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RHIZOBIAL EXOPOLYSACCHARIDES: STRUCTURE AND APPLICATIONS

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Abstract

Microbial exopolysaccharides (EPS) represent an important group of biologically active compounds produced and secreted by bacteria and fungi, which accumulate outside the cells. Recently, research has been focused on the exploration and discovery of new exopolysaccharides of microbial origin, due to their various biotechnological applications. Rhizobium strains produce a wide diversity of exopolysaccharides, with different structures at the species level, and a large field of applications, such as pharmaceutical, food, and cosmetics industries. In this context, this article aims to present a mini-review of the main EPS synthesized by Rhizobium strains, highlighting their structures and potential applications.

***Key words**: applications, exopolysaccharides, Rhizobium spp., structure.*

INTRODUCTION

In recent years, interest in the exploitation of microorganisms for production and obtaining of valuable polysaccharides has significantly increased (Shanmugam & Abirami, 2019).
These biopolymers produced by a variety of microorganisms present commercially relevant properties which are suitable and attractive for a wide range of applications, ranging from several chemical industries to biomedicine and cosmetics (Freitas et al., 2014; Ventorino et al., 2019).
In general, bacterial polysaccharides can be grouped into intracellular polysaccharides, cell wall polysaccharides, and those that are released into the cell culture medium called extracellular polysaccharides (EPS) (Jeong et al., 2022).
In the rhizosphere, polysaccharides play a key role in cell signaling reactions between plants and microorganisms, like plant root nodulation for nitrogen fixation, and also, biofilm formation (Jeong et al., 2022).
Rhizobium genus comprises symbiotic nitrogen-fixing species associated with the roots of legume plants (Gonzalez et al., 2019). *Rhizobium* species are Gram-negative, aerobic, rod shaped bacteria and non-spore forming (Zakhia & de Lajudie, 2001; Ribeiro & Burkert, 2016).
Rhizobial strains can be considered unexplored sources of microbial exopolysaccharides, promising for industrial applications, due to their high morphological, genetic and phylogenetic diversity (Bomfeti et al., 2011; Donot et al., 2012; Sethi et al., 2019). Exopolysaccharides produced by *Rhizobium* species can be obtained through submerged fermentation processes with high yields and controllable operation parameters (temperature, agitation, aeration, pH, dissolved oxygen level, pH correction, substrate addings) (Jeong et al., 2022). In this mini-review, we summarize the current knowledge about exopolysaccharides produced by several *Rhizobium* species, focusing on its structures and possible applications of these biopolymers in industry.

Rhizobial EPS structure

Rhizobium species produce a wide range of EPS, with different types of sugars and their linkages in a single subunit, repeat unit size, and polymerization degree (Bomfeti et al., 2011).
Rhizobial EPS repeating units consist of a variable number of hexose and uronic acid residues linked by alpha or beta glycosidic connections. It can either be linear or side branched. In addition to sugars, non-carbohydrate substituents, such as succinate, pyruvate, or acetate can be found, all of which

contribute to the polysaccharide's acidic nature (Acosta-Jurado et al., 2021).

Several studies regarding the EPS produced by *Rhizobium* spp. have been published in recent years: *Rhizobium radiobacter* S10 (Zhou et al., 2014), *Rhizobium tropici* CIAT899 (commercially known as SEMIA 4077) (Staudt et al., 2011), SEMIA 4080 (Castellane et al., 2014), R. *radiobacter* CAS (Andhare et al., 2017), *Rhizobium* sp. PRIM-18 (Priyanka et al., 2015), and *Rhizobium* sp. M2 (Urai et al., 2017), *Rhizobium tropici* LBMP-C01 (Moretto et al., 2015), *Rhizobium* sp. KYGT 207 (Kaci et al., 2005), *Rhizobium* sp. VMA301 (Mandal et al., 2007), *Rhizobium leguminosarum* bv. *trifolii* (Janczarek et al., 2015) and *Rhizobium leguminosarum* ATCC 10004 (Sellami et al., 2015).

Rhizobium tropici, a legume-symbiont soil bacterium, has the ability to produce extracellular polysaccharides (EPS) (Castellane et al., 2014). *R. tropici* CIAT 899 synthesize an extracellular polysaccharide with a single octasaccharide repeating unit composed of 6D-glucose, 2 D-galactose, 3 pyruvic acid, and 1 acetic acid molecule in the molar ratio of 6:2:1.5:1. Pyruvic acid groups replace half of the terminal groups on 4,6-galactose at position 3, while acetyl O-acetyl groups replace the other half (Oliveira et al., 2012).

Another strain, *Rhizobium meliloti*, which causes nodule formation in alfalfa (*Medicago sativa*) plants, produces two structurally unique EPS: succinoglycan (EPS I) and galactoglucan, which is formed under phosphate deficiency (EPS II) (Janczarek, 2011). EPS I is composed of repeating units of seven D-glucose residues and one D-galactose residue, linked by β-1,3, β-1,4, and β-1,6 glycosidic linkages and replaced with acetyl, pyruvyl, and succinyl groups (Castellane et al., 2015a; Ruiz et al., 2015), while EPS II consists of disaccharide repeating units that are linked by α-1,3 and β-1,3 bonds and include D-glucose and D-galactose in a 1:1 molar ratio. The majority of the glucosyl residues are 6-*O*-acetylated, and all of the galactosyl residues have 4,6-*O*-pyruvyl groups replaced (Janczarek, 2011). The chemical structure of succinoglycan produced by *Rhizobium* is shown in Figure 1. Both EPS I and II are produced in two major fractions: High Molecular Weight (HMW), which consists of hundreds to thousands of repeating units, and Low Molecular Weight (LMW), which consists of monomers, dimers, and trimers in the case of EPS I and oligomers (15-20) in the case of EPS II (Ghosh & Maiti, 2016).

Also, *Rhizobium leguminosarum* is a rhizobial specie that can be divided into three biovars based on the type of legumes infected: *trifolii, viciae*, and *phaseoli*. *R. leguminosarum* strains produce EPS with structures that are similar, but not identical, being composed of repetitive units containing D-glucose, D-glucuronic acid, and D-galactose in a molar ratio of 5:2:1, linked by β-1,3 and β-1,4 glycosidic linkages and modified by acetyl, pyruvyl, and 3-hydroxybutanoyl groups (Janczarek, 2011).

In most *R. leguminosarum* bv. *trifolii* strains, this polymer is composed of octasaccharide repeating units containing D-glucose, D-glucuronic acid, and D-galactose in a molar ratio of 5:2:1, connected by β-1,3 and β-1,4 glycosidic bonds, and are modified by non-sugar (acetyl and pyruvyl) groups, but in other strains, the galactose residue is not present (Skorupska et al., 2006). The EPS repeating unit of *R. leguminosarum* bv. *viciae* is similar to the *R. leguminosarum* bv. *trifolii* octasaccharide, but with an extra D-glucuronic acid residue (Acosta-Jurado et al., 2021).

In the case of *R. leguminosarum* bv. *trifolii* 4S, an EPS subunit consist of seven sugars, but the galactose molecule is absent in this chain, while the EPS subunit of *R. leguminosarum* bv. *viciae* 248 possesses an additional glucuronic acid.

Becker & Pühler (1998) showed that succinoglycan produced by *Rhizobium* sp. NGR234 is composed of repeating units having one galactose and seven glucose molecules coupled β-1,3, β-1,4 and β-1,6 linkages, and succinyl, acetyl, and pyruvyl residues.

Another type of EPS was produced by this strain, which consisted of alternating units of glucose and galactose with α-1,3 and β-1,3 linkages, and residues of acetyl and pyruvyl.

In the study of Guentas et al. (2001), the molecular structure of the EPS produced by a strain of *Rhizobium sp.* B isolated from nodules of alfafa was analysed, and it was shown that it contained high amounts of glucose and rhamnose (1:2), as well as traces of 2-deoxy-D-arabino-hexuronic acid.

Staehelin et al. (2006) found that the acidic EPS produced by *Rhizobium* sp. NGR234 contained glucosyl, galactosyl, glucuronosyl, and 4,6-pyruvylated galactosyl residues with glycosidic linkages of β-1,3, β-1,4, β-1,6, α-1,3 and α-1,4 respectively.

The effect of several carbon sources (sucrose, glucose, glycerol, and galactose) on the composition of EPS generated by *Rhizobium tropici* 4077 and 4080 was investigated by Castellane & Lemos (2007). They found mainly units of glucose, galactose, and glucuronic acid, with differences in their ratios depending on the carbon source utilised. Mannose, rhamnose, and galacturonic acid were also detected.

Zhao et al. (2010) investigated the chemical structure of the EPS produced by *Rhizobium* sp. N613 isolated from the Korshinsk pea shrub. The molecular weight and monosaccharide composition of *Rhizobium* sp. N613 EPS showed that are glucans composed of glucose and β-sugar units.

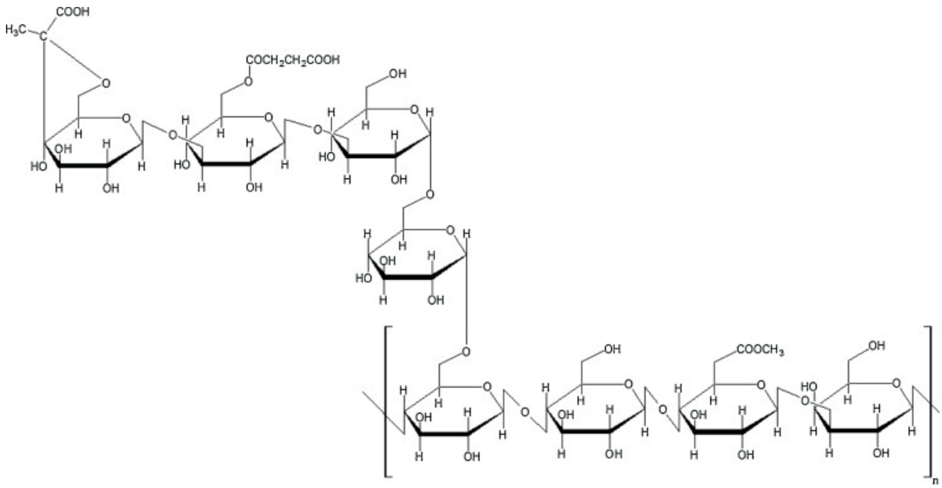

Figure 1. Chemical structure of succinoglycan produced by *Rhizobium*

Potential applications of rhizobial EPS

Succinoglycan produced by *Rhizobium* strains has a significant potential for commercial use as a water-soluble thickener, and the polysaccharide solution has a distinctive high viscosity due to the presence of about 10% succinic acid. It has also been reported to have superior properties under extreme operational situations, such as high temperature, pressure, salt or ionic concentration, and high shear rate (Andhare et al., 2017). These characteristics make the polysaccharide suitable for the use as a thickening, gelling, stabilizing, texturizing, and emulsifying agent in the food, pharmaceutical, and cosmetics industries (Zhou et al., 2014), and as a thickener for oil recovery (Gao et al., 2021). According to Yang et al. (2019) it was demonstrated that dietary succinoglycan successfully reduced diet-induced hypercholesterolemia in rats. The polysaccharide exhibits remarkable anti-inflammatory effects in vivo and in vitro (Cheng et al., 2019). These findings suggest that succinoglycan may be used as a health-promoting dietary ingredient (Gao et al., 2021).

Succinoglycan synthesized by *R. radiobacter* CAS has recently been demonstrated to exhibit useful properties for cosmetic applications, including 95% water solubility at room temperature and binding ability of 4.35 g/g and 3.68 g/g with soybean and peanut oils (Kavitake et al., 2019). Water-in-oil milky lotion, sun screen cream, water-in-oil foundation, sun screen milky lotion, and others are examples of commonly produced succinoglycan composition in cosmetic preparations. Because of its oil-in-water emulsification, thickening, and plasticizing properties, it's a popular cosmetic addition (Halder et al., 2017).

Such properties of rhizobial EPS are important in the production of cosmetic formulations, with hydrating potential, rhizobial polymers being a good option to substitute industrial glycerin derivatives for the development of cosmetics (Vieira et al., 2017).

Furthermore, succinoglycan has been also used as a polymer material in biosensing and drug delivery (Barman et al., 2020).

In another study, Castellane et al. (2014) showed that the wild-type *Rhizobium tropici* SEMIA 4080 strain and the mutant strain (MUTZC3) produce an extracellular polysaccharide with specific properties, that is used as an emulsifying agent.

Rhizobial EPS also showed promising properties, such as cytotoxicity against cancer cells. Zhao et al. (2010) investigated the anticancer activity of the EPS produced by *Rhizobium sp.* N613 isolated from the Korshinsk pea shrub. EPS inhibited the growth of transplantable sarcoma 180 (S180), hepatoma 22 (H22), and Ehrlich ascites carcinoma (EAC) as compared to control, with inhibitory rates of 44.17 % and 55.80% against S180 and H22, respectively, at a dose of 10 mg/kg. At a dosage of 60 mg/kg, the inhibition rate against EAC was found to be 53.10 %.

Also, in the pharmaceutical field, a dermo-pharmaceutical formulation containing a biopolymer produced by *R. meliloti* NCIMB 40472 and an extract of the microalgae *Haematococcus pluvialis* that assures skin nutrition, care, and regeneration has been described (patent number WO 1999013855A1) (Lintner, 1999).

In another study, Priyanka et al. (2015) found that the EPS produced by *Rhizobium* sp. PRIM-18 can be efficiently functionalized to promote cell proliferative and wound healing activity. Therefore, rhizobial EPS could be further explored for its applications in regenerative medicine.

Table 1 presents some information about EPS produced by *Rhizobium.*

Table 1. Overview of production, composition and applications of some EPS produced by *Rhizobium* sp.

Microorganism	**EPS production**	**Monomer units**	**Potential applications**	**References**
Rhizobium radiobacter S10	2.834 mg L^{-1}	Galactose Glucose Glucosamine Mannose	Food industry	Zhou et al., 2014
Rhizobium radiobacter CAS	-	Glucose Galactose	Food processing and product development sector	Kavitake et al., 2019
Rhizobium tropici LBMP-C01	3.48 g L^{-1}	Rhamnose Glucose Galactose	Candidate for food industry	Moretto et al., 2015
Rhizobium tropici Semia 4080, MUTZC3, JAB1, JAB6	-	Glucose Galactose Mannose Rhamnose Glucuronic acid Galacturonic acid	non-Newtonian and pseudoplastic fluid flow	Castellane et al., 2014
Rhizobium tropici Semia 4077	7.45 g L^{-1}	Mannose Rhamnose Glucuronic acid Galacturonic acid Glucose Galactose	It is a good water-solubility, viscous aqueous solutions with shear thinning behaviour, film-forming capacity, and emulsifier agent.	Castellane et al., 2015b
Rhizobium undicola strain N37	-	Galactose Mannose	Good stability Newtonian, fluid behavior	Ribeiro & Burkert, 2016
Rhizobium sp. KYGT207	2.5 g L^{-1}	Glucose Galactose Mannuronic acid	Thickening agent with polyelectrolyte properties	Kaci et al., 2005
Rhizobium sp. PRIM-18	-	Glucose Galactose Mannose	High emulsifying activity, enhanced HDF cell proliferation and wound healing *in vitro*	Priyanka et al., 2015
Rhizobium sp. N613	-	-	Anticancer properties: sarcoma 180 (S180), hepatoma 22 (H22), and Ehrlich ascites carcinoma (EAC)	Zhao et al., 2010

CONCLUSIONS

This mini-review gives an insight into EPS produced by several *Rhizobium* strains.

Rhizobial EPS exhibit significant structural diversity, with novel properties that make them valuable sources of natural polymers for use in a variety of industrial sectors, including medicine.

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MISCELLANEOUS