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SHORT COMMUNICATION

Phage specificity and lipopolysaccarides of stem- and root-nodulating bacteria (*Azorhizobium caulinodans*, *Sinorhizobium* spp., and *Rhizobium spp*.) of *Sesbania spp*.

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Abstract Phage susceptibility pattern and its correlation with lipopolysaccharide (LPS) and plasmid profiles may help in understanding the phenotypic and genotypic diversity among highly promiscuous group of rhizobia nodulating *Sesbania spp.*; 43 phages were from two stem-nodulating bacteria of *S. rostrata* and 16 phages were from root-nodulating bacteria of *S. sesban*, *S. aegyptica* and *S. rostrata*. Phage susceptibility pattern of 38 *Sesbania* nodulating bacteria was correlated with their LPS rather than plasmid profiles. Different species of bacteria (*A. caulinodans*- ORS571, SRS1-3 and *Sinorhizobium saheli*-SRR907, SRR912) showing distinct LPS subtypes were susceptible to different group of phages. Phages could also discriminate the strains of *Si. saheli* (SSR312, SAR610) possessing distinct LPS subtypes. Phages of *Si. meliloti* (SSR302) were strain-specific. All the strains of *R. huautlense* having incomplete LPS (insignificant O-chain) were phage-resistant. In in vitro assay, 100% of the phages were adsorbed to LPS of indicator bacterium or its closely related strain(s) only. These observations suggest the significance of LPS in phage specificity of *Sesbania* nodulating rhizobia. Highly specific phages may serve as biological marker for monitoring the susceptible bacterial strains in culture collections and environment.

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Introduction

Sesbania spp. can successfully colonize extremely diverse habitats and enrich the nutrient deficient soils with nitrogen (146–267 kg N/ha) due to its association with the micro-symbiont (rhizobia). *Sesbania sesban*, *S. aegyptica* and *S. rostrata* are among the major agro-forestry species in India. Among these *S. rostrata* also forms profuse stem-nodules besides the root nodules (Dreyfus and Dommergues 1981). High-ecological amplitude and habitat processing ability of *Sesbania spp.* have been attributed to the highly promiscuous nature of the associated rhizobia (Sharma et al. 2005). Polyphasic methods might be useful to characterize this promiscuous group of rhizobia (Mohmmed et al. 2001). Phage sensitivity pattern has been suggested as one of the powerful methods to discriminate bacteria as: (1) it takes into account the phenotypic and genotypic traits (Lesley 1982; Lindstrom and Lehtomaki 1988); (2) it is comparable to serogroups and is powerful even in the cases where strainal differences are due to the poor immunogenicity of antigens (Sechter et al. 2000); (3) it gives fast and repeatable results for large collection of isolates (Lesley 1982; Wildemauwe et al. 2004); (4) it is economical but still comparable to DNA/genome typing (Lindstrom et al. 1983); (5) it can delimit taxa and assess the genetic relatedness among bacteria (Lindstrom and Lehtomaki 1988); (6) it serves as a stable marker for monitoring of rhizobia and other clinical isolates in the environment through time (5–23 years) and space (Olsson et al. 1984; Lindstrom et al. 1990); (7) it is useful for the

long-term monitoring of purity of the bacterial germplasm collection (Wildemauwe et al. 2004); and (8) it can discriminate and help in the selection of different ecological functional groups of bacteria (Withey et al. 2005).

Specific bacteriophages may serve as biological marker for identification of the particular bacterial strains. The wall associated diversity might have a significance in determining the host specificity of phages (Defives et al. 1996). Outer membrane bound lipopolysaccharides and proteins serve as receptor for bacteriophages (Werts et al. 1994). Plasmid profiles have also been correlated with phage susceptibility in other bacteria (Holmberg et al. 1984). In the present study, phage sensitivity pattern of stem- and root-nodulating bacteria from three *Sesbania spp.* was determined. Correlation of phage susceptibility with LPS and plasmid profiles of bacterial isolates was also examined.

Materials and methods

Bacterial isolates

Five stem nodulating bacterial strains of *Sesbania rostrata* (ORS 571, WE7, SRS1, SRS2, and SRS3) and 33 root-nodulating strains of *Sesbania sesban*, *S. aegyptica* and *S. rostrata* were used. The bacterial strains were maintained on yeast extract mannitol (YEM) and yeast extract lactate (YEL) media (Dreyfus and Dommergues 1981). The taxonomic identity of the isolates was previously determined by 16S rDNA sequence analyses (Sharma et al. 2005).

Phage isolation and host range

Rhizosphere soil (2 g) of different leguminous species was used as a source of phage to infect the actively growing broth culture of indicator bacterial strains (ORS 571, WE7, SSR302, SSR312, SAR608, and SRR907). Phages were enriched by repeated inoculation of 0.2 μm filtered medium (phage lysate) in the fresh bacterial culture (Sharma et al. 2002). Plaque assay was carried out and observations on presence and type of plaque were recorded (Table 1). Purified phage isolates were concentrated with differential centrifugation, negatively stained (1% PTA) and then examined under transmission electron microscope (Philips 300, 80 kV). Host range of the phage isolates was assessed by plaque assay.

Plasmid profiles

Plasmid profiles of bacterial isolates were analyzed by modified Eckhardt's `in well lysis' method (Plazinski et al. 1985) with two-comb system. The molecular masses of these plasmids were determined by calibrating against the relative mobility of plasmids of known molecular masses.

Preparation of LPS samples, electrophoresis and silver staining

The LPS fraction was purified by standard protocol (Sharma et al. 2005). The bacterial cell pellet obtained at late-log phase was washed with phosphate-buffered saline (10 mM phosphate buffer, 0.9% NaCl, pH 7.4) and suspended in 50 mM Tris–HCl, pH 8.5. The cells were disrupted by sonication (3 min) and centrifuged at 1,000*g* for 20 min. Supernatant was treated with lysozyme (0.2 mg/ml) for 30 min at room temperature. KCl (2 M) was added to a final concentration of 0.2 M. The cell envelope was pellet down by centrifugation at 12,000*g* for 60 min at 4°C and washed with sterile distilled water. The pellet was resuspended in 200 μl of 2 mM Tris–HCl, pH 7.8 and treated with proteinase K (0.2 mg/ml) at 60°C for 60 min. LPS sample was appropriately diluted with sample buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 4% SDS and 30% sucrose) and ~10 μg sample was loaded onto the gel. *Salmonella* LPS (Bacto Laboratories, Difco Company, NSW, Australia) was used as a reference for comparison. Electrophoresis was carried out using 12–4% SDS polyacrylamide separating-stacking slab gel system (Mishra et al. 2004). Gels were fixed overnight in fixing solution (25% iso-propanol in 7% acetic acid) and LPS was localized by Ag-LPS preferential staining method (Sharma et al. 2005).

In vitro phage adsorption ability of LPS

Purified LPS samples of stem- and root-nodulating bacteria of *Sesbania* spp. were suspended in 100 μl of assay solution (50 mM Tris–HCl, 100 μM $CaCl_2$, pH 7.5) for 2 h at 30°C (Putnoky et al. 1990). LPS samples (10–25 μg) were incubated with phage isolates (10^5 PFU) representing all plaque-morphotypes in all possible combinations. The phage particles adsorbed on LPS were pellet down by centrifugation at 12,000g for 10 min at 4°C. The number of unadsorbed phage particles in the supernatant was titred by plaque assay using indicator bacterial strains. Phage adsorption ability of LPS was expressed in terms of % PFU loss.

Results and discussion

Variations in *Sesbania* nodulating bacteria

Stem-nodulating bacteria of *Sesbania rostrata Azorhizobium caulinodans* (ORS 571) and *Rhizobium sp.* (WE7) were isolated from Senegal and India, respectively, (Dreyfus

Table 1 Details of phage isolates and their indicator strains

Indicator strain	Phage isolates	Phage morphotype	Plaque characteristics			Phage tail characteristics			Bradley's group	Phage family
			Size (mm)	Clarity	Halo formation	Tail length (mm)	Head length : tail length ratio	Contractility/ flexibility		
Stem nodulating										
Azorhiobium caulinodans (ORS 571)	ACSR304, 305, 351, 352	Fig. 2a	0.3	SC	+	18.0	4.22	Nc, Nf	C1	*Podoviridae*
	ACSR312, 370, 371	Fig. 2a	1.0	SC	+	18.0	4.22	Nc, Nf	C1	*Podoviridae*
	ACCJ109, 121, 123	Fig. 2a	0.3	SC	+	16.0	4.12	Nc, Nf	C1	*Podoviridae*
	ACTP481, 485	Fig. 2a	1.5	Cl	–	18.3	3.93	Nc, Nf	C1	*Podoviridae*
	ACAN413, 461, 462	Fig. 2a	1.0	Cl	–	14.0	5.28	Nc, Nf	C1	*Podoviridae*
	ACTP491, 494, 495	Fig. 2a	1.0	Cl	–	18.3	3.93	Nc, Nf	C1	*Podoviridae*
	ACPC341, 351, 352	Fig. 2a	0.5	Cl	+	17.0	3.88	Nc, Nf	C1	*Podoviridae*
	ACDS505, 551, 556, 557	Fig. 2a	0.2	SC	+	15.0	4.66	Nc, Nf	C1	*Podoviridae*
	ACAN401, 451, 453, 457	Fig. 2a	1.5	Cl	–	16.5	5.28	Nc, Nf	C1	*Podoviridae*
	ACSN1, 2, 3, 4, 5;	Fig. 2a	0.2	SC	+	18.0	4.22	Nc, Nf	C1	*Podoviridae*
	ACSN11, 12, 13, 14	Fig. 2a	1.0	SC	+	18.0	4.22	Nc, Nf	C1	*Podoviridae*
Rhizobium sp. (WE7)	WESR19, 20, 21, 22, 23	Fig. 2b	0.4	Cl	–	118.4	0.93	C, Nf	A1	*Myoviridae*
Root nodulating										
Sinorhizobium meliloti (SSR302)	SSSS304, 305, 307, 308	Fig. 2e	0.5	Cl	–	113.3	0.63	Nc, Nf	B1	*Siphoviridae*
Sinorhiozobium saheli (SSR312)	SSSS313, 323, 325, 326	Fig. 2e	0.2	Cl	–	113.3	0.63	Nc, Nf	B1	*Siphoviridae*
Sinorhiozobium saheli (SAR610)	SASA605, 606, 607, 621	Fig. 2c	0.2	SC	–	134.0	1.17	C, Fl	B2	*Siphoviridae*
Sinorhiozobium saheli (SRR907)	SRSR 911, 912, 913, 914	Fig. 2d	0.3	SC	–	167.4	0.36	Nc, Fl	B1	*Siphoviridae*

SC semi-clear, *Cl:* clear, *Nc* non-contractile, *C* contractile, *Nf* non-flexible, *Fl* flexible

and Dommergues 1981; Olsson and Rolfe 1985). SRS1, SRS2 and SRS3 (*Azorhizobium caulinodans*) were variants of ORS 571, developed after repeated isolation (from stem-nodules) and re-introduction (in the next season) for 16 years in the University of Delhi experimental garden. SRS1-3 showed slightly lower symbiotic efficiency and effectiveness as compared to ORS571 (data not shown). They also showed variations in number and mobility of bands (<15%) in LPS profiles. ORS 571 and its variants lack plasmid and *nod* genes are chromosomally located. WE7 harbours two megaplasmids of 300 and 500 MDa (Olsson et al. 1984; Mohmmed et al. 2001). Thirty-three root nodulating bacteria have also been isolated from three *Sesbania spp*. (*S. sesban*, *S. aegyptica*, and *S. rostrata*) inhabiting six ecologically distinct sites of the Delhi region. Twenty-three isolates were from *S. sesban* inhabiting five sites viz. an experimental garden, an agricultural field, the Aravalli Hill, a fly-ash dump, and the flood plain of the Yamuna river. The isolates included different strains of *Sinorhizobium saheli*, *Si. meliloti* and *R. huautlense* (Table 2). Seven isolates from *S. aegyptica* inhabiting Asola-Bhatti wildlife sanctuary (Delhi) and experimental garden and three isolates from *S. rostrata* inhabiting experimental garden were strains of *Si. saheli*. Based on the variation in number (1–3) and size (30–500 MDa) of plasmids, the isolates were grouped under six plasmid profile classes (Table 2).

Electrophoretic profiles of LPS of *Sesbania* nodulating rhizobia are broadly comprised of slow-moving LPS I (complete LPS molecule) and fast-moving LPS II (incomplete LPS lacking O-antigen) moving close to the running front. To characterize micro-variations, LPS I was categorized

Table 2 Phage sensitivity pattern of *Azorhizobium caulinodans*, *Sinorhizobium saheli*, *Si. meliloti*, *Rhizobium huautlense*, *Rhizobium*. sp., their LPS types and plasmid profiles

Phage susceptible bacterial isolates	Plaque morphotypes (phage isolates)	Phage morphotype	LPS		Plasmid			Species
			Type	Subtype	No.	Size (MDa)	Profile class	
From stem nodules								
ORS571, SRS1, SRS2, SRS3	PM1 (ACSR304, 305, 351, 352)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM2 (ACSR312, 370, 371)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM3 (ACCJ109, 121, 123)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM4 (ACTP481, 485)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM5 (ACTP491, 494, 495)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM6 (ACAN401, 451, 453, 457)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM7 (ACAN413, 461, 462)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM8 (ACPC341, 351, 352)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM9 (ACDS505, 551, 556, 557)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
WE7	PM10 (WESR19, 20, 21, 22, 23)	Fig. 2b	C	C6	2	300, 500	VI	*Rhizobium sp.*
ORS571, SRS1, SRS2, SRS3	PM15 (ACSN1, 2, 3, 4, 5)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
ORS571, SRS1, SRS2, SRS3	PM16 (ACSN11, 12, 13, 14)	Fig. 2a	D	D1	Ab	–	–	*Azorhizobium cauliondans*
From root nodules								
SRR907, 912	PM11 (SRSR 911, 912, 913, 914)	Fig. 2d	D	D2	2	220, 110	III	*Sinorhizobium saheli*
SSR302	PM12 (SSSS304, 305, 307, 308)	Fig. 2e	C	C2	2	180, 60	II	*Sinorhizobium meliloti*
SSR312, 313	PM13 (SSSS313, 323, 325, 326)	Fig. 2e	C	C4	2	220, 110	III	*Sinorhizobium saheli*
SAR608, 610,	PM14 (SASA605, 606, 607, 621)	Fig. 2c	B	B4	2	220, 110	III	*Sinorhizobium saheli*
SSR321, 326, 327, 328	R	NA	A	A1	2	300, 110	IV	*Rhizobium huautlense*
SSR353, 354, 355, 356, 357	R	NA	A	A2	2	300, 110	IV	*Rhizobium huautlense*
SSR335, 336, 337	R	NA	B	B1	3	180, 90, 70	V	*Sinorhizobium meliloti*
SSR341, 342	R	NA	B	B2	2	300, 110	IV	NI
SSR361, 362	R	NA	B	B3	2	300, 110	IV	NI
SAR611	R	NA	B	B4	2	220, 110	III	*Sinorhizobium saheli*
SAR615, 617, 620, 621	R	NA	C	C1	2	220, 110	III	*Sinorhizobium saheli*
SSR329	R	NA	C	C3	2	300, 110	IV	*Rhizobium huautlense*
SRR915	R	*NA*	*C*	C3	2	220, 110	III	*Sinorhizobium saheli*
SSR304, 305	R	NA	C	C4	2	220, 110	III	NI
SSR301	R	NA	C	C5	1	250	I	*Sinorhizobium saheli*

R resistant to all phages; *NI* not investigated; *Ab* absent; *NA* not applicable

into: (1) zone I that comprised bands with high degree of polymerization; and (2) zone II that comprised bands with low degree of polymerization. Based on the presence or absence and continuity of zones I and II and the length of zone II, four major types of LPS (A–D) have been recognized (Fig. 1). Among them the stem-nodulating bacteria *A. caulinodans* (ORS 571 and SRS1-3) showed ladder like profile characteristic of type D, however *Rhizobium* sp. (WE7) showed distinct and widely separated discontinuous zones I and II like type C. The root-nodulating *Si. saheli* strains (SRR907 and 912) also showed type D LPS. Such patterns have been reported in *Sinorhizobium* sp. (Lagares et al. 2001). Type A was represented by *R. huautlense*, which is close to *R. galegae* (Lipsanen and Lindstrom 1989). Types B and C were common among different strains of *Si. saheli* and *Si. meliloti*.

Plaque characteristics and phage morphology

Plaque characteristics and morphology of 59 purified phage isolates were studied. ORS 571 phages (38 isolates) showed clear and semi-clear plaques with presence or absence of halo varying from 0.2 to 1.0 mm in size (Table 1). They formed six different types of plaques. In contrast, WE7 phages formed a distinct plaque type. Phages (16 isolates) of root nodulating bacteria of *S. sesban* (SSR302- *Si. meliloti*, SSR312- *Si. saheli*), *S. aegyptica* (SAR610- *Si. saheli*) and *S. rostrata* (SRR907- *Si. saheli*) also showed distinct plaque types. Transmission electron-micrographs revealed that all phage isolates were non-enveloped and tailed with icosahedral head symmetry. However, substantial differences were observed in their tail characteristics (length and width, flexibility and contractility, base plate, presence or absence or type of tail pins) and head size (Table 1; Fig. 2). All the phages of ORS 571 possessed a very short (14–18 nm) rigid tail with small thick spikes. It is in contrast with another phage (RS2) of ORS 571 reported from Senegal, having an indistinct tail and a base plate (deLajudie and Bogusz 1984). WE7 phages possessed complex rigid long tail (118 nm) consisting of a contractile sheath and a base plate with small thick spikes and a head separated from the tail by a distinct neck region. The head size of WE7 phages (110 nm length, 106 nm diameter) was bigger than that of ORS 571 phages (66–76 nm length, 65–76 nm diameter). Based on these characteristics, phages of ORS 571 and WE7 belonged to family *Podoviridae*

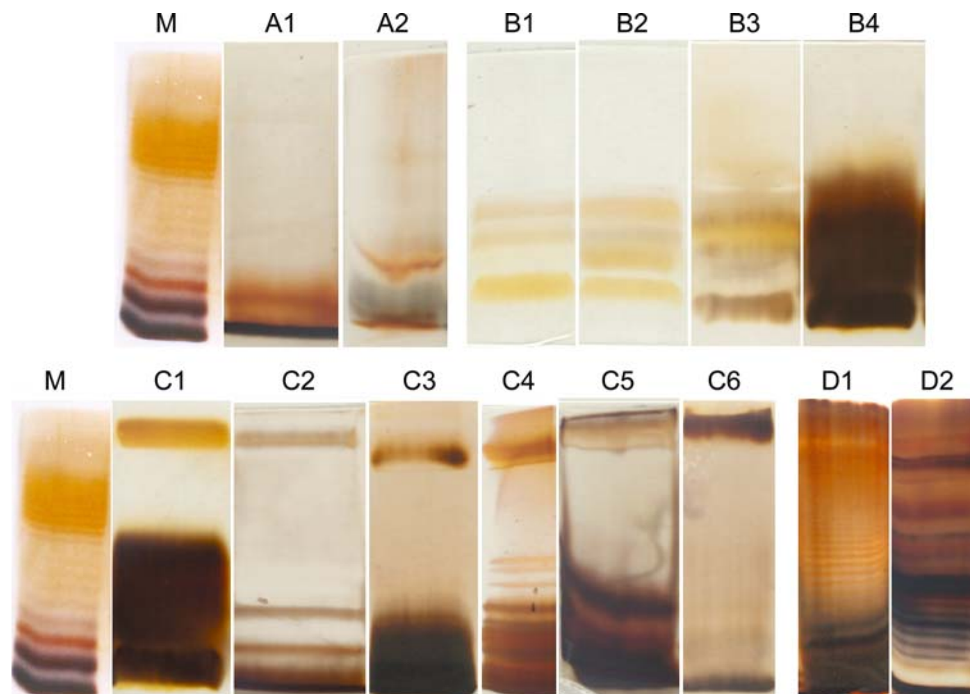

Fig. 1 Lipopolysaccharide types (4) and subtypes (14) of *Sesbania* nodulating bacteria categorized based on microvariations in LPS I region under SDS-PAGE analyses. Electrophoretic profiles are broadly comprised of slow moving LPS I (complete LPS molecule) and fast moving LPS II (incomplete LPS lacking O-antigen) moving close to the running front. LPS I was categorized into: (1) zone I comprised of bands with high degree of polymerization; and (2) zone II comprised of bands with low degree of polymerization. Type A showed a very short inconspicuous zone II, while in type B it was quite distinct and continuous. Type C had distinct and discontinuous zones I and II however type D showed continuous zones I and II. Under different LPS types, profiles showing <80% similarity in bands (zone II) were classified as different LPS subtypes. LPS type A and D included two subtypes each (A1: SSR328, A2: SSR353; D1: SRR907, and D2:ORS571), type B included four (B1: SSR337, B2: SSR342, B3: SSR362, and B4: SAR608) whereas type C included six subtypes (C1: SAR621, C2: SSR302; C3: SRR915; C4: SSR312, C5: SSR301; and C6: WE7). *Salmonella* LPS (M) was used as reference

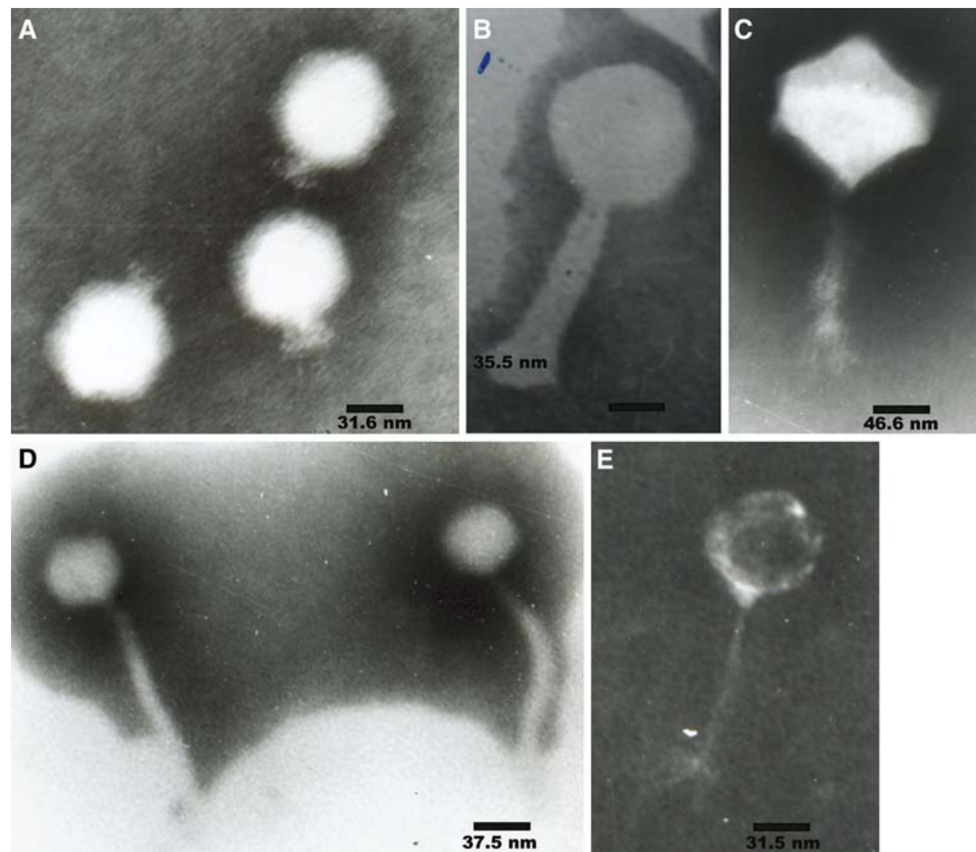

Fig. 2 Transmission electron-micrographs of negatively stained phages of *Azorhizobium caulinodans* (**a**); *Rhizobium sp.* WE7 (**b**); *Sinorhizobium saheli* SAR610 (**c**); *Sinorhizobium saheli* SRR907 (**d**); and *Sinorhizobium meliloti* SSR302 (**e**)

Group C1 and *Myoviridae* Group A1, respectively (Ackermann 2001). Another phage of ORS 571 (RS 1) reported from Senegal also belonged to *Myoviridae* (deLajudie and Bogusz 1984). *Myoviridae* members among cyanophages are known to have a broad host range (Lu et al. 2001) but it does not appear to hold true for phages of *Sesbania* nodulating bacteria. Phages of different strains of *Si. saheli* (SSR312, SRR907, and SAR610) had non-contractile tail and belonged to family *Siphoviridae*. Based on the tail characteristics (Table 1), phages of SSR312 and SRR907 belonged to Bradley's Group B1. However SAR610 belonged to Group B2. Phages of *Si. meliloti* strain (SSR302) also belonged to *Siphoviridae* Group B1.

Three plaque characteristics (size, clarity, halo formation) and five morphological traits (head length and diameter; tail sheath length and width; and tail fiber) were used in all pair-wise combinations for Euclidean distance coefficient and the homology among the phage isolates was assessed. All the phages were segregated into 16 plaque morphotypes (PM) (Table 2). *A. caulinodans* (ORS 571) showed the highest diversity and 38 phage isolates were segregated into 11 plaque-morphotypes (PM1-9; PM15-16). Phage isolates of different strains of *Si. saheli* (SSR312, SAR610, SRR907), *Si. meliloti* (SSR302) and *Rhizobium* sp. (WE7) showed one plaque morphotype each (Table 2).

Phage specificity and lipopolysaccharides (LPS)

Using 59 phage isolates, phage specificity among stem- and root-nodulating bacteria of *Sesbania spp.* was determined. All the phages of ORS 571 could also infect other strains of *A. caulinodans* (SRS1, SRS2, and SRS3). However they did not infect stem-nodulating *Rhizobium* sp. (WE7) (Table 2). Likewise, phages of WE7 failed to infect *A. caulinodans* strains. These phage isolates also failed to infect any of the root nodulating strains of *Si. saheli*, *Si. Meliloti*, and *R. huautlense*. The phages of *S. meliloti* (SSR302) were strain-specific. Phage isolates of *Si. saheli* strains (SSR312, SAR610, SRR907) could discriminate the strains isolated from different species of *Sesbania*, for example phages of SSR312 (from *S. sesban*) failed to infect other strains from *S. aegyptica* (SAR610) and *S. rostrata* (SRR907). The single alternate bacterial hosts (SSR313, SAR608, and SRR912) of these phages were closely related strains of *Si. saheli* from the same *Sesbania* sp. and shared plasmid profile class and LPS subtype with the indicator bacteria (Table 2). The mean nodule number and symbiotic efficiency of the alternate host was also comparable to the indicator bacteria (Sharma et al. 2005). In spite of highly promiscuous nature of *Sesbania* nodulating bacteria the phages were highly specific. Host specificity of these phages makes them useful to discriminate inter- and

intra-specific variations among *Sesbania* nodulating rhizobia. These specific phages can be used for monitoring the purity of culture in in vitro conditions. They also have a potential to be used as biological marker for spatio-temporal tracking of their host bacterium into the environment. In fact, the phages having high specificity to *R. galegae* were used as stable markers comparable to the molecular markers for monitoring the host bacterial strains even after 5 years in the field (Lindstrom et al. 1990).

Variations in host specificity have been suggested due to wall-associated diversity of host bacterium (Holmberg et al. 1984). LPS O-antigen appears to be associated with the phage susceptibility in *Sesbania* nodulating bacteria. All the eight strains of *R. huautlense* representing LPS type A with insignificant O-chain, were phage-resistant (Table 2). In contrast, all the four strains of *A. caulinodans* (ORS571, SRS1-3) and two strains of *Si. saheli* (SRR907, 912) with repeating units of O-antigen represented by LPS Type D were phage-susceptible. Involvement of O-antigen in phage recognition has also been shown by other workers (Defives et al. 1996). Different strains of *Si. saheli*, *Si. meliloti*, *R. huautlense*, *Rhizobium* sp., and other isolates with LPS type B and C represent both phage susceptible and resistant phenotypes.

The grouping of bacterial isolates based on plaque morphotypes strongly correlates with that of LPS subtypes (except C4 subtype). For example, all the isolates representing one plaque morphotype shared similar LPS subtype. Different strains of *R. huautlense* and other isolates of plasmid class IV having mega-plasmid of 300 MDa (except WE7 of plasmid class VI) were phage-resistant. However, plasmid class III included both phage susceptible (SSR312, 313, SAR608, 610) and resistant strains (SAR611, 615, 617, and SRR915) of *Si. saheli*. In fact, phage susceptible strains of *Si. saheli* were heterogeneous, as SRR907, 912; SSR312, 313; and SAR608, 610 were susceptible to distinct group of phages (PM11, PM13, and PM14). This is in contrast with the correlation shown between phage susceptibility and plasmid profiles in other bacteria (Holmberg et al. 1984). Phage susceptibility has been shown to be a plasmid-borne trait (Jun et al. 1993). Lack of such correlation in *Sesbania* nodulating bacteria might be due to high rate of genome flux observed in the promiscuous group (Mohmmed et al. 2001). Therefore, phage specificity in conjunction with micro-heterogeneity in LPS profiles (LPS subtypes) can easily discriminate the cryptic genetic diversity present within the same plasmid class.

The effect of alterations in surface polysaccharides (lipo-, exo-, and capsular-polysaccharides) of *A. caulinodans* (ORS 571) on its hydrophobicity and ability to establish effective symbiosis with *S. rostrata* has been analyzed (Goethals et al. 1994; Gao et al. 2001; D'Haeze et al. 2004). In the present study in vitro phage adsorption ability of purified LPS was tested. A 10 µg of LPS of ORS 571 and WE7 adsorbed 95 and 90% of their homologous free phages, respectively. However, 12 µg LPS of ORS 571 and 14 µg LPS of WE7 lead to 100% adsorption (expressed in terms of complete failure of infection during plaque assay) of homologous phages. LPS failed to adsorb the heterologous phage isolates in cross-reaction study. In case of root nodulating indicator bacterial strains, 10 µg of LPS could adsorb 100% of their homologous phages. LPS of indicator strains and alternate hosts showed similarity in the phage adsorption ability. However, the isolates lacking plaque formation did not show any phage adsorption on their purified LPS. These observations indicate the involvement of LPS in phage recognition among *Sesbania* nodulating rhizobia. LPS alone and LPS–protein complex have been shown to serve as receptors in rhizobia and other Gram-negative bacteria (Defives et al. 1996). These phages with high specificity have potential to serve as biological tools for monitoring the purity of specific rhizobial strains and tracking them in environment.

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