Chapter V: Anabolism and biomass production

1- Amino acids production

Amino acids are organic compounds essential for protein synthesis. While they can be produced chemically, microbial production is preferred industrially due to higher yields, sustainability, and lower cost. Bacteria (like *Corynebacterium glutamicum* and *Escherichia coli*) and some yeasts are commonly used (Ikeda, 2003).

Industrial examples:

- L-glutamate → flavor enhancer (MSG)
- L-lysine → animal feed supplement
- L-threonine, L-valine, L-tryptophan → nutrition and pharmaceuticals

1-1- Microorganisms used

Table 1. Microorganisms used in amino acids production (Paloyan et al., 2022)

Amino Acid	Main Microbe	Special Features
L-glutamate	*Corynebacterium glutamicum*	High tolerance to Na^+; mutations increase excretion
L-lysine	*C. glutamicum*, *E. coli*	Genetic modifications enhance metabolic flux
L-threonine	*E. coli*, *C. glutamicum*	Optimized enzymes in aspartate pathway
L-tryptophan	*E. coli*	Requires feedback inhibition control

1-2- Metabolic pathways for production

Amino acids are synthesized via specific microbial pathways, often branching from glycolysis or the Krebs cycle (Paloyan et al., 2022).

a. L-Glutamate

Precursor: α-ketoglutarate (from Krebs cycle)

Key enzyme: glutamate dehydrogenase (catalyzes reductive amination)

$$\alpha\text{-ketoglutarate} + NH_4^+ + NADPH \rightarrow \text{L-glutamate} + NADP^+ + H_2O$$

Production triggers: biotin limitation, calcium salts (alter cell membrane permeability)

b. L-Lysine

Precursor: aspartate

Pathway: Aspartate → Homoserine → L-Lysine

Key control: Aspartokinase, inhibited by L-lysine

Industrial strategy: mutations prevent feedback inhibition, increasing lysine output.

c. L-Threonine

Precursor: aspartate

Pathway: Aspartate → Aspartyl-phosphate → Homoserine → L-Threonine

Controlled by feedback inhibition of aspartokinase by L-threonine

Optimization: enzyme modifications reduce inhibition.

d. L-Tryptophan

Precursors: phosphoenolpyruvate + erythrose-4-phosphate

Pathway: shikimate → anthranilate → L-tryptophan

Controlled by feedback inhibition by tryptophan

Industrial production uses metabolic engineering to bypass inhibition.

1-3- Fermentation conditions

Methods: batch, fed-batch, or continuous fermentation

Medium: carbon sources (glucose, sucrose), nitrogen (ammonium, urea), mineral salts

pH: neutral to slightly alkaline (6.5–7.5)

Temperature: 30–37°C (depending on strain)

Oxygen: aerobic conditions for strains like *C. glutamicum*

Optimization strategies:

1. Mutate regulatory enzymes to remove feedback inhibition.
2. Adjust medium composition to enhance secretion.
3. Engineer metabolism to increase precursor availability (Paloyan et al., 2022).

1-4- Extraction and purification

After fermentation, amino acids are recovered via:

1. Cell removal – centrifugation or filtration
2. Precipitation – using salts or solvents

3. Crystallization – to purify the amino acid
4. Drying – to obtain a powdered form for industry

1-5- Key biochemical insights

Secretion of amino acids often acts as a detoxification or osmotic regulation mechanism.

Nutrient limitation or excess strongly affects production.

Metabolic engineering is essential to overcome natural regulatory bottlenecks in biosynthetic pathways (Paloyan et al., 2022).

2- Lipid production

Lipids are a diverse group of hydrophobic or amphipathic molecules that play essential roles in cell structure, energy storage, and metabolism. In microbial biochemistry, many microorganisms can synthesize and accumulate lipids either as membrane components or as energy reserves (similar to fats in higher organisms).

Microbial lipid production has become industrially important for:

- Biofuel production (biodiesel from microbial oils)
- Nutraceuticals (polyunsaturated fatty acids, PUFA)
- Cosmetics and pharmaceutical (Costa et al., 2024)

2-1- Types of microbial lipids

Microbial lipids can be divided into two major categories:

a. Structural lipids

- Found in cell membranes (phospholipids, glycolipids, sterols)
- Maintain membrane fluidity, permeability, and functionality
- Commonly produced by bacteria and yeasts under normal growth conditions

b. Storage lipids

- Mainly triacylglycerols (TAGs) and wax esters
- Accumulated as energy reserves in certain microorganisms (especially under nutrient limitation)
- Found in oleaginous microorganisms (lipid-producing microbes)

2-2- Oleaginous microorganisms

These are microbes that can accumulate **more than 20–70% of their dry weight as lipids** (Costa et al., 2024).

Table 2. Microorganisms used in lipid production (Costa et al., 2024).

Type	Microorganism	Typical Product
Yeast	*Yarrowia lipolytica*, *Rhodotorula glutinis*, *Cryptococcus curvatus*	Triacylglycerols
Filamentous fungi	*Mortierella alpina*, *Mucor circinelloides*	Polyunsaturated fatty acids
Bacteria	*Rhodococcus opacus*, *Nocardia sp.*, *Mycobacterium sp.*	Neutral lipids and wax esters
Microalgae	*Chlorella vulgaris*, *Nannochloropsis sp.*	Triglycerides and long-chain fatty acids

2-3- Metabolic pathway of lipid synthesis

a. Precursor formation

Lipid synthesis starts from acetyl-CoA, derived from glycolysis or oxidation of other carbon sources.

In the cytoplasm, acetyl-CoA is carboxylated by acetyl-CoA carboxylase to form malonyl-CoA, the building block for fatty acid elongation (Al Azad et al., 2024).

Reaction:

$$\text{Acetyl-CoA} + CO_2 + \text{ATP} \rightarrow \text{Malonyl-CoA} + \text{ADP} + \text{Pi}$$

b. Fatty acid synthesis

Catalyzed by the fatty acid synthase (FAS) complex

Involves sequential addition of two-carbon units from malonyl-CoA to a growing acyl chain

Produces long-chain fatty acids (C14–C18) (Al Azad et al., 2024).

Key enzymes:

Acetyl-CoA carboxylase (ACC)

Fatty acid synthase (FAS)

Thioesterase (terminates chain elongation)

c. Triacylglycerol (TAG) formation

Fatty acids are esterified to glycerol-3-phosphate through the Kennedy pathway:

Glycerol-3-phosphate + fatty acyl-CoA → lysophosphatidic acid

Lysophosphatidic acid + fatty acyl-CoA → phosphatidic acid

Phosphatidic acid → diacylglycerol (via dephosphorylation)

Diacylglycerol + fatty acyl-CoA → triacylglycerol (TAG) (Ikeda, 2003).

2-4- Conditions affecting lipid accumulation

Lipid synthesis in microbes is tightly regulated by nutrient availability and environmental factors (Ikeda, 2003).

Table 3. Effect of conditions on lipid production (Ikeda, 2003).

Condition	Effect on Lipid Production
Carbon source excess	Promotes lipid accumulation
Nitrogen limitation	Triggers conversion of excess carbon into lipids
Oxygen availability	Required for fatty acid desaturation
pH and temperature	Influence enzyme activity and lipid composition
Carbon source type	Glucose, glycerol, and certain fatty acids enhance yield

2-5- Applications of microbial lipids

Table 4. Applications of lipid producing microorganisms (Ikeda, 2003).

Application	Description
Biofuel	Microbial oils (single-cell oils) can be transesterified to biodiesel
Food & Feed	Source of omega-3 and omega-6 fatty acids
Pharmaceuticals	Used in liposomes, drug carriers, and emulsifiers
Cosmetics	Natural oils and wax esters used in skincare products

3- Nucleotides production

Nucleotides are the building blocks of nucleic acids (DNA and RNA) and also play roles in energy metabolism (ATP, GTP), signaling (cAMP, cGMP), and cofactors (NAD^+, FAD). Industrially, nucleotides are produced microbially for use in:

- Food additives (flavor enhancers: IMP, GMP)
- Nutritional supplements
- Pharmaceuticals (antiviral, anticancer precursors)

Microbial production is preferred because it is cost-effective, environmentally friendly, and yields high purity compared to chemical synthesis (Ledesma-Amaro et al., 2013).

3-1- Used microorganisms

The primary microorganisms for nucleotide production are bacteria and yeasts (Ledesma-Amaro et al., 2013).

Nucleotide	**Microbe**	**Notes**
IMP (inosine monophosphate)	*Corynebacterium ammoniagenes*, *Brevibacterium flavum*	Most industrially important for flavor enhancers
GMP (guanosine monophosphate)	*Corynebacterium glutamicum*	Produced via metabolic engineering of purine pathway
AMP (adenosine monophosphate)	*Bacillus subtilis*	Often co-produced with IMP
UMP (uridine monophosphate)	Yeasts (*Saccharomyces cerevisiae*)	Mainly for nutraceuticals

3-2- Biosynthetic pathways

Nucleotides are synthesized by two main microbial pathways (Ledesma-Amaro et al., 2013):

a. De novo synthesis

Nucleotides are synthesized from basic precursors: amino acids, CO_2, ribose-5-phosphate, and ATP.

Purine nucleotides (IMP, AMP, GMP): synthesized stepwise on ribose-5-phosphate to form the purine ring (Ledesma-Amaro et al., 2013).

- **Simplified steps for IMP (precursor of AMP/GMP):**
    1. Ribose-5-phosphate → PRPP (5-phosphoribosyl-1-pyrophosphate)
    2. PRPP + amino acids → IMP via multi-step enzymatic reactions
    3. IMP → AMP or GMP (depending on enzyme pathway)
- **Pyrimidine nucleotides (UMP, CMP, TMP)**:
    1. Formation of carbamoyl phosphate from glutamine, CO_2, ATP
    2. Carbamoyl phosphate → orotate
    3. Orotate + PRPP → OMP → UMP → CMP/TMP

b. Salvage pathway

Microbes **recycle free bases or nucleosides** to nucleotides.

Uses enzymes like **phosphoribosyltransferases**.

More energy-efficient than de novo synthesis.

3-3- Industrial production process

Microbial nucleotide production is usually carried out in fermentation, often using genetically engineered strains (Roy et al., 2016).

a. Fermentation conditions

- Carbon sources: glucose, sucrose, glycerol
- Nitrogen sources: ammonium salts, amino acids
- pH: slightly acidic to neutral (6.0–7.5)
- Temperature: 30–37°C depending on strain
- Aerobic conditions usually required

b. Optimization strategies

- Block feedback inhibition in purine or pyrimidine pathways (e.g., mutations in IMP dehydrogenase).

- Enhance precursor supply (PRPP, amino acids) through metabolic engineering.
- Nutrient limitation can redirect metabolism to nucleotide accumulation (Roy et al., 2016).

4- Antibiotics production

Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill other microbes. They are not essential for growth but provide a competitive advantage in natural environments. Industrially, microbial production of antibiotics is crucial for medicine, veterinary use, and agriculture (Raaijmakers et al., 2002).

4-1- Microorganisms producing antibiotics

Most antibiotics are produced by bacteria and fungi, especially filamentous bacteria and molds (Raaijmakers et al., 2002).

Class	**Microorganism**	**Example Antibiotics**
Actinomycetes	*Streptomyces spp.*	Streptomycin, Erythromycin, Chloramphenicol
Filamentous fungi	*Penicillium spp.*, *Cephalosporium spp.*	Penicillin, Cephalosporins
Bacteria	*Bacillus spp.*, *Pseudomonas spp.*	Bacitracin, Polymyxin

4-2- Types of antibiotics

β-lactams: Penicillins, cephalosporins – inhibit cell wall synthesis

Aminoglycosides: Streptomycin, Gentamicin – inhibit protein synthesis

Macrolides: Erythromycin – inhibit protein synthesis

Tetracyclines: Broad-spectrum protein synthesis inhibitors

Polyketides: Erythromycin, tetracycline – synthesized via polyketide pathway

Glycopeptides: Vancomycin – inhibit cell wall synthesis (Raaijmakers et al., 2002).

4-3- Biosynthesis of antibiotics

Antibiotic synthesis occurs via secondary metabolism, often after the exponential growth phase. It requires precursors from primary metabolism (amino acids, acetyl-CoA, malonyl-CoA, shikimate derivatives) (Raaijmakers et al., 2002).

a. Precursor pathways

Amino acids → β-lactams, aminoglycosides, glycopeptides

Acetyl-CoA / Malonyl-CoA → polyketides

Shikimate pathway → aromatic antibiotics (chloramphenicol, novobiocin)

b. Enzymatic pathways

Nonribosomal peptide synthetases (NRPS): For peptide antibiotics (vancomycin, bacitracin)

Polyketide synthases (PKS): For macrolides and tetracyclines

Ribosomal modification: Some antibiotics (lantibiotics) are post-translationally modified ribosomal peptides.

4-4- Regulation of antibiotic production

Antibiotic biosynthesis is tightly controlled:

Growth-phase dependent: Usually in stationary phase, not during rapid growth

Nutrient limitation: Nitrogen or phosphate limitation can trigger secondary metabolism

Global regulators: Transcriptional activators/repressors control biosynthetic gene clusters

Feedback regulation: Some antibiotics inhibit their own production if accumulated

Industrial strains are often mutated or genetically engineered to bypass regulatory constraints, increasing yield (Bibb, 1996).

4-5- Applications

Use	**Example**
Medicine	Penicillin, Streptomycin, Erythromycin
Veterinary	Bacitracin in animal feed
Agriculture	Antibiotics for plant disease control
Research	Selection markers and biochemical tools

5- Hormones production

Hormones are chemical messengers that regulate physiological processes in animals and humans. While many hormones were originally extracted from animal tissues, microbial production has become an important industrial approach due to scalability, purity, and ethical considerations. Microorganisms can be engineered to produce peptide hormones, steroid hormones, and analogs (Tsavkelova et al., 2006).

5-1- Microorganisms used

The main microbes used in hormone production are bacteria and yeasts**,** selected for their ability to express recombinant proteins or modify steroid precursors (Tsavkelova et al., 2006).

Table 5. Microorganisms used in hormones production

Hormone	**Microorganism**	**Notes**
Insulin	*Escherichia coli*, *Saccharomyces cerevisiae*	Recombinant expression of human insulin
Growth hormone (GH)	*E. coli*, *Pichia pastoris*	Protein folding and secretion optimized
Glucagon	*E. coli*	Used for diabetes treatment
Steroid precursors	*Corynebacterium*, *Mycobacterium*, fungi (*Rhizopus*)	Microbial conversion of plant sterols to steroid hormones

5-2- Biochemical basis of hormone production

Microbial hormone production generally follows two main strategies, depending on whether the hormone is a peptide (protein) or a steroid (Kumar et al., 2025).

a. Recombinant protein hormones (Peptides)

Microbes can be genetically engineered to produce human peptide hormones. The process involves several steps:

1. The gene encoding the hormone is inserted into a plasmid or vector suitable for microbial expression.
2. The microorganism expresses the hormone, often as a fusion protein or a prohormone.
3. The expressed product undergoes post-translational processing, such as folding and cleavage, to produce the active hormone.

Example: Insulin production in *E. coli* involves separately expressing the A and B chains of insulin. After expression, the two chains are chemically or enzymatically joined to form active insulin (Kumar et al., 2025).

b. Microbial steroid hormone production

Many steroid hormones are derived from **plant sterols**, such as sitosterol or cholesterol. Certain microbes, including *Corynebacterium* and *Mycobacterium*, can convert these sterols into steroid intermediates through enzymatic reactions:

- Hydroxylation: Introduction of hydroxyl (-OH) groups at specific positions
- Dehydrogenation: Oxidation of specific carbon atoms
- Isomerization: Rearrangement of double bonds within the steroid structure

These microbial intermediates can then be further chemically or enzymatically modified to produce hormones like cortisol, testosterone, or progesterone (Kumar et al., 2025).

5-3- Applications

Hormone Type	Application
Peptide hormones	Diabetes (insulin), growth disorders (GH), hypoglycemia (glucagon)
Steroid hormones	Anti-inflammatory drugs (cortisol), contraceptives (progesterone), hormone replacement therapy (testosterone, estrogen)
Research	Cell culture studies, receptor assays

6- Toxins production

Microbial toxins are biologically produced chemical substances that can damage cells, disrupt physiology, or alter ecosystems. They fall into several broad classes depending on source and chemistry (Kumar et al., 2025):

- Exotoxins: proteins secreted by bacteria (e.g., diphtheria toxin, cholera toxin). Often highly specific in their molecular targets.
- Endotoxins: structural components of Gram-negative bacterial outer membranes (lipopolysaccharide, LPS). Released upon cell lysis; trigger strong immune responses.
- Mycotoxins: small-molecule metabolites produced by fungi (e.g., aflatoxins from Aspergillus). Often heat-stable and important in food safety.
- Bacterial small-molecule toxins: non-protein metabolites (e.g., clostridial toxins with enzymatic activity, or secondary metabolites from actinomycetes).
- Phytotoxins and algal toxins: produced by plant-associated microbes or algae (e.g., cyanobacterial microcystins) and affect animals or ecosystems.

6-1- Biological roles and ecological context

Toxin production is typically not for harming humans per se but serves microbial ecological functions:

- Competitive advantage: inhibit growth of competing microbes in the same niche.
- Predation/defense: deter grazers (protozoa, insects), or suppress host defenses in pathogenic interactions.
- Signaling / metabolism: some metabolites play regulatory roles or help in nutrient acquisition.
- Understanding the ecology helps explain why toxins are produced transiently and often under specific environmental conditions (Bernard, 2014).

6-2- Biochemical basis

Toxins arise from different biosynthetic origins:

- Ribosomally synthesized and post-translationally modified peptides (RiPPs): genes encode a precursor peptide that is enzymatically modified to yield the active toxin.
- Nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS): large enzyme assemblies build complex small molecules (common in bacterial and fungal secondary metabolites).
- Primary metabolism derivatives: some toxins are modified products of amino acids, fatty acids, or carbohydrate metabolism.
- Structural components (endotoxin/LPS): built from sugars, lipids, and fatty acyl chains as part of the cell envelope (Bernard, 2014).

At the mechanistic level, toxins act by:

- Enzymatic modification of host proteins (e.g., ADP-ribosylation by diphtheria toxin)
- Pore formation in membranes (e.g., certain hemolysins)
- Interference with signaling pathways (e.g., cholera toxin elevates cAMP)
- Genotoxicity and carcinogenesis (e.g., aflatoxin metabolites forming DNA adducts) (Bernard, 2014).

6-3- Beneficial uses and biotechnology

Some microbial toxins or toxin derivatives have medical or research uses when handled safely and precisely:

Therapeutics: extremely low doses or modified forms of potent proteins (e.g., botulinum neurotoxin used clinically) are used under strict regulatory control.

Research tools: toxins can be powerful probes for cell biology (e.g., to decipher signaling pathways). These applications require extensive safety, regulatory oversight, and specialized facilities (Bernard, 2014).

6-4- Safety and ethical considerations

Because toxins can cause serious harm, discussion of how to produce, purify, formulate, or disseminate them is not appropriate. If your interest is scientific or educational, focus on:

- Mechanisms of action,
- Ecological roles,
- Detection, surveillance, and public-health responses,
- Therapeutic or legitimate research applications under regulation.

If you're working in a legitimate laboratory or public-health role and need guidance about safety, regulations, or approved detection standards, I can provide high-level summaries of:

- biosafety levels conceptually,
- types of regulatory oversight (e.g., food safety agencies, clinical regulations),
- non-actionable descriptions of detection technologies and what they measure (Bernard, 2014).

7- Exopolysaccharides production

Polysaccharides are long-chain molecules composed of repeating units of carbohydrates, commonly known as sugars. While the market has traditionally been dominated by plant- and algae-derived gums, bacterial polysaccharides are increasingly gaining attention (Netrusov et al., 2023).

In marine environments, polysaccharide production is primarily associated with bacterial strains belonging to the genera *Alteromonas*, *Pseudoalteromonas*, *Shewanella*, and *Vibrio*.

In bacteria, these polysaccharides can be found in several locations:

- As a key component of the cell wall**,** forming part of the lipopolysaccharides (LPS) in Gram-negative bacteria.

- On the cell surface**,** forming the capsular polysaccharide that remains attached to the cell.
- Secreted into the surrounding environment as exopolysaccharides (EPS)**,** which are released into the culture medium (Netrusov et al., 2023).

EPS are high-molecular-weight polymers secreted by many bacteria, including lactic acid bacteria (LAB) such as *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Lactococcus*.

EPS play multiple roles in microbial physiology and industry:

- Protect bacteria against stress (acidic conditions, osmotic stress)
- Aid in biofilm formation and cell adhesion
- Enhance the texture, viscosity, and stability of fermented foods like yogurt, kefir, and sourdough (Netrusov et al., 2023).

7-1- Types of EPS in LAB

LAB produce two main types of EPS:

1. **Homopolysaccharides (HoPS)**
    - Composed of a single sugar type (usually glucose or fructose)
    - Examples:
        - Dextran (glucose polymer, α-1,6 linkages)
        - Fructan (fructose polymer)
    - Synthesized extracellularly by glycosyltransferases from sucrose
2. **Heteropolysaccharides (HePS)**
    - Composed of repeating units of different sugars (e.g., glucose, galactose, rhamnose)
    - Often branched and more complex
    - Synthesized intracellularly as repeating units attached to nucleotide sugar precursors (UDP-glucose, UDP-galactose) and secreted via specific transport systems (Donot et al., 2012).

7-2- Biosynthesis of EPS

EPS biosynthesis in LAB generally follows these steps (Rana and Upadhyay, 2020):

a. Precursor formation

Sugars from the growth medium (glucose, sucrose, lactose) are converted to activated sugar nucleotides (e.g., UDP-glucose, dTDP-rhamnose).

b. Polymer assembly

Glycosyltransferases link activated sugar units into repeating units for HePS, or polymerize monosaccharides directly for HoPS.

c. Secretion

EPS are secreted outside the cell, forming a capsule or slime layer, which may remain attached or be released freely into the medium (Rana and Upadhyay, 2020).

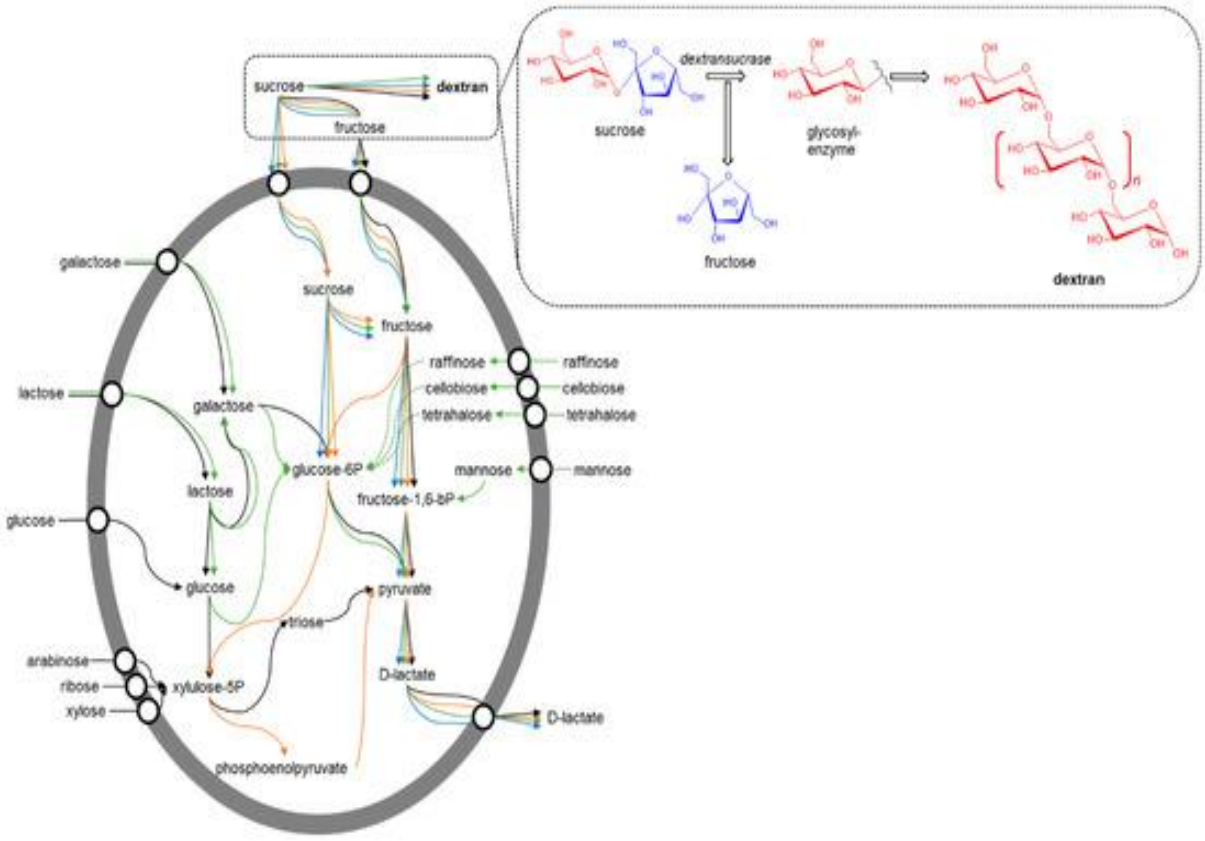

Figure 1. Overview of carbohydrate and dextran synthesis pathways in lactic acid bacteria: *Leuconostoc* (black), *Weissella* (orange), *Lactobacillus* (blue), *Streptococcus* (green) (Díaz-Montes, 2021)

7-3- Functions and applications

Physiological roles for bacteria:

- Protect against desiccation and harsh environments
- Facilitate adhesion and biofilm formation
- Aid in defense against phages and host immune factors

Industrial and biotechnological applications:

- Food industry: Improves texture, viscosity, and mouthfeel in yogurt, kefir, cheese, and fermented beverages
- Health benefits: Prebiotic effects, modulation of gut microbiota, and potential immunomodulatory properties
- Pharmaceuticals: EPS can be used as thickeners, stabilizers, or in drug delivery systems (Chen et al., 2021).

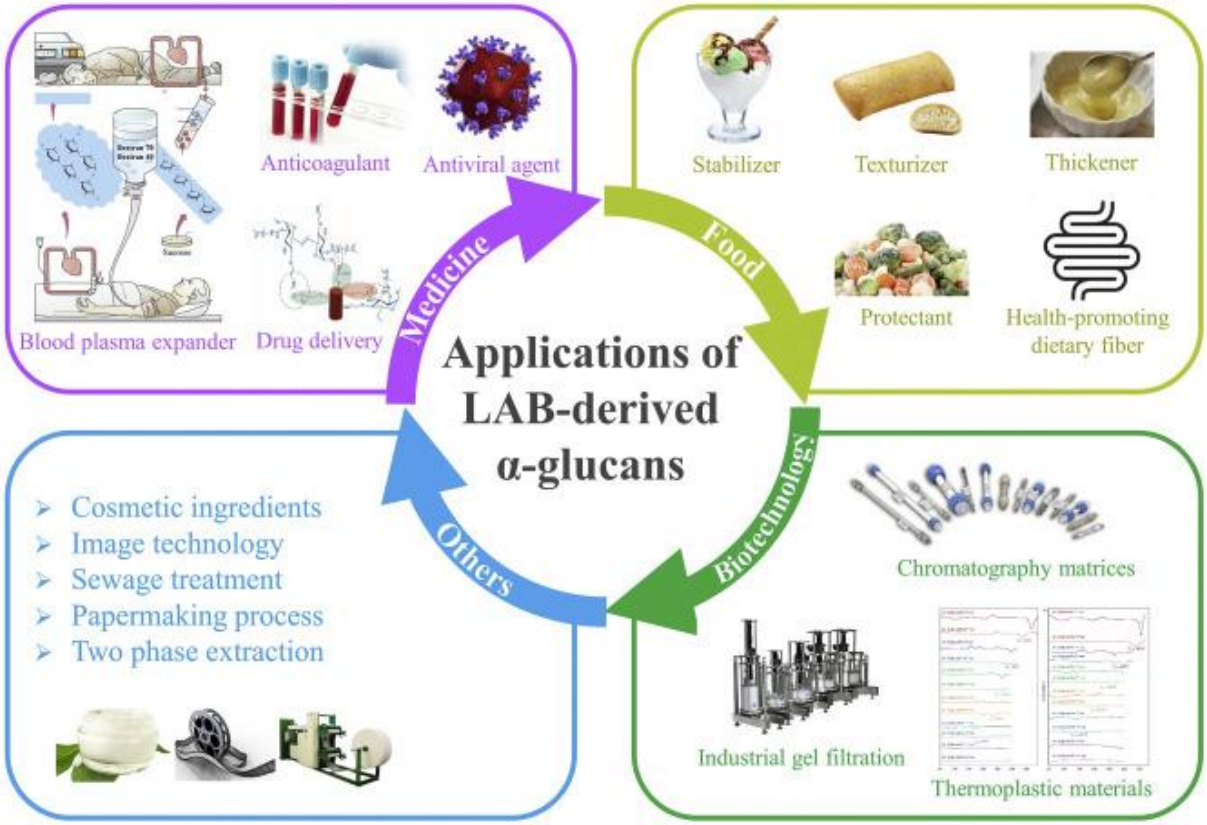

Figure 2. Applications of LAB-derived glucans (Chen et al., 2021).

8- Enzymes production

Microbial enzymes are biocatalysts produced by microorganisms that accelerate chemical reactions without being consumed. They have wide applications in food, pharmaceuticals, textiles, detergents, biofuels, and bioremediation.

Microorganisms, including bacteria, fungi, and yeast, are preferred for industrial enzyme production because of:

- Rapid growth and ease of genetic manipulation
- Ability to secrete large quantities extracellularly
- Stability under diverse environmental conditions (Rana and Upadhyay, 2020).

8-1- Types of microbial enzymes

Microbial enzymes can be classified based on their function or application:

Type	Function	Examples
Hydrolases	Break chemical bonds by adding water	Amylase (starch → sugars), Lipase (lipids → glycerol + fatty acids), Protease (proteins → peptides)
Oxidoreductases	Catalyze oxidation-reduction reactions	Laccase, Glucose oxidase, Catalase
Transferases	Transfer functional groups	Transaminase, Glycosyltransferase
Isomerases	Rearrange molecular structures	Glucose isomerase
Lyases	Remove/add groups to form double bonds	Pectin lyase
Ligases	Join molecules with ATP consumption	DNA ligase

8-2- Microbial Sources

Bacteria: *Bacillus*, *Pseudomonas*, *Escherichia coli*, often produce extracellular enzymes like amylases, proteases, and lipases.

Fungi: *Aspergillus*, *Penicillium*, secrete enzymes like cellulases, pectinases, and glucoamylases.

Yeast: *Saccharomyces*, produce enzymes such as invertase and proteases (Ates, 2015).

8-3- Enzyme production pathways

Microbial enzyme production is part of **primary or secondary metabolism**:

- **Primary enzymes**: produced during active growth (log phase) to support metabolism, e.g., amylases and proteases.
- **Secondary enzymes**: produced in stationary phase, often under stress or nutrient limitation, e.g., antibiotics-degrading enzymes, ligninases (Ates, 2015).

Biosynthesis mechanism:

- **Gene transcription** → mRNA coding for enzyme
- **Translation** → polypeptide chain formation
- **Folding and post-translational modification** (glycosylation, cleavage, disulfide bonds)
- **Secretion or intracellular accumulation** depending on enzyme type (Ates, 2015).

8-4- Fermentation strategies

Microbial enzyme production is usually done via **fermentation**, using two main strategies:

- **Submerged fermentation (SmF)**

Microbes grow in liquid media

Suitable for high-volume, homogeneous enzyme production

Easier to control pH, temperature, and aeration

- **Solid-state fermentation (SSF)**

Microbes grow on solid substrates with low moisture

Often used for fungi to produce cellulases, pectinases, and amylases

Can utilize agro-industrial residues (wheat bran, rice husk)

8-5- Applications

Food industry: Amylases for bread and beer, proteases for cheese

Textile industry: Cellulases for fabric softening and bio-polishing

Detergents: Lipases and proteases for stain removal

Pharmaceuticals: Enzymes for drug synthesis, diagnostics, and therapeutic use

Biofuels: Cellulases and xylanases for biomass conversion (Ates, 2015).