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NOTES

The *katA* Catalase Gene Is Regulated by OxyR in both Free-Living and Symbiotic *Sinorhizobium meliloti*

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The characterization of an *oxyR* insertion mutant provides evidences that *katA*, which encodes the unique H_2O_2-inducible HPII catalase, is regulated by OxyR not only in free-living *Sinorhizobium meliloti* but also in symbiotic *S. meliloti*. Moreover, *oxyR* is expressed independently of exogenous H_2O_2 and downregulates its own expression in *S. meliloti*.

Sinorhizobium meliloti is a ubiquitous soil α-proteobacterium able to establish symbiosis with alfalfa (*Medicago sativa*) and related legumes, characterized by the formation of root nodules. The exchange of sophisticated recognition signals between the plant and the bacteria leads to the entering of the bacteria into the root hairs and to the development of primordial cells in the cortex, where the bacteria are released (21, 37). Inside the nodule, the bacteria differentiate into their symbiotic form, the bacteroids, which are able to reduce nitrogen to ammonia; the ammonia is then assimilated by the plant. The key enzyme of nitrogen fixation, the nitrogenase, is subjected to a fragile equilibrium. To avoid its rapid and irreversible inactivation by oxygen, a diffusion barrier in the cortex of nodules limits permeation by oxygen (40), and the plant oxygen carrier, leghemoglobin, delivers the necessary oxygen to the bacteroids (9). Nevertheless, a high respiration rate is required to support the nitrogen fixation process, and this leads to the generation of large amounts of reactive oxygen species (ROS) such as superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2), which can also inactivate the nitrogenase (31). ROS have also been detected in nodules (34); H_2O_2 accumulation all around bacteria was observed in some infection threads but never inside bacteria or bacteroids, indicating that they contain an efficient H_2O_2-scavenging system.

To cope with H_2O_2, *S. meliloti* possesses three catalases encoded by three different genes: two monofunctional catalases (HPII), KatA (12) and KatC (36), and one bifunctional catalase-hydroperoxidase (HPI), KatB (12). The catalase genes are differentially expressed during free-living growth, oxidative stress, and nodule establishment (13). *katA* expression has been detected during the exponential growth phase of free-living bacteria only, and *katA* is the unique catalase gene inducible by exogenous H_2O_2. In *Escherichia coli*, inducibility by H_2O_2 and expression in exponential phase have been observed for the *katG* catalase gene encoding the catalase-hydroperoxidase HPI (38). The H_2O_2 induction of *katG* requires the positive activator OxyR (26), which directly senses oxidative stress (18, 41).

Analysis of the *oxyR-katA* genetic region. The complete genome sequence of *S. meliloti* (10) revealed a putative *oxyR* gene (SMc00818) in front of *katA*. The *oxyR* homologous gene in *S. meliloti* is located 193 bp upstream of and in the strand opposite to *katA*. The regulation of an HPII-like catalase by OxyR has been described for *Brucella abortus* only (17). The alignment of the *oxyR-katA* intergenic regions from *S. meliloti* and *B. abortus* (Fig. 1A) revealed highly conserved regions, especially in the DNA-binding site described for *B. abortus* (16). The *S. meliloti* sequence (ATAG-N_7-TTAT-N_7-GGCA-N_7-CAAT) is identical to the *B. abortus* sequence, except that GGCA is replaced by AACA. Moreover, the predicted OxyR amino acid sequences (317 amino acids) for *S. meliloti* and *B. abortus* showed 53% identity, and their alignment indicated the location of the OxyR ATG initiation codon of *S. meliloti* to be 18 bp upstream of the annotated translational start codon. Six amino acids (MLTLRQ) were added to the N-terminal region, four of them being identical in both bacteria (MXTXRQ). Thus, the newly annotated *oxyR* gene was determined to be located 175 bp upstream of *katA* and would encode a 317-amino-acid protein in *S. meliloti*. The two critical cysteines, C_{200} and C_{209}, which were implicated in the activation of OxyR, are conserved in the OxyR of *S. meliloti*.

Construction of an *oxyR* insertion mutant and its role in the adaptation to H_2O_2. To determine the role of *oxyR* in the H_2O_2 response in *S. meliloti*, an *oxyR* mutant, named Rm*oxyR*, was constructed using the parental strain Rm1021 by use of insertional inactivation with a *uidA* transcriptional fusion. Genomic

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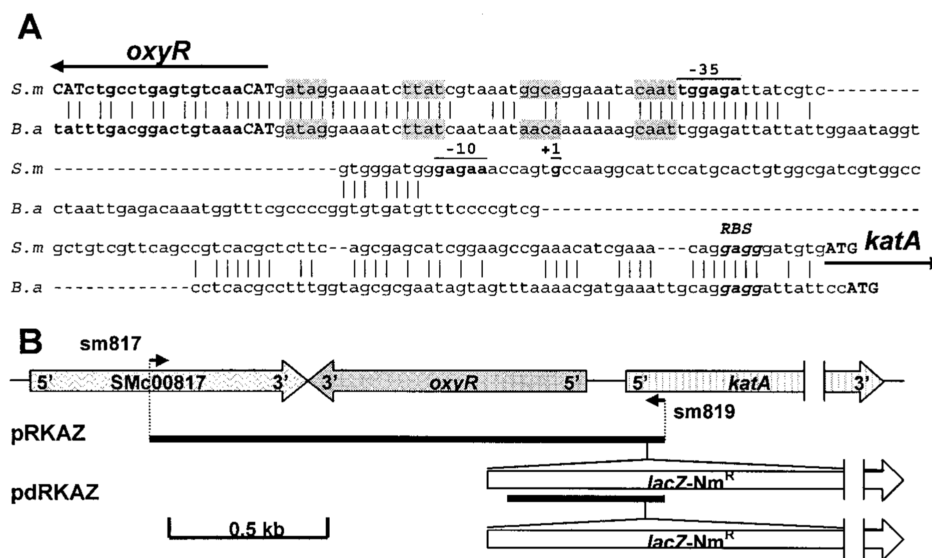

FIG. 1. (A) Alignment of the *oxyR-katA* intergenic sequences of *S. meliloti* (*S.m*) and *B. abortus* (*B.a*). The OxyR DNA-binding site from *B. abortus* is shaded grey. Initial codons from *katA* and *oxyR* are indicated in bold capitals. KatA ribosome binding sites (*RBS*) are indicated in bold italics. The positions of the *katA* promoter from *S. meliloti* are underlined and in bold (+1, −10, and −35 regions). (B) Genetic map of the *oxyR-katA* region and the pRKAZ and pdRKAZ plasmids used in this study. Positions of the primers used for their construction are indicated. Large arrows indicate the locations and directions of transcription of the identified genes. Small arrows indicate the directions and positions of primers.

DNA from strain Rm1021 was amplified by PCR with the oligonucleotides sm818a (5′ CTC GCG GAT GTC GGC AGA TTG G) and sm818b (5′ AAA ACA GCG CCC GGG TAA CGA T) (Fig. 1A). The PCR fragment (271 bp) was cloned into the pCR-TOPO vector (Invitrogen), which was followed by a double digestion with BamHI and XbaI in order to clone this fragment upstream of the present *uidA* gene into the pVO155 suicide vector (29). The mutation was introduced by a single reciprocal recombination into the chromosome of *S. meliloti* Rm1021 by triparental conjugation as described previously (11). The disruption of the *oxyR* gene was confirmed by Southern blotting using vector- and gene-specific probes (data not shown). The bacterial strains and plasmids used in this study are shown in Table 1.

Growth inhibition of strain Rm*oxyR* by H_2O_2 was tested by the halo assay method, which gives a long-term test of resistance to H_2O_2 exposure. Aliquots (200 μl each) of *S. meliloti* cultures with optical densities at 600 nm (OD_{600}) of 0.4 to 0.5 were plated onto Luria-Bertani–MC agar plates (12). Paper disks (6-mm diameter) were impregnated with 5 μl of H_2O_2 (1 M) and placed in the center of the *S. meliloti* plates. After 2 to 3 days of incubation at 30°C, the diameters of the complete growth inhibition zone averaged 26 and 49 mm for the wild-type Rm1021 strain and the Rm*oxyR* mutant, respectively. The Rm*oxyR* strain complemented with the pBBR-*oxyR* plasmid carrying the complete *oxyR* gene showed a growth inhibition zone of 25 mm. To construct the pBBR-*oxyR* plasmid, a PCR fragment carrying the *oxyR* gene, the complete intergenic *oxyR-katA* region, and the beginning of the *katA* gene was amplified using the oligonucleotides sm819 (5′GCGCTCGCGGTTCTG GTG) and sm817 (5′CTTGCGCCCGATTTCCTGTC), subcloned into the pCR-TOPO vector, and cloned in the vector pBBRMCS-3 by use of the SpeI and ApaI sites. In this assay, strain Rm*oxyR* appears to be more sensitive to H_2O_2 than its parental strain, indicating that *oxyR* plays an important role in the protection of *S. meliloti* against the activated species.

To further investigate the role of OxyR in the adaptation of *S. meliloti* to H_2O_2, strains Rm1021 and Rm*oxyR* in exponential phase (OD_{600}, 0.4 to 0.5) were pretreated for 1 h or not with a sublethal dose of H_2O_2 (1 mM), which is known to activate OxyR in *Escherichia coli* (41). Cultures were then treated with a lethal dose of H_2O_2 (20 mM) for various times, and survival was assessed by plating dilutions onto Luria-Bertani–MC agar. With the pretreatment, strain Rm1021 was more resistant to H_2O_2 than Rm*oxyR*, indicating that *oxyR* is required for adaptation to H_2O_2. In contrast, the pretreatment had no effect on the survival of Rm*oxyR* to H_2O_2 treatment. Unexpectedly, without H_2O_2 pretreatment, the Rm*oxyR* mutant was more resistant to H_2O_2 than the parental strain. A similar pattern has been observed for an *oxyR* mutant of *B. abortus*, which was more resistant to H_2O_2 than a wild-type strain when bacteria were not pretreated with H_2O_2 (16).

***oxyR* regulates *katA* expression in free-living conditions.** KatA is the major catalase component of an adaptive response to H_2O_2 (12). To confirm that the results described above were due to KatA deregulation, the Rm*oxyR* mutant and its parental strain were analyzed for total catalase activity spectrophotometrically (Fig. 2A) by monitoring the decomposition of H_2O_2 at 240 nm (15) and on a native polyacrylamide gel by use of negative diaminobenzidine staining (Fig. 2B) as previously described (5, 12) with or without H_2O_2 pretreatment. It clearly appeared that the increases in total catalase activity under the different conditions (Fig. 2A) were due solely to KatA, not to KatB or KatC (Fig. 2B). Upon exposure to H_2O_2, no increase in KatA level was observed in the Rm*oxyR* mutant strain, confirming the hypothesis that OxyR acts as an activator of

TABLE 1. Bacterial strains and plasmids used in this study

Strains and plasmids	Relevant characteristic(s)[a]	Source or reference
Bacteria		
Sinorhizobium meliloti		
RCR2011	SU47, wild type; Nod^+ Fix^+	33
Rm1021	Derivative of RCR2011; Sm^r	23
Rm*oxyR*	Same as Rm1021 but with *oxyR*::*uidA* fusion (*oxyR*); Sm^r Nm^r	This study
1021-pRKAZ	Same as Rm1021 but with plasmid pRKAZ	This study
1021-pdRKAZ	Same as Rm1021 but with plasmid pdRKAZ	This study
oxyR-pRKAZ	Same as Rm*oxyR* but with plasmid pRKAZ	This study
oxyR-pdRKAZ	Same as Rm*oxyR* but with plasmid pdRKAZ	This study
Escherichia coli		
DH5α	F^- *supE44 ΔlacU169* (*ϕ80dlacZΔM15*) *hsdR17*(r_K^- m_K^+) *recA1 endA1 gyrA96 thi-1 relA1*	Bethesda Research Laboratories
MT607	*pro-82 thi-1 hsdR17 supE44 recA56*	8
MT616	MT607(pRK600)	8
Plasmids		
pRK600	ColE1 replicon with RK2 transfer region; Cm^r	8
pBluescriptKS(+)	Derivative of pUC19 with f1(+)*oriR;* Ap^r	Stratagene
pCR-TOPO	Derivative of pUC19 with f1(+)*ori;* Ap^r Km^r	Invitrogen
pGEM-T	T vector with f1 *oriR;* Ap^r	Promega Corp.
pVO155	Derivative of pUC119	29
pKOK5	Derivative of pSUP202, source of *lacZ*-Km^r; Ap^r Km^r	19
pBBR1-MCS-3	Derivative of pBBR1-MCS; Tc^r	20
pCR-TOPO-*oxyR*01	pCR-TOPO vector with 271-bp PCR-amplified fragment of the *oxyR* gene	This study
pCR-TOPO-*oxyR*02	pCR-TOPO vector with 1,780-bp PCR-amplified fragment with the *oxyR* gene and partial *katA* gene	This study
pGEM*oxyR*	pGEM-T, with 920-bp PCR-amplified fragment	This study
pVO-*oxyR*	pVO155, with BamHI-XbaI fragment of pCR-TOPO-*oxyR*01	This study
pBBR-*oxyR*	pBBR IMCS-3, with SpeI-Apal fragment of pCR-TOPO-*oxyR*02	This study
pBBR-d*oxyR*	pBBR-*oxyR,* Sacl-Sacl fragment deleted	This study
pRKAZ	pBBR-*oxyR,* with fusion p*katA-lacZ*	This study
pdRKAZ	pBBR-d*oxyR*, with fusion p*katA-lacZ*	This study
pBSKAI-1	pBluescript, 1.4-kb EcoRI-ApaI fragment with partial *katA*	12

[a] Abbreviations: Tc, tetracycline; Sm, streptomycin; Ap, ampicillin; Km, kanamycin; Cm, chloramphenicol; Sp, spectinomycin.

KatA upon H_2O_2 exposure. Moreover, the basal KatA level in Rm*oxyR* was higher than that in the parental strain Rm1021 without H_2O_2 treatment, which is consistent with the higher resistance observed in strain Rm*oxyR*. No difference was observed in catalase activity patterns and in total catalase activities of Rm1021 and Rm*oxyR* with or without H_2O_2 pretreatment in late stationary phase (data not shown). To verify that KatA deregulations were due to modifications of *katA* transcription, the accumulation of *katA* transcripts was quantified in Rm1021 and Rm*oxyR* by Northern blotting (Fig. 2C). RNAs were isolated and separated by electrophoresis as previously described (1, 3), blotted on a nylon membrane, and probed with a ^{32}P-labeled *katA* probe corresponding to a 450-bp EcoRI-PstI fragment from pBSKA1-1 (12). With or without H_2O_2 treatment, a single 1.6-kb hybridization band was detected in each strain (Fig. 2C). Variations in *katA* RNA levels, measured with a phosphorus imager, and total catalase activities (Fig. 2A) were perfectly correlated, indicating that the observed deregulation is essentially at the transcriptional level. To determine the *katA* transcription start site under oxidative or nonoxidative conditions, primer extension experiments were performed using RNAs from Rm1021 and Rm*oxyR* treated or not with H_2O_2 (Fig. 2D) as described at the Long laboratory website (http://cmgm.stanford.edu/biology/long/protocols.htm#primer), by using primer pextkatA (5′ GGTGGTGGTGATCGTCGGACGATCTGTCAT), which is specific to *katA* labeled with [γ-^{32}P]dATP. DNA sequencing was performed using a CycleReader DNA sequencing kit (MBI Fermentas) with [α-^{33}P]dCTP. The transcription start site is located at G (Fig. 2D), corresponding to a position 103 bp upstream of the *katA* translational start codon, ATG, which is consistent with the hypothetical −35 (TGGAGA) and −10 (GAGAA) boxes and the OxyR binding site (Fig. 1A). The different intensities of the signal for primer extension analysis were also in agreement with the Northern analysis, and no change in the +1 position was observed under the different conditions tested.

An *oxyR* gene has been identified in 31 bacterial genomes (6, 27). The position of *oxyR* is very variable in the different bacterial genomes, but it is often next to a gene involved in oxidative stress protection and regulated by OxyR: for example, *ahpC* in *Mycobacterium tuberculosis* (7) and *Xanthomonas campestris* pv. phaseoli (25), *dps* in *Bacteroides fragilis* (32), *oxyS* in *E. coli* (4), *recG* in *Pseudomonas aeruginosa* (28), *kat* in *B. abortus* (17). Similar genomic relationships between *oxyR* and catalase genes are observed for most members of the *Rhizobiales* studied so far, such as *S. meliloti*, *Agrobacterium tumefaciens*, *B. abortus*, *Mesorhizobium loti*, *Rhizobium etli*, and *Rhizobium leguminosarum* bv. phaseoli. Nevertheless, OxyR potentially regulates a *kat* gene encoding a monofunctional

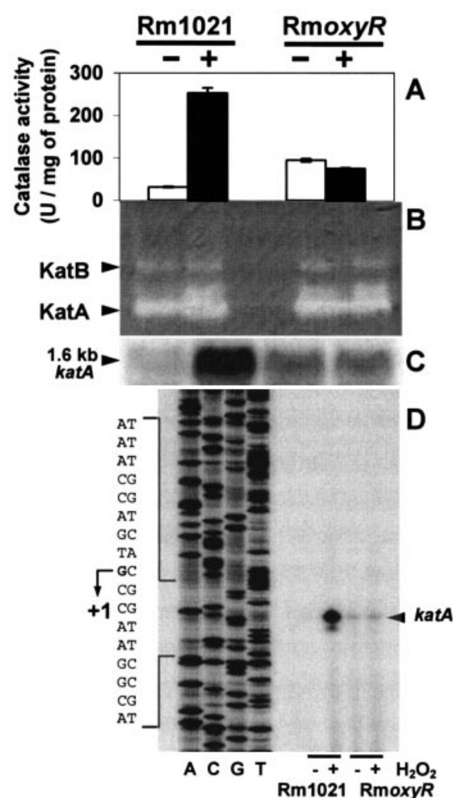

FIG. 2. Effects of H_2O_2 on catalase activity and *katA* gene expression in the parental Rm1021 and the mutant Rm*oxyR* strains. Bacteria were treated (+) or not (−) with 1 mM H_2O_2 for 1 h. Cell extracts were prepared and analyzed for catalase activities spectrophotometrically (A) and on a native polyacrylamide gel (B), using 30 μg of protein per lane and determined with a protein assay kit (Bio-Rad Laboratories GmbH). The positions of KatA and KatB are indicated as described by Sigaud et al. (36). Catalase activities were obtained with triplicate samples from two independent experiments and are given in units per milligram of protein. Data are presented as the means ± standard deviations of results. The expression of the *katA* gene was monitored using Northern blot (C) and primer extension (D) analyses.

catalase (HPII) in *S. meliloti* and *B. abortus* only, whereas in other members of the *Rhizobiales*, *oxyR* is localized in front of a catalase gene encoding a bifunctional catalase-hydroperoxidase (HPI).

***oxyR* expression under free-living conditions.** To analyze the expression of the *oxyR* gene and its regulation by H_2O_2, we monitored the level of β-glucuronidase activity in the Rm*oxyR* strain, which carried an *oxyR*::*uidA* fusion, using *p*-nitrophenyl β-D-glucuronide as the substrate, according to methods previously described (14). With or without H_2O_2 treatment, similar glucuronidase (GUS) activities were observed in Rm*oxyR* (5.9 and 6 U per μg of protein, respectively). In Rm*oxyR* complemented by plasmid pBBR-*oxyR* as well, H_2O_2 treatment did not modify the GUS activity (3.5 and 3.3 U per μg of protein with and without treatment, respectively). This noninduction of *oxyR* expression after H_2O_2 exposure indicates that *oxyR* is constitutively expressed, independently of exogenous H_2O_2. Nevertheless, a reduction in β-glucuronidase activity was observed in Rm*oxyR* complemented by plasmid pBBR-*oxyR* compared to that in the Rm*oxyR* mutant, indicating that *oxyR* downregulates its own expression as in all bacterial species studied so far (27).

TABLE 2. Induction of *katA*-*lacZ* fusion by H_2O_2 in strains 1021-pRKAZ, 1021-pKAZ, *oxyR*-pRKAZ, and *oxyR*-pdRKAZ

Strain	β-Galactosidase activity[a] (Miller units) (mean ± SD)	
	$-H_2O_2$	$+H_2O_2$
1021-pRKAZ	444 ± 97	2,222 ± 265
1021-pdRKAZ	509 ± 130	1,960 ± 320
oxyR-pRKAZ	390 ± 157	1,825 ± 405
oxyR-pdRKAZ	1,338 ± 156	1,292 ± 198

[a] Activity was measured after a 1-h treatment (+) or not (−) with 1 mM H_2O_2.

OxyR regulates *katA* expression in planta. The effects of *oxyR* disruption on the nodulation and fixation capacities of the bacteria were analyzed by infection of *Medicago sativa* and *Medicago truncatula* plantlets with the Rm*oxyR* mutant and the Rm1021 strain as a control. No significant reductions in nodulation and nitrogen fixation capacities were observed in the Rm*oxyR* mutant compared to those in Rm1021 (data not shown).

To analyze the role of the OxyR regulator on *katA* regulation in planta, plants were infected with bacterial strains carrying *katA*-*lacZ* plasmid fusions. By use of the two SacI sites present in the *oxyR* gene and in the polylinker of the vector of the pBBR-*oxyR* plasmid, the *oxyR* gene was disrupted by a deletion, resulting in the pBBR-d*oxyR* plasmid. A 3.7-kb PstI-PstI fragment from the pKOK5 vector containing the promoterless *lacZ*-Km^r cartridge was inserted into the PstI sites of the pBBR-*oxyR* and pBBR-d*oxyR* plasmids. The recombinant plasmids harboring the correctly orientated *katA*-*lacZ* fusions were selected and designated, respectively, pRKAZ and pdRKAZ (Fig. 1B); these plasmids were introduced into the parental strain Rm1021 (resulting in strains 1021-pRKAZ and 1021-pdRKAZ, respectively) and in the Rm*oxyR* mutant (resulting in strains *oxyR*-pRKAZ and *oxyR*-pdRKAZ, respectively). To validate the H_2O_2 inducibility of these *katA*-*lacZ* fusions in the different genetic backgrounds, the β-galactosidase activities were measured for the different strains treated or not with 1 mM H_2O_2 for 1 h (Table 2) using *o*-nitrophenyl-β-D-galactoside as previously described (24). The results clearly indicated that strains 1021-pRKAZ, 1021-pdRKAZ, and *oxyR*-pRKAZ showed similar H_2O_2 induction patterns. In contrast, the *oxyR* mutant harboring the *oxyR*-truncated *katA*-*lacZ* fusion (*oxyR*-pdRKAZ) was deregulated, a result that is consistent with the results presented in Fig. 2. Moreover, the provision of a full-length *oxyR* in *trans* in the Rm*oxyR* mutant (*oxyR*-pRKAZ) restores an essentially wild-type *katA* level when H_2O_2 is not present. In order to keep a ratio of one *oxyR* gene to every *katA* promoter, results obtained with strains 1021-pRKAZ and *oxyR*-pdRKAZ were taken into account only for in planta experiments. Despite the absence of antibiotic selection pressure during the symbiotic process, the stabilities of recombinant plasmids pRKAZ and pdRKAZ in bacteria were determined to be good by testing the levels of antibiotic resistance of bacteria reisolated from 5-week-old root nodules (93 and 95% of earlier resistance levels, respectively). Analysis of total β-galactosidase activity in 5-week-old nodule extracts showed

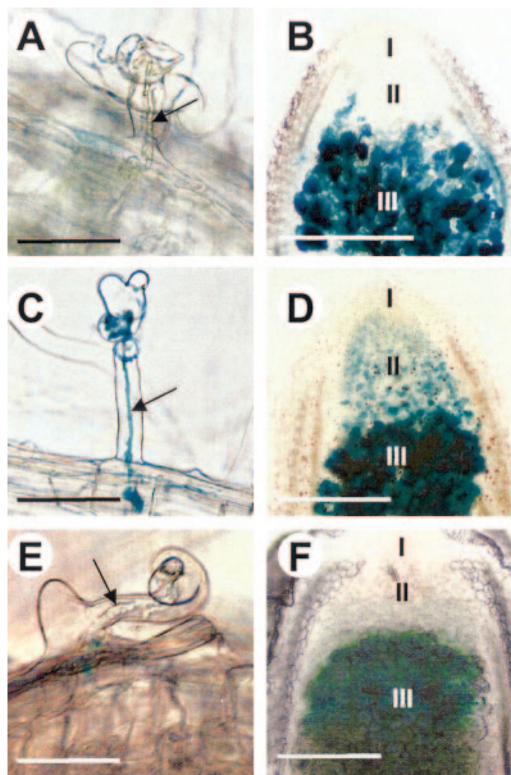

FIG. 3. Histochemical detection of *katA* (A, B, C, and D) and *oxyR* (E and F) expression during *S. meliloti-M. sativa* nodule development. *katA* expression was monitored in the 1021-pRKAZ (A and B) and *oxyR*-pdRKAZ (C and D) strains. β-Galactosidase (A, B, C, and D) and β-glucuronidase (E and F) activities were detected using X-Gal and X-Gluc, respectively. *katA-lacZ* and *oxyR-uidA* fusions were detected in roots hairs 4 days after infection (A, C, and E) and in 5-week-old nodules (B, D, and F). Arrows indicate infection threads. Spatial development zones (I, II, and III) are indicated on nodule cross sections. Scale bars, 50 (A, C, and E) and 200 (B, D, and F) μm.

that the expression of the *katA-lacZ* fusion was higher in 1021-pRKAZ (1,095 ± 106 Miller units per μg of protein) than in *oxyR*-pdRKAZ (469 ± 12 Miller units per μg of protein), indicating that OxyR also acts as an activator for *katA* in symbiotic bacteria. It was noted that equivalent levels of *lacZ* fusion expression were observed in nodule extracts obtained from both strains 1021-pdRKAZ (1,062 Miller units per μg of protein) and *oxyR*-pRKAZ (1,014 Miller units per μg of protein), indicating that strains 1021-pRKAZ, 1021-pdRKAZ, and *oxyR*-pRKAZ showed similar behaviors in planta and in free-living bacteria (Table 2). The histochemical detection of β-galactosidase activity was performed as previously described (2), using X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as a substrate (Fig. 3A to D). Nodule sections (150 μm thick) were obtained with a Vibratome 1000 Plus (Labonord, Lille, France) and mounted on slides for observation and photography with an Olympus microscope. Four days after infection, analysis of root hairs revealed no staining inside most infection threads obtained with 1021-pRKAZ (Fig. 3A), whereas a blue staining in all the infection threads was observed with *oxyR*-pdRKAZ (Fig. 3C). Analysis of 5-week-old nodules showed that the expression of the *katA-lacZ* fusion is strongly detected in nitrogen-fixing bacteroids (zone III) for both the 1021-pRKAZ and *oxyR*-pdRKAZ strains (Fig. 3B and D). In contrast, a significant detection of β-galactosidase was observed in infection zone II with the *oxyR*-pdRKAZ strain only (Fig. 3D). These results are consistent with those observed under free-living conditions: *katA* is constitutively expressed in the Rm*oxyR* mutant. Thus, OxyR is clearly implicated in the regulation of *katA* both in free-living bacteria and in planta.

***oxyR* expression in planta.** To analyze the expression of the *oxyR* gene during the development of the root nodule, *Medicago sativa* plantlets were infected with the Rm*oxyR* mutant strain harboring the *oxyR*::*uidA* fusion. The histochemical detection of β-glucuronidase activity was performed using X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) as the substrate, as previously described (14). Four days after infection, analysis of root hairs revealed no staining (Fig. 3E), suggesting that the *oxyR* expression level was below the threshold detection level at this step of infection. In contrast, analysis of 5-week-old nodules showed that *oxyR*::*uidA* is detectable solely in nitrogen-fixing bacteroids (zone III) (Fig. 3F) and that β-glucuronidase activity from purified Rm*oxyR* bacteroids was exacerbated (36 U per μg of protein) compared to the activity observed in free-living bacteria (5 U per μg of protein). This result indicates an overexpression of *oxyR* gene transcription in bacteroids. Despite the absence of β-glucuronidase activity inside infection threads, analysis of *katA* expression with the 1021-pRKAZ and *oxyR*-pdRKAZ strains showed that OxyR could also repress *katA* expression in most infection threads and in zone II of mature nodules. An expression level of the *oxyR-uidA* fusion below the detection threshold could explain these contradictory results. On the other hand, contrary to our previous observations with the GKAZ01 strain (13), expression of the reporter gene in the 1021-pRKAZ strain has also been detected in some infection threads, which could be correlated with the H_2O_2 accumulation pattern observed during the symbiotic process (34). Recent results suggest that H_2O_2 could be required for the progression of infection threads (30). It may be proposed that these necessary H_2O_2 pulses that occur in infection threads could activate OxyR and periodically derepress *katA* expression.

Despite the fact that the production of a truncated OxyR in the *oxyR* mutant could disturb the regulation of the *katA* gene, our results suggest that OxyR could potentially repress *katA* gene expression in the absence of exogenous H_2O_2 in *S. meliloti*. Indeed, the possibility that OxyR could act as a repressor of a catalase gene has been recently demonstrated in *Neisseria gonorrhoeae* (39). Moreover, OxyR dually regulates *ahpC* expression in *X. campestris* pv. phaseoli (22). In this bacterial plant pathogen, *ahpC* expression is activated by oxidized OxyR and repressed by reduced OxyR. Binding of the reduced form of OxyR blocks the −35 region, preventing binding of RNA polymerase and leading to repression of the gene. Recent results with *E. coli* indicate that alkyl hydroperoxide reductase (Ahp) is also involved in the primary scavenging of H_2O_2 when the concentration is very low (35). It must be pointed out that a sophisticated and unusual regulation of genes involved in the H_2O_2-scavenging system by OxyR has been observed in bacteria interacting with hosts (*S. meliloti*, *B. abortus*, and *X. campestris* pv. phaseoli). It may be suggested that this process optimizes, in all cases, the host-bacterium interactions.

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