JOURNAL OF BACTERIOLOGY, Apr. 2006, p. 3143–3148 Vol. 188, No. 8
0021-9193/06/$08.00+0 doi:10.1128/JB.188.8.3143–3148.2006

BacA-Mediated Bleomycin Sensitivity in *Sinorhizobium meliloti* Is Independent of the Unusual Lipid A Modification

Gail P. Ferguson,[1]* An Jansen,[2] Victoria L. Marlow,[1] and Graham C. Walker[2]*

Institute of Structural and Molecular Biology and Centre for Science at Extreme Conditions, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, United Kingdom,[1] and Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139[2]

Received 25 October 2005/Accepted 27 January 2006

Sinorhizobium meliloti bacA **mutants are symbiotically defective, deoxycholate sensitive, and bleomycin resistant. We show that the bleomycin resistance phenotype is independent of the lipid A alteration and that the changes giving rise to both phenotypes are likely to be involved in the inability of *bacA* mutants to persist within their hosts.**

The BacA protein is essential for *Sinorhizobium meliloti*, a legume symbiont, and *Brucella abortus*, a phylogenetically related mammalian pathogen, to establish chronic intracellular infections in their respective hosts (9, 13). Although the precise function of BacA is unknown, *S. meliloti* and *B. abortus bacA*-null mutants display a range of phenotypes during growth in complex medium, including low-level resistance to bleomycin, a glycopeptide antibiotic, and increased sensitivity to detergents compared with their respective parent strains (7, 10). The detergent sensitivity phenotype led to the discovery that BacA affects the unusual very-long-chain fatty acid (VLCFA) modifications, 27-OHC28:0, 27-OH(βOMeC4:0)C28, and 29-OHC30:0, of the lipid A in both *S. meliloti* and *B. abortus* (5, 7). Thus, in the absence of BacA, only ~50% of the lipid A molecules of *S. meliloti* and *B. abortus* become modified with a VLCFA, in contrast to their respective parent strains, whose lipid A molecules all contain a VLCFA modification (5). However, recent evidence suggests that the unusual lipid A modification observed during growth of wild-type *S. meliloti* in complex medium is important, but not essential, for the legume symbiosis (6). Thus, since additional VLCFA modifications of the lipid A occur during the symbiosis of *Rhizobium leguminosarum* with peas (11), and we observed a similar increase in *S. meliloti* lipopolysaccharide (LPS) hydrophobicity during the alfalfa symbiosis (6), we proposed a model whereby BacA could be involved in host-induced lipid A changes. These BacA-dependent lipid A changes could be essential for the chronic infection of their eukaryotic hosts by *S. meliloti* and *B. abortus* (6).

Nevertheless, there also remained a formal possibility that additional lipid A-independent changes were occurring in the *S. meliloti bacA*-null mutant and that one or more of these changes could also be involved in the inability of *bacA* mutants to persist within their hosts. For example, it seemed unlikely that a reduction in the lipid A VLCFA content could account for the low-level bleomycin resistance phenotype of the *S. meliloti bacA*-null mutant, since deletion of the *bacA* homolog, *sbmA*, in *Escherichia coli* also gives rise to a similar phenotype (10), despite the fact that the lipid A of *E. coli* lacks VLCFA modifications (15). Thus, these data suggested that BacA might have additional effects on *S. meliloti*, resulting in increased sensitization of wild-type *S. meliloti* toward bleomycin relative to the *S. meliloti bacA* mutant.

S. meliloti **mutants completely lacking the lipid A VLCFA modification have increased sensitivity to bleomycin.** Our recent discovery that the lipid A species produced by the *S. meliloti acpXL* and *lpxXL* insertional mutants in LB medium supplemented with 2.5 mM $MgSO_4$ and 2.5 mM $CaCl_2$ (LB/MC medium) completely lacked the lipid A VLCFA modifications (6) provided us with the means to investigate how the absence of the lipid A VLCFA modification in *S. meliloti* influences bleomycin sensitivity. The *acpXL* and *lpxXL* gene products encode a VLCFA-acyl carrier protein (3) and a VLCFA-acyltransferase protein (1), which are directly involved in the biosynthesis of VFCFA-modified lipid A in *S. meliloti*. However, in contrast to *S. meliloti bacA* mutants, which display low-level resistance to bleomycin, the *S. meliloti acpXL* and *lpxXL* insertional mutants displayed increased sensitivity to bleomycin on LB/MC agar relative to the parent strain, Rm1021 (Fig. 1A). Interestingly, consistent with previous stress sensitivity assays (6), the *lpxXL* mutant was more sensitized to bleomycin than the *acpXL* mutant. Since the *acpXL* mutant, but not the *lpxXL* mutant, produces a significant percentage of its total lipid A molecules in the pentaacylated state (6), despite lacking the VLCFA modification, these pentaacylated lipid A molecules must confer some protection against bleomycin. However, these data show that the complete absence of the lipid A VLCFA modification increases the sensitivity of *S. meliloti* to bleomycin and does not lead to increased bleomycin resistance. Thus, these findings provide further support for the notion that the low-level bleomycin resistance phenotype of the *S. meliloti bacA* mutant is unlikely to be due to a reduction in the lipid A VLCFA modification content.

Deletion of *bacA* increases the resistance of *S. meliloti* to bleomycin even in mutants lacking the lipid A VLCFA modification. To further confirm that the low-level bleomycin resistance phenotype of the *S. meliloti bacA*-null mutant could occur independently of the lipid A VLCFA modification, we

* Corresponding author. Mailing address for Graham C. Walker: Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139. Phone: (617) 253-7066. Fax: (617) 253-2643. E-mail: gwalker@mit.edu. Mailing address for Gail P. Ferguson: Institute of Structural and Molecular Biology and Centre for Science at Extreme Conditions, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, United Kingdom. Phone: 44 131 650 7066. Fax: 44 131 6501 3331. E-mail: G.Ferguson@ed.ac.uk.

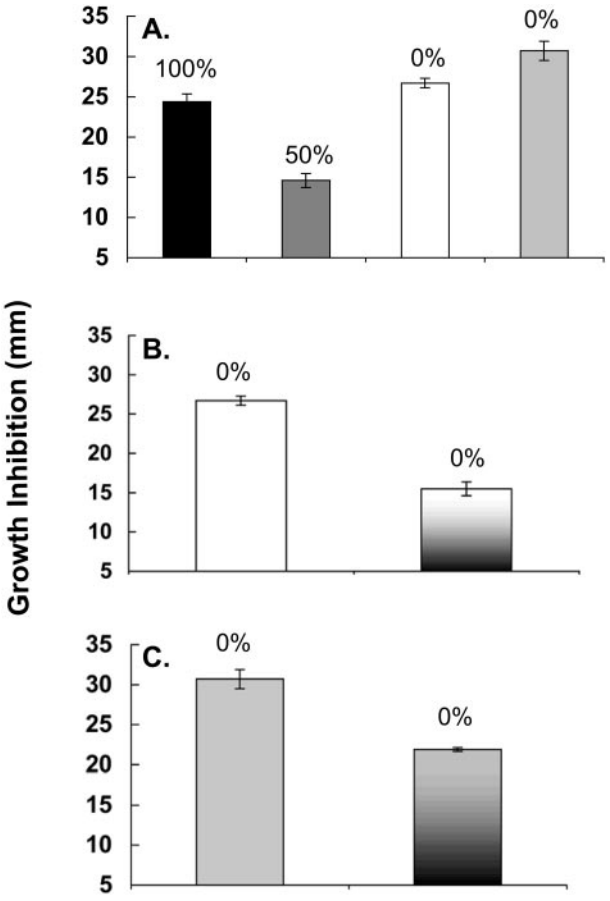

FIG. 1. Deletion of *bacA* confers resistance to bleomycin in strains lacking the lipid A VLCFA modification. (A) The sequenced *S. meliloti* strain Rm1021 (black bar) (8) and a series of isogenic mutants (Δ*bacAsp* [dark gray bar], *acpXL*::pK18mobGII [white bar], *lpxXL*::pJH104 [light gray bar]) (6, 10) were exposed to 5 μl of bleomycin (5-mg ml^{-1} aqueous stock solution) following a standard disk diffusion assay on LB/MC agar (14). The diameter of the growth inhibition zone was recorded after 72 h of growth at 30°C. Experiments were conducted on LB/MC agar, since growth of the *lpxXL* single mutant and the *lpxXL bacA* double mutant was defective on LB agar alone (6). (B) Like panel A, but the sensitivities of isogenic *acpXL*::pK18mobGII (white bar) and Δ*bacAsp acpXL*::pK18mobGII (white and black bar) mutants (6) to bleomycin were assessed. (C) Same as panel B except that isogenic *lpxXL*::pK18mobGII (light gray bar) and Δ*bacAsp lpxXL*::pK18mobGII (gray and black bar) mutants (6) were used. Percentages represent the amount of the lipid A VLCFA modification detected in each strain (6).

compared the bleomycin sensitivities of the *S. meliloti acpXL bacA* and *lpxXL bacA* double mutants to those of the *acpXL* and *lpxXL* single mutants, respectively (Fig. 1B and C, respectively). We demonstrated previously that the *S. meliloti acpXL bacA* and *lpxXL bacA* double mutants had lipid A profiles identical to those of the *acpXL* and *lpxXL* single mutants (6), respectively, and that their lipid A molecules completely lacked the VLCFA modification. However, despite the lack of the lipid A VLCFA modification, the absence of BacA still conferred low-level resistance to bleomycin in the *acpXL bacA* and *lpxXL bacA* double-mutant backgrounds relative to the respective *acpXL* and *lpxXL* single mutants (Fig. 1B and C, respectively). Thus, these data show that deletion of *bacA* increases the resistance of *S. meliloti* to bleomycin, even in mutant strains that completely lack the lipid A VLCFA modification. Additionally, these findings argue that BacA must exert a lipid A-independent effect on the parent strain and that the absence of BacA gives rise to the low-level bleomycin resistance phenotype displayed by the *S. meliloti bacA* mutant.

In the related legume symbiont, *R. leguminosarum*, deletion of *acpXL* results in growth defects in complex medium relative to the parent strain (17). However, we observed that *S. meliloti* mutants with insertions in the *lpxXL* gene, but not the *acpXL* gene, had a reduced growth rate on LB/MC agar without bleomycin (data not shown). Since deletion of *bacA* did not further affect the growth rate of either the *S. meliloti* parent strain or the *lpxXL* and *acpXL* mutants on LB/MC agar without bleomycin (data not shown), these findings rule out the possibility that deletion of *bacA* confers protection of *S. meliloti* against bleomycin due to growth rate alterations.

Transposon insertions in the *bacA* gene alone lead to bleomycin resistance in *S. meliloti*. To gain further insights into resistance to bleomycin in *S. meliloti* and to determine whether bleomycin resistance per se was linked to the inability of *S. meliloti* to form a successful legume symbiosis, a transposon mutant library was constructed in the *S. meliloti* Rm1021 parent strain background using Tn*5-233* delivered on the suicide vector pRK607 (4). Tn*5-233* was used instead of Tn*5* because the latter transposon contains the *ble* gene, which encodes high-level resistance to bleomycin (4). The bleomycin sensitivity of the parental strain Rm1021 was initially determined by plating approximately 5×10^6 stationary-phase cells of Rm1021 onto LB agar supplemented with a range of bleomycin concentrations (0 to 2.5 μg ml^{-1}). We discovered that the growth of the parent strain was severely affected by the inclusion of ≥0.5 μg ml^{-1} bleomycin in the agar. Thus, ~8×10^5 *S. meliloti* Tn*5-233* mutants were subsequently plated onto LB agar containing 0.5 to 2.5 μg ml^{-1} bleomycin, and 9 putative bleomycin-resistant mutants were purified. All nine Tn*5-233* mutants were shown to display a range of bleomycin resistance phenotypes compared to the parent strain, some having a level of resistance similar to that of a *bacA*-null mutant and some having higher levels of resistance (Fig. 2A). However, after transduction of the Tn*5-233* insertions from the bleomycin-resistant mutants into Rm1021, all of the resulting transposon mutants conferred low-level resistance to bleomycin, similar to that observed for the *bacA*-null mutant (Fig. 2A). Thus, these findings suggested that some of the original transposon-induced bleomycin-resistant mutants contained additional unlinked mutations that contributed to their higher-level bleomycin resistance phenotype. Consistent with this hypothesis, we discovered that, like the *bacA*-null mutant, all nine of the transduced Tn*5-233* mutants had increased sensitivity to deoxycholate (DOC) (Fig. 2B) and were defective in alfalfa symbiosis (data not shown). Subsequent analysis by PCR, using a Tn*5-233*-specific forward primer and a *bacA*-specific reverse primer, confirmed that that all nine of the transposon mutants contained insertions disrupting their *bacA* genes (data not shown). Since the *S. meliloti* genome is predicted to encode ~6,000 genes (8) and since we plated ~8×10^5 transposon mutants, our selection was performed under saturating conditions. Additionally, since the *S. meliloti bacA* gene is not arranged in an operon and since a plasmid carrying the *S. meliloti bacA* gene

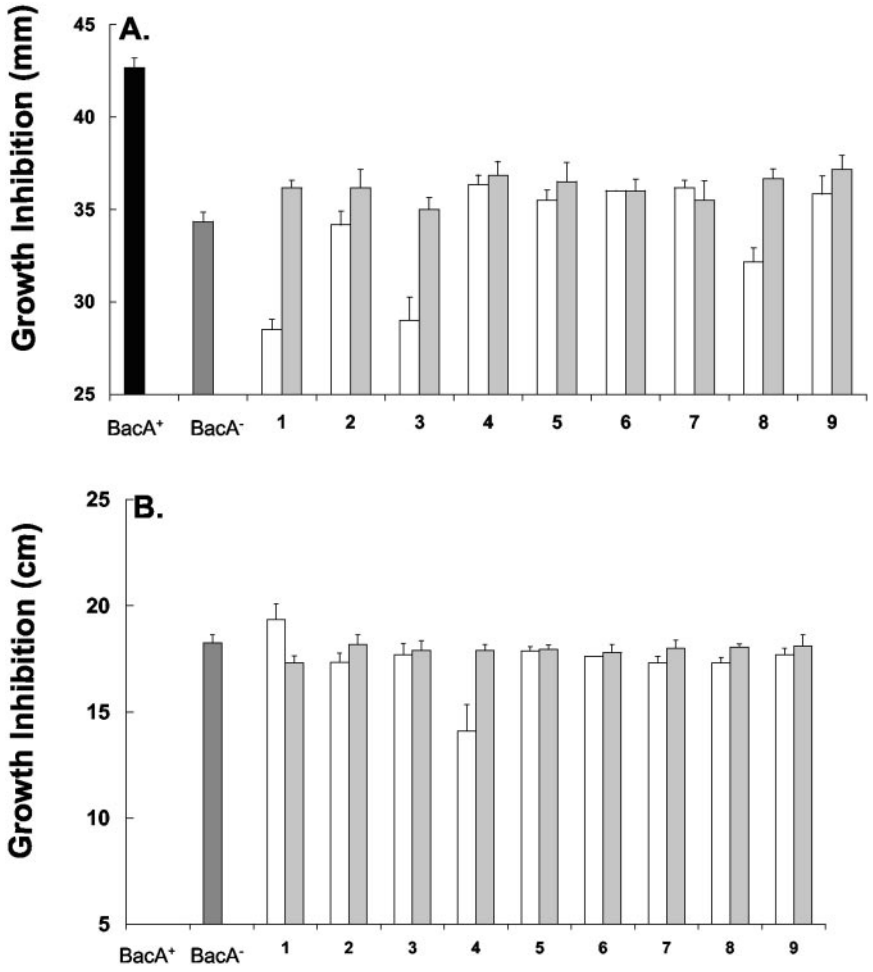

FIG. 2. The bleomycin-resistant transposon mutants are also sensitive to deoxycholate. *S. meliloti* Rm1021 (black bars), a Δ*bacAsp* mutant (dark gray bars), and a series of Tn*5-233* insertional mutants (white bars) were exposed to either bleomycin on LB agar, using the disk diffusion assay (A), or a gradient of 0 to 24 mM deoxycholate on LB agar, as described previously (6) (B), and growth inhibition was recorded. As observed previously (6), Rm1021was not inhibited in the presence of deoxycholate. The bleomycin resistance (A) and deoxycholate sensitivity (B) assays were also conducted after transduction of the Tn*5-233* insertions into Rm1021 (light gray bars).

(9) complemented all the reported phenotypes of the *bacA* transposon insertion mutants (data not shown), this provided evidence that the phenotypes of the transposon mutants were due to disruption of the *bacA* gene and not to polar effects on downstream genes. Thus, under our experimental conditions, only disruption of the *bacA* gene resulted in bleomycin resistance in *S. meliloti* Rm1021. However, we cannot rule out the possibility that essential genes could also affect the resistance of *S. meliloti* to killing by bleomycin.

Bleomycin resistance per se is not necessarily linked with the inability of *S. meliloti* to establish a successful symbiosis. Since only *bacA* mutants were isolated from our transposon mutagenesis study, we were unable to address whether bleomycin resistance per se was linked to the inability of *S. meliloti* to form a successful symbiosis. However, during our earlier assessment of the sensitivity of the parent strain, Rm1021, to bleomycin, we obtained 38 spontaneous bleomycin mutants with various levels of resistance to bleomycin (Fig. 3A). Although we termed these spontaneous mutants, since bleomycin has been shown to be a DNA-damaging agent in *E. coli* (18), it is also possible that some of these 38 bleomycin resistance mutants arose due to bleomycin-induced DNA damage. Interestingly, the level of bleomycin resistance in these 38 mutants did not necessarily correlate with the concentration of bleomycin used for the initial selection conditions (Fig. 3A). Since these mutants displayed a range of bleomycin resistances, despite the selection not being performed in the optimal manner (i.e., independent cultures were not used), they allowed us to investigate whether there was any link between the level of bleomycin resistance per se and the ability of *S. meliloti* to persist within legumes.

Inoculation of the 38 mutants onto individual alfalfa seedlings on Jensen's agar, which lacks a nitrogen and carbon source, enabled us to assess their symbiotic competency (12). Four weeks postinoculation, we discovered that 10/38 mutants were unable to establish a successful symbiosis with alfalfa (data not shown). Thus, in contrast to alfalfa seedlings inoculated with symbiotically competent *S. meliloti*, which were dark green and had elongated pink nodules, indicative of a successful nitrogen-fixing symbiosis, the alfalfa seedlings inoculated

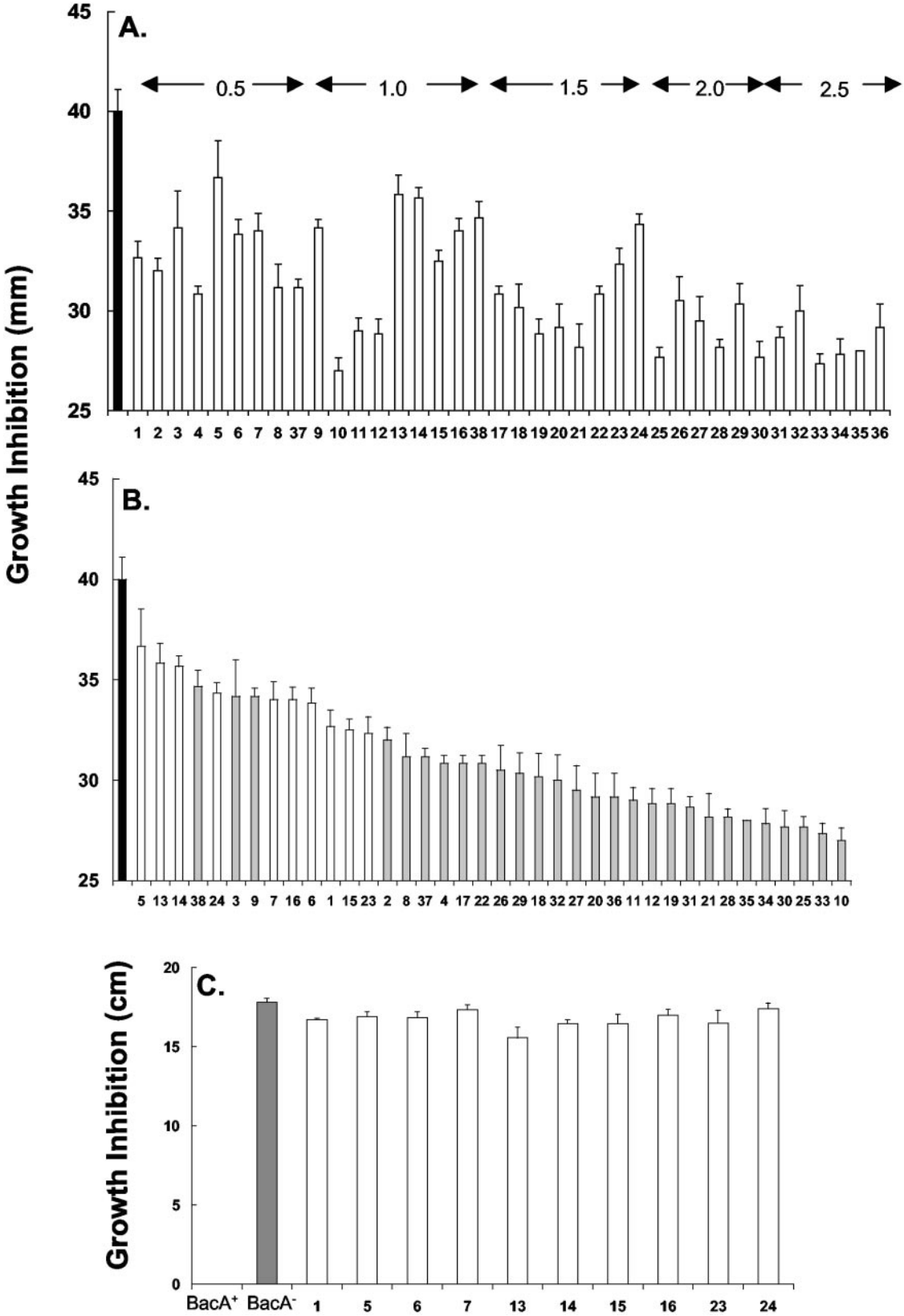

FIG. 3. A subset of the bleomycin-resistant mutants that are defective in alfalfa symbiosis are also deoxycholate sensitive. (A) Growth inhibition of *S. meliloti* Rm1021 (black bar) and the bleomycin-resistant mutants (white bars) was determined on LB agar using the standard bleomycin disk diffusion assay, as described previously (14). The numbers above the bars indicate the bleomycin concentration ($\mu g\ ml^{-1}$) used in the initial mutant selection. (B) Like panel A, except that mutants were arranged occurring to their level of bleomycin resistance. Black bar, Rm1021; white bars,

with the 10 mutants that were defective in establishing a symbiosis were stunted in height and yellowish green, with white nodules. Intriguing, all 10 of the bleomycin-resistant mutants that were unable to form a successful symbiosis with alfalfa (termed class I mutants) displayed low-level resistance to bleomycin (Fig. 3B), similar to the *bacA*-null and transposon mutants (Fig. 2A). However, since several of the mutants (termed class II mutants) also displayed low-level resistance to bleomycin (Fig. 3B) yet were fully competent in alfalfa symbiosis (data not shown), these data showed that the low-level bleomycin resistance phenotype per se was not necessarily linked to the inability of *S. meliloti* to form a successful legume symbiosis. Additionally, since mutants displaying a higher level of resistance to bleomycin (termed class III mutants) (Fig. 3B) were symbiotically proficient (data not shown), these findings provided further evidence that resistance to bleomycin per se is not necessarily linked to the inability of *S. meliloti* to persist within legumes.

Class I mutants all have mutations in their *bacA* genes. The fact that mutations in *bacA* are the only mutations known to date to give rise to low-level bleomycin resistance and symbiotic defects in *S. meliloti* led us to investigate whether our class I mutants contained mutations in their *bacA* genes. To investigate this further, the *bacA* genes and promoter regions from our class I mutants were sequenced, and we determined that all class I mutants had mutations in their *bacA* genes, which would result in the production of truncated BacA proteins (Table 1). Intriguingly, a number of the class I mutants had either an addition or a deletion at position 92 in their *bacA* gene (Table 1). Since the *bacA* gene sequence in this region contains a stretch of guanine residues, which are known to cause problems with DNA replication, leading to an increased frequency of frameshift mutations, this is likely to account for our observations. Additionally, we also observed that a number of the class I mutants had two mutations in their *bacA* gene (Table 1). Thus, it may be that frequently occurring mutations at hot spots in *bacA* lead to a growth disadvantage that results in selection for the acquisition of a second mutation in the *bacA* gene. However, these findings provide evidence that class I mutants produce mutant forms of the BacA protein. Consistent with this, all class I mutants displayed increased sensitivity to DOC on LB agar (Fig. 3C), and all of their phenotypes could be complemented by transformation with a plasmid harboring the intact *S. meliloti bacA* gene (pJG51A) but not with the control plasmid alone (pRK404) (9) (data not shown).

In contrast to the class I mutants, only one of the class II mutants was mutated in the *bacA* coding region and promoter (Table 1). The fact that this class II mutant differed from the class I mutants in being symbiotically proficient suggests that it must produce a functional BacA protein, despite having a mutation in its *bacA* promoter and a frame shift near the 3′ end of the *bacA* coding region. The rest of the class II mutants did not have mutations in their *bacA* coding region or promoter (Table 1). Intriguingly, all the class II mutants were as resistant to DOC as the parent strain, including the class II mutant, which contained mutations in the *bacA* gene (data not shown). Thus, these findings suggest that the changes in the *bacA* mutants giving rise to increased sensitivity to DOC and increased resistance to bleomycin are both involved in the inability of *bacA* mutants to form a successful symbiosis.

TABLE 1. Class I bleomycin-resistant mutants all have mutations in their *bacA* genes

Mutant[a]	Position and nature of mutations[b]	No. of amino acids in mutant BacA protein[c] (no. identical to wild-type BacA)
Class I[d]		
1 and 6	Addition of G at +92, deletion of C at +1251	120 (31)
5 and 6	Addition of G at +92	120 (31)
23	Addition of G at +92, deletion of T at +1260	120 (31)
15 and 24	Deletion of G at +92	120 (31)
13	77-bp C-terminal deletion	396 (382)
14 and 16	Deletion of T at +37 and +1260	96 (12)
Class II[e]		
2, 9, and 38	None	None
3[f]	Deletion of T at +1260	444

[a] All mutants display low-level resistance to bleomycin.
[b] + indicates position in gene relative to start site; the *bacA* promoters and coding regions were sequenced using the forward (5′CCGTCGTTCTCAT GATCTGC3′) and reverse (5′GCGTTGCCGATTATCGAGGC3′) primers.
[c] Wild-type BacA protein has 420 amino acids.
[d] Class I mutants are unable to form a successful alfalfa symbiosis and are sensitive to deoxycholate.
[e] Class II mutants form a successful symbiosis and are deoxycholate insensitive.
[f] This mutant also has an addition of an A at position −63 in the *bacA* promoter.

Conclusions. In this study, we show that the bleomycin resistance phenotype of the *S. meliloti bacA* mutant is independent of the lipid A alteration in this mutant. We also show that bleomycin resistance per se is not necessarily linked to the inability of *S. meliloti* to establish a successful symbiosis. Instead, this study provides evidence that the specific changes in *bacA* mutants resulting in the DOC sensitivity and bleomycin resistance phenotypes appear to be involved in the inability of *bacA* mutants to form an effective legume symbiosis. However, the mechanism by which loss of BacA gives rise to bleomycin resistance is still unresolved.

Interestingly, an *E. coli* mutant lacking the BacA homolog, SbmA, is also resistant to bleomycin and microcin antibiotics (10, 16). Since the *E. coli sbmA* mutant is as sensitive to internally synthesized microcins as the parent strain, this finding led to a model whereby SbmA is proposed to directly transport unusual peptides, such as bleomycin and microcin antibiotics, into the *E. coli* cell (16). Thus, it is possible that *S. meliloti* BacA may also be involved in bleomycin transport. However, there is no direct evidence demonstrating transport

mutants defective in alfalfa symbiosis; light gray bars, mutants proficient in alfalfa symbiosis. (C) Growth inhibition of Rm1021 (black bar) (no growth inhibition observed), an isogenic Δ*bacAsp* mutant (dark gray bar), and the bleomycin-resistant mutants, which were defective in alfalfa symbiosis (white bars), after exposure to a gradient of 0 to 24 mM deoxycholate on LB agar, as described previously (6). The bleomycin-resistant mutants that were symbiotically competent were not inhibited by deoxycholate (data not shown).

of bleomycin by *E. coli* SbmA, and it is difficult to rationalize why loss of a transport system would result only in low-level resistance to bleomycin. Additionally, the fact that bleomycin can still inhibit the growth of the *S. meliloti bacA* mutants suggests that there would have to be another mechanism for bleomycin uptake and/or that bleomycin can cause additional damage to an extracytoplasmic component of *S. meliloti*. However, when we selected for bleomycin-resistant mutants after transposon mutagenesis, we obtained only *bacA* mutants, suggesting either that an additional uptake system does not exist or that BacA is essential for the growth of *S. meliloti* on LB agar. Bleomycin has been shown to induce DNA damage in *E. coli*, and RecA was found to be involved in repair of this damage (18). We also found that an *S. meliloti recA*::Tn*5-233* mutant has increased sensitivity to bleomycin compared to the parent strain (V. L. Marlow, C. Rougier, G. C. Walker, and G. P. Ferguson, unpublished data), suggesting that bleomycin can enter into *S. meliloti* cells and cause DNA damage. However, bleomycin has been shown to damage the cell wall of *Saccharomyces cerevisiae* (2), leading to increased spheroplast production, and thus, bleomycin may also be causing cell wall damage, in addition to DNA damage, in *S. meliloti*. Thus, future studies will be required to determine the precise effects of bleomycin on *S. meliloti*, the molecular basis of BacA-dependent bleomycin sensitivity, and the exact role of BacA in persistent bacteriuml-host interactions. However, these studies provide further evidence that BacA is also capable of exerting lipid A-independent effects in *S. meliloti*.

G.P.F. and A.J. were funded by National Institutes of Health grant GM31030, awarded to G.C.W. G.P.F. is currently supported by a University of Edinburgh Development Trust grant (D54305) and a BBSRC grant (BB/D000564/1). V.L.M. is funded by a BBSRC Ph.D. studentship. G.C.W. is also supported by an American Cancer Society Research Professorship and a Howard Hughes Medical Institute Professorship.

We thank Marty Roop II, Carole Rougier, and the Walker lab for many helpful discussions.

REFERENCES

1. **Basu, S. S., M. J. Karbarz, and C. R. Raetz.** 2002. Expression cloning and characterization of the C28 acyltransferase of lipid A biosynthesis in *Rhizobium leguminosarum*. J. Biol. Chem. **277:**28959–28971.
2. **Beaudouin, R., S. T. Lim, J. A. Steide, M. Powell, J. McKoy, A. J. Pramanik, E. Johnson, C. W. Moore, and P. N. Lipke.** 1993. Bleomycin affects cell wall anchorage of mannoproteins in *Saccharomyces cerevisiae*. Antimicrob. Agents Chemother. **37:**1264–1269.
3. **Brozek, K. A., R. W. Carlson, and C. R. Raetz.** 1996. A special acyl carrier protein for transferring long hydroxylated fatty acids to lipid A in *Rhizobium*. J. Biol. Chem. **271:**32126–32136.
4. **De Vos, G. F., G. C. Walker, and E. R. Signer.** 1986. Genetic manipulations in *Rhizobium meliloti* using two new transposon Tn*5* derivatives. Mol. Gen. Genet. **204:**485–491.
5. **Ferguson, G. P., A. Datta, J. Baumgartner, R. M. Roop II, R. W. Carlson, and G. C. Walker.** 2004. Similarity to peroxisomal-membrane protein family reveals that *Sinorhizobium* and *Brucella* BacA affect lipid-A fatty acids. Proc. Natl. Acad. Sci. USA **101:**5012–5017.
6. **Ferguson, G. P., A. Datta, R. W. Carlson, and G. C. Walker.** 2005. Importance of unusually modified lipid A in *Sinorhizobium* stress resistance and legume symbiosis. Mol. Microbiol. **56:**68–80.
7. **Ferguson, G. P., R. M. Roop II, and G. C. Walker.** 2002. Deficiency of *Sinorhizobium meliloti bacA* mutant in alfalfa symbiosis correlates with an alteration of cell envelope. J. Bacteriol. **184:**5625–5632.
8. **Galibert, F., T. M. Finan, S. R. Long, A. Pühler, P. Abola, F. Ampe, F. Barloy-Hubler, M. J. Barnett, A. Becker, P. Boistard, G. Bothe, M. Boutry, L. Bowser, J. Buhrmester, E. Cadieu, D. Capela, P. Chain, A. Cowie, R. W. Davis, S. Dreano, N. A. Federspiel, R. F. Fisher, S. Gloux, T. Godrie, A. Goffeau, B. Golding, J. Gouzy, M. Gurjal, I. Hernandez-Lucas, A. Hong, L. Huizar, R. W. Hyman, T. Jones, D. Kahn, M. L. Kahn, S. Kalman, D. H. Keating, E. Kiss, C. Komp, V. Lelaure, D. Masuy, C. Palm, M. C. Peck, T. M. Pohl, D. Portetelle, B. Purnelle, U. Ramsperger, R. Surzycki, P. Thebault, M. Vandenbol, F. J. Vorhölter, S. Weidner, D. H. Wells, K. Wong, K. C. Yeh, and J. Batut.** 2001. The composite genome of the legume symbiont *Sinorhizobium meliloti*. Science **293:**668–672.
9. **Glazebrook, J., A. Ichige, and G. C. Walker.** 1993. A *Rhizobium melioti* homolog of the *Escherichia coli* peptide-antibiotic transport protein SbmA is essential for bacteroid development. Genes Dev. **7:**1485–1497.
10. **Ichige, A., and G. C. Walker.** 1997. Genetic analysis of the *Rhizobium meliloti bacA* gene: functional interchangeability with the *Escherichia coli sbmA* gene and phenotype of mutants. J. Bacteriol. **179:**209–216.
11. **Kannenberg, E. L., and R. W. Carlson.** 2001. Lipid A and O-chain modifications cause *Rhizobium* lipopolysaccharides to become hydrophobic during bacteroid development. Mol. Microbiol. **39:**379–391.
12. **Leigh, J. A., E. R. Signer, and G. C. Walker.** 1985. Exopolysaccharide-deficient mutants of *Rhizobium meliloti* that form ineffective nodules. Proc. Natl. Acad. Sci. USA **82:**6231–6235.
13. **LeVier, K., R. W. Phillips, V. K. Grippe, R. M. Roop II, and G. C. Walker.** 2000. Similar requirements of a plant symbiont and a mammalian pathogen for prolonged intracellular survival. Science **287:**2492–2493.
14. **LeVier, K., and G. C. Walker.** 2001. Genetic analysis of the *Sinorhizobium meliloti* BacA protein: differential effects of mutations on phenotypes. J. Bacteriol. **183:**6444–6453.
15. **Raetz, C. R., and C. Whitfield.** 2002. Lipopolysaccharide endotoxins. Annu. Rev. Biochem. **71:**635–700.
16. **Salomón, R. A., and R. N. Farías.** 1995. The peptide antibiotic microcin 25 is imported through the TonB pathway and the SbmA protein. J. Bacteriol. **177:**3323–3325.
17. **Vedam, V., J. G. Haynes, E. L. Kannenberg, R. W. Carlson, and D. J. Sherrier.** 2004. A *Rhizobium leguminosarum* lipopolysaccharide lipid-A mutant induces nitrogen-fixing nodules with delayed and defective bacteroid formation. Mol. Plant-Microbe Interact. **17:**283–291.
18. **Yamamoto, K., and F. Hutchinson.** 1984. The effect of bleomycin on DNA in *Escherichia coli* K12 cells. Chem. Biol. Interact. **51:**233–246.