CHAPTER V: ANABOLISM and PRODUCTION of BIOMASS and METABOLITES

1. Production of Polysaccharides

1. Introduction

Many microorganisms do not perform photosynthesis and are therefore **heterotrophic**. Consequently, they must synthesize their **sugars** from reduced organic molecules rather than from CO_2. **Gluconeogenesis** (Fig. 01) produces fructose-6-phosphate and glucose-6-phosphate. Once these two precursor metabolites are formed, other sugars can then be synthesized.

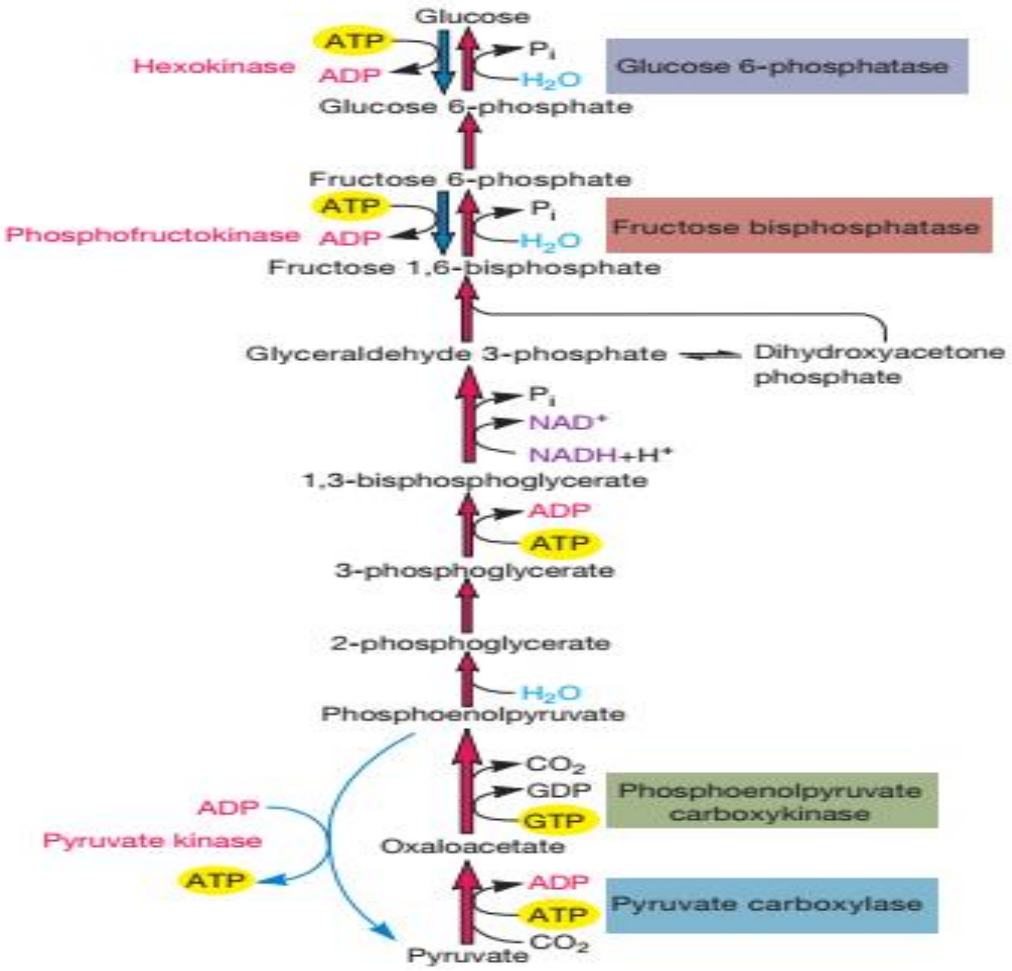

Figure 01: Gluconeogenesis.
[The gluconeogenic pathway used in many microorganisms. The names of the four enzymes catalyzing reactions different from those found in the EmbdenMeyerhof pathway (EMP) are in shaded boxes. EMP steps are shown in blue for comparison].

2. Biosynthesis of monosaccharides

Several simple sugars (monosaccharides) are synthesized when they are linked to **a nucleoside diphosphate**. The most important nucleoside diphosphate sugar is **Uridine Diphosphate Glucose (UDPG)**. Glucose is activated by attachment to the pyrophosphate of uridine diphosphate through a reaction with uridine triphosphate (Fig. 02).

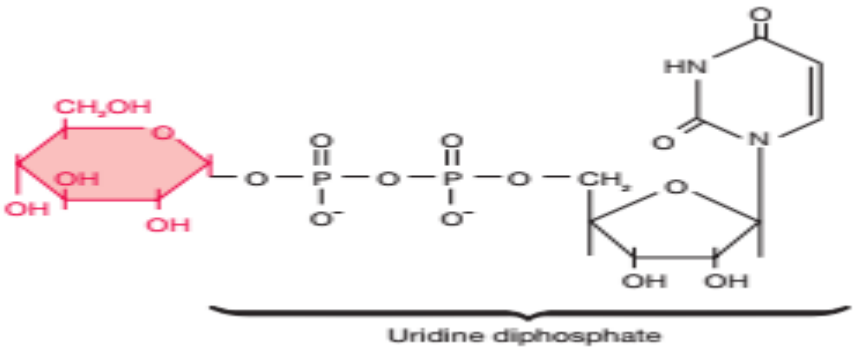

Figure 02: Uridine Diphosphate Glucose (UDPG).
[Glucose is in color].

Most **nucleoside diphosphate sugars** are formed by the condensation of **a nucleoside triphosphate (XTP)** with a **sugar-1-phosphate** (where the sugar may be D-glucose, D-galactose, D-mannose, 2-acetamido-2-deoxy-D-glucose, L-fucose, D-glucuronic acid, or another sugar). This reaction is catalyzed by a specific **pyrophosphorylase enzyme**, as shown in the following reaction:

$$\text{XTP} + \text{glycosyl phosphate} \xleftrightarrow{\text{Pyrophosphorylase}} \text{XDP} - \text{glycose} + \text{PPi}$$

X: may be any nucleoside, such as uridine, guanosine, cytidine, thymidine, or adenosine.

Examples of the biosynthesis of some monosaccharides:

- Glucose synthesis: glucose is activated by binding to uridine diphosphate (UDP) during the reaction between glucose-1-phosphate and uridine triphosphate (UTP) (Fig. 03). The UDP portion of UDP-glucose (UDPG) is recognized by enzymes, and UDP serves as a carrier that transports glucose within the cell, allowing it to participate in enzymatic reactions in a manner analogous to how ADP/ATP transports phosphate.

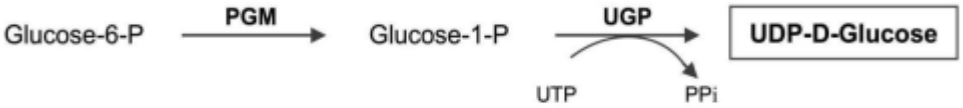

Figure 03: Metabolic pathway leading to UDP-D-glucose.
[**PGM:** phosphoglucose mutase; **UGP:** UDP-glucose pyrophosphorylase; **UTP:** uridine triphosphate; **PPi:** pyrophosphate].

- UDP-galactose synthesis: UDP-galactose is synthesized from UDP-glucose by rearrangement of a hydroxyl group (Fig. 04).

- UDP-glucuronate synthesis: UDP-glucuronate, a component of the bacterial capsule, is produced by oxidation of UDP-glucose (Fig. 04).

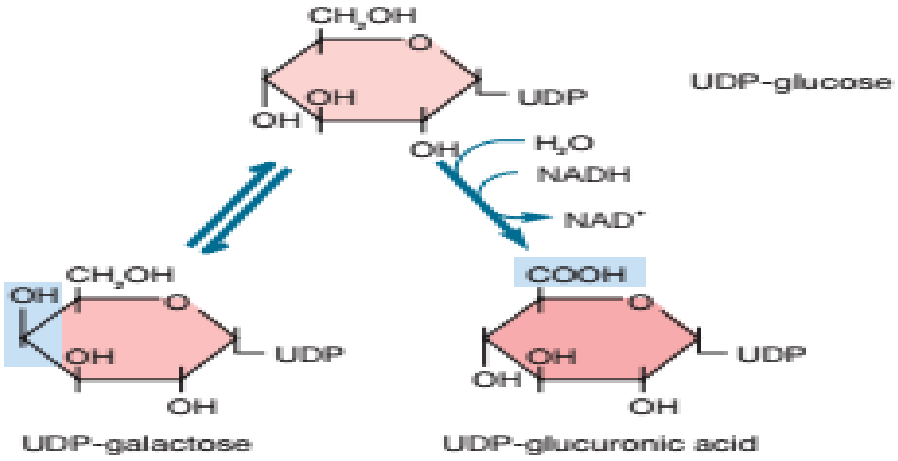

Figure 04: Uridine diphosphate galactose and Glucuronate synthesis.
[The synthesis of UDP-galactose and UDP-glucuronic acid from UDP-glucose. Structural changes are indicated by blue boxes].

- Mannose biosynthesis: mannose biosynthesis is an exception; it is directly derived from fructose-6-phosphate through a simple rearrangement. Mannose is present in many extracellular bacterial polysaccharides (Fig. 05).

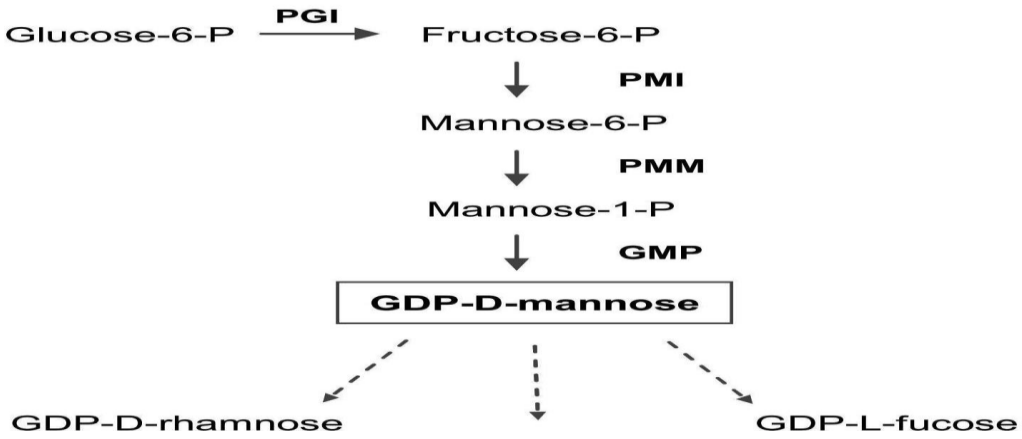

Figure 05: Biosynthetic pathway of mannose and GDP-mannose.
[**PGI:** phosphoglucose isomerase; **PMI:** phosphomannose isomerase; **PMM:** phosphomannose mutase; **GMP:** GDP-mannose pyrophosphorylase].

- GDP-D-rhamnose biosynthesis: the precursor of D-rhamnose, begins with the dehydration of GDP-D-mannose into GDP-4-keto-6-deoxy-D-mannose in a reaction catalyzed by GDP-D-mannose-4,6-dehydratase. The 4-keto group of this intermediate is then reduced to GDP-D-rhamnose

by the enzyme GDP-4-keto-6-deoxy-D-mannose reductase (Fig. 06). This sugar is mainly found in the lipopolysaccharides (LPS) of pathogenic bacteria, where it plays a role in host–bacteria interactions and in the establishment of infection.

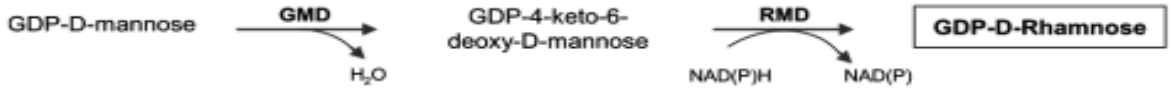

Figure 06: Metabolic pathway leading to GDP-D-rhamnose
[GMD: GDP-D-mannose-4,6-dehydratase; **RMD:** GDP-4-keto-6-deoxy-D-mannose reductase].

- Fucose synthesis: the synthesis of fucose in its activated form (GDP-fucose) also originates from GDP-mannose (GDP-Man). This nucleotide sugar is commonly found in complex carbohydrates that are components of the cell wall and the lipopolysaccharide (LPS) of certain Gram-negative bacteria.

3. Biosynthesis of polysaccharides

3.1. Glycogen synthesis

In prokaryotes, the precursor for glycogen synthesis is **glucose-1-phosphate**. This molecule must first be activated into **ADP-glucose**. Then, **glycogen synthase** transfers the glucose unit from ADP-glucose (ADP-Glc) to the **non-reducing end** of a pre-existing oligosaccharide, which serves as a primer and contains at least four glucose residues (Fig. 07).

However, it is now considered that bacterial glycogen synthase may be capable of initiating glycogen synthesis without a primer, using only ADP-glucose.

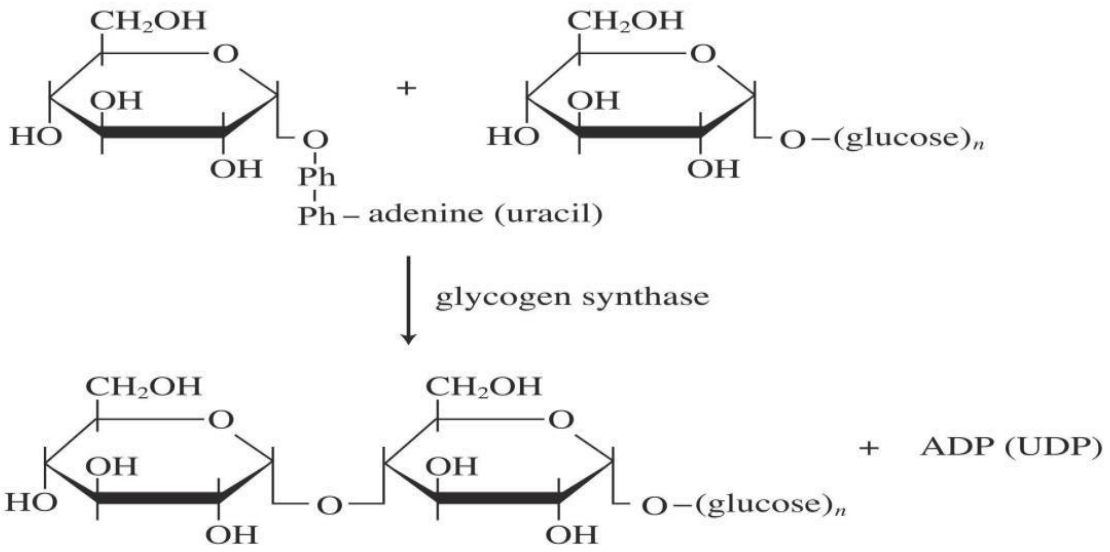

Figure 07: Formation of the α(1→4) glycosidic bond in glycogen by glycogen synthase.

Branching (formation of side chains) is carried out by a branching enzyme, **glycosyl(4→6)transferase**. This enzyme removes an oligosaccharide segment of 5 to 8 glucose residues from the non-reducing end of the growing chain and attaches it to a glucosyl residue of the main chain via an α(1→6) glycosidic bond (Fig. 08).

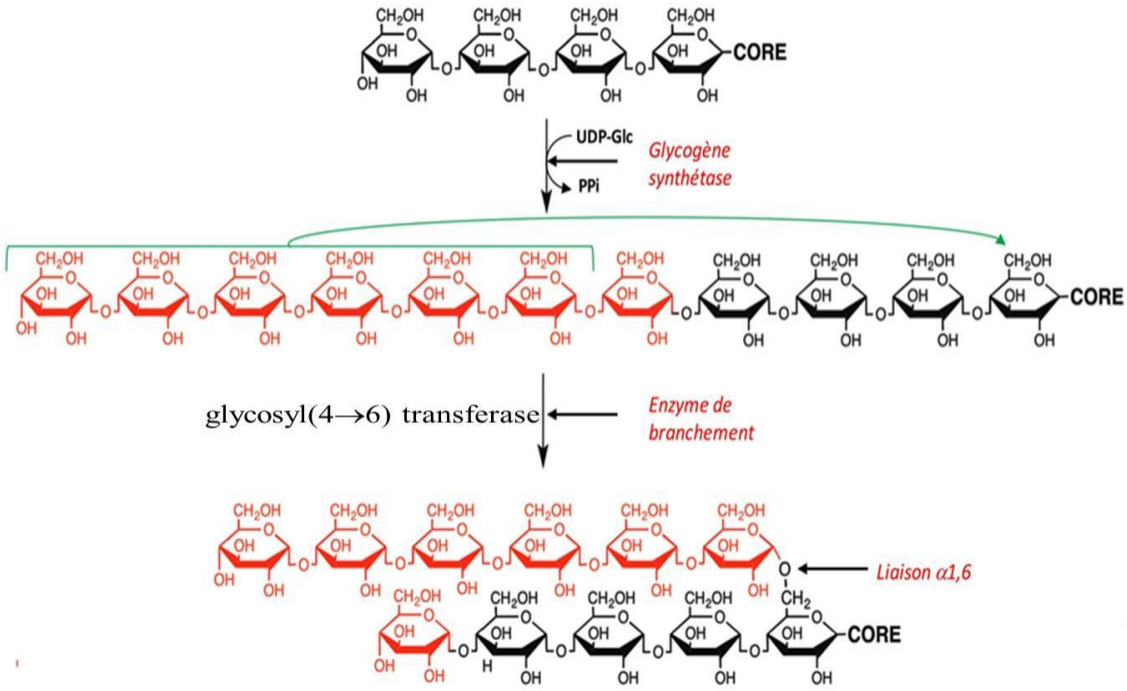

Figure 08: Glycogen elongation and branching.

3.2. Peptidoglycan (Murein) Biosynthesis

Nucleoside diphosphate sugars also participate in the synthesis of peptidoglycan in microorganisms. Peptidoglycan is a large, complex macromolecule composed of long polysaccharide chains made of alternating residues of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG), linked by β(1→4) glycosidic bonds (Fig. 09).

These polysaccharide chains are cross-linked by peptide bridges attached to the NAM residues. These bridges are composed of various amino acids, including D-alanine, L-alanine, glutamic acid, L-lysine, and diaminopimelic acid (a lysine analogue) (Fig. 10). Here, transpeptidation refers to the cross-linking of newly synthesized peptidoglycan to the pre-existing cell wall structure.

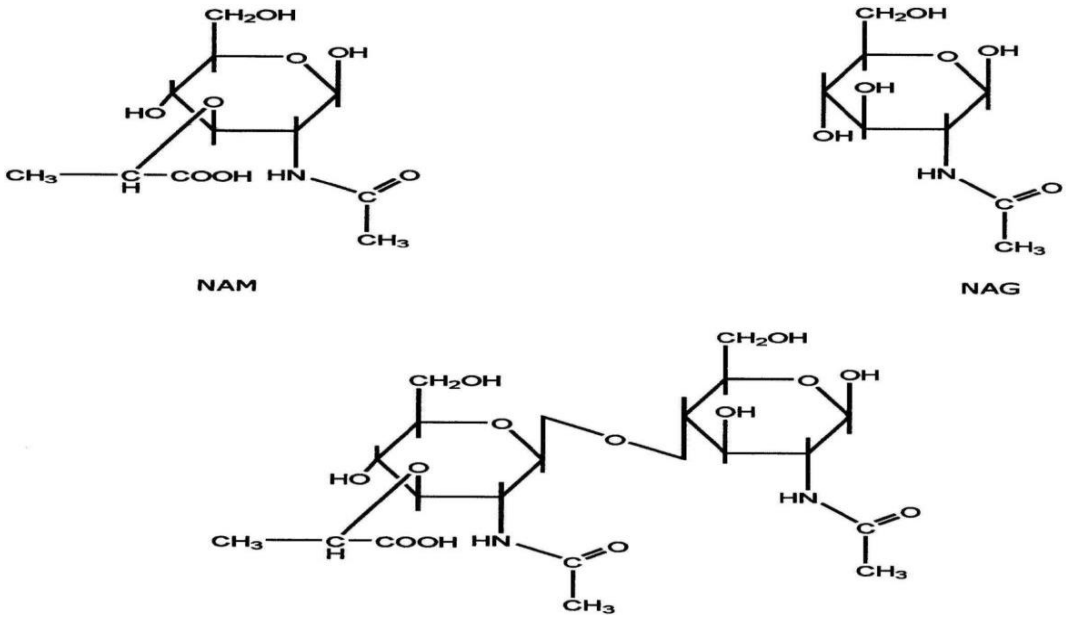

Figure 09: Structures of N-acetylmuramic acid (NAM), N-acetylglucosamine (NAG), and the NAM–NAG dimer.

[A β(1→4) glycosidic bond links NAM to NAG within the dimer, as well as between linear dimers].

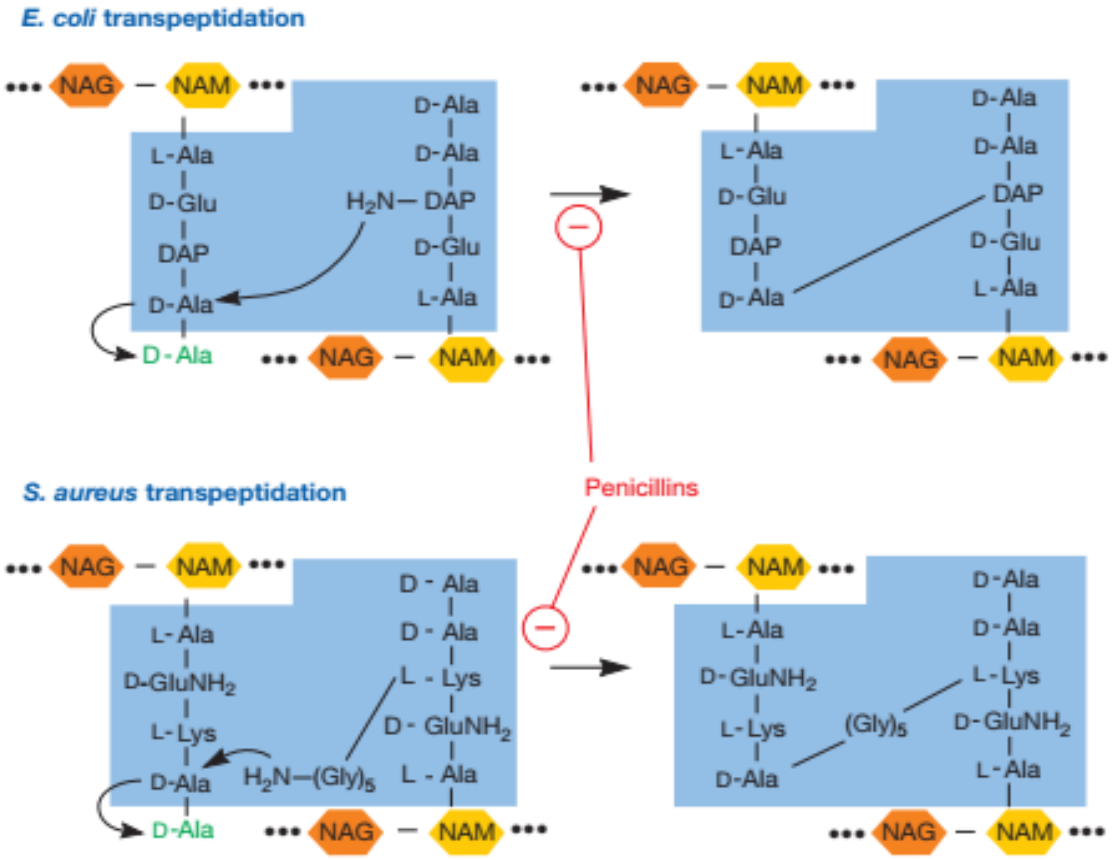

Figure 10: Transpeptidation

[The transpeptidation reactions in the formation of the peptidoglycans of *Escherichia coli* and *Staphylococcus aureus*].

4. Industrial production (Biosynthesis) of polysaccharides

Various microorganisms (and their mutants) synthesize polysaccharides of industrial importance.

- **Dextrans:** dextrans are produced by bacteria such as *Acetobacter*, *Streptococcus*, and *Leuconostoc*. They are high-molecular-weight polysaccharides composed of glucose residues linked mainly by α(1→6) glycosidic bonds, with branching through α(1→4) or α(1→3) linkages. Dextrans are synthesized from sucrose via a transglucosylation reaction, a typical mechanism in polysaccharide biosynthesis. They are used in the manufacture of resins and in the preparation of artificial blood plasma.

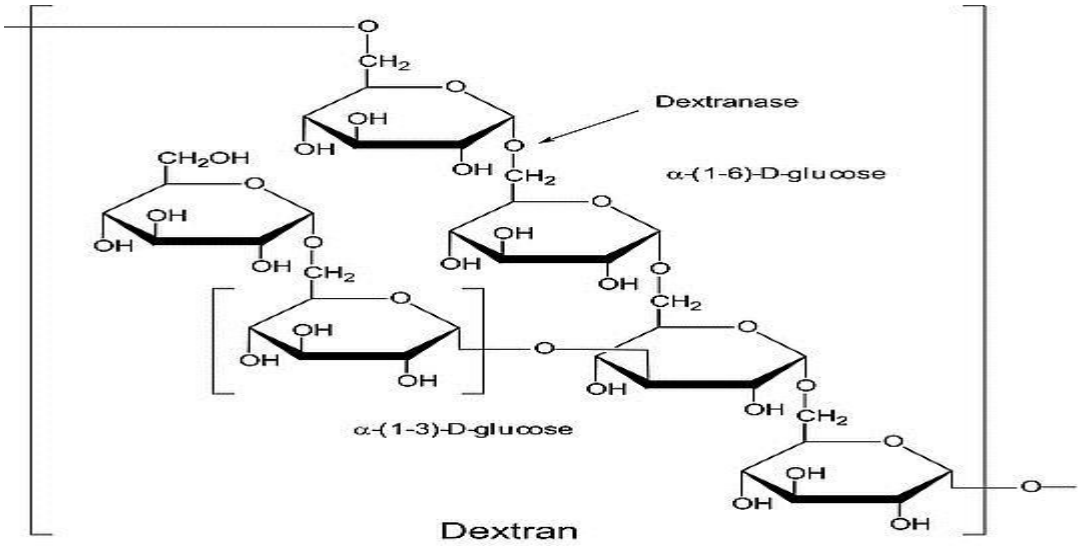

Figure 11: Structure of dextran.

- **Levans:** levans are also produced by bacteria such as *Bacillus*. They are high-molecular-weight fructans (polyfructosans) composed of fructose residues linked by β(2→6) bonds, with β(1→2) branching. Their biosynthesis occurs via transfructosylation from sucrose. They are used in polymer production.

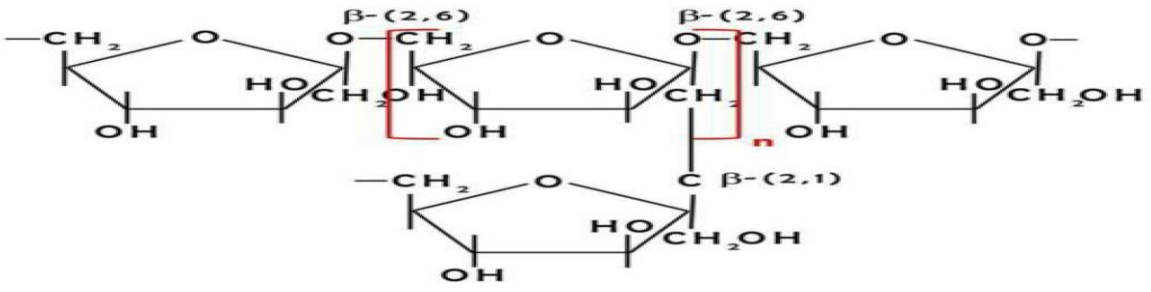

Figure 12: Structure of levan.