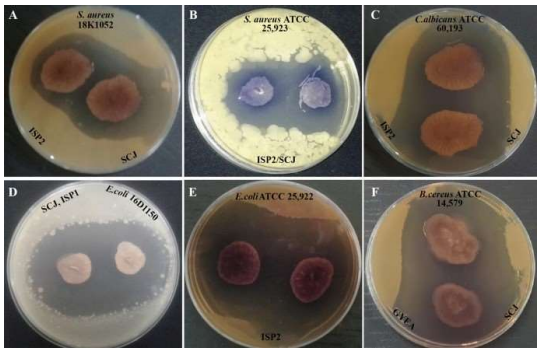
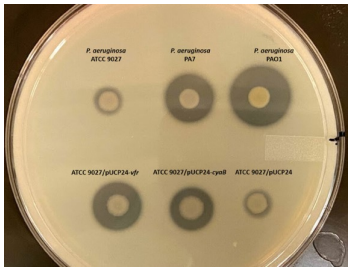
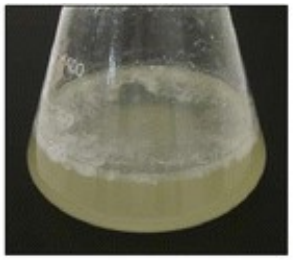
Chapter II. *Industrial microorganisms : isolation, selection, screening and properties*







Paenibacillus sp. #510 growing in MSS supplemented with paraffin.

Emulsifying activity against *n*-hexadecane. A:mineral salt solution B:*Paenibacillus* sp. #510.

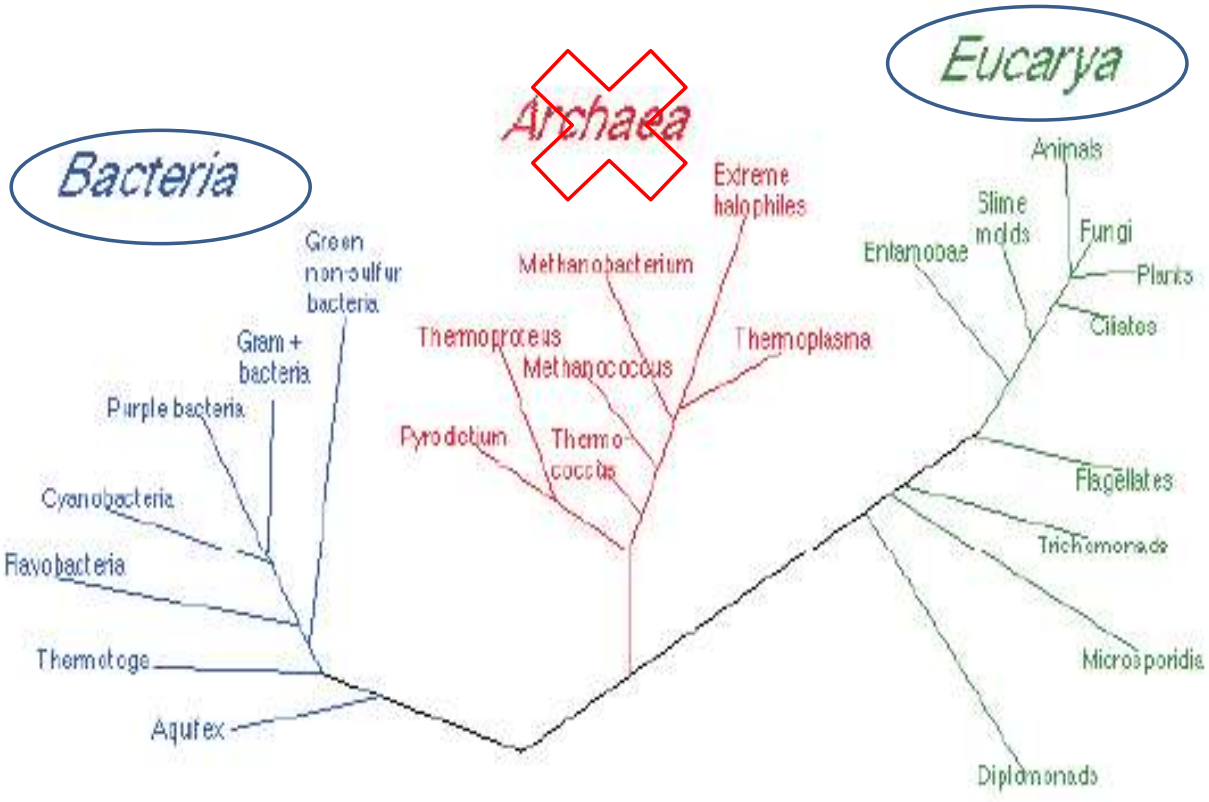


Fig. 2.3 The Three Domains of Living Things Based on Woese's Work

- The microorganisms currently used in industrial microbiology and biotechnology are mainly found in Bacteria and Eukarya, Archaea are not used.
- However, industrial microbiology and biotechnology processes are dynamic, so old processes are systematically discarded when new (more efficient) ones are discovered.
- Currently, Archaea organisms are not used for industrial purposes, but this may change in the future.

I. Bacteria

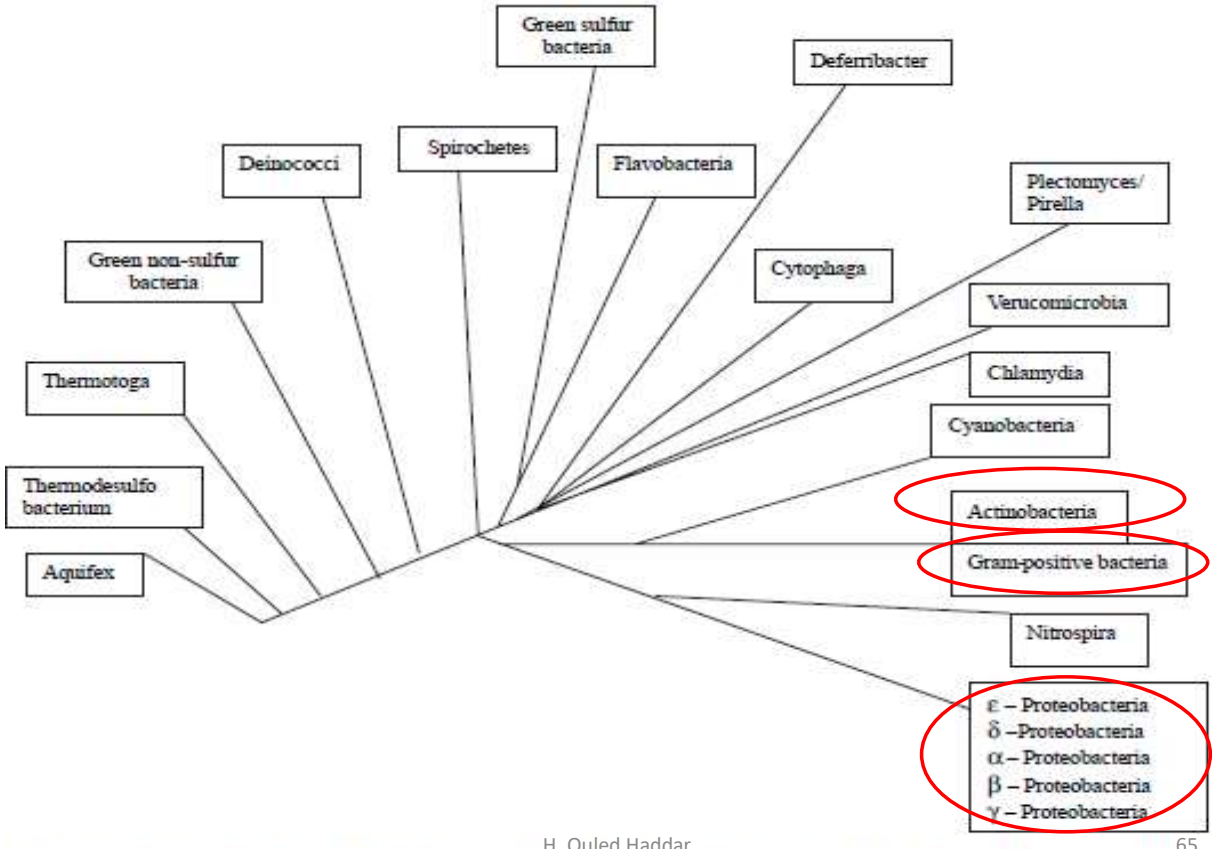
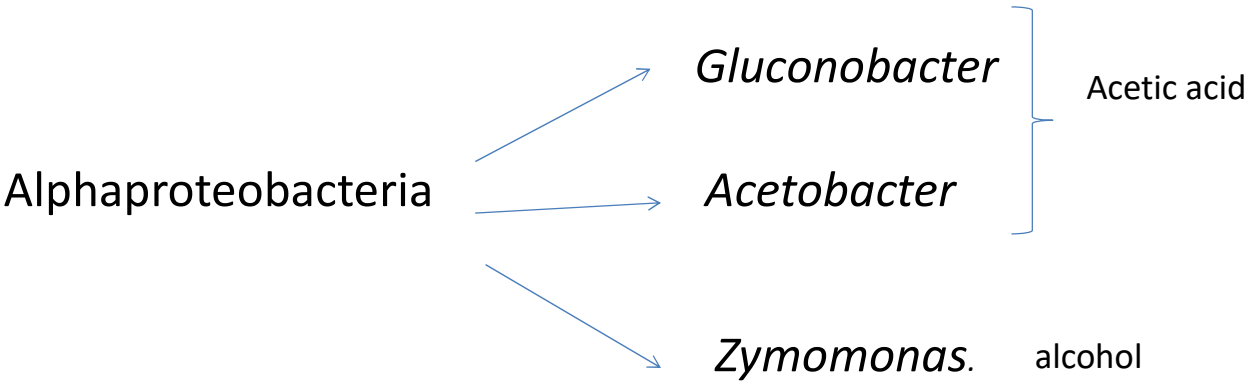


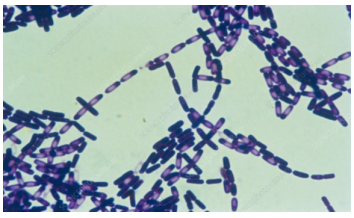
Fig. 2.4 The 18 Phyla of Bacteria Based on 16S RNA Sequences (After Madigan and Matinko, 2006)

I.I. Proteobacteria



I. II. Firmicutes

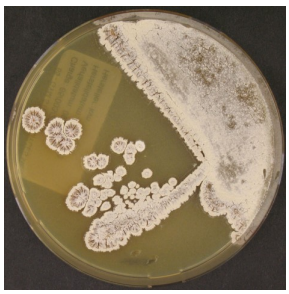
- Firmicutes contain numerous bacteria of industrial interest and are divided into three main groups:



- I. sporulated, (*Bacillus*, *Clostridium*, etc.)
- II. Non-sporulated (lactic acid bacteria, etc.)
- III. wall-less firmicutes (this group contains pathogenic organisms, *Mycoplasma*)

I. III. Actinobacteria

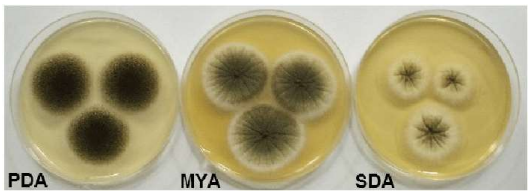
- Actinomycetes (antibiotics and other pharmaceutical products, *Streptomyces*)



- *Corynebacterium* (amino acids)

II.The Eukarya

- Phycomycetes: Rhizopus, Mucor (enzymes)
- Ascomycetes: Yeasts (ethanol and alcoholic beverages), *Claviceps purpurea* (ergot alkaloids)
- Imperfect fungi: *Aspergillus* (aflatoxin) *Penicillium* (penicillin)



- Basidiomycetes, *Agaricus*, mushrooms

Characteristics of microorganisms used in industrial microbiology

- Microorganisms should be capable of growing in a simple medium, without added growth factors (vitamins, nucleotides and acids) except those naturally present in the industrial medium in which they are cultivated.
- It is obvious that additional growth factors increase the cost of fermentation and consequently that of the end-product.

- Microorganisms should be able to grow vigorously and rapidly in the selected medium for the reasons below:

✓The low growth rate exposes the culture to a greater risk of contamination.

✓The profit rate is reduced if low growth rate microorganisms are used.

- Microorganisms should grow quickly, but they should also produce the desired metabolite (cells or metabolic products) as rapidly as possible, for the same reasons indicated above.

- End-products should be free of toxic substances or other unwanted materials, especially when intended for consumption (such as medication or food).

- Microorganisms should exhibit acceptable genetic and physiological stability,

A microrganism that mutates easily can produce undesirable substances, resulting in a reduced yield of the metabolite of interest or the production of a different, potentially toxic substance,

Both situations undermine the industry's primary objective: **profit maximization**

- At the end of fermentation, the selected microorganism should provide an appropriate and cost-effective method for harvesting the product (downstream process).
- For example, if both yeast and bacteria are suitable for producing a given metabolite, it would be preferable to use the yeast if centrifugation is the most suitable harvesting method
- If possible, microorganisms with physiological requirements that protect them against competition from contaminants should be selected.
- For example, a microorganism that has optimal productivity at high temperatures, low pH values, or is capable of secreting antimicrobial agents will be preferred.
- Thus, an efficient thermophilic producer would be favored over a mesophile

- Microorganisms should be sufficiently resistant to predators, such as bacteriophages.
- Therefore, developing phage-resistant strains should be a fundamental part of the research program in the bioindustry
- When possible, microorganisms should have low oxygen requirements; otherwise, the demand for electric energy for agitation of the fermenter and injection of forced air will significantly increase, accounting for approximately 20% of the cost of the end product.

- Finally, the microorganism should be relatively easy to genetically manipulate, allowing for the development of strains with improved properties.

Selection of microorganisms for bioprocess

- The primary task of an industrial microbiologist is to identify an appropriate microorganism (MO) for the desired process. Selecting and utilizing MOs in industrial microbiology and biotechnology requires a strong understanding of their cultivation, manipulation, and interactions with other organisms

- First, it is essential to identify or create a microorganism (MO) capable of performing the desired process as efficiently as possible. This MO can be isolated from the environment or obtained through molecular genetic modification techniques.

- Additionally, MO culture collections also provide relatively efficient strains

1. De novo MO isolation

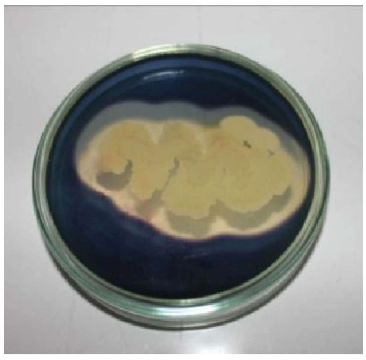
- Although the ubiquity of microorganisms suggests that almost any natural ecological entity (such as water, air, leaves, or tree trunks) can provide them, soil remains the preferred source for isolating microorganisms.
- This is because soil is a vast reservoir of diverse organisms. Indeed, microorganisms capable of utilizing virtually any carbon source can be found in soil when appropriate screening methods are employed.
- Recently, other 'new' habitats, particularly the marine environment, have also been explored in searches for bioactive microbial metabolites.

Enrichment with the substrate used by the desired MO:

- If the desired MO utilizes a particular substrate, the soil will be incubated with that substrate for a certain period (enrichment).
- The incubation conditions are also used to select for a specific organism.
- For example, if a thermophilic organism that hydrolyzes a substrate X, a solid medium containing that substrate is inoculated by an appropriate dilution of the enriched soil and then incubated at high temperatures to facilitate the search for thermophiles

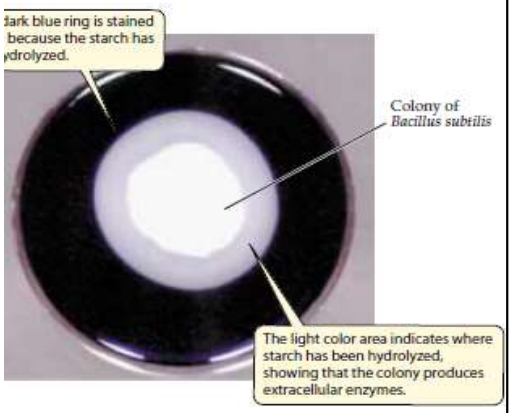
Examples

a. Search for a producer of an extracellular α-amylase:

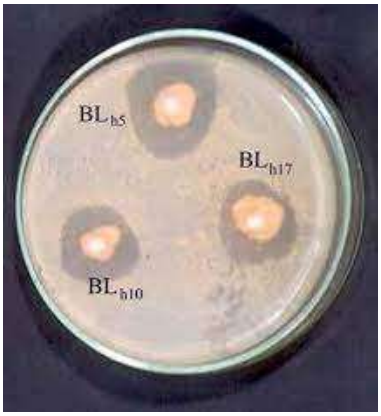


The soil is enriched with starch, and appropriate dilutions of the soil are plated onto agar containing starch as the sole carbon source.

When iodine (stain) is introduced, light areas appear around the colonies on a dark blue background indicating the hydrolysis of starch

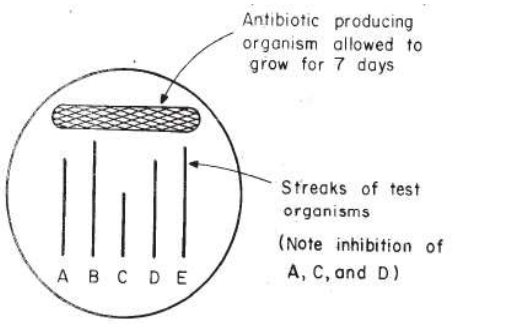


b. Search for an antibiotic producer,



To isolate antibiotic-producing microorganisms, a soil suspension is placed on agar that has been previously inoculated with a reference microorganism which is sensitive to the desired antibiotic.

Zones of inhibition around the colonies indicate the presence of antimicrobial metabolites



The Cross Streak Method for the Primary Search of Antibiotic Producing Organisms

2. Culture collections

There are different types of culture collections (strain banks). Some collections handle a wide variety of organisms, with the most well-known being the American Type Culture Collection (ATCC).

Other collections are specialized and may focus exclusively on pathogenic microorganisms, such as the National Collection of Type Cultures (NCTC) in Colindale, London, or industrial microorganisms, like the National Collection of Industrial Bacteria (NCIB) in Aberdeen, Scotland.

Additionally, some collections manage only one type of organism, such as the Center for Braunschweig (CBS) in the Netherlands, which specializes in fungi

3. Genetic modification of MO

- Genetic manipulations are used to produce MOs with new or improved characteristics.
- Conjugation, transformation and transduction play a vital role in the development of strains,

- Mutation: chemical, UV, Site-directed mutagenesis
- Protoplast fusion
- Cloning
- Metabolic pathway engineering
- ….

Mutation

- Physical mutagenesis: UV
- Chemical mutagenesis:

Base analogues:5,BU

Nitrous acid

Alkylating agents: EMS (ethyl methane sulphonate)

Intercalating agents: acridines

Nitrosoguanidine

The steps

- Exposure of the microorganism to the mutagenic agents
- Selection of mutants (pleotropic mutation, selection on selective medium in the presence of substrate or analogues)
- Screening

Replica plating technique

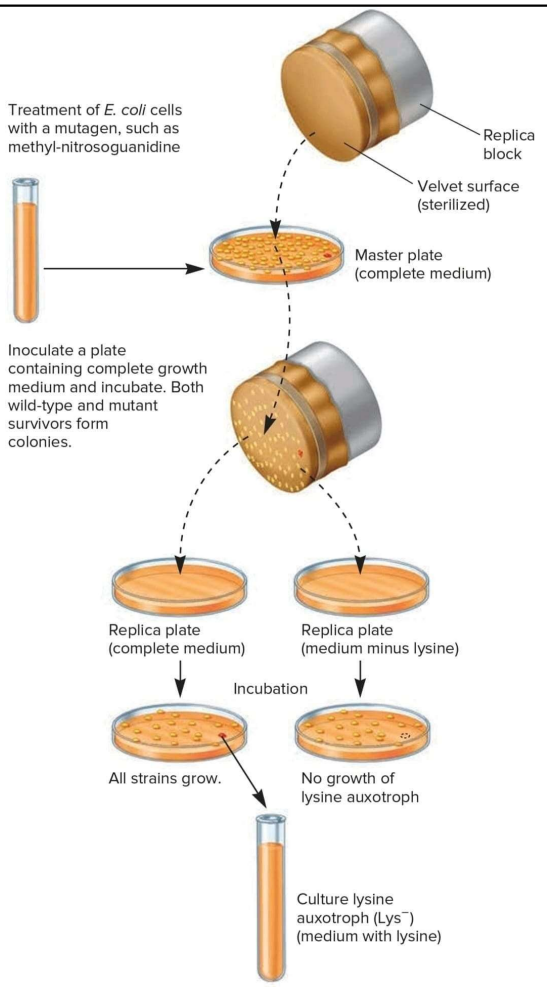
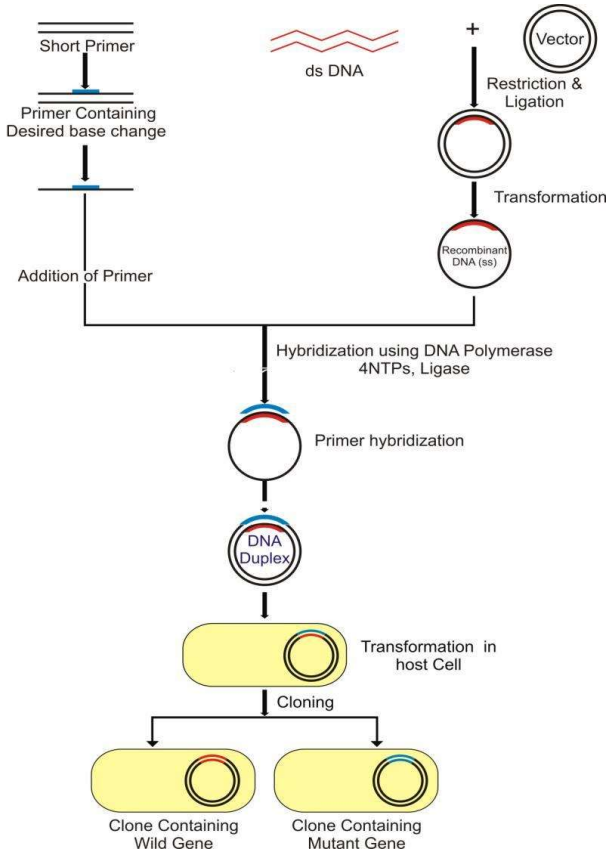


Tableau 3 – Amélioration d'une souche productrice de pénicilline

Identification du micro-organisme	Mutation spontanée ou agent mutagène	Multiplication du rendement par rapport à la souche de Fleming (*Penicillium notatum*)		
Penicillium chrysogenum NRRL 1951		1		
↓ NRRL 1951 B 25	spontanée	× 0,5	perte	Amélioration obtenue par les chercheurs de l'université du Wisconsin
↓ X 1612	UV	× 4		
↓ WIS Q 176	UV	× 4,9		
↓ WIS BL 3 D10	UV	× 9		
↓ 47.1564	spontanée	× 8,5	perte	
↓ 48.701	spontanée	× 8,1	perte	
↓ 49.133	Nitrosoguanidine (3 intermédiaires)	× 12,7		
↓ 51.20	Nitrosoguanidine (10 intermédiaires)	× 22,6		Amélioration réalisée dans les laboratoires pharmaceutiques Eli Lilly
↓ E.15	Nitrosoguanidine	× 50,6		
↓ E.15.1	spontanée	× 55		

Site-directed mutagenesis



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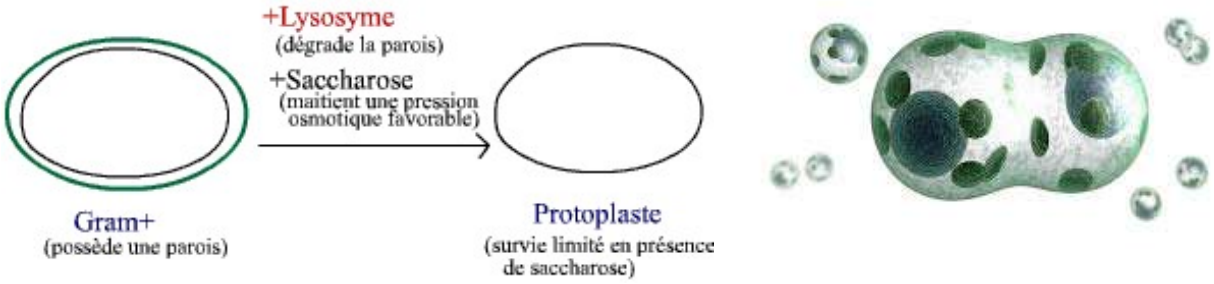
Protoplast fusion



- *Bacillus subtilis and B. megaterium*
- *Streptomyces (S. coelicolor, S. acrimycini, S. olividans, S. pravulies)*
- *Geotrichum and Aspergillus*

Cloning

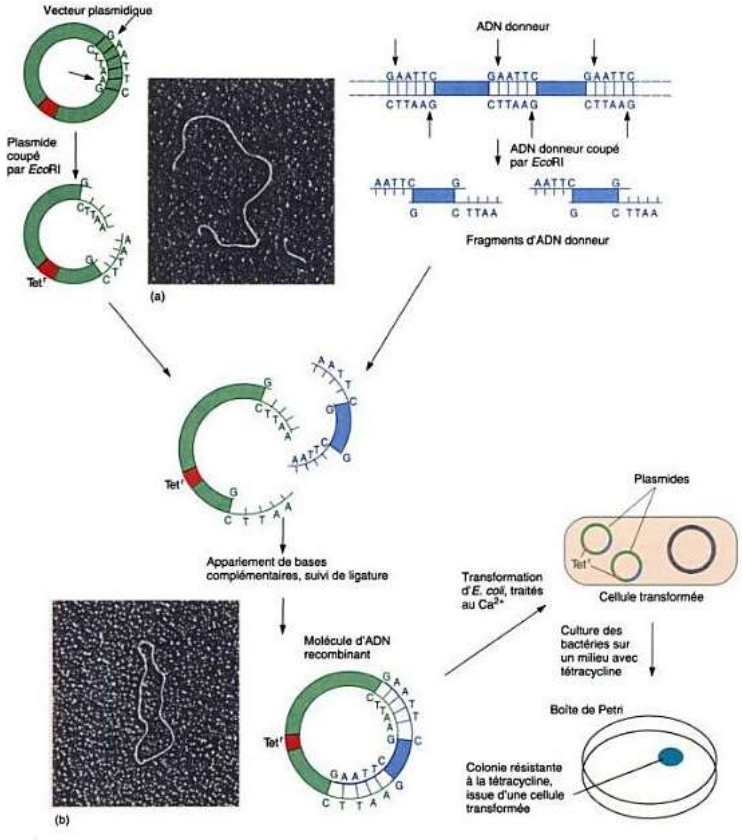


Figure 14.11 La construction et le clonage de plasmides recombinants. La construction et le clonage d'un vecteur plasmidique recombinant exploite l'expression d'un gène de résistance à un antibiotique pour détecter la présence du plasmide. Les bouts cohésifs des fragments et du plasmide ont été agrandis pour mettre en évidence l'appariement des bases complémentaires. **(a)** L'image au microscope électronique montre un plasmide, linéarisé par une enzyme de restriction et un fragment d'ADN donneur. **(b)** L'image au microscope électronique montre un plasmide recombinant. Voir détails dans le texte.

Table 7.5 Some commonly used plasmid cloning vehicles

Plasmid	*Molecular weight ($x\ 10^{-6}$)*	*Marker**	*Single restriction sites*
pSC101	5.8	Tc	BamHI, EcoRI, HindIII, Hpal, SalI, Smal
Col E1	4.2	Colimm	EcoRI
pMB9	3.6	Tc^r, Colimm	BamHI, EcoRI, HindIII, Hpal, SalI Smal
pBR313	5.8	Tc^r, Ap^r Colimm	BamHI, EcorI, HindIII, Hpal, SalI, Smal
pBR322	2.6		BamHI, EcoRI, HindIII, pstI, SalI

*Tc^r: tetracycline resistance. Ap^r: ampicillin resistance
colimm: colicin immunity

TABLE 2.1 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	Stimulate 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 growth hormone secretion	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, anti-inflammatory
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-I)	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)

Metabolic engineering

Production of isoleucine by *Corynebacterium glutamicum*,

Production of ethanol by *E. coli*

Use of lactose by *Corynebacterium glutamicum* (Whey)

Use of xylose by *Klebsiella* sp.

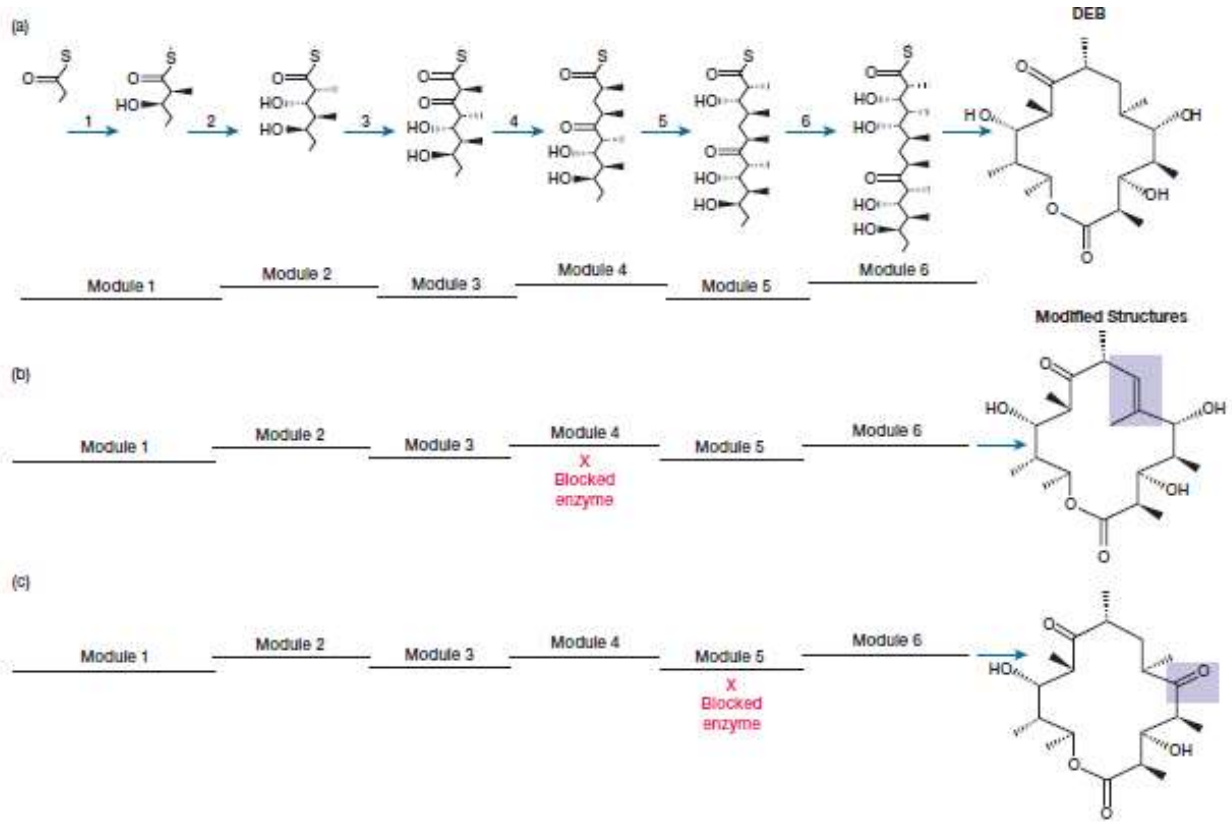


Figure 42.4 **Metabolic Engineering to Create Modified Antibiotics.** (a) Model for six elongation cycles (modules) in the normal synthesis of 6-deoxyerythonilide B (DEB), a precursor to the important antibiotic erythromycin. (b) Changes in structure that occur when the enoyl reductase enzyme of module 4 is blocked. (c) Changes in structure that occur when the keto reductase enzyme of module 5 is blocked. These changed structures (the highlighted areas) may lead to the synthesis of modified antibiotics with improved properties.