



Industrial Microbiology

Introduction & Microorganisms of Industrial Interest

Introduction to Industrial Microbiology



Industrial microbiology exploits microorganisms for commercial large-scale processes: biosynthesis, biotransformation, degradation



Origins: ~7000 years ago (empirical use) for food preservation and production of bread, vinegar, alcoholic beverages, cheese



Development: branch of applied microbiology with advances in discovery and accumulated knowledge

Industrial Microbiology: Products, Organisms, Sectors



Core Output

Produces **protein-rich biomass** or **molecules useful to humans**.

Primary Metabolism

Amino acids

Organic acids

Biogas

Secondary Metabolism

Antibiotics

Vitamins

Polysaccharides

Microorganisms Used

Bacteria

Archaea

Yeasts

Molds

Microalgae

Organism States

- ✓ Natural state
- ✓ Laboratory-selected mutants
- ✓ Genetically modified microorganisms (GMMs)

Industrial Role & Impact

Plays an important role in **Food, Pharmaceutical, Chemical, and Energy** industries.

Provides sustainable and economically viable solutions

Benefits of Using Microorganisms in Industry

Lower Cost

Often lower production costs; cheaper raw materials; simple cultivation conditions

Specific Synthesis and Biotransformation

Certain compounds only effectively synthesized/transformed by microorganisms (e.g., steroids or other complex molecules)

Large-Scale Production

Cultivation in bioreactors enables meeting industrial demands

Health Safety

Can eliminate risk of transmitting animal-borne diseases in certain productions

Conclusion: Economic, technical, and health safety advantages make microorganisms valuable industrial tools

Areas of Activity in Industrial Microbiology

Agri-Food Sector

- **Yogurt:** *Lactobacillus bulgaricus* + *Streptococcus thermophilus*
- **Fresh cheese:** *Lactococcus lactis* (acidifies by fermenting lactose into lactic acid → curd formation)
- **Organic acids:** acetic acid (*Acetobacter*), citric acid (*Aspergillus niger*)

Pharmaceutical Sector

- Microorganisms as medicine sources; bacteria (phylum Actinobacteria) source of 80% of antibiotics on the market

Bioremediation & Decontamination

- Biotransform toxic pollutants; decontamination of polluted environments (bioremediation)

Renewable Energy Sector

- Organic waste → biogas (methane) or bioethanol via microorganisms; used as fuels replacing fossil energy

Functions of Microorganisms



4.1 Technological Function

Fermentation (Transformation of raw materials)

Organoleptic quality (Changes in taste, smell, physical appearance, and consistency/texture (the product's rheological properties))



4.2 Role in Preservation/Improvement of Hygienic Quality

Production of antimicrobial substances:

 Organic acids (Lactic, acetic, propionic acids)

 Bacteriocins / antibiotics

 Alcohols

 Diacetyl

Functions of Microorganisms



4.2 Role in Preservation (continued)

Inhibition of pathogenic and/or spoilage microorganisms and reduction of risk of foodborne microbial poisoning:

Salmonella spp.

Listeria monocytogenes

Escherichia c

Shigella spp.

Staphylococcus aureus

Clostridium

Bacillus cereus



4.3 Nutritional Function

Production of vitamins (B vitamins, folic acid, etc.)

Availability of essential amino acids

Improved digestibility of complex nutrients

Better availability of trace elements (e.g., phytates and iron in bread)



4.4 Health Benefits

Probiotics: beneficial action on human or animal health

Reduction of cholesterol levels in foods

Reduction in severity of lactose intolerance

Colonization of the intestine / useful microorganisms:

✓ Fight against pathogens in the intestine

✓ Anti-cancer role

✓ Degradation of toxins (e.g., lactic acid bacteria and mycotoxines)

Microorganisms of Industrial Interest

Microorganisms are valuable due to:

-  **Ease of cultivation**
-  **Rapid growth**
-  **Ability to use inexpensive substrates**
(sometimes agro-food industry waste)
-  **Adaptability to genetic manipulation**

Main groups used in industry:

Fungi (yeasts and molds)

 **Prokaryotes**
(bacteria, particularly genus *Streptomyces*)

 **Archaea**

These microorganisms have specific properties suitable for particular industrial functions

Archaea



Unicellular prokaryotes
without nucleus and organelles

Diverse Habitats

Vents, hot springs, salt lakes, soils,
intestinal flora

Ecological Role

Key players in carbon and nitrogen cycles



Methane-producing Archaea (Methanoarchaea)

- **Strict anaerobes**
- Live in flooded soils, rice fields,
lake/marine sediments, digestive tracts
- Psychrophilic, mesophilic, thermophilic
- Involved in carbon cycle



Extreme Halophilic Archaea (Haloarchaea)

- Grow only at $\text{NaCl} > 1.8 \text{ M}$
- Facultative or obligate aerobes
- Use amino acids, carbohydrates, or
organic acids



Extreme Thermophilic Archaea

- Grow at temperatures $> 80^\circ\text{C}$
- Found in hot springs, solfataras
- Geothermally heated marine sediments

Archaea: Industrial Applications



Environment and Ecology

- Methanogenic archaea in biogas plants to produce methane from organic waste
- Certain archaea used for wastewater treatment (degradation of organic compounds; water purification)



Food Industry

- Halophilic archaea used in salt production
- Some archaea used in fermentation of sauerkraut and kimchi



Biotechnology

- Extremophilic archaea studied for unique capabilities and enzymes resistant to extreme conditions; used in harsh-condition processes (chemical production or waste treatment)



Scientific Research

- Study models for evolution and adaptation to extreme environments; implications for research on origin of life



Medicine and Pharmacology

- Potential sources of new antibiotics and bioactive compounds
- Studied for role in gut health and microbiome modulation

Bacteria

Morphology & General

Unicellular microorganisms found in many environments; small size; rapid reproduction supports large-scale

production
Shapes:

Cocci, bacilli, coccobacilli, vibrios, spirals, etc.

Grouping patterns:

In clusters, in chains, in grapes, etc.

Genetics

Nuclear apparatus:

Single chromosome (circular, closed on itself; does not contain large repetitive regions) in cytoplasm

Extra-chromosomal genetic material:

Plasmid (circular double-stranded DNA, transmissible)

Metabolism

By carbon/energy source:

- Photoautotrophs
- Photoheterotrophs
- Chemoautotrophs
- Chemoheterotrophs

Applications of Bacteria

Production of enzymes and proteins

Bacillus subtilis: amylase, protease, lipase (food, detergents, biofuel)

Escherichia coli: host for recombinant protein production

Acid production

Lactobacillus acidophilus*, *Lactobacillus casei: lactic acid (food, cosmetic, pharmaceutical)

Acetobacter* and *Gluconobacter: vinegar production

Hydrocarbon degradation

Pseudomonas aeruginosa*, *Alcanivorax borkumensis: degrade hydrocarbons in oil spills

Dairy production

Lactobacillus bulgaricus* + *Streptococcus thermophilus: yogurt

Lactococcus lactis* and *Leuconostoc: cheeses and other fermented dairy products

Vitamin production

Propionibacterium freudenreichii: vitamin B12

Applications of Bacteria



Waste degradation

Bacillus spp. and ***Pseudomonas*** spp.: wastewater treatment plants (break down organic matter; remove pollutants)



Bioplastic production

Cupriavidus necator: polyhydroxyalkanoate (PHA) biodegradable bioplastic (packaging, disposable products)



Drug production

Genetically modified bacteria (e.g., ***Escherichia coli***) produce therapeutic proteins: insulin, growth hormones, antibodies

(Context) Industrial application examples are provided as specific cases of bacterial use in industry

Fungi: Molds



General Characteristics

Molds are generally **multicellular**; most are **heterotrophic**; some have mixed metabolism



Historical Impact

Molds led to discovery of **penicillin** (first truly effective antibiotic), naturally produced by molds of genus *Penicillium*

Aspergillus niger

Filamentous ascomycete fungus (order Eurotiales); appears as black mold on fruits and vegetables. Used in industrial fermentation to produce:



Citric acid

Agri-food acidifier/antioxidant;
enhance flavors; preserve fruit juices



Gluconic acid

Used in medicines, food, detergents,
textiles, leather, etc.



Enzymes

Glucose oxidase, catalase, hydrolases
(cellulase, xylanase, pectinase); used
in production of beers and sweetened
beverages

Penicillium

- *Penicillium* are filamentous fungi.
- They are very common fungi in the environment, capable of causing many degradations.



Cheeses

Penicillium roqueforti,
Penicillium camembertii



Gluconic acid

Penicillium purpurogenum

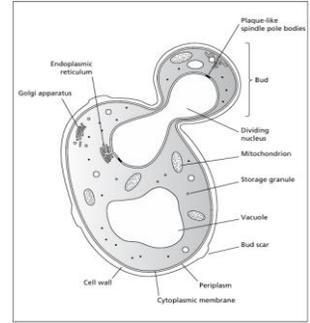


Antibiotics

Penicillin type (*Penicillium notatum*, *Penicillium chrysogenum*)

Fungi: Yeast

- Yeast is a unicellular fungus, reproducing asexually and capable of causing the fermentation of animal or plant organic matter.
- For the most part, they belong to the division Ascomycota, of the kingdom Fungi.
- There are different types of yeasts, among which we can mention:



Brewer's yeast or baker's yeast (*Saccharomyces*)

This is the most common and well-known type of yeast. It is widely used in the manufacture of beer, bread, and wine, such as *Saccharomyces cerevisiae*.



Candida

Some species of *Candida* are types of yeasts that can be pathogenic. They can cause infections in humans, including fungal infections.



Pichia, Hansenula, Kluyveromyces, Metschnikowia

These are other genera of yeasts that present a diversity of species and applications in different fields.

Applications of yeast

Food industry

bread, fermented beverages, ethanol, dairy products, and beer

Human and Animal Health

Production of insulin, probiotics

Cosmetics

Enzyme production

amylases, proteases, and lipases

Bioremediation

Bioethanol

Algae

- Algae are aquatic organisms, often unicellular, which can be classified into two main categories: **macroalgae** and **microalgae**. Macroalgae are large algae, usually visible to the naked eye, while microalgae are unicellular algae, often microscopic, forming an essential component of phytoplankton. They possess chloroplasts, allowing them to convert light energy into chemical energy through photosynthesis. These microorganisms play a crucial role in aquatic ecosystems and are of growing interest in various fields.

The types of microalgae are diverse and fall into two main subfamilies:

1. **Prokaryotic microalgae:** These are unicellular organisms without a nucleus or organelles. They include cyanobacteria, such as spirulina and *Aphanizomenon flosaquae* (Klamath). These microalgae are often used for their nutritional richness and health benefits.
2. **Eukaryotic microalgae:** These organisms possess a nucleus and various organelles. They include different species such as chlorophytes, like *Chlorella*.

Eukaryotic microalgae can be classified by their color:

Green microalgae: Containing chlorophyll, they have a characteristic green color and are used in food and scientific research.

Red microalgae: Their color comes from phycobiliprotein pigments, and they are valued in the food and cosmetic industries.

Blue-Green icroalgae: Also called cyanobacteria, they have the ability to fix atmospheric nitrogen.

Applications

Microalgae have various applications, including:

- **In Food:** Microalgae are a promising source of protein to feed the planet, requiring few resources for their cultivation.
- **In Cosmetics:** They are used in the manufacture of shampoos, body lotions, UV creams, foundations, soaps, and shower gels.
- **In Atmospheric Depollution:** Microalgae can be used to recycle carbon dioxide released by industry, thus helping to reduce CO₂ emissions and combat climate warming; they can be exploited for biofuel production.
- **In Construction:** Projects use microalgae to design Biofacades, walls composed of double-glazed structures containing an aqueous medium where algae are cultivated. These Biofacades allow for the production of electricity, heat, and the recycling of CO₂.
- **As Bioindicators:** Certain microalgae are sensitive to specific pollutants, making them useful as bioindicators of environmental quality.
- **In Wastewater Treatment:** Microalgae cultivated in wastewater can have various applications, including capturing heavy metals and reducing the accumulation of carbon dioxide in the atmosphere.

Viruses

- A virus is an infectious agent **requiring a host** (obligate parasite).
- Viruses are classified based on the nature of the nucleic acid in their genome (DNA or RNA), the structure of the nucleic acid (single-stranded or double-stranded), and the form of the nucleic acid (linear, circular, segmented, or non-segmented).
- They are tools used, for example, to give a cell the ability to produce a protein of interest or to study the effect of introducing a new gene into the genome. Example: Baculoviruses are extensively studied insect viruses; they are means to limit or replace chemical insecticides.

1. Gene Therapy (Delivery Vehicles)

- AAV Vectors:** Treat inherited blindness (Luxturna), spinal muscular atrophy (Zolgensma).
- Lentiviral Vectors:** Cure blood/immune disorders (SCID, "bubble boy" disease).
- Adenoviral Vectors:** Basis for COVID-19 vaccines (AstraZeneca, J&J).

2. Vaccines (Teaching Immunity)

- Live-attenuated:** MMR, chickenpox, yellow fever vaccines.
- Inactivated:** Polio (shot), flu (shot), hepatitis A vaccines.
- Viral Vector Vaccines:** Ebola (VSV-based), COVID-19 (AstraZeneca, Sputnik V).

3. Cancer Treatment (Oncolytic Virotherapy)

- T-VEC (Modified Herpes Virus):** Approved for advanced melanoma.
- Engineered Adenoviruses/Poxviruses:** In trials for liver, colorectal cancers.

4. Research

- Bacteriophages (λ , M13):** Clone DNA, create antibody libraries (phage display).
- Viral Vectors:** Deliver CRISPR or fluorescent tags (GFP) into cells for genetic studies.

5. Agriculture and Biocontrol

- Baculoviruses:** Natural insecticides targeting specific caterpillars.
- Plant Viruses:** Modified to study gene function or engineer crops.

6. Industry and Bioprocessing

- Phage Therapy:** Target antibiotic-resistant bacteria in food safety and medicine.
- Baculovirus Expression:** "Insect cell factories" to produce complex vaccine proteins.

3. The microbial strain for industrial use

3.1. Characteristics of the ideal strain

Irrespective of the origins of an industrial microorganism, it should ideally exhibit:

- 1- genetic stability;
- 2- efficient production of the target product, whose route of biosynthesis should preferably be well characterized;
- 3- limited or no need for vitamins and additional growth factors;
- 4- utilization of a wide range of low-cost and readily available carbon sources;
- 5- amenability to genetic manipulation;
- 6- safety, non-pathogenicity and should not produce toxic agents, unless this is the target product;
- 7- ready harvesting from the fermentation;
- 8- ready breakage, if the target product is intracellular;
- 9- production of limited byproducts to ease subsequent purification problems.

Other features that may be exploited are thermophilic or halophilic properties, which may be useful in a fermentation environment. Also, particularly for cells grown in suspension, they should grow well in conventional bioreactors to avoid the necessity to develop alternative systems. Consequently, they should not be shear sensitive, or generate excessive foam, nor be prone to attachment to surfaces

3.2. Obtaining Strains

→ Isolation and selection of industrial microorganisms "Screening Technique"

The first step in developing a producing "strain" is to isolate the relevant microorganisms from their natural habitat. They are present almost everywhere, for example in air, water, soil, on the surfaces of plants and animals, as well as in plant and animal tissues. But the most common sources of industrial microorganisms are soils, lake and river sediments. Often, the ecological habitat from which a desired microorganism is most likely to be isolated depends on the characteristics of the product one wishes to obtain and the development of the process. The sample is then seeded onto an appropriate medium to isolate the sought-after germ. The next step is to isolate and purify the different clones obtained. Purification is achieved by streak plate isolation. This technique allows for the isolation of colonies and the obtainment of pure cultures.

Strategies for isolation and culture collections

1. Approaches to environmental isolation

There are two main strategies for isolating microorganisms with industrial potential:

- **The "Shotgun" (Random) Approach:** This involves collecting a wide range of samples from diverse habitats (soil, water, biofilms, waste, extreme environments) and then screening them to find a trait of interest.
- **The "Objective" (Targeted) Approach:** Samples are taken from specific sites where natural selective pressure is likely to already favor microorganisms possessing the desired characteristic (e.g., sampling polluted sites to find organisms that degrade a toxic compound).

2. Selection and culture techniques

Once the sample is collected, the challenge is to favor the target microorganism, which is often a minority in the sample:

- **Enrichment cultures:** Using specific media and conditions (batch or continuous culture) that provide a competitive advantage to microbes possessing the desired trait, allowing them to multiply and become dominant.
- **Pure isolation:** Selective agar media are then used to obtain pure cultures of the isolated strains.
- **Screening:** Each isolate is tested for the desired property (enzyme production, inhibition, etc.). At this stage, the initial level of production is not the primary concern, as strain development programs can later optimize it. Other criteria, such as genetic stability and non-toxicity (if required for the application), are also checked.

Note: It is easier to isolate a single organism than to isolate and maintain a stable microbial consortium, although consortia are sometimes more efficient for complex tasks (e.g., complete degradation of recalcitrant compounds).

After isolation, the obtained microorganisms undergo selection based on their biological and technological suitability:

- Specific biological criteria related to the microorganisms and their application. For example, lactic acid bacteria are selected based on their acidifying and flavoring power as well as their production of bacteriocins.
- Non-pathogenic and do not produce undesirable metabolites like toxins.
- Easy to manipulate genetically.
- Can grow on cheap substrates (agri-food industry waste).
- Do not have specific requirements regarding growth factors.
- Genetically stable, especially after preservation by freezing or lyophilization.
- Ability to withstand different technological processes.
- Resistance to bacteriophages.

When a strain is retained and meets the selection criteria, it undergoes molecular identification based on 16S rDNA sequencing.

3. Culture Collections

Culture collections (such as the ATCC in the USA or the UKNCC in the United Kingdom) act as repositories for preserved strains.

- They represent a source of already identified and partially characterized microorganisms, which significantly reduces the time and cost compared to a full environmental isolation campaign.
- **Functions:** To maintain and preserve strains (using cryopreservation, lyophilization/freeze-drying, or storage on glass beads), to continue acquiring new strains, and to supply authenticated culture samples to researchers and industry.
- **Disadvantage:** As these strains are publicly accessible, they do not provide an exclusive competitive advantage to a single company.

Microorganisms can also be obtained as pure cultures from organizations that maintain culture collections ("culture banks"), for example, American Type Culture Collection (ATCC) Rockville, Maryland, USA; Commonwealth Mycological Institute (CMI), Kew, Surrey, England; Fermentation Research Institute (FERM), Tokyo, Japan; Antibiotics Research Institute of the USSR (RIA), Moscow, USSR, etc.

Table 4.3 Examples of some important culture collections useful to industrial microbiologists*

Culture collection	Type of microorganisms held
American Type Culture Collection (ATCC) Manassa, Virginia, USA	All
Centraalbureau voor Schimmelcultures (CBS) Baarn, The Netherlands	Filamentous fungi and yeasts
Collection Nationale de Cultures de Microorganismes (CNCM) Paris, France	All
Deutsche Sammlung von Mikroorganismen und Zellkulture (DSMZ) Braunscheig, Germany	All
UK microbial culture collections	
Culture Collection of Algae & Protozoa (Marine) (CCAP), Dunstaffnage Marine Laboratory, Oban	Algae and protozoa (marine)
Culture Collection of Algae & Protozoa (Freshwater) (CCAP), Institute for Freshwater Ecology, Ambleside	Algae and protozoa (freshwater)
European Collection of Animal Cell Cultures (ECACC), Centre for Applied Microbiological Research (CAMR), Porton Down	Animal cell cultures
CABI Bioscience UK Centre, Egham (formerly International Mycological Institute)	Filamentous fungi
National Collection of Food Bacteria (NCFB), Aberdeen	Food bacteria
National Collection of Industrial & Marine Bacteria (NCIMB), Aberdeen	Bacteria (general, industrial and marine)
National Collection of Pathogenic Fungi (NCPF), Public Health Laboratory, Bristol	Pathogenic fungi
National Collection of Plant Pathogenic Bacteria (NCPFB), Central Science Laboratory, Sand Hutton, York	Plant pathogenic bacteria
National Collection of Type Cultures (NCTC), Central Public Health Laboratory, Colindale	Medical microorganisms
National Collection of Wood Rotting Fungi (NCWRF), Building Research Establishment, Watford	Wood-rotting fungi
National Collection of Yeast Cultures (NCYC), Institute for Food Research, Norwich	Yeasts (other than known pathogens)

* For a comprehensive list see World Data Centre for Microorganisms.

3.3. Strain Improvement

After identifying an organism producing a valuable product, it becomes necessary to increase the fermentation yield to minimize production costs. Therefore, the objectives are:

- Increase the production capacity (of the product) of the microorganisms.
- Improve substrate specificity and production speed.
- Improve new production pathways.
- Improve resistance to unfavorable conditions (temperature, pH, toxins, bacteriophages).

The improvement of industrial strains relies on creating **new genetic combinations**. Three main approaches are used: natural recombination processes in prokaryotes and eukaryotes, classical mutagenesis, and modern genetic engineering techniques.

Table 4.4 Examples of targets for strain improvement

Rapid growth

Genetic stability

Non-toxicity to humans

Large cell size, for easy removal from the culture fluid

Ability to use cheaper substrates

Modification of submerged morphology

Elimination of the production of compounds that may
interfere with downstream processing

Catabolite derepression

Phosphate deregulation

Permeability alterations to improve product export rates

Metabolite resistance

Production of

additional enzymes

compounds to inhibit contaminant microorganisms

heterologous proteins that may also be engineered

with downstream processing 'aids', e.g. polyarginine tails

(see Chapter 7, p. 122)

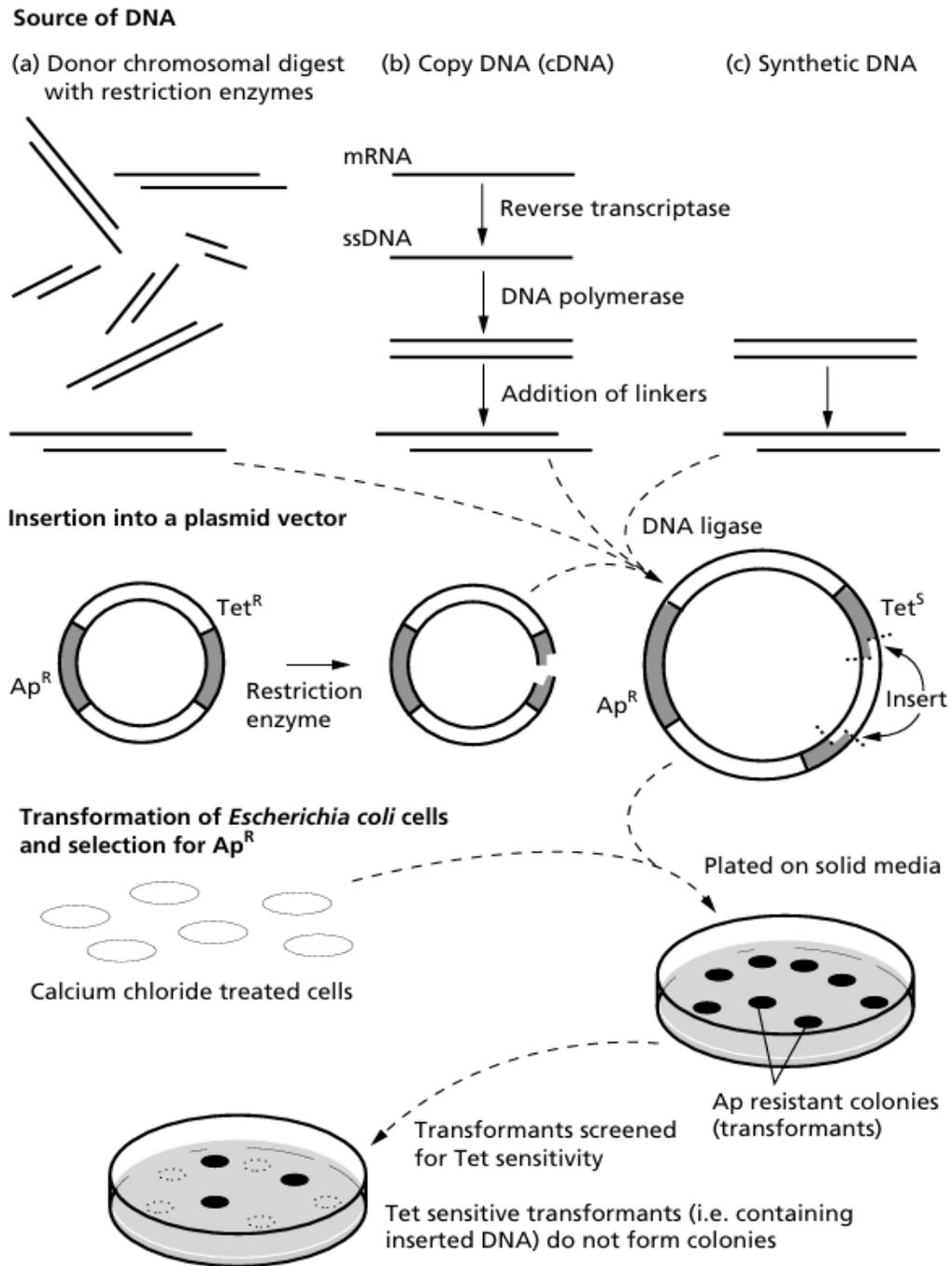


Fig. 4.3 Outline of a strategy for cloning DNA in *E. coli* (after Brown *et al.* (1987)). The source DNA is usually DNA fragments from bacteria, or, for eukaryotic sources, synthetic or cDNA (derived via reverse transcription of mRNA) is used. The DNA fragment, from whatever source, is ligated into a plasmid vector containing suitable markers; in this case, resistance to ampicillin (Ap^R) and tetracycline (Tet^R). Ligation is into the tetracycline resistance gene thus inactivating it (Tet^S). The vector is then used to transform the host cells. All cells successfully transformed exhibit ampicillin resistance. However, of those, only cells containing foreign DNA are tetracycline sensitive. Those containing foreign DNA can then be screened for the desired gene or gene product.

Strain Improvement Methods (1/2)

Natural Selection

- 1 Choose best performing individual
- 2 Cultivate it
- 3 Select best performer among offspring (2nd generation)
- 4 Continue over several generations

Mutagenesis

- ✓ Conventional mutation using mutagens (no foreign DNA)
- ✓ **Mutation frequency:** 10^{-2} (vs 10^{-6} to 10^{-8} for natural mutation)
- ✓ **Mutant screening:** identification and recovery (examples: auxotrophic, resistant, carbohydrate-fermenting mutants)

Physical Agents

Ionizing radiation:
X-rays, gamma rays, alpha particles,
accelerated neutrons

UV radiation (200–300 nm):
Formation of covalent bonds between pyrimidines (C and T)

Chemical Agents

Active on DNA (non-replication):
Nitric acid (sodium nitrate)

DNA analogues:
Replace phosphate backbone to modify chain structure

Frameshift agents:
Intercalate into DNA (ethidium, proflavine)

Strain Improvement Methods (2/2)

Natural Genetic Recombination

- **Sexual reproduction:** possible for Ascomycetes (e.g., yeast); restrictive conditions (same species, opposite mating types)
- **Parasexuality** (case of Fungi imperfecti)

Induced Cell Fusion (Protoplast Fusion)

Obtain recombinations between cells that cannot fuse "naturally"

01

Removal of cell wall by enzymatic attack (isotonic medium)

02

Mixing of the two cell populations in the presence of Ca^{++} ions and polyethylene glycol

03

Regeneration of the cell wall

04

Screening of recombinants (genetic markers)

In Vitro Genetic Recombination (Genetic Engineering)

Recombinant DNA technology / molecular cloning / gene cloning

01

Excision of a specific portion of donor DNA
Insertion of this portion into a replicative DNA (vector, e.g., plasmid)

02

Insertion of this portion into a replicative DNA (vector, e.g., plasmid)

03

Transfer of recombinant DNA into the host cell

04

Isolation of host cells that have effectively received the recombinant DNA

Natural Genetic Recombination

In Bacteria (Prokaryotes)

Bacteria typically possess a single chromosome and **plasmids** (small, circular, autonomous, self-replicating DNA molecules that can be present in up to 1000 copies per cell). These plasmids carry additional, non-essential genes that often provide useful traits (e.g., antibiotic resistance). Bacteria do not undergo sexual reproduction but can exchange genetic material through three main processes:

1. Conjugation: Transfer of DNA (plasmid or chromosomal) from a donor bacterium to a recipient through direct cell-to-cell contact, established by a structure called a **sex pilus**.

2. Transduction: Gene transfer mediated by a bacterial virus (bacteriophage). During its replication cycle, the phage may incorporate fragments of DNA from its host and carry them to a new bacterial cell. Phages, like plasmids, can also carry **transposons** (mobile DNA segments capable of "jumping" from one DNA molecule to another).

3. Transformation: The uptake by a bacterial cell of a free, naked DNA fragment from its surrounding environment (originating from lysed cells). This process only occurs when the bacterium is in a physiological state called **competence**.

In Fungi (Eukaryotes)

- Natural genetic recombination in eukaryotes primarily occurs during sexual reproduction (combining parental chromosomes and **crossing-over** during meiosis).

- For industrially important fungi lacking a sexual phase (e.g., *Penicillium*, *Aspergillus*), a **parasexual cycle** exists: the fusion of hyphae from different strains forms a heterokaryon. Today, this fusion can be induced artificially by fusing **protoplasts** (cells with their walls removed).

- Some eukaryotes (yeasts, molds) also possess natural plasmids (e.g., the 2 μ m plasmid of *S. cerevisiae*), which are useful in genetic engineering.

Mutagenesis: a conventional tool for improvement

Mutagenesis involves inducing physical changes to DNA (deletions, insertions, etc.) to create mutants.

Regulatory advantage: Since mutants can arise spontaneously, obtaining them through mutagenesis generally poses fewer regulatory approval problems compared to genetic engineering.

Mutation rates

•**Spontaneous:** Very low (e.g., $\sim 10^{-10}$ per gene per generation in bacteria).

•**Induced:** Increased by using **mutagens**:

- *Physical:* UV, X-rays, gamma rays.
- *Chemical:* EMS, NTG, nitrous acid, etc.
- *Transposons:* Their insertion into a gene creates a mutation.

• These methods are effective for **eliminating an undesirable trait** (e.g., removal of the yellow pigment chrysogenin from early penicillin preparations) or for **increasing production** by deregulating control mechanisms (e.g., amino acid production, dramatic increases in penicillin yields).

• **Limitation:** They are non-specific. This led to the development of **directed mutagenesis** techniques (requiring a genetic map) and **in vitro mutagenesis** (combined with genetic engineering).

Genetic engineering of microorganisms

The development of recombinant DNA technology has revolutionized the industry.

Advantages over traditional methods

- Specificity:** Allows for precise and controlled modifications.
- Unlimited DNA Source:** Genes can originate from any organism, living or extinct.
- New functionalities:** Completely novel properties can be added to a microorganism.

Applications

- Metabolic pathway improvement:** Removing bottlenecks, amplifying key steps.
- Heterologous protein production:** Enabling microbes to produce proteins of animal or plant origin (e.g., human growth hormone, insulin, interferons).
- Downstream processing facilitation:** Adding "tags" (e.g., polyarginine tails) to proteins to simplify their purification.

3.4. Strain Preservation

- For a desired preservation period: maintain the strain to be preserved viable, available, and unchanged.
- In microbiology, where we work with unicellular organisms, preserving unchanged means preserving viability under conditions that keep the genome identical.
- Preservation must obviously exclude contamination.
- Depending on the case, desired preservation periods vary from a few days to several years, and the choice of a preservation technique will be strongly influenced by this duration parameter.
- It will be necessary to verify that an ensemble of genetic and/or morphological and/or physiological and/or biochemical characteristics that define the pure strain to be preserved and are of interest to the strain's users have been kept unchanged (the characteristics are defined by the user based on the nature of their work on the strain to be preserved).
- Preservation methods involve one or more of the following techniques:
 - Reduction of growth temperature.
 - Desiccation or dehydration of the culture medium.
 - Limitation of nutrients available to the microorganism.
 - Examples of fields using preservation: for taxonomists, in biological engineering, and in the medical field.

Preservation Methods (1/2)

1.1 Ordinary Refrigeration

Temperature

4 – 10°C

Technique: Successive Subculturing

- ✓ Aerobic: Agar slant
- ✓ Anaerobic: Deep agar + paraffin or oil

Storage Duration

3 to 12 months

Pros/Cons: Low cost, but does not completely limit growth. High risk of contamination or mutation

due to repetitive

1.2 Freezing

Principle: Low temperatures stop cellular chemical reactions by crystallization of water; warming restarts life.

 -20°C Not low enough. Bacteria/yeasts/molds preserved months to years.

 -30°C Reactions very slow; changes occur over many months.

 -80°C Virtually no chemical evolution
Good for several years. Requires cryoprotectants (glycerol, DMSO).

 -196°C Liquid Nitrogen
Very long preservation. Complete metabolic arrest.

Preservation Methods (2/2)

2.1 Simple Desiccation

"Water is the solvent for the chemical reactions of life. After desiccation, chemical reactions will be stopped and preservation is effective."

Method

Distribute microorganisms on inert support like filter paper, sand, or silica grains. Facilitated by partial vacuum.

Storage

Store perfectly dry at $0-4^{\circ}\text{C}$ (airtight container with desiccant).

Revivification

Immersion in culture medium.

2.2 Lyophilization (Freeze-drying)

1 Process

Frozen then dehydrated by **sublimation** of ice (solid to

★vapor) under reduced pressure.

▲ Excellent for **long-term storage** (used in major culture banks).



Not compatible with all microorganisms; may cause cellular/genetic alterations.
Revivification by adding culture medium to ampoule.

2 Storage

Lyophilizate sealed and stored in the dark at $0-4^{\circ}\text{C}$.

Suspension Formulations

FOR LYOPHILIZATION

Skim milk, serum-based medium, or polyvinylpyrrolidone (PVP).

FOR FREEZING

Skim milk or blood broth.