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Variations in Outer-membrane Characteristics of Two Stem-nodulating Bacteria of *Sesbania rostrata* and its Role in Tolerance Towards Diverse Stress

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Abstract Outer-membrane characteristics may determine the survivability of rhizobia under diverse abiotic and biotic stresses. Therefore, the role of lipopolysaccharides (LPS) and membrane proteins of two stem-nodulating bacteria of *Sesbania rostrata* (*Azorhizobium caulinodans* ORS571 and *Rhizobium* sp. WE7) in determining tolerance towards abiotic and biotic stresses (hydrophobics and phages) was investigated. Outer-membrane characteristics (LPS and membrane–protein profiles) of ORS571, WE7 and thirteen standard strains were distinct. ORS571 and WE7 also showed susceptibility towards morphologically distinct phages, i.e., ACSR16 (short-tailed) and WESR29 (long-tailed), respectively. ORS571 and WE7 were tolerant to hydrophobic compounds (triton X-100, rifampicin, crystal violet and deoxycholate). To ascertain the role of outer membrane characteristics in stress tolerance, phage-resistant transconjugant mutants of ORS571 (ORS571-M8 and ORS571-M20) and WE7 (WE7-M9) were developed. LPS- and membrane–protein profiles of mutants differed from that of respective wild types (ORS571 and WE7). In in vitro assay, phages got adsorbed onto purified LPS-membrane protein fractions of wild types. Phages did not adsorb onto membrane fraction of mutants and standard strains. Mutant with reduced expression of LPS (ORS571-M20 and WE7-M9) showed reduced tolerance towards hydrophobics. However, the tolerance was unaffected in mutant (ORS571-M8) where expression of LPS was not reduced but pattern was different. The tolerance level of mutants towards hydrophobics varied with the expression of LPS, whereas the specificity towards phages is correlated with the specific LPS pattern.

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Introduction

The ecological success of legume-nodulating bacteria is determined by their ability to tolerate stresses (abiotic and biotic) and maintain minimum rhizobial density required to develop effective nodulation and efficient nitrogen fixation [12, 28]. The cell surface (lipopolysaccharides (LPS), outer membrane protein and LPS-protein complexes) of Gram-negative bacteria protects the cell from harmful toxic substances and also helps in communication with legume host [1, 15]. Lipopolysaccharide (LPS) has been extensively studied by rhizobiologists, but outer membrane proteins (OMPs) are relatively uncharacterized [7, 18, 22].

Sesbania rostrata can colonize ecologically diverse habitats, shows promiscuity and forms nodules (root and stem) with diverse bacterial taxa (*Azorhizobium caulinodans*, *Sinorhizobium teranga*, *S. saheli*, *Rhizobium* sp. and *Methylobacterium* sp.) [14, 19, 21]. Stem-nodulation (besides root-nodulation) makes it an extremely efficient legume green manure crop and a key resource for soil amelioration programme. The role of outer membrane characteristics in the development of stem-nodules in two bacterial strains, ORS571 (*A. caulinodans*) and WE7 (*Rhizobium* sp.), have been investigated [16]. Soil-borne bacteriophages and

man-made hydrophobic compounds pose threats for cellular integrity, viability and effective density of rhizobia, which affect the symbiotic efficiency and effectiveness. Little is known about the involvement of LPS and membrane proteins in rhizobial ability to combat different biotic and abiotic stresses [7, 18]. In fact, their role in stress tolerance in stem-nodulating bacteria has not been investigated. Therefore, in this study, involvement of outer membrane LPS and protein in tolerance to hydrophobic compounds and rhizobiophage susceptibility in two stem-nodulating bacteria (ORS571 and WE7) of *S. rostrata* were tested. Such studies are important to understand the factors affecting the efficacy of rhizobial inoculation technologies under stress environment and selection of appropriate inoculants.

Materials and Methods

Bacterial and Phage Isolates

ORS571 (*Azorhizobium caulinodans*) and WE7 (*Rhizobium* sp.) were isolated from West Africa and India, respectively. Rhizobiophages of ORS571 (ACSR16) and WE7 (WESR29) were isolated from rhizosphere soil of *S. rostrata* by broth-enrichment method [21]. The standard legume-nodulating bacteria used in all the experiments include *Sinorhizobium meliloti* (2011, USDA1002, L5-30) *S. teranga* USDA4894, *S. fredii* USDA205, *Rhizobium galegae* (HAMBI 1143, HAMBI 1141 and HAMBI 540), *R. leguminosarum* biovar *trifolii* (ANU794, ANU1117 and ANU843), biovar *viciae* VF39 and *Agrobacterium tumefaciens*.

Plaque Characteristics, Electronmicroscopy, Host Range and Physico–Chemical Tolerance of Phages

The plaque characteristics of phages were determined on the indicator bacterium as previously described [21]. Phage isolates were concentrated by differential centrifugation and negatively stained with 1% PTA (phosphotungstic acid) for examining under transmission electron microscope (Philips 300, 80 kV). Phage suspensions (2×10^6 PFU/ml) incubated at different temperatures (25–65°C for 1 h with an increment of 5°C) and at different pH (4–11 with an increment of 0.5) were tested for titre value (after every 10 min) by plaque assay [3]. Phage titre in suspensions prepared in different storage media (sterile distilled water, 100 times diluted YM/YL medium, 10 mM Tris–HCl, pH 8.0, saline solution: 0.1 M NaCl and 0.001 M $MgCl_2$) were determined on every tenth day till 60 days.

Isolation of Phage-resistant Mutants and their Sensitivity to Hydrophobic Compounds, (Crystal Violet, Deoxycholate, Rifampicin and Triton X-100)

Mutants of ORS571 (ORS571-M8 and ORS571-M20) and WE7 (WE7-M9) were developed using transposon *Tn*5-*β*-glucuronidase (*Gus*A) fusion assay system [7]. Wild types (ORS571 and WE7), three mutants (ORS571-M8, ORS571-M20 and WE7-M9) and 13 standard strains of *Rhizobium* and related species were tested for their susceptibility to ACSR16 and WESR29 phage isolates. Wild-type and mutant strains were also tested for their sensitivity to hydrophobic compounds (deoxycholate, triton X-100, rifampicin and crystal violet dye), and minimum inhibitory concentrations were determined [7].

Detection of Lipopolysaccharides (LPS) and Membrane Proteins of Wild Types, Mutants and Rhizobial Standard Strains

The LPS and membrane protein fractions of the wild types, mutants and standard strains were purified [6, 22]. For preparation of cell envelope fractions, bacterial cells were grown in YM/YL broth. Bacterial cells harvested at late-log phase were washed with phosphate buffer saline (10 mM NaH_2PO_4–Na_2HPO_4, 0.9% NaCl and pH 7.4) and suspended in 50 mM Tris–HCl, pH 8.5. The cell suspension was subjected to six bursts of 30 s of sonication (heat system ultrasonics, model W-3851) with an intermittent cooling of 2 min. Unbroken cells and large wall fragments were removed by centrifuging at $1{,}000\times g$ for 20 min. Supernatant was treated with lysozyme (0.2 mg/ml) for 30 min at RT followed by the addition of KCl (2 M) to a final concentration of 0.2 M. The cell envelope was pellet down by centrifugation at $12{,}000\times g$ for 60 min at 4°C and thoroughly washed with sterile distilled water. The membrane pellet was resuspended in appropriate volume of 2 mM Tris–HCl, pH 7.8. For LPS, the membrane was treated with proteinase K (0.2 mg/ml) at 60°C for 60 min and appropriately diluted with sample buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 4% SDS and 30% sucrose). For protein, the membrane pellet was treated with sample buffer (62.5 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 0.001% bromophenol blue and 5% 2-mercaptoethanol). Equal amounts of purified samples were subjected to sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE), and the gels were preferentially silver stained for LPS and membrane proteins [13, 22].

Phage Adsorption Abilities of Purified Lipopolysaccharide (LPS)-Membrane Protein Fractions of Wild Types, Mutants and Standard Bacterial Strains

For in vitro phage adsorption assay, purified LPS-membrane protein fractions of wild types (ORS571 and WE7), mutants (ORS571-M8, ORS571-M20 and WE7-M9), and 13 standard strains (2.5–50 µg) were suspended in 100 µl of assay solution (50 mM Tris–HCl, 100 µM $CaCl_2$ and pH 7.5) and incubated with both the phage isolates (10^5 PFU), separately [17]. The phage particles adsorbed onto LPS-membrane protein fraction were pelleted down by centrifugation at 12,000×*g* for 10 min at 4°C. The unadsorbed phage particles in the supernatant were titred by plaque assay and designated as percent survival. In vitro phage adsorption ability of LPS-membrane protein fraction was expressed as % PFU loss.

Results and Discussion

Characterization of Phages of Stem-nodulating Bacteria (*Azorhizobium caulinodans*-ORS571, *Rhizobium* sp.-WE7)

Rhizobiophage of ORS571 (ACSR16) possessed a very short (18 nm) and rigid tail with small thick spikes (*Podoviridae* member) and formed semi-clear plaque with halo formation (Fig. 1). In contrast to RS2 phage of ORS571 from Senegal, the tail of ACSR16 phage (Indian isolate) was distinct. WESR29 phage isolate possessed a long tail (118 nm) with contractile sheath and distinct neck region (*Myoviridae* member) and formed clear plaque (Fig. 1). Another phage of ORS571 (RS1) belonging to *Myoviridae* family has also been reported from Senegal [5]. The range of pH where phage titre remained unchanged in short-tailed phage (ACSR16) (pH 6.0–9.0) was broader than that of the long-tailed phage (WESR29) (pH 6.5–8.0). The cut-off pH value where no effective phage particles were detected was also in extreme in ACSR16 (pH 4.0–11.0) than that of WESR29 (pH 4.5–10.5). This is in contrast to the relatively high pH stability reported for the long-tailed phage of cowpea *Rhizobium* [23]. For ACSR16, the loss of titre showed a linear relationship at 45°C, but it showed two component inactivation curves at 50°C (Fig. 1). In diluted YM/YL broth, the titre for both the phages remained unchanged (after 60 days). In sterile distilled water, the titre loss was pronounced in ACSR16 (16.0%) than WESR26 (2.0%), whereas it was reversed in saline (WESR29: 90%; ACSR16: 66.0%). Such variations in phage titre in different storage media appear to be associated with the role of divalent cations (Ca^{2+} and Mg^{2+}) and carbon source on infectivity of phages. Similar observations have also been reported for other *Rhizobium* phages [23]. Interestingly, the long-tailed phage isolate of *Sesbania Rhizobium* (WESR29) showed high thermostability (T_{50}: 50°C, 40 min) than that of the short-tailed phage (ACSR16) isolate (T_{50}: 45°C, 40 min) (Fig. 1). However, in a recent study, the temperature tolerance of phages specific to *Lens culinaris* rhizobia had no correlation with the tail length [10]. An inverse correlation has also been reported in phages of cowpea rhizobia [23].

Susceptibility to Phages and Variations in Outer-membrane Characteristics of Wild Types and Mutants

Both ACSR16 and WESR29 phages showed high host specificity and they failed to infect any of the thirteen standard strains suggesting their significance as a stable biological marker [11, 20]. Phage specificity has been suggested due to wall-associated factor(s) (lipopolysaccharides, membrane proteins, lipoproteins, etc.) of host bacterium. In SDS-PAGE, LPS of rhizobia generally displays two regions [22]: (i) LPS I: lower mobility band- complete LPS molecules with lipid A, core-oligosaccharide and O-antigenic polysaccharide); and (ii) LPS II: higher mobility band- incomplete LPS molecules with lipid A and core region only). ORS571 showed a ladder like LPS pattern which is characteristic of *A. caulinodans* and *Bradyrhizobia*, whereas WE7 showed two distinct and widely separated bands (Fig. 1), common among *Sinorhizobia* [21]. LPS profiles of different strains of *S. meliloti* (2011 and L5-30) and *R. leguminosarum* bv *trifolii* (ANU794, ANU1117, ANU843) showed variations in O-antigen. Membrane protein profiles of ORS571, WE7 were different from each other and that of the standard strains (Fig. 1). Membrane proteins have been recommended as one of the markers for discriminating different bacterial taxa [4].

Phage-resistant mutants of ORS571 showed two colony morphotypes: (i) semi-mucoid colony (ORS571-M8); and (ii) wrinkled dry colony (ORS571-M20). In case of WE7, the mutant showed only a dry wrinkled colony morphotype (WE7-M9). Except ORS571-M8, the growth rates of mutants were slower than the respective wild types. Similar changes in LPS mutants of other rhizobial species have also been reported [2, 27]. The LPS pattern of ORS571-M8 was marginally altered, but O-antigen was maintained. In membrane protein profile, it showed loss of high molecular weight polypeptides with an altered pattern of low molecular weight polypeptides. In ORS571-M20, the smooth ladder- like LPS profile was lost (Fig. 1). The drastically altered mutant phenotype indicates pleiotropic effect, which needs to be substantiated through future, detailed biochemical investigations and growth kinetics. Its membrane protein profile also showed differences with

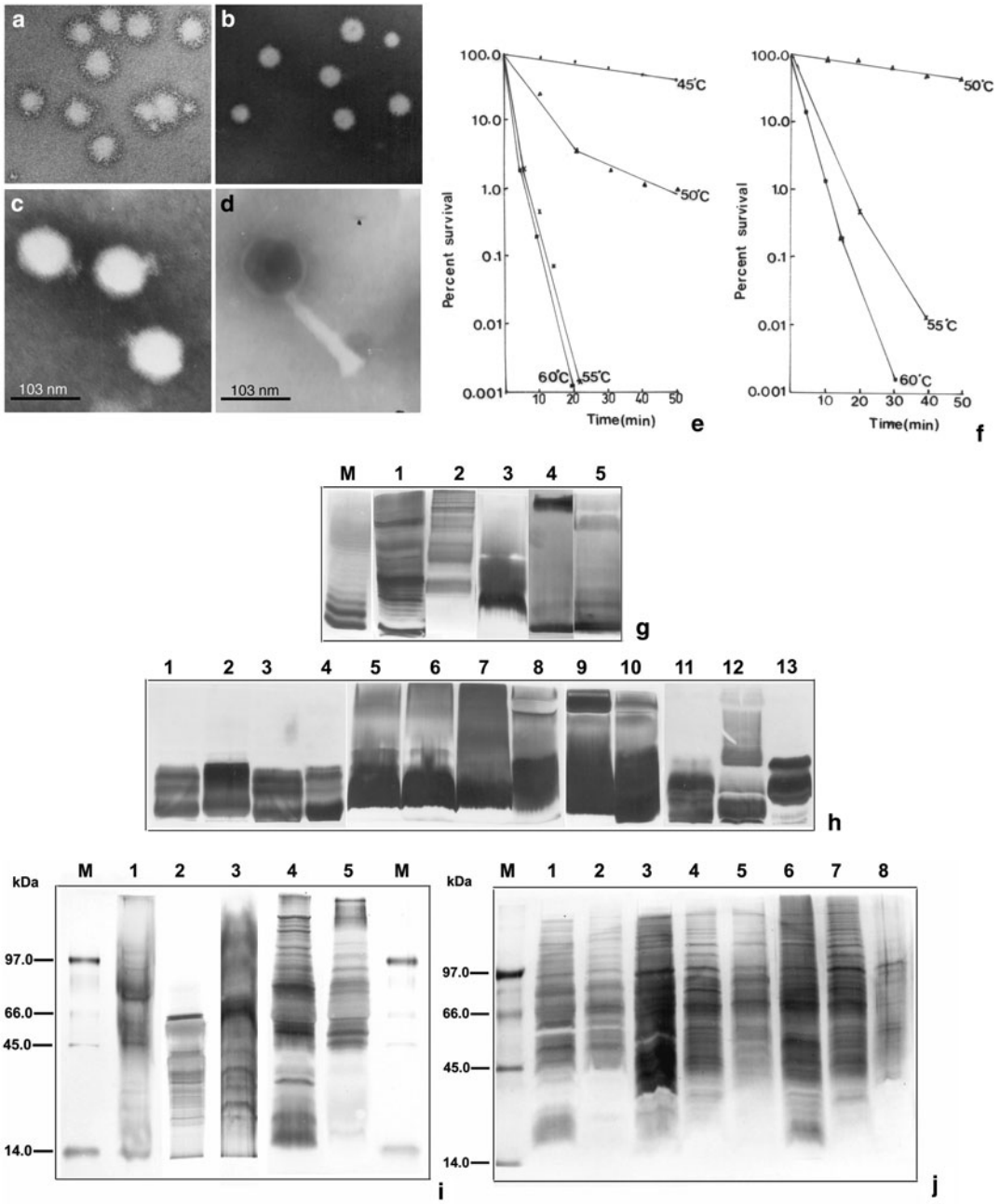

Fig. 1 Phenotypic characteristics of stem-nodulating bacteria of *Sesbania rostrata* and its phages. Photomicrographs of plaques of ACSR16 (semi-clear with halo formation) (**a**), and WESR29 (clear) (**b**). Electronmicrographs of 1% PTA-stained rhizobiophages, ACSR16 (*Podoviridae* short, non-contractile tail) (**c**), and WESR29 (*Myoviridae* a long contractile tail with a distinct neck region) (**d**) (both the virion particles are non-enveloped and possessed icosahedral head (WESR29: head size 110-nm length, 106-nm diameter; ACSR16: 76-nm length, 76-nm diameter). Thermal inactivation pattern of ACSR16 (**e**), and WESR29 (**f**) phage isolates. Variations in SDS-PAGE profiles of outer membrane characteristics (LPS and proteins). LPS profiles of wild type and their transconjugants: (**g**) *Salmonella* (reference) (*lane M*), ORS571 (*A. caulinodans*) (*lane 1*), ORS571-M8 (*lane 2*), ORS571-M20 (*lane 3*), WE7 (*Rhizobium* sp.) (*lane 4*) and WE7-M9 (*lane 5*); and standard strains of *Rhizobium* and its allies: (**h**) *S. meliloti* 2011 (*lane 1*), *S. teranga* USDA4894 (*lane 2*), *S. meliloti* USDA1002 (*lane 3*), *S. fredii* USDA205 (*lane 4*), *R. galegae* HAMBI 1143 (*lane 5*), *R. galegae* HAMBI 1141 (*lane 6*), *R. galegae* HAMBI 540 (*lane 7*), *S. meliloti* L5-30 (*lane 8*), *A. tumefaciens* (*lane 9*), *R. leguminosarum* biovar *trifolii* ANU794 (*lane 10*), *R. leguminosarum* biovar *viciae* VF39 (*lane 11*), *R. leguminosarum* biovar *trifolii* ANU1117 (*lane 12*) and *R. leguminosarum* biovar *trifolii* ANU843 (*lane 13*). Membrane protein profiles of wild type and their transconjugants: (**i**) ORS571 (*lane 1*), ORS571-M8 (*lane 2*), ORS571-M20 (*lane 3*), WE7 (*Rhizobium* sp.) (*lane 4*) and WE7-M9 (*lane 5*) and standard strains (**j**) *S. meliloti* 2011 (*lane 1*), *S. meliloti* L5-30 (*lane 2*), *R. leguminosarum* biovar *trifolii* ANU843 (*lane 3*), *R. leguminosarum* biovar *trifolii* ANU794 (*lane 4*), *R. leguminosarum* biovar *trifolii* ANU1117 (*lane 5*), *R. galegae* HAMBI 540 (*lane 6*), *R. galegae* HAMBI 1141 (*lane 7*), *R. leguminosarum* biovar *viciae* VF39 (*lane 8*) and molecular weight marker (*lane M*)

respect to the intensity and number of polypeptides. In WE7-M9, the mobility and thickness of high molecular weight LPS band was altered (Fig. 1). Similarly, in membrane proteins, the expression of high molecular weight polypeptides was reduced, and certain low molecular weight polypeptides were lost. These observations indicate that the phage resistance in mutants might have correlation with altered wall characteristics (LPS and membrane protein) of ORS571 and WE7. Differences observed in the LPS profiles of ORS571-M8, WE7-M9 and their wild types might be due to variation in the degree of polymerization of O-antigen. Similar changes in the LPS of *A. caulinodans* mutant ORS571-oac have been attributed to a non-polar mutation in the *oac*2 gene that encodes thymidine 5′-diphosphate (dTDP)-L-rhamnose synthase [8].

Phage susceptibility of the nodulating bacteria was strongly correlated with phage adsorption ability of the LPS-membrane protein fraction. In in vitro assay, purified LPS-membrane protein fraction (4 μg) of ORS571 showed 100% adsorption of ACSR16 but failed to adsorb WESR29 phage even in a high dose (up to 50 μg). Similarly, purified LPS-membrane protein fraction (6 μg) of WE7 showed 100% adsorption of WESR29 phage only. LPS-membrane protein fraction (up to 50 μg) of the mutants (ORS571-M8, ORS571-M20 and WE7-M9) and standard strains neither adsorbed the ACSR16 nor the WESR29 phage isolate. These observations indicate that the LPS-membrane protein complex might be involved in phage susceptibility and specificity amongst different species of rhizobia. In a recent study, a large variability in host strain susceptibility and phage host range in *Flavobacterium* hosts has been attributed to variations in their LPS characteristics [9]. The wide host range of P1 bacteriophage for enterobacteria has also been suggested because of LPS core oligosaccharide [24].

Tolerance to Hydrophobics and Variations in Outer-membrane Characteristics of Wild Types and Mutants

The wild types showed high tolerance towards various hydrophobics tested (50 μg/ml rifampicin, 2.0% deoxycholoate, 2.5% triton X-100 and 1.0 ppm crystal violet). The tolerance was significantly reduced in ORS571-M20 (2.0 μg/ml rifampicin, 0.2% deoxycholoate, 0.3% triton X-100 and 0.05 ppm crystal violet) as compared to the WE7-M9 (15 μg/ml rifampicin, 0.5% deoxycholoate, 0.6% triton X-100 and 0.1 ppm crystal violet). However, the tolerance was unaltered in ORS571-M8. The tolerance of wild types and mutants to hydrophobic compounds was positively correlated with the expression of O-antigen of LPS. Mutations that lead to structural defects in the outer membrane often increase outer membrane permeability and sensitivity of cell to hydrophobic antimicrobials [15, 25]. The membrane proteins that have been shown to transport hydrophobic compounds belong to the FadL transporter family [26]. Recently, in *R. leguminosarum* bv. *viciae* VF39SM, the involvement of RopB outer membrane protein in tolerance to hydrophobics has been suggested [7]. This protein imparts stability to outer membrane by interacting with LPS. In stem-nodulating bacteria, LPS-protein complexes seem to play an important role in determining sensitivity towards hydrophobics. However, the presence of O-antigen (altered or unaltered) is must which is in contrast with the recent finding in root-nodulating bacteria, where involvement of lipid A has been emphasized [27]. Maintenance of tolerance to hydrophobic compounds in phage-resistant mutant ORS571-M8 suggests that phage recognition needs a specific pattern of LPS-membrane protein. To have a better understanding about these processes and general underlying mechanisms of abiotic and biotic stresses, other legume-nodulating bacteria need to be further investigated. This study identifies a model system for a detailed molecular investigation on rhizobial sensitivity towards phages and hydrophobic compounds as they form stem nodules and, in contrast to root nodulating bacteria, their ecology is less understood.

In conclusion, variation in outer membrane characteristics (LPS-protein complex) affects the ability of stem-nodulating bacteria to tolerate biotic and abiotic stresses. Unlike phage specificity, tolerance to hydrophobic compounds does not require any specific pattern of LPS and has a correlation with the expression of O-antigen. Such studies form the basis to determine the factors responsible for varying efficacy of rhizobial inoculation technologies under stress environment.

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References

1. Becker A, Fraysse N, Sharypova L (2005) Recent advances in studies on structure and symbiosis-related function of rhizobial K-antigens and lipopolysaccharides. Mol Plant Microbe Inter 18:899–905
2. Bhattacharya I, Das HR (2003) Cell surface characteristics of two halotolerant strains of *Sinorhizobium meliloti*. Microbiol Res 158:187–194
3. Burleson FG, Chambers TM, Wiedbrank DL (1992) Virology a laboratory manual. Academic Press Inc., London
4. Cooper JE, Rao JR (2007) Molecular approaches to soil, rhizosphere and plant microorganism analysis. CABI Publishing, Wallingford
5. de Lajudie P, Bogusz D (1984) Isolation and characterization of two bacteriophages of a stem-nodulating *Rhizobium* strain from *Sesbania rostrata*. Can J Microbiol 30:521–525
6. de Maagd RA, van Rossum C, Lugtenberg BJJ (1988) Recognition of individual strains of fast-growing rhizobia by using

profiles of membrane proteins and lipopolysaccharides. J Bacteriol 170:3782–3785
7. Foreman DL, Vanderlinde EM, Bay DC, Yost CK (2010) Characterization of a gene family of outer membrane proteins (*ropB*) in *Rhizobium leguminosarum* bv. *viciae* VF39SM and the role of the sensor kinase ChvG in their regulation. J Bacteriol 192: 975–983
8. Gao M, D'Haeze W, De Rycke R, Wolucka B, Holsters M (2001) Knockout of an azorhizobial dTDP-L-rhamnose synthase affects lipopolysaccharide and extracellular polysaccharide production and disables symbiosis with *Sesbania rostrata*. Mol Plant Microbe Interact 14:857–866
9. Holmfeldt K, Middelboe M, Nybroe O, Riemann L (2007) Large variabilities in host strain susceptibility and phage host range govern interactions between lytic marine phages and their *Flavobacterium* hosts. Appl Environ Microbiol 73:6730–6739
10. Jaiswal SK, Dhar B (2010) Morphology and general characteristics of phages specific to *Lens culinaris* rhizobia. Biol Fert Soils 46:681–687
11. Lindstrom K, Lipsanen P, Kaijalainen S (1990) Stability of markers used for identification of two *Rhizobium galegae* inoculant strains after 5 year in the field. Appl Environ Microbiol 56:444–450
12. Masson-Bovin C, Giraud E, Batut J (2009) Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? Trends Microbiol 17:458–466
13. Mishra V, Sharma RS, Yadav S, Babu CR, Singh TP (2004) Purification and characterization of four isoforms of Himalayan mistletoe ribosome inactivating protein from *Viscum album* having unique sugar affinity. Arch Biochem Biophys 423:288–301
14. Mohmmed A, Sharma RS, Ali S, Babu CR (2001) Molecular diversity of the plasmid genotypes among *Rhizobium* gene pools of sesbanias from different habitats of a semi-arid region (Delhi). FEMS Microbiol Lett 205:171–178
15. Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593–656
16. Olsson JE, Rolfe BG (1985) Stem and root nodulation of the tropical legume *Sesbania rostrata* by *Rhizobium* strains ORS-571 and WE7. J Plant Physiol 121:199–210
17. Putnoky P, Petrovics G, Kereszt A, Grosskopf E, Ha DT, Banfalvi Z, Kondorosi A (1990) *Rhizobium meliloti* lipopolysaccharide and expopolysaccharide can have the same function in the plant-bacterium interaction. J Bacteriol 172:5450–5548
18. Rasanen LA, Lindstrom K (1997) Stability of short and long O-chain lipopolysaccharide types in *Rhizobium galegae* and their correlation with symbiotic properties and growth conditions, tolerance of low pH, aluminum and salt in the growth medium. FEMS Microbiol Lett 155:17–22
19. Senthilkumar M, Madhaiyan M, Sundaram SP, Kannaiyan S (2009) Intercellular colonization and growth promoting effects of *Methylobacterium* sp. with plant-growth regulators on rice (*Oryza sativa* L. cv CO-43). Microbiol Res 164:92–104
20. Sharma RS, Mishra V, Mohmmed A, Babu CR (2008) Phage specificity and lipopolysachharides of stem- and root-nodulating bacteria (*Azorhizobium caulinodans*, *Sinorhizobium spp*., *and Rhizobium spp*.) of *Sesbania* spp. Arch Microbiol 189:411–418
21. Sharma RS, Mohmmed A, Babu CR (2002) Diversity among rhizobiophages from rhizospheres of legumes inhabiting three ecogeographical regions of India. Soil Biol Biochem 34:965–973
22. Sharma RS, Mohmmed A, Mishra V, Babu CR (2005) Diversity in promiscuous group of rhizobia from three *Sesbania spp*. colonizing ecologically distinct habitats of the semi-arid Delhi region. Res Microbiol 156:57–67
23. Singh RB, Dhar B, Singh BD (1980) Morphology and general characteristics of viruses active against cowpea *Rhizobium* CB 756 and 32H1. Arch Virol 64:17–24
24. Tomas JM, Kay WW (1984) Effect of bacteriophage P1 lysogeny on lipopolysaccharide composition and the lambda receptor of *Escherichia coli*. J Bacteriol 159:1047–1052
25. Vaara M (1993) Outer-membrane permeability barrier to azithromycin, clarithromycin, and roxithromycin in Gram-negative enteric bacteria. Antimicrob Agents Chemother 37:354–356
26. van den Berg B, Black PN, Clemons WM Jr, Rapoport TA (2004) Crystal structure of the long-chain fatty acid transporter FadL. Science 304:1506–1509
27. Vanderlinde EM, Muszyn'ski A, Harrison JJ, Koval SF, Foreman DL, Ceri H, Kannenberg EL, Carlson RW, Yost CK (2009) *Rhizobium leguminosarum* biovar *viciae* 384, deficient in 27-hydroxyoctacosanoate-modified lipopolysaccharide, is impaired in desiccation tolerance, biofilm formation and motility. Microbiology 155:3055–3069
28. Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989