Application of Various Organisms

References

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2004

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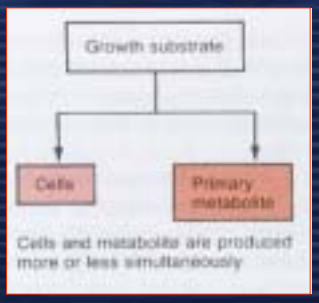
- *The microoganism is* { *always right* / *your friend* / *a sensitive partner* }
- *There are no stupid microorganisms*
- *Microorganisms* { *can do anything* / *will do anything* }
- *Microorganisms are* { *smarter* / *wiser* / *more energetic* } *than chemists, engineers, etc.*

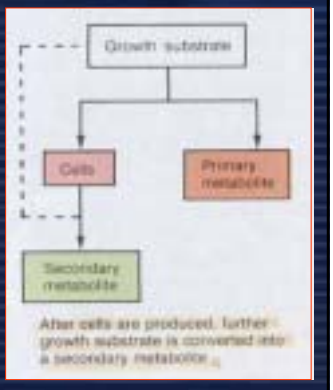
Culture Collections which Supply Cultures of Industrial Microorganisms

Abbreviation	Name	Location
ATCC	American Type Culture Collection	Rockville, MD USA
CBS	Centraalbureau voor Schimmelcultur	Baarn, Netherlands
CCM	Czechoslovak Collection of Microorganisms	J.E. Purkyne University, Brno, Czechoslovakia
CDDA	Canadian Department of Agriculture	Ottawa, Canada
CIP	Collection of the Institut Pasteur	Paris, France
CMI	Commonwealth Mycological Institute	Kew, UK
DSM	Deutsche Sammlung von Mikroorganismen	Göttingen, Federal Republic of Germany
FAT	Faculty of Agriculture, Tokyo University	Tokyo, Japan
IAM	Institute of Applied Microbiology	University of Tokyo, Japan
NCIB	National Collection of Industrial Bacteria	Aberdeen, Scotland
NCTC	National Collection of Type Cultures	London, UK
NRRL	Northern Regional Research Laboratory	Peoria, IL USA

Listed here are just a few of the general culture collections. Many universities and research laboratories maintain collections of specific microbial groups.

Contrast Between Primary and Secondary Metabolites





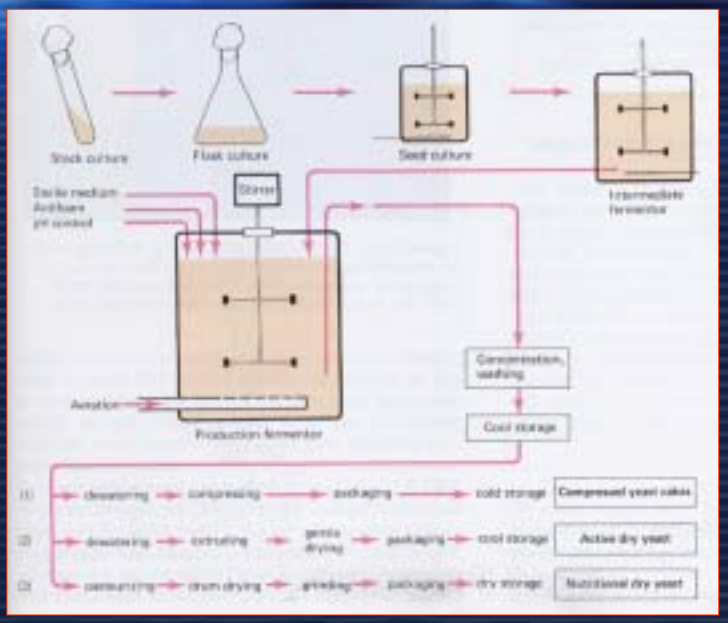
Major Products Dependent on Microbial Biotechnology and Their Primary End Uses Before the Advent of Genetic Engineering

Product	*Major Uses*
Fermented juices and distilled liquors	Beverages
Cheese	Food
Antibiotics	Drugs
Industrial alcohol	Fuel additive (gasohol)
High-fructose syrups	Sweeteners
Amino acids	Feed additives, food enrichment and flavoring agents, artificial sweetener (aspartame), feed preservatives
Baker's yeast	Food additive, enrichment agent
Steroids	Therapeutic agents, animal growth promotion
Vitamins	Feed and food enrichment additives
Citric acid	Food additive
Enzymes	Food processing, laundry detergents
Vaccines	Disease prevention
Polysaccharide gums	Food emulsifiers, thickeners and stabilizers, enhanced oil recovery

Examples of Human Proteins Cloned in E. Coli : Their Biological Functions and Current or Envisaged Therapeutic Use

Protein	*Function(s)*	*Therapeutic Use(s)*
α-1 antitrypsin	Protease inhibitor	Treatment of emphysema
Calcitonin	Influences Ca^{2+} and phosphate metabolism	Treatment of osteomalacia
Colony-stimulating factors	Stimulates hematopoiesis	Antitumor
Epidermal growth factor	Epithelial cell growth, tooth eruption	Wound healing
Erythropoietin	Stimulates hematopoiesis	Treatment of anemia
Factor VIII	Blood-clotting factor	Prevention of bleeding in hemophiliacs
Factor IX	Blood-clotting factor	Prevention of bleeding in hemophiliacs
Growth hormone-releasing factor	Stimulates secretion of growth hormone	Growth promotion
Interferons (α, β, γ)	A family of 20 to 25 low-molecular-weight proteins that cause cells to become resistant to the growth of a wide variety of viruses	Antiviral, antitumor (?), antiinflammatory (?)
Interleukins 1, 2, and 3	Stimulators of cells in the immune system	Antitumor; treatment of immune disorders
Lymphotoxin	A bone-resorbing factor produced by leukocytes	Antitumor
Somatomedin C (IGF-1)	Sulfate uptake by cartilage	Growth promotion
Serum albumin	Major protein constituent of plasma	Plasma supplement
Superoxide dismutase	Decomposes superoxide free radicals in the blood	Prevention of damage when O_2-rich blood enters O_2-deprived tissues; has applications in cardiac treatment and organ transplantation
Tumor necrosis factor	A product of mononuclear phagocytes cytotoxic to certain tumor cell lines	Antitumor
Urogastrone	Control of gastrointestinal secretion	Antiulcerative
Urokinase	Plasminogen activator	Anticoagulant (dissolution of blood clots)

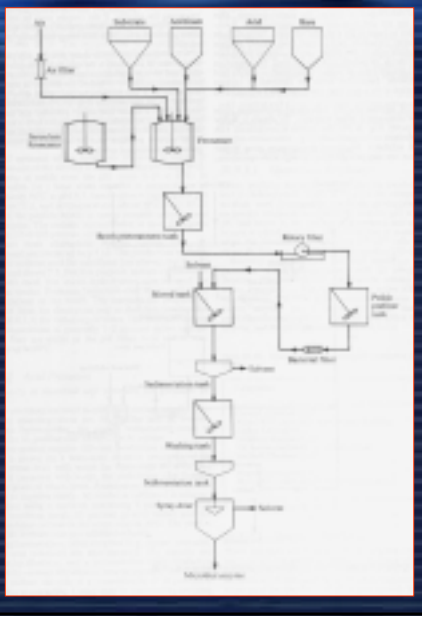
Stages in Commercial Yeast Production



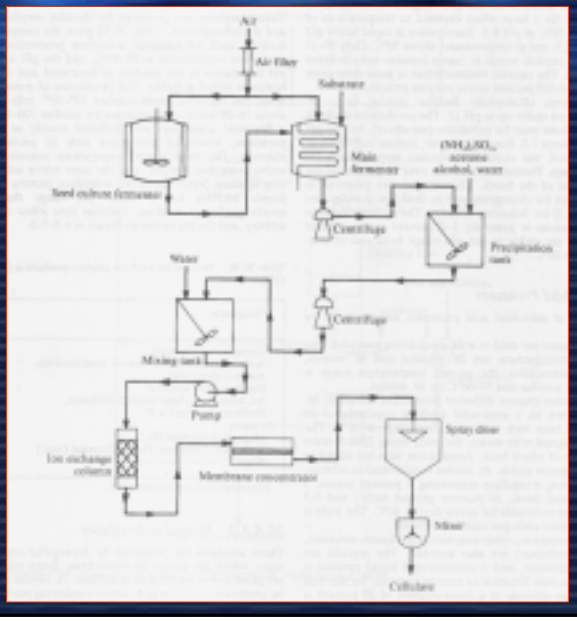
Microbial Enzymes with Industrial-Scale Applications and Some of Their Sources

Enzyme	Source	Action	Application
α-Amylase	*Bacillus subtilis* *Bacillus licheniformis* *Aspergillus oryzae*	Endo-hydrolysis of α-1,4-glycosidic linkages	Starch processing
Glucoamylase	*Aspergillus oryzae* *Aspergillus niger* *Rhizopus oryzae*	Removes glucose from nonreducing end of starch, also splits α-1,6- linkages at branch points but more slowly	Starch processing; brewers' and distillers' malts
Pullulanase	*Klebsiella aerogenes*	Splits α-1,6-glycosidic linkages in pullulan and amylopectin	Starch processing
Glucose isomerase	*Bacillus coagulans* *Streptomyces albus*	Converts D-glucose to D-fructose. This enzyme is actually a xylose isomerase that converts D-xylose to D-xylulose	Production of high-fructose syrups
β-Glucanase	*Bacillus subtilis* *Aspergillus niger* *Penicillium emersonii*	Degrades β-glucan by cleaving β-1,3 (4)-glucosidic linkages	Brewing
Invertase	*Saccharomyces cerevisiae*	Splits sucrose to glucose and fructose	Confectionery industry; baking
Lactase	*Saccharomyces lactis* *E. oryzae, A. niger,* *Rhizopus oryzae*	Splits lactose to glucose and galactose	Dairy industry (treatment of milk and whey)
Pectinase	*E. oryzae, A. niger,* *Rhizopus oryzae*	Degrades pectin, α-1,4-linked anhydrogalacturonic acid with some of the carboxyl groups esterified as the methyl esters	Clarification of fruit juices and wines
Neutral protease	*Bacillus subtilis* *Aspergillus oryzae*	Hydrolyzes peptide bonds in proteins	Flavoring of meat and cheese; baking
Alkaline protease	*Bacillus licheniformis*	Hydrolyzes peptide bonds in proteins	Laundry detergents
Rennin	*Mucor miehei* spp. Recombinant enzyme produced in *E. coli* and fungi	Hydrolyzes a specific bond in κ-casein, leading to coagulation of milk proteins	Cheesemaking
Lipase	*A. oryzae, A. niger,* *Rhizopus oryzae*	Hydrolyzes some linkages in fats	Dairy industry; detergents

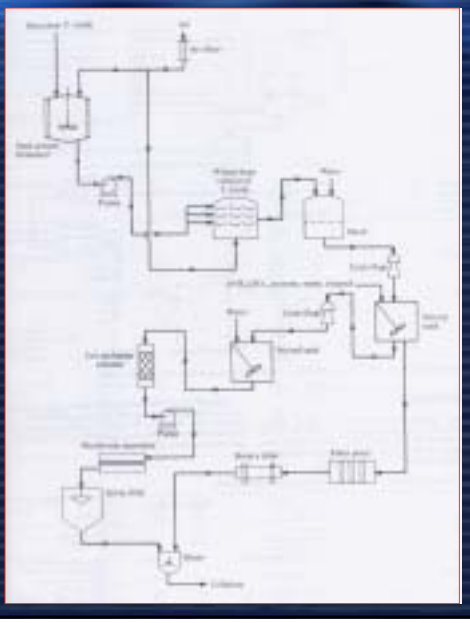
Flowsheet for Solvent Precipitation for Production of Microbial Enzymes



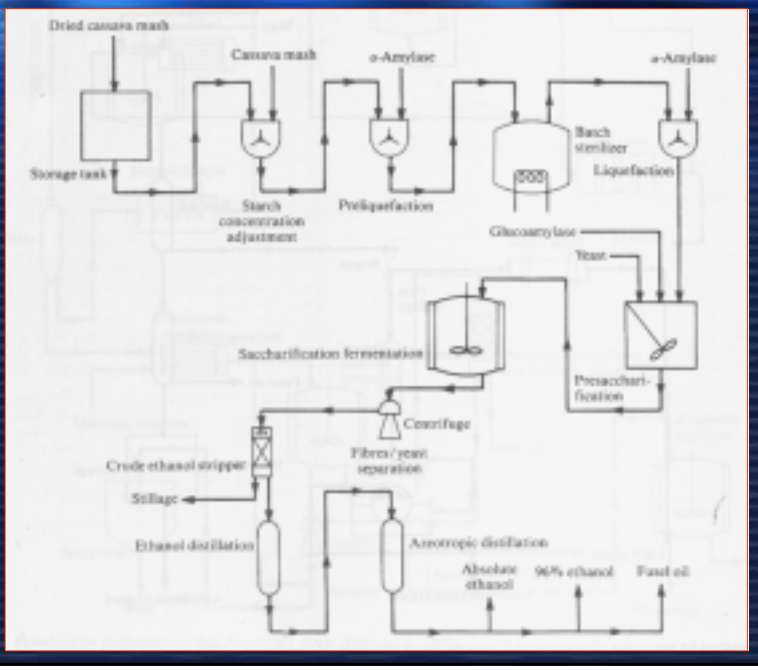
Flowsheet for Submerged-Culture Process for Cellulase Production using *Trichoderma viride*



Flowsheet for Cellulase Production by *Trichoderma viride* using the Koji Technique



Flowsheet for Production of Ethanol from cassava root



Some Antibiotics Produced Commercially

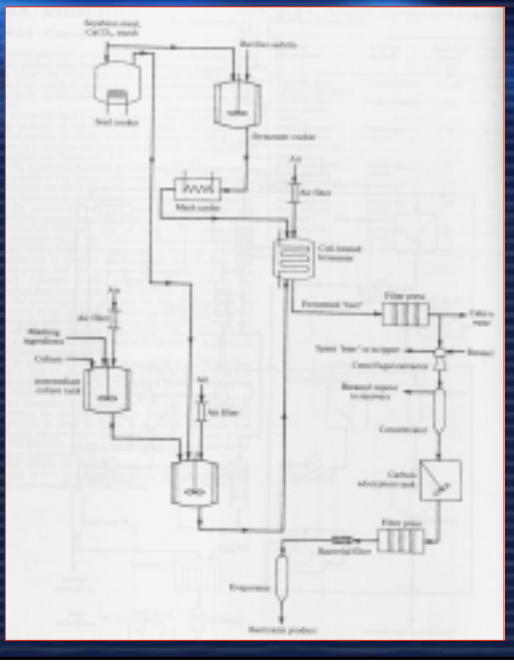
Antibiotic	Producing microorganism	Type of microorganism
Bacitracin	*Bacillus subtilis*	Endospore-forming bacterium
Cephalosporin	*Cephalosporium* sp.	Fungus
Chloramphenicol	Chemical synthesis (formerly *Streptomyces venezuelae*)	
Clindamycin	*Streptomyces griseus*	Actinomycete
Cycloserine	*S. orchidaceus*	Actinomycete
Erythromycin	*S. erythreus*	Actinomycete
Griseofulvin	*Penicillium griseofulvin*	Fungus
Kanamycin	*S. kanamyceticus*	Actinomycete
Lincomycin	*S. lincolnensis*	Actinomycete
Neomycin	*S. fradiae*	Actinomycete
Nystatin	*S. noursei*	Actinomycete
Penicillin	*Penicillium chrysogenum*	Fungus
Polymyxin B	*Bacillus polymyxa*	Endospore-forming bacterium
Streptomycin	*S. griseus*	Actinomycete
Tetracycline	*S. rimosus*	Actinomycete

Classification of Antibiotics according to Their Chemical Structure. An Example of Each is Given in Parentheses.

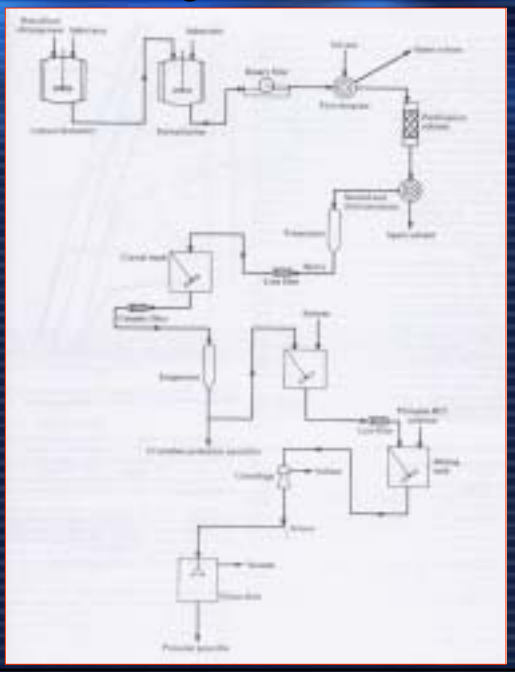
1. **Carbohydrate-containing antibiotics**
   - Pure sugars (Neomycin)
   - Aminoglycosides (Streptomycin)
   - Orthosamycins (Gentamicin)
   - N-Glycosides (Streptothricin)
   - C-Glycosides (Vancomycin)
   - Glycolipids (Moenomycin)
2. **Macrocyclic lactones**
   - Macrolide antibiotics (Erythromycin)
   - Polyene antibiotics (Candicidin)
   - Ansamycins (Rifamycin)
   - Macrotetrolides (Tetronasin)
3. **Quinones and related antibiotics**
   - Tetracyclines (Tetracycline)
   - Anthracyclines (Adriamycin)
   - Naphthoquinones (Actinorhodin)
   - Benzoquinones (Mitomycin)
4. **Amino acid and peptide antibiotics**
   - Amino acid derivatives (Cycloserine)
   - β-Lactam antibiotics (Penicillin)
   - Peptide antibiotics (Bacitracin)
   - Chloramphenicols (Actinomycin)
   - Depsipeptides (Valinomycin)
   - Chelate-forming peptides (Bleomycin)
5. **Heterocyclic antibiotics containing nitrogen**
   - Nucleoside antibiotics (Polyoxin)
6. **Heterocyclic antibiotics containing oxygen**
   - Polyether antibiotics (Monensin)
7. **Alicyclic derivatives**
   - Cycloalkane derivatives (Cycloheximide)
   - Steroid antibiotics (Fusidic acid)
8. **Aromatic antibiotics**
   - Benzene derivatives (Chloramphenicol)
   - Condensed aromatic antibiotics (Griseofulvin)
   - Aromatic ether (Nanaomycin)
9. **Aliphatic antibiotics**
   - Compounds containing phosphorus (Fosfomycin)

Generalized Flowsheet for the Commercial Recovery and Purification of Antibiotics

Flowsheet for Production of Bacitracin



Flowsheet Diagram for Penicillin Production



Biotransformation

- The transformation can be performed by the folowing preparations

1) Growing cells
2) Resting cells
3) Cell-free extracts
4) Immobilized whole cell
5) Immobilized cell-free extracts
6) Spore suspension

Types of Reactions in the Biotransformation of Antibiotics with Examples

Type of reaction	Substrate	Product	Microorganism
Hydrolysis of β-lactams	Penicillin	Penicillanic acid	*Pseudomonas aeruginosa*
Hydrolysis of peptides	Novobiocin	Novenamine	*Alcaligenes* sp.
Hydrolysis of lactones	Actinomycin	Peptides	*Actinoplanes missouriensis*
Hydrolysis of esters	Maridomycin	Desacetylmaridomycin	*Streptomyces* sp.
Acylation	Chloramphenicol	Chloramphenicol-3-acetate	*Streptomyces griseus*
Phosphorylation	Kanamycin	Kanamycin-3′-O-phosphate	*Bacillus circulans*
Formation of nucleoside esters	Tobramycin	4-O-adenylobramycin	*Staph. aureus*
Dehydrogenation	Lapachol	Dehydro-α-lapachone	*Curvularia lunata*
Oxidation	Mycophenolic acid	Several oxidation products	Various microorganisms
Epoxidation	cis-Propenylphosphonic acid	Fosfomycin	*Penicillium spinulosum*
Sulphoxidation	Lincomycin	Lincomycin sulphoxide	*Streptomyces armentosus*
Hydroxylation	Josamycin	3″-hydroxyjosamycin	*Streptomyces olivaceus*
Reduction of ketones	Daunomycinone	Dihydrodaunomycin	*Streptomyces aureofaciens*
Reduction of aldehydes	Maridomycin	18-Dihydromaridomycin	*Nocardia mexicana*
Reduction of nitro groups	Chloramphenicol	Amine	*Streptomyces hygroscopicus*
C-Demethylation	Griseofulvin	Demethylgriseofulvin	*Botrytis alli*
N-Demethylation	Clindamycin	N-demethylclindamycin	*Streptomyces pumpulus*
Deamination	Formycin	Formycin B	*Escherichia coli*
Transglycosylation	Validoxylamine A	Validamycin D	*Rhizoctonia* sp.
Isomerization	Showdomycin	Isoshowdomycin	*Streptomyces* sp.

Media and Fermentation Conditions Used in Microbial Transformations of Steroids

Microorganism	Steroid substrate	Steroid product (approximate yields, % (by wt))	Composition of medium[a]	Length of incubation; temperature; aeration
Alcaligenes faecalis	Cholic acid	Ketocholic acids (90–100%)	A	2 d (monoketo acid), 4 d (diketo acid), 6 d (triketo acid); 37–39°C; surface culture
Corynebacterium rediolaesum	21-Acetoxy-3β-hydroxy-5-pregnen-20-one	21-Hydroxy-4-pregnene-3,20-dione (30%)	B	6 d; 36–37°C; pure oxygen with agitation
Cunninghamella blakesleeana IE334	Compound S	Cortisone (19%), cortisol (65%)	C	3 d; 28°C; rotary shaker (250 rpm)
Cylindrocarpon radicicola ATCC 11011	Progesterone	1-Dehydrotestololactone (50%)	D	3 d; 25°C; reciprocating shaker (120 spm)
Fusarium solani	Progesterone	$\Delta^{1,4}$-Androstadiene-3,17-dione (85%)	E	4 d; 25°C; rotary shaker (100 rpm)
Rhizopus arrhizus ATCC 11145	4-Androstene-3, 17-dione	11α-Hydroxy-4-androstene-3,17-dione (25%)	F	4 d; 28°C; small aerated tank (6–7 mM O_2 l^{-1} min^{-1})
Streptomyces albus	Oestradiol	Oestrone (90–95%)	G	6 h of substrate oxidation with resting cells; 30°C
S. aureus	Progesterone	15α-Hydroxy-4-pregnene-3,20-dione (11%)	H	3 d; 25°C; rotary shaker (280 rpm)

Some Steroid Transformations of Commercial Importance

Reaction	Substrate	Product	Microorganism	Some industrial producers
11α-Hydroxylation	Progesterone	11α-Hydroxyprogesterone	*Rhizopus nigricans*	Upjohn Co.
11β-Hydroxylation	Reichstein compound S	Hydrocortisone (cortisol)	*Curvularia lunata*	Chas. Pfizer & Co.; Glaxo-Brocades NV
16α-Hydroxylation	9α-Fluorocortisol	9α-Fluoro-16α-hydroxycortisol (triamcinolone)	*Streptomyces roseoflavus*	E. R. Squibb & Sons, Leclerc Laboratories
1-Dehydrogenation	Hydrocortisone (cortisol)	Prednisolone	*Arthrobacter simplex, Corynebacterium simplex*	Schering Corp.
	Deoxolol	Triceolol	*Septomyxa affinis*	Upjohn Co.
1-Dehydrogenation, side-chain cleavage and D-ring expansion	Progesterone	1-Dehydrotestololactone (testolactone)	*Cylindrocarpon radicicola*	E. R. Squibb & Sons
Side-chain cleavage	β-Sitosterol	Androstadienedione and/or androstenedione	*Mycobacterium* spp.	G. D. Searle & Co.