AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology®

RESEARCH ARTICLE

PA5339, a RidA Homolog, Is Required for Full Growth in *Pseudomonas aeruginosa*

Jessica Irons,[a] Kelsey M. Hodge-Hanson,[a] Diana M. Downs[a]*

[a]Department of Microbiology, University of Georgia, Athens, Georgia, USA

ABSTRACT The Rid protein superfamily (YjgF/YER057c/UK114) is found in all domains of life. The archetypal protein, RidA from *Salmonella enterica*, is a deaminase that quenches the reactive metabolite 2-aminoacrylate (2AA). 2AA deaminase activity is conserved in RidA proteins from humans, plants, yeast, archaea, and bacteria. Mutants of *Salmonella enterica*, *Escherichia coli*, and *Saccharomyces cerevisiae* that lack a functional RidA exhibit growth defects, suggesting that 2AA metabolic stress is similarly conserved. The PubSEED database shows *Pseudomonas aeruginosa* (PAO1) encodes eight members of the Rid superfamily. Mutants of *P. aeruginosa* PAO1 lacking each of five Rid proteins were screened, and the mutant phenotypes that arose in the absence of PA5339 were dissected. A PA5339::Tn mutant has growth, motility, and biofilm defects that can all be linked to the accumulation of 2AA. Further, the PA5339 protein was demonstrably a 2AA deaminase *in vitro* and restored metabolic balance to a *S. enterica ridA* mutant *in vivo*. The data presented here show that the RidA paradigm in *Pseudomonas aeruginosa* had similarities to those described in other organisms but was distinct in that deleting only one of multiple homologs generated deficiencies. Based on the collective data presented here in, PA5339 was renamed RidA.

IMPORTANCE RidA is a widely conserved protein that prevents endogenous metabolic stress caused by 2-aminoacrylate (2AA) damage to pyridoxal 5′-phosphate (PLP)-dependent enzymes in prokaryotes and eukaryotes. The framework for understanding the accumulation of 2AA and its consequences have largely been defined in *Salmonella enterica*. We show here that in *P. aeruginosa* (PAO1), 2AA accumulation leads to reduced growth, compromised motility, and defective biofilm formation. This study expands our knowledge how the metabolic architecture of an organism contributes to the consequences of 2AA inactivation of PLP-dependent enzymes and identifies a key RidA protein in *P. aeruginosa*.

KEYWORDS RidA, PA5339, motility, biofilm, 2-aminoacrylate

Members of the of the Rid (YjgF/YER057c/UK114) protein superfamily are found in all domains of life, and prokaryotic genomes often encode multiple members. The superfamily has been divided into eight subfamilies based on bioinformatic and phylogenetic analysis (1), but it is unclear whether these divisions reflect biochemical differences (2). RidA, reactive intermediate deaminase A, the archetypal protein of the family, has been primarily studied in *Salmonella enterica* for its role in quenching the reactive metabolite 2-aminoacrylate (2AA), a catalytic intermediate in a number of pyridoxal 5′-phosphate (PLP)-dependent reactions (Fig. 1) (3–5). RidA 2AA deaminase activity requires an active site arginine reside (Arg105 in *S. enterica*), and no other residues have been found to be essential for this activity (3). To date, all RidA proteins tested with an active site Arg residue, including human, plant, and archaeal homologs, have demonstrated 2AA deaminase activity *in vivo* and *in vitro* (6, 7). Some members of

Received 18 July 2018 **Accepted** 27 August 2018

Accepted manuscript posted online 4 September 2018

Citation Irons J, Hodge-Hanson KM, Downs DM. 2018. PA5339, a RidA homolog, is required for full growth in *Pseudomonas aeruginosa*. J Bacteriol 200:e00434-18. https://doi.org/10.1128/JB.00434-18.

Editor George O'Toole, Geisel School of Medicine at Dartmouth

Address correspondence to Diana M. Downs, dmdowns@uga.edu.

* Present address: Diana M. Downs, Department of Microbiology and Immunology, Loyola University Chicago, Maywood, Illinois, USA.

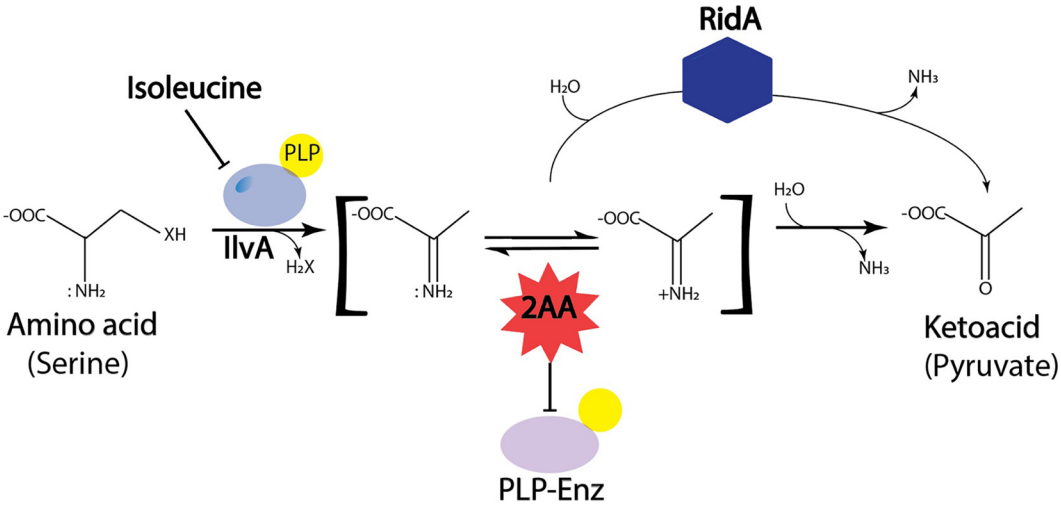


FIG 1 RidA paradigm in *S. enterica*. The framework for 2AA metabolism and its consequences as defined in *S. enterica* are depicted. 2-Aminoacrylate (2AA) is generated from serine by PLP-dependent serine/threonine dehydratases (IlvA or TdcB) and released into the cellular milieu. 2AA is susceptible to spontaneous hydrolysis by H_2O, but in the absence of RidA it persists long enough *in vivo* to damage PLP-dependent target enzymes. The addition of isoleucine allosterically inhibits IlvA, decreasing the formation of 2AA by IlvA and preventing damage to metabolic target enzymes. Exogenous threonine can increase the synthesis of isoleucine which can then inhibit IlvA, reducing the accumulation of 2AA.

Rid subfamilies 1 to 3 also have 2AA deaminase activity *in vivo* and *in vitro*, suggesting at least a partially conserved function of members of the superfamily (2).

Many prokaryotes encode a RidA homolog(s) in addition to one or more representatives from other Rid subgroups (Rid1 to Rid7). The presence of multiple Rid proteins in a prokaryotic genome suggests these proteins have distinct roles *in vivo* and could use diverse substrates important to the metabolism of the relevant organism. Efforts in *S. enterica* have laid the groundwork to dissect both the function of different Rid proteins and the role of 2AA on diverse metabolic networks. *S. enterica* encodes one RidA protein and two additional Rid proteins that do not quench 2AA (2). YoaB, a Rid2 subfamily member, hydrolyzes imines produced from FAD-dependent enzymes *in vitro*, but neither its role *in vivo* nor its true substrate is known (1). The third Rid protein, STM1549, lacks an active site Arg residue and does not deaminate imines. *E. coli* K-12 encodes three Rid proteins, including two RidA homologs that have 2AA deaminase activity: RidA and TdcF (8). Assessing the RidA paradigm in numerous organisms highlighted conserved features and uncovered distinct properties that reflect the consequence of specific metabolic architectures. A *ridA* mutation in *S. enterica* causes a number of growth defects, while growth is unaffected in an *E. coli* double mutant (*ridA tdcF*) unless 2AA accumulation is artificially increased (8). In yeast, inactivating the mitochondrial RidA homolog (Mmf1) results in significant growth and biochemical defects, while the loss of the cytoplasmic homolog (Hmf1) fails to have detectable consequences (9–11).

In *S. enterica*, the PLP-dependent enzymes threonine/serine dehydratase (IlvA; EC 4.3.1.19), cysteine desulfhydrase (CdsH; EC 4.4.1.15), and diaminopropionate ammonia-lyase (DapL; EC 4.3.1.15) generate 2AA from serine, cysteine, and diaminopropionate, respectively. Once released, free 2AA may (i) covalently inactivate specific PLP enzymes, potentially leading to growth defects, (ii) be spontaneously quenched by solvent water, or (iii) be deaminated by RidA as depicted in Fig. 1 (3, 4, 6, 8, 12–17). In the absence of RidA *in vivo*, 2AA can inactivate PLP-dependent enzymes, including serine hydroxymethyltransferase (GlyA; EC 2.1.2.1), alanine racemases (Alr/DadX; EC 5.1.1.1), and transaminase B (IlvE; EC 2.6.1.42) (3, 6, 12, 14, 15). Transcriptomic analysis showed global changes in a *ridA* mutant of *S. enterica* and correctly predicted a motility defect, though the specific enzymatic target responsible for this effect was not found (18).

The biosynthetic threonine/serine dehydratase (IlvA) was the 2AA source responsi-

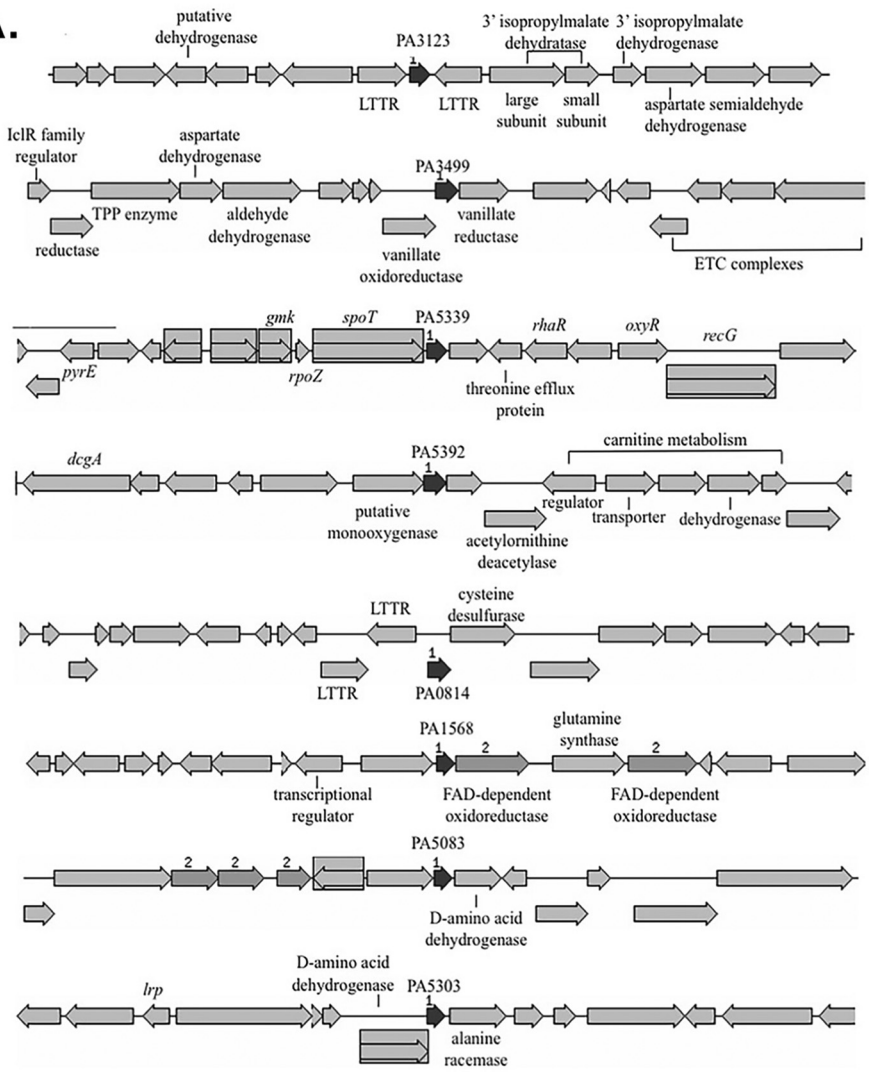
ble for the growth defects in multiple organisms that lacked the relevant RidA homolog, including bacteria (*S. enterica* and *E. coli*), yeasts (9, 11, 15, 19), and plants (7, 20). As such, these defects were corrected by the addition of isoleucine, which allosterically inhibits IlvA to prevent 2AA production. Similarly, exogenous threonine prevents 2AA accumulation, likely by increasing flux to isoleucine, which leads to allosteric inhibition of IlvA. In *S. enterica*, the growth defect caused by 2AA in a *ridA* mutant was reversed by exogenous glycine, which bypassed damage to the serine hydroxymethyl transferase (GlyA) (14, 21). In contrast, in an *Escherichia coli ridA tdcF* double mutant, the 2AA-dependent growth defect was reversed by exogenous aspartate or purines but not glycine. Thus, while the mutant phenotypes that arise without RidA are expected to stem from 2AA inhibition of a PLP-dependent enzyme(s), the global metabolic consequences (i.e., growth phenotype) vary due to the unique metabolic architecture of each organism.

Pseudomonas aeruginosa PAO1 encodes eight bioinformatically identified Rid proteins including, four RidA, one Rid1, and three Rid2 proteins (1, 22). The multiplicity of Rid proteins in *P. aeruginosa* suggests the proteins have distinct roles to adapt to changing conditions and/or act on substrates other than 2AA. For example, the Rid2 proteins (PA0814 and PA5083) increase the rate of FAD-dependent reactions *in vitro* (2). In addition, PA5083 or *dguB* is in the *dguABC* operon and is positively regulated by exogenous D-Glu; however, its role in D-Glu utilization has not been demonstrated (23). Proteomic analyses linked RidA protein (PA5339) to early-stage biofilm formation, while transcriptome analyses implicated RidA (PA3123) in biofilm formation and swarming and showed that Rid2 (PA5303) was induced in response to agmatine and putrescine (24–28). This study was initiated to probe Rid protein function and investigate the impact of 2AA on the *P. aeruginosa* metabolic network.

RESULTS AND DISCUSSION

***Pseudomonas aeruginosa* mutants lacking PA5339 have a growth defect.** There are eight open reading frames (ORFs) identified by the PubSEED database that encode Rid proteins in *P. aeruginosa* PAO1 (Fig. 2). Two of the ORF products defined as RidA proteins (PA3499 and PA5392) do not have an active site Arg, making it unlikely that they catalyze a deaminase reaction, and these proteins were not included in this study. Five mutants of *P. aeruginosa*, each carrying an insertion in a single gene encoding a Rid protein, were obtained from the transposon mutant collection of the Manoil Laboratory (University of Washington). A mutant lacking PA5083, a Rid2, was not available. The correct insertion location for each mutant was validated by PCR using primers flanking the gene of interest. Initially, the mutants relevant to this study were screened for growth on a variety of media, both liquid and solid, including no-carbon E salt (NCE) minimal medium with glucose or glycerol as the sole carbon source and morpholinepropanesulfonic acid (MOPS) minimal medium with glucose or succinate as the sole carbon source. On each of the media tested, the growth of mutants with insertions in PA0814, PA1568, PA3123, or PA5303 was not detectably different from the parental strain (see Fig. S1 in the supplemental material). In contrast, the mutant with an insertion disrupting PA5339 had a significant growth defect compared to wild-type strain PAO1, in each minimal medium, and NCE was used in further liquid growth analyses (Fig. 3A). The growth defect was more severe on solid medium than in liquid, though the reason for this was not pursued. The growth defect caused by inactivating PA5339 distinguished *P. aeruginosa* from *E. coli* and *S. enterica ridA* mutants in three ways. First, although disrupting *ridA* in *S. enterica* causes detectable biochemical effects, it has no significant effect on growth on minimal medium (12, 13, 15, 19). Second, the PA5339::Tn mutant is more sensitive to exogenous serine than a *S. enterica ridA* mutant (Fig. S2). Third, in *E. coli*, both of the RidA homologs present had to be removed before any growth defect was detected (8). The growth defect of *P. aeruginosa* mutants lacking only PA5339 showed that none of the other Rid homologs were functionally redundant under the conditions tested and, as such, PA5339 was renamed *ridA* ($_{PA}$*ridA* here). This

A.



B.

Locus Tag	Assigned Subfamily	Conserved Arg-105	2AA Deaminase Activity	
			in vivo	*in vitro*
PA3123	RidA	Yes	Yes (Figure 4)	No data
PA3499	RidA	No	Not expected	Not expected
PA5339	RidA	Yes	Yes (Figure 4)	Yes (Figure 5)
PA5392	RidA	No	Not expected	Not expected
PA0814	Rid1	Yes	Yes (Figure 4)	Yes (2)
PA1568	Rid2	Yes	No	No data
PA5083	Rid2	Yes	Yes (Figure 4)	Yes (2)
PA5303	Rid2	Yes	No	No data

FIG 2 Genetic organization of Rid superfamily members in *P. aeruginosa*. *P. aeruginosa* strain PAO1 encodes eight Rid subfamily members. (A) In this schematic, taken from the PubSEED website (22), the Rid subfamily member is designated in black among neighboring genes in gray. (B) Characteristics of each Rid family member are listed using data from the listed source or the present study. *In vitro* 2AA deaminase activity indicates activity assays with purified protein. *In vivo* 2AA deaminase activity indicates that the relevant protein complements the defects caused by 2AA accumulation in a *ridA* mutant of *S. enterica*. "No data" indicates that this activity was not tested. (2), reference 2.

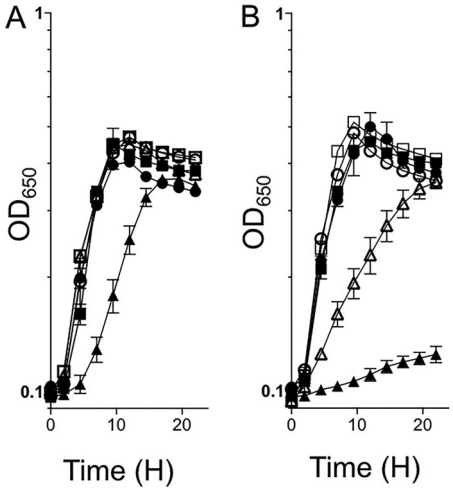


FIG 3 A $_{PA}$*ridA*::Tn mutant is sensitive to exogenous serine. (A) Growth is shown for DM15943, $_{PA}$*ridA*::Tn/pEmpty vector (triangles), DM15944, $_{PA}$*ridA*::Tn/p$_{PA}$*ridA* (squares), and PAO1 (circles) strains. Minimal medium with 11 mM glucose (closed symbols) and medium including 1 mM isoleucine (open symbols) were used. (B) Growth is shown for DM15943, $_{PA}$*ridA*::Tn/pEmpty vector (triangles), DM15944, $_{PA}$*ridA*::Tn/p$_{PA}$*ridA* (squares) and PAO1 (circles) strains. Growth shown was in minimal medium with 11 mM glucose and 5 mM serine (closed symbols) and in medium with serine and 1 mM isoleucine (open symbols).

result could reflect distinct functional roles and/or differential regulation of the additional Rid subfamily members.

Nutritional requirements suggest conservation of Rid paradigm. Characterization of the general growth defect of PW9994 ($_{PA}$*ridA*::Tn) was guided by our understanding of the RidA paradigm in *S. enterica*. Individual vitamins and amino acids were screened for growth stimulation on solid medium. In total, these screens showed that isoleucine, threonine, and (to a lesser extent) glycine, proline, and phenylalanine stimulated the growth of the $_{PA}$*ridA*::Tn mutant, whereas serine and cysteine were inhibitory. Growth of the wild-type PAO1 strain was not affected by the addition of these nutrients. For this reason, DM15943, $_{PA}$*ridA*::Tn/empty vector control, and DM15944 $_{PA}$*ridA*::Tn/pMQ72-$_{PA}$*ridA*, a complemented mutant, were used as the isogenic pair for comparison throughout this study. The individual compounds that impacted the $_{PA}$*ridA*::Tn mutant growth most significantly were assessed in liquid growth curves. In minimal glucose medium (NCE), supplementation with isoleucine improved growth to wild-type levels (Fig. 3A), threonine had a similar effect, and glycine had little effect (Fig. S3). Isoleucine and threonine minimized the impact of a *ridA* mutation in *S. enterica* by allosterically inhibiting IlvA to prevent 2AA synthesis (12, 13, 15). Similar to results for a *S. enterica ridA* mutant, serine (a precursor to 2AA) eliminated the growth of the *P. aeruginosa* mutant lacking PA5339. The addition of isoleucine improved but did not fully restore growth of the *P. aeruginosa ridA* mutant to the level of the complemented $_{PA}$*ridA*::Tn mutant in the presence of serine (Fig. 3B). In total, the behavior of the *P. aeruginosa* $_{PA}$*ridA*::Tn mutant could be explained in the framework of the RidA paradigm if 2AA generated from endogenous (on minimal medium) or exogenous serine damaged a cellular enzyme(s) and caused the growth defects observed. The restoration of growth by isoleucine suggests that at least part of the 2AA was generated by serine/threonine dehydratase (IlvA) activity. *P. aeruginosa* encodes two IlvA proteins that each have both the catalytic and regulatory domains of the *S. enterica* protein, based on protein sequence comparison. Eliminating either one of these genes, *ilvA1* or *ilvA2* (PA0331 and PA1326), alone does not result in an isoleucine requirement, suggesting that they are isozymes with redundant function in isoleucine biosynthesis. The failure of isoleucine to completely reverse the growth defect of the $_{PA}$*ridA*::Tn mutant on serine suggests that (i) the serine/threonine dehydratase(s) is not completely inhibited by isoleucine, (ii) more 2AA is produced by the IlvAs of *P. aeruginosa* than IlvA in *S. enterica*, or (iii) there are other significant sources of 2AA in this organism.

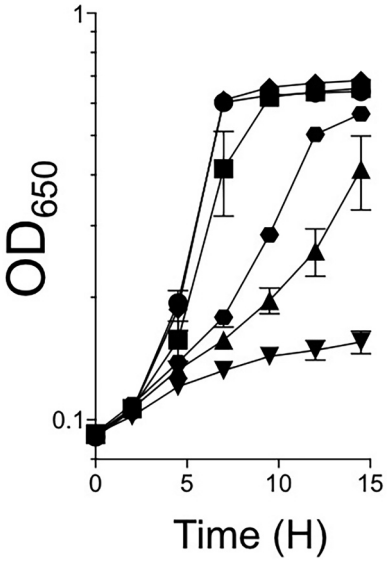


FIG 4 Rid homologs from *P. aeruginosa* complement an *S. enterica ridA* mutant. *S. enterica* ridA mutants carrying pBAD24 plasmids expressing the relevant genes were grown in minimal medium with glucose (11 mM), serine (5 mM), and arabinose (0.2%). Strain DM14846 (circles) expressed *S. enterica ridA*, DM16214 (diamonds) expressed $_{PA}$*ridA*, DM15406 (squares) expressed PA0814, DM15687 (triangles) expressed PA5083, and DM16216 (hexagons) expressed PA3123. Control DM14847 (inverted triangles) carried an empty vector. DM14846 and DM16214 also grew in the absence of arabinose. The error bars represent standard errors of the mean from three biological replicates.

Only PA5339 ($_{PA}$RidA) deaminated 2AA *in vivo* in the *P. aeruginosa* $_{PA}$*ridA*::Tn mutant. One explanation for the $_{PA}$*ridA*::Tn mutant growth defect despite encoding multiple Rid proteins is that the homologs might not be expressed the under conditions tested. This explanation was not supported by complementation analyses in *P. aeruginosa*. The $_{PA}$*ridA*::Tn mutant was transformed with pMQ72 constructs harboring $_{PA}$*ridA*(pDM1533), PA3123/RidA (pDM1566), PA5083/Rid2 (pDM1534), or *S. enterica* RidA (pDM1568), under the control of an arabinose promoter or a no insert control (pMQ72) (29). The growth of the resulting mutants was monitored in minimal medium with 5 mM serine. Plasmids expressing either $_{PA}$*ridA* or $_{Se}$*ridA* fully restored growth to the level of the wild-type PAO1 in the $_{PA}$*ridA*::Tn mutant without inducing expression by arabinose, and no other plasmid consistently improved growth (Fig. S4).

The inability of PA5083, a Rid2, to complement the $_{PA}$*ridA*::Tn mutant was unexpected since this protein had previously been found to deaminate 2AA *in vitro* and complemented a *S. enterica ridA* mutant (2). A *S. enterica ridA* mutant (DM12920) was transformed with pBAD24 vectors encoding each of four Rid proteins from *P. aeruginosa*. The resulting mutants, along with controls DM14846 (pBAD24-$_{SE}$*ridA*) and DM14847 (pBAD24-empty vector), were assessed for the ability to grow in minimal glucose medium with 5 mM serine. pDM1559 (pBAD24-$_{PA}$*ridA*) and pDM1439 (pBAD-$_{SE}$*ridA*) fully restored growth to the *ridA* mutant with or without added arabinose. The remaining *ridA* and *rid1,2*, pDM1561 (pBAD24-PA3123), pDM1464 (pBAD-PA0814), and pDM1534 (pBAD-PA5083), restored the growth of the *S. enterica ridA* mutant to various degrees only when gene expression was induced by arabinose (Fig. 4). In contrast, pDM1580 (pBAD24-PA5303) and pDM1587 (pBAD24-PA1568) failed to restore growth to an *S. enterica ridA* mutant (data not shown). The *S. enterica* complementation data indicated that in addition to PA5083, $_{PA}$RidA, PA3123, and PA0814 had 2AA deaminase activity *in vivo*, while PA5303 and PA1568 did not. Although 2AA deaminases from each domain of life can be substituted for RidA *in vivo*, a survey of *Pseudomonas* Rid1, Rid2, and Rid3 proteins (from *P. aeruginosa*, *P. fluorescens*, or *P. syringae*) found that all five of the tested proteins were active deaminases *in vitro*, yet only three were active *in vivo* (2). As with the other *Pseudomonas* Rid proteins, the inability of PA5303 and PA1568 to

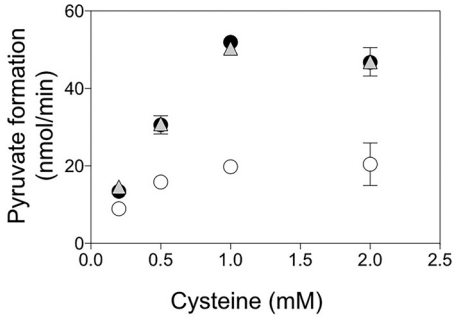


FIG 5 $_{PA}$RidA deaminates 2AA *in vitro*. *S. enterica* cysteine desulfhydrase (CdsH) was purified and used to generate 2AA from cysteine *in situ*. As previously described, coupled pyruvate formation and NADH oxidation are measured with or without the addition of a Rid (either $_{SE}$RidA or $_{PA}$RidA) protein (4). NADH oxidation was used as a measurement of pyruvate formation. The reaction rate was used to determine whether $_{PA}$RidA deaminated 2AA *in vitro*, thus increasing the rate of the reaction. Assay mixtures (100 μl) contained Tris-HCl (100 mM, pH 8), NADH (250 μM), PLP (30 μM), pyruvate kinase/lactate dehydrogenase (5 U), and purified CdsH (0.27 μM). Reaction mixtures contained $_{SE}$RidA (closed circles) or $_{PA}$RidA (triangles) at 0.19 μM or an equal volume buffer as a control (open circles). The reactions were initiated with L-cysteine addition to the indicated final concentrations, and the reaction was monitored by determining the absorbance at 340 nm for 2 min. The initial rate of pyruvate formation was calculated from the rate of NADH oxidation in the first 30 s, along with the molar extinction coefficient (ε = 6,200 M^{-1} cm^{-1}). Experiments were performed in triplicate, and the mean was plotted with error bars representing the standard deviations.

restore metabolic balance to a *S. enterica ridA* mutant might indicate protein stability or folding issues, which could account for the lack of complementation. It is also possible that PA5303 and PA1568 serve alternative roles in *P. aeruginosa* and do not substantially deaminate 2AA *in vivo*. Based on the metabolic defect generated solely by a insertion in $_{PA}$*ridA*, additional work focused on this protein and phenotypes of a $_{PA}$*ridA*::Tn mutant.

***P. aeruginosa* $_{PA}$RidA deaminates 2AA *in vitro*.** $_{PA}$RidA was purified and tested in an activity assay designed for $_{SE}$RidA, using cysteine desulfhydrase (CdsH) from *S. enterica* (4). The short half-life of 2AA requires it to be generated *in situ*, as the substrate for an enamine deaminase, in this case $_{PA}$RidA or $_{SE}$RidA. CdsH converts cysteine to 2AA, which is then deaminated to form pyruvate. The rate of pyruvate formation is a combination of deamination by solvent water and a RidA protein (if present). Inclusion of $_{PA}$RidA in reaction mixtures, with multiple concentrations of cysteine (0.2 to 2 mM), increased the rate of pyruvate formation (Fig. 5). These data showed that $_{PA}$RidA has 2AA deaminase activity *in vitro*, which is consistent with the ability of this protein to complement a *S. enterica ridA* mutant.

2AA accumulates and damages IlvE in a mutant lacking $_{PA}$*ridA*. The activity of the branched-chain amino acid aminotransaminase (transaminase B, IlvE EC 2.6.1.42) has been used as a proxy for *in vivo* 2AA damage both in *S. enterica* (6, 12, 13) and in yeast (9). IlvE carries out the final step in the biosynthesis of isoleucine, leucine, and valine. The activity of IlvE decreases when RidA is absent, due to the accumulation of 2AA, which covalently damages this (and other) PLP-dependent enzymes *in vivo* (15). The *P. aeruginosa* genome encodes a single IlvE candidate, PA5013, with 48% identity to *S. enterica* IlvE. IlvE aminotransferase activity was assessed in mutants DM15943 ($_{PA}$*ridA*::Tn pMQ72-VOC) and DM15944 ($_{PA}$*ridA*::Tn pMQ72-$_{PA}$*ridA*) after growth in minimal glucose medium, or with the addition of isoleucine or threonine as indicated. The data in Fig. 6 show that an insertion in PA5339 (with empty vector) decreased IlvE activity ~2-fold compared to the mutant expressing $_{PA}$*ridA* in *trans*. In contrast, if isoleucine or threonine was added to the growth medium, there was no significant difference in IlvE activity between the two mutants. These data suggest 2AA accumulates in a $_{PA}$*ridA*::Tn mutant and damages PLP-dependent enzymes. Further, the data are consistent with the *S. enterica* model, where the source of 2AA is a serine/threonine dehydratase, IlvA, that is regulated by addition of isoleucine (or indirectly by increasing

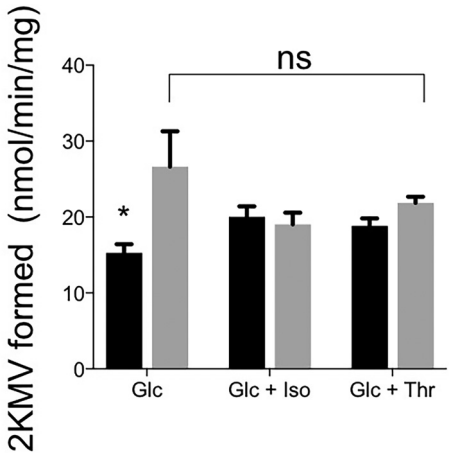


FIG 6 IlvE activity is decreased in a $_{PA}$*ridA*::Tn mutant. A $_{PA}$*ridA*::Tn mutant with pMQ72-empty vector (black) or pMQ72-$_{PA}$ridA (gray) was grown to full density in minimal glucose medium alone (Glc), with isoleucine (Glc + Iso), or with threonine (Glc + Thr). The transaminase B activity was assayed as described in the text. The error bars represent standard errors of the mean from three biological replicates, and the asterisk denotes statistically significant ($P < 0.05$) variation between strains.

threonine). Thus, 2AA accumulation is largely prevented in the uncomplemented $_{PA}$*ridA*::Tn mutant by the addition of exogenous isoleucine or threonine.

2AA accumulation affects motility in a $_{PA}$*ridA*::Tn mutant. *S. enterica ridA* mutants have reduced motility (18), and mutants of *Campylobacter jejuni* (strain 11168) defective in a RidA homolog (Cje1388) are also impaired in motility and autoagglutination (30). The swimming motility of $_{PA}$*ridA*::Tn mutants DM15943 and DM15944 was assessed on minimal NCE and M9 glucose medium with 0.3% agar. The data showed that DM15943, a $_{PA}$*ridA*::Tn mutant carrying an empty vector, had a 3-fold decrease in swimming motility compared to DM15944, a $_{PA}$*ridA*::Tn mutant carrying the $_{PA}$*ridA* complementation vector, on either medium (Fig. 7). The motility of the PAO1 parent strain did not differ from DM15944 in any of the nutrient conditions tested (Fig. S4). Critically, motility was restored by the addition of isoleucine to the medium, supporting the conclusion that reduced motility was due to an accumulation of 2AA. Unlike the relevant mutants in *S. enterica* and *C. jejuni*, the $_{PA}$*ridA*::Tn mutant of *P. aeruginosa* has a slight growth defect on minimal medium that prevents solid conclusions about the extent of the defect in motility caused by the lack of the RidA protein.

2AA accumulation leads to a defect in biofilm formation. A proteomics study found levels of PA5339 ($_{PA}$RidA) to be 5-fold higher at the onset of biofilm formation

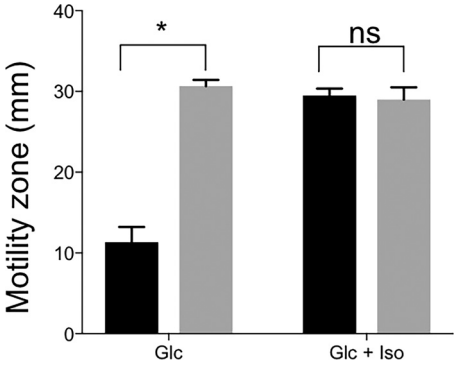


FIG 7 A $_{PA}$*ridA*::Tn mutant is defective in swimming motility. DM15943, a $_{PA}$*ridA*::Tn mutant with pMQ72-empty vector (black), and DM15944, a $_{PA}$*ridA*::Tn mutant with pMQ72-$_{PA}$*ridA* (gray), were inoculated onto minimal motility plates (0.3% agar) with glucose alone (Glc) or in the presence of 1 mM isoleucine (Glc + Iso). The error bars represent standard errors of the mean from three biological replicates, and an asterisk denotes statistically significant ($P < 0.00009$) variation between strains.

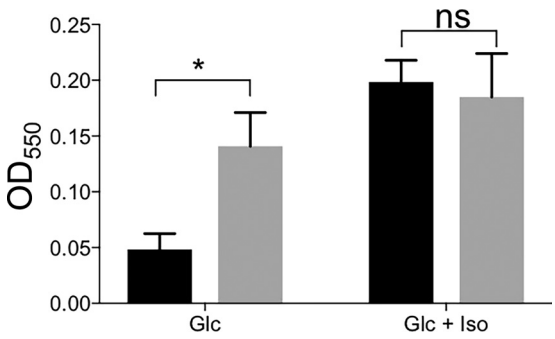


FIG 8 A $_{PA}$*ridA*::Tn mutant is defective in biofilm formation. DM15943, a $_{PA}$*ridA*::Tn mutant with pMQ72-empty vector (black), and DM15944, a $_{PA}$*ridA*::Tn mutant with pMQ72-$_{PA}$*ridA* (gray), were grown in minimal M63 medium with 11 mM glucose as the sole carbon source (Glc) or with 11 mM glucose plus 1 mM isoleucine (Glc + Iso). The error bars represent standard errors of the mean from three biological replicates, and an asterisk denotes statistically significant ($P < 0.009$) variation between strains, as determined by an unpaired Student *t* test performed with GraphPad Prism software, v7.0C.

(i.e., during attachment) (25). Consistent with this result, a $_{PA}$*ridA*::Tn mutation had an effect on biofilm formation. The data show DM15943 had a 3-fold decrease in biofilm formation compared to DM15944 when mutants were grown in minimal M63 medium with 11 mM glucose as the sole carbon source (Fig. 8). While M63 medium was used for consistency with the literature in the field, the stimulatory effect of exogenous isoleucine (1 mM), emphasized the conclusion that the defect was due to 2AA accumulation. As with the motility defects above, it is difficult to distinguish the reduction in biofilm formation in a $_{PA}$*ridA*::Tn mutant from the minor growth defect in this mutant.

Conclusions. The *P. aeruginosa* genome encodes eight Rid superfamily proteins, four of which have demonstrable 2AA deaminase activity *in vitro* and/or *in vivo*. Of the five mutants tested, each lacking one of the Rid homologs, only the mutant without $_{PA}$*ridA* (PW9994) had detectable mutant phenotypes in this study. The $_{PA}$*ridA*::Tn mutant displayed a nutritional phenotype, a biochemical defect, and was impaired in motility and biofilm formation. Each of these phenotypes were attributed to 2AA accumulation based on the exacerbation by serine and/or elimination by isoleucine. The growth defect present in the $_{PA}$*ridA*::Tn mutant complicates any conclusions about a defect in motility or biofilm formation. To confirm that 2AA accumulation causes a defect in either of these processes, like it does in at least *S. enterica* and *C. jejuni*, will require identification of a supplement that restores growth by overcoming the deleterious consequences of 2AA rather than eliminating its production.

In *S. enterica*, 2AA is produced *in vivo* by fold-type II PLP-dependent enzymes, including serine/threonine dehydratase IlvA (EC 4.3.1.19) (3, 6, 16). *P. aeruginosa* carries two IlvA homologs encoded by genes *ilvA1* and *ilvA2* (PA0331 and PA1326). The presence of two IlvA homologues may account for the $_{PA}$*ridA*::Tn mutant increased sensitivity to serine compared to *S. enterica* or *E. coli ridA* mutants (Fig. S2). Both proteins in *P. aeruginosa* PAO1 have an allosteric regulatory domain, consistent with the addition of isoleucine preventing 2AA generation and minimizing the phenotypic defects caused by the lack of $_{PA}$*ridA* (31).

Initial studies suggest *P. aeruginosa* differs from *S. enterica* in the 2AA paradigm. Isoleucine biosynthesis appears to be the major 2AA source in both organisms, but the data suggest there may be another significant source(s) in *P. aeruginosa*. While glycine restores growth to a *ridA* mutant in *S. enterica*, it has little effect on growth in the $_{PA}$*ridA*::Tn mutant when grown in minimal medium without serine (Fig. S4). These data suggest the critical enzyme compromised in *P. aeruginosa* is not always serine hydroxymethyl transferase (GlyA), as it appears to be in *S. enterica*. These findings showed that the *P. aeruginosa* system provides an opportunity to define a third PLP-dependent enzyme critical for growth. Despite the differences in 2AA stress between *S. enterica*, *E. coli*, and *P. aeruginosa*, these three organisms (and *C. jejuni*) appear to share a defect in

TABLE 1 Strains and plasmids

Strain	Genotype	Plasmid	Notes
S. enterica			
DM12920	*ridA1*::Tn*10d* (Tc)	None	
DM14846	*ridA1*::Tn*10d* (Tc)	pDM1439	pBAD24-*S. enterica-ridA*
DM14847	*ridA1*::Tn*10d* (Tc)	pBAD24	pBAD24-empty vector
DM15406	*ridA1*::Tn*10d* (Tc)	pDM1464	pBAD24-PA0814
DM15687	*ridA1*::Tn*10d* (Tc)	pDM1515	pBAD24-PA5083
DM16214	*ridA1*::Tn*10d* (Tc)	pDM1559	pBAD24-PA5339
DM16216	*ridA1*::Tn*10d* (Tc)	pDM1561	pBAD24-PA3123
DM16390	*ridA1*::Tn*10d* (Tc)	pDM1580	pBAD24-PA5303
DM16471	*ridA1*::Tn*10d* (Tc)	pDM1587	pBAD24-PA1568
P. aeruginosa			
PW9994	PA5339::Tn	None	$_{PA}$*ridA*::Tn
PW1611	PA0331::Tn	None	*ilvA1*::Tn
PW3408	PA1326::Tn	None	*ilvA2*::Tn
PW6252	PA3123::Tn	None	RidA
PW2480	PA0814::Tn	None	Rid1
PW3822	PA1568::Tn	None	Rid2
PW9932	PA5303::Tn	None	Rid2
DM15943	$_{PA}$*ridA*::Tn	pMQ72	Empty vector
DM15944	$_{PA}$*ridA*::Tn	pDM1533	pMQ72-PA5339
DM16327	$_{PA}$*ridA*::Tn	pDM1566	pMQ72-PA3123
DM16328	$_{PA}$*ridA*::Tn	pDM1568	pMQ72-ridA (LT2)
DM16443	$_{PA}$*ridA*::Tn	pDM1534	pMQ72-PA5083
E. coli			
DM15055	K-12 Δ*ridA890* Δ*tdcF14* *araBAD*::PBAD-*ilvA219*-cat	None	Fig. S2
DM14931	K-12 *araBAD*::PBAD-*ilvA219*-cat	None	
S. enterica			
DM9404	Wild type	None	
DM3480	*ridA*::MudJ	None	

motility related to 2AA accumulation. If the defect in motility in *P. aeruginosa* is confirmed to be independent of the growth defect, it will suggest a broad role for RidA in protecting a PLP-dependent enzyme involved in the regulatory and/or structural components of motility.

MATERIALS AND METHODS

Bacterial strains, plasmids and media. The strains, plasmids and primers used in this study are listed in Table 1. *P. aeruginosa* PAO1 mutants were obtained from the transposon mutant library collection and included the wild-type strain MPAO1 (32). Transposon location was verified using a transposon-specific primer and a primer annealing to a flanking region of the chromosome. Derivatives of *S. enterica* serovar Typhimurium LT2 were used for complementation studies, and *E. coli* BL21AI was used for protein overexpression.

Lysogeny broth (LB) was used as a rich medium for *P. aeruginosa* and *E. coli*. *S. enterica* was cultivated in Difco nutrient broth (8 g/liter) with NaCl (5 g/liter). All bacterial strains were grown at 37°C. MOPS salts (33), NCE or M9 with $MgSO_4$ (34), and trace minerals (35, 36) were used as a minimal medium base, as indicated. *P. aeruginosa* mutants were grown with 11 mM glucose. Gentamicin was added to 100 μg/ml for *P. aeruginosa* and to 10 μg/ml for *S. enterica*. Ampicillin was added to 150 μg/ml for *S. enterica* and *E. coli*. Supplements were added as indicated, isoleucine, threonine, or glycine (1 mM), and serine (1 or 5 mM). L-Arabinose (250 μM) was added to induce expression of genes inserted in relevant plasmids. Chemicals were purchased from Millipore-Sigma (Sigma-Aldrich, St. Louis, MO).

Growth analysis. Growth on solid medium was evaluated by patching strains to rich medium (LB), incubating the plates for 3 to 4 h at 37°C, and replica printing to plates of the relevant medium. Alternatively, nutrients were spotted on soft agar overlays containing an aliquot of overnight culture (100 μl) that had been pelleted and resuspended in sterile saline solution.

Growth in liquid culture was assessed using a BioTek Elx808 microtiter plate reader monitoring the optical density at 650 nm (OD_{650}) at 37°C with a slow shaking speed. Overnight cultures of *S. enterica* or *P. aeruginosa* in biological triplicate were grown in rich medium at 37°C, pelleted, and resuspended in an equal volume of sterile NaCl. A cell suspension was used to inoculate growth medium (2% inoculum), and growth was monitored for 24 h. The resulting data were plotted using GraphPad Prism 7.0, generating curves in a $\log_{10}$ format that display the means of three replicates and the standard deviations of the

mean. Specific growth rates (μ) were calculated according to the following equation: $\ln(X/X_0)/T$, where X is OD_{650}, X_0 is the starting OD_{650} of the exponential growth period monitored, and T is the time in hours.

Molecular biology. A yeast (*Saccharomyces cerevisiae*) *in vivo* recombineering protocol was used to clone into the pMQ72 vector based on the Gibson cloning method and previously published procedures (29, 37–40). The plasmid pool from *S. cerevisiae* was isolated and electroporated into *E. coli*. Then transformants were selected on LB containing gentamicin. Plasmid inserts were screened via colony PCR with primers PR923 and PR924. Candidate constructs were confirmed via sequence analysis performed by Eton Biosciences (San Diego, CA). Plasmid derivatives of pBAD24 were created using a BspQI restriction cloning method as previously described by Galloway et al. (41) with a modified vector that contained the BspQI site (pCV1) (42). *P. aeruginosa* competent cells were prepared by standard methods, recovered in LB, and plated to selective medium at 37°C (43).

Protein purification. $_{PA}$RidA was purified from *E. coli* strain BL21AI harboring pDM1563 (pET28-$_{PA}$*ridA*) using the $_{SE}$RidA purification scheme described previously (44). Briefly, overnight cultures in LB (10 ml) were used to inoculate two flasks containing a total of 3 liters of superbroth supplemented with ampicillin. Cultures were grown at 37°C with shaking (200 rpm) until the OD_{650} reached 0.5. Protein overexpression was induced by the addition of 0.1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) and 0.2% (wt/vol) arabinose, and the culture temperature was shifted to 30°C overnight. Cells were harvested via centrifugation at 6,000 $\times$ *g* for 15 min, and the cell pellets were kept at −80°C until use. A volume of bind buffer (50 mM potassium phosphate [pH 7.5], 100 mM NaCl, 5 mM imidazole, 10% glycerol) was added to the cell pellets (2 ml/g [wet cell weight]), along with lysozyme (1 mg/ml), DNase (20 U/ml), and phenylmethylsulfonyl fluoride (1 mM). Cells were lysed with a cell disruptor using One Shot Head (Constant Systems, Ltd., Northants, United Kingdom) set to 20 kpsi, and debris was pelleted via centrifugation at 40,000 $\times$ *g* for 40 min. The lysate was clarified with a syringe filter (0.45-μm pore size) prior to being loaded onto a 5-ml HisTrap HP (Amersham Biosciences) column on an ÅKTA fast-performance liquid chromatography apparatus. Protein was eluted with a 0 to 100% gradient of elution buffer (50 mM potassium phosphate [pH 7.5], 100 mM NaCl, 0.5 M imidazole, 10% glycerol) according to the manufacturer's instruction (GE Healthcare). Samples (2 μl) collected from the elution off the HisTrap HP column were analyzed via SDS-PAGE, and relevant fractions were identified by the presence of an ~13-kDa band. Fractions containing $_{PA}$RidA were pooled and concentrated using a centrifugal protein concentrator unit with a 3,000 molecular-weight cutoff (Millipore-Sigma). The protein was transferred into storage buffer (50 mM potassium phosphate [pH 7.5], 10% glycerol) using a PD-10 desalting column (GE Healthcare). The protein concentration was determined with a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL), and dilutions (0.125 to 2 μg) were loaded onto a SDS–12.5% PAGE gel for protein purity analysis. $_{PA}$RidA was enriched to >99% purity, and the preparation was drop-frozen in liquid nitrogen and stored at −80°C.

CdsH assays. 2AA deaminase activity was determined using a coupled assay with purified cysteine desulfhydrase (CdsH)-dependent pyruvate formation and NADH oxidation by lactate dehydrogenase as previously described (4). Reaction mixtures (100 μl) contained Tris-HCl (100 mM; pH 8), NADH (250 μM), pyridoxal 5′-phosphate (30 μM), and pyruvate kinase/lactate dehydrogenase (5 U). Purified CdsH and the indicated Rid proteins were added at monomeric concentrations of 0.27 or 0.19 μM, respectively. The reaction was initiated by the addition of freshly prepared L-cysteine at final concentrations between 0.2 and 2 mM. The absorbance at 340 nm was monitored for 2 min, and the initial rate of pyruvate formation was calculated from the rate of NADH oxidation in the first 30 s, along with the molar extinction coefficient for NADH oxidation (6,200 M^{-1} cm^{-1}).

Transaminase B activity assays. A portion (50 μl) of overnight NB cell culture was inoculated into 5 ml of minimal NCE medium containing 1 mM $MgSO_4$, trace minerals, 11 mM glucose, and, when stated, 1 mM isoleucine or threonine. The cultures were incubated at 37°C with shaking until they reached full density. The cells were harvested by centrifugation and washed with NCE (1 ml). Cell pellets were frozen at −80°C until use. Cell pellets were resuspended in 1.0 ml of 50 mM potassium phosphate (pH 7.5). The cells were lysed using a Constant Systems Limited One Shot (United Kingdom) system by passing the cells through the disrupter one time with the pressure set to 21,000 lb/in^2. The protein concentration was estimated using a bicinchoninic assay reagent kit (Pierce).

The transaminase B activity assay was an adaptation of previously described protocols (6, 45). A 50-μl aliquot of the whole-cell suspension was added to the reaction mixture and allowed to equilibrate at 37°C for 10 min. The reaction mixture contained 50 mM potassium phosphate (pH 7.5), 5 μl of 2 mM PLP, an d4 μl of 0.5 mM α-ketoglutarate in a total volume of 200 μl. Then, 20 μl of L-isoleucine was added to start the reaction (final concentration, 20 mM). The reaction was allowed to proceed for 20 min at 37°C and stopped with 200 μl of 0.3% 2,4-dinitrophenyl-hydrazine. Hydrazone formation was allowed to proceed for 5 min at room temperature, prior to extraction with 1 ml of toluene and shaking for 2 min at 37°C. The two phases were separated by centrifugation, and the aqueous (bottom) layer was removed by micropipette. The toluene layer was washed by adding 0.5 ml of 0.5 N HCl, vortexing, and separating the phases by centrifugation. A 0.8-ml aliquot of the toluene (top) layer was removed and mixed with 1 ml of 1.5 N; the mixture was then vigorously vortexed and centrifuged, and 200 μl was removed from the bottom layer. The absorbance was measured at A_{540}, and the results are reported in nmol/min/mg. The data are presented as means of three biological replicates, and error bars represent the standard errors of the mean. Statistical significance ($P < 0.05$) was determined by conducting one-way analysis of variance and Tukey's posttest using GraphPad Prism (v7.0c).

Motility screens. Motility screens were performed by previously described methods (26, 46). M9 medium (20 mM NH_4Cl, 12 mM Na_2HPO_4, 22 mM KH_2PO_4, 1.0 mM NaCl, 1 mM $MgSO_4$) solidified with Bacto agar (Difco), 0.3% for swimming motility or 0.7% for swarming motility, was prepared (36). The

medium was autoclaved and cooled to 55°C, and trace minerals, 11 mM glucose, and (when applicable) 1 mM isoleucine were added. 25 ml of medium was poured into petri dishes in a single layer and allowed to dry for 24 h. Overnight cultures were grown in triplicate in LB plus antibiotic at 37°C. The cultures were centrifuged, and the pellet was resuspended in NaCl to an OD_{600} of 0.3. Then, 10 μl of bacterial suspension was inoculated onto each plate by gently stabbing into the soft agar; the plates were then incubated at 37°C for 24 h. The diameter of each swimming halo was measured and values are reported in millimeters. The data represent the means of three biological replicates, and error bars represent the standard errors of the mean. Statistical significance ($P < 0.00009$) was determined by using an unpaired Student *t* test with GraphPad Prism 7.0c.

Biofilm formation assays. Static biofilm assays were performed as previously described (47, 48). Briefly, DM15943 and DM15944 were grown in biological triplicates overnight in LB with 100 μg/ml of gentamicin. Cultures were pelleted and resuspended in an equal volume of NaCl, and a 1:100 dilution was made into M63 minimal medium containing 11 mM glucose alone or with the addition of 1 mM isoleucine. Next, 100 μl of cell dilution was used to inoculate a round-bottom 96-well microtiter plate in biological and technical triplicates. The plate was statically incubated for 20 to 24 h at 37°C, rinsed with ddH_2O, dried, and stained with crystal violet. The stain was solubilized with ethanol, and then the ethanol/stain was removed to a flat-bottom 96-well microtiter plate, and the absorbance was read at 550 nm. The data represent averages of technical replicates (within each biological replicate) and the means of three biological replicates. Error bars represent standard errors of the mean from three biological replicates, and one asterisk denotes statistically significant ($P < 0.009$) variation between mutants as determined by an unpaired Student *t* test with GraphPad Prism 7.0c. M63 medium was used for these experiments to be consistent with the literature in the field, although the results are not expected to differ from those determined on NCE (47, 49–52).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JB.00434-18.

SUPPLEMENTAL FILE 1, PDF file, 3.4 MB.

ACKNOWLEDGMENTS

We thank Sherry Kuchma and George O'Toole for helpful discussions, technical assistance in *Pseudomonas* genetic manipulation, and providing *Pseudomonas* plasmids. We thank Rebecca Cox for technical assistance in the biofilm experiments. Strains critical to this study were obtained from University of Washington transposon mutant collection (grant NIH P30 DK089507). CdsH was provided by Dustin Ernst.

This study was supported by competitive grant GM095837 from the National Institutes of Health (D.M.D.).

REFERENCES

1. Niehaus TD, Gerdes S, Hodge-Hanson K, Zhukov A, Cooper AJ, ElBadawi-Sidhu M, Fiehn O, Downs DM, Hanson AD. 2015. Genomic and experimental evidence for multiple metabolic functions in the RidA/YjgF/YER057c/UK114 (Rid) protein family. BMC Genomics 16:382. https://doi.org/10.1186/s12864-015-1584-3.
2. Hodge-Hanson KM, Downs DM. 2017. Members of the Rid protein family have broad imine deaminase activity and can accelerate the *Pseudomonas aeruginosa* D-arginine dehydrogenase (DauA) reaction *in vitro*. PLoS One 12:e0185544. https://doi.org/10.1371/journal.pone.0185544.
3. Lambrecht JA, Flynn JM, Downs DM. 2012. Conserved YjgF protein family deaminates reactive enamine/imine intermediates of pyridoxal 5′-phosphate (PLP)-dependent enzyme reactions. J Biol Chem 287:3454–3461. https://doi.org/10.1074/jbc.M111.304477.
4. Ernst DC, Lambrecht JA, Schomer RA, Downs DM. 2014. Endogenous synthesis of 2-aminoacrylate contributes to cysteine sensitivity in *Salmonella enterica*. J Bacteriol 196:3335–3342. https://doi.org/10.1128/JB.01960-14.
5. Ernst DC, Anderson ME, Downs DM. 2016. L-2,3-Diaminopropionate generates diverse metabolic stresses in *Salmonella enterica*. Mol Microbiol 101:210–223. https://doi.org/10.1111/mmi.13384.
6. Lambrecht JA, Schmitz GE, Downs DM. 2013. RidA proteins prevent metabolic damage inflicted by PLP-dependent dehydratases in all domains of life. mBio 4:e00033-13. https://doi.org/10.1128/mBio.00033-13.
7. Niehaus TD, Nguyen TN, Gidda SK, ElBadawi-Sidhu M, Lambrecht JA, McCarty DR, Downs DM, Cooper AJ, Fiehn O, Mullen RT, Hanson AD. 2014. Arabidopsis and maize RidA proteins preempt reactive enamine/imine damage to branched-chain amino acid biosynthesis in plastids. Plant Cell 26:3010–3022. https://doi.org/10.1105/tpc.114.126854.
8. Borchert AJ, Downs DM. 2017. The response to 2-aminoacrylate differs in *Escherichia coli* and *Salmonella enterica*, despite shared metabolic components. J Bacteriol 199:e00140-17. https://doi.org/10.1128/JB.00140-17.
9. Ernst DC, Downs DM. 2018. Mmf1p couples amino acid metabolism to mitochondrial DNA maintenance in *Saccharomyces cerevisiae*. mBio 9:e00084-18. https://doi.org/10.1128/mBio.00084-18.
10. Kim JM, Yoshikawa H, Shirahige K. 2001. A member of the YER057c/yjgf/Uk114 family links isoleucine biosynthesis to intact mitochondria maintenance in *Saccharomyces cerevisiae*. Genes Cells 6:507–517. https://doi.org/10.1046/j.1365-2443.2001.00443.x.
11. Oxelmark E, Marchini A, Malanchi I, Magherini F, Jaquet L, Hajibagheri MA, Blight KJ, Jauniaux JC, Tommasino M. 2000. Mmf1p, a novel yeast mitochondrial protein conserved throughout evolution and involved in maintenance of the mitochondrial genome. Mol Cell Biol 20:7784–7797. https://doi.org/10.1128/MCB.20.20.7784-7797.2000.
12. Schmitz G, Downs DM. 2004. Reduced transaminase B (IlvE) activity caused by the lack of *yjgF* is dependent on the status of threonine deaminase (IlvA) in *Salmonella enterica* serovar Typhimurium. J Bacteriol 186:803–810. https://doi.org/10.1128/JB.186.3.803-810.2004.
13. Christopherson MR, Schmitz GE, Downs DM. 2008. YjgF is required for isoleucine biosynthesis when *Salmonella enterica* is grown on pyruvate medium. J Bacteriol 190:3057–3062. https://doi.org/10.1128/JB.01700-07.
14. Flynn JM, Christopherson MR, Downs DM. 2013. Decreased coenzyme A levels in *ridA* mutant strains of *Salmonella enterica* result from inacti-

vated serine hydroxymethyltransferase. Mol Microbiol 89:751–759. https://doi.org/10.1111/mmi.12313.
15. Flynn JM, Downs DM. 2013. In the absence of RidA, endogenous 2-aminoacrylate inactivates alanine racemases by modifying the pyridoxal 5′-phosphate cofactor. J Bacteriol 195:3603–3609. https://doi.org/10.1128/JB.00463-13.
16. Downs DM, Ernst DC. 2015. From microbiology to cancer biology: the Rid protein family prevents cellular damage caused by endogenously generated reactive nitrogen species. Mol Microbiol 96:211–219. https://doi.org/10.1111/mmi.12945.
17. Ernst DC, Christopherson MR, Downs DM. 2018. Increased activity of cystathionine beta-lyase suppresses 2-aminoacrylate stress in *Salmonella enterica*. J Bacteriol 200:e00040-18. https://doi.org/10.1128/JB.00040-18.
18. Borchert AJ, Downs DM. 2017. Endogenously generated 2-aminoacrylate inhibits motility in *Salmonella enterica*. Sci Rep 7:12971. https://doi.org/10.1038/s41598-017-13030-x.
19. Enos-Berlage JL, Langendorf MJ, Downs DM. 1998. Complex metabolic phenotypes caused by a mutation in *yjgF*, encoding a member of the highly conserved YER057c/YjgF family of proteins. J Bacteriol 180:6519–6528.
20. Leitner-Dagan Y, Ovadis M, Zuker A, Shklarman E, Ohad I, Tzfira T, Vainstein A. 2006. CHRD, a plant member of the evolutionarily conserved YjgF family, influences photosynthesis and chromoplastogenesis. Planta 225:89–102. https://doi.org/10.1007/s00425-006-0332-y.
21. Ernst DC, Downs DM. 2016. 2-Aminoacrylate stress induces a context-dependent glycine requirement in *ridA* strains of *Salmonella enterica*. J Bacteriol 198:536–543. https://doi.org/10.1128/JB.00804-15.
22. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang HY, Cohoon M, de Crecy-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Glass EM, Goesmann A, Hanson A, Iwata-Reuyl D, Jensen R, Jamshidi N, Krause L, Kubal M, Larsen N, Linke B, McHardy AC, Meyer F, Neuweger H, Olsen G, Olson R, Osterman A, Portnoy V, Pusch GD, Rodionov DA, Ruckert C, Steiner J, Stevens R, Thiele I, Vassieva O, Ye Y, Zagnitko O, Vonstein V. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res 33:5691–5702. https://doi.org/10.1093/nar/gki866.
23. He W, Li G, Yang CK, Lu CD. 2014. Functional characterization of the *dguRABC* locus for D-Glu and D-Gln utilization in *Pseudomonas aeruginosa* PAO1. Microbiology 160:2331–2340. https://doi.org/10.1099/mic.0.081141-0.
24. Chou HT, Kwon DH, Hegazy M, Lu CD. 2008. Transcriptome analysis of agmatine and putrescine catabolism in *Pseudomonas aeruginosa* PAO1. J Bacteriol 190:1966–1975. https://doi.org/10.1128/JB.01804-07.
25. Crouzet M, Claverol S, Lomenech AM, Le Senechal C, Costaglioli P, Barthe C, Garbay B, Bonneu M, Vilain S. 2017. *Pseudomonas aeruginosa* cells attached to a surface display a typical proteome early as 20 minutes of incubation. PLoS One 12:e0180341. https://doi.org/10.1371/journal.pone.0180341.
26. Overhage J, Bains M, Brazas MD, Hancock RE. 2008. Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. J Bacteriol 190:2671–2679. https://doi.org/10.1128/JB.01659-07.
27. Tremblay J, Deziel E. 2010. Gene expression in *Pseudomonas aeruginosa* swarming motility. BMC Genomics 11:587. https://doi.org/10.1186/1471-2164-11-587.
28. Manos J, Arthur J, Rose B, Tingpej P, Fung C, Curtis M, Webb JS, Hu H, Kjelleberg S, Gorrell MD, Bye P, Harbour C. 2008. Transcriptome analyses and biofilm-forming characteristics of a clonal *Pseudomonas aeruginosa* from the cystic fibrosis lung. J Med Microbiol 57:1454–1465. https://doi.org/10.1099/jmm.0.2008/005009-0.
29. Shanks RM, Caiazza NC, Hinsa SM, Toutain CM, O'Toole GA. 2006. *Saccharomyces cerevisiae*-based molecular tool kit for manipulation of genes from gram-negative bacteria. Appl Environ Microbiol 72:5027–5036. https://doi.org/10.1128/AEM.00682-06.
30. Reuter M, Periago PM, Mulholland F, Brown HL, van Vliet AH. 2015. A PAS domain-containing regulator controls flagella-flagella interactions in *Campylobacter jejuni*. Front Microbiol 6:770. https://doi.org/10.3389/fmicb.2015.00770.
31. Lessie TG, Whiteley HR. 1969. Properties of threonine deaminase from a bacterium able to use threonine as sole source of carbon. J Bacteriol 100:878–889.
32. Jacobs MA, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, Will O, Kaul R, Raymond C, Levy R, Chun-Rong L, Guenthner D, Bovee D, Olson MV, Manoil C. 2003. Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 100:14339–14344. https://doi.org/10.1073/pnas.2036282100.
33. Neidhardt FC, Bloch PL, Smith DF. 1974. Culture medium for enterobacteria. J Bacteriol 119:736–747.
34. Vogel HJ, Bonner DM. 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J Biol Chem 218:97–106.
35. Balch WE;, Wolfe RS. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl Environ Microbiol 32:781–791.
36. Tremblay J, Deziel E. 2008. Improving the reproducibility of *Pseudomonas aeruginosa* swarming motility assays. J Basic Microbiol 48:509–515. https://doi.org/10.1002/jobm.200800030.
37. 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.
38. Burke D, Dawson D, Sterans T. 2000. Methods in yeast genetics: a Cold Spring Harbor laboratory course manual. Cold Spring Harbor Laboratory Press, Plainview, NY.
39. Elble R. 1992. A simple and efficient procedure for transformation of yeasts. Biotechniques 13:18–20.
40. Oldenburg KR, Vo KT, Michaelis S, Paddon C. 1997. Recombination-mediated PCR-directed plasmid construction in vivo in yeast. Nucleic Acids Res 25:451–452. https://doi.org/10.1093/nar/25.2.451.
41. Galloway NR, Toutkoushian H, Nune M, Bose N, Momany C. 2013. Rapid cloning for protein crystallography using type IIS restriction enzymes. Crystal Growth Design 13:2833–2839. https://doi.org/10.1021/cg400171z.
42. VanDrisse CM, Escalante-Semerena JC. 2016. New high-cloning-efficiency vectors for complementation studies and recombinant protein overproduction in *Escherichia coli* and *Salmonella enterica*. Plasmid 86:1–6. https://doi.org/10.1016/j.plasmid.2016.05.001.
43. Choi KH, Kumar A, Schweizer HP. 2006. A 10-min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: application for DNA fragment transfer between chromosomes and plasmid transformation. J Microbiol Methods 64:391–397. https://doi.org/10.1016/j.mimet.2005.06.001.
44. Lambrecht JA, Browne BA, Downs DM. 2010. Members of the YjgF/YER057c/UK114 family of proteins inhibit phosphoribosylamine synthesis in vitro. J Biol Chem 285:34401–34407. https://doi.org/10.1074/jbc.M110.160515.
45. Duggan DE, Wechsler JA. 1973. An assay for transaminase B enzyme activity in *Escherichia coli* K-12. Anal Biochem 51:67–79. https://doi.org/10.1016/0003-2697(73)90453-3.
46. Kearns DB. 2010. A field guide to bacterial swarming motility. Nat Rev Microbiol 8:634–644. https://doi.org/10.1038/nrmicro2405.
47. O'Toole GA, Kolter R. 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. Mol Microbiol 28:449–461. https://doi.org/10.1046/j.1365-2958.1998.00797.x.
48. Merritt JH, Kadouri DE, O'Toole GA. 2005. Growing and analyzing static biofilms. Curr Protoc Microbiol Chapter 1:Unit 1B.1. https://doi.org/10.1002/9780471729259.mc01b01s00.
49. Friedman L, Kolter R. 2004. Genes involved in matrix formation in *Pseudomonas aeruginosa* PA14 biofilms. Mol Microbiol 51:675–690. https://doi.org/10.1046/j.1365-2958.2003.03877.x.
50. Merritt JH, Brothers KM, Kuchma SL, O'Toole GA. 2007. SadC reciprocally influences biofilm formation and swarming motility via modulation of exopolysaccharide production and flagellar function. J Bacteriol 189:8154–8164. https://doi.org/10.1128/JB.00585-07.
51. Caiazza NC, Merritt JH, Brothers KM, O'Toole GA. 2007. Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol 189:3603–3612. https://doi.org/10.1128/JB.01685-06.
52. Kuchma SL, Brothers KM, Merritt JH, Liberati NT, Ausubel FM, O'Toole GA. 2007. BifA, a cyclic-Di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol 189:8165–8178. https://doi.org/10.1128/JB.00586-07.