Some forms of hemophilia
Factor IX	Genetics Institute	Hemophilia B
Hirudin	Ciba Novartis, Europharm, Hoechst Marion Roussel	Venous thrombosis, heparin-associated thrombocytopenia
Tissue plasminogen activator	Genentech	Acute myocardial infarction
Truncated tissue plasminogen activator	Galenus Mannheim, Boehringer Mannheim/Centocor	Acute myocardial infarction
Insulin	Eli Lilly, Novo Nordisk, Hoechst AG	Diabetes mellitus
Insulin analogues	Eli Lilly, Novo Nordisk, Aventis	Diabetes mellitus
Human growth hormone	Eli Lilly, Genentech, Biotechnology General, Pharmacia, Upjohn, Novo Nordisk, Serono Laboratories	Growth hormone deficiency in children
Human growth hormone analogue	Genentech	Growth hormone deficiency in children
Human growth hormone	Serono Laboratories	AIDS-associated catabolism and wasting
Glucagon	Novo Nordisk	Hypoglycemia
Thyrotrophin-α	Genzyme	Thyroid cancer
Follicle-stimulating hormone	Ares-Serono, Organon	Anovulation and superovulation
Erythropoietin	Amgen, Ortho Biotech, Boehringer-Mannheim	Anemia
Platelet-derived growth factor	Ortho-McNeil Pharmaceuticals, Janssen-Cilag	Lower-extremity diabetic neuropathic ulcers
DNase I	Genentech	Cystic fibrosis
β-Glucocerebrosidase analogue	Genzyme	Gaucher disease
IFN-α_{2a}	Hoffmann-La Roche, Schering-Plough	Hairy cell leukemia, hepatitis B and C
Synthetic type 1 IFN-α	Amgen, Yamanouchi Europe	Chronic hepatitis C
IFN-α_{2b}	Schering-Plough	Hairy cell leukemia, genital warts, hepatitis B and C
IFN-β_{1b} analogues	Schering AG, Berlex Laboratories, Chiron	Multiple sclerosis
IFN-β_{1a}	Biogen, Ares-Serono	Relapsing multiple sclerosis
IFN-γ_{1b}	Genentech	Chronic granulomatous disease
IL-2 analogue	Chiron	Renal cell carcinoma
IL-11 analogue	Genetics Institute	Prevention of chemotherapy-induced thrombocytopenia

Abbreviations: IFN, interferon; IL, interleukin.

MUST GO THROUGH FDA CLINICAL TRIALS

TIME + EXPENSE!

RECOMINANT VACCINES CAN ALSO BE SYNTHESIZED

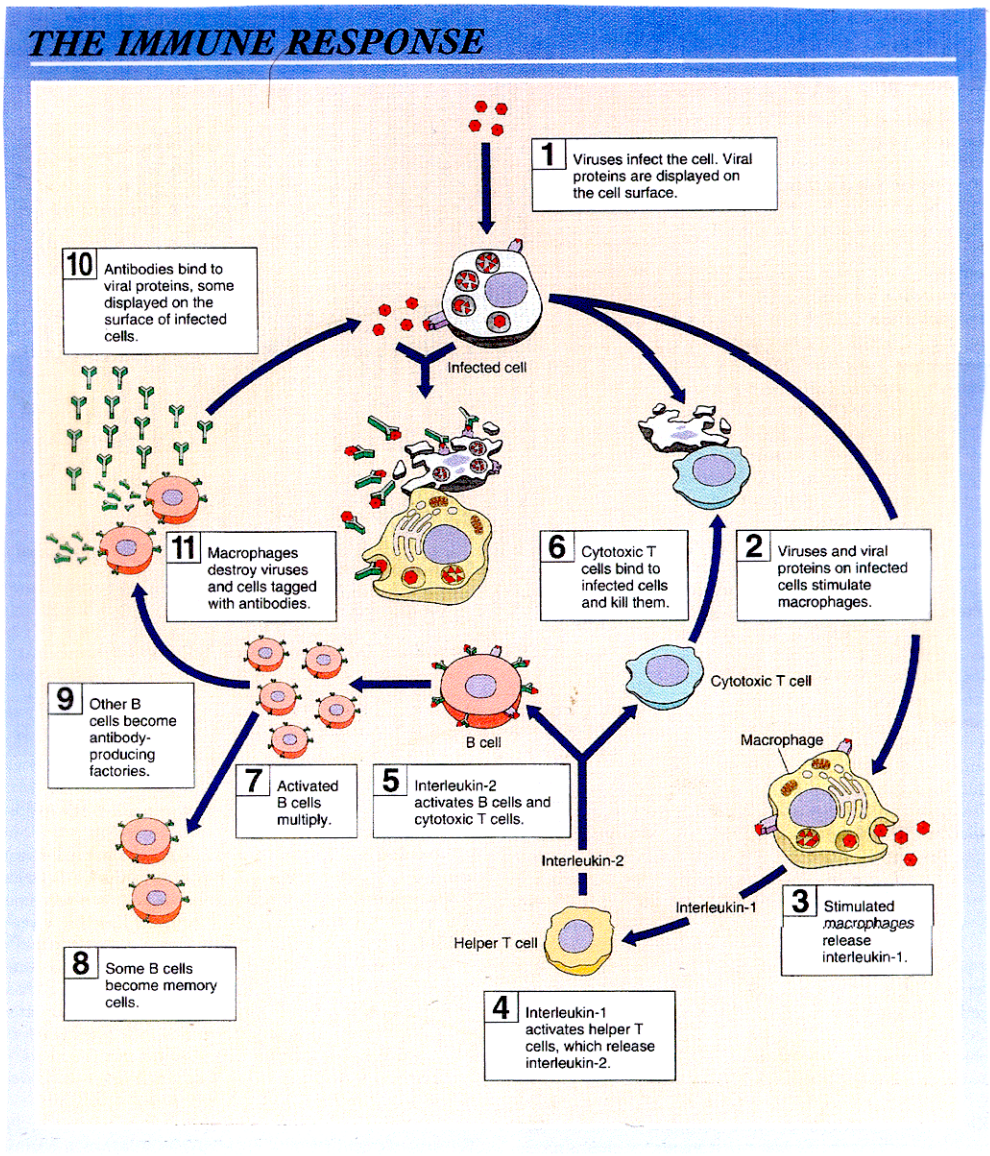


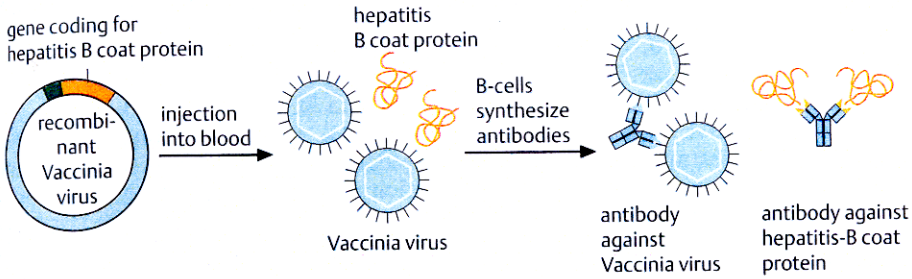
FIGURE 57.20
Overview of the specific immune response.

USING GENETIC ENGINEERING TO MAKE VACCINES

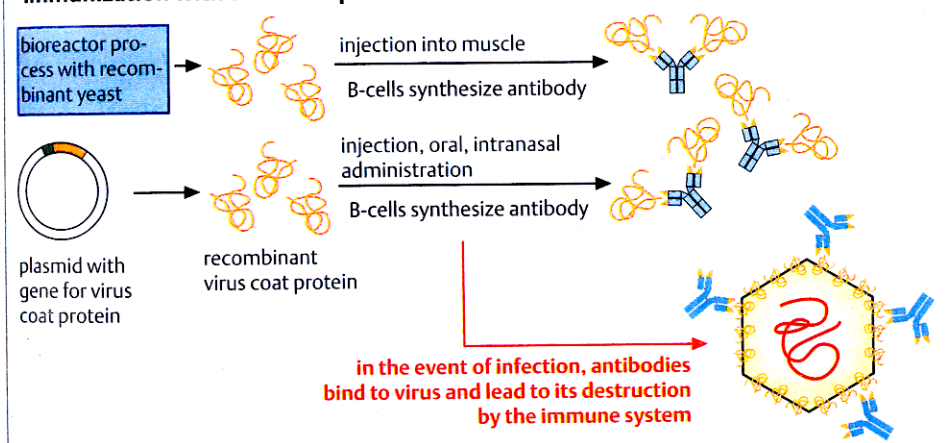
Recombinant vaccines (selection)

		antigen	status
viruses	hepatitis B	surface antigens	registered
	Herpes simplex type 2	surface antigens	clinical studies
	rabies vaccine	surface antigens	not registered
	yellow fever virus	surface antigens	preclinical studies
	AIDS virus	surface antigens	clinical studies
bacteria	*Streptococcus pneumoniae*	polysaccharide conjugate	registered
	Clostridium tetani	tetanus toxin	not registered
	Mycobacterium tuberculosis	surface antigens	clinical studies
parasites	*Plasmodium falciparum*	(malaria)	clinical studies
	Trypanosoma sp.	(sleeping sickness)	clinical studies
	Schistosoma mansoni	(bilharziosis)	clinical studies

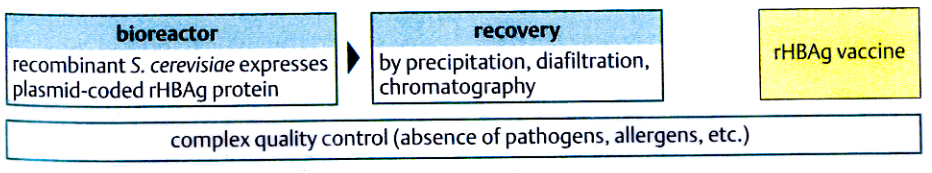
Vaccination by recombinant Vaccinia virus



Immunization with virus coat protein or DNA



Fermentation and recovery of recombinant hepatitis B vaccine



RECOMBINANT VACCINES ARE ALSO BEING DEVELOPED

Table 11.1 Human disease agents for which recombinant vaccines are currently being developed

Pathogenic agent	Disease(s)
Viruses	
Varicella-zoster virus	Chicken pox
Cytomegalovirus	Infection in infants and immunocompromised patients
Dengue virus	Hemorrhagic fever
Hepatitis A virus	High fever, liver damage
Hepatitis B virus	Long-term liver damage
Herpes simplex virus type 2	Genital ulcers
Influenza A and B viruses	Acute respiratory disease
Japanese encephalitis virus	Encephalitis
Parainfluenza virus	Inflammation of the upper respiratory tract
Rabies virus	Encephalitis
Respiratory syncytial virus	Upper and lower respiratory tract lesions
Rotavirus	Acute infantile gastroenteritis
Yellow fever virus	Lesions of heart, kidney, and liver
Human immunodeficiency virus	AIDS
Bacteria	
Vibrio cholerae	Cholera
E. coli enterotoxin strains	Diarrheal disease
Neisseria gonorrhoeae	Gonorrhea
Haemophilus influenzae	Meningitis, septicemic conditions
Mycobacterium leprae	Leprosy
Neisseria meningitidis	Meningitis
Bordetella pertussis	Whooping cough
Shigella strains	Dysentery
Streptococcus group A	Scarlet fever, rheumatic fever, throat infection
Streptococcus group B	Sepsis, urogenital tract infection
Streptococcus pneumoniae	Pneumonia, meningitis
Clostridium tetani	Tetanus
Mycobacterium tuberculosis	Tuberculosis
Salmonella typhi	Typhoid fever
Parasites	
Onchocerca volvulus	River blindness
Leishmania spp.	Internal and external lesions
Plasmodium spp.	Malaria
Schistosoma mansoni	Schistosomiasis
Trypanosoma spp.	Sleeping sickness
Wuchereria bancrofti	Filariasis

CRITICAL TO FIGHT BIOWEAPONS!

LARGE BIOREACTORS + FERMENTORS ARE NEEDED TO GROW RECOMBINANT BACTERIA FOR LARGE SCALE PROTEIN PRODUCTION

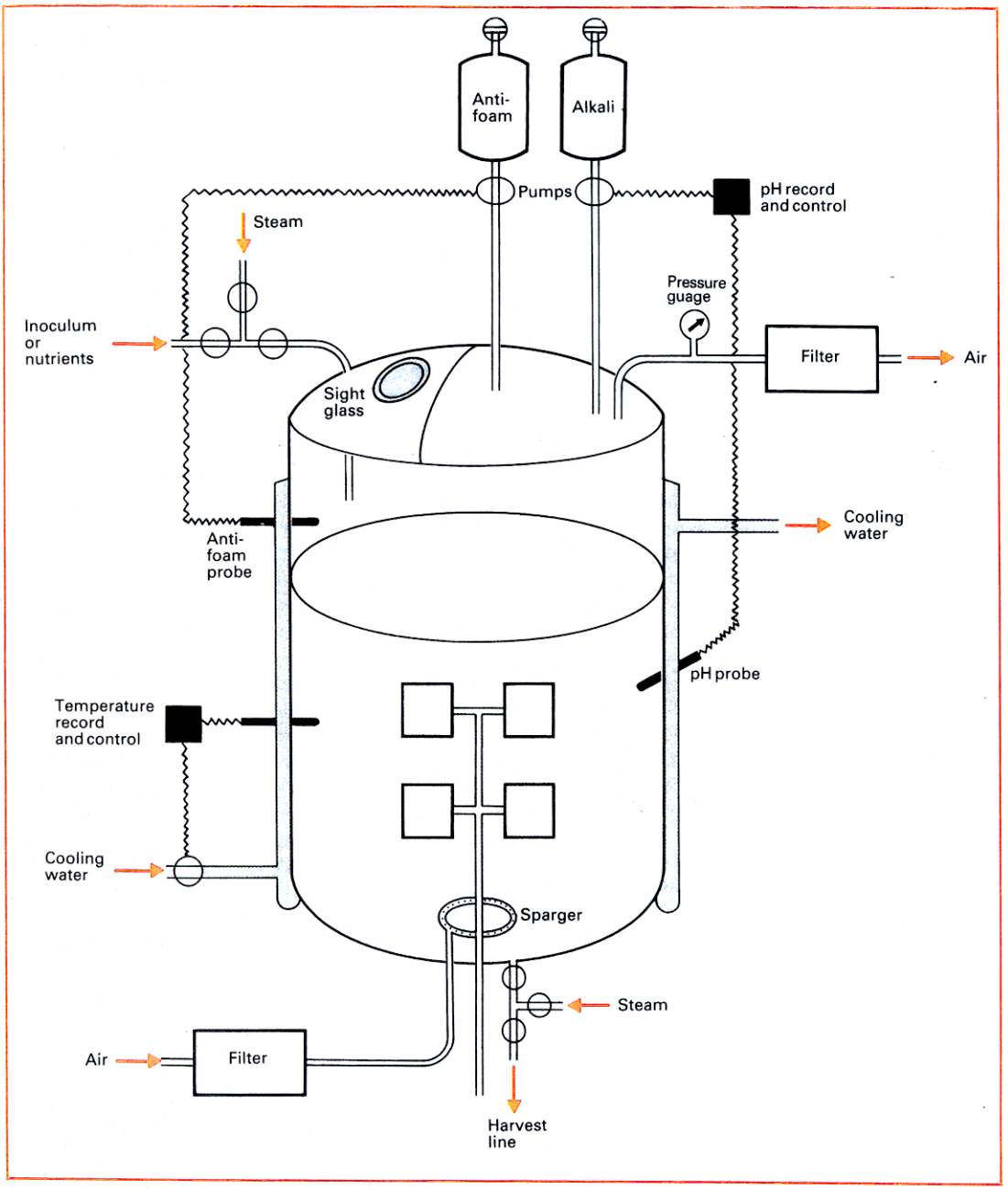


Fig. 5.4 Schematic representation of a stirred tank reactor. For clarity no seal is shown between the agitator shaft and the fermenter body and baffles have been omitted.

INDUSTRIAL-SCALE PROCESSES HAVE BEEN DEVELOPED TO COLLECT BACTERIAL CELLS + ISOLATE HUMAN PROTEINS

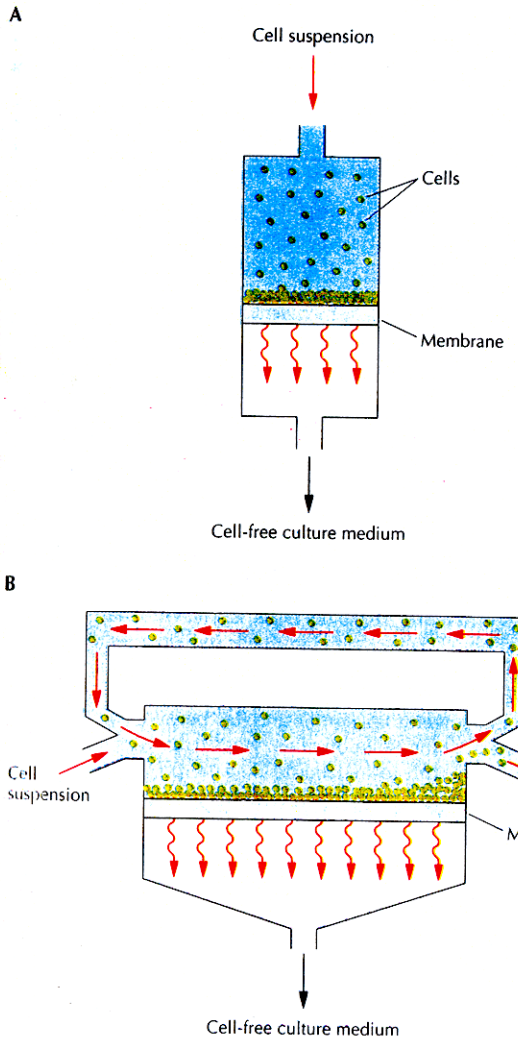


Figure 16.7 Membrane filtration systems for concentrating microbial cells. **A.** Dead-end filtration. **B.** Cross-flow filtration. Arrows within each unit show the direction of the liquid flow.

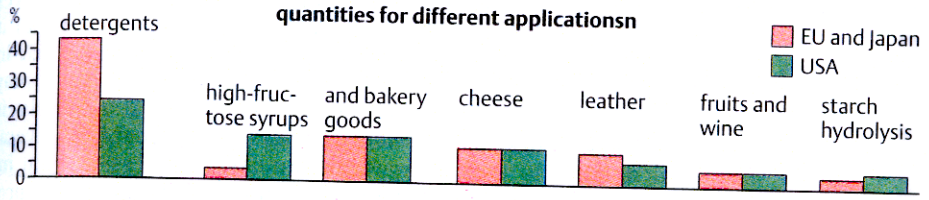
Specific Human Protein Purification e.g. insulin

→ MUST BE 100% Pure for Drug Use + FDA Approval!!

BACTERIA & OTHER MICROBES ARE THE SOURCE OF MANY DIFFERENT PRODUCTS

Enzymes as additives in industry

application	enzyme type	organisms (examples)	market size (% of total)	economic advantage
detergents	proteases, cellulases, lipases	*Bacillus licheniformis* *Aspergillus nidulans* *Trichoderma reesei*	40	1
starch hydrolysis	α-amylase	*Bacillus amyloliquefaciens*	5	3, 4
glucose isomerization	glucose isomerase	*Streptomyces venezuelae*	7	1, 3
beer brewing	amylase	*Bacillus subtilis*	3	3, 4
fruit processing, wine	cellulases, hemicellulases, pectinases	*Aspergillus niger*	5	3, 4, 5, 6
flour, bakery goods	α-amylase, proteases	*Aspergillus oryzae*	8	1, 3
cheese manufacture, aroma	proteases, chymosin, lipases	animal rennin, *Rhizomucor miehei*, *Saccharomyces cerevisiae*	12	2
silage and animal feed	phytases	*Aspergillus niger*	8	3
paper and textiles	α-amylase, lipase	*Bacillus, Humicola*	2	4
leather treatment	proteases	*Aspergillus oryzae*	10	1, 7



quantities for different applicationsn

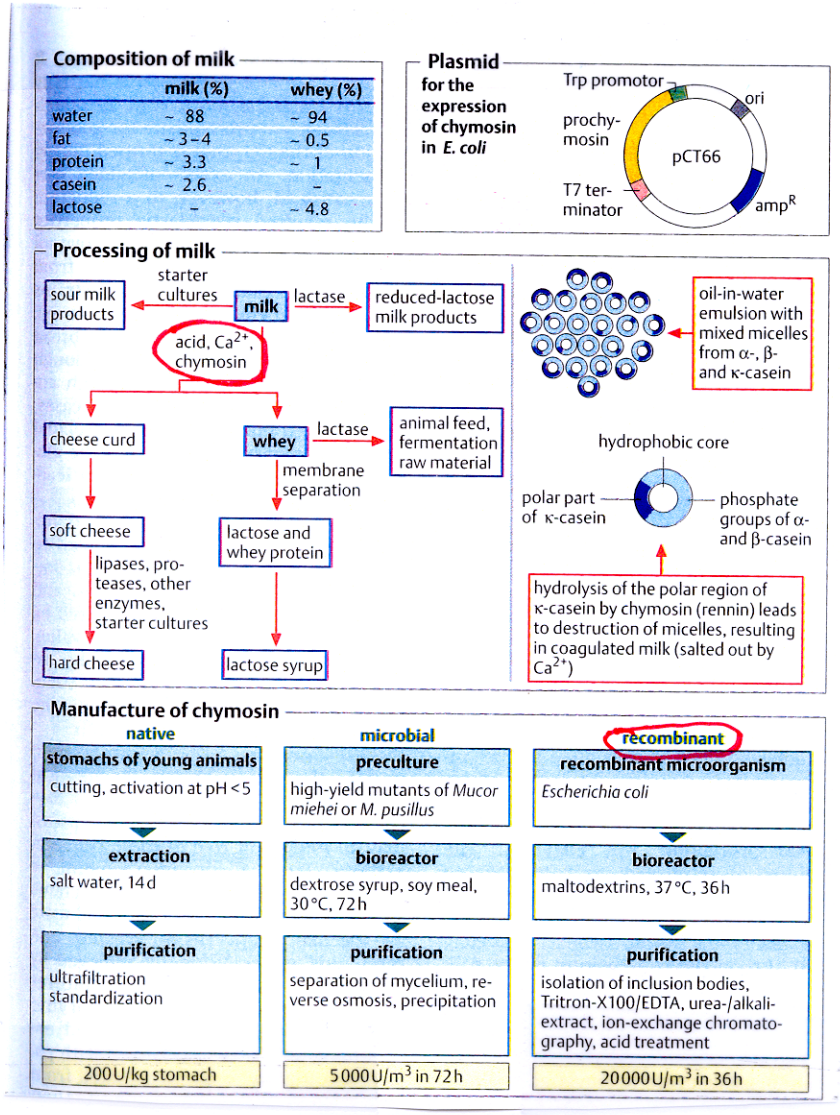
process/application	enzyme cost per unit quantity (US $)
starch liquefaction	ca. $ 2 per t starch
glucose from starch	$ 3.5 per t starch
isomerization of glucose	$ 6 per t starch
HFS in USA	$ 6–7 per t starch
ethanol	$ 1 per t starch
beer	$ 0.1 per 100 L
bakery goods USA	$ 0.1 per 100 kg flour
bakery goods EU	$ 0.1–0.5 per 100 kg flour
fruit juice	$ 0.1–0.5 per 100 L juice
wine	$ 0.1–0.5 per 100 L wine
stabilization of fruit lemonade by glucose oxidase	$ 0.3–0.8 per 1000 L
cheese manufacture	$ 0.05 per 100 L milk
detergents	$ 0.05 per kg detergent
leather tanning	$ 1.2–3 per t skin

important goals in application technology

1 higher product quality
2 improved taste
3 better yields
4 reduced process costs
5 better filtration
6 better conservation
7 improved working conditions, reduced environmental load

IMPROVED and/or MANIPULATED by Recombinant DNA!

RECOMBINANT CHYMOSIN IS USED TO MAKE CHEESE



Chymosin Acts on Milk Protein to coagulate Milk → Cheese!

IS CHEESE A GMO?

Chymosin in Cheesemaking

1. ~ 80-90% of cheeses are made with Recombinant Chymosin
2. Approved for use in Cheesemaking by FDA
3. Not different from non-recombinit Chymosin - ∴ GRAS - Generally Regarded as Safe & not Labeling needed - because not an additive & not different from non-recombinant chymosin!

15 Cheese Made using a GMO?

Industry Adds Claim that Recombinant Chymosin is "Kosher" & "Vegetarian"

Microbes - Including Bacteria - HAVE MANY Useful Metabolites

Table 6.2 Some applications of microbial cells.

Organism	Application
Bacillus thuringiensis and related organisms	Microbial insecticide
Lactobacillus sp., *Streptococcus cremoris* and related species	Starter cultures for the manufacture of dairy products, e.g. yoghurt, cheese
Penicillium roquefortii and related species	Inocula for the production of blue-veined cheeses
Rhizobium sp.	Inoculants for adding to legume seeds to promote nodulation and nitrogen fixation
Pseudomonas syringae	Creation of artificial snow. Ice-nucleation-defective mutants for the prevention of frost damage to crops
Many different organisms	Single-cell protein production

Enzyme	Source	Applications
α-amylase	*Aspergillus oryzae*	Preparation of glucose syrups
	Bacillus amyloliquefaciens	Removal of starch sizes
	Bacillus licheniformis	Liquefaction of brewing adjuncts
β-glucanase	*Aspergillus niger*	Liquefaction of brewing adjuncts
	Bacillus amyloliquefaciens	Improvement of malt for brewing
Glucoamylase	*Aspergillus niger*	Starch hydrolysis
	Rhizopus sp.	
Glucose isomerase	*Arthrobacter* sp.	High-fructose corn syrup
	Bacillus sp.	
Lactase	*Kluyveromyces* sp.	Removal of lactose from whey
Lipase	*Candida lipolytica*	Flavour development in cheese
Pectinase	*Aspergillus* sp.	Clarification of wines and fruit juices
Penicillin acylase	*Escherichia coli*	Preparation of 6-aminopenicillanic acid
Protease, acid	*Aspergillus* sp.	Calf rennet substitute
Protease, alkaline	*Aspergillus oryzae*	Detergent additive
	Bacillus sp.	Dehairing of hides
Protease, neutral	*Bacillus amyloliquefaciens*	Liquefaction of brewing adjuncts
	Bacillus thermoproteolyticus	
Pullulanase	*Klebsiella aerogenes*	Starch hydrolysis

Table 6.8 Sources and applications of some microbial enzymes.

Polysaccharide	Producing organism	Uses
Xanthan gum	*Xanthomonas campestris*	**1** Food additive for stabilizing liquid suspensions and gelling soft foods, e.g. ice cream, cheese spreads **2** Lubrication in, for example, toothpaste preparations **3** Enhanced oil recovery
Gellan	*Pseudomonas* sp.	**1** Solidification of food products
Emulsan	*Acinetobacter calcoaceticus*	**1** Cleaning oil spills
	Arthrobacter	**2** Enhanced oil recovery
Pullulan	*Aureobasidium pullulans*	**1** Biodegradable material for food coating and packaging
Dextrans	*Leuconostoc mesenteroides*	**1** Blood expander **2** Adsorbents for pharmaceutical preparations

Table 6.7 Commercially available microbial polysaccharides and their uses.

BACTERIAL METABOLIC PATHWAYS CAN BE ENGINEERED TO OPTIMIZE PRODUCTION OF NOVEL INDUSTRIAL PRODUCTS

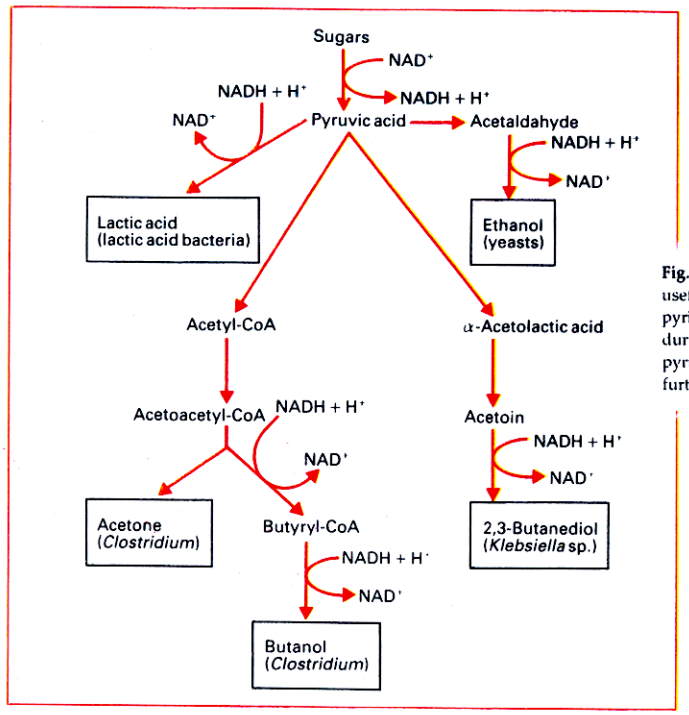


Fig. 6.5 The formation of commercially useful metabolic end-products. Note that pyridine nucleotide cofactors are reduced during the conversion of sugars to pyruvate and subsequently oxidized by further metabolism of pyruvate.

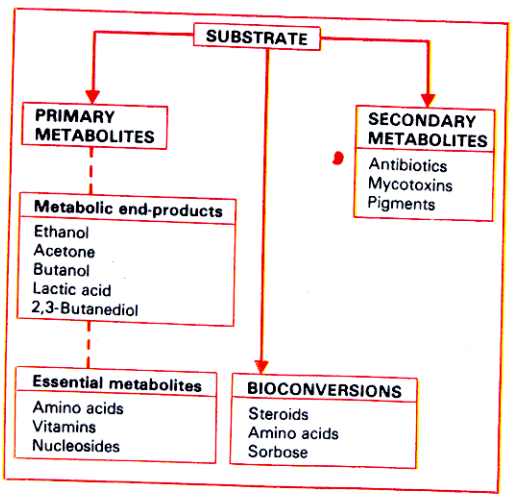


Fig. 6.4 The different classes of low-molecular-weight compounds synthesized by microorganisms.

These pathways can be optimized and/or changed by adding genes on plasmids that encode novel enzymes

e.g., Maxygen® gene shuffling protein evolution