Journal of Genetic and Environmental Resources Conservation, 2021, 9(2):85-95.
www.gercj.com Print ISSN: 2306-8663, Online ISSN: 2306 – 8280

Exo-Polysaccharides and his role symbiotic relationship between Rhizobium and leguminous plants: A review article

Taghreed N Ahmed[1], Islam Yasir Abdullah[2], Aryan Mohammed Hamed[3] and Raghad Nawaf Al-zaidy[4]*
[1,2,4] College of Education for Pure Science, Department of biology, Mosul University and [3] College of Education, Department of psychology, Alhamdanyia University, Iraq.
***Corresponding author: raghadnawaf@uomosul.edu.iq**
Received: May 7th 2021; Accepted: May 30th 2021

Abstract

Exo-Polysaccharides is a compound of an extracellular acid nature with high molecular weights, an exo-cellular product of an acid nature with high molecular weights that accumulate on the surface of the cell and secrete to the cell's circumference, Its main function is to protect the cell from environmental pressures as well as its important role in the association of link bacterium with the roots of legumes plants and then in the formation of the root nodules during the symbiotic relationship that results in the stabilization of atmospheric nitrogen to ammonia.
Exopolysaccaride (EPS) can be classified as polysaccharides depending on the type of units that make up the polysaccharide. Correlation and size of component units and degree of polymerization it is called Homopolysaccharides or Heteropolysaccharide and the type of Exo-Polysaccharides produced varies between types of the Rhizobium bacterium, the type of sugars, the method of association and the size of the constituent units and the degree of their composition. The production of Exopolysaccaride is influenced by various factors, including the composition of the media environment and the culture conditions , which have been of great importance in the growth of microbiology and stimulate their production of Exo-Polysaccharides and provide them with the energy necessary for biological processes. One of the most important factors affecting the production of the bacterial strain ,the components of the dietary medium and pH.

Keywords: Rhizobium, Exo-Polysaccharide,Homopolysaccharides, Heteropolysaccharide, legumes plants, symbiotic relationship.

Introduction

Under nitrogen-limiting conditions, bacteria belonging to genera *Rhizobium, Sinorhizobium, Phylorhizobium, Mesorhizobium, Azorhizobium* and *Bradyrhizobium*, which named rhizobia, have the ability to builds root symbiosis with certain legumes plants. Upon stimulation by flavonoids detachment from legume roots into soil, rhizobia synthesize the mission molecules that are responsible for nodule formation (Broughton *et al* .,2000; Spaink, 2000). Exopolysaccaride (EPS) can be classified depending on the type of units that are multiple sugars, either similar or differentiated to its syntheticunits, called Homopolysaccharides or Heterospolysaccharide, These mission molecules, named Nodfactors, have been identified aslipochito oligosaccharides (LCOs) having diverse chemical substitutions. Nod factors are sufficient for initiation of root hair distortion, infection thread formation and activation of division operation of cortical cells(Schulze *et al* .,1998). Polysaccharides forms in the rhizobium bacteria it includes several types Exo-Polysaccharides(EPS), Lipo-Polysaccharides (LPS)and Capsular Polysaccharides(KPS) (Becker and Puhler, 1998).

1-Polysaccharides forms in the rhizobium bacteria

1.1Exo-Polysaccharides

Exo-Polysaccharides is an extra-cellular acid compound with high molecular weights, and has frequent units between 7-9 hexagon sorority, hexose sugar, And they are not associated with some of them by linkage glycoside, In addition, it has non-carbohydrate supplenate, Pyruvate or Acetate products, which are an acid-based extracellular product with high molecular weights that accumulate on the cell surface and are excreted to the cell's circumference, as it is noted that their association with the surface of the outer bacterial membrane is weak (Spaink, 2000). The nature of these sugars is specific to the type or strain and there are several chemical compositions based on the size of the recurring units as well as the non-carbohydrate totals in them (Becker and Puhler, 1998).

Their acidic nature is due to the following organic acids uronic, Pyruvate ketals and Succinate (Gray *et al.*, 1991). Bacteria *S.meliloti* produces two types of Exo-polysaccharide name (EPSI) Succinoglycan Eps container on the Succing 1 range as well as Acety 1, Pyruvy 1 and units of glucose and (EPSII) Eps Gabctoglcan which consists of From repeated units of two types of sugars, clocose, calactose, acety 1 and Pyruvy 1, its main function is to protect the cell from environmental pressures as well as its important role in the link of Rhizobium bacterium with the roots of legumes plants and then in the formation of the root nodules during the symbiotic relationship that results in the stabilization of Air nitrogen to Ammonia (Fraysse *et al*., 2005).

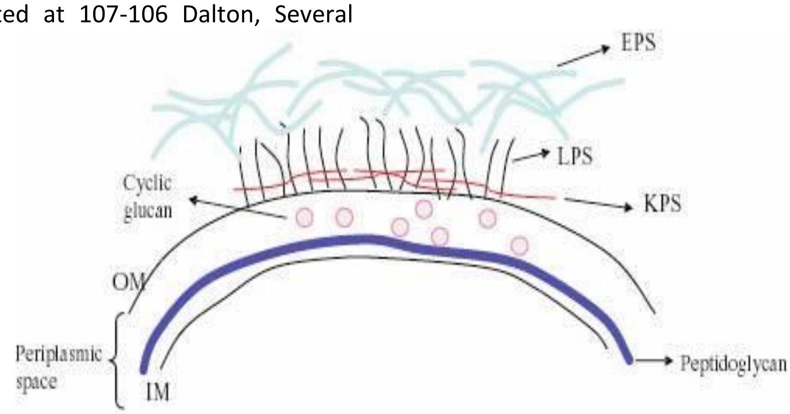
1.2 Lipo-Polysaccharides (LPS)

Lipo-Polysaccharides (LPS) are a complex, greasy, sugary compound, usually released during the normal growth cycle of the bacterium or after cell death or crash by host defenses (Kanman *et al*., 1997), also known as Lipo-glycans or endotoxins. They are large molecules usually made up of fats and Polysaccharides that are associated with each other by covalent bonds. Found in the outer membrane of the gram negative bacterium, it also provokes strong immune responses in animals, and uses the term Lipo-OligoSaccharide to refer to the low molecular weight of some of its types, noting that the molecular weight of the Lipo-Polysaccharides molecule is estimated at 107-106 Dalton, Several studies have indicated that Lipo-Polysaccharides sugar is boiling-down for (30) minutes, and it breaks down when treated with strong oxidizing agents such as Hypoclorite. Superoxide, Perocide and not digested with protein-analyzed enzymes (Todar, 2002).

Chemically consists of 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, which consists of repeated units of Polysaccharides, which communicates with Lipid-A by KDO, and the third part consists of a series of recurring units called O- antigen, which consist mainly of sugar units (D'Antuon *et al*. , 2005).

1.3 Capsular Polysaccharides

It is a collar that surrounds these bacteria and gives them resistance against germs that are charged bacteriophages because they contain hydrate hydrogen, may be released into the area around the root, It is composed of molecules (Kdo)2,3 - deoxy - a - manno -2 - Octuuvsnic acid which is similar to the composition of K-antigen in *Escherichia coli* bacteria and therefore called (KPS) K-antigen Polysaccharide, and has an indirect role in the symbiotic relationship of bacteria Ak,31 *S.melilot*, which is limited in growth in Alfalfa plants and works on the synthesis of bacterial cells with each other (D'Antuon *et al*. , 2005). Figure 1 shows these three types of sugars and their locations in the Risobiom bacteria.



Figure(1): The three forms of polysaccharides located on the surface of the Rhizobium bacteria (D'Antuon *et al*. , 2005)

1.4 Cyclic glucans

These are ring particles that accumulate mainly in the region surrounding the plasma (Breedveld *et al.,* 1993), and were first isolated from *Sinorhizobium meliloti*. Cyclo-glucan is a symmetric neutral polymer containing 20 glucose molecules bound together - often substituted with phosphoglucerol, phosphocholin, and succinyls (Chen *et al.,* 1985*).* It is found in □--proteobacteria such as *M. Loti, S.meliloti*, *Agrobacterium tumefaciens* and *Brucella spp*. All represent the type (1→ 2) clocan species (Briones *et al*. 2001) and also the presence of and β (1→ 3) and (1→ 6)clocan in *Bradyrhizobium japonicum* bacteria. (Mithofer, 2002) is formed in specific and unspecified nodules and that the absence of cyclic glucan affects the ability of the bacteria to invade and infect (D'Antuono *et al*., 2005). It contributes to protection against high osmosis, and its ability to protect the membrane against the degradation effect of calcium (Chen *et al*., 1985).

2- Chemical Structure of Exopolysaccaride

Exopolysaccaride (EPS) can be classified as polysaccharides depending on the type of units that make up the polysaccharide. Either it is the same or differential in its structural units, it is called Homopolysaccharides or Heteropolysaccharides, as the first consists of glucan units while the second is called polyanionic due to the presence of uronic acid. And the extra-cellular polysaccharides produced by most types of rhizobia bacteria by repeating units of hex sugars such as glucose, galactose, mannose, ramose, ketoyuronic acid, pyruvate, acetyl and succinyl. The type of extra-cellular sugars produced varies among the types of rhizobia bacteria, as they vary in the type of sugars, the method of attachment, and the size of their component units Its polymerization (VanWorkun *et al*., 1998). The basic structure of exogenous polysaccharides varies in different types of bacteria and within different genus , in *R. leguminosarum*. Although there are different types of biovars, they have the ability to form root nodules on different plant hosts. Like alfalfa, peas, and beans, they are characterized by having extra-cellular sugars consisting of glucose and glucuronic acid and lactose, with perentage of 1: 2: 5, respectively (Robertsen *et al*., 1981). While *S. meliloti* bacteria have the ability to produce a different extra-cellular polysaccharide called (EPS II) or Galactoglucan, which consists of a duplicate unit of glucose with which the acetyl group is attached and one unit of calcium lactose attached to it by the Pyruyl group that binds to the glycosidic binds α - (1→ 3) or β - (1→ 3) (Her *et al*., 1990) .As for the second type, which is called (EPS I) which consists of one unit of galactose and one unit of glucose (Reinhold et al., 1994). As for the Bradyrhizobium bacteria, which has the ability to produce multiple sugar extracellular, which is composed of mannose sugars, galactose, glucose, and calectoernic acid, with proportions 1:2:1:1 As the Figure (2).

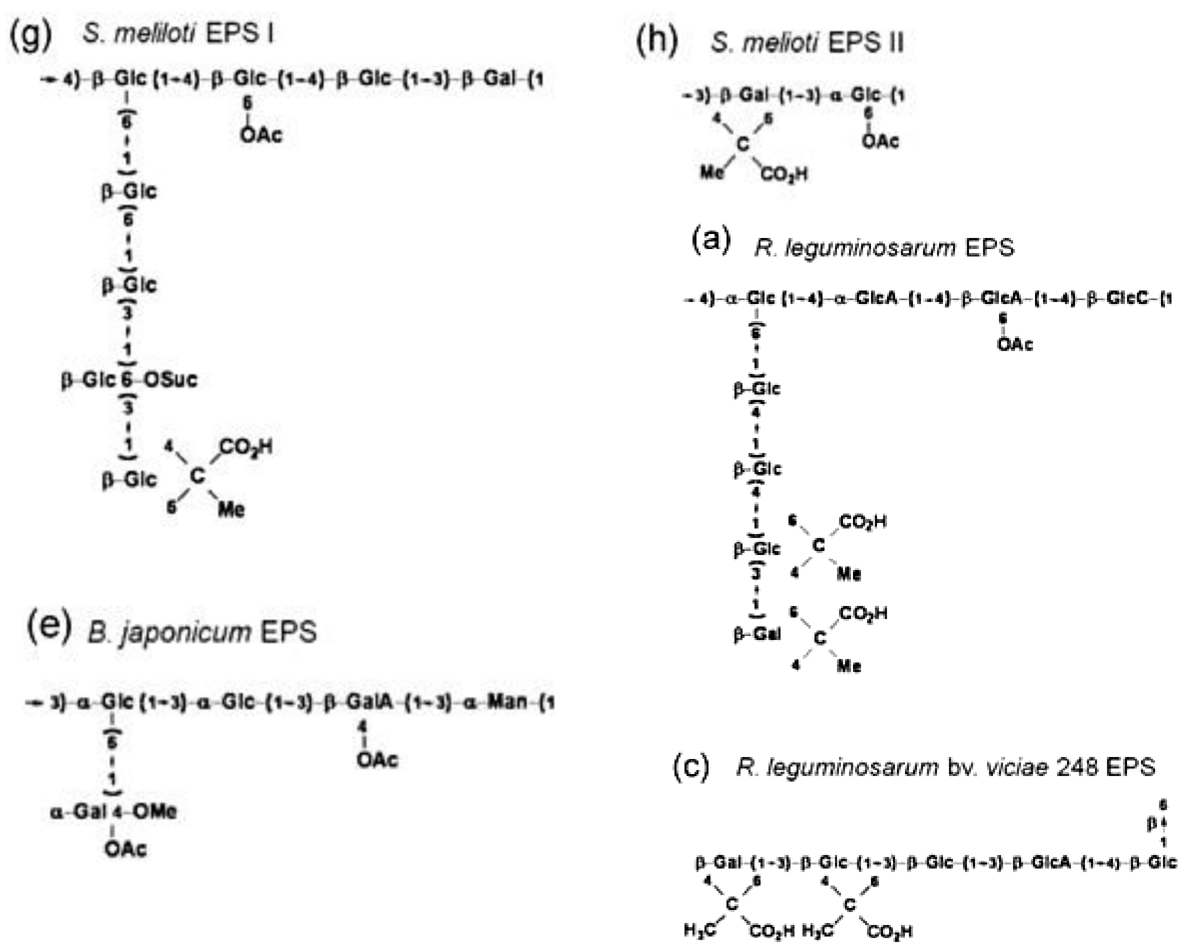


Figure (2): The chemical composition of the Exo-Polysaccharides to Risobium Bacteria (Bomfeti *et al*., 2011) .

3- Biosynthetic pathways of exopolysaccharides synthesis

The genes that control or guide the process of manufacturing external polysaccharides are exo/exs or pss genes, which are large clusters of genes located either on the chromosome or on large symbiotic plasmids (Finan *et al.*, 2001). Among the enzymes encoded by these genes is the transferase enzyme responsible for arranging the recurrent units of external polysaccharides, and it works to polymerization and export the developing external polysaccharides chain to the cell surface (Whitfield and Paiment, 2003). In the *S. meliloti* bacterium, the gene cluster directs the bio-manufacturing of Succinoglycan (EPSI), which is located on the large plasmid 2 (pSymB) (Megaplasmid 2) Arnold *et al*.,1992).

Studies after the completion of the norwegian sequencing project in the *S. meliloti* bacterial genome showed that of the 11 genetic regions only two regions responsible for the manufacture of external polysaccharides previously diagnosed with pSymB (Gsluckmann *et al.,*1993). 14% of the size of plasmids enters their genes in the manufacture of polysaccharides (Finan *et al*., 2001). Although all EPSI biosynthesis genes cluster on pSymB plasmids, there are some other genes important for the manufacture of Succinoglycan and their regulated genes (exoC, exoR, exoS, mucR and exoD) located on the chromosome (Keller *et al*.,1995). The exo/exs genes are organized in several(Becker *et al*., 1995).

When manufacturing Succinoglycan Are manufacturing nucleotide sugar, the first exoC gene encrypts phosphoglucomutase, which stimulates the conversion of glucose-6-phosphate to Glucose-1-phosphate, and the exoB gene is encoded for UDP-glucose-4-epimerase, which converts UDP-glucose to UDP-galactose (Buendia *et al*.,1991). Mutations in the exoB and exoC genes lead to the loss of EPSI production and also affect the productivity of other polymers (EPSII, LPS and ⍰-glucans). The enzyme encoded by the exoN gene leads to the effectiveness of the enzyme UDP-glucose pirophosphorylase . Mutations in the exoN gene is sped into a decrease in EPSI production (Becker *et al*.,1993).

The aggregation of recurrent units of exopolysaccharides is initiated by the *exo*Y galactosyltransferase (Reuber and Walker, 1993) (Fig. 3). The exoY gene, which cannot produce succinoglycan, has created a failed symbiotic relationship because it cannot form the thread of infection. The exoF gene is an enzyme that is necessary to add galactose to the fatty carrier, The subsequent addition of glucose units is carried out by the enzymatic complex glucosyl transferase and encoded by the following genes: exoA, exoL, exoM, exoO, exoU and exoW (Reuber and Walker, 1993). Studies have shown that Mutations in the exoF, exoA, exoL and exoM genes completely stop the production of Succinoglycan, which results in a mutation that stimulates the formation of a radical unproven contract of nitrogin (Fix-) (Zhan and Leigh, 1990).

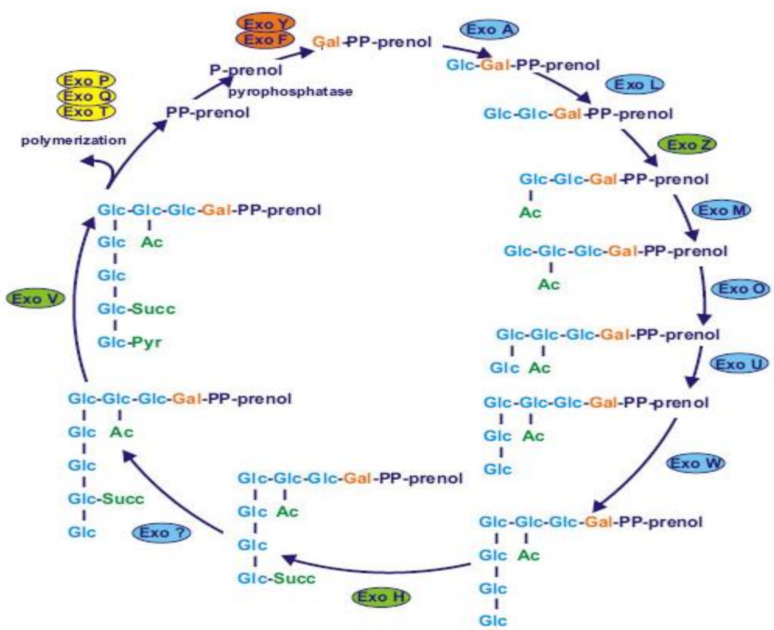


Figure (3): Biosynthetic pathways of exopolysaccharides synthesis
Glc: Clocos, Gal: Calacotus, Ac: Acet, Pyr: Pyr, Succ: Sexnet, (Skorupska *et al.*,2006)

The polymerization process of the recurrent units that make up Succinoglycan and the secretion of this polymer is based on enzymes encoded by the exoPQT gene (Glucksmann *et al*., 1993). The second multi- Exopolysaccaride molecule (EPSII) galactoglucan is directed by exp genes. The size of the exp genes in the form of a cluster is 23 kb and is located on pSymB plasmid and is separated from the exo/exs gene cluster in an area of about 200 kb. The manufacture of Nucleotide diphosphosugar prefixes depends on the efficacy of enzymes ExpA7, ExpA8, ExpA9 and ExpA10 that are involved in the dTDP-rhamnose configuration, and the intermediate compound of this pathway is dTDP-glucose, which acts as a glucose as an endothesis when manufacturing EPSII. The UDP-glucose, which is a profix of glucose when manufacturing EPSI (Skorupska *et al*., 2006).

4- Factors affect production of exopolysaccharides

The production of exopolysaccharides is influenced by various factors, including the composition of the food environment, and the nutritional conditions are of great importance in the growth of microbiology, stimulating the production of exopolysaccharides and providing them with the energy necessary for bio-synthesize processes (Dunn, 1985).

The aim of changing the conditions of the implant sought to identify the factors affecting the increase and decrease of the productivity of exopolysaccharides and to know the response of bacteria to these changes, where more than one plant factor may overlap to give the best response and it is not possible to separate each influential factor individually (Pinto *et al*.,2002). The most important factors affecting production are:

4.1 Strain variation

There are clear differences in the susceptibility of bacteria isolates to the production of exopolysaccharides, as well as the quality of the product from a chemical and physical point of view, mainly due to the strain of bacteria isolated from different agricultural areas, where each strain has properties that make it independent in itself from other strains, and this explains to us the conflicting and differentiated results related to the production of exopolysaccharides , as this is due to the different breeds used in research (Duta *et al*.,, 2004)

4.2 Culture component

The components of the implanted medium play an important role in the growth of micro-organisms (Pinto *et al*., 2002) and affect metabolic products by these microorganisms, the most important of which are:

4.2.1 Carbon source

Carbohydrates are a general source of carbon, such as glucose, which is exploited by micro-organisms more than any other sugar and is a general carbon source for them (Al-Saad, 1990) and carbohydrates are common sources used by micro-organisms in the manufacture of exopolysaccharides, as the production and quality of exopolysaccharides depends on the carbon source used, which in turn affects production (Vandam *et al*., 1997). Carbon accounts for about half of the dry weight of micro-organisms cells, Carbon is essential for cell material synthesis, but its use depends on a micro-organism capable of equipping itself with energy, but if it does not produce all the basic intermediate compounds necessary for the synthesis process, it is not valid to use to feed these micro-organisms as a single source of carbon (Sajdi and Aly, 1987). Carbon serves two important purposes for micro-organisms, where carbon is essential for building essential compounds such as carbohydrates, proteins, lipids and nucleic acids first. Secondly, carbon oxidation provides a source of energy that is employed to complete biological processes for micro-organisms (Al-Khafaji, 2008)

The best medium for the production of exopolysaccharides is found to contain manitol, sucrose and clocose (Duta *et al*., 2006). The concentration of carbon source also affects the efficiency of its transformation to exopolysaccharides, for example the efficiency of sucrose-polymer conversion by Rhizobium bacteria is reduced with increased concentrations of carbonic sucrose source (Ghosh and Basu, 2001), as these researchers pointed out that the different mono-and bilateral sugars used in the production of exopolysaccharides sugar support its production in varying proportions

4.2.2 Nitrogen source

Natrogin is included in multiple structures of cell parts, so a source containing nitrogin must be available in the environment so that micro-organisms can exploit it, and these sources play an important role in providing this element for use in structural units rather than the role it plays as sources of energy and enter ingerogin in the synthesis of amino acids, protein, nucleotides and certain vitamins and forming nitrogin 8-14% of the dry weight of microscopic cells (Dunn, 1985)

There is a wide range of organic and inorganic sources of nitrogen, for example inorganic salts NH4 + and NO3- and organic such as types of amino acids, urea and proteins, as well as natural products such as yeast extract and casamino acid milk amino acids, which are used to meet the needs of the microorganism from nitrogen Nitrogen. is also included in the synthesis of many enzymes necessary for the representation of these sugary compounds and other compounds (Sanford, 1979). The nitrogen source dominates the production of microbial polysaccharides in liquid culture (Roberson *et al*., 1995)

The production of rhizobium exopolysaccharidesic sugar is influenced by various organic and inorganic sources, where naergonos are important for the production of polysaccharides by microbiology (Ghosh and Basu, 2005). One of the most commonly used nitrogin sources used by microbiology in the production of polysaccharides is ammonia salts, amino acids, nitrates and nitrogin gas, the first and second sources are among the most used nitrogen sources in agricultural circles, but they must be added in balanced proportions because increasing the concentration of these sources stops the ability of bacteria to produce polysaccharides while encouraging bacterial growth (Wenster-Botz, 2000)

4.2.3 Inorganic nutrient

Although the energy, carbon and nitrogin sources of the teraper cell are available, they cannot grow in the absence of mineral elements (Ghosh and Basu, 2005). Therefore, the presence of mineral elements in the media in general is necessary for the growth of microbiology as well as for the production of various metabolic substances, whether primary or secondary, as they act as a catalyst for certain enzymes that play an important role in metabolic processes, and they play an important role in controlling the permeability of the cellular membrane, including the transmission of energy sources. Therefore, the presence of some positive ions such as potassium, magnesium, iron and calcium in the media is necessary to give optimal growth. Microbiology requires negative ions such as Cl- which may act as a catalyst for certain enzymes involved in metabolic processes or may play an important role in the permeability of the cell membrane and the transmission of energy sources, but these elements may be bidirectional as their concentration above the optimum limit discourages production (Al-Khafaji, 2008).

4.2.3.1 Phosphor

Living cells, including bacteria, need phosphorus, which plays an important role in metabolism and other vital events, phosphorus enters high-energy compounds such as ATP, GTP, etc., and is used in the synthesis of fats –containing phosphorous and nuclear acids (Al-Khafaji, 2008).

(Breedveld *et al*., 1995) studied the effect of the decrease of phosphorus on the glucans (1→ 2) in the bacterium *S. meliloti* and *Agrobacterium tumefaciens* and these researchers found that when the phosphorus element decreases phosphoglycerol will not be converted into (1→ 2) ring. Phosphorus was used in the form of kh2PO4 potassium phosphate by Souw and Demain (1979), where they noted that phosphorus was an important factor in the production of polymers in microbiology.

4.2.3.2 Magnesium

Magnesium Mg is an important inorganic nutrient needed by microbiology in sufficient quantities, it is involved in the synthesis of some important enzymes, and enters into the processes of energy production and transformations of ATP to ADP (energy-rich compound) and also in the synthesis of some important cell components such as membranes and cellular walls of the bacterium. It also assists in the installation of ribosom. (Al-Khafaji, 1990)

4.2.3.3 Sulfur

Sulfur is an important mineral needed by microbiology to produce polysaccharides, as the role of sulfur for microscopic cells is more than the role it plays for other types of cells (Al-Khafaji, 1990). Sulfur is involved in the synthesis of amino acids such as cysteine, cysteine and methionine. Sulfur may be one of the important elements needed by microbiology to perform various metabolic events, and ammonium sulfate (NH4)2SO4, which is used as a common source of nitrogin, is the usual source of sulfur used by micro-organisms in the media intended to produce different compounds. Dunn (1985) noted that sulfur is found in some types of vitamins such as thamine, biotin and pyrodoxin.

4.2.3.4 Potassium

Potassium is an accompaniment to certain enzymes ,that it helps stimulate growth and is needed by the cell in carbohydrate synthesis and in the stages of transport (Dunn, 1985). Potassium K+ is usually added inorganic salt in the form of K2HPO4 or KH2PO4 to media intended for the development of certain types of micro-organisms.

4.2.4 Elements and other factors

micro-organisms in its media needs to add growth factors as these substances stimulate the growth of micro-organisms, the growth factors include three groups: vitamins, amino acids, naitrogine bases and other factors such as certain types of alcohol (ethanol,methanol). These compounds are used with very little concentrations in media to stimulate the growth of micro-organisms and to produce important compounds such as citric acid, antibiotics and polysaccharides (Al-Khafaji, 2008).

Yeast extract is added to the media intended for the growth of micro-organisms and the production of polysaccharides as one of the compounds containing various growth factors and vitamins in order to stimulate the production of polysaccharides by micro-organisms, vitamins have been used in the preparation of sugars that use enzyme facilities in biological systems such as nicotine acid, which is enters into fitting of DNA and other vitamins (Al-Khafaji, 1987).

4.2.5 pH value

PH is one of the important factors in the production of polysaccharides, during the subsequent phases of polysaccharides fermentation it has been observed that the concentration of hydrogen ion decreases due to the formation of polymer and organic and inorganic acids (Duta *et al*., 2004). Research indicates the role of pH in influencing the growth of micro-organisms and its production of primary or secondary metabolic substances, particularly Exo polysaccharides .Berry (1975) explained the effect of pH in the growth of micro-organisms and He mentioned that there are multiple factors that change pH during biological processes, including acid-producing compound , nutrient taking, oxidative reactions, reduction and changes to the equivalent medium solution Moraine and Rogovin (1997) said the effect of pH in the production of polysaccharides was more than the growth of micro-organisms. Rose (1976) has shown its effect on cell permeability and the nature of vital events occurring in the cell.Commercially important polysaccharides show a range of hydrogen ion concentrations between (6.0-7.5) for polysaccharides building (Kang and Cottrel, 1979). The rise or fall of the final pH of the fermentation medium depends on the type of bacterial strain and on the composition of the medium (DeVuyst *et al*., 1998).

5- Physical properties of exopolysaccharides

5.1 Viscosity

Microbial polysaccharides solutions are of a sticky nature, with a degree of viscosity varying depending on the type of micro-organism producing polysaccharides and even different strains of the same type. Viscosity is one of the main features that determine the rheological properties of polysaccharides, and the unit used to express viscosity is poise, which is known to be a single dane-sufficient force to move a 1 cm^2 magnitude flat surface 1 cm away from another similar surface, giving an acceleration to the moving surface of 1cm/s (Maron and Lando, 1974).

The viscosity of polysaccharides depends on several factors, including the components of the medium, the source and concentration of carbon and nitrogin, mineral salts and fermentation conditions (pH, oxygen concentration and the degree product movement) (Wenster-Botz, 2000). The researcher (Navarini *et al*., 1997) noted that the specific viscosity of polysaccharides produced by the bacterium Rhizobium hedysari isolation HCNT1 amounted to 3.2 boise

5.2 Specific optical rotation

Specific optical rotation is one of the common physical characteristics of polysaccharides and this characteristic varies depending on the type of polysaccharides. Sugars are visually effective materials and are characterized by the characteristic of rotating the level of polarized light during the passage of the solution containing the visually effective particles and are of two types either to be ⍰⍰and⍰⍰. This is caused by the difference of the coefficient of refractive light polarized circularly to the right as in the case of the⍰ - glucan and the boat is called the Dextrorotatory and gives it the symbol (+ or d) while the left-hand vessel is called the Levorotatory as in the case of ⍰ - glucan and symbolizes it (-or L). The variation of the phase between the polarized light waves circularly to the right or left turns into a rotation of the polarization level of light passing through the diabetic solution and varies according to the type of sugar and is (Al Fleih, 2000). Researcher Ghai (1980) pointed out that the degree of optical rotation of polysaccharides produced from *Rhizobium trifolii* bacterium is 23-20^D[⍰].

6- Role of rhizobial exopolysaccharide in symbiosis

Although the information obtained from many researches is available, the role of exopolysaccharides in symbiotic remains not fully understood, but the role of exopolysaccharides in the symbiotic relationship between Rhizobium bacteria and leguminous plants is inevitable, which includes the mechanical role in protecting bacteria against environmental stress,

Surface adhesion, nutrient aggregation (Gray and Rolfe, 1990). It also played a role in the early events of infection of the root nodules on leguminous of the host plant,which includes the binding of bacteria to the roots of the host plant and has a synthetic role in the formation of the infection thread, frees bacteria from the thread of infection, develops the formation of bacteroids, suppresses the defensive responses of the host plant, and protects the Rhizobium bacteria against the antimicrobial compounds produced by the plant (Becker and Puhler, 1998).

Dazzo *et al*.1984 observed two steps in the process of bacteria sticking to the root hairs of the host plant. The initial adhesion, which is based on lectins recognition by the surface of the special carbohydrates of the Rhizobium bacterium. This may enhance the association of bacteria with the top of the developing root barley and are necessary to infect the root hairs. In the second phase, the association of Rhizobium with roots is stronger and difficult to remove from the root barley (Cheng and Walker, 1998).

Many research suggests that exopolysaccharides has a role in determining the specificity of the host plant for the formation of the root nodules and works to twist the root hair completely and form the infection thread , release bacteria, and develop bacteroids and thus stimulate an effective root nodules (Gray and Rolfe, 1990). The researchers concluded that the defect caused by exo mutants was repaired in terms of twisting the root hair and the formation of the infection thread by adding small amounts of EPS (Gray *et al*., 1990).

Research has shown that exo mutants for *S. meliloti* bacterium cannot invade the tissue of the root noduls, and when EPSI or EPSII are present, a radical contract was made of a radical bacteroids installed for root nodules (Gonzalez *et al*., 1996; Wang *et al*., 1999). But In the case of bacteria *R. leguminosarum bv. trifolii*, the purified exopolysaccharides has repaired the imbalance in the symbiotic relationship produced by exo mutent (Djordjevic *et al*., 1987). The researchers have concluded that the EPS composite can be one of the determinants in the specialized relationship between Rhizobiume and the host plant, But not as a determinant, especially at the initial stages for infection the root hairs of leguminous plants (Skorupska *et al*., 2006).

The imbalance in the possibility of stimulating the formation of the root nodules of exo bacteria , *Rhizobium* sp. NGR234, *S. meliloti* and *R. leguminosarum bv trifolii* has *been* repaired when pollinated with these mutant insulation synopsis other similar isolates cannot stimulate the formation of a root nodules but produces EPS (Van Workum and Kijne, 1998). These findings confirm the role of exopolysaccharides in a successful symbiotic relationship.

Research studies have shown that exopolysaccharidess play a role in avoiding the Rhizobiume bactreia response to the plant's defensive response, which represents antimicrobial compounds and Phytoalexins, where the study showed that polysaccharidess found on the surface of the riseopia bacteria such as EPS play An important role in the protection against plant defenses (D'Haeze and Holsters, 2004). And in the study the researcher (Pellock *et al.*,2000) concluded that the correct chemical formula for exopolysaccharides sugar is necessary in order to suppress the defensive responses of the plant, and that changes in the formula The composition of the exopolysaccharides on the surface of the Rhizobiume bactreia generally leads to increased sensitivity to the antibacterial compounds produced by the host plant.The study showed that exo *S. meliloti* bacterial are capable of producing EPSI but stimulate the formation of a Pseudonodules root this stimulated the defensive responses to the Alfalfa plant (Niehaus *et al*., 1996).

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 exopolysaccharides that exopolysaccharides has a role in determining the specificity of the host plant for the formation of the root nodules and works to twist the root hair completely and form the infection thread , release bacteria, and develop bacteroids and thus stimulate an effective root nodules.

References

Al-Fleih, Khawla Ahmed. (2000). Introduction to Biochemistry. Second edition, Book House for Printing and Publishing, Mosul University, Iraq.

Al-Khafaji, Zahra Mahmoud. (1987). Vital events of the bacterium. Directorate of Dar al-Books For Printing and Publishing, Mosul University, Iraq.

Al-Khafaji, Zahra Mahmoud.(1990). Biotechnology. Dar al-Hikma Printing and Publishing Press, Mosul, Iraq.

Al-Khafaji, Zahra Mahmoud. (2008). Microbial Biotechnology (Molecular Orientations). Institute of Genetic and Biotechnology Studies for Graduate Studies, University of Baghdad, Ministry of Higher Education and Scientific Research, Iraq.

Al-Saad, Maha Rauf Mahmoud. (1990). Principles of microbiology. Higher education presses. Mosul University. Iraq

Arnold, W., Keller, M., Becker, A., Roxlau, A., Niehaus, K., Quandt, J., ... & Pühler, A. (1992). the surface polysaccharides of rhizobium-meliloti and their role in forming n-2-fixing alfalfa nodules. *endocytobiosis and cell research*, *8*(2-3).

Becker, A. and Puhler, A. (1998). *Production of exopolysaccharides. In:* Rhizobioceae Edited by: Spaink, H.P.; Kondorosi, A. and Hooykaas, P.J.J.

Kluwer. Acad. Publ. Dordrecht, Boston, London, uk., pp. 97-118.

Becker, A.; Kleickmann, A.; Keller, M.; Arnold, W. and Puhler, A. (1993). Identification and analysis of the Rhizobium meliloti exoAMONP genes involved in exopolysaccharide biosynthesis and mapping of promoters located on the exoHKLAMONP fragment. Mol. Gen. Genet, 241, 367-379.

Becker, A.; Kuster, H.; Niehaus, K. And Puhler, A. (1995). Extension of the Rhizobium meliloti succinoglycan biosynthesis gene cluster: identification of the exsA gene encoding an ABC transporter proteins, and the exsB gene which probably codes for a regulator of succinoglycan biosynthesis. Mol. Gen. Genet, 249,487-497

Berry, D.R. (1975). The environmental control of the physiology of filamentous fungi. In : The filamentous fungi. 1: 30-31. Smith, J.E. and Boil, F.I. Eds. UK.

Bomfeti, C. A.; Florentino, L. A.; Guimaraes, A. P.; Cardoso, P. G.; Guerreiro, M. C.; and Moreira, F. M.(2011) .Exopolysaccharides produced By the Symbiotic Nitrogen–Fixing Bacteria of Leguminosae. Network of Scientific Journals from Latin America, The Caribbean, Spain and Portugal. 657 R- Bras. Ci. Solo. 35: 657-671.

Breedveld, M. W., Benesi, A. J., Marco, M. L., & Miller, K. J. (1995). Effect of phosphate limitation on synthesis of periplasmic cyclic (beta)-(1, 2)-glucans. *Applied and environmental microbiology*, *61*(3), 1045-1053.

Breedveld, M.W.; Canters Cremers, H.C.J.; Batley, M.; Posthumus, M.A.; Zevenhuizen, L.P.T.M.; Wijffelman, C.A. and Zehnder, A.J.B. (1993). Polysaccharides synthesis in relation to nodulation behavior *Rhizobium leguminosarum*. J. Bacteriol, 175, 750-757.

Briones, G.; inon de Iannino, N.; Roset, M.; Vigliocco, A.; Silva, P.P. and Ugalde, R. (2001). Brucella abortus cyclic -1,2-glucan mutants have reduced virulence in mice and are defective in intracellular replication in Hela cell. Infect. Immun, 69, 4528-4535.

Broughton WJ, Jabbouri S, Perret X: Keys to symbiotic harmony.
J Bacteriol 2000, 182,5641-5652.

Buendia, A.M.; Enenkel, B.; Koplin, R.; Niehaus, K.; Arnold, W. and Puhler, A. (1991). The Rhizobium meliloti exoZ/exoB fragment of megaplasmid 2: ExoB function as a UDP-glucose 4-epimerase and ExoZ shows homology to NodX of Rhizobium leguminosarum biovar viciae strain TOM. Mol. Microbiol, 5,1519-1530.

Chen, H.; Batley, M.; Redmond, J. and Rolfe, B.G. (1985). Alteration of effective nodulation properties of a fast-growing broad host rang Rhizobium due to changes in exopolysaccharide synthesis. J. Plant Physiol, 120, 331-349.

Cheng, H.P. and Walker, G.C. (1998). Succinglycan is required for initiation and elongation of infection threads during nodulation of alfalfa by Rhizobium meliloti. J. Bacteriol, 180, 5183-5191.

D'Antuono, A.L.; Casabuono, A.; Couto, A.; Uglade, R.A. and Lepek, V.L. (2005). Nodule development induced by Mesorhizobium loti mutant strains affected in polysaccharide synthesis. Mol. Plant-Microbe Int, 8, 155-164.

D'Haeze, W. and Holsters, M. (2004). Surface polysaccharides enable bacteria to evade plant immunity. Trends Microbiol, 12, 555-561.

Dazzo, F.B.; Truchet, G.L.; Sherwood, J.E.; Hrabak, E.M.; Abe, M. and Pankratz, S.H. (1984). Specific phases of root hair attachment in the Rhizobium trifolii-clover symbiosis. Appl. Environ. Microbiol, 48,1140-1150.

De Vuyst, L.; Vanderverken, F.; Van de Van, S. and Deggest, B. (1998). Production by and isolation of exopolysaccharides from Streptococcus thermophilus grown in a milk medium and evidence for their growth-associated biosynthesis. J. Appl. Microbiol, 84,1059-1068.

Djordjevic, S.P.; Chen, H.; Batley, M.; Redond, J.W. and Rolfe, B.G. (1987). Nitrogen fixation ability of exopolysaccharide synthesis mutants of Rhizobium sp. strain NGR234 and Rhizobium trifolii is restored by the addition of homologous exopolysaccharide. J. Bacteriol, 169, 53-60

Dunn, G.M. (1985). Nutritional requirement of microorganisms. In: Comprehensive Biotechnology, 1, 113-125. Moo-Young M., Ed. USA.

Pereira Duta, F., Pessôa de França, F., & de Almeida Lopes, L. M. (2006). Optimization of culture conditions for exopolysaccharides production in Rhizobium sp. using the response surface method. *Electronic Journal of Biotechnology*, *9*(4), 0-0.

Duta, F. P., De França, F. P., Sérvulo, E. F. C., Lopes, L. M. D. A., Da Costa, A. C. A., & Barros, A. (2004). Effect of process parameters on production of a biopolymer by Rhizobium sp. In *Proceedings of the Twenty-Fifth Symposium on*

Biotechnology for Fuels and Chemicals Held May 4–7, 2003, in Breckenridge, CO (pp. 639-652). Humana Press, Totowa, NJ.

Finan, T. M., Weidner, S., Wong, K., Buhrmester, J., Chain, P., Vorhölter, F. J., ... & Pühler, A. (2001). The complete sequence of the 1,683-kb pSymB megaplasmid from the N2-fixing endosymbiont Sinorhizobium meliloti. *Proceedings of the National Academy of Sciences*, *98*(17), 9889-9894.

Fraysse, N., Lindner, B., Kaczynski, Z., Sharypova, L., Holst, O., Niehaus, K., & Poinsot, V. (2005). Sinorhizobium meliloti strain 1021 produces a low-molecular-mass capsular polysaccharide that is a homopolymer of 3-deoxy-D-manno-oct-2-ulosonic acid harboring a phospholipid anchor. *Glycobiology*, *15*(1), 101-108.

Ghai, S. K. (1980). Intra and inter generic homology in polysaccharide structure ofRhizobium andAlcaligenes. *Journal of Biosciences*, *2*(4), 329-335.

Ghosh, A. C., & Basu, P. S. (2001). Extracellular polysaccharide production by Azorhizobium caulinodans from stem nodules of leguminous emergent hydrophyte Aeschynomene aspera.

Ghosh, A. C., Ghosh, S., & Basu, P. S. (2005). Production of extracellular polysaccharide by a Rhizobium species from root nodules of the leguminous tree Dalbergia lanceolaria. *Engineering in life sciences*, *5*(4), 378-382.

Glucksmann, M. A., Reuber, T. L., & Walker, G. C. (1993). Family of glycosyl transferases needed for the synthesis of succinoglycan by Rhizobium meliloti. *Journal of Bacteriology*, *175*(21), 7033-7044.

González, J. E., Reuhs, B. L., & Walker, G. C. (1996). Low molecular weight EPS II of Rhizobium meliloti allows nodule invasion in Medicago sativa. *Proceedings of the National Academy of Sciences*, *93*(16), 8636-8641.

Gray, J. X., & Rolfe, B. G. (1990). Exopolysaccharide production in Rhizobium and its role in invasion. *Molecular microbiology*, *4*(9), 1425-1431.

Gray, J. X., Djordjevic, M. A., & Rolfe, B. G. (1990). Two genes that regulate exopolysaccharide production in Rhizobium sp. strain NGR234: DNA sequences and resultant phenotypes. *Journal of bacteriology*, *172*(1), 193-203.

Gray, J. X., Zhan, H. J., Levery, S. B., Battisti, L., Rolfe, B. G., & Leigh, J. A. (1991). Heterologous exopolysaccharide production in Rhizobium sp. strain NGR234 and consequences for nodule development. *Journal of bacteriology*, *173*(10), 3066-3077.

Her, G. R., Glazebrook, J., Walker, G. C., & Reinhold, V. N. (1990). Structural studies of a novel exopolysaccharide produced by a mutant of *Rhizobium meliloti* strain Rm1021. *Carbohydrate research*, *198*(2), 305-312.

Kang, S. K. (1979). Polysaccharides. *Microbial technology*, *1*, 442-464.

Keller, M., Roxlau, A., Weng, W. M., Schmidt, M., Quandt, J., Niehaus, K., ... & Puhler, A. (1995). Molecular analysis of the *Rhizobium meliloti* mucR gene regulating the biosynthesis of the exopolysaccharides succinoglycan and galactoglucan. *MPMI-Molecular Plant Microbe Interactions*, *8*(2), 267-277.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. (1997). Diagnostic microbiology. *The nonfermentative gram-negative bacilli. Philedelphia: Lippincott-Raven Publishers*, 253-320.

Maron, S.H. and Lando, J.B. (1974). *Fundementals of Physical Chemistry*. MacMillom Publishing Co. Inc. NewYork, USA.

Mithöfer, A. (2002). Suppression of plant defence in rhizobia–legume. symbiosis. *Trends in plant science*, *7*(10), 440-444.

Moraine, R. A., & Rogovin, P. (1971). Xanthan biopolymer production at increase concentration by pH control. *Biotechnology and Bioengineering*, *13*(3), 381-391.

Navarini, L., Stredansky, M., Matulova, M., & Bertocchi, C. (1997). Production and characterization of an exopolysaccharide from Rhizobium hedysari HCNT 1. *Biotechnology letters*, *19*(12), 1231-1234.

Niehaus, K., Baier, R., Becker, A., & Pühler, A. (1996). Symbiotic suppression of the Medicago sativa defense system-the key of Rhizobium meliloti to enter the host plant. *Biology of Plant-Microbe Interactions*, 349-352.

Pellock, B. J., Cheng, H. P., & Walker, G. C. (2000). Alfalfa root nodule invasion efficiency is dependent on Sinorhizobium meliloti polysaccharides. *Journal of bacteriology*, *182*(15), 4310-4318.

Pinto, E., Moreira, A., & Vendruscolo, C. T. (2002). Influence of pH, addition of salts and temperature in the viscosity of biopolimers produced by Beijerinckia sp. 7070 and UR4. *Revista Brasileira de Agrociência*, *8*(3), 247-251.

Reinhold, B. B., Chan, S. Y., Reuber, T. L., Marra, A., Walker, G. C., & Reinhold, V. N. (1994). Detailed structural characterization of succinoglycan, the major exopolysaccharide of Rhizobium meliloti Rm1021. *Journal of bacteriology*, *176*(7), 1997-2002.

Reuber, T. L., & Walker, G. C. (1993). Biosynthesis of succinoglycan, a symbiotically important exopolysaccharide of *Rhizobium meliloti*. *Cell*, *74*(2), 269-280.

Roberson, E. B., Shennan, C., Firestone, M. K., & Sarig, S. (1995). Nutritional management of microbial polysaccharide production and aggregation in an agricultural soil. *Soil Science Society of America Journal*, *59*(6), 1587-1594.

Robertsen, B. K., Åman, P., Darvill, A. G., McNeil, M., & Albersheim, P. (1981). Host-symbiont interactions: V. The structure of acidic extracellular polysaccharides secreted by Rhizobium leguminosarum and Rhizobium trifolii. *Plant physiology*, *67*(3), 389-400.

Rose, A.H. (1976). *Chemical Microbiology. An Introduction to Microbial Physiology*. Butter. Worths, London, Uk.

Sajidi, Adel George and Ali, Alaa Yahya Mohamed.(1987) *Industria Microbiology - Part 1 Of Industrial Fermentation Fundamentals*. Basra University Press, Iraq.

Sanford, P.A. (1979). Exocellular microbial Polysaccharides, Adv. Carbohydr. *Chem. Biochem,36* ,295-312.

Schulze M, Kondorosi E, Ratet P, Buire M, Kondorosi A .(1998) Cell and molecular biology of *Rhizobium*-plant interaction. *Int Rev Cytol* , 156,1-75.

Skorupska, A., Janczarek, M., Marczak, M., Mazur, A., & Król, J. (2006). Rhizobial exopolysaccharides: genetic control and symbiotic functions. *Microbial cell factories*, *5*(1), 1-19

Souw, P., & Demain, A. L. (1979). Nutritional studies on xanthan production by *Xanthomonas campestris* NRRL B1459. *Applied and Environmental Microbiology*, *37*(6), 1186-1192.

Spaink, H. P. (2000). Root nodulation and infection factors produced by rhizobial bacteria. *Annual Reviews in Microbiology*, *54*(1), 257-288.

Todar, K. (2015). Textbook of bacteriology.

van Workum, W. A., & Kijne, J. W. (1998). Biosynthesis of rhizobial exopolysaccharides and their role in the root nodule symbiosis of leguminous plants. In *Phytochemical Signals and Plant-Microbe Interactions* (pp. 139-166). Springer, Boston, MA.

van Workum, W. A., van Slageren, S., van Brussel, A. A., & Kijne, J. W. (1998). Role of exopolysaccharides of Rhizobium leguminosarum bv. viciae as host plant-specific molecules required for infection thread formation during nodulation of Vicia sativa. *Molecular plant-microbe interactions*, *11*(12), 1233-1241.

Vandam, M.C.E.; deu, W.H.; Currell, B.R. and Patmore, R.A. (1997). *Biotechnological Innovation in Chemical Synthesis*, Butter Worth: UK.

van Workum, W. A., van Slageren, S., van Brussel, A. A., & Kijne, J. W. (1998). Role of exopolysaccharides of Rhizobium leguminosarum bv. viciae as host plant-specific molecules required for infection thread formation during nodulation of Vicia sativa. *Molecular plant-microbe interactions*, *11*(12), 1233-1241.

Wang, L. X., Wang, Y., Pellock, B., & Walker, G. C. (1999). Structural characterization of the symbiotically important low-molecular-weight succinoglycan of *Sinorhizobium meliloti*. *Journal of bacteriology*, *181*(21), 6788-6796.

Weuster-Botz, D. (2000). Experimental design for fermentation media development: statistical design or global random search?. *Journal of bioscience and bioengineering*, *90*(5), 473-483.

Whitfield, C., & Paiment, A. (2003). Biosynthesis and assembly of Group 1 capsular polysaccharides in Escherichia coli and related extracellular polysaccharides in other bacteria. *Carbohydrate research*, *338*(23), 2491-2502.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. (1997). Diagnostic microbiology. *The nonfermentative gram-negative bacilli. Philedelphia: Lippincott-Raven Publishers*, 253-320

Zhan, H. J., & Leigh, J. A. (1990). Two genes that regulate exopolysaccharide production in Rhizobium meliloti. *Journal of Bacteriology*, *172*(9), 5254-5259.