AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology and Molecular Biology Reviews®

REVIEW

MarR Family Transcription Factors from *Burkholderia* Species: Hidden Clues to Control of Virulence-Associated Genes

Ashish Gupta,[a] Anuja Pande,[b] Afsana Sabrin,[b] Sudarshan S. Thapa,[b] Brennan W. Gioe,[b] Anne Grove[b]

[a]Novavax, Inc., Process Development Department, Gaithersburg, Maryland, USA

[b]Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, USA

SUMMARY Species within the genus *Burkholderia* exhibit remarkable phenotypic diversity. Genomic plasticity, including genome reduction and horizontal gene transfer, has been correlated with virulence traits in several species. However, the conservation of virulence genes in species otherwise considered to have limited potential for infection suggests that phenotypic diversity may not be explained solely on the basis of genetic diversity. Instead, differential organization and control of gene regulatory networks may underlie many phenotypic differences. In this review, we evaluate how regulation of gene expression by members of the multiple antibiotic resistance regulator (MarR) family of transcription factors may contribute to shaping the physiological diversity of *Burkholderia* species, with a focus on the clinically relevant human pathogens. All *Burkholderia* species encode a relatively large number of MarR proteins, a feature common to bacteria that must respond to environmental changes such as those associated with host invasion. However, evolution of gene regulatory networks has likely resulted in orthologous transcription factors controlling disparate sets of genes. Adaptation to, and survival in, diverse habitats, including a human or plant host, is key to the success of *Burkholderia* species as (opportunistic) pathogens, and recent reports suggest that control of virulence-associated genes by MarR proteins features prominently among the survival strategies employed by these species. We suggest that identification of MarR regulons will contribute significantly to clarification of virulence determinants and phenotypic diversity.

Citation Gupta A, Pande A, Sabrin A, Thapa SS, Gioe BW, Grove A. 2019. MarR family transcription factors from *Burkholderia* species: hidden clues to control of virulence-associated genes. Microbiol Mol Biol Rev 83:e00039-18. https://doi.org/10.1128/MMBR.00039-18.

Address correspondence to Anne Grove, agrove@lsu.edu.

Published 28 November 2018

KEYWORDS BifR, MftR, OhrR, ROS, TctR, gene regulation, oxidative stress, transcriptional regulation

INTRODUCTION

Members of the genus *Burkholderia* (originally classified as *Pseudomonas*) are versatile in terms of their ecological niches. *Burkholderia* species use oxygen as the primary terminal electron acceptor during respiration; however, some species can survive hypoxic environments, and some can perform anaerobic respiration with nitrate as the terminal electron acceptor or use fermentation to produce ATP (1). The majority of species inhabit the rhizosphere, where they utilize plant-derived compounds as nutrients; some species fix nitrogen and are beneficial to the plants, and others are efficient bioremediation agents (2). For example, several species in the genetically related but phenotypically diverse *Burkholderia cepacia* complex (Bcc) are useful as plant pest antagonists, plant growth-promoting rhizobacteria, or degraders of toxic substances (3, 4). However, others are plant pathogens, including *B. cepacia*, which was originally identified by Walter Burkholder as the causative agent of soft-rot disease in onion (5). Subsequently, *B. cepacia* emerged as an opportunistic human pathogen that can survive intracellularly and remain metabolically active (6). In addition to *B. cepacia*, the related Bcc members *B. cenocepacia* and, more recently, *B. multivorans* have received much attention as some of the most serious pathogens of immunocompromised individuals, such as patients with cystic fibrosis (CF) and chronic granulomatous disease (CGD). Bcc infections in CF patients have highly unpredictable outcomes that range from largely asymptomatic infections to the potentially fatal necrotizing pneumonia and sepsis known as cepacia syndrome (7).

While Bcc members are considered opportunistic pathogens, other species of this genus, the facultative intracellular pathogen *B. pseudomallei* and the obligate mammalian pathogen *B. mallei*, are the causative agents of melioidosis (Whitmore's disease) and glanders, respectively; a low infectious dose is sufficient for transmission of disease, rendering *B. mallei* in particular highly infectious. Despite its inability to persist in the environment, *B. mallei* was used in the past for biological warfare on account of the low infectious dose, capacity for latency, and likelihood of causing lethal infections (for example, to target livestock during World War I), and both species have been categorized by the Centers for Disease Control and Prevention (CDC) as category B biological agents (8).

The availability of complete genome sequences (as opposed to relying on 16S rRNA) for *Burkholderia* species led to a reevaluation of phylogenetic relationships. Such analysis prompted the division of *Burkholderia* species into separate clades. Species within clade I include all plant and human pathogens and represent the clinically relevant species: one group comprises Bcc species; a second group consists of the closely related species of the *B. pseudomallei* complex (Bpc), a group previously referred to as the Bptm group, as it was named for the originally identified members, *B. pseudomallei*, *B. thailandensis*, and *B. mallei*; and a third group comprises phytopathogens such as *B. glumae* and *B. gladioli*. A second clade, for which the new genus *Paraburkholderia* was adopted, consists mainly of environmental species such as *B. xenovorans* (9–12).

While genetic diversity has been correlated with virulence traits in several species, the conservation of virulence genes in species without a marked potential for virulence suggests that phenotypic diversity may not be explained solely on the basis of such genetic variability. However, differential evolution of gene regulatory networks may underlie many phenotypic differences. The purpose of this review is to evaluate how members of the multiple antibiotic resistance regulator (MarR) family of transcription factors may contribute to shaping the physiological diversity of *Burkholderia* species, with a focus on the clinically relevant human pathogens. Since the majority of MarR proteins contain one or more cysteine residues, and since bacterial defenses against host-generated reactive oxygen species (ROS) are key to successful host colonization,

the role of oxidant responses is considered. In addition, *Burkholderia* species may periodically encounter hypoxic conditions that demand metabolic adjustment, conditions that may, for instance, be present in moist soil or in oxygen-deprived host microenvironments such as abscesses or the CF lung. Both circumstances, the addition of an oxidant or adjustment to microaerobic conditions, have been shown to elicit global changes in gene expression, including changes in the expression of genes encoding specific MarR family proteins (13, 14).

BURKHOLDERIA SPECIES

The Bpc Group Members *B. thailandensis*, *B. mallei*, and *B. pseudomallei*

At least seven closely related species belong to the Bpc group (11), of which *B. pseudomallei* and *B. mallei* have been shown to cause severe and potentially fatal human disease. In contrast, *B. thailandensis* is a soil saprophyte and only rarely associated with human infection. Prior to its classification in the late 1990s, *B. thailandensis* was often mistaken for *B. pseudomallei* due to similarity in the biochemical, morphological, and antigenic profiles (15). Key traits that differentiate these strains include the ability of *B. thailandensis* to assimilate L-arabinose, which suppresses its type 3 secretion system (T3SS), an important factor contributing toward rendering this species relatively nonpathogenic to humans and animals (16, 17). *B. pseudomallei* K96243 and *B. thailandensis* E264 display high genomic synteny: they have $<$10 nucleotide differences between their 16S rRNA sequences, and $\sim$85% of their genes are conserved (18, 19). *B. mallei* is believed to have evolved from a *B. pseudomallei* isolate by selective genome reduction (20). Although the *B. mallei* ATCC 2344 genome (5.8 Mb) is 20% smaller than the *B. pseudomallei* K96243 genome (7.2 Mb), the two genomes share 99% nucleotide sequence identity (21, 22). During evolution, *B. mallei* appears to have lost genes that are necessary for environmental survival while preserving those required for persistence in the host (20, 21).

Despite exhibiting reduced virulence, *B. thailandensis* encodes homologs of known virulence factors, including lipopolysaccharide, the T3SS, and quorum-sensing systems that are expressed in *B. pseudomallei* and *B. mallei* (23, 24). For this reason, *B. thailandensis* is commonly used as a model system to investigate virulence mechanisms. It also highlights the lack of an obvious correlation between gene content and virulence and suggests that differential transcriptional control contributes to phenotypic differences (as exemplified by the above-mentioned downregulation of genes encoding T3SS components upon expression of the arabinose assimilation operon). These *Burkholderia* species also share resistance to many common antibiotics; this feature, along with facile aerosol transmission of the pathogenic species and no availability of effective vaccines, forms the basis for their categorization as potential bioterror agents (25).

The host-pathogen interaction does not always result in disease. The outcome depends on whether the initial steps of the interaction, namely, commensalism, colonization, persistence, and infection, result in host damage. As noted above, *B. thailandensis* conserves a number of genes associated with virulence in the pathogenic species, yet it is largely considered nonpathogenic, likely due in part to a failure to express virulence determinants such as the T3SS. The T3SS is a highly specialized virulence system that plays a vital role in the host-pathogen interaction by facilitating events such as bacterial invasion and escape from endocytic vesicles (26). *B. pseudomallei* and *B. mallei* encode three and two T3SS systems, respectively, and they both express one Bsa (*Burkholderia* secretion apparatus) T3SS that is required for virulence (27, 28). While *B. thailandensis* is rarely pathogenic to humans, a few cases have been reported (29), showing that *B. thailandensis* is capable of causing human infection, and it has been suggested that the *B. thailandensis*-encoded Bsa T3SS has a similar function in virulence as in *B. pseudomallei* and *B. mallei* (23). A transcriptome analysis of *B. pseudomallei* grown intracellularly in a human macrophage-like cell line showed differential expression of a large number of genes, including repression of the virulence-associated T3SS, indicating that the T3SS is vital during the initial phase of invasion but not at later stages (30).

Bcc: *B. cenocepacia*

The monophyletic Bcc group currently comprises more than 20 members (11). *B. cenocepacia* and *B. multivorans* are the most prevalent species, accounting for ~90% of Bcc infections in CF patients, and they have the potential to cause epidemic outbreaks because of transmissibility from one infected patient to another (31). A main contributing factor is intrinsic resistance to antibiotics and antiseptics, which confounds both treatment and disinfection protocols. *B. cenocepacia* is considered particularly dangerous due to the number of epidemic strains and the risk of developing fatal cepacia syndrome (31).

In *B. cenocepacia* J2315, genomic islands associated with virulence occupy 9.3% of its 8.06-Mb chromosome (19). A number of virulence factors have been experimentally verified. Examples include proteins involved in iron homeostasis, such as proteins responsible for the generation of ornibactin and pyochelin, both of which are siderophores that scavenge free iron from the environment (32). *B. cenocepacia* also encodes members of all five major families of efflux systems that may contribute to intrinsic resistance to polymyxins, aminoglycosides, and beta-lactams (19, 31). An intriguing link between iron uptake and antimicrobial resistance is that upregulation of RND (resistance-nodulation-division) efflux pumps may be required for siderophore secretion and that the bacteria "hit two birds with one stone" by simultaneously promoting antibiotic efflux while adjusting to an iron-limiting environment (31). Another factor contributing to antibiotic resistance is biofilm formation, in which surface-adherent cells are encased in a protective extracellular matrix. In the lungs of CF patients, *B. cenocepacia* may even exist together with the opportunistic pathogen *Pseudomonas aeruginosa* to form persistent biofilm infections (33).

B. xenovorans

B. xenovorans (now *Paraburkholderia*; previously known as *B. fungorum*) is more distantly related to the pathogenic strains. It was isolated from a landfill contaminated with polychlorinated biphenyl (PCB), and it has received much attention due to its ability to degrade PCB and other aromatic compounds. A genome comparison of *B. xenovorans* with *B. pseudomallei* and *B. cenocepacia* revealed 77.5% and 76.8% average nucleotide identities, respectively (34). It has three replicons, the large chromosome 1, chromosome 2, and the megaplasmid, and many core functions are encoded on the larger chromosome 1, while there is much greater genetic diversity among the smaller replicons, a feature that is common for *Burkholderia* species. Degradation of aromatic compounds typically generates intermediates that are processed in the conserved β-ketoadipate pathway, and many peripheral pathways that feed into this central pathway have been identified (34, 35). Although *B. xenovorans* possesses various genes required for *in vivo* survival, it lacks several genes that encode virulence factors and is therefore considered to have little potential for being infectious (34).

MULTIPLE ANTIBIOTIC RESISTANCE REGULATORS (MarR)

The MarR transcription factor was first identified in *Escherichia coli* K-12 and shown to regulate resistance to diverse antibiotics, organic solvents, and oxidative stress agents (36, 37). More than 54,000 genes that encode MarR proteins in bacteria and archaea have since been annotated according to Ensembl Bacteria, with an average of ~7 paralogs per genome (38). MarR family proteins, which have been suggested to have originated before the divergence of bacteria and archaea (39), belong to the very common winged helix-turn-helix (wHTH) subset of HTH proteins. The wHTH proteins are characterized as having at least one β-sheet (or wing) adjacent to the HTH motif (α2-α3-α4) (Fig. 1), and DNA binding typically involves the insertion of recognition helices into DNA major grooves, with the wing contacting the neighboring minor groove. MarR proteins are further characterized as being obligate dimers in which both N- and C-terminal helices are intertwined to form a dimer interface that is connected to the wHTH motif by the long helices α2 and α5 (Fig. 1). Thus, a signature of MarR family proteins is that they form a single, compact globular fold with the DNA-binding

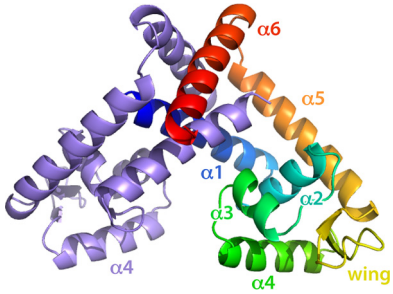

FIG 1 Prototypical MarR family protein. Shown is a predicted model of *B. thailandensis* BifR, created using SwissModel in the automated mode using the structure under PDB accession number 2FBH as the template. One monomer is in purple, and the other is colored blue to red (amino terminus to carboxy terminus, with helices α1 to α6 identified). The DNA recognition helices (α4) are identified in both subunits.

region composed of central helices, in contrast to many other wHTH-type proteins, in which the DNA-binding domain is separate from a regulatory or ligand-binding domain and located at either the N or C terminus (40–43).

The MarR protein family is named for *E. coli* MarR, which indirectly controls the expression of a multidrug efflux pump via repression of the *marRAB* operon, which encodes the transcriptional activator MarA (44). The multiple-antibiotic resistance phenotype arises from the inactivation of MarR by oxidation, an event, for example, brought about by antibiotic-induced envelope stress, which results in the release of redox-active Cu^{2+} from membrane proteins (45). While several other MarR family proteins have been functionally characterized and shown to play vital roles in the control of antibiotic efflux, other events, such as oxidative stress responses, the control of genes involved in virulence, and catabolism of aromatic compounds, have also been reported to be under the control of MarR family transcriptional regulators (for examples, see Table 1 and references 38, 40, and 43). Based on either functional characteristics, sequence features, or a combination thereof, subtypes of MarR family proteins have been identified; examples include SlyA, which is considered to have arisen from gene duplication and which positively regulates gene expression by a mechanism that involves remodeling of repressive H-NS–DNA complexes, and urate-responsive transcriptional regulators (UrtR), which feature characteristic sequence elements, including an N-terminal α-helical extension (46–48).

MarR family proteins most often bind DNA to prevent RNA polymerase from accessing cognate promoters, thereby repressing gene expression (Fig. 2). Upon binding of a small-molecule ligand or specific cysteine oxidation, DNA binding is attenuated, resulting in gene expression (for a review of this and other modes of gene regulation by MarR proteins, see references 40 and 43). MarR proteins are often autoregulatory; their cognate sites are palindromic sequences (reflecting binding of pairs of recognition helices in consecutive DNA major grooves), and such sites may be frequently identified in their gene promoters. Genes encoding MarR family proteins are typically adjacent to (and often divergent from) a gene under MarR control, and MarR proteins may in addition control the expression of distant genes in their regulon (Fig. 2). By responding to environmental changes, MarR proteins are ideally poised to transduce such cues into

TABLE 1 Examples of MarR homologs, classified according to their regulatory role

Regulatory role(s)	MarR homologs (reference)
Antibiotic and oxidative stress responses	MarR (45), MexR (106), EmrR (107), PecS (108), HucR (109), MftR (67), TamR (110), OhrR (81), SarA (111), SarZ (112), MosR (113)
Production of virulence factors	SlyA (114), PecS (108), NadR (115)
Catabolism of aromatic compounds	HpaR (85), CinR (116), BadR (117), HucR (109), HcaR (118)
Master regulator	MgrA (119), SarZ (112), PecS (108), MftR (64)

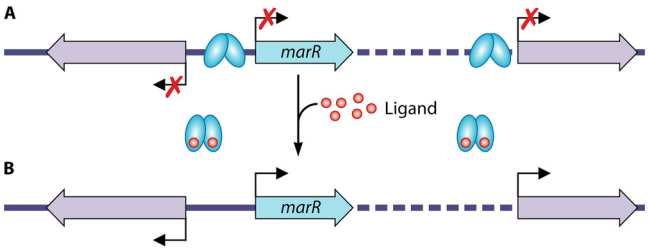

FIG 2 Typical mode of gene regulation by MarR homologs. (A) In the absence of a small-molecule ligand or oxidant, the gene encoding the MarR family protein (*marR*) (cyan arrow) and a divergently oriented gene(s) are repressed by the MarR family protein binding cognate sites in gene promoters (cyan ovals). MarR may also control distant members of its regulon (dotted line) (genes under the control of the MarR family protein are shown as purple arrows). Repression of gene expression is denoted with red crosses. (B) Ligand binding or specific oxidation of the MarR family protein (with red dots representing ligand) relieves repression, as the ligand-bound MarR family protein dissociates from cognate sites.

changes in gene expression, and many that regulate the production of virulence factors in response to host-derived signals have been identified (Table 1).

ROLE OF REACTIVE OXYGEN SPECIES IN HOST DEFENSES

When a bacterium infects a host, reactive oxygen species (ROS) are produced as a first defense (49, 50). The primary source of ROS is NADPH oxidase, which produces a superoxide anion by transferring an electron from NADPH to molecular oxygen. Superoxide in turn dismutates to H_2O_2 and oxygen, and H_2O_2 may be converted to hypochlorous acid by myeloperoxidase or react with transition metals to produce highly reactive hydroxyl ions (OH^-). Lipid peroxidation may also occur by the abstraction of hydrogen from polyunsaturated fatty acids, with the resulting organic hydroperoxides causing further damage to cellular components (51). In chronic granulomatous disease (CGD), a defect in NADPH oxidase impairs the phagocytic production of ROS, a result of which is that patients suffer recurring infections, such as infections with Bcc pathogens (52).

In the absence of a functional NADPH oxidase, xanthine oxidase becomes important for bacterial clearance (53). Xanthine dehydrogenase functions in purine degradation, transferring electrons to NAD^+ to generate NADH and in the process converting hypoxanthine to xanthine and xanthine to urate (54). In mammals, xanthine dehydrogenase is converted to xanthine oxidase by reversible sulfhydryl oxidation or by irreversible proteolytic modification, and this form of the enzyme instead transfers electrons to molecular oxygen to generate superoxide (55). In plants, the urate that is produced has been shown to act as an antioxidant to protect host cells from the adverse effects of ROS (56).

While not part of the innate host defense, it should also be noted that treatment with antibiotics has been linked to bacterial production of ROS. For example, bactericidal antibiotics such as fluoroquinolones, which are known for their inhibition of the bacterial gyrase, resulting in cell death because of the accumulation of DNA double-strand breaks, were reported to elicit oxidative stress due to the production of hydroxyl radicals (57, 58). Similar antibiotic-mediated production of ROS was also reported in Bcc species (59). However, whether or not such ROS contribute to antibiotic-mediated cell killing is subject to debate, and it may depend on specific circumstances (60, 61). As noted above, another potential consequence of antibiotic treatment is envelope stress, in which damaged or misfolded membrane proteins may release Cu^{2+}; in *E. coli*, a consequence of such Cu^{2+} accumulation is the oxidation of MarR to generate disulfide bonds between two protein dimers, thereby precluding DNA binding (45).

MarR PROTEINS IN *BURKHOLDERIA* SPECIES

All *Burkholderia* species encode a relatively large number (greater than the average of ~7 per bacterial genome [38]) of MarR family proteins. A correlation between large genome size and a greater number of transcriptional regulators is a general feature and

a common characteristic of bacteria with a more complex lifestyle that may require responses to environmental changes (41). The MarR homologs in the surveyed *Burkholderia* species were identified in an iterative approach, starting with proteins annotated as a MarR family transcriptional regulator in the *Burkholderia* Genome Database (http://www.burkholderia.com/) (62). This was followed by a search of the same database for orthologs of the annotated MarR family proteins; for example, 9 MarR homologs were found in *B. thailandensis* based on annotation alone, with an additional 3 being identified as orthologs of MarR family transcriptional regulators annotated in other *Burkholderia* genomes. Sequences of select proteins, including any orthologs annotated as a "hypothetical protein," were submitted to Pfam for verification. This analysis revealed that *B. thailandensis* encodes 12 annotated MarR homologs, all of which are conserved in the Bpc group members *B. pseudomallei* and *B. mallei*, and this conservation extends to the neighboring gene(s), which may be under the control of the respective MarR protein (Table 2). *B. pseudomallei* and *B. mallei* encode an additional 3 MarR family proteins, whereas *B. cenocepacia* and *B. xenovorans* encode totals of 26 and >30 MarR family proteins, respectively.

A phylogenic tree of MarR family proteins from the surveyed *Burkholderia* species was constructed (Fig. 3), with sequences of MarR and SlyA from *E. coli* K-12 included for reference. This analysis indicated the close evolutionary relationship between orthologs from the different *Burkholderia* species (Table 2). This includes several MarR orthologs that are conserved across Bpc, Bcc, and *Paraburkholderia* (*B. xenovorans*) species, such as HpaR, OhrR, BifR, and TctR. Others, such as MftR, are absent from *B. xenovorans*, perhaps reflecting a gene loss event after the divergence of the genus *Paraburkholderia*. That other MarR family proteins exist in only a few *Burkholderia* species suggests frequent gene loss/duplication and/or horizontal gene transfer events.

Evolution of gene regulatory networks may result in orthologous transcription factors controlling disparate sets of genes, although they may maintain a constant set of core members of the regulon (63). This is an important source of phenotypic diversity; even closely related species may have rather different gene contents, requiring rewiring of the regulons for orthologous transcription factors. Considering the plasticity of *Burkholderia* genomes and the variable genome sizes, such diversity of regulons is likely; for example, *B. cenocepacia* J2315 was isolated from a CF patient, and ~21% of its genome differs from other *B. cenocepacia* genomes, perhaps reflecting optimization for persistence in the CF lung (19). Among the annotated *Burkholderia* MarR homologs, only four (*B. thailandensis* MftR, BifR, and OhrR and *B. pseudomallei* TctR) have been characterized (64–69).

Major Facilitator Transport Regulator (MftR) Controls Virulence-Associated Genes

The *B. thailandensis*-encoded MftR protein is divergently oriented from an operon encoding a major facilitator transport protein (MftP) and FenI (Fig. 4A). MftP, for which the substrate remains unknown, belongs to the major facilitator superfamily, and FenI is a predicted glycosyl hydrolase. This genomic locus (along with the two palindromes in the *mftR-mftP* intergenic region identified as MftR-binding sites [67]) is conserved in the closely related species *B. mallei* and *B. pseudomallei*, while only *mftR* and *mftP* (and the binding sites) are conserved in *B. cenocepacia*, and the entire locus is absent from *B. xenovorans* (Table 2). MftR is a negative regulator of both *mftR* and *mftP-fenI*, and binding of urate to MftR results in attenuation of DNA binding and upregulation of gene expression (64, 66, 67). Since urate is produced by host xanthine oxidase in response to bacterial infection, the implication is that MftR would be important for controlling gene expression after host colonization.

The absence of FenI results in clumping of bacterial cells in culture (64). Since FenI is predicted to be a glycosyl hydrolase, one possibility is that it may be involved in cleavage of the glycosidic bond between sugars in exopolysaccharides, thereby promoting detachment of cells. The specific function of FenI notwithstanding, the derepression of *mftP-fenI* that is associated with ligand (urate) binding to MftR should

TABLE 2 Annotated MarR proteins in select *Burkholderia* genomes[a]

Protein(s) encoded by adjacent gene(s)	MarR protein encoded by:				
	B. pseudomallei K96243	*B. mallei* ATCC 23344	*B. thailandensis* E264	*B. cenocepacia* J2315	*B. xenovorans* LB400
EmrB, RND transporters	**BPSL0021**	**BMA2771**	**BTH_I0021**	**BCAL3512**	**Bxe_A4487**
Allophanate hydrolase	**BPSL0260**	**BMA3312**	**BTH_I0231**	**BCAL0553**	**Bxe_A0196**
Short-chain dehydrogenase	**BPSL0378**	**BMA0089**	**BTH_I0350**	—	—
EmrB efflux pump; LasA protease	**BPSL0626**	**BMA0174**	**BTH_I0542 (BifR)**	**BCAL0862**	Bxe_A4061 (no *lasA*)
Glycerate kinase	**BPSL1400**	**BMA1469**	**BTH_I2116**	**BCAL1999**	**Bxe_A2299**
MFS efflux pump; Fen1 glycosidase	**BPSL1752**	**BMA1154**	**BTH_I2391 (MftR)**	BCAL1732 (no *fen1*)	—
RND, EmrA, MDR efflux pumps	**BPSL1912**	**BMA1055**	**BTH_I2558**	**BCAL1513**	**Bxe_A2814**
Glutamine amidotransferase	**BPSL3431**	**BMA2918**	**BTH_I3344 (TctR)**	**BCAL0003**	**Bxe_A0003**
Isochorismatase, fusaric acid resistance	**BPSS1908**	**BMAA0181**	**BTH_II0468**	—	—
Organic hydroperoxide reductase	**BPSS1781**	**BMAA0304**	**BTH_II0598 (OhrR)**	**BCAM0897**	**Bxe_B2842**
ClC chloride channel	**BPSS1013**	**BMAA1198**	**BTH_II1396**	**BCAM1724**	**Bxe_A3929**
hpa operon (homoprotocatechuate degradation)	**BPSS0691**	**BMAA1141**	**BTH_II1736 (HpaR)**	**BCAM1365**	**Bxe_B2027**
ABC transporter-related substrate-binding protein	**BPSL1750**	**BMA1152**	—	—	—
None	**BPSS0772**	**BMAA0619**	—	BCAM2794 (gluconolactonase; *p*-hydroxycinnamoyl CoA hydratase-lyase)	—
Glyoxalase	**BPSS1556**	**BMAA1561**	—	—	—
Glutathione-dependent formaldehyde-activating enzyme	—	—	—	**BCAL1761**	—
Hypothetical protein	—	—	—	**BCAM0588**	—
SnoaL-like protein	—	—	—	**BCAM0731**	—
RND, EmrB, NodT transporters	—	—	—	BCAM0793	
LysR, hypothetical proteins (DUF521, DUF126)	—	—	—	BCAM0795	Bxe_0734
MFS transporter	—	—	—	**BCAM1139**	—
Tetratricopeptide repeat protein	—	—	—	BCAM1254	Bxe_A0659 (MFS transporter)
Hypothetical, MFS transporter, dioxygenase	—	—	—	**BCAM1437**	**Bxe_C0762**
Hydrolase	—	—	—	**BCAM1568**	—
Isochorismatase, MFS transporter	—	—	—	**BCAM1750**	**Bxe_B2020**
Oxoacid dehydrogenase	—	—	—	**BCAM1943**	—
Monooxygenase	—	—	—	**BCAM2162**	—
Vanillate *O*-demethylase (*vanAB*)	—	—	—	**BCAM2435**	—
Fusaric acid resistance	—	—	—	**BCAS0018**	**Bxe_B2611**
Pentapeptide repeat protein	—	—	—	**BCAS0126**[b]	—

[a]Boldface type indicates conservation of the adjacent gene(s). — indicates that there is no corresponding gene for the indicated organism. MFS, major facilitator superfamily; MDR, multidrug resistance.

[b]Annotated as a MarR-acetyltransferase fusion protein. BCAM0866 was annotated in the *Burkholderia* Genome Database as a MarR protein, but it was omitted because it was classified as an HxlR protein by Pfam. For *B. thailandensis* OhrR (encoded by *BTH_II0598*), the correct coordinates are positions 698839 to 699291 (minus strand; the *Burkholderia* Genome Database currently identifies a longer coding region). *B. pseudomallei* TctR and *B. thailandensis* BifR, MftR, and OhrR were experimentally characterized; HpaR is named based on homology to orthologs from different species.

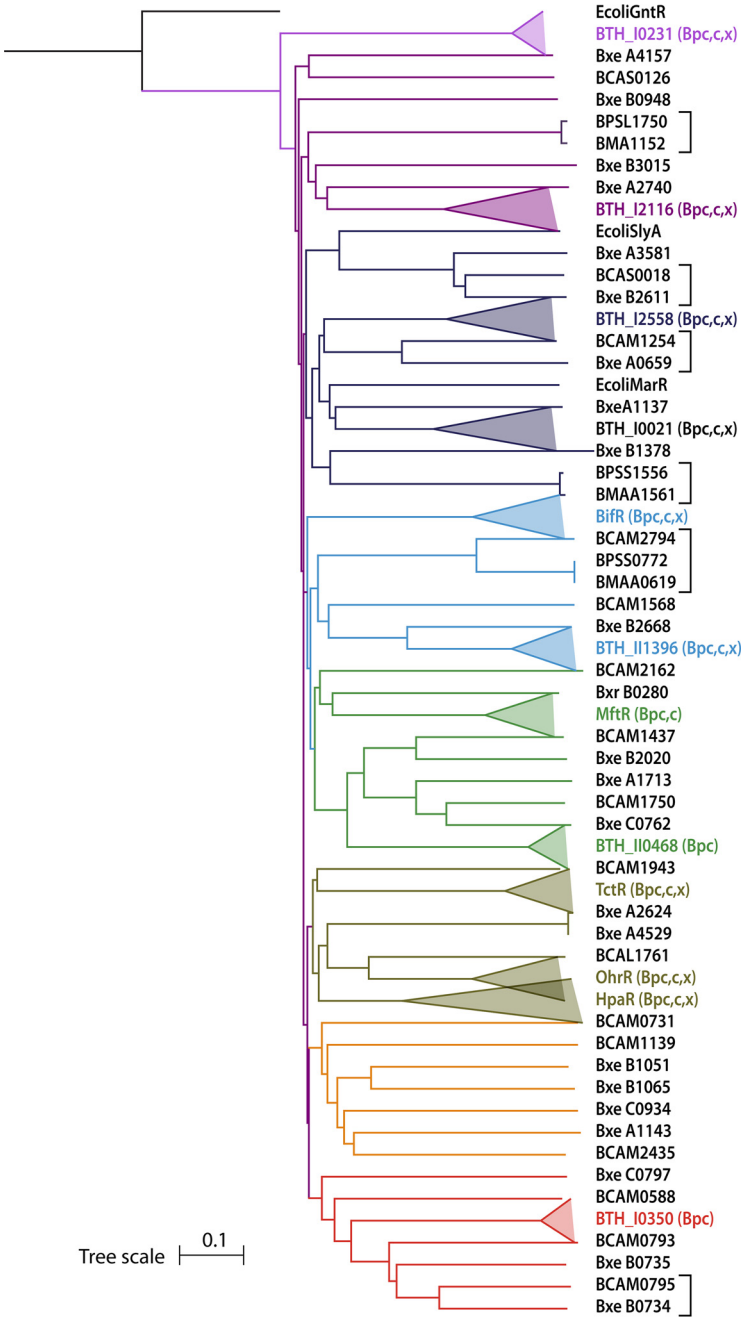

FIG 3 Phylogenetic tree of MarR family proteins encoded by the surveyed *Burkholderia* species. Sequences were aligned using Clustal Omega, and the tree was visualized using iTOL (104, 105). Clades corresponding to orthologous proteins are collapsed and identified with the protein name where available or with the respective locus in *B. thailandensis*. Orthologs identified by collapsed clades are present in all surveyed Bpc species (denoted Bpc); the presence of a given ortholog in *B. cenocepacia* or *B. xenovorans* is denoted with c and x, respectively. Orthologs in other species are identified by brackets. *E. coli* K-12 GntR (an unrelated HTH protein) was used as an outgroup.

promote dispersal of cells, an important step toward colonization of a new environment.

A genome-wide expression analysis revealed that MftR controls a number of genes that are associated with survival in a host environment, genes that are also differentially expressed upon the addition of urate (64). For example, genes associated with survival under hypoxic conditions and the production of siderophores are upregulated in Δ*mftR* cells, whereas the large gene clusters that encode T3SS components and effectors are

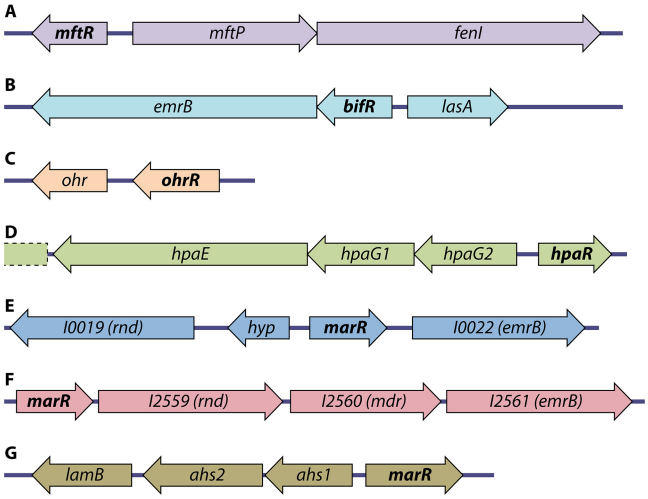

FIG 4 Representative genomic loci that are conserved among *Burkholderia* species. Genes encoding named MarR family transcriptional regulators or uncharacterized MarR family transcriptional regulators (with the latter denoted *marR*) are identified in boldface type. All examples represent *B. thailandensis* genes. (A) MftR controls *mftR* and the divergent *mftP-fenI* operon (66). (B) BifR controls the *bifR-emrB* operon and the divergent *lasA* gene (originally annotated *ecsC*) (65). (C) OhrR represses expression of both *ohr* and *ohrR* (68). (D) The *hpa* operon is conserved in many bacterial species and has been shown to be under the control of HpaR, which responds to hydroxyphenyl acetate (HPA). Only part of the *hpa* gene cluster is shown. The *B. xenovorans hpa* genes are induced by HPA (86). (E) The MarR family protein encoded by *BTH_I0021* has three Cys residues per monomer and may respond to the cellular redox state. The gene encoding the RND efflux system outer membrane component is upregulated in *B. cenocepacia* upon the addition of an oxidant (13). (F) The MarR family protein encoded by *BTH_I2558* has two Cys residues per monomer. *BTH_I2558* is upstream of genes encoding an RND efflux system outer membrane component, a multidrug resistance protein, and an EmrB family drug resistance transporter. The expression of genes encoding transporters is reduced ~50% in *B. cenocepacia* H111 under low-oxygen conditions (14). (G) The MarR family protein encoded by *BTH_I0231* is divergent from an operon encoding a predicted allophanate hydrolase. The expression of this operon is linked to virulence and T3SS expression in *R. solanacearum* (93).

repressed. This suggests that MftR mediates differential gene expression at later stages of infection, and it rationalizes the absence of MftR in environmental isolates such as *B. xenovorans*. That MftR (directly or indirectly) activates the expression of genes encoding T3SS components while repressing other virulence-associated genes is intriguing, and it speaks to a complex regulatory network.

Biofilm Regulator (BifR)

The *B. thailandensis*-encoded redox-sensitive BifR protein is named for its role in controlling biofilm formation (65). BifR is encoded as part of the *emrB-bifR* operon, which is divergently oriented from a gene encoding LasA protease (Fig. 4B); LasA is a virulence factor in *P. aeruginosa*, where it contributes to elastin degradation, thus facilitating invasion of epithelial cells (70). Δ*bifR* cells exhibit enhanced elastin degradation, suggesting that *B. thailandensis* LasA conserves this function (65). The complete genomic locus *lasA-emrB-bifR* is conserved in *B. cenocepacia*, *B. pseudomallei*, and *B. mallei*, and while the *emrB-bifR* operon is conserved in *B. xenovorans*, the divergent gene encoding LasA is not, consistent with its role in virulence (Table 2 and Fig. 3). The *emrB* gene is predicted to encode an EmrB family drug resistance transporter for which the substrate remains unknown.

B. thailandensis BifR binds two adjacent 16-bp palindromes in the *emrB-bifR* promoter, the sequences of which are conserved in *B. pseudomallei*, *B. mallei*, *B. cenocepacia*, and *B. xenovorans* (30/32, 30/32, 22/32, and 19/32 bp conserved, respectively), indicating conservation of the regulatory function yet reflecting divergence in the more distantly related organisms *B. cenocepacia* and *B. xenovorans*. A phylogenetic analysis also supports a common ancestor for BifR in the surveyed *Burkholderia* species (Fig. 3). BifR represses the expression of *emrB-bifR* and *lasA*, and the already low expression

level is further reduced upon the addition of H_2O_2, conditions under which BifR forms a cross-linked dimer of dimers (BifR has a single Cys residue in the DNA-binding region). Such oxidant-mediated repression was also reported in *B. cenocepacia* J2315, whereas expression was increased under microaerobic conditions in the H111 strain, suggesting a conserved regulatory mechanism (13, 14). These observations suggest that oxidized BifR functions as a "superrepressor" that competes more effectively with RNA polymerase for DNA binding. Notably, the expression level of the *phz* operon (*BTH_I0953* to *BTH_I0949*) encoding enzymes required for the synthesis of phenazine derivatives is ~28-fold higher in the Δ*bifR* strain (65); in *P. aeruginosa*, such compounds act as alternative electron acceptors within a biofilm, where they support survival in a low-oxygen environment, and they contribute to maintaining iron homeostasis (71–73). Thus, efficient repression would be expected when oxygen is abundant, conditions under which BifR is in superrepressor mode. Taken together, these data show that BifR links biofilm formation to the cellular redox state. The absence of the virulence-associated *lasA* gene from *B. xenovorans* indicates that the BifR regulon varies among species.

Response to Organic Hydroperoxides: OhrR

Several genes encoding MarR family proteins are adjacent to genes encoding proteins with likely roles in association with a mammalian host. For example, the organic hydroperoxide-sensing OhrR protein, which has been characterized in numerous bacterial species, including *B. thailandensis*, is conserved among *Burkholderia* species (Fig. 3 and 4C). OhrR is oxidized by organic hydroperoxides, results of which are that conformational changes occur, DNA binding is attenuated, and expression of the adjacent *ohr* gene is enhanced; organic hydroperoxide reductase (Ohr) degrades the damaging hydroperoxides, which promotes survival (68, 74–76).

Organic hydroperoxides may be produced upon infection, linking OhrR to virulence; fatty acid (mainly linoleic acid) hydroperoxides are produced in plants (77, 78), and mammalian cells can release the polyunsaturated fatty acid arachidonic acid, which is subsequently oxidized by lipoxygenase enzymes (79, 80). In sessile *B. cenocepacia*, *ohr* was surprisingly shown to be markedly upregulated upon the addition of inorganic oxidants, and in *B. thailandensis*, a modest upregulation of *ohr* by inorganic oxidants was reported to depend on OhrR (13). Such a response to inorganic oxidants is not a common feature of OhrR proteins and may reflect optimization of individual OhrR proteins for bacterial survival in specific oxidative environments. The accumulation of plant exudates in the rhizosphere rationalizes the need to retain *ohrR-ohr* in nonpathogenic soil dwellers such as *B. xenovorans*.

Notably, deletion of *ohrR* has been reported to reduce virulence in some bacterial species, including *B. thailandensis* (despite increased Ohr production, which leads to enhanced survival *in vitro* upon exposure to organic hydroperoxides), and this reduced virulence was inferred to derive from OhrR-mediated control of genes other than *ohr* (68, 81, 82). In *B. thailandensis*, another counterintuitive observation is that deletion of *ohr* results in increased bacterial killing of *Caenorhabditis elegans* and in modestly enhanced survival compared to wild-type cells upon exposure to organic hydroperoxides *in vitro*. This observation suggests that the higher cellular levels of organic hydroperoxides may more efficiently induce genes associated with survival and repair of oxidant-mediated damage and that the *B. thailandensis* Ohr-OhrR system is optimized to ensure that cellular levels of organic hydroperoxides remain high enough for such induction (68). Accordingly, the OhrR regulon may well differ among *Burkholderia* species.

Control of Genes Encoding Type 6 Secretion System Components by TctR

A genetic screen for regulators of genes encoding components of a *B. pseudomallei* K96243-encoded type 6 secretion system (T6SS-2) uncovered a MarR family protein encoded by *BPSL3431*, which was named TctR (for T6SS cluster 2 regulator) (69). In general, T6SSs are contact-dependent systems that inject effectors directly into target

cells, either competing bacterial cells or eukaryotic cells, thereby participating in establishing bacterial communities and in virulence (83). Among the *Burkholderia* T6SSs, T6SS-2 has been implicated in interaction with bacterial cells, not virulence (84). Salient observations of that recent report include the ability of TctR to repress the expression of the gene cluster encoding T6SS-2 components (locus tags BPSS0515 to BPSS0533). Using a representative T6SS promoter-*lacZ* transcriptional fusion, subinhibitory concentrations of antibiotics such as fluoroquinolones were reported to induce expression (including the expression of other T6SS clusters) but only in sessile cells and not in planktonic cells; the mechanism was not identified (69).

The gene encoding TctR is part of a conserved operon that also encodes a glutamine amidotransferase; TctR has four Cys residues per monomer, suggesting the potential for regulation by oxidation. In *B. cenocepacia* H111, this operon is repressed ~2-fold under microaerobic conditions, consistent with regulation by the redox state (14). Glutamine amidotransferases participate in a wide range of biosynthetic processes by transferring an amino group from glutamine to a specific substrate. That T6SS genes under TctR control appear to be sensitive to subinhibitory levels of antibiotics may not be due to a direct interaction of antibiotics with the transcription factor. In analogy with the release of redox-active Cu^{2+} as a consequence of antibiotic-induced envelope stress in *E. coli* (45), one possibility is that the effect of antibiotics on the expression of genes encoding T6SS components in *B. pseudomallei* is due to oxidation of TctR.

Degradation of Aromatic Compounds

HpaR. Degradation of aromatic compounds, including compounds deriving from lignin degradation, root exudates, and xenobiotics, generally occurs via peripheral pathways that feed into central pathways. The homoprotocatechuate pathway in which homoprotocatechuate undergoes ring cleavage and conversion to citric acid cycle intermediates has been functionally characterized in several bacterial species, including *B. xenovorans* (85, 86). In *B. xenovorans* (and other species), expression of *hpa* genes is induced by 3- and 4-hydroxyphenylacetate (3-HPA and 4-HPA, respectively) (86). In *E. coli*, HpaR was shown to repress both the *hpa* operon as well as its own expression (85). Indeed, several MarR family proteins for which related aromatics induce the expression of the adjacent catabolic enzymes have been characterized (38). Based on the conservation of the *hpa* gene locus (Fig. 4D), it is therefore a reasonable prediction that *B. xenovorans* HpaR likewise responds directly to 3-HPA and 4-HPA to induce *hpa* expression and that HpaR serves an equivalent function in other *Burkholderia* species.

The *vanAB* operon. In response to pathogens, plant roots may release *de novo*-synthesized hydroxycinnamates, such as ferulate and *p*-coumarate, into the rhizosphere. Hydroxycinnamates have broad antimicrobial activity, as they disrupt membrane integrity and decouple the respiratory proton gradient (87). As an example of the relevance of such compounds, mutations in the plant pathogen *Ralstonia solanacearum* that render it deficient in the degradation of hydroxycinnamates also cause it to be less virulent (88). Degradation of ferulate proceeds through vanillate, which is in turn converted to protocatechuate. An operon annotated *vanAB* is oriented divergently from a gene encoding a MarR homolog (*BCAM2435*) in *B. cenocepacia*. The *vanAB* operon is predicted to encode a vanillate *O*-demethylase that converts vanillate to protocatechuate. A predicted function of the associated MarR family protein is therefore to respond to vanillate or other precursors to induce *vanAB* expression; a potential binding site for the MarR family protein consisting of 7-bp half-sites separated by 3 bp (ACTGAATctcATTCAGT) may be identified 59 bp upstream of the start codon. That this locus is found in *B. cenocepacia* and phytopathogens such as *B. glumae* (but not in Bpc and Bcc species) may be related to their success as plant pathogens.

Degradation of hydroxycinnamates. *B. cenocepacia BCAM2794* encodes a MarR family transcription factor, and it is flanked by a gene encoding *p*-hydroxycinnamoyl CoA hydratase-lyase and a gene encoding gluconolactonase; these three genes are repressed 3- to 10-fold in *B. cenocepacia* H111 during growth under low-oxygen

conditions (14). This locus is not conserved in the other surveyed species (Table 2). The *p*-hydroxycinnamoyl CoA hydratase-lyase enzyme participates in the degradation of hydroxycinnamates by converting feruloyl-CoA to acetyl-CoA and vanillin (89). In *Sphingobium* sp. strain SYK-6, the genes encoding enzymes involved in ferulate degradation are repressed by a MarR protein (FerC), which responds to feruloyl-CoA and related CoA derivatives (90). Ferulate esters function as antioxidants (91), which could rationalize the downregulation of enzymes involved in ferulate degradation when oxygen levels are low.

Regulation by Reactive Oxygen Species

While gene regulation by *B. thailandensis* BifR, MftR, and OhrR and *B. pseudomallei* TctR has been experimentally demonstrated, and the functional role of HpaR may be predicted with some confidence based on functional characterization of orthologs from other bacterial species, the functions of the remaining MarR family proteins are more speculative. An intriguing characteristic is that the vast majority of *Burkholderia*-encoded MarR homologs have at least one cysteine, raising the possibility that some may be sensitive to ROS (as discussed above for BifR, OhrR, TctR, and BCAM2794). Proteins such as OhrR clearly respond to host-derived organic hydroperoxides; however, other redox-sensitive MarR family proteins could potentially respond to both endogenous and exogenous ROS, depending on their reactivity with various oxidants. Among the Bpc group members, MftR and *B. pseudomallei* BPSL1400 have no Cys residues, and several MarR family proteins that are unique to *B. cenocepacia* have no Cys residues, including BCAM2435, which would be predicted to bind aromatic compounds, as noted above.

A microarray analysis of genes that are differentially expressed upon the addition of an oxidant to sessile *B. cenocepacia* J2315 cells showed an ~3-fold increase in the expression of *BCAL3514*, which is predicted to encode an RND efflux system outer membrane component (13). *BCAL3514* is located downstream of a gene encoding a small hypothetical protein and as part of a conserved locus that also includes divergent genes encoding a MarR family transcription factor and an EmrB family drug resistance transporter (Fig. 4E and Table 2). The MarR family protein has three Cys residues per monomer and is therefore likely to sense the cellular redox state. Another conserved MarR family regulator predicted to control the expression of transporters is encoded by *BTH_I2558*, which is upstream of genes encoding an RND efflux system outer membrane lipoprotein, a multidrug resistance protein, and an EmrB/QacA family drug resistance transporter (annotated as a possible operon in *B. thailandensis* but not in other species) (Fig. 4F); this MarR family protein has two Cys residues per monomer. The expression of the corresponding genes encoding transporters is reduced ~50% in *B. cenocepacia* H111 under low-oxygen conditions and in strain J2315 during stationary phase in minimal medium (14, 92).

Of the remaining MarR family transcription factors that are conserved among species, the protein encoded by *BTH_I0231* is divergent from an operon encoding a predicted allophanate hydrolase (Fig. 4G); this MarR family protein has four Cys residues per monomer. Allophanate hydrolase is required for the cells to use urea as a nitrogen source, and it converts allophanate to ammonia and carbon dioxide. In the plant pathogen *R. solanacearum*, allophanate hydrolase was also shown to be required for pathogenicity and for optimal expression of T3SS components (93). The latter observation highlights the fact that even though the locus is conserved in environmental bacteria such as *B. xenovorans*, expression may still selectively promote survival in a host environment.

Another conserved operon that is repressed (2- to 5-fold) in *B. cenocepacia* H111 and J2315 grown under low-oxygen tension corresponds to the *BTH_II1396-BTH_II1397* operon, which encodes a MarR family protein with one Cys residue per monomer and a ClC chloride channel (14, 92). In *E. coli*, the ClC chloride channel functions as a Cl^-/H^+ exchanger and is involved in acid resistance (94).

MarR Family Proteins Unique to Certain Species

BTH_II0468 is predicted to be part of an operon that includes a gene encoding isochorismatase and a fusaric acid resistance protein (FusC_2, an inner membrane transporter). This operon is conserved in Bpc species, but it is absent from both *B. cenocepacia* and *B. xenovorans*. The fusaric acid resistance protein is involved in resistance to the nonspecific fungal toxin fusaric acid, which is produced by *Fusarium* species and considered a virulence factor in their interaction with susceptible plants (95). Resistance to fusaric acid would therefore benefit inhabitants of the rhizosphere. Indeed, several *Burkholderia* species preferentially colonize the rhizosphere of plants infected with *Fusarium* spp. compared to noninfected control plants, likely because they utilize fungal exudates as a source of nutrients. In addition, the bacteria have the ability to restrict fungal growth *in vitro* (96). Isochorismatase catalyzes the conversion of isochorismate into 2,3-dihydroxybenzoate and pyruvate. Isochorismate is a precursor to several siderophores, which contribute to virulence by mediating the uptake of iron; for example, *P. aeruginosa* PhzD was identified as an isochorismatase that participates in the biosynthesis of the siderophore phenazine (97).

B. cenocepacia instead encodes a predicted isochorismatase downstream of *BCAM1750*, which encodes a MarR family protein. This locus is conserved in *B. xenovorans*. Also conserved between *B. cenocepacia* and *B. xenovorans* is a locus consisting of a gene encoding a MarR homolog (BCAS0018/Bxe_B2611) followed by a gene encoding a fusaric acid resistance protein (FusC), a small hypothetical protein, an efflux system transport protein, and an outer membrane efflux protein. It has been reported that the ability of bacteria (with pseudomonads exhibiting the greatest resistance) to survive in the presence of fusaric acid correlates with the copy number of genes encoding FusC. Among the *Burkholderia* species analyzed in this particular survey were *B. glumae* and *B. cepacia*, both of which encode two FusC proteins; one of these FusC proteins is a homolog of *B. cenocepacia* BCAS0018 (95). Expression of the *B. cepacia* fusaric acid resistance locus in *E. coli* conferred resistance to fusaric acid, indicating that the enzyme is functional (98). Genes corresponding to *BCAS0018* and the adjacent *fusC* gene (which overlaps *BCAS0018* by 3 bp) are repressed ~5-fold in *B. cenocepacia* H111 under low-oxygen conditions; an almost perfectly conserved 16-bp palindrome (TGTC AtCC-GGgTGACA) may be identified in the *BCAS0018* promoter (14). While the MarR family protein encoded by *BCAS0018* has two Cys residues per monomer, consistent with regulation by the redox state, the homolog encoded by *Bxe_B2611* has none, indicating that inducing signals may be different.

A saturating transposon insertion screen that aimed to predict essential genes in *B. pseudomallei* K96243 was reported (99). In this transposon-directed insertion sequencing (TraDIS) approach, putative essential genes are identified by the absence of a transposon insertion. Among the genes predicted to be essential were *BPSL1750*, encoding a MarR family transcription factor, and the adjacent gene encoding an ABC transporter (*BPSL1751*) for which the substrate is unknown. This locus is conserved in *B. mallei* but not in any of the other surveyed species. However, a previous characterization of *B. pseudomallei* strain 708a, which was identified based on susceptibility to aminoglycoside antibiotics, revealed that this strain lacks an ~131-kb region that includes not only genes encoding the AmrAB-OprA efflux system responsible for aminoglycoside efflux but also a number of other genes (100). The region deleted in strain 708a includes genes corresponding to *BPSL1750* and *BPSL1751* as well as several other genes identified in the TraDIS screen as potentially being essential. While it is conceivable that the genetic background may impact which genes are essential, it is more likely that this underrepresentation in the TraDIS screen reflects that the genes in question confer a fitness advantage, possibly combined with some insertion bias. The MarR homolog encoded by *BPSL1750* has two Cys residues per monomer and could potentially be responsive to the cellular redox state.

Several MarR homologs are absent from the Bpc species, including the *B. cenocepacia*-borne *BCAL1761* gene, which is downstream of a gene encoding a pre-

dicted glutathione-dependent formaldehyde-activating enzyme; this enzyme is involved in the processing of the toxic formaldehyde that is produced during various metabolic reactions (101, 102). Both genes are upregulated ~6-fold during growth under microaerobic conditions (14). A TraDIS approach to the prediction of essential genes in *B. cenocepacia* J2315 identified *BCAL1761* as a conditionally essential/critical gene for growth on minimal medium (but not in LB) (103). In contrast, the MarR family protein encoded by *BCAM0731* is repressed ~2-fold under microaerobic conditions, whereas the divergent gene *BCAM0730*, which encodes a SnoaL-like protein (a polyketide cyclase), is upregulated ~3-fold (14).

The largest number of MarR homologs is encoded by *B. xenovorans* (>30, based on a search of the *Burkholderia* Genome Database). *B. xenovorans* LB400 has one of the largest bacterial genomes, with an estimated 20% of genes having been recently acquired by lateral gene transfer (34). MarR homologs that are not encoded by Bpc and Bcc species include several that are predicted to control the expression of transporters and biosynthetic operons (not shown).

OUTLOOK

The *modus operandi* of MarR family transcription factors is to sense changes in the environment, either in the form of binding a small-molecule ligand or metal ion or by oxidation of specific cysteines, and to transduce such signals into differential gene expression. As such, they are ideally suited to sense host-derived signals and effect the requisite expression of virulence-associated genes. Among the MarR family proteins encoded by *Burkholderia* species, some that are highly conserved and predicted to perform the same function in all species may be identified, such as HpaR, which is predicted to control the production of enzymes that function in the central homoprotocatechuate pathway. Other conserved MarR family proteins are likely to conserve the control of a core regulon but also to regulate other genes that differ between species, as exemplified by BifR and most likely OhrR, which has been implicated in virulence. However, others are encoded only by Bpc and Bcc species, most notably MftR, which has been shown to control virulence-associated genes. While many predictions may be reliably made about the regulation of genes located adjacent to genes encoding MarR family transcriptional regulators, it is clear that determination of individual regulons from different species is liable to uncover important and unexpected clues to their role in shaping individual phenotypes. That some MarR homologs may be conditionally critical for growth is particularly intriguing and should serve as an added incentive to define their mode of action.

ACKNOWLEDGMENTS

We gratefully acknowledge support of research in our laboratory from the National Science Foundation (MCB-1714219 to A. Grove).

We have no conflicts of interest with the contents of this article.

REFERENCES

1. Palleroni NJ. 18 March 2015. Burkholderia. *In* Bergey's manual of systematics of archaea and bacteria. John Wiley & Sons, Chichester, United Kingdom. https://doi.org/10.1002/9781118960608.gbm00935.
2. Compant S, Nowak J, Coenye T, Clement C, Ait Barka E. 2008. Diversity and occurrence of *Burkholderia* spp. in the natural environment. FEMS Microbiol Rev 32:607–626. https://doi.org/10.1111/j.1574-6976.2008.00113.x.
3. Fries MR, Forney LJ, Tiedje JM. 1997. Phenol- and toluene-degrading microbial populations from an aquifer in which successful trichloroethene cometabolism occurred. Appl Environ Microbiol 63:1523–1530.
4. Ramette A, LiPuma JJ, Tiedje JM. 2005. Species abundance and diversity of *Burkholderia cepacia* complex in the environment. Appl Environ Microbiol 71:1193–1201. https://doi.org/10.1128/AEM.71.3.1193-1201.2005.
5. Burkholder WH. 1950. Sour skin, a bacterial rot of onion bulbs. Phytopathology 40:115–117.
6. Martin DW, Mohr CD. 2000. Invasion and intracellular survival of *Burkholderia cepacia*. Infect Immun 68:24–29. https://doi.org/10.1128/IAI.68.1.24-29.2000.
7. Loutet SA, Valvano MA. 2010. A decade of *Burkholderia cenocepacia* virulence determinant research. Infect Immun 78:4088–4100. https://doi.org/10.1128/IAI.00212-10.
8. Aschenbroich SA, Lafontaine ER, Hogan RJ. 2016. Melioidosis and glanders modulation of the innate immune system: barriers to current and future vaccine approaches. Expert Rev Vaccines 15:1163–1181. https://doi.org/10.1586/14760584.2016.1170598.
9. Sawana A, Adeolu M, Gupta RS. 2014. Molecular signatures and phylogenomic analysis of the genus Burkholderia: proposal for division of this genus into the emended genus Burkholderia containing pathogenic organisms and a new genus Paraburkholderia gen. nov. harboring environmental species. Front Genet 5:429. https://doi.org/10.3389/fgene.2014.00429.

10. Estrada-de los Santos P, Vinuesa P, Martinez-Aguilar L, Hirsch AM, Caballero-Mellado J. 2013. Phylogenetic analysis of *Burkholderia* species by multilocus sequence analysis. Curr Microbiol 67:51–60. https://doi.org/10.1007/s00284-013-0330-9.
11. Sahl JW, Vazquez AJ, Hall CM, Busch JD, Tuanyok A, Mayo M, Schupp JM, Lummis M, Pearson T, Shippy K, Colman RE, Allender CJ, Theobald V, Sarovich DS, Price EP, Hutcheson A, Korlach J, LiPuma JJ, Ladner J, Lovett S, Koroleva G, Palacios G, Limmathurotsakul D, Wuthiekanun V, Wongsuwan G, Currie BJ, Keim P, Wagner DM. 2016. The effects of signal erosion and core genome reduction on the identification of diagnostic markers. mBio 7:e00846-16. https://doi.org/10.1128/mBio.00846-16.
12. Dobritsa AP, Samadpour M. 2016. Transfer of eleven species of the genus *Burkholderia* to the genus *Paraburkholderia* and proposal of *Caballeronia* gen. nov. to accommodate twelve species of the genera *Burkholderia* and *Paraburkholderia*. Int J Syst Evol Microbiol 66:2836–2846. https://doi.org/10.1099/ijsem.0.001065.
13. Peeters E, Sass A, Mahenthiralingam E, Nelis H, Coenye T. 2010. Transcriptional response of *Burkholderia cenocepacia* J2315 sessile cells to treatments with high doses of hydrogen peroxide and sodium hypochlorite. BMC Genomics 11:90. https://doi.org/10.1186/1471-2164-11-90.
14. Pessi G, Braunwalder R, Grunau A, Omasits U, Ahrens CH, Eberl L. 2013. Response of *Burkholderia cenocepacia* H111 to micro-oxia. PLoS One 8:e72939. https://doi.org/10.1371/journal.pone.0072939.
15. Wuthiekanun V, Smith MD, Dance DA, Walsh AL, Pitt TL, White NJ. 1996. Biochemical characteristics of clinical and environmental isolates of *Burkholderia pseudomallei*. J Med Microbiol 45:408–412. https://doi.org/10.1099/00222615-45-6-408.
16. Galyov EE, Brett PJ, DeShazer D. 2010. Molecular insights into *Burkholderia pseudomallei* and *Burkholderia mallei* pathogenesis. Annu Rev Microbiol 64:495–517. https://doi.org/10.1146/annurev.micro.112408.134030.
17. Smith MD, Angus BJ, Wuthiekanun V, White NJ. 1997. Arabinose assimilation defines a nonvirulent biotype of *Burkholderia pseudomallei*. Infect Immun 65:4319–4321.
18. Yu Y, Kim HS, Chua HH, Lin CH, Sim SH, Lin D, Derr A, Engels R, DeShazer D, Birren B, Nierman WC, Tan P. 2006. Genomic patterns of pathogen evolution revealed by comparison of *Burkholderia pseudomallei*, the causative agent of melioidosis, to avirulent *Burkholderia thailandensis*. BMC Microbiol 6:46. https://doi.org/10.1186/1471-2180-6-46.
19. Holden MT, Seth-Smith HM, Crossman LC, Sebaihia M, Bentley SD, Cerdeno-Tarraga AM, Thomson NR, Bason N, Quail MA, Sharp S, Cherevach I, Churcher C, Goodhead I, Hauser H, Holroyd N, Mungall K, Scott P, Walker D, White B, Rose H, Iversen P, Mil-Homens D, Rocha EP, Fialho AM, Baldwin A, Dowson C, Barrell BG, Govan JR, Vandamme P, Hart CA, Mahenthiralingam E, Parkhill J. 2009. The genome of *Burkholderia cenocepacia* J2315, an epidemic pathogen of cystic fibrosis patients. J Bacteriol 191:261–277. https://doi.org/10.1128/JB.01230-08.
20. Losada L, Ronning CM, DeShazer D, Woods D, Fedorova N, Kim HS, Shabalina SA, Pearson TR, Brinkac L, Tan P, Nandi T, Crabtree J, Badger J, Beckstrom-Sternberg S, Saqib M, Schutzer SE, Keim P, Nierman WC. 2010. Continuing evolution of *Burkholderia mallei* through genome reduction and large-scale rearrangements. Genome Biol Evol 2:102–116. https://doi.org/10.1093/gbe/evq003.
21. Nierman WC, DeShazer D, Kim HS, Tettelin H, Nelson KE, Feldblyum T, Ulrich RL, Ronning CM, Brinkac LM, Daugherty SC, Davidsen TD, Deboy RT, Dimitrov G, Dodson RJ, Durkin AS, Gwinn ML, Haft DH, Khouri H, Kolonay JF, Madupu R, Mohammoud Y, Nelson WC, Radune D, Romero CM, Sarria S, Selengut J, Shamblin C, Sullivan SA, White O, Yu Y, Zafar N, Zhou L, Fraser CM. 2004. Structural flexibility in the *Burkholderia mallei* genome. Proc Natl Acad Sci U S A 101:14246–14251. https://doi.org/10.1073/pnas.0403306101.
22. Song H, Hwang J, Yi H, Ulrich RL, Yu Y, Nierman WC, Kim HS. 2010. The early stage of bacterial genome-reductive evolution in the host. PLoS Pathog 6:e1000922. https://doi.org/10.1371/journal.ppat.1000922.
23. Haraga A, West TE, Brittnacher MJ, Skerrett SJ, Miller SI. 2008. *Burkholderia thailandensis* as a model system for the study of the virulence-associated type III secretion system of *Burkholderia pseudomallei*. Infect Immun 76:5402–5411. https://doi.org/10.1128/IAI.00626-08.
24. Rainbow L, Hart CA, Winstanley C. 2002. Distribution of type III secretion gene clusters in *Burkholderia pseudomallei*, *B. thailandensis* and *B. mallei*. J Med Microbiol 51:374–384. https://doi.org/10.1099/0022-1317-51-5-374.
25. Rhodes KA, Schweizer HP. 2016. Antibiotic resistance in *Burkholderia* species. Drug Resist Updat 28:82–90. https://doi.org/10.1016/j.drup.2016.07.003.
26. Deng W, Marshall NC, Rowland JL, McCoy JM, Worrall LJ, Santos AS, Strynadka NCJ, Finlay BB. 2017. Assembly, structure, function and regulation of type III secretion systems. Nat Rev Microbiol 15:323–337. https://doi.org/10.1038/nrmicro.2017.20.
27. Stevens MP, Haque A, Atkins T, Hill J, Wood MW, Easton A, Nelson M, Underwood-Fowler C, Titball RW, Bancroft GJ, Galyov EE. 2004. Attenuated virulence and protective efficacy of a *Burkholderia pseudomallei bsa* type III secretion mutant in murine models of melioidosis. Microbiology 150:2669–2676. https://doi.org/10.1099/mic.0.27146-0.
28. Ulrich RL, DeShazer D. 2004. Type III secretion: a virulence factor delivery system essential for the pathogenicity of *Burkholderia mallei*. Infect Immun 72:1150–1154. https://doi.org/10.1128/IAI.72.2.1150-1154.2004.
29. Glass MB, Gee JE, Steigerwalt AG, Cavuoti D, Barton T, Hardy RD, Godoy D, Spratt BG, Clark TA, Wilkins PP. 2006. Pneumonia and septicemia caused by *Burkholderia thailandensis* in the United States. J Clin Microbiol 44:4601–4604. https://doi.org/10.1128/JCM.01585-06.
30. Chieng S, Carreto L, Nathan S. 2012. *Burkholderia pseudomallei* transcriptional adaptation in macrophages. BMC Genomics 13:328. https://doi.org/10.1186/1471-2164-13-328.
31. Drevinek P, Mahenthiralingam E. 2010. *Burkholderia cenocepacia* in cystic fibrosis: epidemiology and molecular mechanisms of virulence. Clin Microbiol Infect 16:821–830. https://doi.org/10.1111/j.1469-0691.2010.03237.x.
32. Visser MB, Majumdar S, Hani E, Sokol PA. 2004. Importance of the ornibactin and pyochelin siderophore transport systems in *Burkholderia cenocepacia* lung infections. Infect Immun 72:2850–2857. https://doi.org/10.1128/IAI.72.5.2850-2857.2004.
33. Fazli M, Almblad H, Rybtke ML, Givskov M, Eberl L, Tolker-Nielsen T. 2014. Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species. Environ Microbiol 16:1961–1981. https://doi.org/10.1111/1462-2920.12448.
34. Chain PS, Denef VJ, Konstantinidis KT, Vergez LM, Agullo L, Reyes VL, Hauser L, Cordova M, Gomez L, Gonzalez M, Land M, Lao V, Larimer F, LiPuma JJ, Mahenthiralingam E, Malfatti SA, Marx CJ, Parnell JJ, Ramette A, Richardson P, Seeger M, Smith D, Spilker T, Sul WJ, Tsoi TV, Ulrich LE, Zhulin IB, Tiedje JM. 2006. *Burkholderia xenovorans* LB400 harbors a multi-replicon, 9.73-Mbp genome shaped for versatility. Proc Natl Acad Sci U S A 103:15280–15287. https://doi.org/10.1073/pnas.0606924103.
35. Romero-Silva MJ, Mendez V, Agullo L, Seeger M. 2013. Genomic and functional analyses of the gentisate and protocatechuate ring-cleavage pathways and related 3-hydroxybenzoate and 4-hydroxybenzoate peripheral pathways in *Burkholderia xenovorans* LB400. PLoS One 8:e56038. https://doi.org/10.1371/journal.pone.0056038.
36. George AM, Levy SB. 1983. Amplifiable resistance to tetracycline, chloramphenicol, and other antibiotics in *Escherichia coli*: involvement of a non-plasmid-determined efflux of tetracycline. J Bacteriol 155:531–540.
37. Ariza RR, Cohen SP, Bachhawat N, Levy SB, Demple B. 1994. Repressor mutations in the *marRAB* operon that activate oxidative stress genes and multiple antibiotic resistance in *Escherichia coli*. J Bacteriol 176:143–148. https://doi.org/10.1128/jb.176.1.143-148.1994.
38. Grove A. 2017. Regulation of metabolic pathways by MarR family transcription factors. Comput Struct Biotechnol J 15:366–371. https://doi.org/10.1016/j.csbj.2017.06.001.
39. Perez-Rueda E, Collado-Vides J. 2001. Common history at the origin of the position-function correlation in transcriptional regulators in archaea and bacteria. J Mol Evol 53:172–179. https://doi.org/10.1007/s002390010207.
40. Deochand DK, Grove A. 2017. MarR family transcription factors: dynamic variations on a common scaffold. Crit Rev Biochem Mol Biol 52:595–613. https://doi.org/10.1080/10409238.2017.1344612.
41. Perez-Rueda E, Collado-Vides J, Segovia L. 2004. Phylogenetic distribution of DNA-binding transcription factors in bacteria and archaea. Comput Biol Chem 28:341–350. https://doi.org/10.1016/j.compbiolchem.2004.09.004.
42. Rivera-Gomez N, Segovia L, Perez-Rueda E. 2011. Diversity and distribution of transcription factors: their partner domains play an important role in regulatory plasticity in bacteria. Microbiology 157:2308–2318. https://doi.org/10.1099/mic.0.050617-0.

43. Perera IC, Grove A. 2010. Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. J Mol Cell Biol 2:243–254. https://doi.org/10.1093/jmcb/mjq021.
44. Martin RG, Jair KW, Wolf RE, Jr, Rosner JL. 1996. Autoactivation of the *marRAB* multiple antibiotic resistance operon by the MarA transcriptional activator in *Escherichia coli*. J Bacteriol 178:2216–2223. https://doi.org/10.1128/jb.178.8.2216-2223.1996.
45. Hao Z, Lou H, Zhu R, Zhu J, Zhang D, Zhao BS, Zeng S, Chen X, Chan J, He C, Chen PR. 2014. The multiple antibiotic resistance regulator MarR is a copper sensor in *Escherichia coli*. Nat Chem Biol 10:21–28. https://doi.org/10.1038/nchembio.1380.
46. Perera IC, Grove A. 2011. MarR homologs with urate-binding signature. Protein Sci 20:621–629. https://doi.org/10.1002/pro.588.
47. Will WR, Bale DH, Reid PJ, Libby SJ, Fang FC. 2014. Evolutionary expansion of a regulatory network by counter-silencing. Nat Commun 5:5270. https://doi.org/10.1038/ncomms6270.
48. Will WR, Brzovic P, Le Trong I, Stenkamp RE, Lawrenz MB, Navarre WW, Main-Hester K, Miller VL, Libby SJ, Fang FC. 2018. The evolution of SlyA/RovA transcription factors from repressors to counter-silencers in *Enterobacteriaceae*. bioRxiv https://doi.org/10.1101/369546.
49. Nguyen GT, Green ER, Mecsas J. 2017. Neutrophils to the ROScue: mechanisms of NADPH oxidase activation and bacterial resistance. Front Cell Infect Microbiol 7:373. https://doi.org/10.3389/fcimb.2017.00373.
50. Sies H, Berndt C, Jones DP. 2017. Oxidative stress. Annu Rev Biochem 86:715–748. https://doi.org/10.1146/annurev-biochem-061516-045037.
51. Refsgaard HH, Tsai L, Stadtman ER. 2000. Modifications of proteins by polyunsaturated fatty acid peroxidation products. Proc Natl Acad Sci U S A 97:611–616. https://doi.org/10.1073/pnas.97.2.611.
52. Porter LA, Goldberg JB. 2011. Influence of neutrophil defects on *Burkholderia cepacia* complex pathogenesis. Front Cell Infect Microbiol 1:9. https://doi.org/10.3389/fcimb.2011.00009.
53. Segal BH, Sakamoto N, Patel M, Maemura K, Klein AS, Holland SM, Bulkley GB. 2000. Xanthine oxidase contributes to host defense against *Burkholderia cepacia* in the p47*phox*$^{-/-}$ mouse model of chronic granulomatous disease. Infect Immun 68:2374–2378. https://doi.org/10.1128/IAI.68.4.2374-2378.2000.
54. Martin HM, Hancock JT, Salisbury V, Harrison R. 2004. Role of xanthine oxidoreductase as an antimicrobial agent. Infect Immun 72:4933–4939. https://doi.org/10.1128/IAI.72.9.4933-4939.2004.
55. Nishino T, Okamoto K, Eger BT, Pai EF, Nishino T. 2008. Mammalian xanthine oxidoreductase—mechanism of transition from xanthine dehydrogenase to xanthine oxidase. FEBS J 275:3278–3289. https://doi.org/10.1111/j.1742-4658.2008.06489.x.
56. Ma X, Wang W, Bittner F, Schmidt N, Berkey R, Zhang L, King H, Zhang Y, Feng J, Wen Y, Tan L, Li Y, Zhang Q, Deng Z, Xiong X, Xiao S. 2016. Dual and opposing roles of xanthine dehydrogenase in defense-associated reactive oxygen species metabolism in Arabidopsis. Plant Cell 28:1108–1126. https://doi.org/10.1105/tpc.15.00880.
57. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. Cell 130:797–810. https://doi.org/10.1016/j.cell.2007.06.049.
58. Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, Chan CT, Lobritz MA, Braff D, Schwarz EG, Ye JD, Pati M, Vercruysse M, Ralifo PS, Allison KR, Khalil AS, Ting AY, Walker GC, Collins JJ. 2014. Antibiotics induce redox-related physiological alterations as part of their lethality. Proc Natl Acad Sci U S A 111:E2100–E2109. https://doi.org/10.1073/pnas.1401876111.
59. Van Acker H, Gielis J, Acke M, Cools F, Cos P, Coenye T. 2016. The role of reactive oxygen species in antibiotic-induced cell death in *Burkholderia cepacia* complex bacteria. PLoS One 11:e0159837. https://doi.org/10.1371/journal.pone.0159837.
60. Liu Y, Imlay JA. 2013. Cell death from antibiotics without the involvement of reactive oxygen species. Science 339:1210–1213. https://doi.org/10.1126/science.1232751.
61. Zhao X, Drlica K. 2014. Reactive oxygen species and the bacterial response to lethal stress. Curr Opin Microbiol 21:1–6. https://doi.org/10.1016/j.mib.2014.06.008.
62. Winsor GL, Khaira B, Van Rossum T, Lo R, Whiteside MD, Brinkman FS. 2008. The *Burkholderia* Genome Database: facilitating flexible queries and comparative analyses. Bioinformatics 24:2803–2804. https://doi.org/10.1093/bioinformatics/btn524.
63. Perez JC, Groisman EA. 2009. Evolution of transcriptional regulatory circuits in bacteria. Cell 138:233–244. https://doi.org/10.1016/j.cell.2009.07.002.
64. Gupta A, Bedre R, Thapa SS, Sabrin A, Wang G, Dassanayake M, Grove A. 2017. Global awakening of cryptic biosynthetic gene clusters in *Burkholderia thailandensis*. ACS Chem Biol 12:3012–3021. https://doi.org/10.1021/acschembio.7b00681.
65. Gupta A, Fuentes SM, Grove A. 2017. Redox-sensitive MarR homologue BifR from *Burkholderia thailandensis* regulates biofilm formation. Biochemistry 56:2315–2327. https://doi.org/10.1021/acs.biochem.7b00103.
66. Gupta A, Grove A. 2014. Ligand-binding pocket bridges DNA-binding and dimerization domains of the urate-responsive MarR homologue MftR from *Burkholderia thailandensis*. Biochemistry 53:4368–4380. https://doi.org/10.1021/bi500219t.
67. Grove A. 2010. Urate-responsive MarR homologs from *Burkholderia*. Mol Biosyst 6:2133–2142. https://doi.org/10.1039/c0mb00086h.
68. Pande A, Veale TC, Grove A. 2018. Gene regulation by redox-sensitive *Burkholderia thailandensis* OhrR and its role in bacterial killing of *Caenorhabditis elegans*. Infect Immun 86:e00322-18. https://doi.org/10.1128/IAI.00322-18.
69. Losada L, Shea AA, DeShazer D. 2018. A MarR family transcriptional regulator and subinhibitory antibiotics regulate type VI secretion gene clusters in *Burkholderia pseudomallei*. Microbiology 164:1196–1211. https://doi.org/10.1099/mic.0.000697.
70. Cowell BA, Twining SS, Hobden JA, Kwong MS, Fleiszig SM. 2003. Mutation of *lasA* and *lasB* reduces *Pseudomonas aeruginosa* invasion of epithelial cells. Microbiology 149:2291–2299. https://doi.org/10.1099/mic.0.26280-0.
71. Wang Y, Kern SE, Newman DK. 2010. Endogenous phenazine antibiotics promote anaerobic survival of *Pseudomonas aeruginosa* via extracellular electron transfer. J Bacteriol 192:365–369. https://doi.org/10.1128/JB.01188-09.
72. Okegbe C, Price-Whelan A, Dietrich LE. 2014. Redox-driven regulation of microbial community morphogenesis. Curr Opin Microbiol 18:39–45. https://doi.org/10.1016/j.mib.2014.01.006.
73. Wang Y, Wilks JC, Danhorn T, Ramos I, Croal L, Newman DK. 2011. Phenazine-1-carboxylic acid promotes bacterial biofilm development via ferrous iron acquisition. J Bacteriol 193:3606–3617. https://doi.org/10.1128/JB.00396-11.
74. Fuangthong M, Atichartpongkul S, Mongkolsuk S, Helmann JD. 2001. OhrR is a repressor of *ohrA*, a key organic hydroperoxide resistance determinant in *Bacillus subtilis*. J Bacteriol 183:4134–4141. https://doi.org/10.1128/JB.183.14.4134-4141.2001.
75. Sukchawalit R, Loprasert S, Atichartpongkul S, Mongkolsuk S. 2001. Complex regulation of the organic hydroperoxide resistance gene (*ohr*) from *Xanthomonas* involves OhrR, a novel organic peroxide-inducible negative regulator, and posttranscriptional modifications. J Bacteriol 183:4405–4412. https://doi.org/10.1128/JB.183.15.4405-4412.2001.
76. Newberry KJ, Fuangthong M, Panmanee W, Mongkolsuk S, Brennan RG. 2007. Structural mechanism of organic hydroperoxide induction of the transcription regulator OhrR. Mol Cell 28:652–664. https://doi.org/10.1016/j.molcel.2007.09.016.
77. Klomsiri C, Panmanee W, Dharmsthiti S, Vattanaviboon P, Mongkolsuk S. 2005. Novel roles of *ohrR-ohr* in *Xanthomonas* sensing, metabolism, and physiological adaptive response to lipid hydroperoxide. J Bacteriol 187:3277–3281. https://doi.org/10.1128/JB.187.9.3277-3281.2005.
78. Prost I, Dhondt S, Rothe G, Vicente J, Rodriguez MJ, Kift N, Carbonne F, Griffiths G, Esquerre-Tugaye MT, Rosahl S, Castresana C, Hamberg M, Fournier J. 2005. Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. Plant Physiol 139:1902–1913. https://doi.org/10.1104/pp.105.066274.
79. Guillemot L, Medina M, Pernet E, Leduc D, Chignard M, Touqui L, Wu Y. 2014. Cytosolic phospholipase A2alpha enhances mouse mortality induced by *Pseudomonas aeruginosa* pulmonary infection via interleukin 6. Biochimie 107(Part A):95–104. https://doi.org/10.1016/j.biochi.2014.08.018.
80. Leslie CC. 2015. Cytosolic phospholipase A(2): physiological function and role in disease. J Lipid Res 56:1386–1402. https://doi.org/10.1194/jlr.R057588.
81. Atichartpongkul S, Fuangthong M, Vattanaviboon P, Mongkolsuk S. 2010. Analyses of the regulatory mechanism and physiological roles of *Pseudomonas aeruginosa* OhrR, a transcription regulator and a sensor of organic hydroperoxides. J Bacteriol 192:2093–2101. https://doi.org/10.1128/JB.01510-09.
82. Previato-Mello M, Meireles DA, Netto LES, da Silva Neto JF. 2017. Global

transcriptional response to organic hydroperoxide and the role of OhrR in the control of virulence traits in *Chromobacterium violaceum*. Infect Immun 85:e00017-17. https://doi.org/10.1128/IAI.00017-17.
83. Sana TG, Lugo KA, Monack DM. 2017. T6SS: the bacterial "fight club" in the host gut. PLoS Pathog 13:e1006325. https://doi.org/10.1371/journal.ppat.1006325.
84. Schwarz S, West TE, Boyer F, Chiang WC, Carl MA, Hood RD, Rohmer L, Tolker-Nielsen T, Skerrett SJ, Mougous JD. 2010. *Burkholderia* type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. PLoS Pathog 6:e1001068. https://doi.org/10.1371/journal.ppat.1001068.
85. Galan B, Kolb A, Sanz JM, Garcia JL, Prieto MA. 2003. Molecular determinants of the *hpa* regulatory system of *Escherichia coli*: the HpaR repressor. Nucleic Acids Res 31:6598–6609. https://doi.org/10.1093/nar/gkg851.
86. Mendez V, Agullo L, Gonzalez M, Seeger M. 2011. The homogentisate and homoprotocatechuate central pathways are involved in 3- and 4-hydroxyphenylacetate degradation by *Burkholderia xenovorans* LB400. PLoS One 6:e17583. https://doi.org/10.1371/journal.pone.0017583.
87. Fitzgerald DJ, Stratford M, Gasson MJ, Ueckert J, Bos A, Narbad A. 2004. Mode of antimicrobial action of vanillin against *Escherichia coli*, *Lactobacillus plantarum* and *Listeria innocua*. J Appl Microbiol 97:104–113. https://doi.org/10.1111/j.1365-2672.2004.02275.x.
88. Lowe TM, Ailloud F, Allen C. 2015. Hydroxycinnamic acid degradation, a broadly conserved trait, protects *Ralstonia solanacearum* from chemical plant defenses and contributes to root colonization and virulence. Mol Plant Microbe Interact 28:286–297. https://doi.org/10.1094/MPMI-09-14-0292-FI.
89. Mitra A, Kitamura Y, Gasson MJ, Narbad A, Parr AJ, Payne J, Rhodes MJ, Sewter C, Walton NJ. 1999. 4-Hydroxycinnamoyl-CoA hydratase/lyase (HCHL)—an enzyme of phenylpropanoid chain cleavage from *Pseudomonas*. Arch Biochem Biophys 365:10–16. https://doi.org/10.1006/abbi.1999.1140.
90. Kasai D, Kamimura N, Tani K, Umeda S, Abe T, Fukuda M, Masai E. 2012. Characterization of FerC, a MarR-type transcriptional regulator, involved in transcriptional regulation of the ferulate catabolic operon in *Sphingobium* sp. strain SYK-6. FEMS Microbiol Lett 332:68–75. https://doi.org/10.1111/j.1574-6968.2012.02576.x.
91. Masuda T, Yamada K, Maekawa T, Takeda Y, Yamaguchi H. 2006. Antioxidant mechanism studies on ferulic acid: identification of oxidative coupling products from methyl ferulate and linoleate. J Agric Food Chem 54:6069–6074. https://doi.org/10.1021/jf060676z.
92. Sass AM, Schmerk C, Agnoli K, Norville PJ, Eberl L, Valvano MA, Mahenthiralingam E. 2013. The unexpected discovery of a novel low-oxygen-activated locus for the anoxic persistence of *Burkholderia cenocepacia*. ISME J 7:1568–1581. https://doi.org/10.1038/ismej.2013.36.
93. Zhang Y, Kiba A, Hikichi Y, Ohnishi K. 2011. *prhKLM* genes of *Ralstonia solanacearum* encode novel activators of *hrp* regulon and are required for pathogenesis in tomato. FEMS Microbiol Lett 317:75–82. https://doi.org/10.1111/j.1574-6968.2011.02213.x.
94. Iyer R, Iverson TM, Accardi A, Miller C. 2002. A biological role for prokaryotic ClC chloride channels. Nature 419:715–718. https://doi.org/10.1038/nature01000.
95. Crutcher FK, Puckhaber LS, Stipanovic RD, Bell AA, Nichols RL, Lawrence KS, Liu J. 2017. Microbial resistance mechanisms to the antibiotic and phytotoxin fusaric acid. J Chem Ecol 43:996–1006. https://doi.org/10.1007/s10886-017-0889-x.
96. Bevivino A, Peggion V, Chiarini L, Tabacchioni S, Cantale C, Dalmastri C. 2005. Effect of *Fusarium verticillioides* on maize-root-associated *Burkholderia cenocepacia* populations. Res Microbiol 156:974–983. https://doi.org/10.1016/j.resmic.2005.05.007.
97. Parsons JF, Calabrese K, Eisenstein E, Ladner JE. 2003. Structure and mechanism of *Pseudomonas aeruginosa* PhzD, an isochorismatase from the phenazine biosynthetic pathway. Biochemistry 42:5684–5693. https://doi.org/10.1021/bi027385d.
98. Utsumi R, Yagi T, Katayama S, Katsuragi K, Tachibana K, Toyoda H, Ouchi S, Obata K, Shibano Y, Noda M. 1991. Molecular cloning and characterization of the fusaric acid-resistance gene from *Pseudomonas cepacia*. Agric Biol Chem 55:1913–1918.
99. Moule MG, Hemsley CM, Seet Q, Guerra-Assuncao JA, Lim J, Sarkar-Tyson M, Clark TG, Tan PB, Titball RW, Cuccui J, Wren BW. 2014. Genome-wide saturation mutagenesis of *Burkholderia pseudomallei* K96243 predicts essential genes and novel targets for antimicrobial development. mBio 5:e00926-13. https://doi.org/10.1128/mBio.00926-13.
100. Trunck LA, Propst KL, Wuthiekanun V, Tuanyok A, Beckstrom-Sternberg SM, Beckstrom-Sternberg JS, Peacock SJ, Keim P, Dow SW, Schweizer HP. 2009. Molecular basis of rare aminoglycoside susceptibility and pathogenesis of *Burkholderia pseudomallei* clinical isolates from Thailand. PLoS Negl Trop Dis 3:e519. https://doi.org/10.1371/journal.pntd.0000519.
101. Goenrich M, Bartoschek S, Hagemeier CH, Griesinger C, Vorholt JA. 2002. A glutathione-dependent formaldehyde-activating enzyme (Gfa) from *Paracoccus denitrificans* detected and purified via two-dimensional proton exchange NMR spectroscopy. J Biol Chem 277:3069–3072. https://doi.org/10.1074/jbc.C100579200.
102. Chen NH, Djoko KY, Veyrier FJ, McEwan AG. 2016. Formaldehyde stress responses in bacterial pathogens. Front Microbiol 7:257. https://doi.org/10.3389/fmicb.2016.00257.
103. Wong YC, Abd El Ghany M, Naeem R, Lee KW, Tan YC, Pain A, Nathan S. 2016. Candidate essential genes in *Burkholderia cenocepacia* J2315 identified by genome-wide TraDIS. Front Microbiol 7:1288. https://doi.org/10.3389/fmicb.2016.01288.
104. Letunic I, Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res 44:W242–W245. https://doi.org/10.1093/nar/gkw290.
105. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539. https://doi.org/10.1038/msb.2011.75.
106. Chen H, Hu J, Chen PR, Lan L, Li Z, Hicks LM, Dinner AR, He C. 2008. The *Pseudomonas aeruginosa* multidrug efflux regulator MexR uses an oxidation-sensing mechanism. Proc Natl Acad Sci U S A 105:13586–13591. https://doi.org/10.1073/pnas.0803391105.
107. Lomovskaya O, Lewis K, Matin A. 1995. EmrR is a negative regulator of the *Escherichia coli* multidrug resistance pump EmrAB. J Bacteriol 177:2328–2334. https://doi.org/10.1128/jb.177.9.2328-2334.1995.
108. Hommais F, Oger-Desfeux C, Van Gijsegem F, Castang S, Ligori S, Expert D, Nasser W, Reverchon S. 2008. PecS is a global regulator of the symptomatic phase in the phytopathogenic bacterium *Erwinia chrysanthemi* 3937. J Bacteriol 190:7508–7522. https://doi.org/10.1128/JB.00553-08.
109. Wilkinson SP, Grove A. 2004. HucR, a novel uric acid-responsive member of the MarR family of transcriptional regulators from *Deinococcus radiodurans*. J Biol Chem 279:51442–51450. https://doi.org/10.1074/jbc.M405586200.
110. Huang H, Grove A. 2013. The transcriptional regulator TamR from *Streptomyces coelicolor* controls a key step in central metabolism during oxidative stress. Mol Microbiol 87:1151–1166. https://doi.org/10.1111/mmi.12156.
111. Cheung AL, Nishina KA, Trotonda MP, Tamber S. 2008. The SarA protein family of *Staphylococcus aureus*. Int J Biochem Cell Biol 40:355–361. https://doi.org/10.1016/j.biocel.2007.10.032.
112. Chen PR, Nishida S, Poor CB, Cheng A, Bae T, Kuechenmeister L, Dunman PM, Missiakas D, He C. 2009. A new oxidative sensing and regulation pathway mediated by the MgrA homologue SarZ in *Staphylococcus aureus*. Mol Microbiol 71:198–211. https://doi.org/10.1111/j.1365-2958.2008.06518.x.
113. Brugarolas P, Movahedzadeh F, Wang Y, Zhang N, Bartek IL, Gao YN, Voskuil MI, Franzblau SG, He C. 2012. The oxidation-sensing regulator (MosR) is a new redox-dependent transcription factor in *Mycobacterium tuberculosis*. J Biol Chem 287:37703–37712. https://doi.org/10.1074/jbc.M112.388611.
114. Dolan KT, Duguid EM, He C. 2011. Crystal structures of SlyA protein, a master virulence regulator of *Salmonella*, in free and DNA-bound states. J Biol Chem 286:22178–22185. https://doi.org/10.1074/jbc.M111.245258.
115. Brier S, Fagnocchi L, Donnarumma D, Scarselli M, Rappuoli R, Nissum M, Delany I, Norais N. 2012. Structural insight into the mechanism of DNA-binding attenuation of the neisserial adhesin repressor NadR by the small natural ligand 4-hydroxyphenylacetic acid. Biochemistry 51:6738–6752. https://doi.org/10.1021/bi300656w.
116. Dalrymple BP, Swadling Y. 1997. Expression of a *Butyrivibrio fibrisolvens* E14 gene (*cinB*) encoding an enzyme with cinnamoyl ester hydrolase activity is negatively regulated by the product of an adjacent gene (*cinR*). Microbiology 143(Part 4):1203–1210. https://doi.org/10.1099/00221287-143-4-1203.
117. Hirakawa H, Hirakawa Y, Greenberg EP, Harwood CS. 2015. BadR and

BadM proteins transcriptionally regulate two operons needed for anaerobic benzoate degradation by *Rhodopseudomonas palustris*. Appl Environ Microbiol 81:4253–4262. https://doi.org/10.1128/AEM.00377-15.
118. Parke D, Ornston LN. 2003. Hydroxycinnamate (hca) catabolic genes from Acinetobacter sp. strain ADP1 are repressed by HcaR and are induced by hydroxycinnamoyl-coenzyme A thioesters. Appl Environ Microbiol 69:5398–5409. https://doi.org/10.1128/AEM.69.9.5398-5409.2003.
119. Chen PR, Bae T, Williams WA, Duguid EM, Rice PA, Schneewind O, He C. 2006. An oxidation-sensing mechanism is used by the global regulator MgrA in *Staphylococcus aureus*. Nat Chem Biol 2:591–595. https://doi.org/10.1038/nchembio820.

Ashish Gupta received his Ph.D. degree in Biochemistry from Louisiana State University, Baton Rouge, LA. Working in Anne Grove's laboratory, his Ph.D. was mainly focused on understanding the role of *B. thailandensis*-encoded MarR homologs in response to host-derived signals. He characterized two *B. thailandensis* MarR proteins (MftR and BifR). He continued in the same laboratory as a postdoc to study genome-wide changes in gene expression elicited by the host-derived signal urate. During this the time, he developed an interest in vaccine development and related fields. Keeping his career path in mind, he joined the NIAID, NIH, for a postdoc. After a short postdoctoral period at NIH, he moved to Novavax, Inc., as Scientist, Process Development (PD). Now as a member of the downstream group (DSP), he is providing analytical support to all PD groups at Novavax.

Anuja Pande received her master's in Biological Sciences in 2011 from the School of Science, NMIMS University (India). There she developed an antioxidative herbal sunscreen, made using curry leaf extract, against oxidative stress induced upon exposure to UVB radiation in mice. She pursued her enthusiasm for research further by completing another master's degree followed by a Ph.D. in Biochemistry at Louisiana State University (LSU) in 2018, under the guidance of Anne Grove. During her graduate tenure, she studied the protective role of the *B. thailandensis* MarR family protein OhrR (organic hydroxide peroxide reductase regulator) against host-derived oxidative species (especially during infection). It primarily involved *in vitro* and *in vivo* characterization of OhrR in response to organic and inorganic oxidants (some commonly found in disinfectants). Currently, as a postdoctoral fellow in Biological Sciences, LSU, she is continuing her work on other MarR proteins, emphasizing those involved in virulence.

Afsana Sabrin received her M.S. in Biochemistry and Molecular Biology from the University of Dhaka, Bangladesh. Her M.S. thesis research focused on the effect of *in utero* arsenic exposure on the gene expression profile in placenta and cord blood and on the child thymus index. She is currently doing her Ph.D. (2015 to present) in Biological Sciences at Louisiana State University. Her projects involve expression, purification, and characterization of transcriptional regulators belonging to the MarR family. She is currently characterizing the response of one of the 12 *B. thailandensis* MarR proteins to oxidants and metals. This regulator controls two important transport systems, RND and EmrB, which are involved in drug resistance, one of the burning issues in science. She has expertise in expression and purification of proteins, biophysical and biochemical characterization of proteins, gene expression analysis, and analytical techniques using *in vitro* and *in vivo* methods. She is expected to graduate by Spring 2019.

Sudarshan S. Thapa received his bachelor's degree in Biological Sciences and is currently enrolled in the Ph.D. program, both at Louisiana State University. He joined the Grove laboratory in 2016 and has been working with the *B. thailandensis* MarR homolog MftR. MftR serves as an environmental sensor and can bring about differential expression of genes to counteract the stress that may be induced in a host environment. He contributed to research to establish MftR as a global regulator. Deciphering the mechanism by which MftR regulates various genes is his current objective.

Brennan W. Gioe is a third-year undergraduate student, pursuing a B.S. in Biological Sciences from Louisiana State University. He joined the Grove laboratory as soon as he enrolled at LSU as a participant in the President's Future Leaders in Research Program. He has been participating in the characterization of an oxidant-sensing MarR family protein, including identification of oxidant-sensing cysteine residues.

Anne Grove earned her Ph.D. in Molecular Biology from the University of Copenhagen, Denmark. She completed postdoctoral research at University of California, San Diego, with Peter Geiduschek, which is where her interest in gene regulation was sparked. She then accepted a faculty position at Louisiana State University, Baton Rouge, LA, where she is currently the Ouachita Parish Chapter Alumni Professor. Her research primarily concerns the changes in gene expression that occur when cells are exposed to stress. Main focus areas include the mechanisms by which bacterial pathogens subvert host defenses by utilizing host-derived signals to trigger upregulation of virulence genes.