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Exopolysaccharide production by *Rhizobium leguminosarum* and its role in biofilm formation

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Abstract

The acidic exopolysaccharide (EPS) produced by *Rhizobium leguminosarum* is required for the foundation of viable advantageous interaction with good host plants. In the rhizobium legume interaction, early stages of root infection and nodules development have been well studied from a hereditary point of view. However, vital factors for colonization of a few surfaces by rhizobia, including soil particles and roots, have not yet been completely explored. The main aim of this research work was building up of environmental variables influencing production of EPS by *Rhizobium leguminosarum* isolates and the role of this polysaccharide in bacterial surface properties and capacity of attachment. Among rhizobial strains RLB1, its subsidiaries varying in the degree of EPS synthesis were utilized to these investigations. The capacity of attachment to abiotic and biotic surfaces of these strains was built up utilizing colony forming unit test (CFU).

It was confirmed that the capacity of *Rhizobium leguminosarum* to produce EPS significantly influenced bacterial connection and biofilm formation on both abiotic and biotic surfaces. Also, the presence of this polysaccharide affected the zeta potential of rhizobial cells. EPS facilitated connection of bacterial cells to the tested surfaces most presumably because of hydrophobic associations and heterogeneity of the envelope surface. EPS synthesized by *Rhizobium leguminosarum* assumes a significant contribution in attachment and formation of biofilm to both abiotic and biotic surfaces just as bacterial surface properties.

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R*hizobium leguminosarum* is a nitrogen fixing bacterium that can either exist as a free-living organism in the soil or live in symbiotic association with leguminous plants[7,8]. The foundation of advantageous interaction is an intricate process, which includes exchange of numerous molecular signals between both the host plant and the microsymbionts; among them, flavonoids and bacterial lipochito oligosaccharides, called Nod factors, are the best characterized molecules[4,21]. Rhizobia are able to induce nodule formation, particular organs on roots and stems of leguminous plants offering the microorganisms an exclusive ecological niche where they convert environmental di-nitrogen to ammonium. This type of nitrogen is then made accessible to the host plant, which in turn gives carbon sources to the microsymbiont. Besides flavonoids and Nod factors, rhizobial acidic exopolysaccharides (EPS) assume a huge job in the foundation of a powerful advantageous interaction particularly with leguminous plants that form nodules of intermediate type (for example clover, pea, vetch, and hay)[1,33]. This polysaccharide is vital for protection against host plant resistance responses and initiation and elongation of infection threads, extraordinary tubular structures through which rhizobia colonize root nodules[21]. EPS insufficient mutant strains of *R. leguminosarum* bvs. *trifolii* and *viciae*, and *Sinorhizobium meliloti* induce formation of vacant or just somewhat partialy infected nodules like structures on roots of compatible host plants that are inadequate in nitrogen fixation[15,18]. Then again, EPS overproducing *R. leguminosarum* and *S. meliloti* strains are described by altogether enhanced occupancy of the host plant nodules and symbiotic effectiveness[20]. In free-living rhizobia, a few different capacities are likewise attributed to this polysaccharide, including gathering of nutrient, biofilm formation, and security against drying up (desiccation) and ecological stresses, which guarantee adjustment of these microbes to changing soil conditions[3,19]. The structure of EPS synthesized by *R. leguminosarum* has been organized in detail. This polymer is made out of repeating octasaccharide units which contain D-glucose, D-galactose, and D-glucuronic acid residues in a molar ratio 5:1:2, additionally adjusted with O-acetyl and pyruvyl gatherings[2,26,27,32]. Additionally, a dominant part of the EPS synthesis pathway has been established in this bacterium and biological functions of a few proteins have been tentatively affirmed. The biosynthesis of this heteropolymer in *R. leguminosarum* is led by a huge multi-enzymatic complex situated in the bacterial internal membrane. The synthesis of the EPS repeating units is initiated by a chemical encoded by the pssA quality which moves UDP-glucose to an isoprenyl phosphate lipid bearer situated in the inward layer[28]. The second step of the unit synthesis is led by a glucuronosyl-(β1-4) - glucosyl transferase PssDE, while a glucuronosyl-(β1-4)-glucuronosyl transferase PssC catalyzes the third step of this procedure. Based on sequence similarities of Pss proteins to enzymes accessible in databases and phenotypes of a few pss mutants, Ivashina and Ksenzenko[15] hypothesized that subsequent steps of the subunit assembly are carried out by PssS, PssF, PssI/PssG and PssH/PssI glycosyl-transferases, separately.

As described above the effect of carbon sources on EPS production, the nitrogen source also influence the growth and production EPS by rhizobial isolates. Dextrose and yeast has greater influence on growth and EPS production. However, different scientists like Breedveld *et al.,*[2], Ghosh and Ghosh[12] reported that KNO_3 used as a nitrogen source and mannitol as a carbon source helps in maximum EPS production. Further, Datta and Basu[5] reported that the maximum EPS production was obtained by the isolates of *Cajanus cajan* when KNO_3 and sucrose were used as nitrogen and carbon sources. The biosynthesis of EPS in rhizobia is a complex process affected by a few stresses and nourishment factors, which to date have been examined most broadly in *S. meliloti*. Soil conditions, for example, accessibility of nitrogen, phosphate and sulphur, and osmotic pressure influence production of EPS in this bacterium (for details see survey of Janczarek[19]). Rather than *S. meliloti*, the information because of natural factors on EPS creation in *R. leguminosarum* and its job in bacterial surface properties are still rare. Up to now, it was prove that phosphate and legume plant roots exudate influence the degree of EPS synthesis in this rhizobial species[19]. The point of this investigation was to set up natural elements influencing generation of EPS by *R. leguminosarum* and the job of this polysaccharide in bacterial surface properties and attachment capacity to both biotic and abiotic surfaces. This was assessed utilizing, aside from the rhizobial isolate, its subordinates varying in the degree of EPS delivered (EPS-deficient and EPS-overproducing strains).

Bacterial strains utilized in this examination are recorded in Table 1. *R. leguminosarum* strain RLB1, RLS1, RLS2, RLC1, RLC2 subordinates conveying extra duplicates of these genes were developed in 79CA medium with 1 % glycerol as a carbon source at 28°C (Vincent 1970).When required, antibiotic agents were utilized at the accompanying last focuses: kanamycin 40 µg mL^{-1}, nalidixic corrosive 20 µg mL^{-1}, and rifampin 40 µg mL^{-1}. To contemplate the impact of different carbon sources on the development of the RLB1 strain and its effectiveness in EPS creation, a 1% grouping of these mixes and a 72-h time of development were utilized. So as to set up the impact of pH on the development and the degree of EPS delivered by RLB1 after 72 h, a lot of 79CA media with pH running from 3 to 10 was tested.

Isolation of EPS and its quantification :

After the isolation, EPS was quantified as explained already[20]. Quickly, microscopic organisms were developed in proper 79CA medium for as long as 4 days at 28°C in a rotating shaker (160rpm). Then, the optical density OD^{600} of the rhizobial culture was estimated and culture supernatants were utilized for EPS precipitation with 96 % cold ethanol (1:4 vol/vol). Along these lines, EPS^s were collected by centrifugation (20 min, 14000 rpm), and after re-dissolving in deionized water examined for sugars (Carbohydrates). The complete sugar content was determined as glucose reciprocals per unit of OD^{600}. Each trial was rehashed multiple times with three repetitions for every treatment.

Determination of dry weight of biofilm (Biofilm assay) :

With some modification in the protocol of dry weight of biofilm we determined the dry weight of biofilm. Incubate bacterial culture in polypropylene microfuge tube (Eppendorf tube) with Luria–Bertani broth (LB broth) and incubate at 37 °C for 24 hrs. After incubation wash biofilm with distill water and di-methyl sulphoxide and measured dry weight of biofilm. For these purpose, we weigh empty microfuge tube and then weigh those microfuge tube in which biofilm developed. By subtracting in the value of empty microfuge we observed dry weight of biofilm.

Sand assays :

The sand assay of rhizobial cell adhesion to quartz sand was performed according to the technique of Fujishige *et al*.[9,10] with certain adjustments (modifications). Quickly, rhizobial strains were developed in 79CA medium containing 1 % glycerol at 28 °C for 24 h. For the RLB1 strains, kanamycin (20 µg mL^{-1}) was included into the medium. At that point, the bacterial cultures were centrifuged (10 min, 8,000 rpm), washed, and diluted in a similar medium to OD^{600}=0.2 (ca. 2×108 cells mL^{-1}). 500 µl of the bacterial suspension was included into an Eppendorf tube containing 150 mg of sterile sand or a similar volume of the medium just as a control. The cylinders were left on a horizontally at 28°C for 1 and 48 h. Next, the samples were further centrifuged (30 s, 2,000 rpm), the fluid was decanted, and the sand was repeatedly washed three times with 10mM $MgSO_4$ and broadly vortexed to separate cells from the sand particles. At last, 1 ml 79CAwas added to each sample and serial dilutions were made in this medium. 100-µl aliquots were put on plates with 79CA agar (with kanamycin when required) to get CFUs (estimations of CFU were standardized per mg of sand). The examination was performed in triplicate with three natural repetitions for each strain.

Attachment assay of Rhizobia to chick pea roots :

The rhizobial strain RLB1 and its subsidiaries (RLS1, RLS2, RLC1, RLC2) were utilized to set up the capacity of rhizobial strains to connect to roots of chick pea. For this analysis, the strategy depicted by Fujishige *et al*.,[9] was applied with a slight change. Suspensions of rhizobial strains were made in Fåhraeus fluid medium with OD^{600}=0.1. Roots of 3-day-old chick pea seedlings were immersed into the bacterial suspensions for 1 h or incubated for 48 h (100 µl of bacterial suspension per seedling). At that point, the plants were put on sterile Whatman paper to evacuate the abundance fluid. Next, the roots were washed with 0.05 % Tween-20 on a shaking stage shaker (100 rpm) to expel loosely associated microbes, and weighed in the wake of expelling the overabundance fluid. To set up the quantity of microbes appended to the root surface, every seedling was homogenized in 300 µl of sterile water. The homogenates acquired were centrifuged for a short period (30 s, 8,000 rpm), serially diluted in water, and 100-µl aliquots were put on 79CA plates for CFU value. The test was multiple times repeated and 10 seedlings for each individual strain were utilized in every repetition.

Statistical analysis :

The outcomes are mean±SD from three investigations performed in triplicate. Correlation of qualities between various bacterial strains in similar conditions was performed utilizing a single direction ANOVA to discover factually huge contrasts. In situations where the null hypothesis (all populations implies are equivalent) was rejected at the alpha=0.05 level. Significant differences between the tested strains were considered at the degree of p values <0.05.

Factors affecting bacterial growth and EPS production :

There are different methods available for EPS screening. Identification of EPS can be done through colony morphology, precipitation and dye based coverslip assay. All of these processes does not require any equipment and also have their own limitations and benefits. Extracellular polysaccharide discharged by *R. leguminosarum* into the environment plays a vital role in the symbiotic association of this bacterium with its host plant, for example the chick pea (*Cicer arietinum*). Subsequently, we chose to build up which ecological variables influence the production of this polysaccharide in *R. leguminosarum*. Various parameters, for example, the sort of the carbon source, time of bacterial development, temperature and pH were examined utilizing a well-portrayed strain RLB1.

At first, the influence of the type of the carbon source on both the bacterial development rate and effectiveness of EPS blend was considered. EPS production by all of the isolates was investigated with different sources of carbon. The samples were decanted after equal interval of time and quantitative estimation of EPS was carried out. These results show that maximum level of EPS production was produced by RLB1 rhizobial isolates with glycerol. Among the tested mixes, glycerol demonstrated to be the best carbon source for the development of the investigated strain (Fig. 1). An elevated level of development was likewise seen within the sight of maltose, sucrose, mannitol, mannose, glucose, galactose and sorbitol, though the level of utilization of the remaining compounds was essentially lower. In addition, in presence of glycerol, RLB1 produced highest amounts of EPS. Additionally, different mixes, as sorbitol, glucose, succinate, mannitol, and glucosamine were carbon sources, in presence of which significant levels of EPS were produced by this strain. Based on the presented data, it may be noticed that the levels of EPS synthesis correlated with bacterial culture density under a few development conditions. This was observed for such carbon sources as glycerol, glucose, sorbitol, rhamnose, and glucosamine. On the other hand, in spite of the fact that strain RLB1 adequately utilized some carbon sources for the growth (maltose, lactose, mannitol, mannose), the production of EPS by this bacterium within the sight of these mixes persevered at moderately low levels. An opposite situation was observed for succinate. Culture density in the presence of this carbon source was extremely low yet the measure of EPS production was high. These results indicate complex regulation of the process of EPS synthesis in rhizobia, in which carbon sources and their metabolism are most likely locked in.

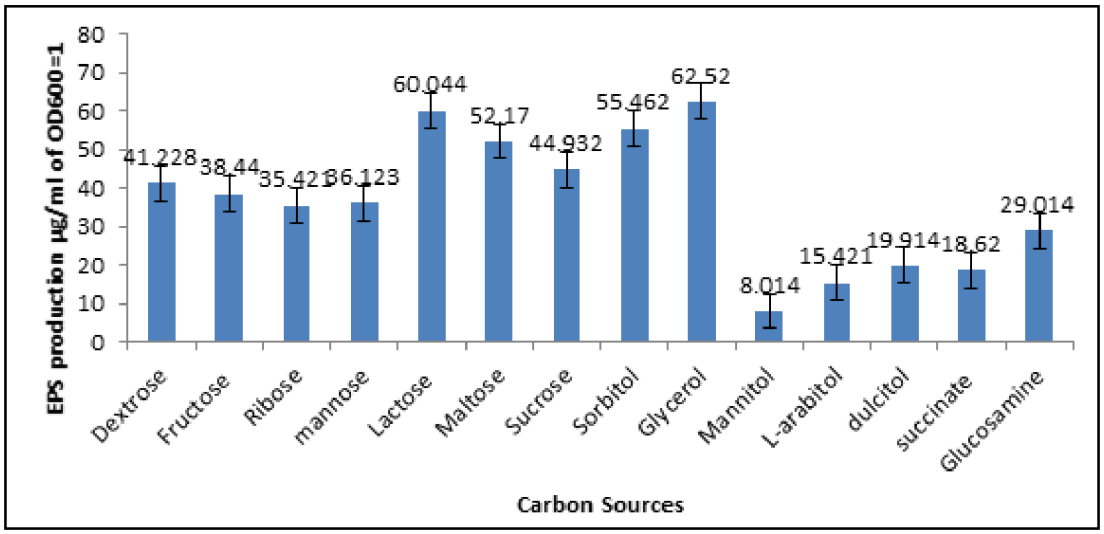


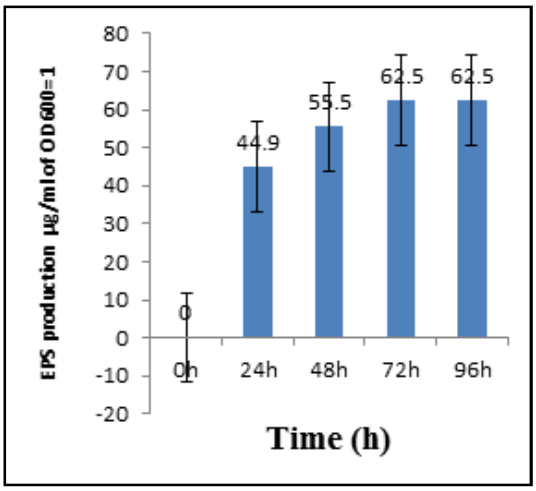
Fig. 1 Impact of different carbon sources on the growth and exopolysaccharide productions in *R. leguminosarum* RLB1. Optical densities (OD600) of rhizobial cultures are displayed as blue squares and measures of EPS production by the RLB1 strain with specific carbon sources are appeared as blue color columns. The displayed information is mean±SD for three experiments. Statistically, there are significant differences in the amount of EPS production.

At last, since glycerol demonstrated to be the best carbon source for both the bacterial development and EPS generation, this compound was utilized in every further investigation.

Another parameter of this research was the time taken by bacteria to grow. For this reason, an experiment of time course was performed, in which the RLB1 growth and efficiency of EPS production were resolved after 24, 48, 72 and 96h.The growth kinetics and amounts of EPS produced by this bacterium are shown in Fig. 2a. The RLB1 strain developed adequately in the tried culture medium and reached a stationary phase after 72h. Further incubation time did not affect the density of the bacterial cultures. It was seen that the RLB1 strain synthesize high amounts of EPS during the tried time periods, and the most elevated level of the synthesis was accomplished after 72 h of development (Fig. 2a). The longer incubation time (96 h) did not increase the production of EPS. Hence, incubation period of 72-h time was utilized in further examinations.

Also, the impact of temperature on the bacterial development and effectiveness of EPS production was analyzed. For this reason, the RLB1 strain microorganisms were developed at different temperatures (20–30 °C) for 3 days and culture densities and measures of synthesized EPS were set up (Fig. 2b). Among the tested conditions, the range of 24–28 °C was ideal for both the growth rate and efficiency of EPS synthesis. At a higher temperature (30 °C), the bacterial development

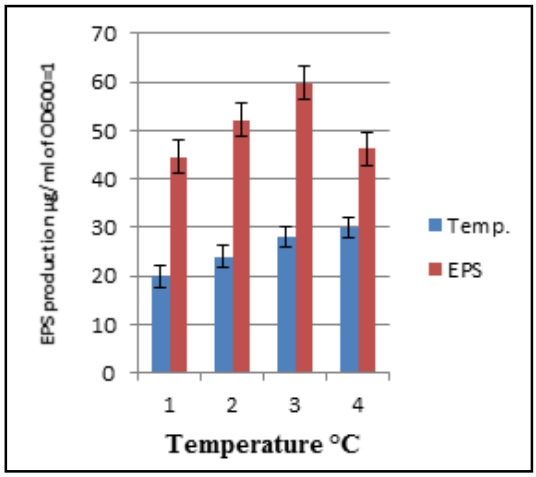
and the degree of created EPS diminished altogether. Strangely, in spite of the fact that the low temperature (20 °C) reduced the development rate most altogether among the entirety of the tested conditions, the creation of EPS was not significantly diminished. These outcomes demonstrate that the temperature required for the highest production of EPS by the RLB1 strain is like that for its optimal growth.



(Fig. 2a) It shows the influence of time on the growth and production of EPS in *R. leguminosarum*. The amount of EPS formed by rhizobial strains were shown by columns. The displayed data is mean±SD for three independent experiments. Statistically significant differences observed in the amounts of EPS ($p<0.05$; ANOVA).

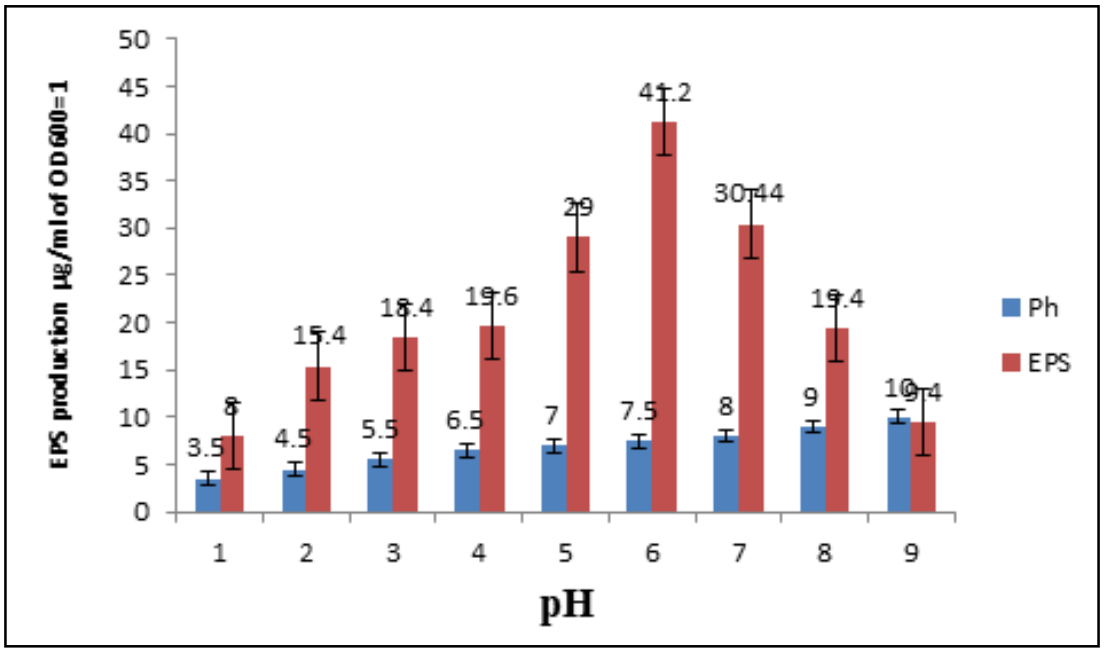
The next growth parameter followed by temperature was pH of the medium. The range of the pH was tested from 3.5 to 10 (Fig. 2c). Under the conditions used, RL01 strain grows efficiently at a range of pH 6.5 to 8.5, and the optimal growth of this strain was achieved at pH 7. At a pH lower than 6.5 and higher than 8.5, the bacteria grew inadequately and the cultures reached low optical density significantly after 72 h growth. Considering EPS production, the optimum degree of synthesis of this polymer was acquired at pH, from 7 to 9 with the highest amounts obtained at pH 7. These data indicate that the pH range for high production of EPS by the RL01 strain is larger than that required for the optimal bacterial development.

Taking everything into consideration, all the exhibited information show that different components influence production of EPS in *R. leguminosarum*



(Fig. 2b) It shows the influence of temperature on the growth and production of EPS in *R. leguminosarum*. The amount of EPS formed by rhizobial strains were shown by big columns. The displayed data is mean±SD for three independent experiments. Statistically significant differences observed in the amounts of EPS ($p<0.05$; ANOVA).

including, the sort of the carbon source, temperature, pH, and the age of the bacterial culture. For the RLB1 strain, the highest level of EPS production was calculated with



(Fig. 2c) It shows the influence of pH on the growth and production of EPS in *R. leguminosarum*. The amount of EPS formed by rhizobial strains were shown by big columns. The displayed data is mean±SD for three independent experiments. Statistically significant differences observed in the amounts of EPS ($p<0.05$; ANOVA).

glycerol as the carbon source; at the temperature extend 24–28 °C, pH 7.5, and the stationary development stage (72–96 h).

Dry Weight of biofilm :

To determine the ability of biofilm formation by *Rhizobium*, the isolates were grown in the LB broth. The growth of bacteria was calculated by all selective bacterial strains on polypropylene surface. On the basis of biofilm formation by *R. leguminosarum* strains, the dry weight of biofilm formed on polypropylene surface by RLB1, RLS1, RLS2, RLC1, RLC2, are shown below (Fig. 3). The maximum dry weight of biofilm was shown by Rhizobial strains RLS1 and RLS2. The rest of the isolates show less than .05g of dry weight of biofilm while the control does not show any biofilm formation.

Attachment of Rhizobial strains to chick pea roots and sand :

Without good host plants, *R. leguminosarum* has to survive long periods of time under soil conditions. Along these lines, it appeared important to determine the attachment and biofilm forming capacity of the RLB1 strain and its subsidiaries on surfaces, for example, quartz sand and host plant roots. Quartz sand was picked in light of the fact that it is a characteristic environment for some soil microbes including rhizobia, and furthermore

it is less complex than soil, consequently allowing us to build up the connection capability of the tested strains in a defined, but still natural material. In this experiment, the RLB1 microbes showed high proficiency in attachment to sand particles, since 3×10^4 cells per1mg of sand were attached after 1 h and 12.5×10^4 cells inside 48 h post vaccination (Fig. 4). Conversely, the other strains (RLS2) showed a decreased ability to adhere to this material, since the quantities of the cells connected after 1-h and 48-h incubation were 28.5 and 33.4 %, respectively, of those joined by the wild-type strain. In the case of the RLS1, an even stronger negative effect was watched. This strain completely lost the capacity of connection and biofilm arrangement on this surface, since only few cells were adherent to sand particles after 1-h incubation and they represented 5 % of those identified for the control strain. Then again, the RLS1 strains secreting more EPS than the remaining microbes displayed essentially higher efficiency in adhesion (Fig. 4). This was detected particularly after 48 h of the examination, when the numbers of the follower microscopic organisms were 2 fold higher than the remaining strains. These information show that EPS happening outside of the rhizobial cells is significant for effective and productive bond of the microscopic organisms to surfaces present in the earth. Rhizobia interact with compatible legume host plants in the soil so as to build up beneficial interaction. The initial step of this interaction is attachment to and biofilm formation on the host root surface including root hairs, which are a target for legume root infection. In this way, we can check the ability of the *R. leguminosarum* strain, its EPS-insufficient and EPS-overexpressing derivatives to attach to chick pea roots. In this test, it was seen that the RLB1 attached with high productivity to the host roots after the principal hour of the

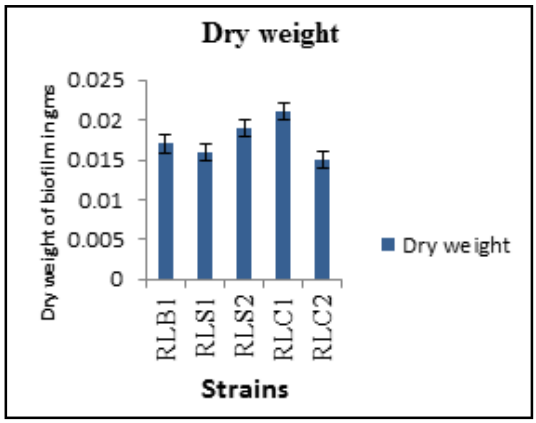
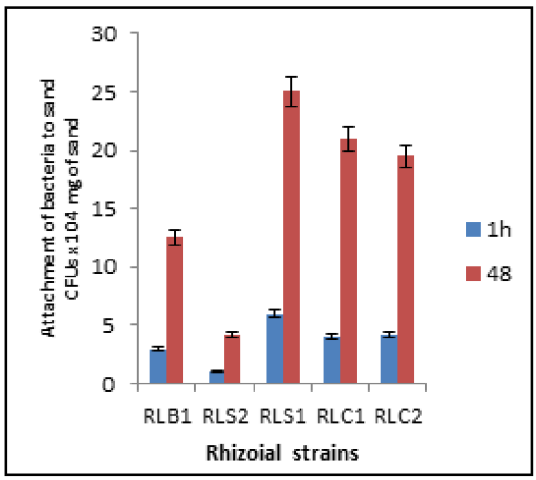


Fig. 3: Dry weight of biofilm formed by rhizobial strains



(Fig. 4) It shows the attachment of *R. leguminosarum* strains to the sand paricles observed after 1h and 48h. The displayed data is mean±SD for three independent experiments. Statistically significant differences were obtained between the strains of 1h experiment and 48h experiment ($p<0.05$; ANOVA).

test, and further incubation still persistently expanded the quantity of microbes attached. Likewise in this test, the RLC1 strains displayed a most minimal attachment capacity,

among all the broke down strains. A low number of microscopic organisms were attached to the host roots 1 h post inoculation. Then again, both EPS-overproducing strains RLC1 and RLC2 showed more effective connection to chick pea roots. A similar inclination and comparative contrasts in the adhesion ability between the tested strains were likewise seen after 48-h incubation. The above outcomes confirm the significant role of EPS in biofilm formation and attachment on the tested surfaces by *R. leguminosarum* and demonstrate that the absence of this polysaccharide or its lacking sum bring about influences in these procedures.

Many researchers showed that the higher population of beneficial microorganisms in soil could increase nutrient retention. This led to germination up to 20% yield from 10 to 40%, increase the availability and uptake of nitrogenous and phosphorus in plants, improve the status of soil fertility, maintain good soil health and crop productivity, suppress harmful and pathogenic soil microorganisms. They are ecofriendly and non-polluting (Khanafari *et al*., 2012).

Rhizobia are the Gram negative and aerobic bacteria that symbiotically form nodules with roots of leguminous plants. A Dutch scientist, Beijernck in 1888 first isolated a bacterium from root nodules of legumes and named it Bacillus *radicicola*, which is now named as *Rhizobium*. Most of them produce extracellular polysaccharides. To confirm the isolates plant infection test is carried out to observe nodulation. From 750 genera and 19,000 species of leguminous plant about 15% plants have been examined for nodulation and less than 0.5% has been studied for their symbiotic relationships with nodule bacteria. Most of plants examined have been of agriculture importance and the huge reservoir of wild tropical leguminous plants is only now beginning for investigation. They fix atmospheric nitrogen and render it into combined forms resulting in high amount of protein in roots. The proteins are transported along the plants and also secreted in rhizosphere region. In recent years, rhizobia are used as biofertilizer for selected crops.

The morphology and physiology of *Rhizobium* will vary from free living condition to the asteroid of nodules. In addition to fixing the atmospheric nitrogen through nodulation, it shares many characteristics with other PGPRs including hormone production and solubilization of organic and inorganic phosphate. Through plant growth promoting substances, it helps in root expansion, improve uptake of plant nutrients, protect plants from root diseases and most important improves biomass production of fast growing at wasteland[13].

They are nitrogen fixing microorganisms ready to set up beneficial interaction with leguminous plants and giving them a nitrogen source, when they grow in soils deficient in nutrient[21,25]. The procedure of organic nitrogen obsession is done in specific structures, called nodules, which are shaped on vegetable roots.

Strong infection of host roots is subject on a reciprocal “molecular dialogue” between the plant and the microsymbiont, in which, among others, rhizobial exopolysaccharides excreted into environment play a vital role [4,19]. The initial step of the infection procedure is

attachment to and formation of biofilm on host root surfaces[6]. Subsequently, the attachment capacity of the rhizobial strains is a crucial feature for their capability of host plant disease. *R. leguminosarum* strains can build up a harmonious interaction with chick pea plants. We have indicated that the strain RLB1 creating a lot of EPS had a high ability of attachment to roots of a perfect host plant just as to abiotic surfaces. Additionally, growth conditions ideal for a maximal degree of EPS production were established for this bacterium (Figs. 1 and 2). In contrast, the strains analyzed in this work, in which the synthesis of EPS was maximum for some strains and was significantly reduced for other strains, showed decrease in the attachment with abiotic and biotic surfaces, decrease in biofilm formation which ultimately shows that EPS is essential for this whole process. Likewise, Williams *et al.*,[35] have shown that the *R. leguminosarum* bv. *viciae* strain, having a gene mutation in a quality homologous to *R. leguminosarum,* formed immature biofilm on polystyrene plates, obvious as a level inexactly loosely attached lawn. Besides, this strain demonstrated decreased connection to root hairs of pea plants. On the other hand, the results of this study indicated that rhizobial strains RLS2 and RLS1, which overproduced EPS, had a fundamentally expanded capacity to get attached to various surfaces, including soil particles and the host roots, which guaranteed better survival and adjustment to changing ecological conditions. Similarly in *S. meliloti*, EPS is required for biofilm improvement, since an *exoY* strain non-producing this polymer frames an immature biofilm[9,10]. In this bacterium, a symbiotically dynamic low molecular weight division of galactoglucan demonstrated to be significant for biofilm arrangement and colonization of host plant roots[31]. Also, the level of EPS polymerization is significant for appropriate advancement of biofilms in rhizobia. Interestingly, pH and outrageous temperature adversely affected this procedure. Additionally in *R. leguminosarum*, a few supplements influenced biofilm formation[19].

Apart from being a signal molecule in the early stages of the rhizobium legume interactions, EPS is likewise an objective site for plant lectins emitted by host roots and connecting with the sugar residues of rhizobial polysaccharides. As of late, Xie and co-workers[36] have recognized a plant arabinogalactan like glycoprotein, which is engaged in a novel type of polar surface attachment by *R. leguminosarum*. This sort of connection required EPS and demonstrated to be significant for development of these microbes on the underlying foundations of the two legumes and non-legumes. In this work, a few ecological elements were tried and conditions ideal for both bacterial development and synthesis of EPS by *R. leguminosarum* strain were set up (Figs. 1 and 2). We have tried 14 carbon sources, and among them glycerol, maltose, sorbitol, and lactose demonstrated to be the best mixes for the bacterial development. Considering the yield of EPS blend, the most elevated levels of generation of this polysaccharide were identified when the RLS1 strain developed within the sight of glycerol, sorbitol, and glucose. Among the tried compounds, glycerol demonstrated to be the best carbon source for both the EPS union and the development of this strain. The outcomes got for other carbon sources demonstrated that the degree of the bacterial development didn’t

constantly connect with the degree of delivered EPS. We have discovered that other development parameters, for example, time, temperature and pH likewise impacted the yields of EPS creation. These discoveries are in concurrence with the outcomes for other rhizobial species, which indicated that the degrees of EPS synthesis were reliant on the kind of the carbon source, culture time, pH, temperature, and the shaking rate[14]. Likewise, Quelas *et al*.,[29] depicted for *Bradyrhizobium japonicum* strain USDA 110 that culture conditions such as the type of the carbon source, nitrogen availability, and culture age can modify the measure of created EPS. The creators tried mannitol and malate as carbon sources and indicated that the measure of orchestrated EPS was higher in the nearness of the former than the latter carbon source, whereas EPS organization was the equivalent with both these carbon sources. In contrast, Karr *et al*.,[22] found for another *B. japonicum* strain 2143, the kind of the carbon source likewise influence the amount of this polysaccharide. For instance, EPS synthesized by this bacterium developing within the sight of glucose contained rhamnose connected deposits, which were absent when this bacterium developed in the presence of arabinose.

Another explanation of these perceptions could be the way that, contingent upon the development conditions and strategy of EPS disengagement utilized, the part of extracellular polysaccharides could contain small amounts of other types of sugar polymers. For model, *S. meliloti* produces two kinds of EPS, succinoglycan and galactoglucan, contingent upon environmental conditions[38,34] and *R. leguminosarum* produces extra kinds of surface polysaccharides (nonpartisan glucomannan and gel-forming polysaccharide)[23]. This shows the intricacy of the external surface of rhizobial cells and recommends a complex administrative system engaged in the synthesis of polysaccharides[11,19].

Our outcomes demonstrate that EPS modified the electro kinetic properties of rhizobial cells and impacted attachment just as biofilm arrangement. The EPS charge in the primary (physicochemical) advance of grip appears to have minor contribution, though the hydrophobic properties of this polysaccharide assume a progressively prevailing role in this procedure. In a few papers, the role of EPS in early periods of symbiotic interaction (infection of host plant roots) is very much reported[18,33]. Different authors affirmed that rhizobial EPS was required for attachment to idle substrates, and extra bacterial parts (adhesins, glucomannan, cellulose fibrils) are occupied with the further steps of this procedure[23,35]. Likewise, some environmental factors (pH of soil, root exudates) influence bacterial connection to have root hairs[6].

The results of this work have demonstrated that the capacity of *R. leguminosarum* isolates to synthesize EPS essentially influences bacterial connection with both biotic and abiotic surface, formation of biofilm. EPS produced by rhizobia encourage attachment of bacterial cells to both biotic and abiotic surfaces through hydrophobic associations and heterogeneity of the envelope surface instead of electrostatic interactions, which for this situation are emphatically repulsive.

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