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EXPERIMENTAL ARTICLES

Analysis of *Azospirillum brasilense* Plasmid Loci Coding for (Lipo)Polysaccharides Synthesis Enzymes

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Abstract—Lipopolysaccharides LpsI and LpsII containing the same O-specific polysaccharide (OPS), yet different in antigenic structure and charge, have been revealed in the rhizobacterium *Azospirillum brasilense* Sp245. In the present work, four putative glycosyltransferase genes were identified in a 14-kbp fragment of a 120-MDa plasmid of Sp245, p120-*lps*KM348X. Insertional mutagenesis of one of them, encoding the predicted ADP–heptose:LPS–heptosyltransferase, resulted in LpsI loss. By means of DNA hybridizations and PCR with primers specific towards several sites of p120-*lps*KM348X, it was demonstrated that homologous segments of 120-MDa plasmids of *A. brasilense* strains Sp245 and Sp107, which are characterized by identical structures of the OPS repeating units, are practically identically organized. In an 85-MDa plasmid of Sp245, a locus was identified with high homology to the plasmid genes of glycosyltransferases and conserved membrane-bound proteins from a wide range of soil bacteria.

Key words: Azospirillum brasilense, plasmid genes, lipopolysaccharides, glycosyltransferases, insertional mutations.

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Lipopolysaccharides (LPSs) are the major cell wall polymers of *Azospirillum brasilense*; they mediate the first stages of the associative interaction between these bacteria and host plants [1, 2]. Bacterial LPSs consist of the lipid A, the core oligosaccharide, and an O-specific polysaccharide (OPS) [3]. Recently, the structure of the OPS repeating units in *A. brasilense* Sp245, SR75, Sp107, and a number of other strains was determined [2, 4–6]. We found no data on the structures of lipid A and core of *A. brasilense* LPS in the literature.

So far, there have been few studies on the genetic aspects of LPS formation in azospirilla. As in many other alpha-proteobacteria, large plasmids constitute a considerable part of the multicomponent genome of azospirilla. *In silico* analysis of the nucleotide sequence of a 90-MDa plasmid (pRhico) of *A. brasilense* Sp107 revealed numerous open reading frames (or *orf*), the predicted products of which seem to participate in polysaccharide (PS) biosynthesis and export [7].

Fine differences in the structure and charge of OPS and/or core between LpsI and LpsII of *A. brasilense* Sp245 were detected by immunochemical reactions with polyclonal antibodies against LPSs and by ion-exchange chromatography [2, 8]. Meanwhile, OPSs of LpsI and LpsII consist of identical repeating units, each containing five D-rhamnose (D-Rha) residues [4]. Using an artificial transposon, Omegon-Km, mutants of *A. brasilense* Sp245 incapable of synthesis of either LpsI or LpsII were obtained. In all the mutants, single Omegon-Km insertions were localized in different sites of a 120-MDa plasmid (p120) of *A. brasilense* Sp245 [8].

The aim of this work was to characterize the plasmid loci coding for the polysaccharide biosynthesis enzymes in *A. brasilense* Sp245 and in the serologically related strain Sp107.

MATERIALS AND METHODS

Bacterial strains, plasmids, and cultivation conditions. The strains and plasmids used in the work are presented in Table 1. *Escherichia coli* was grown in Luria–Bertani (LB) medium [13] at 37°C, while *A. brasilense* was grown on malate–salt medium [16] supplemented with 1 g/l NH_4Cl (MSM) at 30°C. When needed, kanamycin (Km) and ampicillin (Ap) were added to the medium to the final concentration of 50 μg/ml.

DNA treatment techniques, nucleotide and amino acid sequence analysis. Plasmid DNA isolation and purification, hydrolysis by restriction endonucleases, gel-electrophoresis, elution of DNA fragments from agarose gels, DNA ligation, and *E. coli* cell transformation were performed according to the standard procedures [13]. Peroxidase labeling of DNA and Southern hybridization under strict conditions using an

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Table 1. Bacterial stains and plasmids used in the work

Strain or plasmid	Characteristics	Source
Strains:		
A. brasilense:		
Sp245	Wild type, isolated from surface-sterilized wheat roots in Brazil	[9]
Sp7	Wild type, isolated from *Digitaria decumbens* rhizosphere in Brazil	[10]
Sp107	Wild type, isolated from wheat roots in Brazil	[11]
SR75	Wild type, isolated from wheat seedlings in Russia	[12]
Escherichia coli:		
DH1	*supE44*, *hsdR17*, *recA1*, *endA1*, *gyrA96*, *thi-1*, *relA1*. Recipient of pOmegon-Km-*lps*348X, pOmegon-Km-*fla*048X, and pEK248X	[13]
TOP10	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lac* Δ*M15* Δ*lacX74 deoR recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL*(Sm^R) *endA1 nupG*. Recipient of pGEM-4Z and its derivatives	Promega, United States
Plasmids:		
pGEM-4Z	Contains promoters of SP6 and T7 bacteriophages; 2871 bp, Ap^R. Used for cloning of Omegon-Km and a 2.4-kbp *Eco*RI fragment of pEK248X	Promega, United States
pOmegon-Km-*lps*348X	Self-ligated Km^R *Xho*I fragment of p120 with an insertion of Omegon-Km from *A. brasilense* KM348 (an $LpsI^-$ mutant of Sp245), 17.8 kbp	[8]
pOmegon-Km-*fla*048X	Self-ligated Km^R *Xho*I fragment of p120 with an insertion of Omegon-Km from *A. brasilense* SK048 (an immotile mutant of Sp245), 12.1 kbp	[14]
pEK248X	Self-ligated Km^R *Xho*I fragment of p85::pJFF350 from *A. brasilense* SK248 (an immotile mutant of Sp245), ~25 kbp	[15]

enhanced chemiluminescence (ECL) gene detection system were performed according to the manufacturer's (Amersham Pharmacia Biosciences) recommendations. Recombinant plasmids, pOmegon-Km-*lps*348X and pEK248X, containing the fragments of p120 and p85 were sequenced by primer walking [13] in forward and reverse directions. The templates for sequencing were prepared using a Wizard Plus SV Minipreps (Promega, United States) DNA purification system. DNA sequencing was performed on an ABI Prism 3130 Avant automated DNA sequence analyzer (Applied Biosystems, United States) according to the manufacturer's instructions. Since the nucleotide sequence of the Omegon-Km transposon was unavailable [17], we sequenced its ends. Sequencing of p120 DNA flanking the omegon insertion in pOmegon-Km-*lps*348X was initiated using primers to the transposon ends, OmeF212 (5'-TCGGGCCT-TGATGTTACC-3') and OmeRuni (5'-AGGCTG-GCTTTTTCTTGTTATCG-3'). Sequencing of pEK248X was started from its 2.4-kbp *Eco*RI fragment subcloned in pGEM-4Z (Promega, United States). In the latter case, first sequencing reactions were performed with the primers M13F (5'-TTCAGGGAGC-CTGCGGTCC-3') and M13R (5'-AACAGCTAT-GACCATG-3'). For further rounds of pOmegon-Km-*lps*348X and pEK248X sequencing, oligonucleotides complementary to the DNA region at least 70 bp distant from the 3'-end of the sequence determined in the previous stage were used. Nucleotide sequences were translated in six possible reading frames and all *orf* of more than 100 bp were analyzed. Nucleotide and amino acid sequences were analyzed with the BLAST (URL http://www.ncbi.nlm.nih.gov/blast) and Fasta (URL http://www.ebi.ac.uk/fasta33) algorithms. Position of *orf* start codons was determined according to the distance from predicted Shine–Dalgarno (SD) sequences and to the results of alignment of corresponding amino acid sequences with similar proteins found in the databases (when possible). The properties of *orf* protein products were studied using the tools available on the following servers: Pfam (URL http://pfam.sanger.ac.uk), PSORTb v.2.0 (URL http://www.psort.org/psortb), ProDom (URL http://www.toulouse.inra.fr/prodom.html), Predict-Protein (URL http://www.predictprotein.org), SCOP (URL http://scop.mrc-lmb.cam.ac.uk/scop), SignalP (URL http://www.cbs.dtu.dk/services/SignalP), SMART (URL http://smart.embl-heidelberg.de), and SUPERFAMILY (URL http://supfam.org/SUPERFAMILY/hmm.html). The annotated nucleotide sequences of p120-*lps*KM348X and two p85 *orf* coding for glycosyltransferases were deposited in GenBank under accession numbers EU194338 and EU595706, respectively.

Polymerase chain reactions (PCR). In order to compare DNA of four *A. brasilense* strains, primers were designed mainly for those regions of a 13984-bp *Xho*I fragment of p120 (p120-*lps*KM348X) cloned in pOmegon-Km-*lps*348X, which are enriched with A/T and contain numerous direct and inverted nucleotide repeats. Such DNA organization may increase the

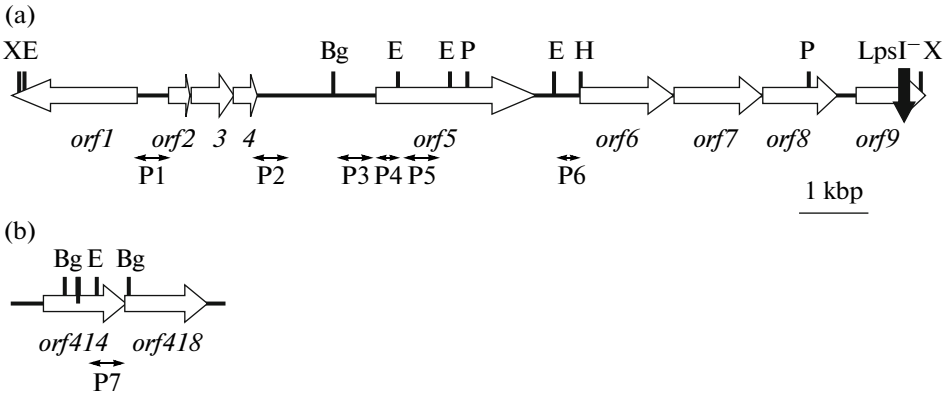

Fig. 1. Scheme of the sequenced segments of 120- and 85-MDa plasmids from *A. brasilense* Sp245. Open reading frames are shown as horizontal arrows, oriented in the direction of transcription. Restriction sites: Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; P, *Pst*I; and X, *Xho*I. Bidirectional arrows designate targets for PCR amplification using primer pairs P1–P7. Scale bar equals to 1 kbp. (a) Physical and genetic map of a 13 984-bp *Xho*I fragment of p120, p120-*lps*KM348X. The site of Omegon-Km insertion in p120 of an LpsI⁻ *A. brasilense* mutant, KM348 is marked with a vertical arrow. (b) Physical and genetic map of a 3264-bp segment of p85.

probability of genetic rearrangements. Six pairs (P) of forward (F) and reverse (R) primers were used to amplify the following regions of p120-*lps*KM348X: 523 bp between *orf1* and *orf2* (P1), 492 bp between *orf4* and *orf5* (P2), 504 bp between *orf4* and *orf5* (P3), 424 bp upstream *orf5* and at its beginning (P4), 581 bp in *orf5* (P5), and 416 bp between *orf5* and *orf6* (P6). To amplify a 436-bp internal segment of *orf414* localized in p85, the primer pair P7 was used (Figs. 1a and 1b). Nucleotide sequences of the primers synthesized by Syntol (Moscow, Russia) are presented in Table 2. For PCR, bacterial cultures were suspended in 20 μl of freshly prepared lysis buffer (50 mM NaOH, 0.25% SDS) and boiled for 15 min. The lysate was diluted with 200 μl Milli-Q water and centrifuged for 5 min. Four μl supernatant supplemented with primers, 25 pM each, four dNTP (Fermentas, Lithuania), 0.2 mM each, and 1.25 U *Taq*-polymerase (Fermentas) in the final volume of 50 μl was used for PCR. The following amplification conditions were used: 10 min at 95°C, *Taq*-polymerase addition; 25 cycles with the temperature profile: 30 s at 94°C, 1 min at 61°C (for P1), 63°C (P2 and P6), 58°C (P3), 64°C (P4), 68°C (P5), or 69.3°C (P7); and 4 min at 70°C. After 25 cycles, the reaction mixtures were incubated for additional 5 min at 70°C. A Tercyc automated thermocycler (DNA-Technology, Russia) was used for DNA amplification. For PCR products electrophoresis, 2% agarose gels were used.

Table 2. Primers specific toward DNA of plasmids p120 (P1–P6) and p85 (P7) from *A. brasilense* Sp245 used for azospirilla DNA amplification

Primer pair	Nucleotide sequence of the forward (F) and reverse (R) primers
P1	F1: 5'-GCGCCGCGGTCAGAAAC-3'
	R1: 5'-TGGCTCAGGCACGAAAAA-3'
P2	F2: 5'-GCATCCGCTACACCGACCTTTACG-3'
	R2: 5'-GCTTATCCGCGACCTCCTTATCCA-3'
P3	F3: 5'-AAGAATTACGCCGTTTTATGC-3'
	R3: 5'-ATGTTATCCCGACGCCTATTG-3'
P4	F4: 5'-TGCCTTACAACCTTTCCATTCC-3'
	R4: 5'-ATTCCGGTCGGGGGCAGGTG-3'
P5	F5: 5'-GCCCCCGCTTTCCCGATGTGATG-3'
	R5: 5'-AAGCCGCAATGACGTGGGAAGAGC-3'
P6	F6: 5'-CACCGCGCCGATCGTTTATTTACT-3'
	R6: 5'-AACCCGCATCGCCCGCTTCAGG-3'
P7	F7: 5'-CGGCACGGTGATGGAAGACG-3'
	R7: 5'-CGACGGGGAGGCGCTGGTGGACT-3'

RESULTS AND DISCUSSION

Analysis of a 14-kbp segment of *A. brasilense* Sp245 120-MDa plasmid, the mutagenesis of which is concurrent with LPS structure alterations. Earlier, an *Xho*I fragment of p120, p120-*lps*KM348X, with a single Omegon-Km insertion was cloned from *A. brasilense* KM348, an LpsI⁻ mutant of Sp245, in a recombinant plasmid pOmegon-Km-*lps*348X [8]. Nucleotide sequence analysis of p120-*lps*KM348X performed in the work revealed nine *orf*, from p120-*lps*KM348X-*orf1* to p120-*lps*KM348X-*orf9*, with properties of azospirilla coding sequences (Fig. 1a). Further on, these *orf* will be termed simply *orf1–orf9*. The *orf1* and *orf9* flanking p120-*lps*KM348X end beyond the fragment.

The proteins encoded by *orf1, orf7, orf8*, and *orf9* have been annotated as predicted glycosyltransferases

Table 3. Characteristics of the products of *orf* localized in *A. brasilense* Sp245 plasmids p120 (*orf1*–*orf9*) and p85 (*orf414*, *orf418*)

Orf (length, bp)	Characteristics of the protein product of *orf*	
	Domain coordinates in amino acid sequence, its name; E-value*	GenBank accession no., name and source of the most similar protein; identity/similarity percent (aligned amino acid sequences coordinates) between an *orf* product and a similar protein; E-value
orf1 (>1890)	(20–204) and (239–590), GT** superfamily RfaG; $3e^{-09}$ and e^{-09}	ZP_01116121, O-antigen biosynthesis enzyme from *Reinekea* sp.; 34/55 (1–204:307–517); $6e^{-27}$
	(107–149) and (411–573), GT group 1, pfam00534; $8e^{-03}$ and $2e^{-07}$	YP_376137, a GT-like protein from *Synechococcus* sp.; 28/44 (225–587:3–373); $3e^{-28}$
orf2 (321)	(14–102), Family alanine dehydrogenase/pyridine nucleotide transhydrogenase, N-terminal domain, pfam05222; $9e^{-04}$	NP_214669, an alpha-subunit of a putative NADP-transhydrogenase PntAa from *Mycobacterium tuberculosis*; 34/48 (14–102:28–107); $9e^{-05}$
orf3 (666)	(1–23), a signal peptide	Q6QW91, an Omp-like protein pRhico011 from *A. brasilense* Sp7; 33/50 (70–220:307–450); $4e^{-07}$
	(85–220), an Omp-like protein Q6QW91_AZOBR, PDA0E7B0; $4e^{-14}$	
orf4 (303)	(1–99), Q6QW90_AZOBR, PD940841; $3e^{-20}$	Q6QW90, a hypothetical protein pRhico012 from *A. brasilense* Sp7; 49/63 (1–100:1–99); $6e^{-15}$
orf5 (2436)	(28–804), Superfamily TolB, C-terminal domain, SCOP 1.69; $1.2e^{-06}$	Q6J7Z7, a predicted O-methyltransferase Pas34 from *Actinoplanes* phage *phi*Asp2; 29/42 (6–162:294–455); $6e^{-64}$
		Q2DZR5, Alanyl dipeptidyl peptidase (amino acid transport and metabolism) from *Acidothermus cellulolyticus* 11B; 32/45 (398–541:214–355); e^{-40}
		Q1NBG2, Oligogalacturonate lyase (periplasmic component of the Tol biopolymer transport system) from *Sphingomonas* sp.; 24/38 (600–804:208–430); $4e^{-79}$
orf6 (1454)	ND***	ND
orf7 (1293)	(7–326), GT, RfaG; $6e^{-10}$	ABQ91728, GT family 2 from *Roseiflexus* sp.; 20/42 (19–337:335–610); $2e^{-09}$
	(186–389), GT group 1, pfam00534; $6e^{-09}$	
orf8 (1098)	(123–364), GT, RfaG; $9e^{-23}$	NP_948682, a putative mannosyltransferase from *Rhodopseudomonas palustris*; 33/48 (6–353:11–350); $2e^{-38}$
	(179–345), GT group 1, pfam 00534; $2e^{-21}$	
orf9 (>1047)	(1–310), Superfamily of ADP–heptose:LPS–heptosyltransferases, RfaF; $2e^{-19}$	Q7M8S5, an ADP–heptose:LPS–heptosyltransferase from *Wolinella succinogenes*; 23/51 (1–308:1–328); $2.2e^{-06}$
	(57–291), GT family 9 (heptosyltransferase), pfam01075; e^{-09}	
orf414 (1245)	(10–391), Predicted GT, COG4671; $2e^{-100}$	ZP_02190374, a predicted GT from *Stappia aggregata*, 59/72 (9–393:7–394); $6e^{-127}$
orf418 (1257)	(1–397), GT, RfaG, $3e^{-54}$	ZP_01545197, a putative GT from *S. aggregata*; 59/73 (3–406:4–405); $3e^{-131}$
	(212–385), GT group 1, pfam00534; $4.6e^{-30}$	

Notes: * Expected number of accidental matches of the same statistical weight. E-value $\le e^{-05}$ indicates a high degree of reliability of the prediction. Results with E-value $\ge e^{-03}$ are usually neglected.

** Glycosyltransferase.

*** Not detected.

(GTs) performing synthesis of LPS and exopolysaccharides (EPS) (Table 3) (GenBank accession no. EU194338). To comment the results of their analysis presented in Table 3, it should be noted that GTs from the same family have similar secondary structures but, as a rule, rather low amino acid sequence homologies [18].

It is quite possible that *orf1* codes for a bifunctional GT. The product of *orf1* contains over 630 amino acid residues and is larger than many bacterial GTs involved in EPS and LPS biosynthesis. Two ORF1 segments comprising amino acid residues from 1 to 204 and from 225 to 587 are the most similar to various GT (Table 3).

Despite the fact that ORF8 protein is similar to predicted mannosyltransferases from an alpha-proteobacterium *R. palustris* (Table 3) and other bacteria, we did not class it as a mannosyltransferase. ORF8

exhibits significant similarity (21% identity, 36% similarity; E-value = $2e^{-26}$) to the WbpY rhamnosyltransferase from a gamma-proteobacterium *Pseudomonas aeruginosa* as well (GenBank accession no. NP_254135). WbpY transfers two α1 ⟶ 3-linked D-Rha residues onto the OPS of *P. aeruginosa*, a D-rhamnan. D-rhamnose is a 6-deoxy derivative of D-mannose; thus, WbpY may be structurally similar, for example, to O9a mannosyltransferases of *E. coli* responsible for D-mannan synthesis [19].

The product of *orf9* belongs to the superfamily of ADP–heptose:LPS–heptosyltransferases (RfaF) and to GT family 9, which includes heptosyltransferases and LPS–*N*-acetylglucosamine transferases (Table 3). A gene of GT RfaF involved in the inner LPS core synthesis was initially characterized in *Enterobacteriaceae* and later in many other bacteria. Mutagenesis of *rfaF* is known to cause total loss of OPS and production of R-type LPS [3]. Omegon-Km mutagenesis of *orf9* allowed us to identify the functional activity of its product in *A. brasilense* Sp245 cells. In an LpsI$^-$ mutant *A. brasilense* KM348, Omegon-Km insertion starts after the 594th bp of *orf9*. In two other LpsI$^-$ mutants, KM127 and KM134, single omegon insertions were mapped in the same region of p120 [8]. Interestingly, Omegon-Km transposition into *orf9* resulted in loss of only one of the LPSs [8]. The full-length OPS retention in the mutants KM348, KM127, and KM134 [8] with damaged *orf9* may be explained by assuming that at least two different O-antigen acceptors are produced. This phenomenon is known. For example, in an alpha-proteobacterium *Rhizobium leguminosarum*, a trisaccharide and a tetrasaccharide were detected in the core [20]. In a gamma-proteobacterium *Pasteurella multocida*, two inner cores are formed and two different heptosyltransferases exhibit specificity toward the corresponding acceptor molecule [21].

Searching in the databases revealed no proteins with significant similarity to the *orf6* product. However, four amino acid motifs $E(X)_7E$ (where X stands for any amino acid) revealed in ORF6 are present in practically all enzymes involved in LPS biosynthesis [23]. The same motifs are present in ORF1, ORF7, ORF8, ORF9, and ORF5 proteins.

ORF5 is structurally similar to the C-terminal domain of a model periplasmic protein TolB from *E. coli* (Table 3). Tol proteins are essential for the maintenance of the outer membrane integrity and transport of a number of high-molecular components of the outer membrane to their location [24]. The PSI-BLAST algorithm revealed significant similarity between three ORF5 segments and O-methyltransferase, alanyl dipeptidyl peptidase, and oligogalacturonate lyase (Table 3). It is important in this connection that in a number of bacteria, one of the key mechanisms of OPS length control is O-methylation of terminal glycosides. For example, in *E. coli* strains O8 and O9, 3-O-methylation of D-mannan is required for polymerization termination, chain length regulation, and OPS export from the cytoplasm [25]. In an electrophoresis picture of the LPS from *A. brasilense* Sp245, a limited number of high-molecular bands are visualized, evidencing a rather strict regulation of D-rhamnan chain length [8, 22]. It is of interest whether ORF5 is a multifunctional protein, for example, a methyltransferase and a component of Tol transport system (Table 3), involved in LPS modification, OPS length regulation, and OPS transport to the surface of *A. brasilense* Sp245 cells.

Identification of predicted glycosyltransferase genes in an 85-MDa plasmid of *A. brasilense* Sp245. Full annotation of the sequence of pEK248X plasmid is beyond the framework of the present publication. However, as a result of pEK248X sequence analysis (see Table 1), two *orf* (named *orf414* and *orf418* according to the number of amino acids in their protein products) coding for predicted GT (Fig. 1b, Table 3) were also identified in p85. In addition to significant similarity between amino acid sequences of ORF414, ORF418, and a number of GTs, high identity level (65–69%) was revealed between the nucleotide sequences of *orf414* and *orf418* and the genes of GT and conserved membrane-bound proteins localized in plasmids pRL11 of *R. leguminosarum* bv. *viciae* (GenBank accession no. AM236085), p42e of *Rhizobium etli* (GenBank accession no. CP000137), pSymB of *Sinorhizobium meliloti* (GenBank accession no. AL591985), and others. In this connection, horizontal transfer of related plasmids or gene clusters between various bacterial genera and species in the rhizosphere seems quite probable.

Search for homology to the sequenced p120 and p85 segments in the DNA of other *A. brasilense* strains. Proteins ORF1, ORF3, ORF4, ORF7, and ORF8 encoded by p120 of *A. brasilense* Sp245 are structurally and/or functionally similar to predicted products of pRhico genes of the type strain *A. brasilense* Sp7 [7], the OPS of which contains xylose, galactose, and fucose, in addition to rhamnose [26]. However, only three short sequences homologous to pRhico (in the region of *orf2–orf4*) were detected in p120-*lps*KM348X. None of the PCRs on *A. brasilense* Sp7 DNA with primers P1–P6 specific to p120-*lps*KM348X yielded products (Fig. 2a–2f). A locus homologous to *orf414* was found in Sp7 with P7 primers (Fig. 2g) supporting the data on affinity between a 115-MDa plasmid of the strain and p85 of Sp245 [22, 27].

Negative results of PCR with P7 primers (Fig. 2g) allowed us to conclude that there are no homologous genes in the DNA of *A. brasilense* SR75 and Sp107, which belong to the same serogroup as Sp245 [6]. Apparently, GT encoded by *orf414* is not crucial for LPS biosynthesis.

Earlier, we observed distinct homology between p120-*lps*KM348X and two plasmids of 120 and over 300 MDa from strain *A. brasilense* SR75 possessing the OPS identical to that of Sp245 [5]. In the present work, in Southern hybridization reactions of p120-

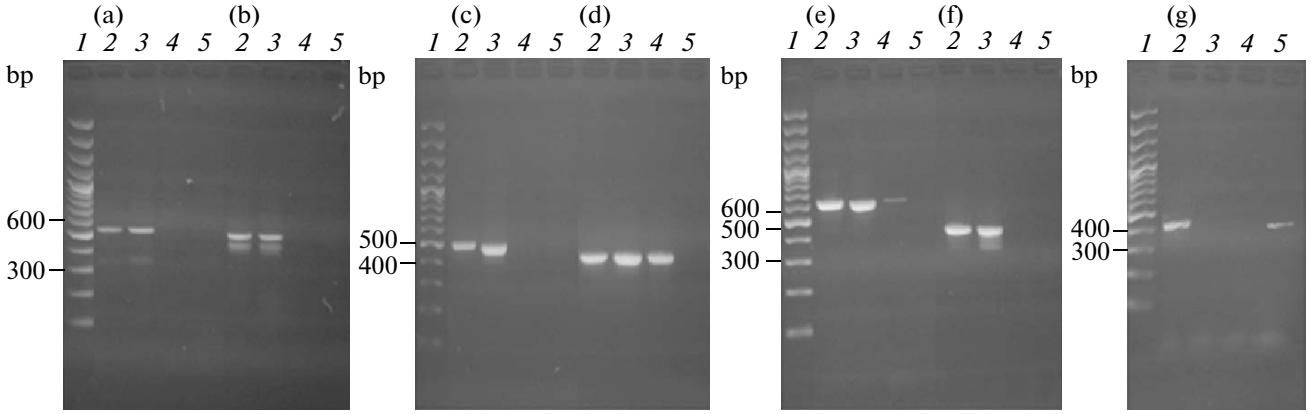

Fig. 2. Results of PCR on total DNA of *A. brasilense* Sp245 (*2*), Sp107 (*3*), SR75 (*4*), and Sp7 (*5*) using primers P1 (a), P2 (b), P3 (c), P4 (d), P5 (e), P6 (f), and P7 (g) specific toward p120 (P1–P6) and p85 (P7). DNA marker, 100 bp+ DNA ladder (*1*). DNA fragment length is given at the left.

*lps*KM348X with plasmids of *A. brasilense* Sp107, another strain with OPS identical to that of Sp245 [6], we also detected a strong positive signal in a 120-MDa plasmid of Sp107 (data not shown). On the contrary, in hybridization reactions of the plasmids and total DNA of strains SR75 [5] and Sp107 (present work) with the probes developed on the basis of fragments of p120 and p85 carrying no genes for PS synthesis (namely, an ~8.3-kbp p120 fragment from pOmegon-Km-*fla*048X and a 2.4-kbp p85 fragment from pEK248X) negative results were obtained.

All PCRs on *A. brasilense* Sp245 and Sp107 DNA templates with primers specific to p120-*lps*KM348X resulted in formation of amplification products of practically equal lengths (Figs. 2a–2f). Two amplicons in PCRs with primers P1 and P2 (Figs. 2a and 2b) evidence existence of homologous DNA in Sp245 (and Sp107) beyond p120-*lps*KM348X. Therefore, in serologically related strains Sp245 and Sp107, even potentially variable regions of homologous segments of 120-MDa plasmids are similar.

In PCRs on an SR75 DNA template, positive results were obtained only with primers P4 and P5 to the segments localized upstream/in *orf5* (Figs. 2d and 2e). The absence of PCR products on SR75 DNA with primers P1, P2, P3, and P6 specific to non-coding regions of p120-*lps*KM348X confirmed the earlier elucidated differences in physical organization of p120-*lps*KM348X and SR75 plasmid segment highly homologous to this p120 region [5]. The organization of loci corresponding to p120-*lps*KM348X in *A. brasilense* strains Sp107 and Sp245 isolated from wheat roots in Brazil apparently is practically identical and different from the one in a serologically related *A. brasilense* strain SR75 isolated from wheat seedlings in Russia.

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