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Unveiling a Novel Purine Auxotroph in *Sinorhizobium meliloti*: Insights into Defective Exopolysaccharide Production

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ABSTRACT

In the establishment of symbiotic relationships between leguminous plants and rhizobial strains, exopolysaccharides (EPS) play a pivotal role in molecular signaling. This study investigates the consequences of EPS deficiency by examining *Sinorhizobium meliloti* strains Rmd 201(WT) and the pleiotropic mutant Rmd 1102(A_2) Exo^- and Pur^- generated by employing NTG mutagenesis. Rmd 201(WT) induced typical indeterminate nodules, while mutant Rmd 1102(A_2) Exo^- and Pur^-formed small, white, spherical anddeterminate pseudonodules. Calcofluor staining of Rmd 201(WT) exhibited strong fluorescence under UV light, confirming the presence of normal EPS, while mutant Rmd 1102(A_2) lacked fluorescence, confirming its EPS deficient phenotype. HPLC analysis of rhizobial exopolysaccharide especially tightly bound sugars revealed that Rmd 201(WT) contained peaks for xylose and sucrose, whereas Rmd 1102(A_2) lacked the xylose peak and exhibited a slightly modified sucrose peak, indicating minor changes in EPS composition. Further, when Rmd 1102(A_2) was streaked on minimal medium containing xylose as the sole carbon source, it exhibited phenotypic suppression of the calcofluor dark phenotype, with the appearance of fluorescent colonies only in proximity of Rmd 201(WT). This observation hints on a qualitative change in EPS of Rmd 1102(A_2) which is restored in the presence of xylose and is influenced by diffusible substance/s secreted by parent strain Rmd 201(WT). Similar unique exopolysaccharide has been reported in same strain of *Sinorhizobium meliloti* which contains xylose sugar.

This study highlights the dual impact of a pleiotropic mutation, affecting both EPS synthesis and purine metabolism, while shedding light on the role of xylose sugar utilization in the symbiotic relationship.

KEYWORDS: *Sinorhizobium meliloti*, NTG mutagenesis, Exopolysaccharide, pleiotropic mutants, xylose sugar, Symbiotic relationship.

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INTRODUCTION

Rhizobia, are gram-negative bacteria, establishing a symbiotic relationship with legumes by colonizing the area around their roots and forming specialized plant structures known as nodules[1]. Within these nodules, they play a crucial role fixing nitrogen. Exopolysaccharides are essential for the successful establishment of this symbiosis and can be either species-specific homopolymers or heteropolymers composed of different carbohydrates[2].

The successful initiation of the Rhizobium-legume symbiosis relies on the interplay of various signaling molecules. Leguminous plants, members of family Fabaceae , release plant exudates, such as flavonoids, which serve as signals to chemotactically attract compatible bacteria from the rhizosphere[3]. These flavonoids activate Nod regulatory proteins, which in turn, induce the expression of rhizobial nodulation genes. Flavonoids can passively diffuse through the bacterial membrane. Bacteria recognize these signals through positively acting transcription factors, often encoded by bacterial nod gene.These genes encode enzymes required for the synthesis of specific lipochitooligosaccharides collectively known as Nod factors[4]. The binding of Nod factors to Nod factor receptors in plants triggers the initial plant response, including the curling of root hairs, which traps rhizobia as micro colonies. Rhizobia then invade the root hairs and ultimately the roots, forming tubular structures referred to as infection threads. These infection threads are characterized by the progressive ingrowth of plant cell membranes containing a matrix of cell wall material[5,6]. Within the root hairs, bacteria multiply and stimulate the division of root cortex cells, leading to the formation of nodule primordia. Inside these nodule primordia, rhizobia are released via endocytosis from infection threads into compartments called symbiosomes. Within symbiosomes, rhizobia differentiate into bacteroids, which are surrounded by membrane derived from the plant. Once bacteroids are formed, they carry out the essential task of converting atmospheric dinitrogen into ammonia, which is then utilized by the host plant. This is particularly important as these host plants often grow in nitrogen-deficient soils[7].In return, the bacteria receive carbon and energy sources from the plant photosynthates, creating an ideal niche for their multiplication and growth within the root nodule. The process of symbiotic nitrogen fixation in root nodules is genetically and biochemically complex, involving genes related to nodule development (nod), nitrogen fixation (nif, fix), and host range determination (hsn)[8]. Additionally, genes responsible for biosynthetic pathways for amino acids, nitrogenous bases, vitamins, and bacterial cell surface components play crucial roles in establishing effective symbiosis. Effective symbiosis also involves the induction of genes responsible for the production of various cell surface polysaccharides, including lipopolysaccharides (LPS), capsule polysaccharide (CPS), exopolysaccharides (EPS), gel-forming polysaccharide (GPS), K-

antigen polysaccharide (KPS), cyclic ß-(1,2) glucans, and high-molecular-weight neutral polysaccharide (NP)[2].

The attachment of rhizobia to the root hairs of legume plants is a crucial step in initiating the symbiosis, involving secreted proteins and surface polysaccharides. Proximity to the roots provides a nutrient supply for bacterial growth, enabling the formation of a biofilm on root hairs. While root-hair deformation can occur in response to Nod-factor signals. Root hair curling, where the root hair curls back on itself to entrap bacteriais only possible when induced by attached rhizobia[9]in the rhizosphere. This curling is crucial for most successful infections in many legumes. The genomic sequence of *S. meliloti* 1021 reveals a tripartite structure consisting of a 3.65-Mb chromosome, 1.35-Mb pSymA megaplasmid and 1.68-Mb pSymB megaplasmid[10]. Nodulation genes are found on pSymA, while genes responsible for symbiotically important polysaccharides are distributed across the chromosome and pSymB.

The regulation of nod genes is intricate and involves multiple NodD copies (NodD1, NodD2, NodD3) and another regulator, SyrM. NodD1 and NodD2 are constitutively expressed and activate nod genes in response to flavonoids and plant betaines, while NodD3 activates nod genes independently but is also regulated by SyrM[11], forming a feedback loop. SyrM also activates SyrA, a gene encoding a protein that promotes the biosynthesis of a specific type of EPS (EPSI).

NodD3 in *S. meliloti* is involved in the production of two important symbiotic signals: Nod factors and EPS I. The biosynthesis of both EPS I and EPS II relies on distinct gene clusters on pSymB. The EPS I biosynthesis involves a 27 kb region with 19 exo and 9 exs genes[12].

Since cytokinins are related to purines, it is speculated that certain genes are influenced by various growth regulators[13]. EPS II production relies on a 30 kb cluster with 30 wgx genes. Both clusters encode proteins for precursor production, repeating unit synthesis, polymerization, and transport[14]. The chromosomal exoC gene codes for phosphoglucomutase required for converting glucose-6-phosphate into glucose-l-phosphate, a precursor for EPS I. The regulation of the production of both *S. meliloti* EPS is extremely complex, taking place at the transcriptional, post-transcriptional, and post-translational levels, and is subjected to external signals, such as plant flavonoids, abiotic stresses, and the level of nutrients like nitrogen and phosphate[14].Presence of novel surface exopolysaccharide has been reported in *Sinorhizobium meliloti* which involves a new fatty acid synthase like gene cluster and is involved in symbiotic nitrogen fixation[15]. Kdo rich exopolysaccharide has been detected in *Sinorhizobium fredii* and is analogous to group II K antigen of *Escherichia coli*[16]. This polysaccharide contains glucose, glucoronic acid, galactose, mannose, xylose, glucosamine and Kdo in the ratio 12:1:1:1:2:0.5:,05:1.5.This unique exopolysaccharide has

been detected in two strains of *Sinorhizobium fredii* and several *S. meliloti* species and is neither EPS I or EPS II[17,18].

Recent findings challenge the conventional understanding that Nod factors are essential for legume nodulation. Some unique rhizobia-legume combinations have demonstrated the ability to form nodules without using Nod factors[19]. The specific bacterial signals involved and how plants respond to these signals is still not clear. There is suggestive evidence that purine-based signals might be implicated in this process. In typical nodulation, the activation of a cytokinin pathway enables legumes to induce nodule formation even in the absence of rhizobia[20]. Since cytokinins are related to purines, it is speculated that certain rhizobia may have evolved to bypass the conventional Nod-factor signaling pathway and trigger nodule development at the level of cytokinins. It has been previously recognized that a purine precursor, aminoimidazole carboxamide ribonucleotide (AICAR), plays a role in aiding rhizobia in infecting legumes[9].

In present study, several purine auxotrophs were isolated using NTG mutagenesis in a strain of *S. meliloti* Rmd201. Interestingly one purine auxotroph Rmd1102(A_2) appeared to be pleiotropic mutant because it exhibited Exo^- Pur^- phenotype and was forming symbiotically defective, small determinate white pseudonodules that lacked detectable nitrogenase activity on *Medicago sativa*. Rmd 1102(A_2) showed normal infection threads, shepherd crook formation, normal infection but nodules could not fix nitrogen.

Prototrophic revertants showed restoration of symbiotic ability and mutation of Rmd 1102(A_2) was a point mutation[21]. Rmd 1102(A_2) showed a block at some step between conversion of AICAR (amino imidazole carboxamide ribonucleotide) to IMP (inosine monophosphate) (Fig. 1).

This indicates that point mutation in Rmd 1102(A_2) is either at the step of conversion of AICAR (amino imidazole carboxamide ribonucleotide) to FAICAR (5- formamido-4- imidazole carboxamide ribonucleotide) or conversion of FAICAR (5- formamido-4- imidazole carboxamide ribonucleotide) to IMP (inosine monophosphate). This indicates that point mutation could be in PurH, PurJ, PurP or Pur0 genes or some other gene affecting these genes[22]. However, point mutation in Rmd 1102(A_2) is a pleiotropic mutation that is simultaneously affecting exopolysaccharide synthesis, purine biosynthesis and forming ineffective determinate nodules. External supplementation of plant growth medium with AICAR or IMP at the time of inoculation of the seedlings with bacterial strains did not cause the formation of nitrogen fixing nodules. Mutant bacteria can be recovered from nodules of 30-day-old seedlings.

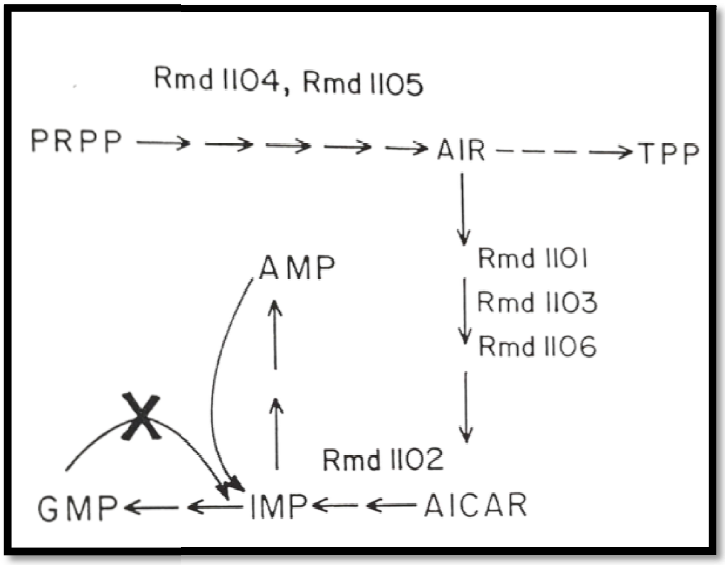

Fig. 1. Schematic diagram representing *de novo* purine biosynthetic pathway in *Sinorhizobium meliloti* along with relevant intermediates. Rmd 1102(A_2) Exo^- Pur^- exhibits a block at the step of conversion of AICAR to IMP. PRPP = phosphor ribosyl pyrophosphate; AIR = amino imidazole riboside; TPP = thiamine pyrophosphate; AICAR = amino imidazole carboxamide; IMP = inosine mono phosphate; AMP = Adenosine mono phosphate; GMP = guanosine mono phosphate. Note that GMP is not converted to IMP in *Sinorhizobium meliloti*

MATERIALS AND METHODS

***Bacterial strains*:** The wild type strain Rmd 201 is a streptomycin resistant derivative of AK 631, which forms indeterminate nodules on host *Medicago sativa* and nodules, are able to fix nitrogen. Rmd 201(WT) can grow well on minimal media. The purine auxotroph Rmd 1102(A_2) was isolated using NTG mutagenesis and could grow on minimal media only when supplemented with adenine and produces defective exopolysaccharide.

***Media and culture conditions*:** Complete medium (MSY) and minimal medium (RMM) for rhizobium were used[23].

***Calcofluor staining*:** The bacterial strains to be tested were either streaked or spotted on MSY medium plates containing calcofluor white and incubated at 28°C for 4 days. Calcofluor staining was observed under UV light and positively stained samples showed fluorescence.

***Qualitative analysis of exopolysaccharide sugar composition*:** The qualitative analysis of sugars was carried out using Waters 501millipore HPLC (High Performance Liquid Chromatography) unit comprising of automated gradient controller, with two pumps, a differential refractometer and a data module for recording peaks. A completely degassed acetonitrile (80%) solvent system was used as

mobile phase. The carbohydrate analysis column (3.9mm x 30cm stainless steel column) was used in present investigation. The detector used was a refractive index detector specific for carbohydrates. The concentrated free sugars and hydrolysate were dissolved in HPLC grade water and clarified by passing through a sample clarifier to eliminate salts and impurities. The pump was run in isocratic mode. Before injection, the column was thoroughly washed with 80% acetonitrile solvent system for 30 minutes by keeping flow rate at 2 ml/min. The samples were degassed and subsequently injected 1 ml of sample into the injector port. The peaks were identified by comparison of their retention time with those of standard sugars. For preparing individual standards of sugars, 100mg of sugar powder (Himedia) was added to 2m1 of HPLC grade water. Same concentration of sugar was maintained for all sugars. Names and retention times of standard sugars used are depicted in the Table 1.

Table1: Retention times of Standard Sugars.

SNo.	Sugar	Retention time (minutes)
1.	Xylose	3.1
2.	Adonitol	3.58
3.	L- Arabinose	3.47
4.	Cellobiose	7.33
5.	Dextrose	4.25
6.	Ducitol	4.45
7.	GalactoseN	4.68
8.	Fructose	3.75
9.	Inulin	13.67
10.	Inositol	7.81
11.	Lactose	9.87
12.	Trehalose	8.34
13.	Sucrose	6.27
14.	Rhamnose	2.76
15.	Salicin	2.15
16.	Raffinose	13.61
17.	Melibiose	10.14
18.	Mannose	4.11
19.	Mannitol	4.63
20.	Maltose	7.39
21.	Sorbitol	4.53

***Isolation of extracellular exopolysaccharide*:** 10 ml of log phase culture of wild type strain Rmd 201(WT) and Rmd 1102(A_2)Exo^- were separately centrifuged at 5000rpm for 15 minutes. Collected the supernatant in fresh centrifuge tubes. Washed the pellet with 10 ml distilled water at 60°C twice and the washings were added to the supernatant fraction. Then volume was reduced to 10 ml using lyophilizer. To this was added 10% TCA (w/v) to precipitate proteins, nucleic acids etc. Centrifuged this fraction at 5000 rpm for 10 minutes and retained the supernatant. To the above supernatant added three volumes of chilled ice-cold absolute alcohol and incubated at -20°C overnight. Next day centrifuged at 5000 rpm for 10 minutes and reduced the supernatant fraction to 5 ml. To isolate complex sugars treated the pellet. The supernatant fraction containing free sugars was dialyzed and 1ml was injected for HPLC analysis.

***Hydrolysis of complex exopolysaccharides*:** For detection of complex exopolysaccharides, 2m1 of 0.7% H_2SO_4 was added to the pellet obtained from isolation of extracellular polysaccharide and transferred to hydrolysis tubes. The hydrolysis tubes were carefully sealed and incubated at 120°C for 6 hours. After hydrolysis, neutralized acid with barium carbonate and the entire constituents were centrifuged. The supernatant volume was reduced using lyophilizer, dialyzed and lml of sample was injected for HPLC analysis.

RESULTS

***Microscopic examination of infection process*:** Normal infection thread formation and root hair bending was seen in both Rmd 201(WT) and Rmd 1102(A_2) exopolysaccharide defective mutant. Rmd 201(WT) induced normal indeterminate nodules. Rmd 1102(A_2) formed symbiotically defective, small, white and determinate pseudonodules that lacked detectable nitrogenase activity on *Medicago sativa* (Fig. 2).

***Calcofluor staining*:** Reaction of wild type strain Rmd201(WT) on calcofluor staining clearly exhibited fluorescence when examined under UV light and Rmd 1102(A_2) did not exhibit any fluorescence under UV light.

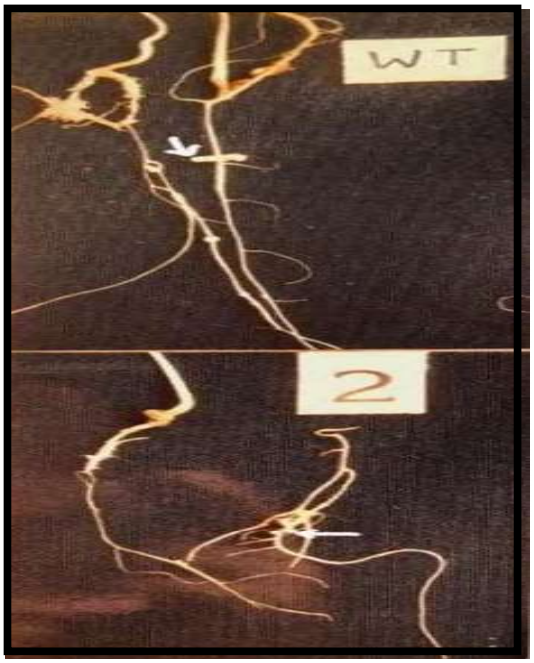

Fig. 2. Label WT depicts morphology of indeterminate nodules elicited by WT strain *Rmd*201. Label 2 depicts small and determinate nodules elicited by *Rmd* 1102 mutant, which is $Pur^-$and Exo^-.

***HPLC analysis of sugars of rhizobial exopolysaccharide*:** Since Rmd 1102(A_2) showed Cal^- phenotype, this indicated that it produced a defective exopolysaccharide as compared to wild type Rmd 201(WT)Cal^+. On analyzing free sugars in the supernatant only could be one peak, could be observed corresponding to fructose in both Rmd 1102(A_2) and Rmd 201(WT) (Fig.3).

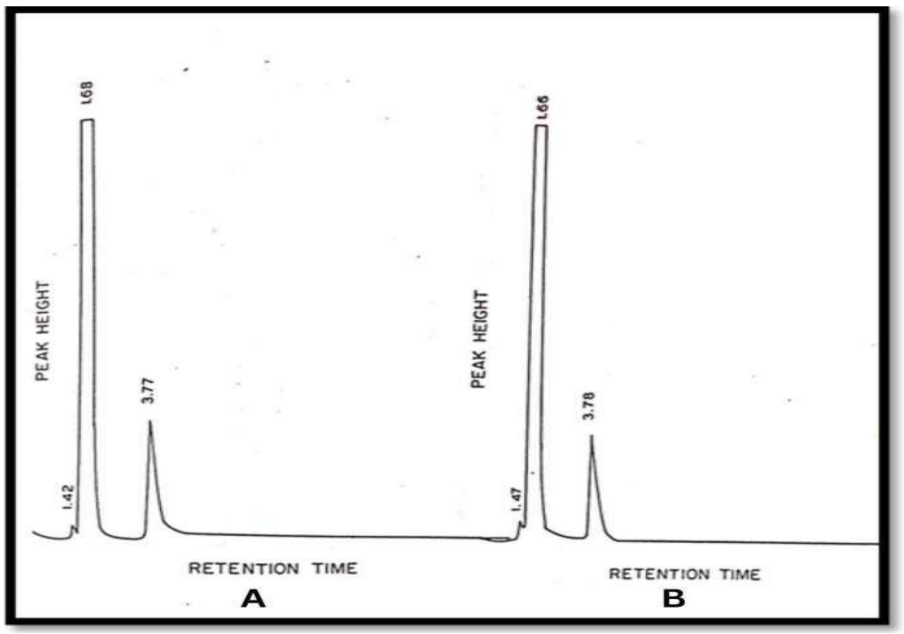

Fig.3. HPLC Analysis of free sugars in the supernatant fraction of both (A) Mutant *Rmd*1102and(B) WT strain*Rmd*201.

On analysis of tightly bound sugars after acid hydrolysis, two peaks were observed in Rmd 201(WT). One was at retention time 3.11minutes corresponding to xylose and another peak at retention time 6.25 minutes corresponding to sucrose. However, in Rmd 1102(A_2) which is Exo^- and Pur^-, the xylose peak was absent and peak corresponding to sucrose was slightly modified indicating some minor change in sucrose (Fig. 4).

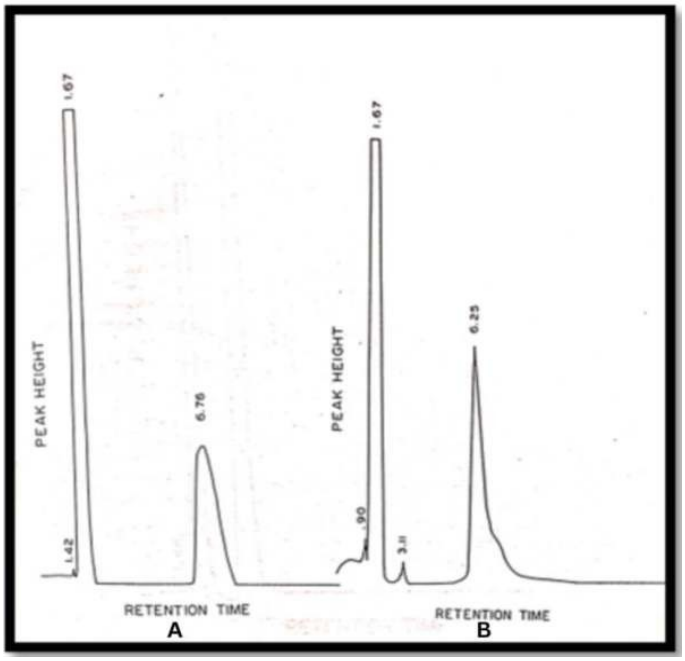

Fig. 4. HPLC Analysis of tightly bound sugars in pellet fraction of both (A) Mutant *Rmd*1102 and (B) WT Strain *Rmd*201.

From the difference in composition of sugars of Rmd 201(WT) and Rmd 1102(A_2), it is clear that the mutation has affected the synthesis of exopolysaccharide as well as it is causing purine mutation.

***Media and sugar supplementation experiments with strain Rmd 1102(A_2)*:**

The mutant strain Rmd 1102(A_2) was examined for growth and calcofluor binding ability of its exopolysaccharide on minimal medium containing different carbon sources. Growth on xylose containing medium was distinguishable in terms of exopolysaccharide quality. On streaking Rmd 1102(A_2) along with wild type Rmd 201(WT) on minimal media containing xylose as sole carbon source, phenotypic suppression of calcofluor dark phenotype occured as evident from (Fig. 5B). However, calcofluor positive (fluorescent) colonies appeared only in the region of Rmd 1102(A_2) streak close to Rmd 201(WT), while the major part of the growth away from Rmd 201(WT) was still

dark and showed no calcofluor binding. However, on minimal medium containing glucose as sole carbon source, wild type Rmd 201(WT) did not suppress calcofluor dark phenotype of Rmd 1102(A_2) (Fig. 5A). This probably indicates a qualitative change in the exopolysaccharide of Rmd 1102(A_2) on xylose containing medium due to some diffusible substance(s) secreted by wild type strain Rmd 201(WT) reverting the Exo^- mutation. On comparing HPLC analysis data of the wild type and mutant strain, these results appear to be highly significant and coherent.

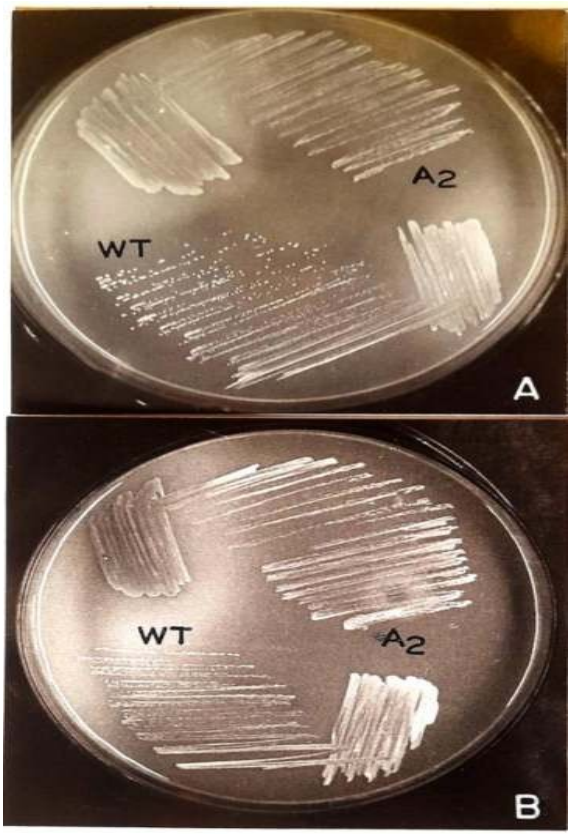

Fig. 5: Effect of media and sugars on the growth of *Rmd* 201 (WT) and *Rmd* 1102 (A_2).

Label A depicts Cal^-Phenotype of *Rmd*1102 is not suppressed in the presence of *Rmd*201when streaked on RMM + adenine + glucose.

Label B depicts Cal^- Phenotype of *Rmd* 1102 is suppressed in the presence of *Rmd*201 when grown on RMM +adenine+xylose.

DISCUSSION

Present study involves a mutant strain, Rmd 1102(A_2), and its characteristics in relation to exopolysaccharide production compared to wild type strain Rmd 201(WT). Rmd 1102(A_2) is both a purine mutant and exhibits Exo^- phenotype[21]. This phenotype is characterized by its inability to bind

to calcofluor dye and unable to fluorescence under UV light. In contrast, the wild type strain Rmd 201(WT)effectively binds to calcofluor dye and fluoresces under UV light, indicating the production of normal exopolysaccharide[24]. Rhizobia are known to produce various types of exopolysaccharides[25], which are crucial for initiating infection thread formation[5]and suppressing host plant defense responses. Specifically, the exopolysaccharide EPS I, which can bind calcofluor stain, does so by attaching to a succinoglucan residue[26]. It is likely that the mutant strain Rmd 1102(A_2) lacks this succinoglucan residue in its exopolysaccharide, distinguishing it from the wild type parental strain.

The composition of the exopolysaccharide in wild type strain Rmd 201(WT) was analysed through sulfuric acid hydrolysis, with subsequent HPLC analysis of the hydrolyzed exopolysaccharides and free sugars in the supernatant. Interestingly, there was no change in the content of free sugars between the wild type strain Rmd 201(WT) and the purine mutant Rmd 1102(A_2), which exhibited the Exo^- phenotype. However, differences appeared in the composition of tightly bound polysaccharides. In Rmd 201(WT), the polymer was composed of xylose and sucrose in a molar ratio of 1:92, whereas in the mutant Rmd 1102(A2), the xylose peak was absent, and the sucrose peak seemed to have been modified. The exact nature of this modification could not be detected through HPLC analysis.

The exopolysaccharide structures in*S. meliloti*, specifically EPS Iand EPS II, have different compositions of glucose and galactose[27]. Also in current investigation it appears that,there is significant difference in the exopolysaccharide composition ascompared to previous findings. The estimates of exopolysaccharide composition represent the overall sugar content, considering other carbohydrates present on bacterial cell surface of strain Rmd 201. There is similar mention of exopolysaccharide analysis in blue-green algae *Anabaena*, which had a different composition[28]. There is a need for a detailed understanding of the chemical structure of the exopolysaccharide under investigation before definitive comments can be made about its structure, particularly regarding the defect observed in the mutant strain Rmd 1102(A_2).

Many findings have emphasized differences in the composition of exopolysaccharides in *S. meliloti*[29]and the importance of further research to establish the detailed chemical structure of the exopolysaccharides. This also holds true for exopolysaccharide of mutant in question.

The role of xylose is extremely important as observed in the mutant strain Rmd 1102(A_2) in *S. meliloti*. It is evident from cross-feeding experiments where the mutant's ability to fluoresce was restored only when exposed to xylose in presence of parental strain. The proposed hypothesis

suggests that diffusible product(s) from the parent strain, in the presence of xylose, restore the normal exopolysaccharide structure, allowing the mutant to bind to calcofluor dye and fluoresce under UV light. Calcofluor dye binds to the succinate moiety of EPS Iand the hypothesis proposes that the exudate and other compounds provide this succinyl moiety in the presence of xylose. There is a possibility that the block in Rmd 1102(A_2) occurs before the synthesis of the succinyl moiety, similar to the mutant exoB of EPS I[27].Unique surface polysaccharide has been detected in several species of *Sinorhizobium* and this unique exopolysaccharide is different from EPS I and EPSII. This polysaccharide also contains xylose sugar. We have probably isolated similar exopolysaccharide and presence xylose sugar in medium as well as exudate from the parental strain may be helping in restoring normal exopolysaccharide synthesis, with xylose possibly serving as an essential substrate in the mutant strain Rmd 1102(A_2). The exudate from the parental strain may be diffusing to some extent in the medium therefore the mutant streak closest to streak of parental strain converted from Cal^- to Cal^+. This may be because the exudates secreted into environment by the parental strain exhibited limited diffusion in nearby areas on the medium. Since xylose was essential requirement of mutant strain it was utilized by the mutant and mutant produced normal exopolysaccharide reverting the Exo^- mutation in the mutant but only in close proximity to streak of parental strain.

The role of xylose and potential mechanisms for restoring exopolysaccharide structure in a mutant strain of *S. meliloti*, along with the significance of studying mutants in understanding the symbiotic relationship with plants and nodulation further needs to be explored. In addition,this single gene mutation in the mutant has pleiotropic effects[29,30,31,32].

There also seems to be an important connection between the purine pathway and exopolysaccharide synthesis in *Sinorhizobium meliloti*[9,22,33]. Impairment in the synthesis of nucleotide sugar diphosphate intermediates of purine biosynthetic pathway is also leading to defective exopolysaccharide production. The purine mutation in Rmd 1102(A_2) disrupts a critical step in the purine pathway, potentially affecting the production of necessary intermediates for exopolysaccharide synthesis. Different exopolysaccharides in *S. meliloti* are known to compensate each other, suggesting their functional redundancy[29]. The mutation in Rmd1102(A_2) may involve a distinct exopolysaccharide as it cannot be complemented by plasmid constructs carrying genes for EPS I synthesis. This implies differences from the known EPS I chemical structure. Further studies, including the creation of a double mutant with exoB[34,35], are required to confirm this distinction.

S. meliloti appears to produce multiple exopolysaccharides, possibly due to its co-evolution with various legume hosts, indicating the presence of more than one exopolysaccharide in this

bacterium. Further research is required to understand clearly the structure and function of various exopolysaccharides of produced by this bacterium.

Exopolysaccharides are essential for the formation of nodules on legumes, and they serve multiple roles in the symbiotic interaction between rhizobium and legume plants[31,36]. These roles include, aiding invasion, nodule development, bacterial release from infection threads, bacteroid development, suppressing host plant defense responses, and protecting against antimicrobial compounds[37].

Genes known as exo/exs, exp, or pss clusters control the synthesis, modification, polymerization, and export of exopolysaccharides[29]. Exopolysaccharide deficiency has been extensively researched regarding its impact on nodule development. Environmental factors like phosphate, nitrogen, and sulfur influence exopolysaccharide production, and the production of various symbiotically active polysaccharides helps rhizobial strains to adapt to changing conditions and interact effectively with legumes.

Succinoglycan (EPS I) is a well-known rhizobial exopolysaccharide found in some *S. meliloti* strains, consisting of octasaccharide repeating units containing one galactose and seven glucose residues. It is decorated with acetyl, pyruvyl, and succinyl groups. *S. meliloti* can also produce another exopolysaccharide called galactoglucan (EPS II), which differs in structure and is synthesized under phosphate starvation or due to specific regulatory gene mutations. EPS II consists of a disaccharide repeating unit composed of acetylated glucose and pyruvylated galactose[29].

In summary, exopolysaccharides are vital for legume nodule formation and are greatly impacted by environmental factors. Different exopolysaccharides, such as succinoglycan (EPS I) and galactoglucan (EPS II), play important roles in the interaction between rhizobia and legumes. Additionally, *S. meliloti* can produce capsular polysaccharides like the K antigen when exopolysaccharides are absent, contributing to symbiotic interactions with legumes.

CONCLUSION

The research on exopolysaccharides in *S. meliloti* and the mutant strain Rmd 1102(A_2) highlights critical differences in exopolysaccharide composition, suggesting the importance of xylose and potential restoration mechanisms. The proposed hypothesis suggests that diffusible product(s) from the parent strain, in the presence of xylose, may contribute to this restoration of Cal^+phenotype. Further research, including cross-feeding studies with various mutant strains, is essential to validate this hypothesis and obtain a detailed understanding of exopolysaccharide structure in question.Other researchers have reported unique exopolysaccharide containing xylose in same strain of *S. meliloti*

further validates the importance of this study in accordance with results obtained by other workers. In conclusion, the study underscores the importance of exploring the role of xylose and potential mechanisms for restoring exopolysaccharide structure in *Sinorhizobium meliloti* mutant. These findings underscore the need for furtherinvestigations into mutants, especially pleiotropic mutants, and the interplay between the purine pathway and exopolysaccharide synthesis. Moreover, exopolysaccharides are vital for legume nodule formation and the symbiotic relationship with legumes, with several distinct exopolysaccharides contributing to these interactions.

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ABBREVIATIONS

EPS: Exopolysaccharides

WT: Wild Type

Exo-: Exopolysaccharide-deficient

Pur-: Purine-deficient

NTG:N-methyl-N'-nitro-N-nitrosoguanidine

HPLC: High Performance Liquid Chromatography

Nod: Nodulation genes rhizobia

LPS: Lipopolysaccharides

CP: Capsule Polysaccharide

GPS: Gel-Forming Polysaccharide

KPS:K-Antigen Polysaccharide

ß-(1,2) glucans: Beta-(1,2) glucans

NP: Neutral Polysaccharide

NodD: Nodulation genes D

NodDI: Nodulation genes DI

NodD2: Nodulation genes D2

NodD3: Nodulation genes D3

SyrM: SyrM regulator

EPS I: Exopolysaccharide I

EPS II: Exopolysaccharide II

pSymB: Plasmid SymB

wgx:EPS II gene cluster

exoC: Exopolysaccharide C

S. meliloti*:Sinorhizobium meliloti*

AICAR: Aminoimidazole carboxamide ribonucleotide

IMP: Inosine monophosphate

FAICAR:5-formamido-4-imidazole carboxamide ribonucleotide

RMM: Rhizobium minimal medium

MSY: Mannitol soya medium

TCA: Trichloroacetic acid

UV: Ultraviolet

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