Sequence and regulation of *psrA*, a gene on the Sym plasmid of *Rhizobium leguminosarum* biovar *phaseoli* which inhibits transcription of the *psi* genes

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The *psr* region of *Rhizobium leguminosarum* biovar *phaseoli* had originally been recognized on the basis of its ability to repress the transcription of the *psi* genes, one of which, *psiA*, inhibits exopolysaccharide synthesis when cloned in multi-copy plasmids. Both *psr* and *psi* are located on the symbiotic plasmid pRP2JI. The *psrA* gene was localized and sequenced. The deduced amino acid sequence of PsrA was shown to have similarity to the DNA-binding region of a family of other transcriptional regulators, consistent with its known effects on the expression of *psi*. The transcription of *psrA* itself appears to be constitutive in free-living *Rhizobium*, but is regulated by another gene on the Sym plasmid pRP2JI.

Keywords: *Rhizobium*, exopolysaccharide, *psi*, *psr*, transcriptional regulation

INTRODUCTION

Strains of *Rhizobium* synthesize large amounts of extra-cellular, high molecular mass, acidic exopolysaccharide (EPS). Genetic studies have shown that, at least in certain strains or species of *Rhizobium*, this polymer is important for the induction of normal, nitrogen-fixing nodules on leguminous plants (reviewed in Leigh & Coplin, 1992; Gray & Rolfe, 1990; Reuber *et al.*, 1991b). Thus, in *R. meliloti*, (*exo*) mutants, defective in EPS production induce nodules on alfalfa which are devoid of bacteria and which fail to fix N₂ (e.g. Leigh *et al.*, 1985, 1987) and those in *R. leguminosarum* biovar (*bv.*) *viciae* fail to induce nodules at all on peas (Borthakur *et al.*, 1986). Interestingly, though, *exo* mutants of *Rhizobium* strains which infect plants with determinant nodules, as, for example, those on *Phaseolus* beans by *R. leguminosarum* *bv. phaseoli*, appear to be unaffected in symbiotic nitrogen fixation [e.g. Borthakur *et al.* (1986) and see, also, Hotter & Scott (1991)].

In *R. meliloti*, where the most detailed genetic studies in *Rhizobium* on *exo* genes have been conducted, most of them are clustered (Long *et al.*, 1988) on a large indigenous

plasmid, different from the so-called pSym which contains many of the *nod*, *nif* and *fix* genes (Finan *et al.*, 1986; Hynes *et al.*, 1986).

In a strain of *R. leguminosarum* *bv. phaseoli*, a gene termed *psi* was identified on pRP2JI, the pSym of this biovar, which has significant effects on the production of EPS and on nodulation and nitrogen fixation (Borthakur *et al.*, 1985). The *psi* region is close to several *nod* and *nif* genes on the symbiotic plasmid pRP2JI (Lamb *et al.*, 1985; Borthakur *et al.*, 1985; Davis & Johnston, 1990a, b; Hawkins & Johnston, 1988). It was initially identified because, when it was cloned in a wide-host-range, multi-copy plasmid vector such as pLAFR1 or pKT230, it inhibited production of EPS by *R. leguminosarum*; hence its name, *polysaccharide inhibition*. One gene, *psiA*, was sufficient for this phenotype. The product of *psiA* is a small (10 kDa) protein that is probably located in the inner membrane of *Rhizobium* (Borthakur & Johnston, 1987; Latchford *et al.*, 1991). *psiA* is part of a larger transcriptional unit which contains at least one upstream gene whose product is also associated with the bacterial membrane(s) or periplasm (Latchford *et al.*, 1991). Mutations in *psiA* do not appear to affect EPS synthesis but such mutants are defective in symbiotic nitrogen fixation on beans, inducing non-fixing nodules that are devoid of bacteria (Borthakur *et al.*, 1985).

A gene, termed *exoX*, with similar properties to *psiA* was identified in *R. meliloti* (Reed *et al.*, 1991a) and *Rhizobium*

Abbreviations: EPS, exopolysaccharide.

The GenBank accession number for the sequence reported in this paper is L26581.

strain NGR234 (Gray *et al.*, 1990). When present in multi-copy, *exoX* also inhibits EPS synthesis; further, the hydropathy profiles of ExoX and PsiA are very similar (extremely hydrophobic N half and hydrophilic C half) although actual sequence similarities of the two proteins were limited to a small tract of 14 amino acids in the middle of the proteins. Note that in contrast to *psiA*, the *exoX* gene of *R. meliloti* is not on the Sym plasmid but is among the large cluster of other *exo* genes. It has been suggested that PsiA/ExoX functions as a post-translational inhibitor of PssA/ExpY, a structural protein involved in EPS synthesis (Gray *et al.*, 1990; Borthakur *et al.*, 1988; Reuber *et al.*, 1991b).

It was proposed that the function of *psiA* (*exoX*) was to inhibit EPS synthesis by *R. leguminosarum* during normal nodule development. Bacteroids within nodules make little or no EPS (Tulley & Terry, 1985) and it was suggested (Borthakur *et al.*, 1985) that, normally, *psi* was expressed only during infection of legumes and not in the free-living state. Direct evidence to support this was subsequently obtained using *psi-uidA* fusions (Latchford *et al.*, 1991). Also, consistent with this was the observation that *psi* mutants of *R. leguminosarum* bv. *phaseoli* induced non-fixing nodules that were devoid of bacteria (Borthakur *et al.*, 1985) indicating that if EPS synthesis were not switched off, an aberrant infection would ensue.

It was further proposed that by cloning *psi* in a multi-copy vector, its expression became 'deregulated' and that its influence on EPS production would be seen in free-living bacteria. (Borthakur *et al.*, 1985). It is certainly clear that transcription of *psi* is under regulatory control. A region of DNA on pRP2JI approximately 13 kb from *psi* was identified which had the following two properties. When this region was itself cloned in one plasmid (pLAFR1) and introduced into a non-mucoid strain containing multi-copy *psi* on a compatible plasmid (pKT230), the trans-conjugants regained their ability to make EPS (Borthakur & Johnston, 1987). The gene(s) responsible for this was termed *psr* (polysaccharide restoration). Further, using *psi-lacZ* and *psi-phoA* fusions, it was shown that this effect was because *psr* repressed the transcription of *psi*; thus by having 'extra' copies of *psr*, this overcame the inhibition of EPS synthesis caused by multi-copy *psi* (Borthakur & Johnston, 1987; Latchford *et al.*, 1991).

A strain of *R. leguminosarum* bv. *phaseoli* with a *psr::Tn5* mutation induced normal nitrogen-fixing nodules on *Phaseolus* beans. Strains with multi-copy *psr*, though, induced non-fixing 'empty' nodules which could be interpreted as being due to the lack of expression of *psi* in the bacteria during the infection; i.e. strains with multi-copy *psr* would mimic those with *psi* mutations (Borthakur & Johnston, 1987).

In this paper, we describe the detailed location of *psr*, show that its product has a striking similarity to other transcriptional regulatory proteins, and that the expression of *psr* is, in turn, regulated by another gene on the Sym plasmid.

METHODS

Bacterial strains, plasmids and growth conditions. These are shown in Table 1. *E. coli* strains were routinely cultured on Luria Broth (LB), *Rhizobium* on TY solid or liquid media (Beringer, 1974). For *Rhizobium*, antibiotics were added as appropriate as follows in $\mu\text{g ml}^{-1}$: kanamycin (20), tetracycline (5), streptomycin (200). 5-Bromo-4-chloro-3-indolyl galactoside (X-Gal) was incorporated at $40 \mu\text{g ml}^{-1}$. β -Galactosidase was assayed according to Rossen *et al.* (1985). EPS phenotypes were scored on TY solid medium.

Genetic manipulations. Plasmids were transferred by conjugation from *E. coli* to *Rhizobium* in tri-parental matings as described by Buchanan-Wollaston *et al.* (1980) using the plasmid pRK2013 as a helper to mobilize the pMP220-based recombinant plasmids.

DNA manipulations. Restriction digestions, ligations and transformations were done essentially as described by Maniatis *et al.* (1982). Subcloning various fragments (see Fig. 1) of the *psr* region into pMP220 usually involved some 'shuttling' in which relevant fragments were obtained from pIJ1426, cloned into appropriate sites of pUC18 before recloning into the polylinker of pMP220. Details of plasmid construction are as follows.

pBIO132/133. The region of DNA containing *psrA* was cloned as a 1240 bp *SphI/EcoRV* DNA fragment into pUC19 *SphI/SmaI*. The resulting plasmid, pBIO138, was then digested with *HindIII/SacI* to release the DNA fragment and subsequently filled-in with T4 DNA polymerase. This DNA fragment was cloned in both orientations into the *SmaI* restriction site of Bluescript SK- to give the plasmids pMLM152 and pMLM153, which were digested with *EcoRI/XbaI* to release the *psrA*-containing DNA fragments which were finally cloned into pMP220 to give the resulting plasmids pBIO132/133.

pBIO137. pBIO138 was digested with *BglII* and filled-in using T4 DNA polymerase and checked for loss of the unique *BglII* restriction site in the region of the *psrA* gene by restriction endonuclease digestion and sequencing. This plasmid, pMLM42, was further digested with *EcoRI/HindIII* to release the *psrA*-containing DNA fragment which was cloned into pLAFR3 to generate pBIO137.

pBIO134. pMLM153 (see above) was digested with *DraI/EcoRI* releasing the *psrA* DNA fragment which was cloned into Bluescript SK- *SmaI/EcoRI* to give pMLM169; this was digested with *EcoRI/XbaI* and the fragment was cloned into pMP220 to generate pBIO134.

pBIO135. pMLM153 was digested with *BglII/EcoRI* to release a DNA fragment cloned into pMP220 *BglII/EcoRI* to generate the plasmid pBIO135.

pBIO136. pBIO138 was digested with *EcoRI/PstI* and the *psrA* fragment was cloned into pMP220 to generate the plasmid pBIO136.

The *psrA-lacZ* translational fusion plasmid pBIO142 was made as follows. The 1240 bp *SphI/EcoRV* *psrA* fragment was cloned into the *HincII* site of pUC18. The resulting plasmid was digested with *EcoRI/PstI* (the *PstI* site is within *psrA*) and the truncated *psrA* was ligated into Bluescript SK before being taken out as a *BamHI* fragment and ligated to *BamHI*-cut pIJ1363. The authenticity of the fusion was checked by sequencing: it was confirmed that *psrA* and *lacZ* were fused in-frame and in the correct orientation. To form the 'promoter-trap plasmid' pBIO143, a fragment containing the 5' end of *psrA* plus a region immediately upstream of it was made by PCR

Table 1. Strains and plasmids used in this study

Strain/plasmid	Relevant features	Source
<i>Escherichia coli</i>		
803	Used for routine transformations	Wood (1966)
JM101	Used as a host for pUC18- and pUC19-derived plasmids	Maniatis <i>et al.</i> (1982)
<i>Rhizobium leguminosarum</i>		
8401	Str ^r , cured of its Sym plasmid	Lamb <i>et al.</i> (1982)
KH1014	8401 pRL1JI, <i>lac</i> ::Tn5	Rossen <i>et al.</i> (1985)
4292	Wild-type <i>R. leguminosarum</i> bv. <i>phaseoli</i> , <i>rif</i>	Lamb <i>et al.</i> (1982)
JW167	Nod ⁻ mutant of <i>R. leguminosarum</i> bv. <i>phaseoli</i> contains 50–60 kb deletion in pRP2JI	Lamb <i>et al.</i> (1982)
Plasmids		
pRP2JI	Sym plasmid of <i>R. leguminosarum</i> bv. <i>phaseoli</i> strain 8002	Lamb <i>et al.</i> (1982)
pKT230	Q-group cloning vector; Kan ^r Str ^r	Bagdasarian <i>et al.</i> (1981)
pIJ1393	Contains <i>psi</i> in pKT230	Borthakur <i>et al.</i> (1985)
pIJ1363	P1-group <i>lacZ</i> translational fusion vector	Rossen <i>et al.</i> (1985)
pMP220	P1-group cloning vector	Spaink <i>et al.</i> (1987)
pML122	Q-group cloning vector	Labes <i>et al.</i> (1990)
pIJ1426	<i>psr</i> region cloned in pKT230	Borthakur & Johnston (1987)
pBIO142	<i>lacZ-psrA</i> fusion in pIJ1363	This work
pBIO143	<i>psrA</i> promoter in pML122	This work
pBIO132	Derivatives of pMP220 or pLAFR3 containing various fragments of the <i>psr</i> region See Fig. 1	This work
pBIO133		
pBIO134		
pBIO135		
pBIO136		
pBIO137	<i>SphI-EcoRI</i> fragment containing <i>psrA</i> , cloned in pUC19	This work
pBIO138		

amplification using the oligonucleotide primers J82 and J84 shown in Fig. 2. This fragment was cloned first into *HincII*-cut pUC18 and thence into the wide-host-range plasmid pML122 (*HindIII/BamHI*) to form pBIO143.

The sequence of the 833 bp *DraI-SphI psrA*-containing fragment was determined for both strands by sequencing out in both directions from the *DraI*, *BglII*, *Clal*, *PstI* and *SphI* sites shown in Fig. 1, relevant fragments having been cloned in pUC18 or pUC19. Clones that spanned these restriction sites were also obtained (G. F. Hong, unpublished) to complete the sequence of the *psr* region. Sequencing was done using the Sanger, dideoxy method using a Pharmacia ALF automated sequencer according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Location of *psrA*

Borthakur & Johnston (1987) isolated two pLAFR1-based cosmids, pIJ1399 and pIJ1400, both of which overcame the EPS⁻ phenotype of a strain containing multi-copy *psi*. By subcloning fragments of pIJ1399 into pKT230, the *psr* gene(s) was located within a 4 kb *EcoRI-SacI* fragment. This fragment, cloned in pKT230

to form pIJ1426 suppressed the effect of multi-copy *psi* on EPS synthesis and was also shown to be sufficient to repress transcription of *psi-lacZ* and *psi-phoA* fusions (Borthakur & Johnston, 1987; Latchford *et al.*, 1991). Further, a *psr*::Tn5 mutation was located within this same region of pIJ1399.

A more detailed restriction map of the 4 kb *psr* DNA was compiled, and individual fragments were cloned in the wide-host-range vector pMP220 (Fig. 1). The various resulting plasmids were each mobilized into *R. leguminosarum* strain 8401 containing pIJ1393 (*psi* cloned in pKT230). The transconjugants were scored for their mucoid colony morphology and by such means *psr* was localized to a region of just under 1 kb (see Fig. 1) defined by the region between the *DraI* and *SphI* sites shown in Fig. 1. Thus, fragments lacking DNA to the left of the *BglII* site (pBIO135) or to the right (pBIO136) of the *PstI* site (as shown in Fig. 1) did not suppress the Psi phenotype.

The vector pMP220 does not have a promoter at its polylinker cloning site. Thus any plasmid expressing *Psr* activity must not only contain the *psr* gene, but upstream

Fig. 1. Map of the *psr* region. Relevant restriction sites in the *psr* region are shown. The numbering refers to positions in the sequence presented in Fig. 2. The dimensions of the fragments cloned in pMP220 and the *Psr* phenotype of the resulting plasmids are shown. In pBIO132 and pBIO133 the location of the vector *lacZ* gene relative to the cloned fragment is presented. The open boxed region defines the extent of the *psrA* open reading frame and the hatched shaded region defines the position of the DNA-binding domain within the *psrA* gene. In pBIO137 the (■) refers to the modified *Bgl*II site.

sequences responsible for its transcription. Immediately downstream of the polylinker is the *lacZ* gene of *E. coli*. The *Eco*RI–*Sph*I fragment had been cloned in both orientations to form pBIO132 and pBIO133 each of which was transferred to *R. leguminosarum* strain KH1014, which lacks the constitutive, *R. leguminosarum* β -galactosidase. The transconjugants were streaked on medium containing X-Gal. The strain containing pBIO132 formed pale-blue colonies whereas those with pBIO133 were white. This indicated that transcription of the cloned DNA was from left to right as drawn in Fig. 1 (and see below).

Sequence determination of *psrA*

The 833 bp *Dra*I–*Sph*I fragment containing *psr* was sequenced (Fig. 2). One ORF in the appropriate orientation (left to right as drawn in Fig. 1) was identified which spanned both the *Pst*I and the *Bgl*II sites that had been shown to be required for *psr* function (see above). It is proposed that the ORF corresponds to a gene termed *psrA* which starts at a GTG at position 95, just 10 bp upstream of the *Bgl*II site. To confirm that the DNA at this *Bgl*II site was needed for *psr* function, a plasmid (pBIO138) containing the *Sph*I–*Eco*RI fragment, cloned in pUC19, was cleaved with *Bgl*II, the ends were filled in and blunt-end ligated (the *Bgl*II site in *psrA* is the only one present in pBIO138). The modified *Sph*I–*Eco*RI fragment was then ligated to pMP220 and the resulting plasmid, pBIO137, was tested for *Psr* function as described above. As indicated in Fig. 1, pBIO137 no longer suppressed the EPS[−] phenotype caused by multi-copy *psi*,

confirming the requirement for the sequence immediately spanning the *Bgl*II site.

Note that the *Pst*I site lies only 48 nt upstream of the 3' end of *psrA*. The failure of pBIO136 (Fig. 1) to suppress the *Psi* phenotype thus shows that the C-terminal 16 amino acids of *PsrA* are essential for its function.

PsrA resembles other transcriptional regulators

The deduced polypeptide product of *psrA* has molecular mass of 18092 Da and its sequence was compared with those in databases. As shown in Fig. 3, it is clear that *PsrA* is a member of a family of transcriptional regulators, consistent with its known ability to repress *psi* expression. Largely, this similarity was confined to the helix-turn-helix DNA-binding domains of these regulatory proteins, the regions in which they themselves show greatest similarity to each other. There were, though, other intermittent matches elsewhere in the proteins, the most pronounced being that between *PsrA* and Gene C, a repressor in bacteriophage P2.

The group of regulatory proteins to which *PsrA* is related comprises those that are repressors (e.g. *cI*), those that are activators [e.g. *UhpR*; see Friedrich & Kadner (1987)] and those that can have either function at different promoters (e.g. *Fnr*). To date, the only regulatory property attributed to *PsrA* is its ability to repress the *psi* operon and further work will be required to establish if it can act positively or negatively on other *Rhizobium* promoters.

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DraI   J82
1  TTTAAACGGTTGTAGTCTGGCGATGGCAAGTGGTTCAAATCGGTTGAGTTGGCTCAAG 60
                                     BglII
61  TCTACAACGAGTGGCAGGAAATAGGACGTGCGGTGACGGAAGATCTAGCAAGCTACAG 120
                                     RBS      V T E D L A S Y R
121 AAGTTCGAGAGGCCTCGTCAGCACCGCGATCCGGAGGTTGACAAGCGCATCTATCGAAA 180
    S S R G L V S T A D P E V D K A I Y R K
                                     J84
181 GCCAGGTTTCGGCGGCGAAATTACATCGTTGGGTACATGGAGGAATTGATCAGCGGTT 240
    P G F G G E I T S L G H M E E L I S A F
241 CCTGAAAAAGACACCGCAAGCGCAGGGCCCTGTCCCGCGCAGACGTTGCTCCAATGCTTGG 300
    L K K T R E A Q G L S R A D V A P M L G
301 TCTGTCCATCCCTGTATACGGACGCTACGAGCGGGCATTTCCTCAAATGACTGTTACTCG 360
    L S I P V Y G R Y E R A F S K M T V T R
                                     ClaI
361 GATGATCCATCTGTGTGAGATTCTTGGCTTCATGCCTATCGATATGATCTTTGAAGCTGC 420
    M I H L C E I L G P M P I D M I F E A A
421 GCCACATCTTTGGGCGCGACATCGGAGGAGGCGGAGGATTGTCTCACACTGGCGAGGAT 480
    P H L W G R T S E E A E D C L T L A R I
                                     PstI
481 TCTTAGGAGACTTCCGAACGGCAGCAGCGCGATCTTATTCGGCTCTCGACGGAATGAC 540
    L R R L P N G T T R D L I R L L Q R M T
541 CCCCGATGACGACGAGAGCGCAGAGTATCAGTGATGTAGCGGAGCCCGTGAAGTGAGCT 600
    P D D D E S D R V S V M *
601 GGGTTTGTCTATGACGCCCCAGTAGTTAGCAGTATTGGTGATAGCTGTGCGAGCCGACG 660
661 CGCTGTCGTCGGTGTGCAGACCCGCAATCTCACAACCTAGTTTTCACTCGAATTTCC 720
721 GTCTGATCAACAATACGCGCGGATTGCCCAATTTTTCGATCTTTGGTTGGCGTTGATCCT 780
                                     SphI
781 CCTCGATGCTCGAGCGATTCTGTCGGCGAGCGACGCCAAGTCTATTTGCATGC 833

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Fig. 2. Nucleotide sequence of the *psr* region. The nucleotide sequence extending for 833 bp from the *DraI* site to the *SphI* site is presented. The predicted amino acid sequence for the *psrA* gene is indicated in the one-letter code. The proposed ribosome binding site (RBS) is indicated and the position of the putative DNA-binding domain is underlined. The dimensions of the primers J82 and J84 used to make a fragment containing the *psrA* promoter are shown. Relevant restriction sites are also indicated.

Protein	Starting residue	Sequence	Reference
PsrA	59	<u>L S R A</u> <u>D V A</u> P M <u>L G L</u> S I P V Y G R Y E R	
cl	31	L S Q E S V A D K M G M G Q S G V G A L F N	1
Cro	17	F G Q T K T A K D L G V Y Q S A I N L A I H	1
Cro434	19	M T Q T E L A T K A G V K Q Q S I Q L I E N	2
P2 gene C	18	L S R Q Q L A D L T G V P Y G T L S Y Y E S	3
CAP	167	I L R Q E I G Q I V G C S R E T V G R I L K	4
Fnr	195	M T R G D I G N Y L G L T V E T L S R L L G	4
DeoR	22	L H L K D A A A L L G V S E M T I R R D L N	5
AsnC	23	T A Y A E L A K Q F G V S P G T I H V R V E	6
UhpA	153	M A V K E I A A E L G L S P K T V H V H R A	7
TrpR	66	M S Q R E L K N E L G A G I A T I T R G S N	1
OmpR	88	E V D R I V G L E I G A D D Y I P K P F N P	8
PhoB	199	I R R L R K A L E P G G H D R M V Q T V R G	9

Fig. 3. Comparison of PsrA with other transcriptional regulators. The boxed regions indicate the extent of the sequence similarity within the DNA-binding domain of these regulatory proteins. Amino acid residues shown underlined in the PsrA protein indicate positions in which the same residue occurs in at least two other proteins. References: 1, Pabo & Sauer (1984); 2, Gicquel-Sanzey & Corssart (1982); 3, Ljungquist *et al.* (1984); 4, Shaw *et al.* (1983); 5, Valentin-Hansen *et al.* (1985); 6, Kolling & Lother (1985); 7, Friedrich & Kadner (1987); 8, Makino *et al.* (1986).

To date, no gene analogous or homologous to *psrA* has been found within the rhizobia. It has no similarity in sequence to *exoR* (Doherty *et al.*, 1988; Reed *et al.*, 1991a),

a regulatory gene in *R. meliloti* which can repress transcription of several *exo* 'structural' genes in that species.

psrA is regulated by another gene on Sym plasmid pRP2JI

Sequence analysis and expression of *lacZ* from the cloned *psrA* region in pMP220 (see above) strongly indicated that *psrA* was transcribed from left to right as shown in Fig. 1 and that the *psrA* promoter was located between the *DraI* site and the start of *psrA*. To confirm this, the *psrA-lacZ* translational fusion plasmid pBIO142 was made (see Methods).

pBIO142 was mobilized into *R. leguminosarum* bv. *phaseoli* wild-type strain 4292 and also into its derivative 8401 in which the Sym plasmid pRP2JI is missing. The transconjugants were plated on media supplemented with X-Gal. The derivative of strain 4292 stained more brightly blue than that of strain 8401 indicating that pRP2JI contained a gene or genes that activated transcription of *psrA*. In quantitative assays it was found that in the wild-type background the fusion expressed β -galactosidase at a level equivalent to 250 units. In contrast, in the cured strain 8401, there was only 31 units of β -galactosidase, little above background (12 units).

This regulatory gene was shown to be in the vicinity of the symbiotic (*nod/nif*) region of pRP2JI; when pBIO142 was mobilized into strain JW167, which has a large (50–60 kb) deletion that removes *nod* and *nif* genes, the transconjugants were pale-blue on X-Gal.

We obtained evidence that the regulatory gene in pRP2JI specifies a product that binds to sequences upstream of *psrA* in free-living *R. leguminosarum* bv. *phaseoli*. A plasmid, pBIO143 containing the region upstream of *psrA* (see Fig. 1 and Methods) was introduced into the wild-type *R. leguminosarum* bv. *phaseoli* strain 4292 containing the *psrA-lacZ* fusion plasmid pBIO142; the presence of pBIO143 reduced the level of β -galactosidase from 250 to 60 Miller units. Since pBIO143 contains the *psrA* promoter cloned in the relatively high copy number plasmid pKT230, we interpret this reduction of expression of the *psrA-lacZ* fusion as being due to the binding of the activator protein to the promoter in pBIO143, thus reducing the level available for inducing transcription of the *psrA-lacZ* fusion.

The *psrA-lacZ* fusion plasmid pBIO142 was also introduced into the *R. leguminosarum* bv. *viciae* strain 8401 pRL1JI. The levels of β -galactosidase were the same as those obtained when pBIO142 was present in strain 8401, which lacks any Sym plasmid. Thus it would appear that there is not a corresponding gene on the Sym plasmid of biovar *viciae* which can activate transcription of *psrA*.

It is not clear why the regulation of *psi* in *R. leguminosarum* bv. *phaseoli* appears to be so different from that of its analogue, *exoX*, in *R. meliloti*. There are, for example, no striking similarities in the sequences upstream of *exoX* and the *cis*-acting regulatory region of *psi*.

It is clear that *psrA*, whose product has similarity to a family of transcriptional regulators, is sufficient to repress transcription of the *psi* operon. Since, under normal circumstances, *psi* is expressed in the bacteroids but not in

the free-living bacteria, it might have seemed reasonable to suppose that the role of *psrA* is to keep transcription of *psi* quiescent in the latter form but that, in some way, in the bacteroids *psi* is no longer subject to *psrA*-mediated repression. However, it is also apparent that the regulation and the effects of expression of *psi* must be more complex than being simply subject to repression by *psrA*. We can deduce this from the fact that *psr* mutants are phenotypically normal for EPS synthesis in *in vitro* *Rhizobium*. Thus, other factors must be required for the high level expression of *psi* in *Phaseolus* nodules. These may be mediated by factors which control expression of *psrA* itself. It is clear from results obtained here that the Sym plasmid pRP2JI contains a gene or genes that, in turn, regulate transcription of *psrA*; it will be of interest to identify the nature of this regulatory gene.

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