A Burkholderia thailandensis Acyl-Homoserine Lactone-Independent Orphan LuxR Homolog That Activates Production of the Cytotoxin Malleilactone

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ABSTRACT

Burkholderia thailandensis has three acyl-homoserine lactone (AHL) LuxR–LuxI quorum-sensing circuits and two orphan LuxR homologs. Orphans are LuxR-type transcription factors that do not have cognate LuxI-type AHL synthases. One of the orphans, MalR, is genetically linked to the mal gene cluster, which encodes enzymes required for production of the cytotoxic polyketide malleilactone. Under normal laboratory conditions the mal gene cluster is silent; however, antibiotics like trimethoprim induce mal transcription. We show that trimethoprim-dependent induction of the mal genes requires MalR. MalR has all of the conserved amino acid residues characteristic of AHL-responsive LuxR homologs, but in B. thailandensis, MalR activation of malleilactone biosynthesis genes is not responsive to AHLS. MalR can activate transcription from the mal promoter in E. coli without addition of AHLS or trimethoprim. Expression of malR in B. thailandensis is induced by trimethoprim. Our data indicate that MalR binds to a lux box-like element in the mal promoter and activates transcription of the mal genes in an AHL-independent manner. Antibiotics like trimethoprim appear to activate mal gene expression indirectly by somehow activating malR expression. MalR activation of the mal genes represents an example of a LuxR homolog that is not a receptor for an AHL quorum-sensing signal. Our evidence is consistent with the idea that mal gene activation depends solely on sufficient transcription of the malR gene.

IMPORTANCE

LuxR proteins are transcription factors that are typically activated by acyl-homoserine lactone (AHL) signals. We demonstrate that a conserved LuxR family protein, MalR, activates genes independently of AHLS. MalR is required for transcription of genes coding for synthesis of the cytotoxic polyketide malleilactone. These genes are not expressed when cells are grown under normal laboratory conditions. In laboratory culture, MalR induction of malleilactone requires certain antibiotics, such as trimethoprim, which increase malR expression by an unknown mechanism. At sufficient levels of malR expression, MalR functions independently of any external signal. Our findings show that MalR is an activator of the silent malleilactone biosynthesis genes and that MalR functions independently of AHLS.
**MATERIALS AND METHODS**

Bacterial strains, culture conditions, and reagents. We used *B. thailandensis* strain E264 (14) and *E. coli* strain DH5α or MG4 for genetic manipulations and recombinant DNA expression, respectively (see Table S1 in the supplemental material). Our *B. thailandensis* malA-lacZ chromosomal insertion mutant (BT01447) was from a sequence-defined transposon mutant library (15). This mutant has a transposon insertion in the *malA* coding sequence after bp 5704 (out of 8,379) relative to the predicted *malA* translational start site. The other strains are listed in Table S1 in the supplemental material.

All *E. coli* growth was in Luria-Bertani (LB) broth at 37°C with shaking, and *B. thailandensis* was grown in morpholinepropanesulfonic acid (MOPS)-buffered LB broth (LB-MOPS) at 30°C with shaking. When appropriate, the following antibiotics were used (per milliliter): 100 μg trimethoprim (E. coli) and *B. thailandensis* and 15 μg gentamicin and 100 μg ampicillin (E. coli). We added IPTG (isopropyl-β-D-thiogalactopyranoside) as indicated.

We measured β-galactosidase activity with a Tropix Galacto-Light Plus chemiluminescence kit according to the manufacturer’s protocol (Applied Biosystems, Foster City, CA). β-Galactosidase activity in *B. thailandensis* is reported as light units relative to the optical density at 600 nm (OD<sub>600</sub>). Genomic DNA, PCR, and DNA fragments, and plasmid DNA were purified by using a DNeasy blood and tissue kit, PCR, plasmid purification kit, or gel extraction kit (Qiagen) according to the manufacturer’s protocol.

**Transcription reporter assays.** To assess MalR activation of *malA* expression in recombinant *E. coli*, we used *E. coli* MG4 with arabinose-inducible malR (pNJ105.malR) and either pQF50.PmalA with lacZ fused to the wild-type *malA* promoter or pQF50.mutPmalA with lacZ fused to the T4C and G5A mutant *malA* promoter. Overnight cultures were used as starters by diluting them to an OD<sub>600</sub> of 0.05. When experimental cultures reached an OD<sub>600</sub> of 0.5, 1-β-arabinose was added at the concentrations indicated to induce MalR expression. These cultures were added to sterile 16-mm test tubes or test tubes containing dried AHls, as indicated. The volume of culture in each tube was 0.5 ml. After 2 h at 37°C with shaking, β-galactosidase activity was measured as described above.

To assess MalR activation of *malA* in *B. thailandensis*, we used a *B. thailandensis* malA-lacZ reporter strain (BT01447) (15). Logarithmic-phase cultures were diluted to an OD<sub>600</sub> of 0.05 in growth medium supplemented with antibiotics or IPTG at the concentrations indicated. When used, AHls were added to the culture tube and dried prior to adding inoculated LB broth. The final AHL concentrations were 5 μM. After 24 h at 30°C with shaking, β-galactosidase activity was measured as described above.

**Purification of malleilactone.** Malleilactone was purified from *B. thailandensis* as follows. A stationary-phase culture of *B. thailandensis* E264 was used as the inoculum for 8 640-ml cultures in 4-liter Erlenmeyer flasks. Cultures were diluted to an OD<sub>600</sub> of 0.05 in growth medium supplemented with antibiotics or IPTG at the concentrations indicated to induce MalR expression. These cultures were added to sterile 16-mm test tubes or test tubes containing dried AHls, as indicated. The volume of culture in each tube was 0.5 ml. After 2 h at 37°C with shaking, β-galactosidase activity was measured as described above.

**An AHL-Independent LuxR Homolog**

*dus symbiotica* PauR, show conservation in the AHL binding region but vary with respect to at least one of the conserved residues. OryR and PauR do not respond to AHLs. Rather, they respond to host-derived factors or an endogenously produced secondary metabolite, respectively. Our results are consistent with a model whereby certain malR genes are required for Caenorhabditis elegans infections (12). There is a lux box-like sequence in the promoter region of the *mal* operon. One might imagine that MalR is an AHL-responsive activator of the *mal* operon. However, the *mal* operon is silent under laboratory conditions where AHls are produced. Perhaps MalR binds to a signal other than an AHL. This would be unusual, but perhaps not unique (13), for a LuxR family member showing complete identity with the conserved residues in the AHL binding and DNA binding regions. We seek to understand how the *mal* operon is regulated because its product, malleilactone, appears to be a virulence factor and because it may be regulated by MalR in an unusual manner.

Here, we show MalR is required for trimethoprim activation of the malleilactone gene and *mal* gene transcription is stimulated by several, but not all, antibiotics. Activation of the *mal* genes does not involve any of the AHls tested in *B. thailandensis* or in recombinant *Escherichia coli*. We also show that induction is not the consequence of a general antibiotic stress response, nor does it involve a direct interaction of MalR with a malleilactone-inducing antibiotic. Our results are consistent with a model whereby certain antibiotics activate expression of the silent *malR* gene and the *malR* gene product in turn activates transcription of genes for malleilactone synthesis.

We are interested in the *B. thailandensis* MalR protein for several reasons. First, it shows complete identity in all of the residues conserved among AHL-responsive LuxR family members. Second, it is adjacent to and divergently transcribed from the *mal* gene cluster, which is required for the production of the *B. thailandensis* cytoxolytic polyketide malleilactone. The genes for MalR and malleilactone biosynthesis are silent when *B. thailandensis* was grown in standard laboratory media but expressed in cells grown with certain antibiotics, such as trimethoprim (11). Third, *malR* and the *mal* genes are required for *Caenorhabditis elegans* infections (12). There is a lux box-like sequence in the promoter region of the *mal* operon. One might imagine that MalR is an AHL-responsive activator of the *mal* operon. However, the *mal* operon is silent under laboratory conditions where AHls are produced. Perhaps MalR binds to a signal other than an AHL. This would be unusual, but perhaps not unique (13), for a LuxR family member showing complete identity with the conserved residues in the AHL binding and DNA binding regions. We seek to understand how the *mal* operon is regulated because its product, malleilactone, appears to be a virulence factor and because it may be regulated by MalR in an unusual manner.

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flasks. The growth medium was LB-MOPS plus 9 μg/ml trimethoprim. Cells were inoculated to an OD$_{600}$ of 0.05. After 32 h at 30°C with shaking (200 rpm), the cultures were extracted with 1 volume of ethyl acetate, and the extract was dried over Na$_2$SO$_4$ and evaporated completely in a vacuum. The remaining residue was resolved through a Kupchan liquid extraction scheme. Briefly, the dried extract was dissolved in 200 ml of methanol (MeOH)-H$_2$O (9:1) and extracted four times with 200 ml hexanes. The remaining methanolic extract was diluted in 800 ml of MeOH-H$_2$O (6:4), which was extracted four times with an equal volume of CH$_2$Cl$_2$. High-performance liquid chromatography–mass spectrometry (HPLC-MS) analysis of the three resulting fractions (MeOH-H$_2$O, CH$_2$Cl$_2$, and hexanes) showed that malleilactone was in the CH$_2$Cl$_2$ fraction. This fraction was dried, suspended in a small volume (2 to 3 ml) of CH$_2$Cl$_2$, and resolved on a silica gel column (16 g; diameter, 1.25 cm; length, 20 cm). The column was equilibrated in CH$_2$Cl$_2$, loaded with the malleilactone-containing mixture, and eluted with 3 column volumes (CV) of CH$_2$Cl$_2$, followed by 3 CV (each) of 2.5%, 5%, 7.5%, and 10% MeOH in CH$_2$Cl$_2$. Fractions containing malleilactone, as judged by thin-layer chromatography (TLC) with 5% MeOH in CH$_2$Cl$_2$ as a solvent, were pooled; the pooled fractions consisted of a number of malleilactone analogs. Pure malleilactone A was obtained by a final HPLC step consisting of a preparative Eclipse XCB-C8 column (Agilent; 7 μm) at a higher concentration (15 μg/ml), malleilactone reduced growth by 70 to 80% and showed potency as a THF pathway inhibitor. In addition to trimethoprim, several other antibiotics activate the mal operon. They include fluoroquinolone DNA gyrase inhibitors and the cell wall biosynthesis inhibitors piperacillin, ceftazidime, and cefotaxime (11). The fact that these antibiotics are structurally distinct and target different activities suggests that they might not interact with MalR directly. An alternative explanation is that they affect the growth rate of B. thailandensis at the sublethal concentrations tested, and this might correlate with activation of the mal genes. We first tested the influence of trimethoprim, the strongest mal gene inducer (11), on growth and mal gene induction by using our B. thailandensis malA-lacZ reporter. At concentrations that induced mal expression, trimethoprim also inhibited bacterial growth (Table 1 and Fig. 2), and in fact, the level of mal gene expression was inversely correlated with growth over a range of trimethoprim concentrations (Fig. 2). We also tested other antibiotics at sub-MIC but growth-slowing concentrations. Not all of the antibiotics we tested served to activate malA-lacZ. Kanamycin, for example, did not activate lacZ expression, but it did inhibit growth (Table 1 and Fig. 2). Thus, slow growth is not sufficient for activation of the mal genes, but it may be necessary.

Our malA-lacZ reporter disrupts malA, which is a predicted polyketide synthase thought to be critical for malleilactone synthesis (12). Thus, malleilactone itself is likely not required for trimethoprim and other antibiotics to activate the mal genes. Nevertheless, malleilactone might influence mal gene expression. To address this possibility, we purified malleilactone from cultures of trimethoprim-treated B. thailandensis cells, where it was estimated to be at a concentration of 0.8 μg/ml (see Materials and Methods). At this concentration, our purified malleilactone had a minimal effect on growth or mal gene expression in the B. thailandensis reporter strain (Fig. 2). However, at a higher concentration (15 μg/ml), malleilactone reduced growth by 70 to 80% and showed potency as a malA activator comparable to that of trimethoprim (Fig. 2).

Transcription from the malA promoter in recombinant E. coli requires MalR and the lux box-like sequence. We engi-

These results show that MalR is required for trimethoprim activation of the mal biosynthesis genes.

**Influence of antibiotics on mal gene expression and the B. thailandensis growth rate.** Trimethoprim is a dihydrofolate reductase inhibitor. In addition to trimethoprim, several other antibiotics activate the mal operon. They include fluoroquinolone DNA gyrase inhibitors and the cell wall biosynthesis inhibitors piperacillin, ceftazidime, and cefotaxime (11). The fact that these antibiotics are structurally distinct and target different activities suggests that they might not interact with MalR directly. An alternative explanation is that they affect the growth rate of B. thailandensis at the sublethal concentrations tested, and this might correlate with activation of the mal genes. We first tested the influence of trimethoprim, the strongest mal gene inducer (11), on growth and mal gene induction by using our B. thailandensis malA-lacZ reporter. At concentrations that induced mal expression, trimethoprim also inhibited bacterial growth (Table 1 and Fig. 2), and in fact, the level of mal gene expression was inversely correlated with growth over a range of trimethoprim concentrations (Fig. 2). We also tested other antibiotics at sub-MIC but growth-slowing concentrations. Not all of the antibiotics we tested served to activate malA-lacZ. Kanamycin, for example, did not activate lacZ expression, but it did inhibit growth (Table 1 and Fig. 2). Thus, slow growth is not sufficient for activation of the mal genes, but it may be necessary.

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**Transcription from the malA promoter in recombinant E. coli requires MalR and the lux box-like sequence.** We engi-

### RESULTS

MalR is required for trimethoprim induction of mal biosynthesis genes. MalR is a LuxR orphan with all of the LuxR family conserved amino acids and 35% amino acid sequence identity to the P. aeruginosa AHL-responsive orphan QscR. MalR is genetically linked to a 13-gene cluster, the malleilactone (mal)-biosynthetic genes. The mal genes are transcriptionally activated by trimethoprim (11). To address the question of whether MalR is required for trimethoprim to induce expression of the mal genes, we used a B. thailandensis strain with a chromosomal lacZ fusion to malA, the first gene in the mal cluster (15). We compared malA-lacZ activation in a malR mutant to that in wild-type malR. The lacZ reporter was activated by trimethoprim in the wild type, as previously reported (11), but not in the malR mutant (Fig. 1).

**TABLE 1** Abilities of antibiotics to activate malA-lacZ in B. thailandensis

| Antibiotic | Antibiotic class | Relative malA-lacZ activity
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Trimethoprim (15)</td>
<td>THF pathway inhibitor</td>
<td>9.7 (0.4)</td>
</tr>
<tr>
<td>Kanamycin (115)</td>
<td>Ribosomal inhibitor</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>Zeocin (1,200)</td>
<td>DNA intercalator</td>
<td>1.5 (0)</td>
</tr>
<tr>
<td>Mitomycin C (10)</td>
<td>DNA cross-linker</td>
<td>4.3 (0.1)</td>
</tr>
<tr>
<td>Sulfamethoxazole (3,000)</td>
<td>THF pathway inhibitor</td>
<td>10 (0.7)</td>
</tr>
<tr>
<td>Malleilactone (15)</td>
<td>Unknown</td>
<td>12.3 (1.5)</td>
</tr>
</tbody>
</table>

*The concentration of antibiotic used is indicated in parentheses, and in each case, the concentration resulted in a 70 to 80% reduction in the growth yield, as determined by the OD$_{600}$ taken at the time of induction of the reporter. At concentrations that induced mal expression, trimethoprim also inhibited bacterial growth (Table 1 and Fig. 2), and in fact, the level of mal gene expression was inversely correlated with growth over a range of trimethoprim concentrations (Fig. 2). We also tested other antibiotics at sub-MIC but growth-slowing concentrations. Not all of the antibiotics we tested served to activate malA-lacZ. Kanamycin, for example, did not activate lacZ expression, but it did inhibit growth (Table 1 and Fig. 2). Thus, slow growth is not sufficient for activation of the mal genes, but it may be necessary.*

*Trimethoprim was previously shown to activate malA-lacZ (11).*

*THF pathway, tetrahydrofolate reductase pathway.
neered a plasmid with a 500-bp fragment containing the presumed malA promoter fused to a promoterless lacZ (from positions −1 to −500 with respect to the malA translation start codon) and a plasmid with an arabinose-inducible malR. In E. coli, lacZ expression was dependent on the presence of both plasmids and on arabinose (Fig. 3A).

The lux box-like sequence is centered 63.5 bp upstream of the malA translational start site (Fig. 3B and C). We tested the hypothesis that MalR requires this lux box-like sequence to activate malA by introducing the base substitutions T4C and G5A (Fig. 3B), which are critical for transcription factor binding in LuxR-LuxI-type systems (22). In E. coli containing this construct, the basal level of lacZ expression was similar to that in E. coli containing the full-length malA-lacZ fusion; however, the T4C and G5A substitutions abolished MalR-dependent activation of malA-lacZ (Fig. 3A). Our results are consistent with the idea that MalR binds to the lux box-like element upstream of malA and that this binding leads to transcriptional activation of malA. Activation of reporter transcription in E. coli did not require AHLs or antibiotics.

MalR-dependent activation of the mal genes is not influenced by AHLs. Although activation of the malA-lacZ reporter in E. coli does not require an AHL, it might nevertheless be influenced by AHLs either positively or negatively. Thus, we measured lacZ expression in our E. coli reporter containing both the malA-lacZ expression vector and the arabinose-dependent malR expression vector as follows. We grew the E. coli reporter strain in the presence of arabinose at a concentration that elicits maximal or half-maximal MalR-dependent malA-lacZ activation (0.4 or 0.004% arabinose, respectively) plus the following AHLs (5 μM each): octanoyl-homoserine lactone (HSL), 3-hydroxy-octanoyl-HSL, 3-hydroxy-decanoyl-HSL, and 3-hydroxy-hexanoyl-HSL (C4-, C6-, C10-, C12-, C14-, and 3OHC6-HSL); and p-coumaroyl-HSL. Addition of any of the above-listed AHLs to the growth medium did not positively or negatively affect lacZ transcription (Fig. 3A and data not shown).

It is possible that AHLs do not affect MalR or transcription
from the malA promoter in recombinant E. coli but that they do have an influence on malA expression in B. thailandensis. To test this possibility, we measured lacZ expression from the malA-lacZ reporter in the parent B. thailandensis strain, a malR mutant, and a mutant incapable of producing any AHLs. Cells were grown both with and without added AHLs (Fig. 4). As previously shown (Fig. 1), trimethoprim induced lacZ expression in the malR wild type but not in the malR mutant. Trimethoprim also induced malA-lacZ activity in the AHL synthesis mutant (Fig. 4). In the absence of trimethoprim, self-produced or exogenously added AHLs modestly repressed malA-lacZ reporter activity by about 2-fold (Fig. 4). Because malR is not expressed under these conditions (11), we posit that this may be due to cross-regulation by one of the other LuxR homologs encoded in the B. thailandensis chromosome. Our results with both recombinant E. coli and B. thailandensis are consistent with our conclusion that trimethoprim activates malA-lacZ expression in a MalR-dependent fashion and show that the AHLs we tested, including the B. thailandensis AHLs, are not required for MalR-mediated activation of the mal operon.

MalR-dependent activation of malA transcription correlates with malR transcription. Our results with recombinant E. coli (Fig. 3) suggest the possibility that MalR does not require a ligand to activate malA gene expression. It may be that a sufficient level of MalR is the only requirement for activation of malA gene expression and that trimethoprim and certain other antibiotics somehow activate malR transcription. In fact, trimethoprim, pipercillin, and malleilactone all induce malR expression in B. thailandensis (11) (see Fig. S1 in the supplemental material). Furthermore, we constructed a strain with plac-malR in a neutral site (glmS1) on the chromosome of a B. thailandensis malR mutant containing the malA-lacZ reporter. We monitored malA expression in this strain, grown with or without trimethoprim and increasing concentrations of IPTG to induce plac-malR expression. We found that activation of malA-lacZ required IPTG but not trimethoprim (Fig. 5).

Although trimethoprim was not required for malA-lacZ activation, it had a modest stimulatory influence on IPTG-dependent activation of malA-lacZ. This raised the possibility that trimethoprim does interact with MalR directly to affect its ability to activate mal gene expression. We addressed the possibility that trimethoprim is a ligand for MalR by introducing a gene coding for a trimethoprim-resistant dihydrofolate reductase (dhfrIIb) into our reporter strain. We found that dhfrIIb product changes the target of trimethoprim and does not affect trimethoprim itself, we conclude that although trimethoprim activation of the mal operon is MalR dependent, the trimethoprim effect does not result from a direct interaction of trimethoprim and MalR. This is consistent with the hypothesis that trimethoprim serves to activate malR transcription and that elevated levels of MalR are sufficient to activate the otherwise silent mal genes in laboratory cultures of B. thailandensis.

DISCUSSION
Here, we describe an unusual LuxR family protein, MalR, which retains all of the conserved amino acid residues in the AHL binding domains of LuxR family members involved in AHL quorum sensing but does not require an AHL for activity. Generally, LuxR family members that do not respond to AHLs have one or more substitutions in the conserved residues of the AHL binding domain. For example, X. oryzae OryR responds to a plant-derived signal (9), P. asymbiotica PauR responds to dialkylresorcinols (9, 10), and Serratia sp. CarR appears to be like MalR in that it does not seem to require an AHL or any exogenously added ligand for activity (23). CarR activity may also correspond to its level of transcription (24, 25). Although CarR has a change in one of the conserved AHL binding residues, genetic evidence supports the idea that the conversion to AHL independence occurred through mutations in the C terminus (23). The C-terminal mutations in CarR (23) are in a region that is not present in MalR.

The B. thailandensis mal gene cluster is silent in laboratory-grown cultures, but mal gene expression can be activated by certain antibiotics. We have shown that antibiotic activation of the mal genes requires MalR. Our results also indicate that antibiotics activate mal gene expression indirectly by driving malR expres-
sion. This conclusion is based on our results with \textit{B. thailandensis} (Fig. 1 and 4) and a \textit{B. thailandensis} AHL signal synthesis mutant and a trimethoprim-resistant mutant (Fig. 4), as well as with \textit{E. coli} (Fig. 3), showing that sufficient levels of \textit{malR} expression are all that is needed to activate \textit{mal} gene expression. In our experiments, MalR does not appear to require an AHL or a ligand added to the culture medium to influence \textit{mal} gene expression. It is possible that a common metabolite in \textit{B. thailandensis} and \textit{E. coli} serves as a ligand. Recently, the \textit{E. coli} LuxR orphan SdiA was shown to bind a conserved metabolite (1-octanoyl-\textit{rac}-glycerol) in the absence of its cognate AHL (13). SdiA has some activity in the absence of A\textit{HLs}, as do a few other AHL-responsive LuxR homologs (e.g., \textit{Burkholderia cenocepacia} CepR2) (26, 27). It is not known if any other LuxR proteins bind common metabolites, like SdiA. In ways other than the AHL requirement, MalR is similar to LuxR quorum-sensing signal receptors. For example, MalR requires an intact \textit{lux} box-like sequence to activate the promoter of the malleilactone-biosynthetic genes.

Transcription of \textit{malR} is activated by several, but not all, of the antibiotics we tested. In every case, antibiotics that activated \textit{mal} gene expression also slowed the growth of \textit{B. thailandensis}, with growth and \textit{mal} activation inversely correlated. However, the influence of an antibiotic on growth was not in itself sufficient for \textit{mal} gene activation. Some antibiotics slowed growth but did not affect \textit{mal} gene activation. We imagine that the presence of certain antibiotics activates a specific cellular response pathway, which in turn activates expression of \textit{malR}. Malleilactone may activate the \textit{mal} genes through a similar pathway, although the cellular target of malleilactone is unknown. We find it interesting that antibiotic modulation of AHL-responsive LuxR family members has been reported elsewhere (28, 29). In fact, several classes of antibiotics activate transcription of the \textit{P. aeruginosa} orphan QscR (29). MalR might be a degenerate AHL quorum-sensing signal receptor. There is increasing evidence that AHL receptors play an important role in interspecies competition (30, 31). Antibiotics and cytotoxic factors are often activated by AHL quorum sensing (31–33), and as discussed above, MalR (11) and QscR (29) are antibiotic activated. The \textit{mal} genes code for synthesis of malleilactone, which is a cytotoxic that also has antibiotic activity against several Gram-positive bacteria (12), and as we show here (Fig. 2), malleilactone even slows the growth of \textit{B. thailandensis} at high concentrations. Activation of the \textit{mal} genes by low, nonlethal concentrations of antibiotics might be an alarm response whereby \textit{B. thailandensis} reacts to danger by mounting an attack against competitors.

\textit{B. thailandensis} is closely related to two human pathogens, \textit{Burkholderia pseudomallei} and \textit{Burkholderia mallei}. The malleilactone-biosynthetic gene clusters are conserved (>80% identity at the amino acid sequence level), and MalR shows 100% identity across these three species. \textit{B. pseudomallei} is the causative agent of melioidosis, an often fatal emerging infectious disease that is prevalent in northeast Thailand (34). One of the difficulties in treatment is the high level of antibiotic resistance in \textit{B. pseudomallei}. Trimethoprim and sulfamethoxazole are two of the few clinically useful antibiotics (35). Both of these antibiotics activate malleilactone production in \textit{B. thailandensis} (Table 1). These findings, together with the finding that malleilactone is important for \textit{B. thailandensis} virulence in \textit{C. elegans} (12), suggest the need to evaluate how antibiotics regulate virulence and the gene homologs for malleilactone and MalR in \textit{B. pseudomallei}. Finally, it is interesting that at sufficiently high concentrations (15 \textmu g/ml) malleilactone itself inhibits the growth of \textit{B. thailandensis}. Members of the genus \textit{Burkholderia} are quite insensitive to most antibiotics, and this creates clinical difficulties (36). It is not unreasonable to think that malleilactone might provide a scaffold for anti-\textit{Burkholderia} agents or that discovery of the malleilactone target might be helpful in drug discovery.

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