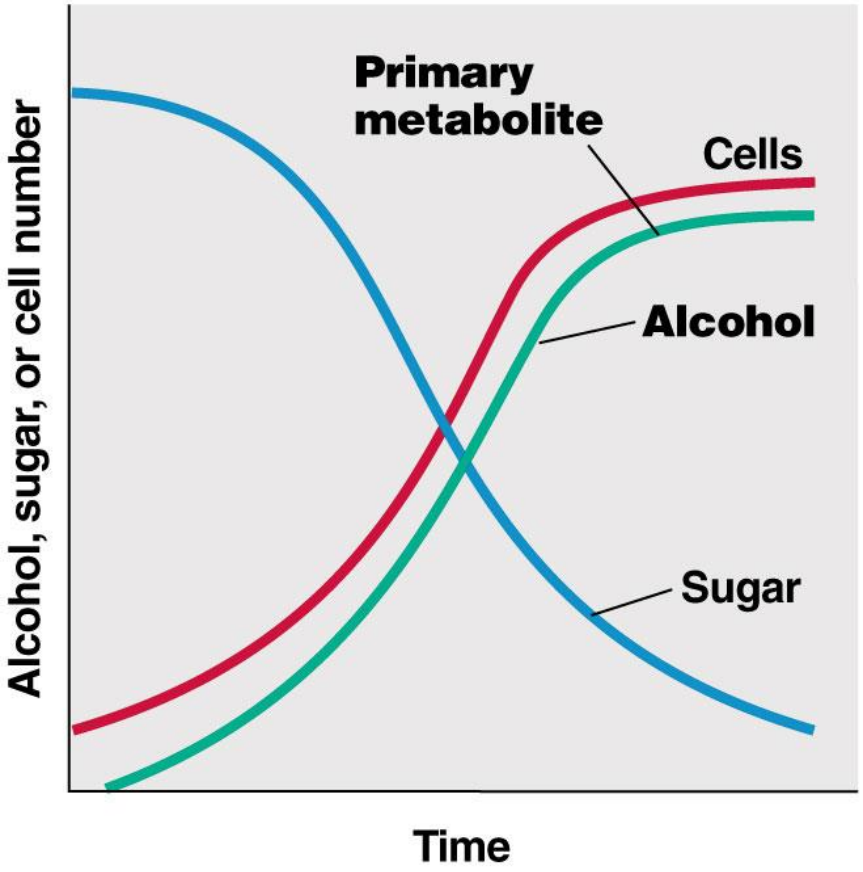
Chapter 15

Commercial Microbial Products and Biotechnology

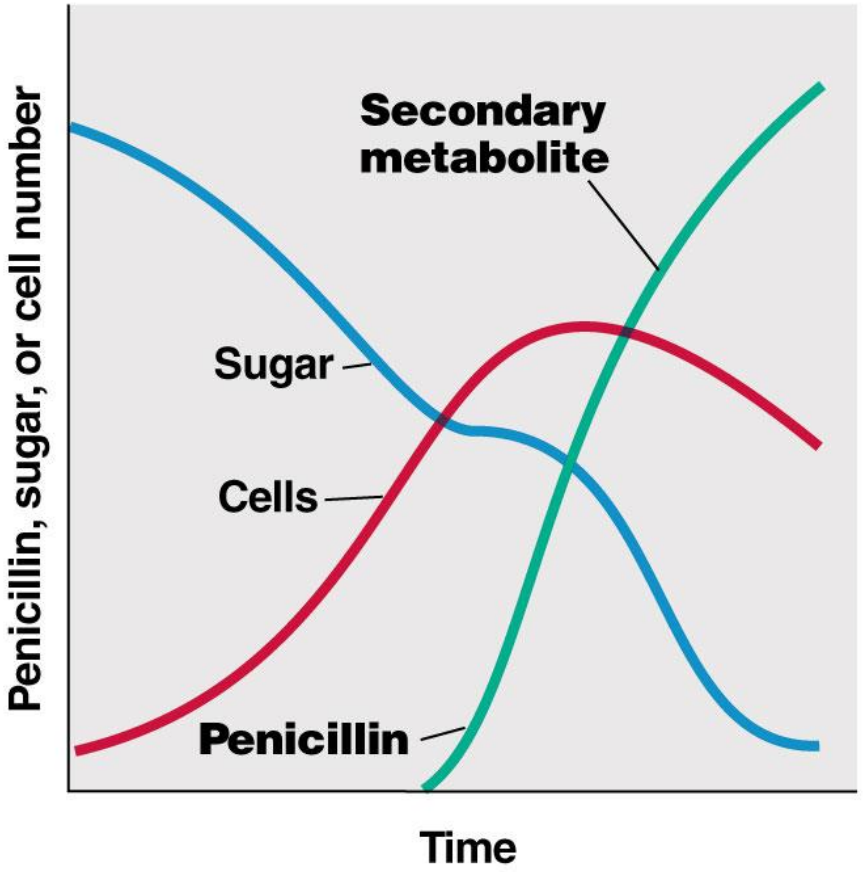
Table 15.1 *Major products of industrial microbiology*

Product	*Example*
Antibiotics	Penicillin, tetracycline
Enzymes	Glucose isomerase, laundry proteases and lipases
Food additives	Vitamins, amino acids
Chemicals	Biofuels (alcohol and biodiesel), citric acid
Alcoholic beverages	Beer, wine, distilled spirits

Primary and Secondary Metabolites



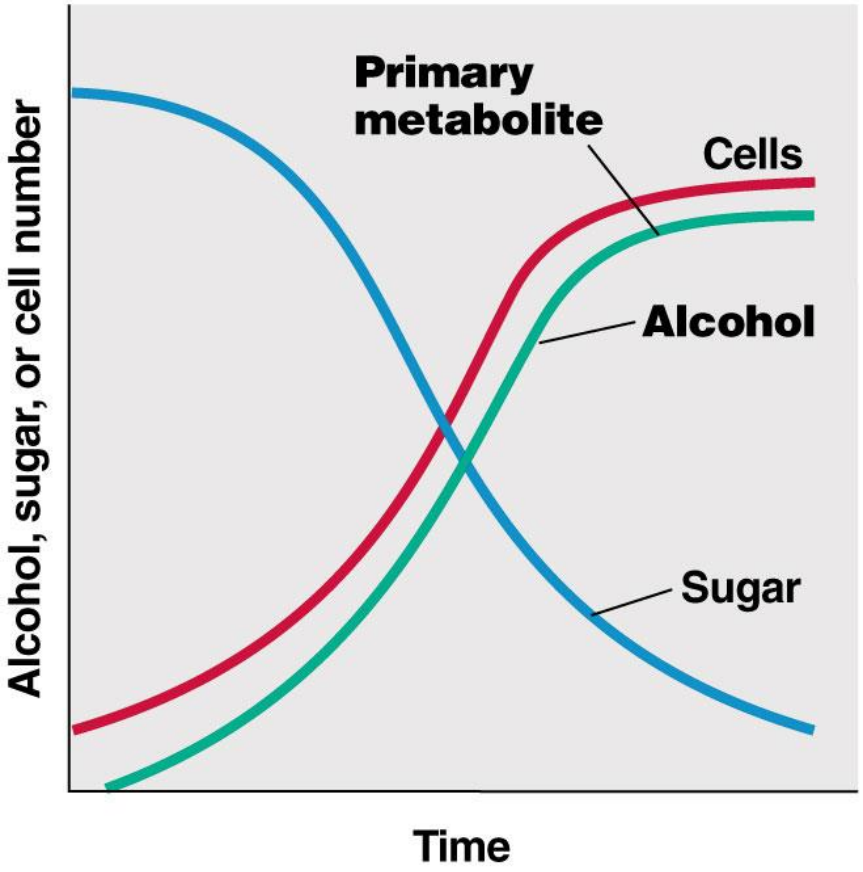
(a) Yeast Fermentation



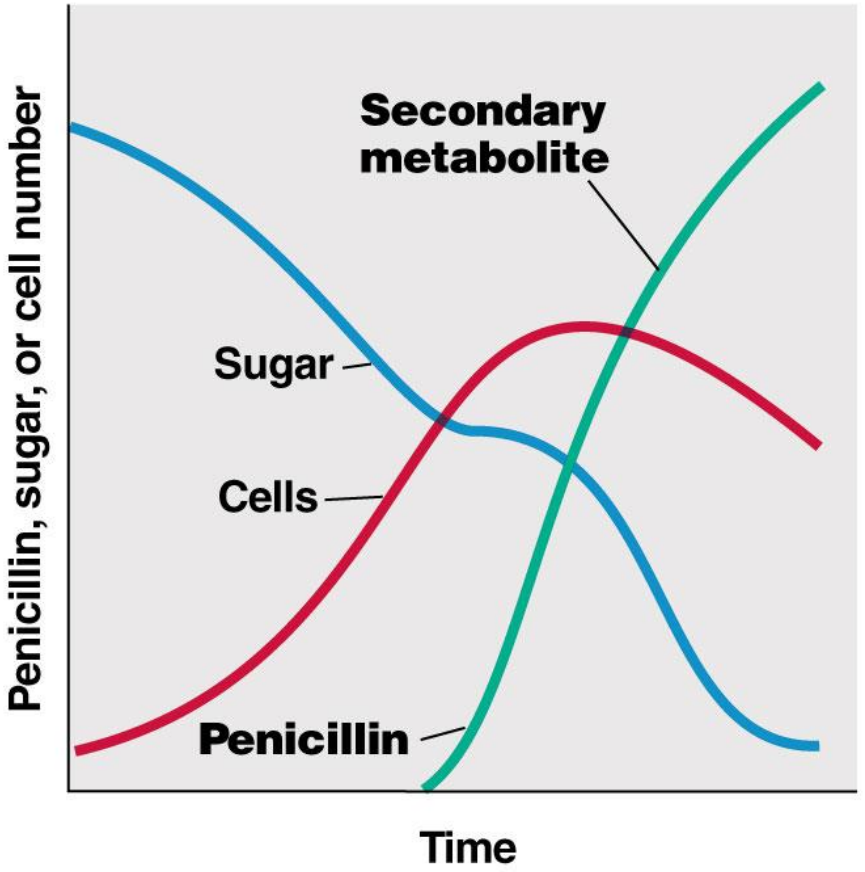
(b) Antibiotic Production: ***P. chrysogenum***

Clicker Question:

Primary and Secondary Metabolites



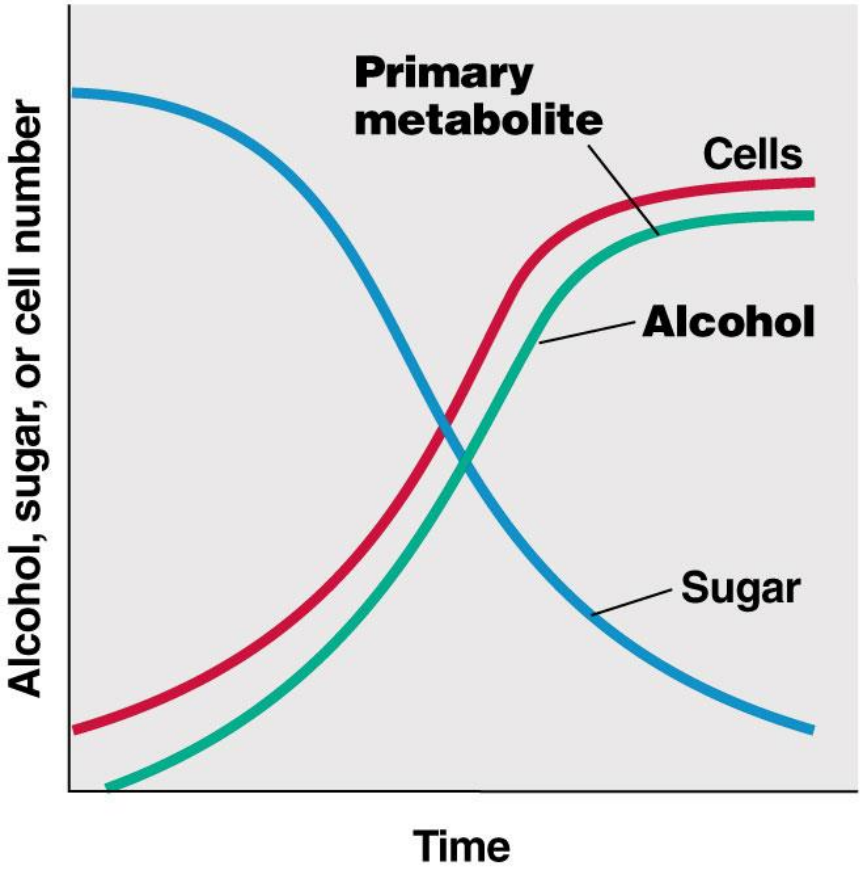
(a) Yeast Fermentation



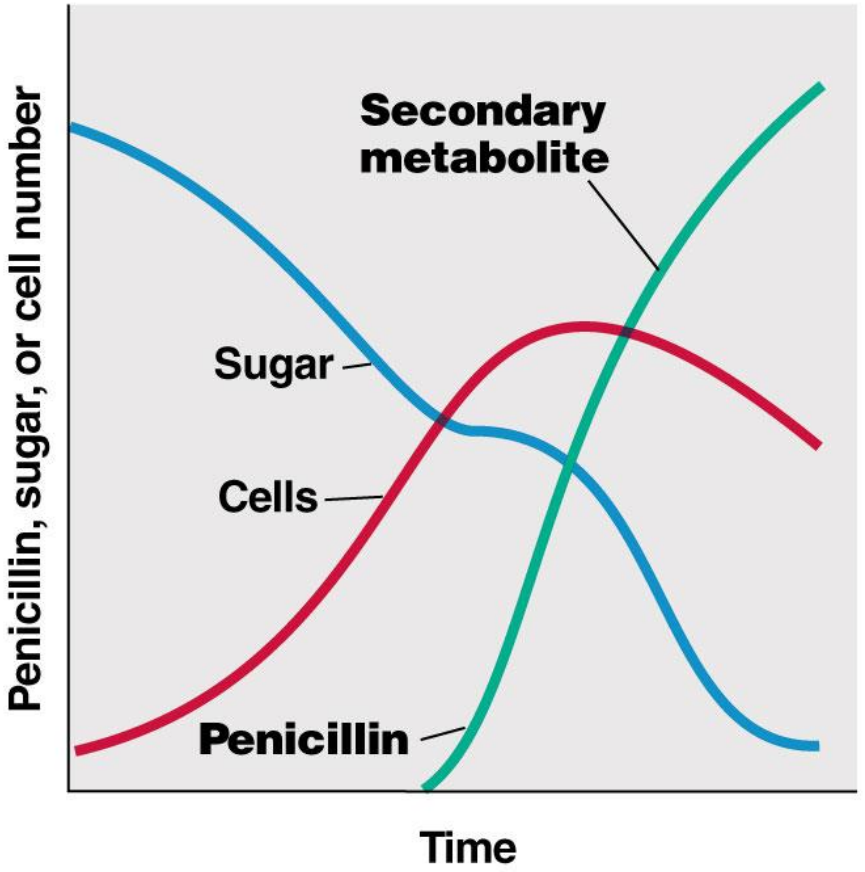
(b) Antibiotic Production: *Penicillium chrysogenum*

Clicker Question:

Primary and Secondary Metabolites



(a) Yeast Fermentation



(b) Antibiotic Production: *Penicillium chrysogenum*

Clicker Question:

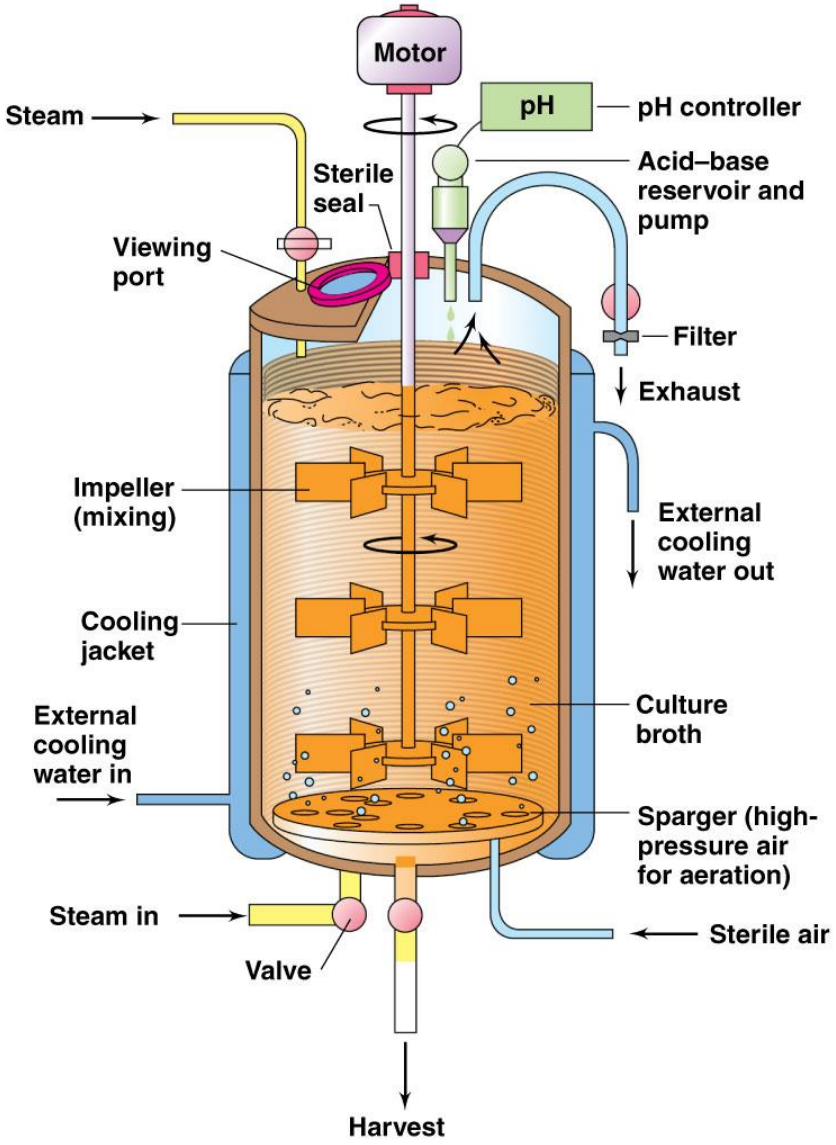
Fermenters

Queue Systems, Inc.

(a)

Novo Nordisk

(c)



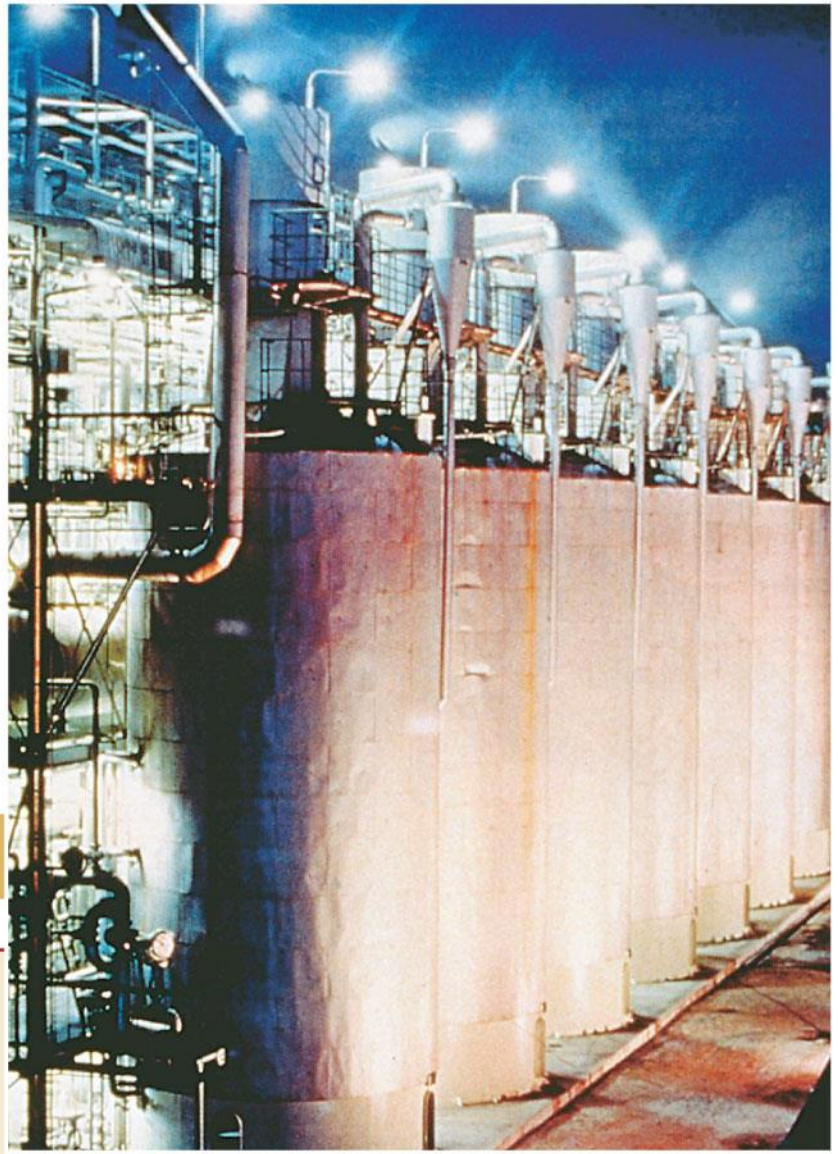
(b)

Research to Industrial Size Fermenters

Elmer L. Gaden, Jr.

Table 15.2 *Fermentor sizes for various industrial fermentations*

Size of fermentor (liters)	*Product*
1,000–20,000	Diagnostic enzymes, substances for molecular biology
40,000–80,000	Some enzymes, antibiotics
100,000–150,000	Penicillin, aminoglycoside antibiotics, proteases, amylases, steroid transformations, amino acids, wine, beer
200,000–500,000	Amino acids (glutamic acid), wine, beer



Elmer L. Gaden, Jr.

(b)

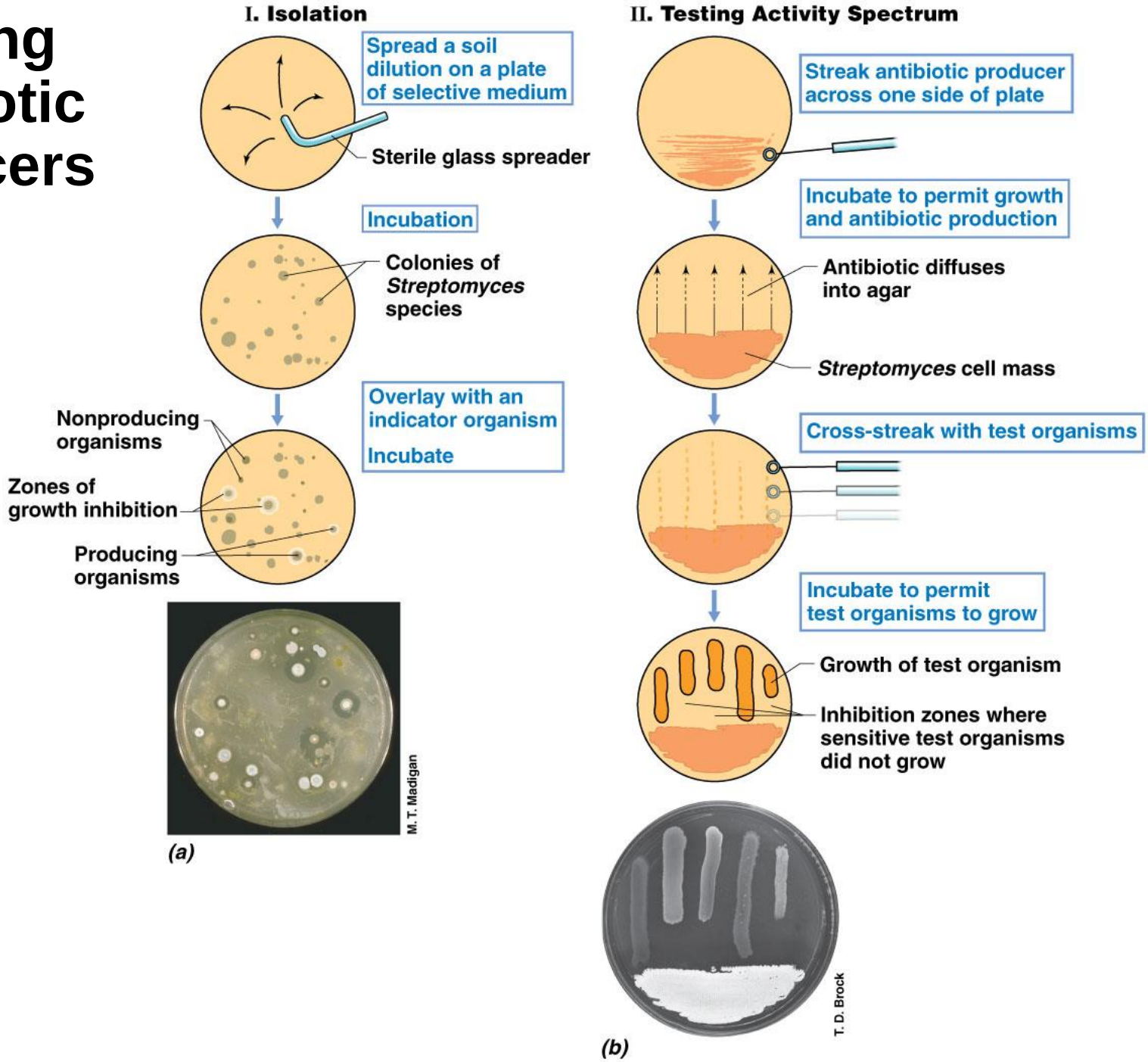
Table 15.3 *Some antibiotics produced commercially*[a]

Antibiotic	*Producing microorganism*[b]
Bacitracin	*Bacillus licheniformis* (EFB)
Cephalosporin	*Cephalosporium* spp. (F)
Cycloheximide	*Streptomyces griseus* (A)
Cycloserine	*Streptomyces orchidaceus* (A)
Erythromycin	*Streptomyces erythreus* (A)
Griseofulvin	*Penicillium griseofulvum* (F)
Kanamycin	*Streptomyces kanamyceticus* (A)
Lincomycin	*Streptomyces lincolnensis* (A)
Neomycin	*Streptomyces fradiae* (A)
Nystatin	*Streptomyces noursei* (A)
Penicillin	*Penicillium chrysogenum* (F)
Polymyxin B	*Bacillus polymyxa* (EFB)
Streptomycin	*Streptomyces griseus* (A)
Tetracycline	*Streptomyces rimosus* (A)

[a]See Chapter 26 for structures and more discussion of these antibiotics.

[b]EFB, endospore-forming bacterium; F, fungus; A, actinomycete.

Finding Antibiotic Producers



After Finding an Antibiotic Producing Microbe

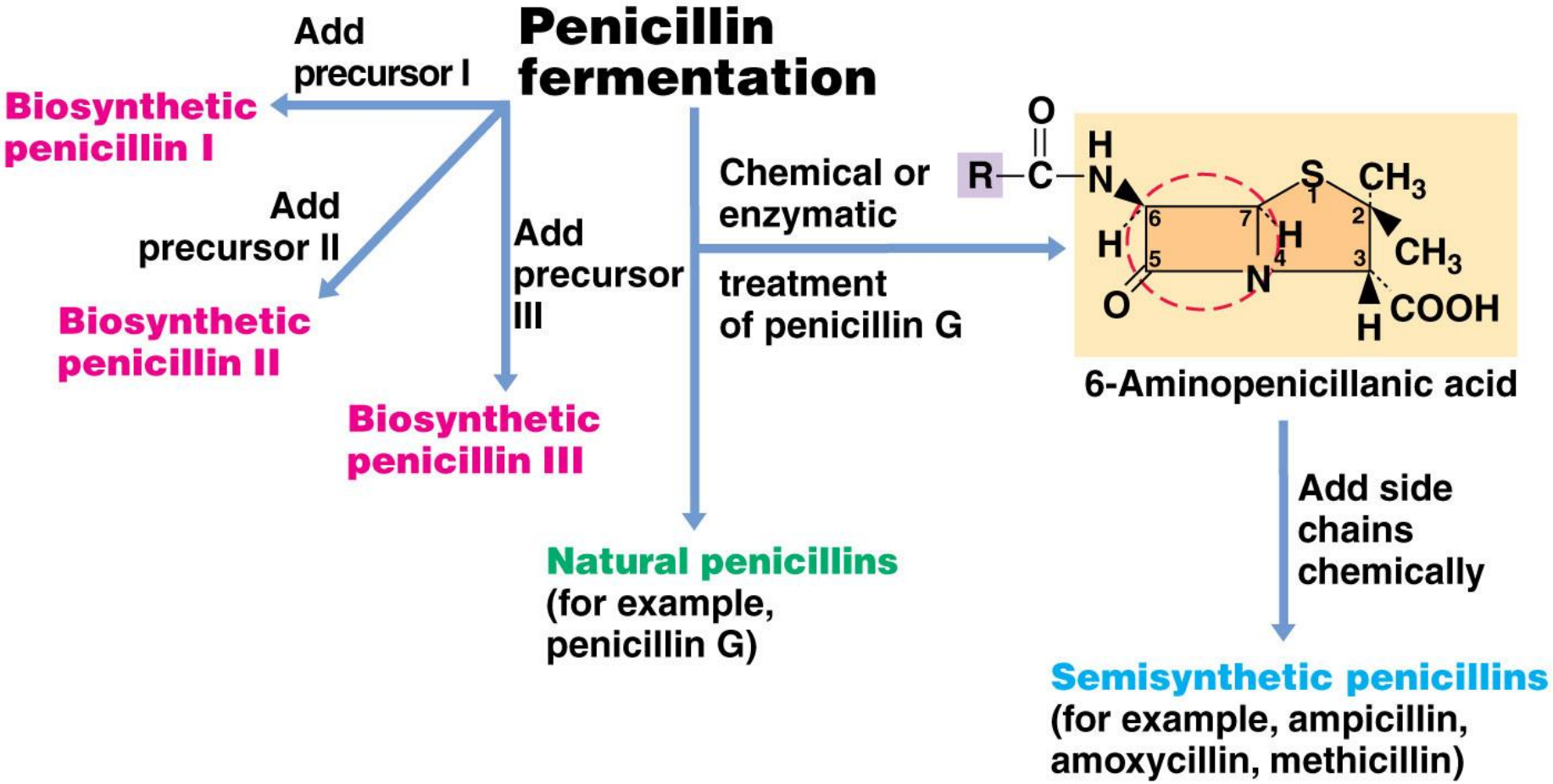
1. **Test for antibiotic yield…need high yielding strains to produce enough for the next steps.**
2. **Purification so that a highly pure crystalline product.**
3. **Chemical Identification, and testing tolerance in animal models.**

After Finding an Antibiotic Producing Microbe

1. **Test for antibiotic yield…need high yielding strains to produce enough for the next steps.**
2. **Purification so that a highly pure crystalline product.**
3. **Chemical Identification, and testing tolerance in animal models.**

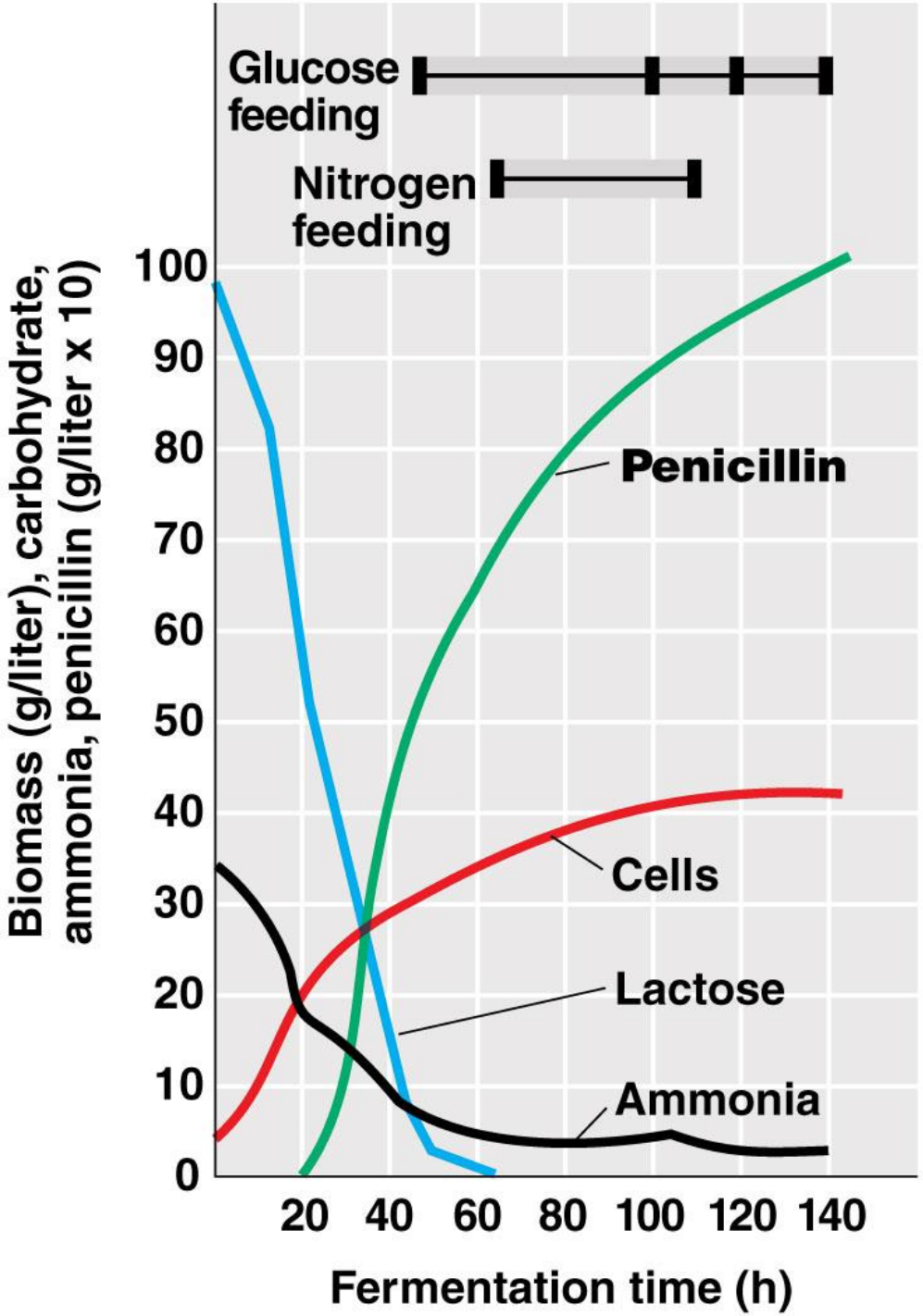
Clicker Question:

Industrial Production of Penicillin



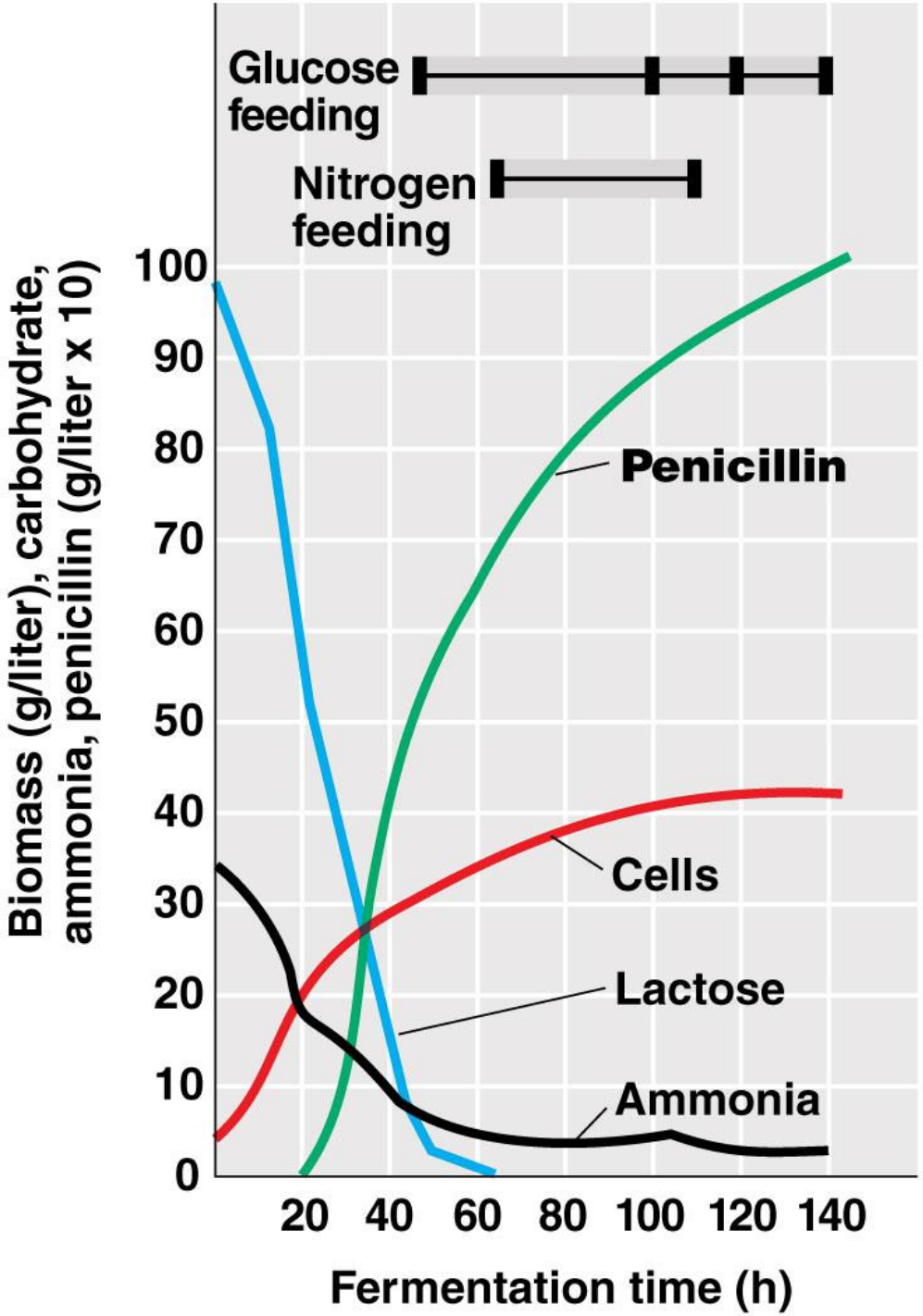
Fermenter Kinetics with *Penicillium chrysogenum*

Clicker Question:



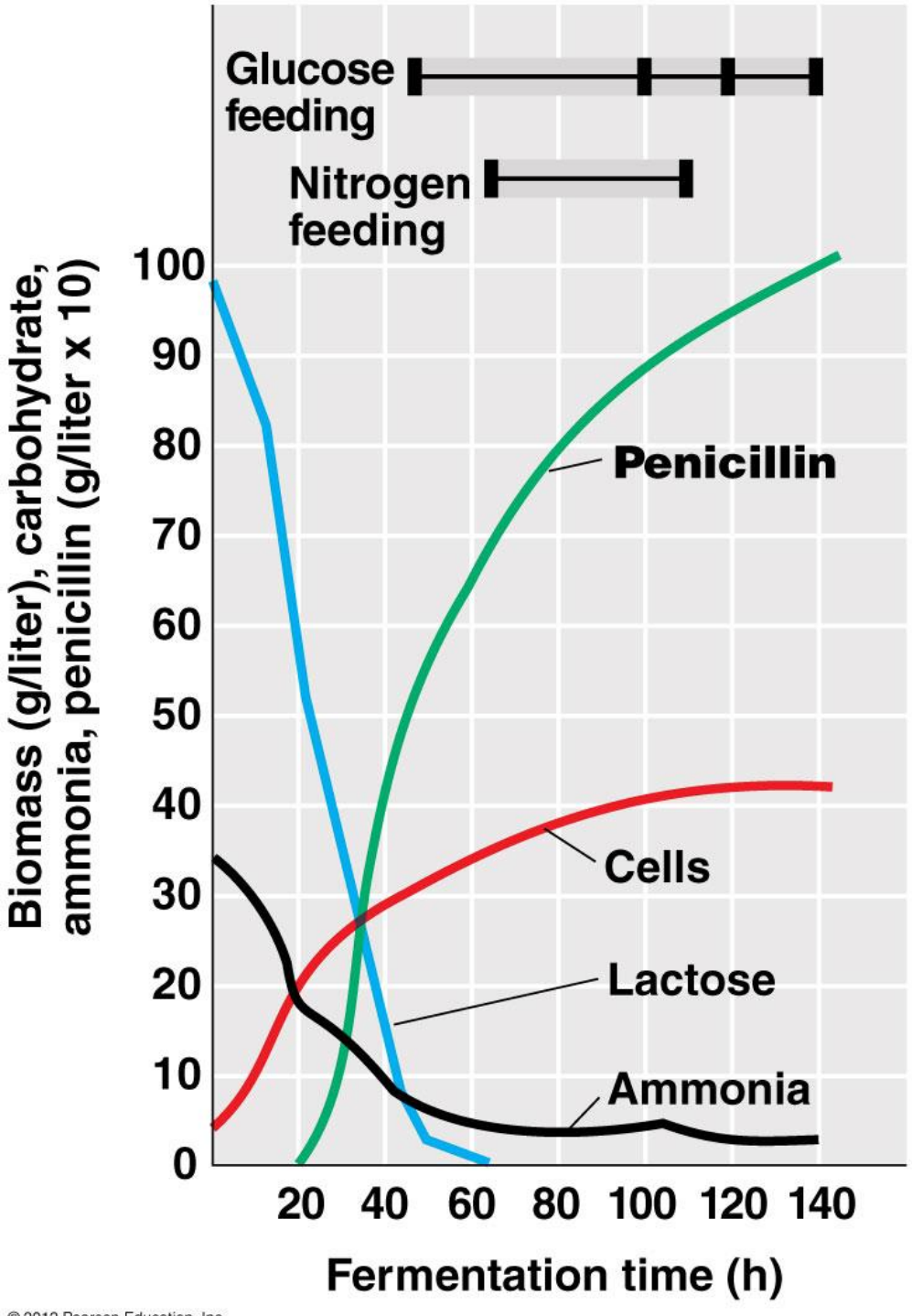
Fermenter Kinetics with *Penicillium chrysogenum*

Clicker Question:



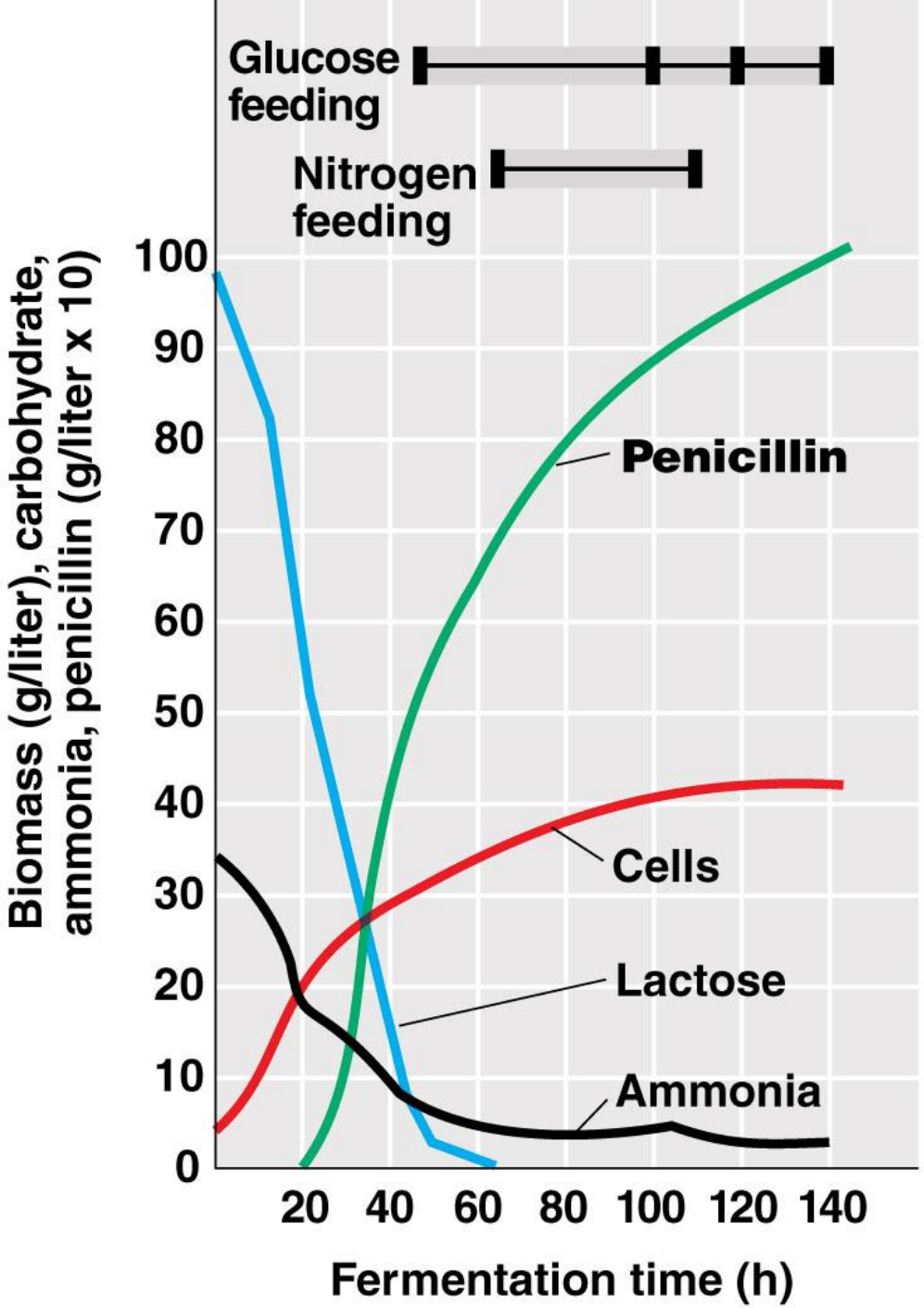
Fermenter Kinetics with *Penicillium chrysogenum*

Clicker Question:

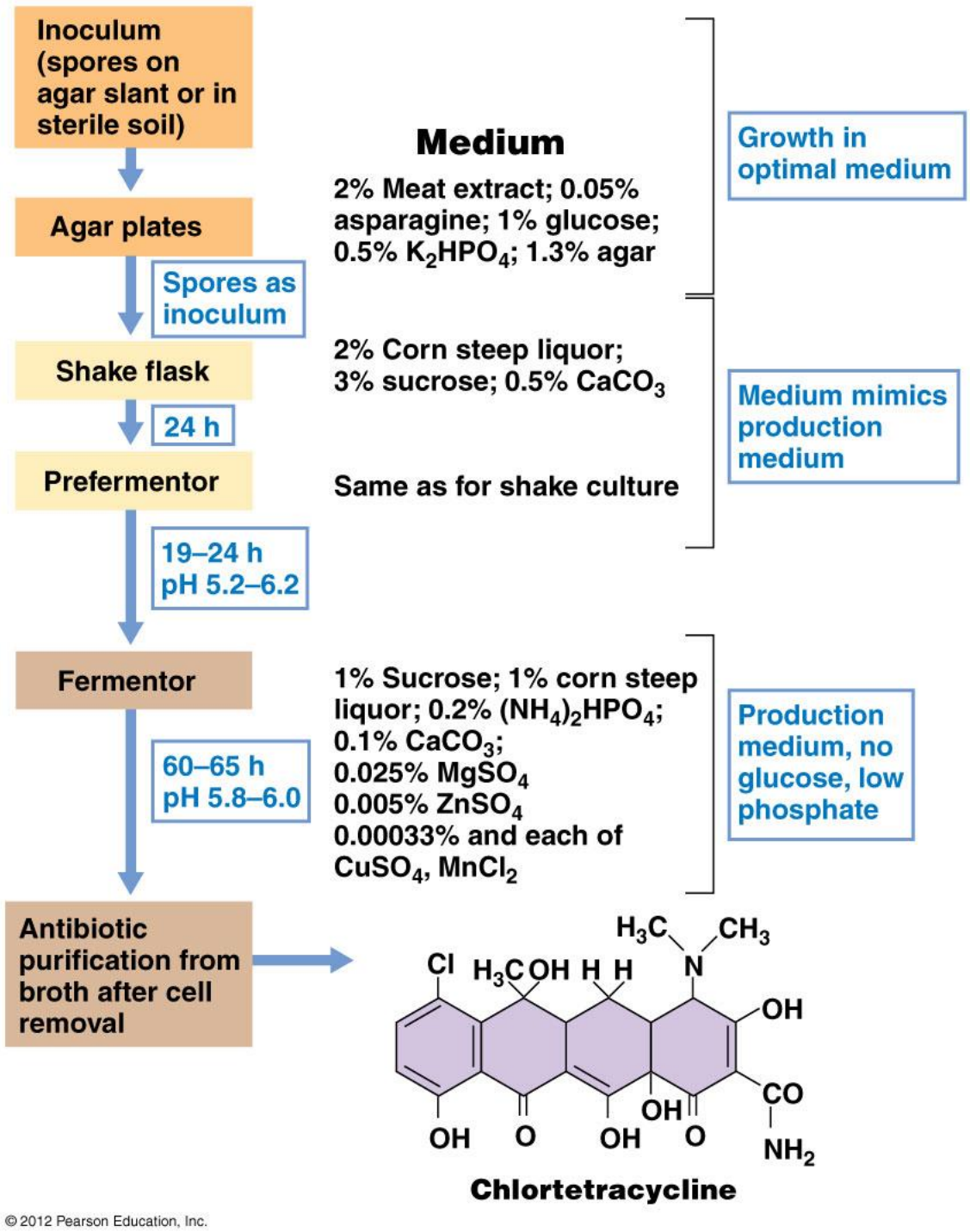


Fermenter Kinetics with *Penicillium chrysogenum*

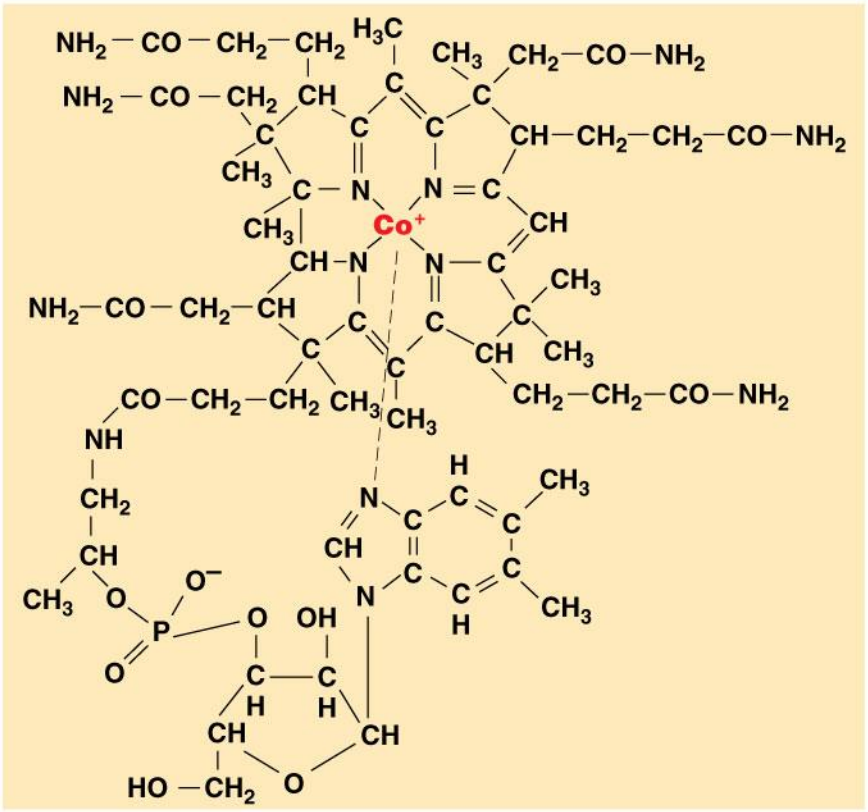
Clicker Question:



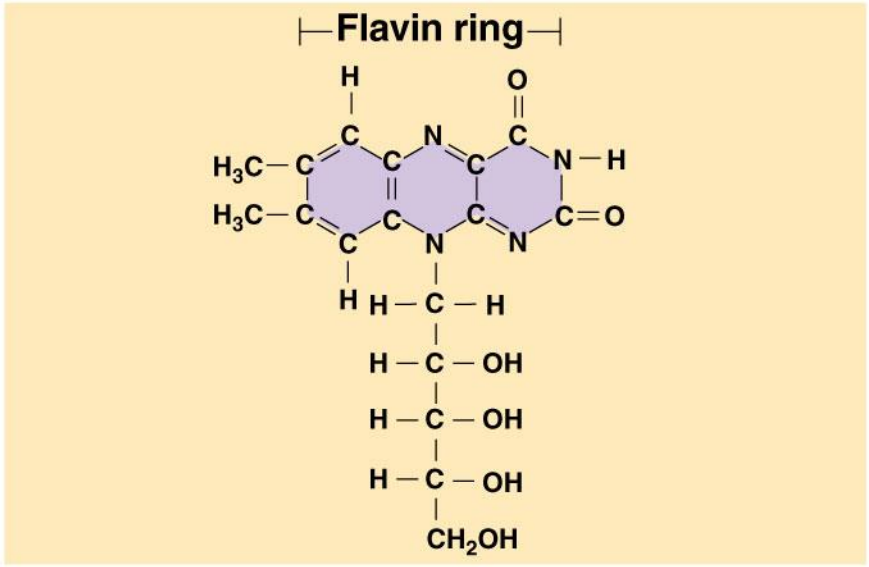
Tetracycline Production



Vitamin Production



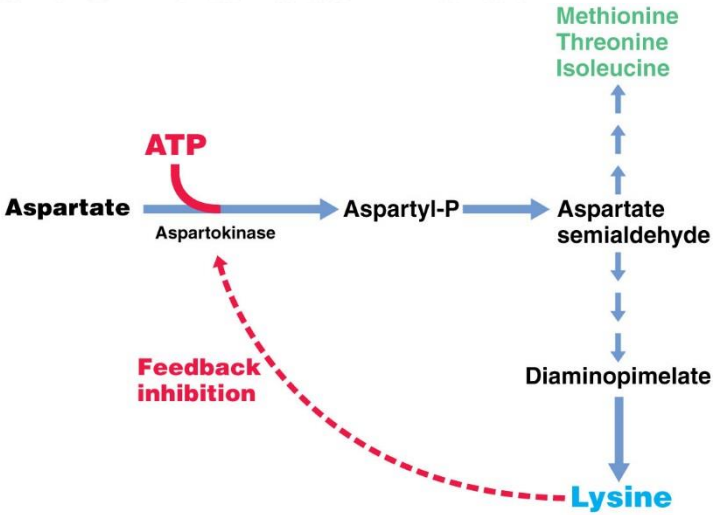
(a) B_{12}



(b) Riboflavin

Table 15.4 *Amino acids used in the food industry*[a]

Amino acid[b]	*Annual production worldwide (tons)*	*Uses*	*Purpose*
L-Glutamate (monosodium glutamate, MSG)	1,000,000	Various foods	Flavor enhancer; meat tenderizer
L-Aspartate and L-alanine	13,000	Fruit juices	"Round off" taste
Glycine	6,000	Sweetened foods	Improves flavor; starting point for organic syntheses
L-Cysteine	700	Bread	Improves quality
		Fruit juices	Antioxidant
L-Tryptophan + L-Histidine	400	Various foods, dried milk	Antioxidant, prevent rancidity; nutritive additives
Aspartame (made from L-phenylalanine + L-aspartic acid)	7,000	Soft drinks, chewing gum, many other "sugar-free" products	Low-calorie sweetener
L-Lysine	800,000	Bread, cereal, and feed additives	Nutritive additive
DL-Methionine	70,000	Soy products, feed additives	Nutritive additive



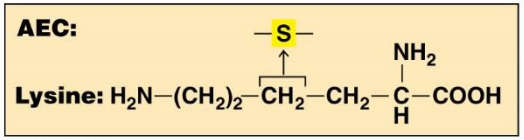


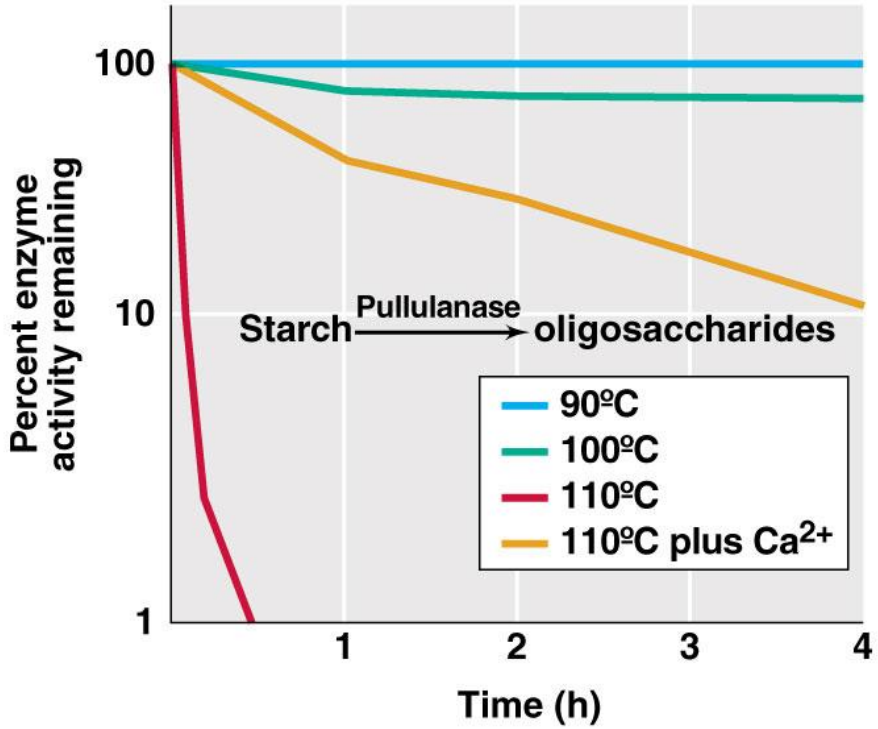
Table 15.5 *Microbial enzymes and their applications*

Enzyme	*Source*	*Application*	*Industry*
Amylase (starch-digesting)	Fungi	Bread	Baking
	Bacteria	Starch coatings	Paper
	Fungi	Syrup and glucose manufacture	Food
	Bacteria	Cold-swelling laundry starch	Starch
	Fungi	Digestive aid	Pharmaceutical
	Bacteria	Removal of coatings (desizing)	Textile
	Bacteria	Removal of stains; detergents	Laundry
Protease (protein-digesting)	Fungi	Bread	Baking
	Bacteria	Spot removal	Dry cleaning
	Bacteria	Meat tenderizing	Meat
	Bacteria	Wound cleansing	Medicine
	Bacteria	Desizing	Textile
	Bacteria	Household detergent	Laundry
Invertase (sucrose-digesting)	Yeast	Soft-center candies	Candy
Glucose oxidase	Fungi	Glucose removal, oxygen removal	Food
		Test paper for diabetes	Pharmaceutical
Glucose isomerase	*Bacteria*	High-fructose corn syrup	Soft drink
Pectinase	Fungi	Pressing, clarification	Wine, fruit juice
Rennin	Fungi	Coagulation of milk	Cheese
Cellulase	*Bacteria*	Fabric softening, brightening; detergent	Laundry
Lipase	Fungi	Break down fat	Dairy, laundry
Lactase	Fungi	Breaks down lactose to glucose and galactose	Dairy, health foods
DNA polymerase	*Bacteria, Archaea*	DNA replication in polymerase chain reaction (PCR) technique (↻ Section 6.11)	Biological research; forensics

Extremozymes

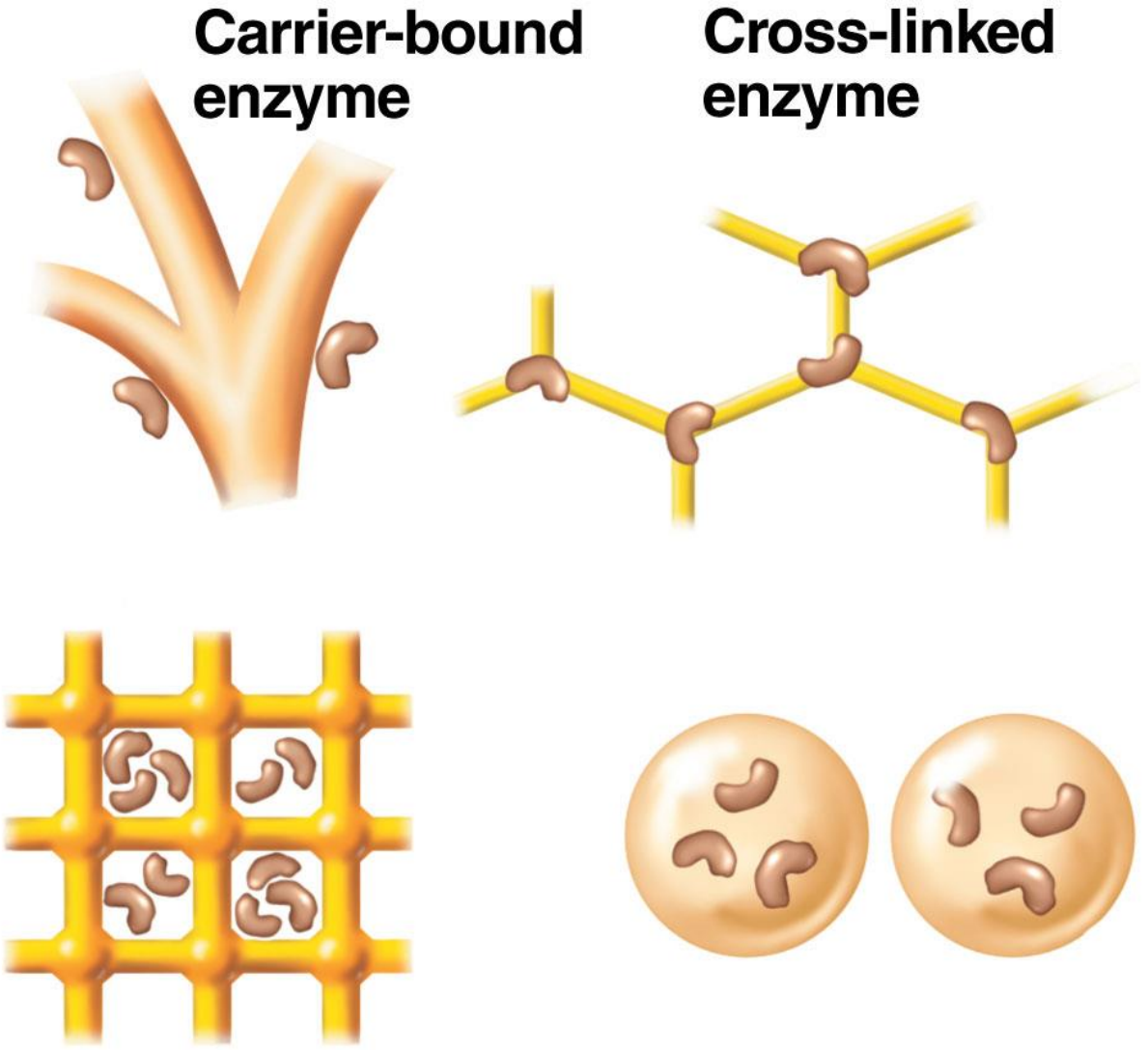
Finnfeeds International

(a) Acid Tolerant Enzymes to Digest Fibrous Foods



(b) Pullulanase from *Pyrococcus woesei* which has a growth opt temp = 100ºC

Immobilized Enzymes: Reactor Columns



Wine Fermentation

The Christian Brothers Winery

(a)

The Christian Brothers Winery

(b)

The Christian Brothers Winery

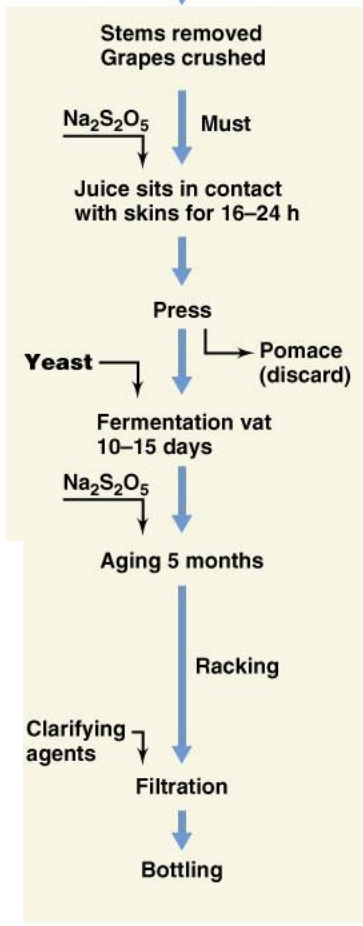
(c)

M.T. Madigan

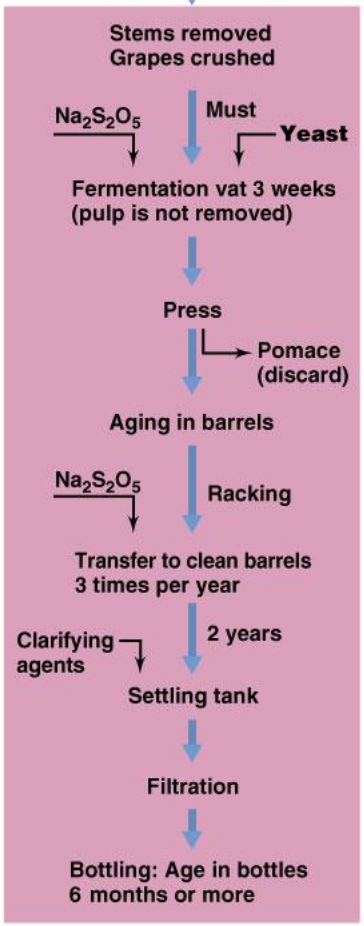
(d)

1. It starts with the type of grape, *Vitis vinefera*.
2. The yeast: *Saccharomyces ellipsoideus.*
3. Then finished with the malolactic fermentation (acid tolerant *Oenococcus, Lactobacillus* and *Pediococcus*).

M.T. Madigan



(a) White wine



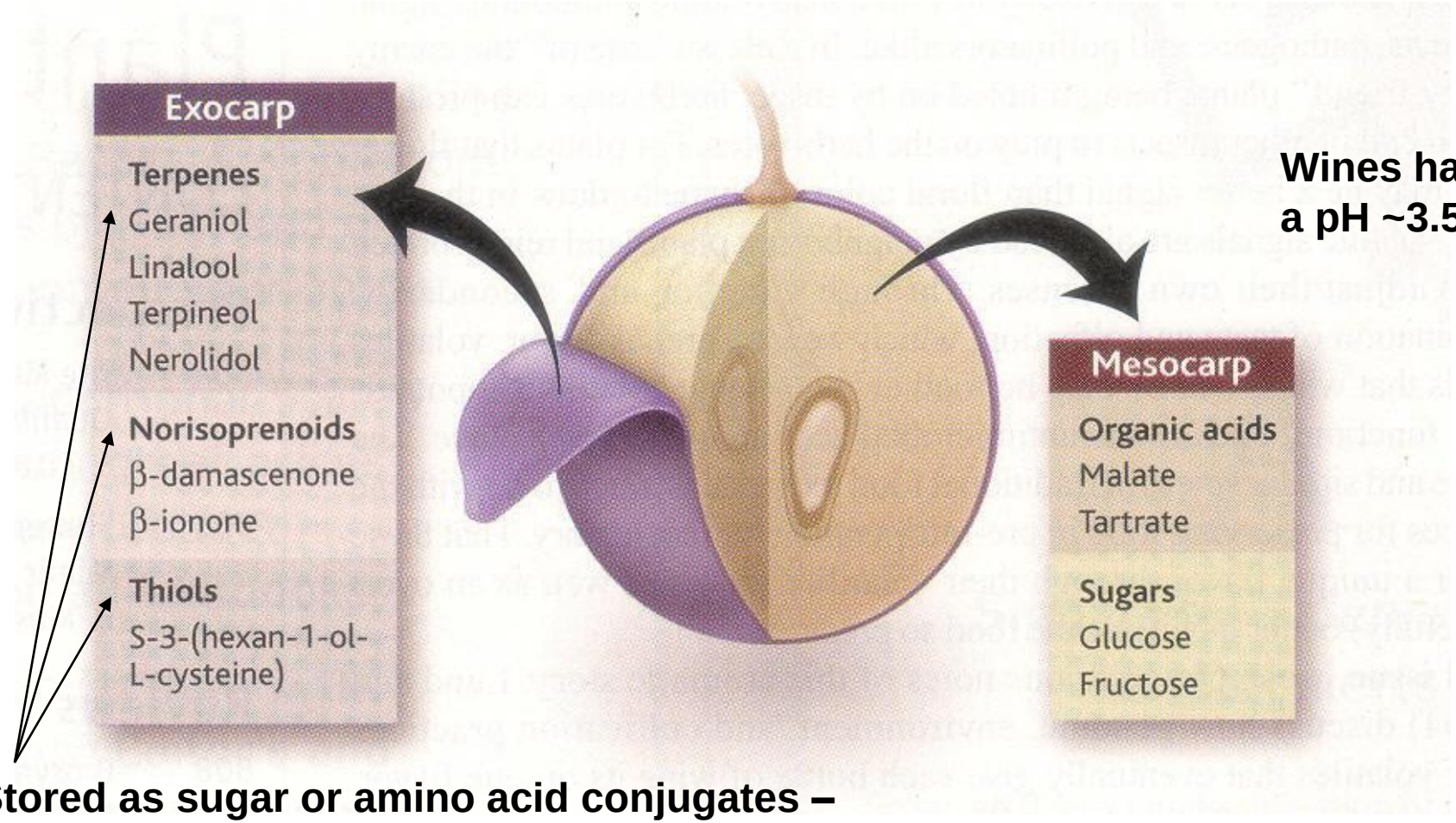
(b) Red wine

Vitis vinefera, what a beautiful plant!

Sauvingon Blanc **Cabernet Sauvingon** **Pinot Noir**

Cultivars

Grape Chemistry



Wines have a pH ~3.5

Stored as sugar or amino acid conjugates – liberated during crushing and enzymatic attack during fermantation

Science, 10 February 2006 vol 311 pg 804

Aging Barrels in Beringer Winery Caves

Malolactic Fermentation: Convert Malate ➔ Lactate + CO_2

Uncontrolled vs Controlled Fermentation

The Evolution of Wine Making ?

How did it start? **- required non-leaking vessels, and**
- making juice…with the discovery that old juice became a bit different, and what seemed to be a great idea.

Clicker Question:

Uncontrolled vs Controlled Fermentation

The Evolution of Wine Making ?

How did it start? - required non-leaking vessels, and
- making juice…with the discovery that old juice became a bit different, and what seemed to be a great idea.

Some how – they could control the fermentation by using the gooey stuff at the bottom of the barrel to start the next round of fermentation.

This reduced tremendous variation in batches.

This continued for a few thousands of years.

Then came the pure culture technique (~1880's)

Wine is Good for You

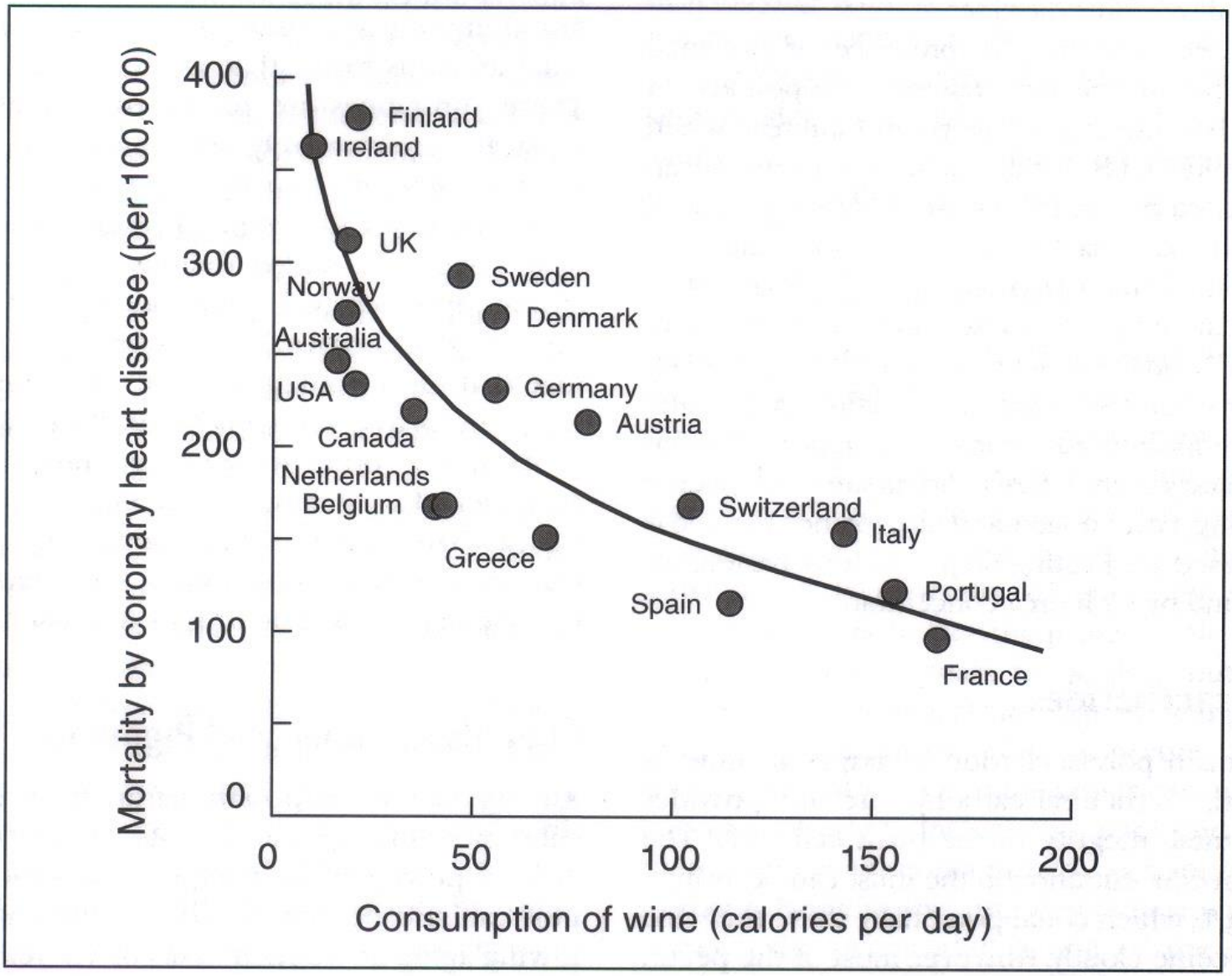


Figure 1. Correlation between wine consumption in eighteen industrialized countries and mortality by coronary heart disease. Adapted from Renaud and de Lorgeril, 1992.

Beer is Made from Malted Barley

"Beer is proof the God loves us and wants us to be happy"
….Benjamin Franklin

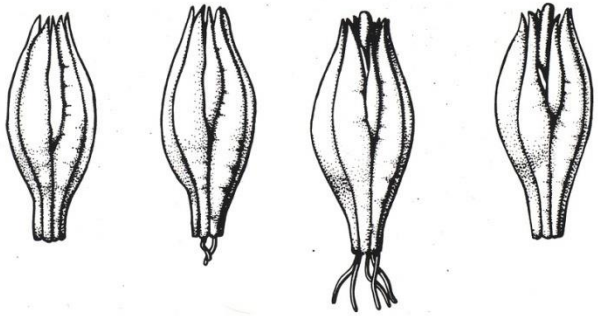


FIGURE 15–19
In the process of malting, barley is permitted to germinate until the emerging embryonic seedling is one-third the length of the fruit. At that point the fruits are kiln-dried (which shrivels the emerging rootlet) and shipped to breweries.

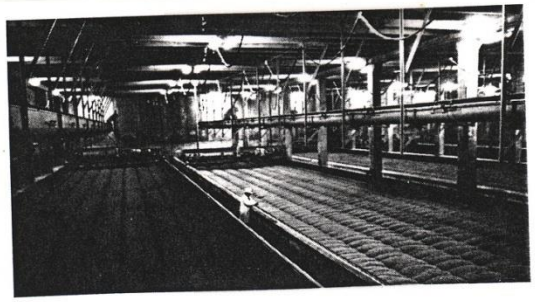
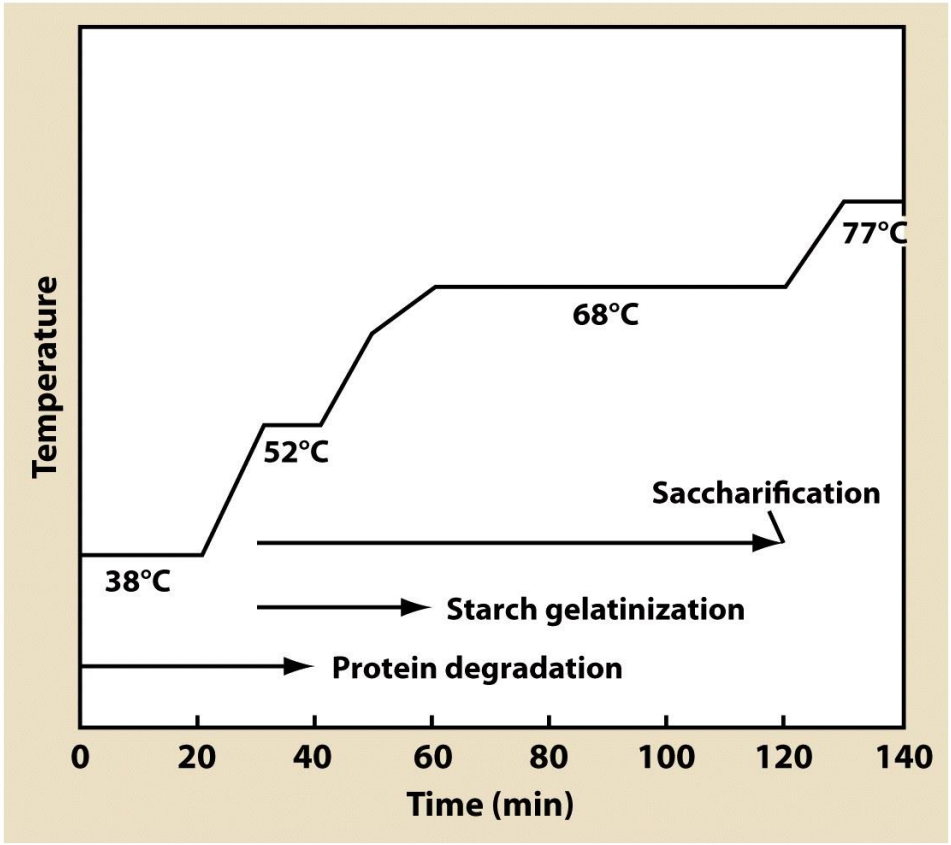


FIGURE 15–18
Barley in the malting room is turned continuously to ensur even germination. *(Courtesy the Rahr Malting Company.)*

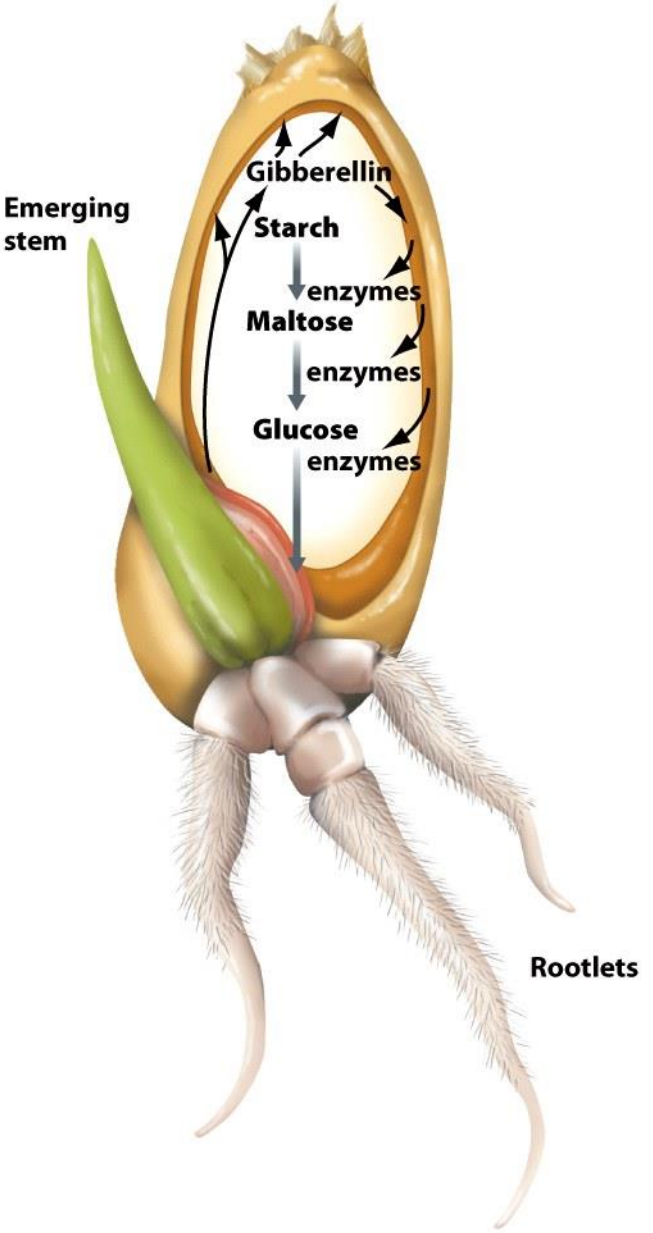
FIGURE 15–17
Modern beer making is an exacting science, as the elaborate equipment and computerized control board of the Lone Star Brewing Company in San Antonio, Texas, show.

Mashing – Temp Regulation

Making the WORT

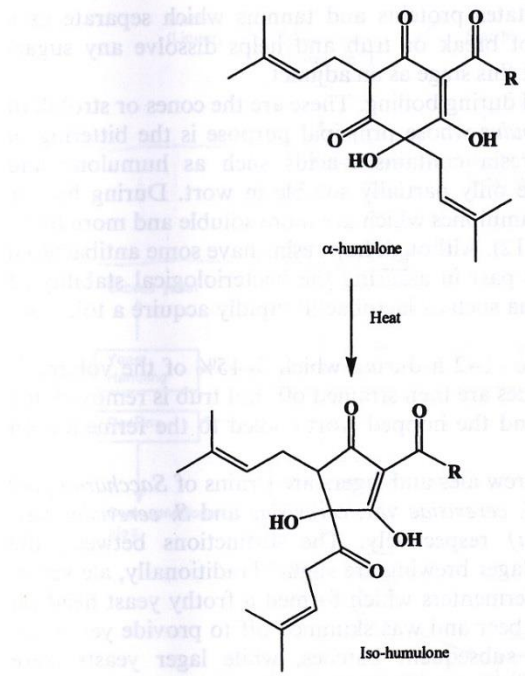


Special Topic 16.2 figure 3 Microbiology: An Evolving Science



Special Topic 16.2 figure 2 Microbiology: An Evolving Science

Hops



$R = -CH_2.CH(CH_3)_2$ humulone

$R = -CH(CH_3)_2$ cohumulone

$R = -CH(CH_3).CH_2.CH_3$ adhumulone

Figure 9.12 *Isomerization of hop α- and β-acids*

Beer

Busch Creative Services, Anheuser Busch Company

(a)

Busch Creative Services, Anheuser Busch Company

(b)

Busch Creative Services, Anheuser Busch Company

(c)

Busch Creative Services, Anheuser Busch Company

(d)

Home Brew

Bryon Burch

(a) **Boiling the Wort**

Bryon Burch

(b) **Fermentation**

Bryon Burch

(c) **Bottling**

Barton Spear

(d) **Pils** **Dark**

Whiskey's

Barley or Grape Fermentation ➔ Distilled, usually to 100 proof (50% ethanol).

Distillate ➔ Aging Barrels (oak) for 10-12 years ending up about 80 proof along with a great change in flavor.

Barley fermentation (beer) distillate ➔ aging to make **Scotch**.

Barley, Corn + Rye (beer) distillate ➔ aging in charred barrels =

Bourbon if done in the State of Kentucky

Others named **Whiskey** if done out of Kentucky.

Barley, Wheat + Rye (beer) distillate ➔ aging to make **Irish Whiskey**

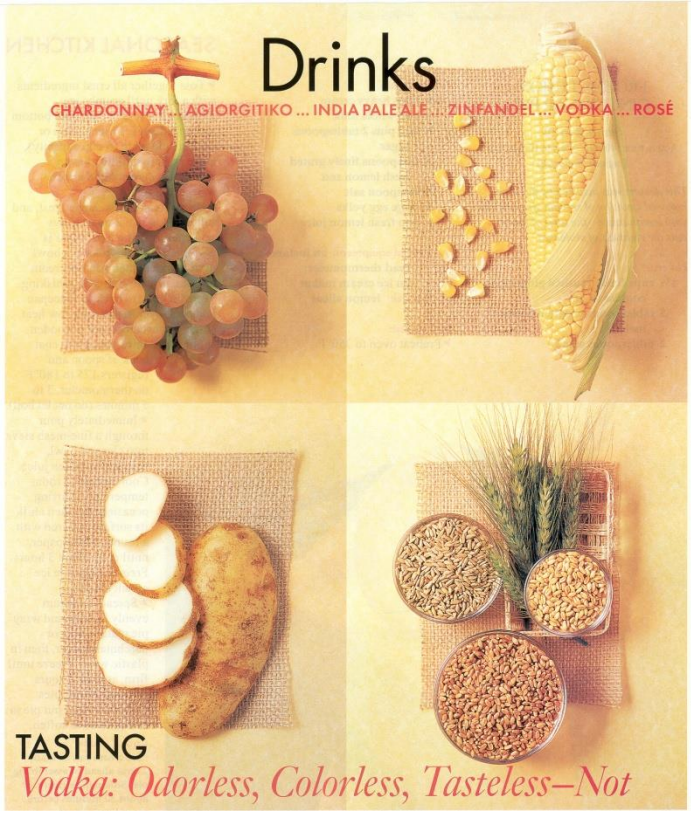
Barley + Rye (beer) distillate ➔ aging to make **Canadian Whiskey**.

Brandies and Vodkas

Grape wines distillate ➔ aging to **Cognac** (only in the Cognac Department of France), **Brandies** everywhere else.

Classical Vodka from potato….with little flavor other than ethanol.

New trendy vodkas:



BRAND	MADE FROM	$	COMMENTS
Stolichnaya Gold, Russia	Wheat	$30	Big, round, slightly citrus nose. Sweet and velvety, long and complex.
Ultimat, Poland	Rye, wheat, potato	$56	Lively chocolate and citrus aromas. Extremely smooth.
Pearl, Canada	Wheat	$24	Very clean nose. Chocolaty-smooth in mouth, but still stands up to the tonic.
Teton Glacier, Idaho	Potato	$20	Crisp lemon nose, peppery. Nice viscosity, rich, with a hint of licorice. Very long.
Türi, Estonia	Rye	$26	Clean, crisp chocolate aromas. Delicate, with a sweet finish.
Ketel One, The Netherlands	Wheat	$22	Slightly medicinal smell. Very elegant and rich. Smooth, with a touch of spice and a long finish.
Grey Goose, France	Grain	$30	Interesting pine and root beer notes on nose. Almost gin-like flavor.
Citadelle, France	Wheat	$24	Anise and black-currant aromas. Complex and quite distinctive flavors

Ethanol as a Biofuel

Chris Standlee and DOE/NREL

(a) Corn based Ethanol

(b) Switch Grass

Clicker Question:

Ethanol as a Biofuel

Chris Standlee and DOE/NREL

(a) Corn based Ethanol

(b) Switch Grass

Clicker Question:

Ethanol as a Biofuel

Chris Standlee and DOE/NREL

(a) Corn based Ethanol

(b) Switch Grass

Clicker Question:

Ethanol as a Biofuel

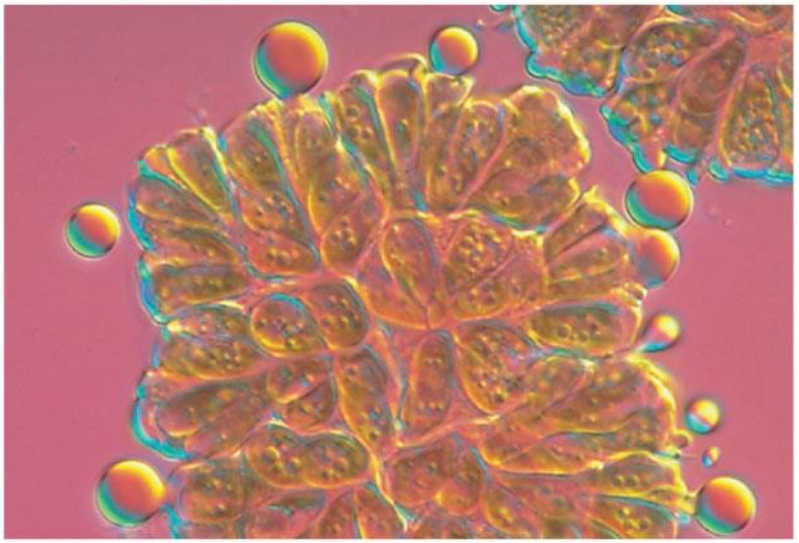
Chris Standlee and DOE/NREL

(a) Corn based Ethanol

(b) Switch Grass

Clicker Question:

Oil from Algae



(c) *Botryococcus braunii.*

The oil droplets are evident in this phase micrograph, some have been expelled and are droplets in the medium. These oil droplets have 30-36 carbon hydrocarbons.

Clicker Question:

Expressing Mammalian Genes in Bacteria

There are a few Problems … that can be overcome:

1. **Expressing intron-free genes in bacteria.**

Clicker Question:

Expressing Mammalian Genes in Bacteria

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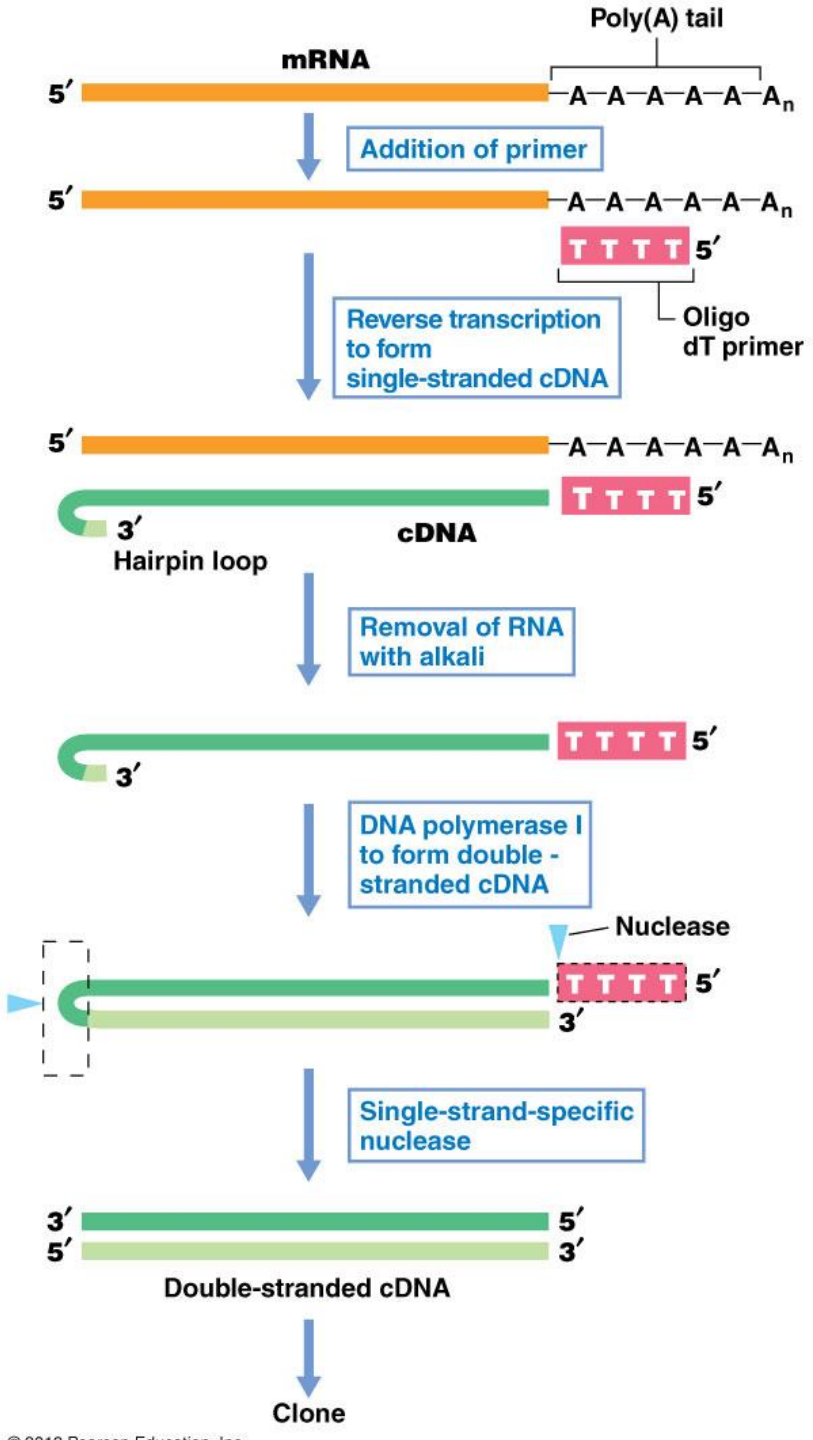
1. **Expressing intron-free genes in bacteria.**

Clicker Question:

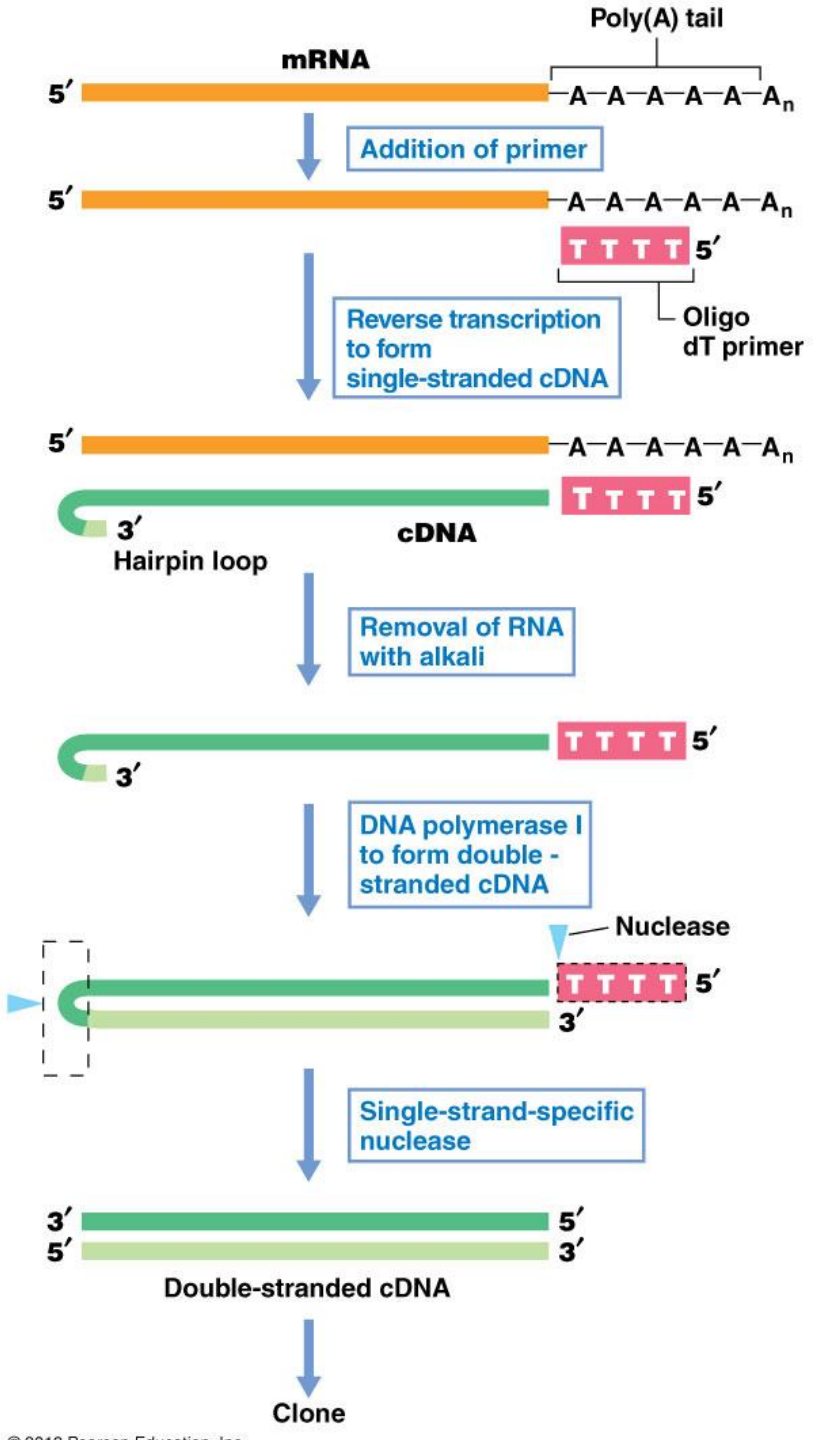
Expressing intron-free genes in bacteria.

Clicker Question:



Expressing intron-free genes in bacteria.

Clicker Question:



Expressing Mammalian Genes in Bacteria

There are a few Problems … that can be overcome:

1. **Expressing intron-free genes in bacteria.**

2. **Purification of the Protein.**
   a. **Is it expressed in the cytoplasm or exported to the medium**
   b. **Fusion proteins and other ways to simplfy purification.**

3. **Testing the purity AND safety.**

Table 15.6 *A few therapeutic products made by genetic engineering*

Product	*Function*
Blood proteins	
Erythropoietin	Treats certain types of anemia
Factors VII, VIII, IX	Promotes blood clotting
Tissue plasminogen activator	Dissolves blood clots
Urokinase	Promotes blood clotting
Human hormones	
Epidermal growth factor	Wound healing
Follicle-stimulating hormone	Treatment of reproductive disorders
Insulin	Treatment of diabetes
Nerve growth factor	Treatment of degenerative neurological disorders and stroke
Relaxin	Facilitates childbirth
Somatotropin (growth hormone)	Treatment of some growth abnormalities
Immune modulators	
α-Interferon	Antiviral, antitumor agent
β-Interferon	Treatment of multiple sclerosis
Colony-stimulating factor	Treatment of infections and cancer
Interleukin-2	Treatment of certain cancers
Lysozyme	Anti-inflammatory
Tumor necrosis factor	Antitumor agent, potential treatment of arthritis
Replacement enzymes	
β-Glucocerebrosidase	Treatment of Gaucher disease, an inherited neurological disease
Therapeutic enzymes	
Human DNase I	Treatment of cystic fibrosis
Alginate lyase	Treatment of cystic fibrosis

Recombinant Vaccines

Pathogenic Bacteria and Viruses have many genes, a great diversity of proteins.

Clicker Question:

Recombinant Vaccines

Pathogenic Bacteria and Viruses have many genes, a great diversity of proteins.

Clicker Question:

Transgenic Animals and Plants

Wu-Shinn Chih

(a)

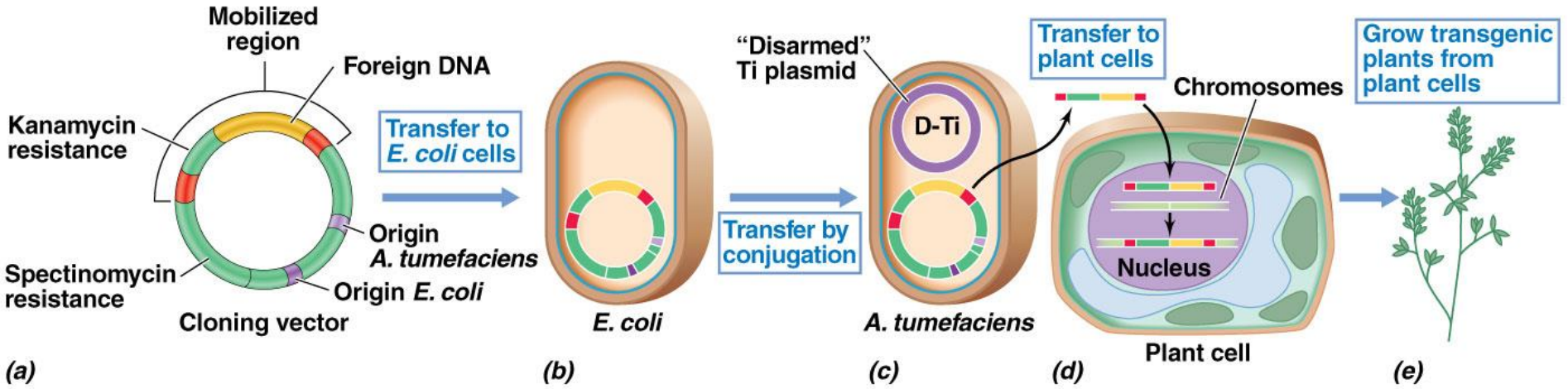
Transgenic salmon is 4.5 kg.

Control salmon is 1.5 kg.

Aqua Bounty Technologies

(b)

Getting a Gene into a Plant

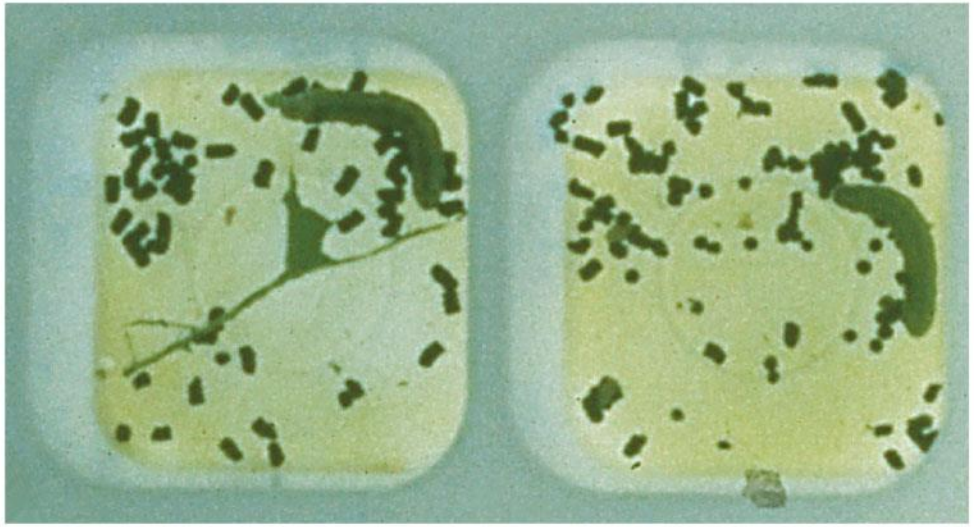


Stephen R. Padgette, Monsanto Company

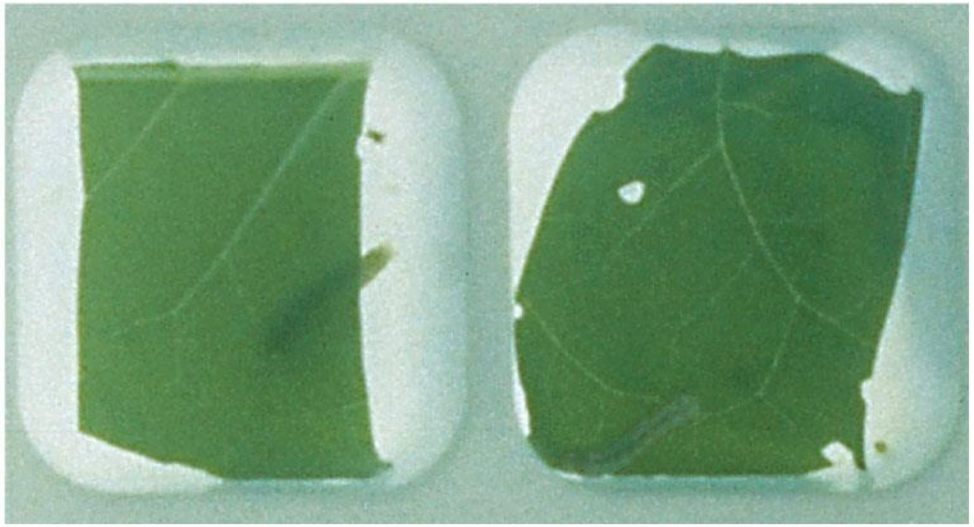
Transgenic Plants

Tobacco plants, one a Bt toxin transgenic plant. Both exposed to armyworm larvae.

Clicker Question:



(a)



(b)

Kevin McBride, Calgene, Inc.