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# *Sinorhizobium meliloti* BACTERIUM AS A PERSPECTIVE OBJECT FOR BIOTECHNOLOGY

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*Sinorhizobium meliloti* is a Gram-negative soil nitrogen-fixing bacterium that increases the yield of legumes. There is information in the literature about the complete genome sequence of this bacterium. In addition, the polysaccharide composition of the biofilm, which is actively involved in nitrogen fixation, has been studied. The well-known nucleotide sequence, as well as the genetic and biochemical features of *S. meliloti* bacterium makes this organism an ideal model for biotechnological research. The purpose of this work was to analyze the current data provided in the literature on the symbiotic interaction of *Sinorhizobium meliloti* bacterium with the host plant, and to characterize the main directions of the use of this bacterium in agriculture, bioremediation and medicine.

***Key words:*** *Sinorhizobium meliloti* bacterium, symbiosis, biotechnology.

More than half of all nitrogen required for successful farming is currently provided by nitrogenous chemical fertilizers [1]. However, these fertilizers are expensive both economically and environmentally [2]. The application of synthetic N fertilizers has greatly enhanced crop production but also has caused serious environmental problems, such as groundwater contamination and surface water eutrophication [3]. On the other hand, symbiotic nitrogen-fixing microorganisms are a sustainable nitrogen source for agriculture.

Nodule bacteria belong to microaerophilic microorganisms that can develop at a low partial pressure of oxygen in the environment. One of the most studied nitrogen-fixing bacteria, *Sinorhizobium meliloti*, has distinguished features of metabolism and structure. The complete genome of *S. meliloti* was sequenced and annotated in 2001. All strains analyzed so far contain three replicons: one chromosome and two inherently stable megaplasmids [4]. Plasmid pSymA is considered as a symbiotic accessory megaplasmid, as it can be cured without affecting *S. meliloti* viability. It contains genes for nodule formation and nitrogen fixation. Most sequences located on pSymA are transcribed only at the bacteroid stage. It was shown that pSymA had a role in the regulation of the expression of genes from the other replicons (3.5 Mbp chromosome and the 1.7 Mbp pSymB plasmid) presented in the *S. meliloti* cells [5]. Plasmid pSymB contains both plasmid and chromosomal features and is designed as a second chromosome. Genes involved in polysaccharide biosynthesis were identified in this megaplasmid. It was shown that 14% of the pSymB sequence is dedicated to polysaccharide synthesis [6]. Other recognizable gene clusters include many involved in catabolic activities. *S. meliloti* genome architecture was shown to be highly dynamic, as the three replicons continuously cointegrate and excise. A detailed study of the bacterial genotype allowed scientists to identify the genes responsible for the key stages of symbiosis. Thus, it was possible to purposefully influence the genetic material in order to obtain viable symbionts that showed resistance to adverse environmental conditions [7].

On the other hand, *S. meliloti* has found application in biotechnology as a source of polysaccharides, which due to their ability to change the rheological properties of water

systems, can be considered as emulsifiers, suspending and gel-forming agents [8–9].

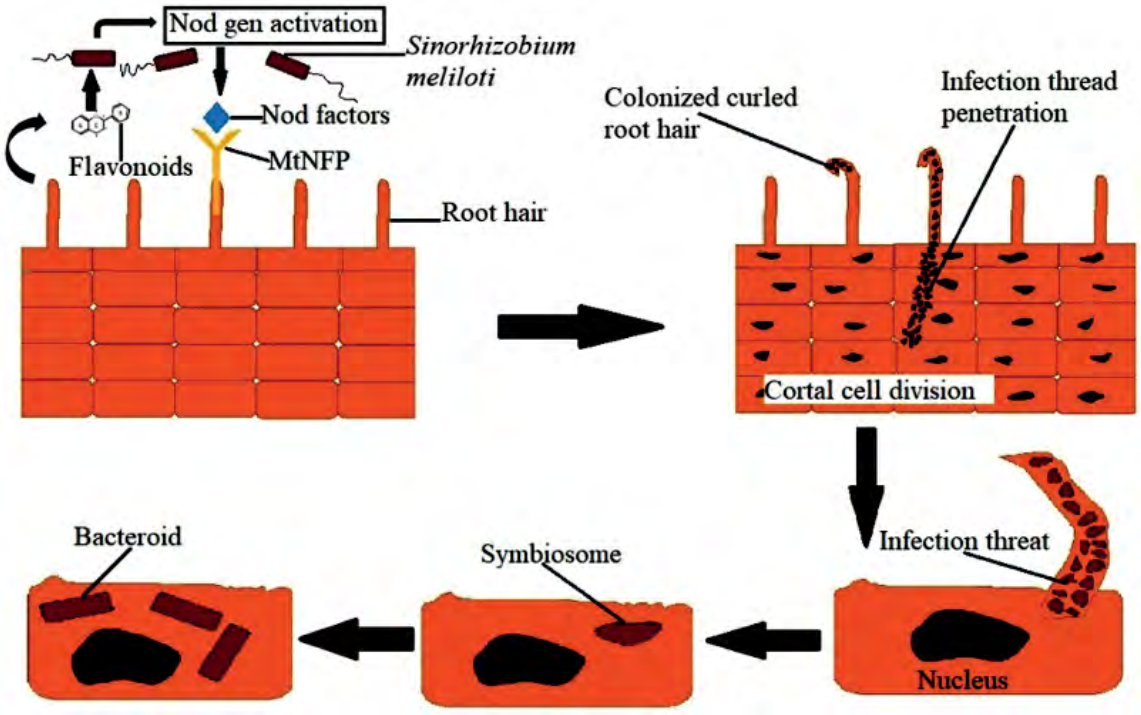
This paper describes the main steps of symbiosis of *Sinorhizobium meliloti*, taking into account the data obtained over the last decade, and concludes with application for this microorganism in biotechnology.

*The mechanism of symbiosis*

The molecular reactions underlying symbiotic relationships are well described in the modern literature, however, taking into account the latest data, the following stages can be distinguished (see Figure).

It is known that the roots of leguminous plants secrete a diverse cocktail of flavonoids and isoflavonoids into the soil. Flavonoids in the exudates of legume roots act as chemotactic signals for rhizobia in conditions of low nitrogen content [10]. It is difficult to determine, which flavonoid in the rhizosphere is perceived by a compatible bacterium, since plants, as a rule, secrete a complex mixture. It is likely that the determining factor for host specificity is the spectrum of flavonoids secreted by this legume plant. Some rhizobia are chemotactic with respect to compatible flavonoids. Hence, it is assumed that aspects of host specificity are established before the bacterium and its host physically interact [11]. Flavonoids of plant origin cause a transcriptional response in bacteria, in particular, they lead to the activation of genes that are responsible for the synthesis of nod factors (NF), the expression of these genes is suppressed in the presence of ammonium [12].

Among the rhizobial genes induced by activated flavonoids, there are mainly genes encoding enzymes necessary for the production of lipochytooligosaccharide Nod factors [13]. Nod factor is a complex signaling molecule that can be represented as a chitinous backbone modified on a non-reduced terminal residue at the C2 position by a fatty acid; however, the size and saturation state of this lipid chain vary depending on the species [14]. The NF molecule can be further modified by various chemical substituents. A certain type of rhizobia has its own mixture of node factor compounds, approximately from 2 to 60 molecules, this is especially characteristic of bacteria with a wide range of hosts [15]. *Nod* genes are divided into common and specific. The common nod genes (including *nod*A, B and C), which are found in almost all rhizobia species, are responsible for the synthesis of the NF chitin framework. On the contrary, host-specific nod genes ensure the formation of nodules in a particular host and are involved in various modifications of the chitin framework. However, the presence of the rhizobial bacterium itself is not a prerequisite. If a




**Stages of development of legume-rhizobium symbiosis**

cluster of biosynthetic genes of node factors is transferred into the Gram-negative bacterium *Escherichia coli*, then this bacterium gets the ability to cause several early reactions of the host plant to these factors, in particular a spike in the concentration of calcium ions [16]. The movement of the bacterium towards the host plant is due to the presence of chemoreceptors. The periplasmic region of transmembrane chemoreceptors of *S. meliloti* acts as a sensory input module for chemotaxis systems directly or indirectly through the binding of specific ligands. The methyl-accepting chemotaxis protein has a periplasmic sensory domain for binding the chemoeffector ligand, as well as a cytoplasmic signaling domain, which is reversibly methylated by glutamic acid residues. *S. meliloti* demonstrated positive chemotaxis against seven carboxylates found in the host alfalfa seed exudates, namely — α-ketobutyrate, citrate, glyoxylate, malate, malonate, oxalate and succinate [17]. The interaction of the receptor with the ligand leads to a rapid response at the level of the flagellar motor. Recently some interesting results, which contradict the previous investigations concerning the role of flavonoids, have been obtained. Compton K. et al. analysed exudate from germinating alfalfa seedlings for composition and quantities of different flavonoid compounds using mass spectrometry. They found four prevalent flavonoids in germinating alfalfa seed exudates (hyperoside, luteolin, luteolin-7-glucoside, and chrysoeriol). Using quantitative chemotaxis capillary assays, they did not detect chemotaxis of motile *S. meliloti* 1021 cells to these, and two other flavonoids identified in seed exudates. In support of these findings, the flavonoid fraction of seed exudates was found to be an insignificant attractant relative to the more hydrophilic fraction. Authors have proposed that the role flavonoids play in *S. meliloti* chemotaxis is insignificant relative to other components released by alfalfa seeds [11].

*The plant's response to the action of node factors*

Initial reactions of the root epidermis include cytosol alkalinization and plasma membrane depolarization within a few minutes after root inoculation. Purified NF can also cause root hair deformation and root hair twisting within a few hours after application. The deformation of the root hair probably depends on calcium-induced changes in the organization of the actin cytoskeleton, which lead to a reorientation of cell growth. These reactions are followed by twisting of the root hairs, which detains rhizobial bacteria in the tightly colonized twisted root hair [18]. At the same time, the NF stimulate the cells of the root cortex to resume mitosis, resulting in the formation of cells that will form the primary nodule and ensure the invasion of bacteria [19]. For a complete plant response to NF, several receptors containing an extracellular domain are required. One of these MtNFP receptors is a member of the LysM receptor family and is required for root hair twisting and induction of transcriptional changes. Some experiments have demonstrated the additional role of MtNFP at a later stage of bacterial penetration into the root hair, namely, the formation of an infectious thread. It is known that another receptor of the LysM family is necessary for twisting the root hair and fine-tuning plant reactions of plants to the NF during the formation of an infectious thread [20]. However, the interaction of NF with their receptors is not the only event at this stage. It has been shown that NF stimulate the formation of reactive oxygen species. Reactive oxygen species have a regulating effect on the process of symbiosis between legumes and nitrogen-fixing bacteria. Rapid and temporary production of these forms has been reported after treatment of the root hairs of leguminous plants with NF [21]. In addition to NF, bacterial surface exopolysaccharides (EPS) and their receptors play an important role in the recognition of the symbiont bacterium by the plant. Thus, the exopolysaccharide receptor LJEPR3 contains a LysM domain, which is not required for an early response to the NF, but is necessary for the formation of a microcolony. The LJEPR3 receptor is differentially regulated in the epidermis and primary nodules. However, in the question of the combined action of EPS and NF, much remains unclear [22]. It has been suggested that exopolysaccharides produced by rhizobial species are actively involved in suppressing the protective reactions of the host plant [23].

*Infection tread development*

Bacteria trapped in a twisted root hair and capable of producing NF and a symbiotically active exopolysaccharide induce ingrowth of the root hair cell membrane, which leads to bacterial invasion into the internal plant tissue. Effective invasion occurs even if the NF and the exopolysaccharide are supplied separately by *S. meliloti* strains, which are jointly captured in the same twisted hair [24].

*S. meliloti* produces the exopolysaccharides succinoglycan and galactoglucan, which contribute to the formation of infection filaments. As soon as this thread penetrates into the base of the root hair cell, the bacteria must induce new cycles of formation of the infection thread in each subsequent cell layer. Several hormon signaling pathways intersect with NF signals to control nodule formation [25]. Organogenesis of nodules is controlled by hormones. Concentrations and ratios of auxins and cytokinins determine where and when cells divide. Cytokinins regulate cell division, leading to the formation of a primary nodule, and their signals are mainly perceived by the MTCRE1/LJLHK1 cytokinin receptor [26]. Direct targets of cytokinin signaling include MTNSP2 and the main gene MtbHLH476 TF, both encoding positive regulators of nodule organogenesis. Genes for the biosynthesis and homeostasis of cytokinins are necessary for the normal development of nodules. Cytokinin biosynthesis is mediated by isopentenyltransferase and cytokinin homeostasis during nodule development is supported by cytokinin oxidase/ dehydrogenase [27]. Plant cytokinin and Nod-dependent cell cycle reinitiation are involved in the spread of infection into the root hairs of the plant. Depletion of the cytokinin receptor of *M. truncatula* leads to blocking of the re-initiation of cell division and interruption of infection filaments [28]. The earliest infection threads penetrating the growing nodule of *M. truncatula* or *M. sativa* should germinate past actively dividing cells in the developing primary nodule. As a result, stable nodule meristem is formed.

*Symbiosome and bacteroid formation*

Each bacterial cell is endocytosed by a target cell. A system consisting of a single bacterium and the surrounding endocytic membrane is known as a symbiosome. In nodules, the bacterial cell and the surrounding membrane divide synchronously before the bacteria differentiate into nitrogen-fixing bacterioids, which can fix atmospheric nitrogen into ammonia, establishing an intricate metabolic interchange with the host plant [29].

Biochemical markers of the symbiosome membrane were determined. These include identified nodule-specific proteins, energy and transport proteins, bacterial proteins and proteins that will participate in the docking of vesicles on the target membrane. According to the data concerning the structure of symbiosome membrane proteins, protein syntaxin plays an important role in this process. Syntaxin may be crucial for the transformation of a vesicle containing rhizobium into a mature symbiosome [30]. Once the bacteria are absorbed by the host cell membranes, they must survive in the symbiosomal compartment and differentiate into a nitrogen-fixing bacterioid. Both bacterial and plant factors are involved in these processes. One of the important defense mechanisms that Gram-negative bacteria use to resist the extracellular environment is lipopolysaccharide (LPS). It has been shown that *S. meliloti* produces lipid A of the appropriate structure that will ensure its survival in host cells [31].

*Symbiosis control*

The host plant controls the survival of bacteria in the symbiosome and must not only provide nutrition and the right microaerobic environment necessary for nitrogen fixation, but also provide an opportunity for bacterial differentiation. In unformed nodules, the captured bacteria and the symbiosome membrane divide simultaneously before the formation of bacteroids. The bacteroids in the nodules increase the DNA content and cell size, which may allow them to achieve a higher metabolic rate necessary for nitrogen fixation. The intensive DNA synthesis required for endoreduplication in bacterioids requires a large number of dideoxynucleotides, which must be supplied by ribonucleotide reductase. For many bacterial species, DNA synthesis in an oxygen-rich environment (an infection thread) and in an oxygen-depleted environment (a symbiosome) would pose a significant problem [32]. However, some bacterial species, including rhizobia, have adaptations that solve this problem: they have a B12-dependent ribonucleotide reductase that functions independently of oxygen concentration [33]. Bacteria that are enclosed in symbiosomes are provided with a low-oxygen environment and complete the bacteroid differentiation program. They can express the enzymes of the nitrogenase complex and begin to fix nitrogen. An oxygen-sensitive bacterial regulatory cascade controls the expression of the nitrogenase complex and microaerobic respiratory enzymes, which are necessary to provide energy and a nitrogenase reducing agent. This cascade is induced by a low oxygen content in the differentiating bacteroid. Bacterial regulators include the oxygen-sensitive two-component regulatory system FIXL and FIXJ, NIFA, σ54 and FixK98. These regulators are responsible for

many changes in the expression of genes and proteins were found during the differentiation of bacteroids [33].

As an example of the mutual adaptation of legumes and bacteria, the reaction of legumes to chitin can be considered. Chitin is a pathogen that induces innate immune responses of plants, such as an oxidative explosion. This reaction helps plants to protect themselves from fungal attack, since chitin is a component of the cell walls of fungi [34] Interestingly, the chitin receptor of rice (*Oryza sativa*) is a protein containing the LysM domain, as are Nod factor receptors such as MTNFP and MtLYK3131. Perhaps in the same way legumes recognize bacterially produced lipochitooligosaccharide NF [35]. At the same time, there are protective reactions of plants that limit the number of nodules on the colonized root. After the initial round of nodule formation has begun, subsequent nodule formation events undergo auto-inhibition. Signaling is mediated by plant hormones ethylene and jasmonic acid, which are involved in protective reactions during other interactions of plants and microorganisms. There is an assumption that auto-inhibition is controlled by the plant by interrupting infection threads [36].

*Defensive reactions of the plant*

In parallel with the choice of a partner dependent on the NF, the immune system of plants helps to exclude other soil microorganisms from the roots of legumes [37]. The host legume plant uses multiple control points throughout this process to differentiate between symbionts and pathogens. Protective receptor kinase complexes, including LRR-RLKS and LysM-RLKS, recognize microbial molecules on the surface of plant cells, while microbial "effectors" injected into cells to remove plant protection are recognized and neutralized by proteins NBS-LRR ((nucleotide binding site leucine-rich repeat) R (Resistance) proteins) R [38]. It has been shown that the compatibility of bacteria and plants is regulated by peptides with a high content of cysteine named NCR (nodule-specific cysteine-rich), which are produced in the nodules of the plant. The role of these peptides as effectors of differentiation of endosymbionts into nitrogen-fixing bacteroids was previously shown. NCR peptides were detected as a result of transcriptomic analysis of *M. truncatula.* In non-lethal doses, NCR peptides have several effects on rhizobial cells: they promote genome endoreduplication and stimulate cell branching, promote efficient nitrogen uptake, nutrient exchange and inhibition of rhizobia proliferation [39]. It has recently been shown that a genetic disorder of specific genes encoding NCR peptides leads to a violation of the ability of the bacteroid to fix nitrogen [40]. The effect of host-derived NCR peptides on nodule-associated bacteria has led to the creation of a model, in which the corresponding bacterial peptidases can modulate this effect [41].

It was later discovered that NCR peptides cause bacterial cell death and early nodule aging in the case of incompatible microsymbionts [42]. The reaction of host plants to lipopolysaccharide (LPS) of bacteria deserves special attention. In most plant species, the reaction to bacterial LPS differs from the toxic shock that can be caused in animals. The lipid component A of *S. meliloti* can suppress both the oxidative spike and the expression of protective genes in cultured host cells of *M. truncatula* and *M. sativa* [43]. A promising area of research is to determine, which LPS epitopes from rhizobial bacteria and plant pathogens cause or suppress protective reactions in plants of various lines.

*Bacterial exopolysaccharides and their role in symbiosis*

Speaking about the symbiotic relationship of *S. meliloti* with plants, special emphasis should be placed on the elements of exopolysaccharide nature, their regulation of their biosynthesis. It is known that *S. meliloti* secretes two acidic exopolysaccharides (EPSs), succinoglycan (EPSI) and galactoglucan (EPSII), which differentially enable it to adapt to a changing environment. Succinoglycan is essential for invasion of plant hosts and, thus, for the formation of nitrogen-fixing root nodules. Galactoglucan is critical for population-based behavior such as swarming and biofilm formation and can facilitate invasion in the absence of succinoglycan on some host plants. The biosynthesis of galactoglucan is not as completely understood as that of succinoglycan. NMR analysis of EPS isolated from a mutant of the WGAE gene revealed a new pyruvyltransferase that modifies galactoglucan. [44].

It was suggested that succinoglycan plays an important role in the survival of *S. meliloti* at low pH levels. When *S. meliloti* Rm 1021 was grown at pH 5.75, synthesis of succinoglycan increased, whereas synthesis of galactoglucan decreased. Succinoglycan that was isolated from cultures grown at low pH had a lower degree of polymerization relative to that, which was isolated from cultures grown at

neutral pH, suggesting that low–molecular weight (LMW) succinoglycan might play a role in adaptation to low pH. The data suggest that the role for LMW succinoglycan in nodule development may be to enhance survival in the colonized curled root hair [45].

The SYRM and PHOB proteins are positive regulators of EPS I and EPS II production, respectively [46]. Among the identified regulators, MUCR appears to be a global regulatory protein that plays a key role in both the positive regulation of EPS I synthesis and the negative regulation of EPS II synthesis, thus linking these two biosynthetic pathways [46].

The production of *S. meliloti* EPS is affected by several nutritional and stress conditions. Limitations of some non-carbon nutrients, such as nitrogen and sulfur, very high concentrations of phosphates and hyperosmotic stress stimulate the synthesis of EPS I [47].

On the other hand, phosphate starvation stimulates the production of EPS II, indicating that the concentration of this nutrient is an important signal affecting, which type of EPS will be produced by *S. meliloti*. In addition, different osmotic conditions alter the biosynthesis of EPS in this bacterium. Low osmotic pressure leads to the formation of mainly low-molecular EPS I, while the production of the high-molecular fraction of this polymer is stimulated by increased osmotic pressure. Jofre and Becker reported that the polymerization of EPS I is influenced by the ionic strength of the medium, and not by osmolarity [48].

Although there are quite a lot of studies that consider the role of EPS in symbiosis, it is still far from a complete understanding of the functions of EPS in the formation and functioning of symbiotic systems. It has been shown that the quality and quantity of polysaccharides, especially EPS and lipopolysaccharides produced by rhizobia, can affect both their agglutination of these bacteria and the formation of biofilms on the surface of plant roots, which plays a crucial role in the initial stages of symbiosis [49,50].

Mutants that do not produce EPS could cause twisting of root hairs, but do not form infectious filaments and nodules. EPS has also been shown to be involved in various stages of the development of the infection thread, bacteroids and suppression of the immune response of the host plant [51, 52]. There are several papers that have been devoted to the specificity of bean-rhizobial symbiosis due to the structure of EPS [22, 53]. It has been suggested that the amount of EPS produced by rhizobia is associated with the optimization of the interaction of the microsymbiont with the macrosymbiont [54]. One of the latest achievements in this field, using optical control of the expression of EPS II biosynthesis genes, demonstrated spatial control of structured biofilm formation [55]. Thus, EPS is one of the key factors for achieving successful interaction between symbiotic partners [56].

However, there are still no effective ways to modify the synthesis of EPS in rhizobial cells for the purpose of further application of transformed strains. Most articles describe mutant strains, in which the expression of certain genes involved in EPS biosynthesis is blocked. Often, such strains lose their competitiveness and cannot enter into a full-fledged symbiotic relationship [57].

*Application of Sinorhizobium meliloti in biotechnology*

There are several directions for the use of nitrogen-fixing bacteria, in particular, *S. meliloti*, in biotechnology. The first direction is the creation of strains that provide the plant with a sufficient amount of nitrogen and the enrichment of the soil with nitrogen. However, as noted by a number of authors, in the practical use of the results the scientists face some problems. Despite the advantages of microbial inoculant technology, there are some success-limiting factors against a universal utilization [58]. In fact, the efficiency of microbe-based biofertilizers depends on many factors including the targeted crop, edaphic (pH, salinity, and soil type), biotic (competition between introduced and indigenous strains, microbial parasites and predators), and climatic factors [59, 60]. Competition among microbial strains for resources and plant nodulation, partner fidelity and specificity mediated by genetic and molecular mechanisms are among the success-limiting factors against a universal utilization of microbial inoculants [61, 62]. On the other hand, commercial inoculants were often made with one or at most two strains, while under field conditions, plants are associated with many strains, which provide them diverse benefits through functional complementarity. Nevertheless, the poor performance of biofertilizers is primarily linked to inappropriate strains and inefficient production technology. Herrmann et al., studying the microbial quality of 65 commercial inoculants manufactured in seven different countries, showed that only 36% of the products could be considered as “pure”.

Among the remaining 64% some contained one or several strains of contaminants and some products did not contain any strains [63].

Particular attention is paid to the use of symbiotic bacteria in various climatic zones and under the influence of stress factors, such as soil salinity. The effectiveness of symbiotic interaction under salinization conditions depends on the effectiveness of isolates under standard conditions, on the number of nodules formed by rhizobia on the roots of the host plant, but did not correlate with the source of rhizobia release (soil, nodule) and their salt resistance [64]. The data obtained indicate the possibility of identifying strains of nodule bacteria that provide high efficiency of symbiosis in conditions of salinity of the soil.

However, the use of nodule bacteria is not limited to the earth's soil. Nodule plants and their N-fixing symbionts may play a role in increasing the fertility of the Martian soil. This approach is due to an increase in population growth and a reduction in arable land on Earth. It is known that on Earth clover (*Melilotus officinalis*) forms a symbiotic relationship with the N-fixing bacteria *S. meliloti*. It was assumed that an increase in plant biomass would be observed in the Martian regolith inoculated with the corresponding N-fixing bacteria, and the excess nitrogen available to plants would be deposited in the surrounding regolith, as is the case on Earth. Experiments have shown that the growth of shoots and roots of plants increased by more than 75% with inoculation of *S. meliloti* 1021compared with plants grown in non-inoculated regolith. This study highlights the importance of nitrogen as the main limiting factor for plant growth in regolith, suggesting that nitrogen-fixing bacteria can be used to reduce this restriction. The authors of the study suggest that their experience can become the basis for future research on food production in the conditions of the Martian soil [65].

The second most significant and developed direction of biotechnological use of *S. meliloti* is bioremediation. It means the elimination, neutralization or conversion into a less toxic form of eco-pollutants using biological processes. This method is often used in cases of soil contamination with heavy metals, using microorganisms and plants to restore the biological productivity of the ecosystem. Soil contamination by heavy metals has become a serious worldwide environmental problem. Nitrogen-fixing rhizobia with high intrinsic metal resistance have been investigated widely for their potential to improve plant growth, reduce metal toxicity, and change metal availability in soil, which may help in the development of microbe-assisted phytoremediation [66]. In recent years, more attention has been paid to the potential use of legume-rhizobium symbiosis for bioremediation of contaminated soil and to the responsible biochemical and molecular mechanisms [67]. *S. meliloti* CCNWSX0020 displayed tolerance to high levels of multiple metals, such as Cu, Zn, Cd, and Pb. Moreover, it could promote the growth, metal uptake, and antioxidant responses of *M. lupulina* in copper-contaminated soil [68, 66]. Extracellular polymeric substances were found to immobilize Cu ions and were predicted to play a role as a first protective barrier to prevent copper from reaching the cytoplasm [69]. Several genes conferring copper resistance were identified and a putative copper-transporting P1B-type ATPase and a zinc-transporting P1B-type ATPase were identified and shown to be involved in Zn, Cd, and Pb resistance [70]. Additionally, the genome sequence of *S. meliloti* CCNWSX0020 revealed several putative molecular chaperones, metal binding proteins, and unspecific divalent cation transporters predicted to have a role in Cu and Zn resistance [71]. The transcriptome profiles of the *S. meliloti* CCNWSX0020 responses to Cu and Zn stresses were analyzed to investigate *S. meliloti* CCNWSX0020 Cu and Zn resistance mechanisms [72].

Szewczuk-Karpisz et al. made the attempt to determine flocculating properties of exopolysaccharide (EPS) synthesized by the bacteria *Sinorhizobium meliloti* 1021, which would increase the efficiency of chromium(III) oxide removal from sewages and wastewaters [8]. Chromium(III) oxide is an amphoteric, dark green solid. This most stable dye is widely used in construction and ceramic industries as well as in painting. Due to its extensive use in many industries, the $Cr_2O_3$ presence in wastewaters is inevitable. The obtained results showed that EPS of *Sinorhizobium meliloti* 1021causes chromium(III) oxide suspension destabilization in the whole examined pH range. The largest change in the system stability before and after the polymer addition was observed at pH 9. It is probable that under these conditions bridging flocculation occurs in the examined system [73].

EPSs of *S. meliloti* can have a protective role against the exposure to toxic metals. In this sense, in presence of either As or Hg, the Rm8530 WT strain was able to reach OD or cfu·mL$^{-1}$ similar to control condition

without metal, whereas mutants defective on the synthesis of EPSs were not capable, in presence of metals, of achieving the growth parameters reached under control conditions. These results support that the EPS II would be more relevant that the EPS I in dealing with the toxicity of heavy metals/metalloids [74]. These results are quite promising, but the following things should be taken into consideration. There must be strict monitoring and regulation of EPS-metal ion sorption experimental conditions in order to yield maximum possible removal. The subsequent notable point is the reusability and selectivity of this polymeric adsorbent. Also, it has been evident that the sorption through EPS is generally non specific. Overcoming this pitfall requires technological advancements as well as deeper understanding about the polymer and its mechanism of metal uptake. EPS modification and immobilization can be a good idea, but it is still much unexplored field [75].

Another approach is the use of genetically modified strains to expand the range of bioremediation action. This approach allows *S. meliloti* to be used for bioremediation of polychlorinated biphenyls (PCBs). Polychlorinated biphenyls are a class of potent environmental toxicants. The toxicological properties of a class of PCB congeners are largely influenced by the aqueous solubility and subsequent bioavailability. A variety of human health effects have been attributed to PCB exposure, including reproductive and birth defects, damage to the kidney, the nervous system and the immune system, and cancer. It is known that the genome of *S. meliloti* does not possess genes for bioremediation of aromatic pollutants. However, the genetically modified bacterium has the ability to enhance fertility of soil in association with the leguminous alfalfa plant while simultaneously enhancing bioremediation of PCB-contaminated soils. Enhanced bioremediation of PCB and robust alfalfa plant growth was also noted when uncharacterized mixed cultures containing alfalfa plant nodule formers were used [76].

The third direction of biotechnological use of *S. meliloti* has been developed relatively recently. It is known that lipolysaccharides of Gram-negative bacteria have a pronounced biological activity, including therapeutic activity. The experimental study of hematopoietic activity of four lipopolysaccharide (LPS) fractions isolated from *S. meliloti* L2 under induced immunodeficiency was carried out in mice. It was shown that administration of the lipolysaccharide preparation to mice with secondary experimental immunodeficiency was associated with decreased count of stab neutrophils and monocytopenia; LPS-1 fraction increased the count of segmented neutrophils; LPS-2 decreased the count of stab neutrophils and induced lymphocytosis; LPS-3 decreased the count of stab neutrophils and induced lymphocytosis associated with a significant increase in the count of segmented neutrophils; LPS-4 induced basophilia, decreased count of stab neutrophils, and lymphocytosis associated with a significant increase in the count of segmented neutrophils. It was made the conclusion that lipolysaccharide fractions of *S. meliloti* L2 exerted modulating effects similar to the mechanisms of "emergency myelopoiesis" in the physiological course of bacterial infections [77].

On the other hand, the study of rhizobial succinoglycan for its application in biotechnological and biomedical developments deserves attention. It can be used in drug delivery, biomedical imaging and nano-biosensor. A number of studies on biomedical applications of dextran-nanoparticle conjugate indicated a paradigm shift in bacterial exopolysaccharide based nanobiotechnology [78]. These conjugates are widely used in organ specific drug delivery, biosensor, drug carrier and encapsulation, haemoglobin-conjugate as blood substitute etc. [79]. Recently, modifications of succinoglycan using alginate beads with functionalized polydiacetylene vesicles are developed to assess barium (II) as a tangible fluorogenic sensor system [80]. As bacterial exopolysaccharides are unique group of biopolymers, which is both biodegradable and non-toxic, thus more research on application of succinoglycan as nanobiomaterial may open a new era in biomedical field. Till date, due to the non-toxic and good viscosifying nature, succinoglycan is used in food or cosmetic industry as commercial emulsifying agent. Production and commercialization of raw EPS is an intensive process; thus, easy downstream processing technique is needed for fast and better sales [81].

## Conclusions

Analyzing the research of *S. meliloti*, some prospective directions of the further investigations can be considered:

- in the field of agricultural research: selection of optimal genotypes of bacterial strains and host plants for effective symbiosis;

**Application of Sinorhizobium meliloti in biotechnology**

| Strain of *S. meliloti* | Application area | References |
|---|---|---|
| *Sinorhizobium meliloti* p221 | Destructor of polycyclic aromatic hydrocarbons | [82] |
| *Sinorhizobium meliloti* АК130 | Nitrogen fixator for normal and saline soils | [83] |
| *Sinorhizobium meliloti* АК55 | Nitrogen fixator for various agro-climatic conditions | [84] |
| *Sinorhizobium meliloti* 1021 | Chromium(III) oxide removal from sewages and wastewaters | [73] |
| *Sinorhizobium meliloti* 1021 | Cosmobiology | [65] |
| *Sinorhizobium meliloti* Rm8530 WT | Binding toxic metals (As or Hg) | [74] |
| *Sinorhizobium meliloti* MS-125 | Heavy metals adsorption (Pb, Ni, Zn) | [75, 85] |
| transformed *Sinorhizobium meliloti* (pE43) containing PCB-degrading genes | Bioremediation of polychlorinated biphenyls | [76] |
| *Sinorhizobium meliloti* L-14 | Medecine | [77] |
| *Sinorhizobium meliloti* 1021 | Stabilizing agent | [81, 86] |
| *Sinorhizobium meliloti* A2 | Emulsifier | [87] |

the study of the genetic regulation of the resistance of symbiont bacteria and host plants to stress factors for the targeted design of symbiotic systems with a given adaptive potential;

- in bioremediation field: strict monitoring and regulation of EPS-metal ion sorption;
- in medicine: modifications of bacterial exopolysaccharides to create the effective nanobiomaterials.

The data on the practical application of this bacterium in biotechnology are summarized in Table.

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## БАКТЕРІЯ *Sinorhizobium meliloti* ЯК ПЕРСПЕКТИВНИЙ ОБ'ЄКТ ДЛЯ БІОТЕХНОЛОГІЇ

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Бактерія *Sinorhizobium meliloti* належить до Грам-негативних грунтових азотфіксувальних бактерій, здатних підвищувати врожайність бобових рослин. У літературі є відомості про повну послідовність геному цієї бактерії, крім того, вивчено полісахаридний склад біоплівки, яка бере активну участь у фіксації нітрогену. Відома нуклеотидна послідовність, а також генетичні та біохімічні особливості бактерій *S. meliloti* роблять цей організм ідеальною моделлю для біотехнологічних досліджень. Метою роботи було проаналізувати сучасні дані, наведені в літературі щодо симбіотичної взаємодії бактерій *Sinorhizobium meliloti* з рослиною-господарем, й охарактеризувати основні напрями їх використання в сільському господарстві, біоремедіації та медицині.

***Ключові слова:*** *Sinorhizobium meliloti*, симбіоз, біотехнологія.

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## *Agrobacterium rhizogenes* — ОПОСЕРЕДКОВАНА ТРАНСФОРМАЦІЯ ЯК СПОСІБ СТИМУЛЮВАННЯ СИНТЕЗУ АНТИОКСИДАНТНИХ СПОЛУК У *Artemisia absinthium* L.

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Рослини *Artemisia absinthium* L. відомі як продуценти речовин з антиоксидантними властивостями. Зокрема, в них виявлено поліфеноли та флавоноїди. Активізувати синтез цих сполук можна шляхом генетичної трансформації навіть без перенесення специфічних генів, які беруть участь у біосинтезі. Так, «бородаті» корені, одержані після *Agrobacterium rhizogenes*-опосередкованої трансформації, можуть бути продуцентами комплексу цінних метаболітів.

*Метою* роботи було одержати «бородаті» корені *A. absinthium* як продуценти поліфенольних сполук.

*Методи.* «Бородаті» корені одержували шляхом культивування листків з суспензією *A. rhizogenes* з вектором *pCB124*. Наявність перенесених генів підтверджували методом ПЛР. Для визначення вмісту флавоноїдів та поліфенолів використовували реакції з $AlCl_3$ та реактивом Фоліна-Чокальте. Антиоксидантну активність оцінювали за здатністю екстрактів відновлювати DPPH радикал.

*Результати.* ПЛР аналіз виявив наявність бактеріальних *rol* генів та відсутність генів плазміди pCB124. Лінії коренів відрізнялися між собою за швидкістю росту. «Бородаті» корені характеризувалися більшим вмістом поліфенолів, зокрема, флавоноїдів (до 4.784±0.10 мг/г ВМ) та вищим рівнем антиоксидантної активності ($EC_{50}$ = 3.657 мг) у порівнянні з контролем (3.861±0,13 мг/г СМ та $EC_{50}$ = 6.716 мг відповідно).

*Висновки.* Трансформацію *A. absinthium* із застосуванням *A. rhizogenes* може бути використано для одержання ліній з підвищеним вмістом поліфенольних сполук та більшою антиоксидантною активністю.

*Ключові слова: Artemisia absinthium* L., *Agrobacterium rhizogenes*-опосередкована трансформація, «бородаті» корені, флавоноїди, поліфенольні сполуки, антиоксидантна активність.