

BIOLOGICAL ACTIVITY OF *Rhizobium* LIPO-POLYSACCHARIDES: A REVIEW ARTICLE

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ABSTRACT

Lipo-polysaccharides are important substances that consist of LipidA, Core and O-antigen, and are formed in several metabolic pathways that include nine enzymes encoded by special genes called Custer genes. These carbohydrates are characterized by many physical properties, including viscosity. It's viscosity of polysaccharides viscosity ranges from 0.0020 to 0.0033 seconds/cm², Optical rotation, right-side dextrorotatory and levorotatory. Lipo-polysaccharides have a prominent role in many fields. In the agricultural, they contribute to the formation of root nodules on leguminous plants of great importance in nitrogen fixation processes. In addition to its role in the processes of creation and differentiation of callus leguminous plants, such as clover, alfalfa, and non-leguminous plants, such as tobacco. As for the medical and pharmaceutical fields, it has clear importance in the immune response processes through its presence as one of the important antigens present on the surface of bacterial cells. In addition to its role in the adhesion process leading to the formation of bacterial colonies and as barriers that prevent the penetration of anti-bacterial agents into the bacterial cell.

Key Words: *Rhizobium*, Lipo-polysaccharide, Lipid-A, Core regin, O-antigen

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INTRODUCTION

The Gram-negative bacteria possesses an internal membrane surrounded from the outside by the plasma membrane and from the inside of the cytoplasm, which plays an important role in protecting bacteria from external conditions and is the point of contact of bacteria to the outer environment, as well as its importance in various biological activities of bacteria such as movement and contact with host (Lavery, *et al.*, 2014). This membrane is called Lipo-Polysaccharide (LPS), which is chemically synthesized from three regions: lipid-A, core, and O-antigen, the Lipid-A region is the part responsible for the association with the bacterial membrane, while core, is consists of frequent units of multiple sugars that communicate with lipid-A by a poly-2-keto-3-deoxyoctulosonic (Kdo), and the third part O-antigen consist mainly of sugar units (D' Antuono *et al.*, 2005). O-antigen is also formed in *Ensifer meliloti* from four repeating units of glucose amine linked together by β -1,4-linkage to form the N-acetyl-D-glucosamine chain, carrying the sulfate group as sulfated carbohydrates responsible for choosing the right legume host for it, known for its role in suppressing plant defense systems by protecting bacteria from the full effect of phytotoxins secreted in the area around the roots (Lerouge and Vanderleyed, 2002; and Campbell *et al.*, 2002). Several studies have also been conducted on its use (LPS) in the field of tissue culture on some leguminous and non-leguminous plants (Tang *et al.*, 2014).

1- The types of polysaccharides in rhizobium bacteria:

Researchers Noel and Duelli (2000) indicated that polysaccharides exist in three forms on the outer membrane of *rhizobium* bacteria. Figure (1) shows these three types of sugars and their locations on the surface of *rhizobium* bacteria.

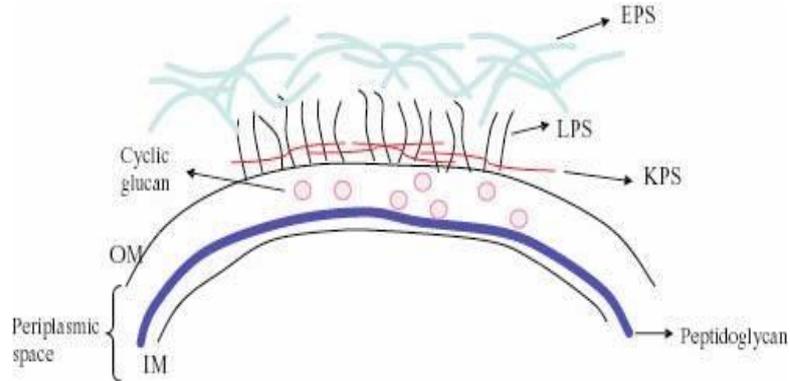


Figure (1): The three forms of polysaccharides located on the surface of *rhizobium* bacteria (Lepek and D'antuono, 2005).

1-1 Exopolysaccharides: It is a compound of an acidic nature outside of cellular differential composition with high molecular weights, and possesses repeating units between (7-9) of hexose sugar, and is not associated with each other by linkage glycoside bonds, furthermore having four non-carbohydrate substitutes such as succinate, pyruvate, or acetate, their acidic nature is due to the organic acids uronate, pyruvate and succinate, Pyruvyl, units of glucose (Spaink, 2000), and (EPSII) galactoglycan EPS, which consist of repeating units of two types of sugars, glucose and lactose, group acetyl and pyruvyl (Moreira *et al.*, 2000). Its main function is to protect the cell from environmental stresses in addition to its function in the association of rhizobial bacteria with the roots of leguminous plants and then in the formation of root nodules during the symbiotic relationship that results in the fixation of atmospheric nitrogen to ammonia (Frayse *et al.*, 2005).

1-2 Capsular polysaccharides: It's a collar that surrounds these bacteria and gives them the characteristic of resistance against bacteriophages because they contain hydrate. Which is similar to the structure of K-antigen present in *Escherichia coli* bacteria and that is why it is called K-antigen Polysaccharides (KPS) polysaccharide K-antigen (Sachdeva, *et al.*, 2017), and it has an indirect role in the symbiotic relationship of *Ensifer meliloti* bacteria, the strain Ak31, which forms an indefinite nodule. Alfalfa plants also have a role in the sticking of bacterial cells to each other on the outer membrane (Lepek and D'Antuono, 2005).

1-3 Lipo-PolySaccharides (LPS): a lipid-glycans complex, usually liberated during the normal growth cycle of the bacterium or after the death of the cell or its breakdown by the host's defenses (Todar, 2002), also known as lipo-glycans or endotoxins. They are large molecules that usually consist of fats and multiple sugars that are bound together by covalent bonds. It is found in the outer membrane of the Gram-negative bacteria, and it provokes strong immune

responses in animals. The term lipo-oligosaccharide (LOS) is used to refer to the low molecular weight of some of its types (Kilar *et al.*, 2013), given that the average molecular weight of the sugar molecule poly-fatty acids are estimated at 07-106 dalton, on the other hand, many studies indicated that poly-fatty sugar is stable at boiling point for (30) minutes and that it breaks down when treated with strong oxidizing agents such as hypochlorite, superoxide, peroxide and is not digested with proteolytic enzymes (Todar, 2002), these molecule importance in bacteria is to help helping the cell membrane to be more stabilize, as well as to be a barrier against anti-bacterial compounds such as base peptides, on the other hand, its composition varies from one strain to another (Lerouge and Venderlegden, 2002). Several researchers have found that it has specific roles in the late stages of root nodules formation processes such as penetration of infection thread into cortical cells or of the nitrogen fixation phenotype formation (Frayse *et al.*, 2003).

1-4 Lipo-polysaccharide structure: It is a complex compound consisting of hydrophobic and hydrophilic molecules, and it is found in all the outer membranes in the Gram-negative bacteria, and it consists of two chemically different regions, the hydrophilic region, which includes the core region, O-antigen, and the hydrophobic region is called the Lipid-A region (Raetz and Whitfield, 2002), as shown in Figure (2).

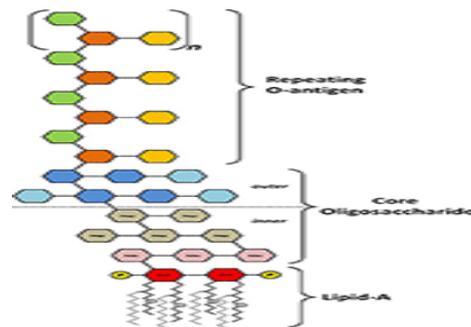


Figure (2): Chemical structure of Lipo-polysaccharide (Serrato, 2014).

1-5 O-Antigen sidechain -region: is a polymeric glucan, whose composition varies from one strain to another, and there are 160 types of it (Raetz and Whitfield, 2002). The presence or absence of this chain depends on the type of LPS, as it is present in the form of a long chain in the case of smooth LPS and is absent or reduced in the rough LPS, as it contains repeated secondary units of oligosaccharide consisting of 3-5 sugars (Rittig *et al.*, 2003). Also, these chains may differ in their lengths exceeding 40 repeated units of sugars, so that, the O-antigen polysaccharide is longer than the sugar present in the core region, and in general, the response against the LPS is often attributed to the O-antigen (Lerouge and Vanderleyden, 2002) because the principle antigenic in terms of antibody binding to the Gram-negative bacterial wall (Todar, 2002).

1-6 Core-Region: It is a short chain of low-unit sugars covalently attached to the site (6) of (N-acetylglucosamine), which in turn binds to lipid-A, noting that this core region consists of two types of unusual sugars, namely heptose Keto -3-deoxy octanoic acid (Kdo), it is a distinct and exceptional compound that is permanently present in LPS, so it is positive evidence for the presence of the Core-

region in multiple sugar tests, It is also found in all types of the same bacterial genus, but it differs morphologically between the different genus of Gram-negative bacteria such as *Shigella*, *Escherichia*, and *Salmonella*, and these types are similar in which the Core-region is not identical (Todar, 2002). The Core-region of the lipo-polysaccharide in *E. coli* consists of five distinct structures of oligosaccharides (Amor *et al.*, 2000).

1-7 Lipid-A Region: This region is responsible for the endotoxin in bacteria, and its chemical composition has been determined in different bacterial types, including *E. coli* (Figure 3), (Silipo *et al.*, 2010). The core region of these bacteria was considered as the lowest structure capable of exhibiting endotoxin activities represented by the molecule containing residues of two or two groups of D-glucoseamin, two phosphorylated groups, and six fatty acids (Kato *et al.*, 2000). Although it is stable at high temperatures in Gram-negative bacteria, its activity depends on its chemical composition, chain length, and a number of sites of acetyl group in addition to the number of phosphate groups (David, 2001).

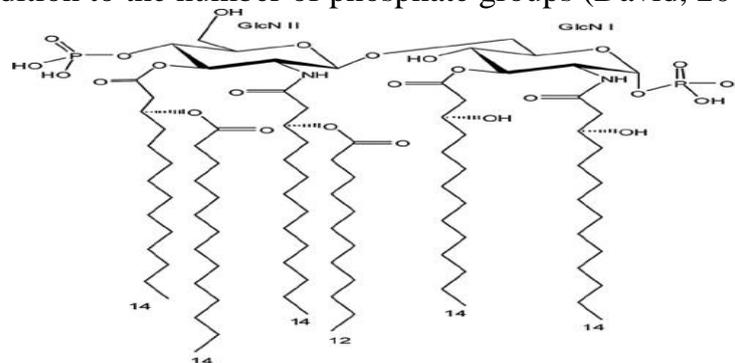


Figure (3): The chemical composition of lipid-A from the Lipo-polysaccharide of *Escherichia coli* (Silipo *et al.*, 2010)

1-8 Metabolic pathways for the manufacture and transfer of Lipo-polysaccharides:

The synthesis of LPS begins in the cytoplasm and goes through several consecutive polymerization processes until it reaches the final formula of its chemical composition. Its synthesis and metabolic pathways are among the characteristics of the Gram-negative bacteria. The synthesis process of it begins at the inner side of the plasma membrane and is carried out with the involvement of nine different enzymes, including enzymes LPXL, LPXC, LtdA, LPXK, LPXB, LPXH, LPXD, LPXC and LPXA. It begins to form Uridine diphosphate - diacyl-D-glucosamine (UDP - diacyl - GlcNAc) from Uridinediphoshat N-acetylglucosamine (UDP-GlcNAc) which is plays a key role in the biosynthesis of lipid-A in *R. leguminosarum*, different enzymes;

(i) UDP-GlcNAc3-O-acyltransferase;

(ii) UDP-3-O-(R-3-hydroxymyristoyl)-lcNAcdeacetylase;

(iii) UDP-3-O-(R-3-hydroxymyristoyl)-GlcNN-acyltransferase;

(iv) disaccharide synthase;

(v) 4'-kinase; and (vi) Kdo transferase (Price *et al.*, 1994), that catalyze the early steps of *E. coli* lipid-A biosynthesis were also present in extracts of *R. leguminosarum*, and all these enzymes of the pathway leading to the formation of the intermediate 3-

deoxy-D-manno-2-octulosonic acid (Kdo₂)-lipid IV_A are functional in both *R. leguminosarum* biovars *phaseoli* and *viciae*, thus giving the final KDO₂-lipid-A by four enzymes; 1-phosphatase, a mannosyl transferase, a galactosyl transferase, and a long chain acyl transferase, and three enzymes; Lauroyl transferase (HtrB), Myristoyl transferase (MsbB) and Heptosyl transferase (RfaC), in *R. leguminosarum* and *E. coli*, respectively, Fig.(4), (Brozek *et al.*, 1996).

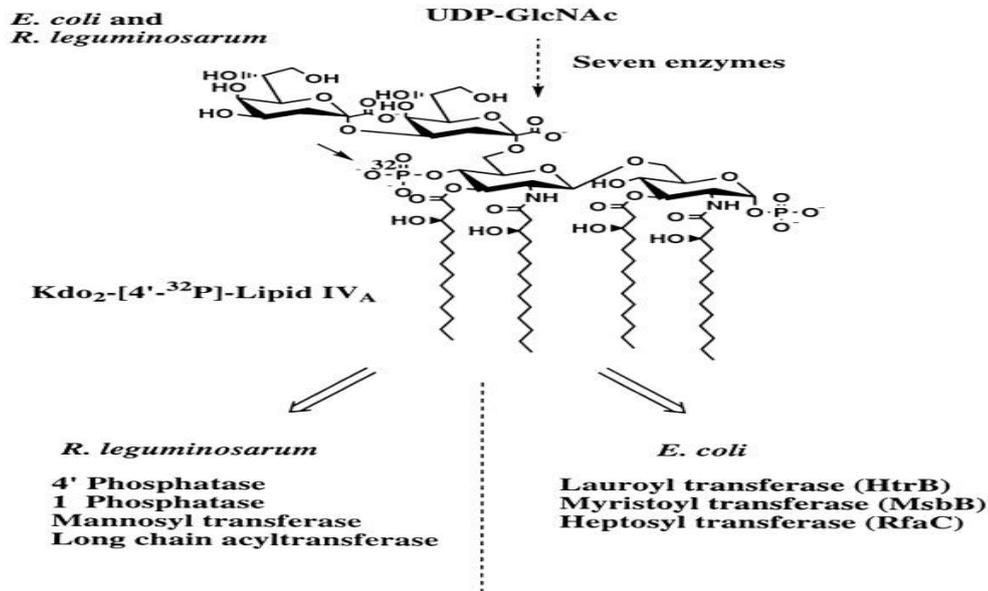


Figure: (4): Conserved biosynthesis followed by divergent processing of (Kdo)₂-lipid IV_A in extracts of *R. leguminosarum* and *E. coli*. (Brozek *et al.*, 1996).

As shown in Figure (5), Lipid A, which is made in the cytoplasm and inner membrane, passes another stage by glycosyltransferases, where it joins the membrane with losing a sugar molecule to form Core Oligo-saccharides, which consists of 10-15 single sugar molecules, and then splits into two molecules to form Lipid-A and O antigen and thus the formation of lipo-polysaccharides (LPS), (Wang and Quinn, 2010), to be transported through the cytoplasmic space between the two membranes to reach the outer membrane by many protein vectors such as LPTD, LPTG, LPtF, LPtC, LPtB, LPLA, LPtE (Sperando *et al.*, 2007). Sometimes it may be required to send it back to the inner membrane to re-polymerize it if there is any deficiency in its components (Bos *et al.*, 2004), this means that the LPS manufacturing process can be summed up in two paths: the first includes the formation of Lipid-A and the core, and the second includes the formation of the O-antigen (Wang and Quinn, 2010). A large number of cluster genes located on the bacterium chromosome and its plasmids may participate in its synthesis, including the LPXD - Fabz - LPXA - LPXB Cluster genes, as the LPXB and LPXD gene group is split to manufacture N-acyltransferases, which add fatty acids to the glucosamine compounds, while the LPXB gene encodes for the synthesis of disaccharide of Lipid-A from UDP-2-3 diacyl glucosamine, and the Fabz gene encodes for an enzyme that reduces the (3R)-hydroxyacyl-Acp complex to trans-2-acyl-Acp used as precursors of amino acids to manufacture phospholipids (Wang and Quinn, 2010). The Waa cluster genes encode to make several modifications to Lipid-A by adding an Mg component, and the Pmr A and

Pmr B genes make modifications to LPS by adding Phospho ethanolamine (PETN) to the Core region of the LPS complex, and the operon *D-gmh* genes encode for building the synthesized LPS transporter proteins and link Polysaccharide to Lipid-A and the Core region (Gunn, 2008). The genes responsible for the synthesis of O-antigen were also classified, the first involved in the manufacture of internal sugars and involved in the synthesis of O-antigen, the second stimulates the manufacture of primary sugars to form Oligosaccharid, and the third worked to terminate the process of manufacturing O-antigen and its transmission through the membrane (Reeves and Wang, 2002), these genes encoding proteins active in these processes include the *gulf* and *gnd* genes and the genes encoding man B and C enzymes that convert mannose-6-p into GDP-mannose (Ma *et al.*, 2008).

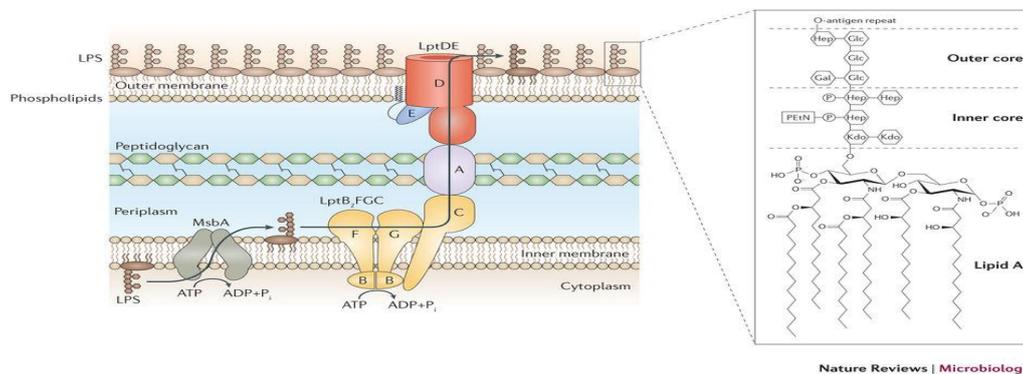


Figure.(5):Metabolic pathways for the manufacture and transport of Lipo-Polysaccharides (Schmid *et al.*, 2015).

1-9 Factors affecting the production of polysaccharides:

The process of producing lipo-polysaccharides is affected by various factors, including the composition of the nutrient medium, and the Incubation conditions are of great importance in the growth of microorganisms and stimulating their production from lipo-polysaccharides and providing them with the necessary energy for the processes of biological construction (Duta *et al.*, 2004), It is known that the main goal of changing the cultural conditions attempts to find out the factors affecting the increase and decrease in the productivity of Lipo-polysaccharides and to know the response of the bacteria to these changes, as more than one cultural factor may interfere to give the best response, and it is not possible to separate each factor affecting separately (Pinto *et al.*, 2002). The most important factors affecting production are:

1-9-1 Strain variation: There are clear differences in the ability of bacterial isolates to produce multiple lipo-polysaccharides, as well as the quality of the product from a chemical and physical point of view, and this difference is mainly due to the strain of bacteria isolated from different cultural areas as each strain has properties that make it independent of the strains. The other, and this explains to us the conflicting and disparate results related to the production of polysaccharides when it comes to the different strains used in this research (Duta *et al.*, 2004).

1-9-2 Culture component: The components of the culture media play an important role in the growth of microorganisms (Pinto *et al.*, 2002), and affect the metabolites of these microorganisms, the most important of these components are:

- A- The carbon source:** Carbohydrates are a general source of carbon, such as glucose, as it is used by micro-organisms more than any other sugar and is a general carbon source for them, as well as carbohydrates, are among the common sources used by microorganisms in the manufacture of polysaccharides. Since the production of multiple sugar and its quality depends on the carbon source used, which in turn affects the production (Basu and Ghosh, 2001). However, if it does not produce all the basic intermediate compounds necessary for the synthesis process, it is not correct to use it to feed these micro-organisms as the only source of carbon (Pereira Duta *et al.*, 2006), this is because the carbon element performs two important purposes for microorganisms. First, it is necessary to build essential compounds such as carbohydrates, proteins, lipids, and nucleic acids, and secondly, it is important in the process of carbon oxidation and provides a source of energy that is used to accomplish the biological processes of microorganisms (Al-Khafaji, 2008). It was found that the best medium for the production of LPS by that contains mannitol, sucrose, and glucose (Pereira Duta *et al.*, 2006). Also, the concentration of the carbonic source affects the efficiency of its transformation into multiple sugars. For example, the efficiency of the conversion of sucrose into a polymer by the rhizobium bacteria decreases with the increase in the concentrations of the carbonic source of sucrose. (Basu and Ghosh, 2001) pointed out that the different monosaccharides and disaccharides used in the production of the LPS from *Rhizobium* support its production in varying percentages.
- B- Nitrogen source:** Nitrogen constitutes about 8-14% of the dry weight of microorganisms and is included in the composition of their cellular components, especially amino acids, protein, nucleotides, some vitamins, and many enzymes necessary for the representation of these sugary compounds and other compounds (White, 2000). It also controls the production of microbial polysaccharides in liquid medium (Wenster-Botz, 2000). Therefore, a source containing nitrogen must be available in the environment so that microorganisms can exploit it, and these sources play an important role in providing this element for use in building units. More than the role they play as energy sources, (Burgin *et al.*, 2011). There is a wide range of organic and inorganic nitrogen sources that are used to meet the needs of the microorganism from nitrogen, and inorganic salts such as NH_4^+ and NO_3^- and organics such as amino acids, urea as well as non-synthetic sources of nitrogen such as yeast extract and casamino acid of milk. Among the most important nitrogen sources that are exploited by micro-organisms in the production of polysaccharides are ammonia salts, amino acids, nitrates, and nitrogen gas. The first and second sources are among the most used nitrogen sources in cultural circles, but they must be added in balanced proportions because increasing the concentration of these sources stops the ability of bacteria to produce multiple sugars on despite its stimulatory effect on increasing bacterial growth (Wenster-Botz, 2000).
- C- Inorganic nutrient:** Despite the availability of energy sources, carbon, and nitrogen for the microbial cell, it cannot grow in the absence of mineral elements (Duta *et al.*, 2004). Therefore, its presence in food circles, in general, is necessary for the growth of micro-organisms as well as for the production of various metabolic materials, whether primary or secondary metabolites, as they act as a

catalyst for some enzymes that play an important role in the metabolic processes, and play an important role in controlling cell membrane permeability, including the transfer of energy sources, so the presence of some positive ions such as potassium, magnesium, iron, and calcium in the culture media is necessary to give an ideal growth. On the other hand, the micro-organisms need negative ions such as Cl^- which may act as a catalyst for some enzymes involved in the metabolic processes or it may play an important role in the permeability of the cell membrane and the transmission of energy sources. However, these elements may be bidirectional, as increasing concentration to an optimal level inhibits production (Al-Khafaji, 2008). Inorganic nutrients include:

- 1. Phosphorous:** living cells, including bacteria, need phosphorous, which plays an important role in metabolism and other vital activities. Phosphorous is included in high-energy compounds such as ATP, GTP and others. It is also used in the synthesis of fats containing phosphorous and acids. (Al-Khafaji, 2008). Xu *et al.*, (2012) also studied the effect of phosphorus deficiency on cyclic (12) gluconate in *Agrobacterium tumefaciens*, and they was found that this leads to a loss of the bacteria's ability to convert the phosphoglycerol compensator into β (1 \rightarrow 2) Cyclic, One of the studies (Rehm, 2010) has indicated the possibility of using phosphorous to form KH_2PO_4 and he concluded from this study that phosphorus is an important factor in the production of polymers in microorganisms.
 - 2. Magnesium:** It is an important inorganic nutrient that micro-organisms need in adequate quantities. Important cell components such as the membranes and cell walls of bacteria, as well as help in the synthesis of ribosomes (Al-Khafaji, 2008).
 - 3. Sulfur:** It is one of the important minerals that microorganisms need to produce polysaccharides. The role that sulfur plays in microorganisms is more than the role it plays for the rest of the cell types. Sulfur enters in the synthesis of amino acids such as cysteine, cystine, and methionine (Martin *et al.*, 2011). It may be one of the important elements that micro-organisms need to carry out various metabolic activities, and ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$, which is used as a nitrogen source, represents the usual source of sulfur that is used by microorganisms in agricultural media prepared to produce various compounds. Al-Khafaji (2008) indicated that sulfur is found in some types of vitamins such as thymine, biotin, and pyridoxine.
 - 4. Potassium:** It is entered as a companion to some enzymes as it helps in stimulating growth and the cell needs in the synthesis of carbohydrates and the transport stages (Etesami *et al.*, 2017). Potassium ions may be added, usually in an inorganic form, in the form of $(\text{K}_2\text{HPO}_4$ or KH_2PO_4) to the prepared nutrient media to develop some types of microorganisms.
- D- Elements and other factors:** The micro-organisms in their own culture media need to add growth factors as these substances stimulate the growth of micro-organisms. The growth factors to include the groups: vitamins, amino acids, nitrogenous bases, and other factors such as some types of alcohols (ethanol, methanol) or fats, fatty acids and some types of plant hormones. These compounds are used in very low concentrations in nutrient media to stimulate the growth of micro-organisms and produce important compounds such as citric acid, antibiotics, and polysaccharides (Al-Khafaji, 2008). Yeast extract is added to the

food media preparation for the growth of micro-organisms and the production of polysaccharides as one of the compounds containing various growth factors and vitamins to stimulate the production of polysaccharides by micro-organisms (Hakobyan, *et al.*, 2012).

E- pH value: The pH is one of the important factors in the production of polysaccharides. During the subsequent stages of fermentation of polysaccharides, it was observed that the concentration of hydrogen ion decreases due to the formation of polymers and organic and inorganic acids, where research indicates the role of pH in influencing the growth of micro-organisms and its production from primary or secondary metabolites, in particular extracellular polysaccharides. Duta *et al.*, (2004) explained the effect of the pH on the growth of micro-organisms and stated that multiple factors change the pH during biological processes, including acid-producing components and some types of nutrients, as well as oxidation and reduction reactions and changes in neutral nutrient solutions, he also showed that the effect of the pH in the production of polysaccharide is more than its effect on the growth of micro-organisms, and explained its effect on the permeability of cells and the nature of the vital activities that occur in the cell. The hydrogen ion concentration and its fall between (6.0-7.5) is very important in the process of building commercial polysaccharides of bacteria (Gorret *et al.*, 2001), and the rise or fall of the final pH of the fermentation medium depends on the type of bacterial strain and the composition of the culture medium.

5- Physical Properties of Lipo-polysaccharides:

5-1 Viscosity: The polysaccharide microbial solutions are characterized by their viscous nature, as their viscosity varies according to the type of the micro-organism producing the polysaccharide and even with the different strains of the same type, and this viscosity is one of the main parameters determining the rheological properties of this compound (Moretto *et al.*, 2015). Poise is used to express the viscosity relative to the world Poiseuille, and it is defined as the force estimated in the unit of one dyne, sufficient to move a flat surface of an area of 1 cm² away from a distance of 1cm from another surface similar to it, which gives acceleration to the moving surface of 1cm/second, the viscosity of multiple sugars depends on several factors including the components of the culture medium, the source and concentration of the elements of carbon and nitrogen, mineral salts and fermentation conditions such as pH, oxygen concentration and the degree of product movement (Duta *et al.*, 2004). The viscosity of this compound was measured by Al-Zuhairi (2002) by preparing aqueous solutions from (LPS) and measuring it with a viscometer, which ranged between 0.0020 - 0.0033 (dyne. Sec/cm²).

5-2 Specific optical rotation: Optical rotation is one of the common physical characteristics of polysaccharides and this characteristic varies according to the type of polysaccharide. Where sugars are considered optically active substances and are distinguished by the special rotation of the level of polarized light circularly to the right, as in the case of α -glucan, and this compound is called dextrorotatory, so is given the symbol (+ or D), while the left-spinning compound is called Levorotatory, as in the case of β -glucan, and it is symbolized by it (- or

L). The phase difference between the waves of polarized light circularly to the left or right lead to the rotation of the level of polarization of the light passing through the sugar solution, and it varies according to the type of sugar, it is usually symbolized by it $[\alpha]_D^{20}$ where (a) represents the degree of optical rotation and (D) represents the wavelength (Al-Fleih,2000). The optical rotation of the lipopolysaccharides produced from the rhizobium bacteria is performed to detect the active groups using the infrared ray of the IR device (Al-Zuhairi, 2002), this assay is used to detect that the LPS compound contains the carbonyl group, the ester group, and the OH hydroxyl group, respectively.

6-LPS and its role in the symbiotic relationship between *Rhizobium* and leguminous plants

The LPS compound plays an important role in the success of the symbiotic relationship between *Rhizobium* bacteria and leguminous plants, as these compounds consist of (4-5) repeated units of glucosamine tetra or penta Emeric N-acetyl glucosamine (GlcNAc) to form a two-ended chain, the first end is reduced, bearing compensators specific to the rhizobia species are important for forming root nodules on leguminous plants specialized in their infection, and they may be either in the form of N-acetyl-glucosyl or in the form of a sulfate group (Broughton *et al.*, 2000). The second non-reducing end holds the fatty acid chain at carbon atom No. 2 and the acetyl group at carbon atom No. 6 of the Glucosamine molecule (Cullimore *et al.*, 2001). Nodulation (Nod) factors are produced by two types of genes: the common genes and host-specific *nod* genes. The common genes *nodABC* are found in all types of rhizobia. Together, by the enzyme N-acetyl glucosamine 1 transferase encoded by the *odc* gene, to build the chitin portion (UDP - N-acetylglucosamine) which is the backbone of the compounds of nodule-forming factors (René and Ton, 2002). The *nodA* and *nodB* genes are responsible for the transfer of the Acuity 1 group and the fatty acyl chain into the free amine group that extends at the non-reducing endpoint, respectively (Galibert *et al.*, 2001), while the host-specific genes, including the *nodL* gene, encoding to build acetyl group, while *nodFE* genes encode to build the unsaturated fatty acyl chain at the non-reducing end of the N-acetylglucosamine (GlcNAc) chain (Geurts, and Bisseling, 2002). Fatty acids differ in the number of saturated and unsaturated carbon atoms that form them according to the type of rhizobia bacteria. In *Ensifer meliloti* bacteria, it consists of 16 carbon atoms, two of which are unsaturated (16: 2), while in *R. Leguminosarium* bv. *viciae* consists of 18 carbon atoms, one of which may be saturated and four unsaturated (18: 1, 18: 4), and in *Mezorhizobium loti* bacteria, this chain is made of 18 carbon atoms, one of which is saturated (18:1), (Diaz *et al.*, 2000). The other genes, the *nodHPQ* genes that are also specialized in the host, encode the production of specialized compensators located on the carbon atom No. 6 of the glucose amine molecule at the reduced end of the GlcNAc chain (Keatling *et al.*, 2002). Nodulation factors agents are associated with recipients located on the root hairs of plasma membrane (Cullimore *et al.*, 2001), two types of them (NFBS1 and NFBS2) were distinguished in both *Medicago* and *Phaseolus* beans, the first site having little interest in binding and not having any role in the symbiotic relationship, and the second site with high affinity for binding to the fatty acyl chain present in the nodulation factors and had an important role in the success of the specialized symbiotic relationship as well as, the known role of these

factors in the deformation of the root hairs and the occurrence of divisions of cells of the cortex infected with bacteria to form the primary nodules called primordium nodules(Perret *et al.*, 2000).

7-The role of lipopolysaccharides (LPS) in tissue culture of plants:

Many of the studies indicated that the LPS compound consisting of (4-6) sugar units has a positive effect in stimulating cell division more effectively than the compound consisting of less than 4 units of monosaccharide, and the first evidence of its effectiveness has indicated that it is directly responsible for abnormal growth and development of somatic embryos in plant pieces of *Medicago sativa*(Hoori *et al.*, 2007). As well as its ability to induce plant pieces of *Pimpinella anisum* seedlings to divide and create and differentiate callus from it alone or in combination with growth regulators (AL-Barhawi and AL-Zaidy,2020), while other studies have indicated the importance of the lipo-polysaccharides isolated from *Ensifer meliloti* bacteria and added to solid MS medium, in callus formation from hairy roots cultures formed on *M. sativa* by infection with *Agrobacterium rhizogenes* R1601 (Habash, 2002), and in this context Al-Zuhairi (2002) succeeded in isolating callus from pieces of stems and root of *Trifolium repens* when supplementing Murashige and Skoog medium(MS)with concentrations (4 and 6) mg/L of lipo-polysaccharides isolated from *Rhizobium leguminosarium* biovar *trifolii*.

The study conducted by (Al-Qusaymy, 2006) indicated a high rate of nodule numbers and a high protein content of *S. meliloti*-infected seedlings *Medicago sativa*, the tops of the root hairs swelled and the protoplast was released from them when grown this seedlings on NFree medium (NF) supplemented with LPS compound , and the growth rates increased of callus for pieces plant grown on MS medium supported with interfering concentrations of 1-Naphthaleneacetic acid (NAA) and LPS,and formation of green-colored callus, due to the development of stems of fenugreek seedlings on the surface of solid MS medium supported by growth regulator NAA and LPS extracted from this bacteria as well.It was also observed that LPS extracted from *Ensifer meliloti*and *Rhizobium leguminosarum* *bv. trifolii* when added to solid NF medium had a stimulating role represented by the formation of nodules-like structures free of atmospheric nitrogen-fixing bacteria on the roots of the *Medicago sativa* and *Trifollum repens* seedlings specialized in infecting them (Gressent *et al.*, 2002). By following up the effect of nodulation factors known as lipo-polysaccharides isolated from this bacterium(*Rhizoium* sp.) on cell divisions in cell suspension cultures of legume plants, and in the field of identifying the effect of Lipid-A fatty acid extracted from LPS of *Ensifer meliloti*, noted it has a Stimulating effect on cell division from cell suspension cultures of alfalfa plants (Scheidle *et al.*, 2005) and on obtaining secondary metabolites and saponin steroids such as diosgenin from fenugreek(AL-Zubaidi, 2004).

8-Immuno-modulatory effects of Lipo-polysaccharides:

Lipo-polysaccharides is polycyclic one of the many components present on the surface of bacterial cells, which has the ability to prevent the devouring of bacteria by specialized antibodies to them, and is responsible for the formation of infection shock, so it is a major target in treatment strategies against infection with pathogenic bacteria producing it. Endotoxins derived from different bacterial species share a common basic structure(Pulendran *et al.*, 2001; Todar, 2002).The Lipo-

polysaccharides isolated from bacteria, especially *Escherichia coli*, are one of the endotoxins that have a role in the vitality and virulence of the bacteria (Nagao *et al.*, 2001 and Pulendran *et al.*, 2001). The biological activity of endotoxins is usually related to the Lipo-polysaccharide, while the Toxic activity of the Lipo-polysaccharide is related to the lipid-A. The immune activity is related to the components of the multiple sugars. The cell wall antigens (O antigens) of Gram-negative bacteria are actually components of the LPS, so it induces different inflammatory responses inside the host, and it is located on the outer sides of the membrane where contact with the external environment and the LPS is necessary for the function of the outer membrane as one of the components of the cell, and it may play many roles in the pathogenesis of Gram-negative bacteria by preventing it from bile salts from penetrating the bacteria or any other toxic particles in the gastrointestinal tract, LPS also acts as a hindrance to hemolytic enzymes and anti-bacterial agents. Besides, it prevents bacterial cell breakdown using serum components and macrophages. It plays an important role as a surface compound in the interaction of the pathogen with its host, as it may be related to adhesion (colony formation), resistance to phagocytosis, or antigenic changes that determine the duration and outcome of infection (Todar, 2002).

LPS is a strong stimulator of natural immunity in its host because it increases the cellular immune response (Yaseen, 2009), so it is an immunomodulator for both humoral and cellular immunity, and this property plays a role in the growth and development of the chronic immune response to treating micro-organisms. It also stimulates, especially the extract from *E. coli* bacteria, dendritic cell groups, and T cells to produce different cytokines and induce acquired immunity (Pulendran *et al.*, 2001). It also stimulates monocytes and macrophages to produce cytokines, including interleukin 8, 6, and 1, which is the cancer necrosis factor and platelet-activating agent, and at the same time it stimulates macrophage cells to promote macrophage and cytotoxicity by stimulating them to produce and release enzymes such as cytokines and release interleukin 1 causes fever. The Lipo-polysaccharide activates the complement chain, especially C_{5a} and C_{3a}, which release histamine leading to vasodilation, as well as lead to the chemical attraction of the neutrophil cells that result in inflammation. This compound also activates the clotting chain that results in internal bleeding and the ultimate effect is to stimulate inflammation, intravascular thrombosis, hemorrhage, and shock (Todar, 2002). The LPS activates beta cell to produce polyclonal antibodies, and multiply the (B) cells to produce the immunoglobulins IgG and IgM, noting that the physiological effects of LPS are caused by Lipid-A (Nagao *et al.*, 2001), there are different reductive types of reactions in the host called endotoxin-toxic activities including fever, hypotension, internal organ failure, and in standard cases haematological shock and vascular thrombosis through interaction with humoral and cellular factors of mammalian hosts and high concentrations of this toxin to prepare cells of inflammatory vascular systems (Abd and Abdul-Hameed, 2012).

CONCLUSION

The difference in Gram-negative bacteria, especially *Rhizobium* bacteria, at the level of species and strain, is due to their difference in terms of chemical composition

and biological effects of the Lipo-polysaccharides that these bacteria contain, in stimulating the formation of pseudo-root nodules without bacteria, as well as, stimulating the cells of plant pieces plant to division and production callus under laboratory condition, and we hope in the coming years to discover its positive and security effect on human life, given that these bacteria are non-pathogenic sympathetic species for living organisms, especially humans.

الفعالية البايولوجية لعدد السكريات الدهنية في بكتريا الرايزوبيوم

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الخلاصة

تعد السكريات المتعددة الدهنية من المواد الحيوية المهمة والتي تتألف غالباً من ثلاثة مكونات أساسية هي LipidA: واللب و O-antigen ويتم عملية تكوينها بعدة مسارات ايضية تتضمنها تسعة انزيمات تشفر من قبل جينات خاصة تسمى بالجينات العنقودية Cluster genes، وتتميز هذه السكريات بالعديد من الخواص الفيزيائية ومنها خاصية اللزوجة Viscosity، التي تتراوح ما بين 0.0020 الى 0.0033 دايين ثانية/سم²، فضلاً عن خاصية الدوران البصري وبنوعيه الأيمن dextrorotatory والأيسر levorotatory. وللسكريات المتعددة الدهنية دور بارز في العديد من المجالات، ففي المجال الزراعي تسهم في تكوين العقد الجذرية على النباتات البقولية ذات الأهمية الكبيرة في عمليات تثبيت النتروجين الجوي وزيادة المحتوى البروتيني للنباتات البقولية، فضلاً عن دورها في عمليات استحداث وتمايز كالس النباتات البقولية كالجبت والبرسيم وغير البقولية مثل التبغ. أما في المجال الطبي والصيدلاني فلها أهمية واضحة في عمليات الاستجابة المناعية من خلال وجودها كأحد المستضدات المهمة الموجودة على أسطح الخلايا البكتيرية، فضلاً عن دورها في عملية الالتصاق المؤدية لتكوين المستعمرات البكتيرية وكحواجز تمنع نفاذ العوامل المضادة لداخل الخلية البكتيرية.

الكلمات المفتاحية: *Rhizobiumm, Lipopolysaccharide, Lipid-A, Core regin, O-antigen*

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