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SHORT COMMUNICATION

Exopolysaccharide biosynthesis is important for *Mesorhizobium tianshanense*: plant host interaction

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Abstract *Mesorhizobium tianshanense* is a nitrogen-fixing bacterium that can establish symbiotic associations with *Glycyrrhiza uralensis* in the form of root nodules. Nodule formation in rhizobia often requires various secreted carbohydrates. To investigate exopolysaccharide (EPS) production and function in *M. tianshanense*, we performed a genome-wide screen using transposon mutagenesis to identify genes involved in EPS production. We identified seven mutants that produced significantly lower amounts of EPS as well as a two-component sensor kinase/response regulator system that is involved in the activation of EPS synthesis. EPS mutants formed significantly less biofilm and displayed severely reduced nodulation capacity than wild type bacteria, suggesting that EPS synthesis can play important roles in the symbiosis process.

Keywords *Mesorhizobium tianshanense* · Exopolysaccharide production · Biofilms · Nodulation

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Introduction

Nitrogen-fixing soil bacteria that establish mutualistic associations with legumes are referred to as rhizobia. Rhizobia produce polysaccharides, such as exopolysaccharides (EPSs), capsular polysaccharides (KPSs), lipopolysaccharides (LPSs), and cyclic β-(1,2)-glucans which are necessary for establishing symbiotic associations (Kannenberg and Brewin 1994; Hoang et al. 2004). In addition to protecting bacteria against environmental stresses, polysaccharides of the rhizobia play vital but poorly understood roles in infection of legume roots (Skorupska et al. 2006). EPS may aid in the attachment of bacteria to roots, and play a structural role in infection thread formation (Skorupska et al. 2006). In addition, numerous pieces of evidence indicate that rhizobial extracellular polysaccharides are crucial to protect bacteria against host defenses (Niehaus and Becker 1998; D'Antuono et al. 2005). The symbiotic phenotype of mutants defective in elements of EPS production depends on the type of nodule ontogeny (Gray et al. 1991; Fraysse et al. 2003). For example, specifically, acidic EPS seems to be essential for the establishment of rhizobium–legume symbiosis on legumes developing an indeterminate type of nodule (Fraysse et al. 2003).

Exopolysaccharide biosynthesis represents a multi-step process, in which many gene products are involved. The synthesis of succinoglycan (EPSI) of *Sinorhizobium meliloti* has been extensively studied. Many *exo* genes responsible for EPSI biosynthesis in *S. meliloti* have been identified, including all the glycosyl transferase genes involved in the synthesis of whole repeating carbohydrate units, as well as some genes involved in secretion (*exsA*) and polymerization (*exoQ*, *exoT*, and *exoP*) (Heidstra et al. 1994; Gonzalez et al. 1996). Genes homologous to those involved in biosynthesis of EPS II of *S. meliloti* have also been reported

in the synthesis of EPSs of other rhizobial species, such as *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* (Heidstra et al. 1994). The process of rhizobial EPS biosynthesis is complicated and regulated at both transcriptional and post-translational levels, with multiple regulatory systems identified in *S. meliloti* and *R. leguminosarum* (Heidstra et al. 1994; Skorupska et al. 2006). It is known that environmental factors such as the media osmolarity, and phosphate and nitrogen concentrations can influence the synthesis of the EPS (Bardin and Finan 1998; Mendrygal and Gonzalez 2000), but it is not clear how bacteria recognize and respond to these environmental signals to affect EPS synthesis.

To date there have been few detailed studies about EPS in the *Mesorhizobium* genus (Chen et al. 1995) of moderately growing rhizobium. In *M. loti*, exopolysaccharide mutants are fully effective on *Lotus uliginosus* (determinate nodulating host) but are ineffective on *Leucaena leucocephala* (indeterminate nodulating host) (Hotter and Scott 1991). In this study, we used transposon mutagenesis to identify seven EPS production defective mutants in *Mesorhizobium tianshanense*, a bacterium originally isolated from arid, saline, desert soil in northwestern China in 1995 (Chen et al. 1995), and later widely found in dry soils, acting as a nitrogen-fixing symbiont for at least eight different plant species, including species of *Glycyrrhiza* (licorice) (Tan et al. 1997), whose roots are one of the most important crude medicines in Asia and Europe. We found that exopolysaccharide production in *M. tianshanense* is important for the rhizobial biofilm-formation and nodulation.

Materials and methods

Bacterial strains, plasmids, and culture conditions

Bacterial strains and plasmids used in this work are shown in Table 1. *M. tianshanense* strains were grown at 28°C in TY medium (Vincent 1970) and *Escherichia coli* were grown at 37°C in LB medium (Sambrook et al. 1989). *Mesorhizobium* exopolysaccharide production defective mutants were screened at 28°C in YMA medium (Vincent 1970). Antibiotics were added to the following final concentrations: streptomycin, 100 mg/ml; kanamycin, 50 μg/ml; chloramphenicol, 30 μg/ml; and ampicillin, 100 μg/ml. The *mtpC* insertional deletion was constructed by cloning the *mtpC* internal fragment into pVIK112 (Kalogeraki and Winans 1997). The resulting plasmid, pZJ2, was then introduced into the *M. tianshanense* chromosome by single crossover homologous recombination. The insertional mutants were confirmed by PCR using a *mtpC* upstream primer and a *lacZ* primer. This also simultaneously generated a *mtpC–lacZ* transcriptional fusion. The *mtpR* and *mtpS* deletion mutants were constructed by cloning the internal fragments of *mtpR* and *mtpS* into the plasmid pEX18Gm (Hoang et al. 1998), resulting pWP2, and pWP6, respectively. These two plasmids were then transferred into *M. tianshanense* strains by conjugation and single-crossover events were selected. The insertional mutants were confirmed by PCR.

Screening for exopolysaccharide-deficient mutants

Random transposon mutagenesis of *M. tianshanense* Sm^R was carried out by introducing a mariner transposon

Table 1 Bacterial strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Source
Strains		
Mesorhizobium tianshanense		
CCBAU 3306	Wild-type	CCBAU
Es0	Derivative of CCBAU3306, spontaneous Sm^R	This work
ZJ55	Derivative of Es0, *mtpC*$^-$, *mtpC-lacZ*	This work
WP50	Derivative of Es0, *mtpR*$^-$ (insertional mutation)	This work
WP40	Derivative of Es0, *mtpS*$^-$ (insertional mutation)	This work
WP55	Derivative of ZJ55, *mtpR*$^-$	This work
WP45	Derivative of ZJ55, *mtpS*$^-$	This work
Plasmids		
pVIK112	R6K, promoterless *lacZ*, Km^R	Kalogeraki and Winans (1997)
pEX18Gm	R6K, *sacB*, Gm^R	Hoang et al. (1998)
pKGL3	Mariner transposon, Km^R	Xu and Mekalanos, unpublished
pSC137	Mariner transposon, Cm^R	Chiang and Mekalanos (1999)
pZJ2	*mtpC* internal fragment in pVIK112	This work
pWP2	*mtpR* internal fragment in pEX18Gm	This work
pWP6	*mtpS* internal fragment in pEX18Gm	This work

CCBAU Culture Collection of Beijing Agricultural University, Beijing, China

containing a promoterless *lacZ* gene (pKGL3) (Xu and Mekalanos, unpublished) from *E. coli* SM10λpir using filter mating (Sambrook et al. 1989). The *trans*conjugants were selected on YMA medium with kanamycin and streptomycin. Nonmucoid colonies were selected from ten libraries of transposon insertion mutants for further characterization.

Arbitrary PCR and nucleotide sequence analysis

Arbitrary PCR was performed as described previously (Gao et al. 2006) to obtain short fragments of chromosomal DNA flanking transposon ends. The PCR products of the second round were sequenced with the transposon primer used in the second round, and the sequences were compared with the Genbank DNA sequence database using the BLASTX program. The full sequences were obtained by subcloning the transposon insertion flanking regions into pBluescript (Clonetech) and sequencing using the primer walking method.

β-Galactosidase activity assays

Bacteria containing *mtpC–lacZ* transcriptional reporters were grown in TY medium with appropriated antibiotics. Samples were withdrawn at the time points indicated and β-galactosidase activity was measured as described previously (Miller 1972).

Screening for exopolysaccharide regulatory genes

The *M. tianshanense* strain containing a *mtpC–lacZ* transcriptional fusion was mutagenized by the mariner transposon pSC137 (Chiang and Mekalanos 1999) as described previously (Sambrook et al. 1989). *Trans*conjugants were selected on TY plates with appropriate antibiotics and 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) (100 μg/ml) and white colonies, which lost the ability to activate *mtpC–lacZ*, were isolated.

Biofilm formation assay

Borosilicate glass tubes were filled with 1 ml of MM medium containing 10 μl of saturated overnight cultures as inoculum and appropriate antibiotics (Wang et al. 2004). After 3 days incubation at 28°C with circular agitation, the content of each tube was removed gently. The tubes were rinsed with water and stained with 1.2 ml of 1% crystal violet for 5 min, and tubes were then washed and dried and photographs were taken.

Nodulation assays

Glycyrrhiza uralensis (Asian licorice) seeds were sterilized and germinated as previously described (Zheng et al. 2006). After 6 days, plants were inoculated with 300 μl cultures of wild-type or mutant *M. tianshanense* grown in TY medium (OD_{600} = 2.0). The plants were pulled out to count the number of nodules at the time indicated. There were at least six replicates for each inoculation.

Nucleotide sequence accession number

The *M. tianshanense* polysaccharide biosynthesis gene DNA sequence reported here has been deposited in the GenBank database under accession number EU034647 and EU034648.

Results and discussion

Screening of exopolysaccharide defective mutants in *M. tianshanense*

Exopolysaccharides play important roles in establishing rhizobium–plant interactions. However, little is known of the regulation of EPS biosynthesis in rhizobia such as *M. tianshanense*. To study the regulation of EPS production, a transposon insertion mutant library of *M. tianshanense* was screened for mutants defective in exopolysaccharide production. By screening approximately 10,000 random transposon insertion mutants on YMA agar, we isolated seven nonmucoid mutants (Es2, Es3, Es4, Es5, Es6, Es8, Es10). The colony morphology of these mutants is distinct from that of wild type (Fig. 1a, left panel).

We then used arbitrary PCR followed by subcloning and DNA sequencing to identify transposon insertion sites in these seven EPS mutants. Sequence analysis revealed that those transposons inserted in two clusters (Fig. 1b). The first gene cluster is similar to *pssNOPT* of *R. leguminosarum* bv. *viciae* (Young et al. 2006). All these genes are highly conserved in many *Rhizobium* species and involved in the translocation of polysaccharides and polymerization of the repeating subunits of EPS (Skorupska et al. 2006). We thus propose to name these genes *mtpABCD* (*Mesorhizobium tianshanense* polysaccharide genes ABCD). Another gene isolated from the transposon screen was located upstream of the *mtpABCD* operon and divergently transcribed (Fig. 1b). This gene, which we named *mtpE*, was similar to the *exo5* gene in *R. leguminosarum* bv. *trifolii*. *exo5* encodes a UDP-glucose dehydrogenase responsible for the oxidation of UDP-glucose to UDP-glucuronic acid (Krol and Skorupska 1997); mutations in *exo5* mutant causes a pleiotropic phenotype and affects the production of glucuronic acid- and galacturonic acid-containing polysaccharides (Laus et al. 2004). Of note, our screen for EPS mutants may not be saturating, as all seven mutants have different insertion sites and we failed to obtain the regulators

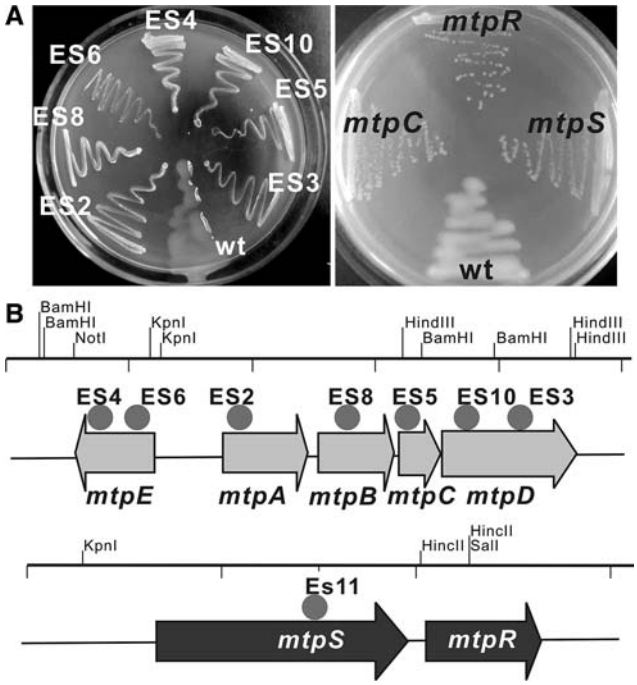

Fig. 1 Colony morphology and chromosomal locations of transposon insertions of the exopolysaccharide-deficient mutants. **a** *M. tianshanense* and its EPS mutants were streaked on MM agar plates at 28°C for 2 days. **b** Genetic maps of genes involved in *M. tianshanense* EPS production and transposon insertion locations (*circles*). The *mtp* stands for *M. tianshanense* polysaccharide gene

isolated from our second screen (see below). It is likely that more genes are involved in EPS biosynthesis in *M. tianshanense*.

To confirm our screen results, we constructed a strain containing a *lacZ* insertion (see "Materials and methods") in one of the EPS genes, *mtpC*, and measured the exopolysaccharide production in the mutant. As expected, the production of exopolysaccharides was completely abolished in the *mtpC::lacZ* mutant (Fig. 1a, right panel), indicating that the *mtpC* gene product is essential in *M. tianshanense* EPS production.

M. tianshanense eps genes are regulated by a two-component regulatory system

Various genes have been found to regulate the synthesis of exopolysaccharides, but the mechanisms behind their regulatory roles are not very clear. In order to investigate the regulatory genes of exopolysaccharides in *M. tianshanense*, we constructed a chromosomal *mtpC–lacZ* transcriptional reporter fusion and examined β-galactosidase activity at the different time points of bacterial growth. Expression of *mtpC* reached maximal levels at the late-log phase and slightly declined by stationary phase (data not shown).

To investigate how EPS genes are regulated in *M. tianshanense*, we mutagenized the *M. tianshanense* strain containing the *mtpC–lacZ* reporter using a transposon and screened for LacZ$^-$ transconjugants (white colonies on X-gal plates). From approximately 10,000 transconjugants screened, five mutants displayed low β-galactosidase activity. Sequence analysis of arbitrary PCR products from those strains indicated that the mariner transposon disrupted the *lacZ* gene in four mutants, while the transposon insertion in one mutant (ES11) disrupted the sensor histidine kinase gene of a two-component regulatory system. Further sequencing indicated that a response regulator gene is located immediately downstream of the senor kinase. We therefore named them *mtpS* and *mtpR*, respectively (Fig. 1b). The predicted gene products are similar to the sensor kinase/regulator ActS–ActR of *R. leguminosarum* bv.*viciae* (86% identity at amino acid levels), which has previously been studied for its function in low pH conditions (Boesten et al. 2000). In *S. medicae*, ActSR has been shown to regulate many genes, including *cbbS* and *narB*, and is required for the low pH and microaerobic induction of the nitrogen fixation regulators *fixK* and *nifA* (Fenner et al. 2004). To confirm the role of MtpRS in regulation of EPS biosynthesis in *M. tianshanense*, we constructed *mtpR* and *mtpS* insertional deletions in wild type and strains containing *mtpC–lacZ*, respectively. Both *mtpR* and *mtpS* mutants did not produce exopolysaccharides, similar to that of *mtpC* mutants (Fig. 1a, right panel). Mutations in either *mtpR* or *mtpS* gene also abolished *mtpC–lacZ* activity (Fig. 2), indicating that this two-component regulatory system is crucial for *mtpC* activation. Interestingly, both MtpR and MtpS did not affect *mtpE* transcription, as *mtpE–lacZ* β-galactosidase activity in *mtpRS* mutants was similar to that in wild type bacteria (data not shown). The actual external signals required to modulate MtpRS activity is currently under investigation.

Influence of EPS biosynthesis on biofilm formation and nodulation

Rhizobia can form dense, structurally complex biofilms on abiotic surfaces as well as on root surfaces, and biofilm formation is important for rhizobium–legume symbiosis (Ramey et al. 2004). *S. meliloti* exopolysaccharide-deficient mutants exhibit reduced biofilm phenotypes that correspond to their reduced nodulation abilities (Fujishige et al. 2006). To examine whether EPS biosynthesis is involved in biofilm formation in *M. tianshanense*, we compared the biofilm formation of wild type and EPS mutants on glass surfaces. Figure 3 shows that while wild type strains produced significant amount of biofilm, while *mtpC*, *mtpR*, and *mtpE* mutants all formed little biofilms. These data indicate that EPS is indispensable in forming biofilms in *M. tianshanense* and are accordance with exopolysaccharide mutants in many other bacterial species, such as *E. coli* (Danese et al. 2000),

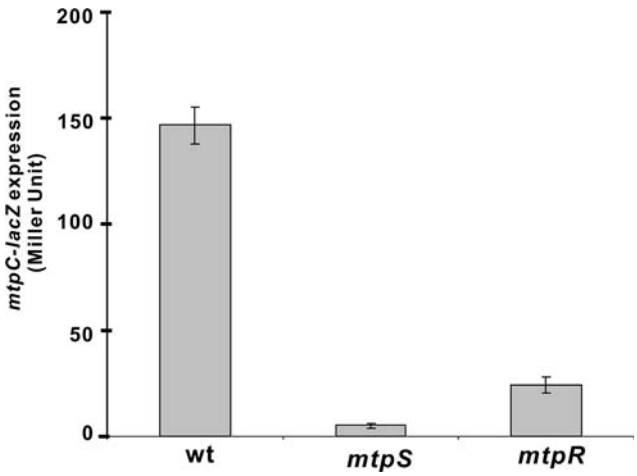

Fig. 2 The effect of the MtpR/MtpS two-component regulatory system on EPS production and *mtpC* transcription. Both MtpR and MtpS are required for *mtpC* expression. Strains containing *mtpC–lacZ* reporters were grown to late-log at 28°C and *β*-galactosidase activity was measured and reported as Miller Units. The results are average of three experiments ± SD

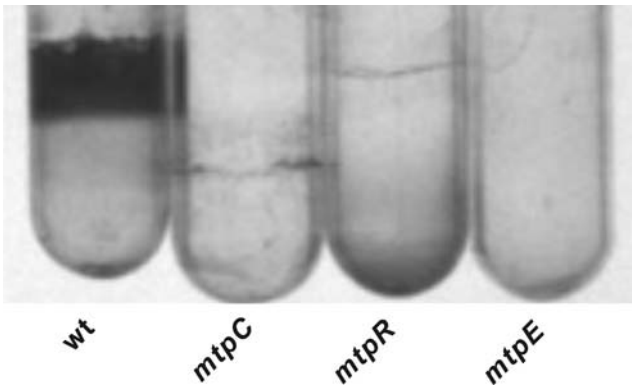

Fig. 3 The effect of EPS production on *M. tianshanense* biofilm formation. Biofilm formation of wild type and EPS mutants. Strains were grown in TY medium at 28°C for 3 days. Biofilms were visualized by staining with 1% crystal violet

Pseudomonas aeruginosa (Friedman and Kolter 2004), and *Vibrio cholerae* (Yildiz and Schoolnik 1999).

To test whether EPS production in *M. tianshanense* plays a role in symbiosis, a nodulation assay on Asian licorice (*Glycyrrhiza uralensis*) was performed using wild type and EPS mutant *mtpC*, *mtpR* strains. On average 11.3 nodules were formed per plant about 1 month after inoculation of wild type bacteria. In contrast, no nodules formed on the plant roots inoculated with either *mtpC* or *mtpR* mutant cultures, indicating that *M. tianshanense* EPS mutants are profoundly defective in nodulation. These results are not unexpected as in many other rhizobia mutants that are defective in EPS production usually induce abnormal nodules or fail to nodulate at all (Becker and Pühler 1998; Wielbo et al. 2004).

Mesorhizobium tianshanense, capable of nodulating and fixing nitrogen on various plants grown in arid environments, was only recently discovered, and the genes and gene regulation involved in bacterium–plant symbiosis are largely unknown. We have previously demonstrated that LuxR/LuxI-type quorum sensing regulation is crucial for root hair adherence and nodulation by this bacterium (Zheng et al. 2006). In this study, we discovered genes involved in *M. tianshanense* exopolysaccharides production and regulation. Given the significant role played by this bacterium in the growth of important plant species, a better understanding of exoploysaccaride function in *M. tianshanense* symbiotic associations is important from both economic and environmental points of view.

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References

Bardin SD, Finan TM (1998) Regulation of phosphate assimilation in *Rhizobium* (*Sinorhizobium*) *meliloti*. Genetics 148:1689–1700

Becker A, Pühler A (1998) Production of exopolysaccharides. In: Spaink HP, Kondorosi A, Hooykaas PJJ (eds) The Rhizobiaceae. Molecular biology of model plant-associated bacteria. Kluwer, Dordrecht, pp 97–118

Boesten B, Schlüter A, Prell J, Krämer M, Lipka V, Priefer UB (2000) Identifying genes suitable for constructing pH and salt tolerant Rhizobium inoculants for improving French bean cultivation under semiarid conditions. In: Olivares J, Palomares AJ (eds) Proceedings of the fourth european nitrogen fixation conference, Viceconsejería, p 319

Chen W, Wang E, Wang S, Li Y, Chen X, Li Y (1995) Characteristics of *Rhizobium tianshanense* sp. nov., a moderately and slowly growing root nodule bacterium isolated from an arid saline environment in Xinjiang, People's Republic of China. Int J Syst Bacteriol 45:153–159

Chiang SL, Mekalanos JJ (1999) rfb mutations in *Vibrio cholerae* do not affect surface production of toxin-coregulated pili but still inhibit intestinal colonization. Infect Immun 67:976–980

D'Antuono AL, Casabuono A, Couto A, Ugalde RA, Lepek VC (2005) Nodule development induced by *Mesorhizobium loti* mutant strains affected in polysaccharide synthesis. Mol Plant Microbe Interact 18:446–457

Danese PN, Pratt LA, Kolter R (2000) Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. J Bacteriol 182:3593–3596

Fenner BJ, Tiwari RP, Reeve WG, Dilworth MJ, Glenn AR (2004) *Sinorhizobium medicae* genes whose regulation involves the ActS and/or ActR signal transduction proteins. FEMS Microbiol Lett 236:21–31

Fraysse N, Couderc F, Poinsot V (2003) Surface polysaccharide involvement in establishing the rhizobium–legume symbiosis. Eur J Biochem 270:1365–1380

Friedman L, Kolter R (2004) Genes involved in matrix formation in *Pseudomonas aeruginosa* PA14 biofilms. Mol Microbiol 51:675–690

Fujishige NA, Kapadia NN, De Hoff PL, Hirsch AM (2006) Investigations of *Rhizobium* biofilm formation. FEMS Microbiol Ecol 56:195–206

Gao Y, Zhong Z, Sun K, Wang H, Zhu J (2006) The quorum-sensing system in a plant bacterium *Mesorhizobium huakuii* affects growth rate and symbiotic nodulation. Plant Soil V286:53–60

Gonzalez JE, York GM, Walker GC (1996) *Rhizobium meliloti* exopolysaccharides: synthesis and symbiotic function. Gene 179:141–146

Gray JX, Zhan HJ, Levery SB, Battisti L, Rolfe BG, Leigh JA (1991) Heterologous exopolysaccharide production in *Rhizobium* sp. strain NGR234 and consequences for nodule development. J Bacteriol 173:3066–3077

Heidstra R, Geurts R, Franssen H, Spaink HP, Van Kammen A, Bisseling T (1994) Root hair deformation activity of nodulation factors and their fate on *Vicia sativa*. Plant Physiol 105:787–797

Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP (1998) A broad-host-range Flp–FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. Gene 212:77–86

Hoang HH, Becker A, Gonzalez JE (2004) The LuxR homolog ExpR, in combination with the Sin quorum sensing system, plays a central role in *Sinorhizobium meliloti* gene expression. J Bacteriol 186:5460–5472

Hotter GS, Scott DB (1991) Exopolysaccharide mutants of *Rhizobium loti* are fully effective on a determinate nodulating host but are ineffective on an indeterminate nodulating host. J Bacteriol 173:851–859

Kalogeraki VS, Winans SC (1997) Suicide plasmids containing promoterless reporter genes can simultaneously disrupt and create fusions to target genes of diverse bacteria. Gene 188:69–75

Kannenberg EL, Brewin NJ (1994) Host–plant invasion by *Rhizobium*: the role of cell-surface components. Trends Microbiol 2:277–283

Krol J, Skorupska A (1997) Identification of genes in *Rhizobium leguminosarum* bv. *trifolii* whose products are homologues to a family of ATP-binding proteins. Microbiology 143(Pt 4):1389–1394

Laus MC et al (2004) Involvement of exo5 in production of surface polysaccharides in *Rhizobium leguminosarum* and its role in nodulation of *Vicia sativa* subsp. *nigra*. J Bacteriol 186:6617–6625

Mendrygal KE, Gonzalez JE (2000) Environmental regulation of exopolysaccharide production in *Sinorhizobium meliloti*. J Bacteriol 182:599–606

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, New York

Niehaus K, Becker A (1998) The role of microbial surface polysaccharides in the rhizobium–legume interaction. Subcell Biochem 29:73–116

Ramey BE, Koutsoudis M, von Bodman SB, Fuqua C (2004) Biofilm formation in plant–microbe associations. Curr Opin Microbiol 7:602–609

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, New York

Skorupska A, Janczarek M, Marczak M, Mazur A, Krol J (2006) Rhizobial exopolysaccharides: genetic control and symbiotic functions. Microb Cell Fact 5:7

Tan ZY, Xu XD, Wang ET, Gao JL, Martinez-Romero E, Chen WX (1997) Phylogenetic and genetic relationships of *Mesorhizobium tianshanense* and related rhizobia. Int J Syst Bacteriol 47:874–879

Vincent JM (1970) A manual for the practical study of root nodule bacteria. Blackwell Scientific, Oxford

Wang H, Zhong Z, Cai T, Li S, Zhu J (2004) Heterologous overexpression of quorum-sensing regulators to study cell-density-dependent phenotypes in a symbiotic plant bacterium *Mesorhizobium huakuii*. Arch Microbiol 182:520–525

Wielbo J, Mazur A, Krol J, Marczak M, Kutkowska J, Skorupska A (2004) Complexity of phenotypes and symbiotic behaviour of *Rhizobium leguminosarum* biovar *trifolii* exopolysaccharide mutants. Arch Microbiol 182:331–336

Yildiz FH, Schoolnik GK (1999) *Vibrio cholerae* O1 El Tor: identification of a gene cluster required for the rugose colony type, exopolysaccharide production, chlorine resistance, and biofilm formation. Proc Natl Acad Sci USA 96:4028–4033

Young JP et al (2006) The genome of *Rhizobium leguminosarum* has recognizable core and accessory components. Genome Biol 7:R34

Zheng H, Zhong Z, Lai X, Chen WX, Li S, Zhu J (2006) A LuxR/LuxI-type quorum-sensing system in a plant bacterium, *Mesorhizobium tianshanense*, controls symbiotic nodulation. J Bacteriol 188:1943–1949