SUPPLEMENTARY MATERIALS

PA5339, a RidA homolog, is required for full growth in *Pseudomonas aeruginosa*

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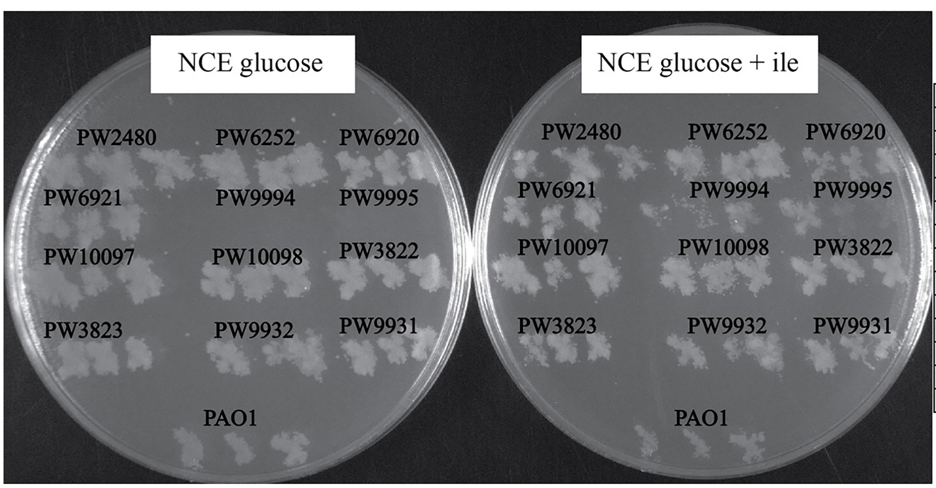
RUNNING TITLE: 2-aminoacrylate stress in *P. aeruginosa*

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

Primer Name	Gene to amplify	Plasmid	Sequence
PR863	PA5339	NA	CGAGTACCGCATGGAAGTCT
PR864	PA5339	NA	CCCATGATCTCGTTGACCTT
PR857	PA3123	NA	GCCATGAGCACCAACTACCT
PR858	PA3123	NA	GTCGATATGAGCCAGGGTTG
PR851	PA0814	NA	GCTGTTGGAACCGTATTCGT
PR852	PA0814	NA	CGACAGAGGACGAGGAAGTC
PR869	PA1568	NA	GTATAGAGGGTGCCGTCCAG
PR870	PA1568	NA	GCTGTTCATGATGGGACTGA
PR871	PA5303	NA	CTCATCATCACGATCTTCGC
PR872	PA5303	NA	CCAGCACACTAACGAGCGTA
PR923	Sequencing	pMQ72 F	CCGCCAAAACAGCC
PR924	Sequencing	pMQ72 R	ACCCGTTTTTTTGGGCT
Universal	Sequencing	pBAD F	ATGCCATAGCATTTTTATCC
Universal	Sequencing	pBAD R	GATTTAATCTGTATCAGG
PR1037	LT2 ridA	pMQ72-*ridA* F	ccaagcttgcatgcctgcaggtcgactctagaggatccccTTAGCGACGAACAGCGA
PR1038	LT2 ridA	pMQ72-*ridA* R	tacccgtttttttgggctagcgaattcgagctcggtacccCATTATGTGGTGCTGGCC
PR897	PA5339	pMQ72-*PAridA* F	TACCCGTTTTTTTGGGCTAGCGAATTCGAGCTCGGTACCCGATTTCAGCAAGGAGTTCCC
PR898	PA5339	pMQ72-*PAridA* R	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCTTACTCGAGGACGACGATG
PR1033	PA3123	pMQ72-PA3123 F	ccaagcttgcatgcctgcaggtcgactctagaggatccccCTCAGCCCTCGCCGACCCACGCC
PR1034	PA3123	pMQ72-PA3123 R	tacccgtttttttgggctagcgaattcgagctcggtacccCGCAGTGCTACATGAATACGC
PR917	PA5083	pMQ72-dguB F	TACCCGTTTTTTTGGGCTAGCGAATTCGAGCTCGGTACCCCCCTCGACTGGAGCACTCC
PR918	PA5083	pMQ71-dguB R	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCtcaGCCCCTGGCCGC
PR978	pBAD24-PA5339 F	PA5339	NNGCTCTTCNTTCATGACCAAGACCGTTATCCAC
PR979	pBAD24-PA5339 R	PA5339	NNGCTCTTCNTTACTCGAGGACGACGATGG
PR976	pBAD24-PA3123 F	PA3123	NNGCTCTTCNTTCATGAGCGATGACATCCAGCGTTA

PR977	pBAD24-PA3123 R	PA3123	NNGCTCTTCNTTAGCCCTCGCCGACCCA
PR503	pBAD24-PA0814 F	PA0814	NNGCTCTTCNTTCATGCAGACATCCCCCGCTCCG
PR504	pBAD24-PA0814 R	PA0814	NNGCTCTTCNTTATCATGGCCGCACCGCCGC
PR1020	pBAD24-PA1568 F	PA1568	NNGCTCTTCNTTCATGCATATCGAACGTTTCGAAGT
PR1021	pBAD24-PA1568 R	PA1568	NNGCTCTTCNTTATCACTGCGGGCGGGC
PR1022	pBAD24-PA5303 F	PA5303	NNGCTCTTCNTTCATGCCCATCCAGCGCCAG
PR1023	pBAD24-PA5303 R	PA5303	NNGCTCTTCNTTATCAGGGCAGCGCAGCGAC
PR729	pBAD-PA5083 F	PA5083	NNGCTCTTCNTTCATGGAACCGACCCGTATCG
PR730	pBAD-PA5083 R	PA5083	NNGCTCTTCNTTATCAGCCCCTGGCCGCCAC

Table S1: List of primers used in this study



Strain ID	Genotype	Rid
PW2480	PA0814::Tn	Rid1
PW6252	PA3123::Tn	RidA
PW6920	PA3499::Tn	RidA
PW6921	PA3499::Tn	RidA
PW9994	PA5339::Tn	RidA
PW9995	PA5339::Tn	RidA
PW10097	PA5392::Tn	RidA
PW10098	PA5392::Tn	RidA
PW3822	PA1568::Tn	Rid2
PW3823	PA1568::Tn	Rid2
PW9932	PA5303::Tn	Rid2
PW9931	PA5303::Tn	Rid2
MPAO1	Wild type	

Figure S1: Only PA5339::Tn mutants have a growth defect under conditions tested. Mutants were streaked from frozen glycerol stocks to LB agar. Single colonies were patched in triplicate to LB agar and incubated four hours at 37˚C. The LB agar plate was replica printed to NCE/glucose (11 mM) and NCE/glucose/isoleucine (0.3 mM). Only PW9994 and PW9995 (PA5339:Tn) strains had a growth defect on NCE/glucose, which was corrected with the addition of isoleucine.

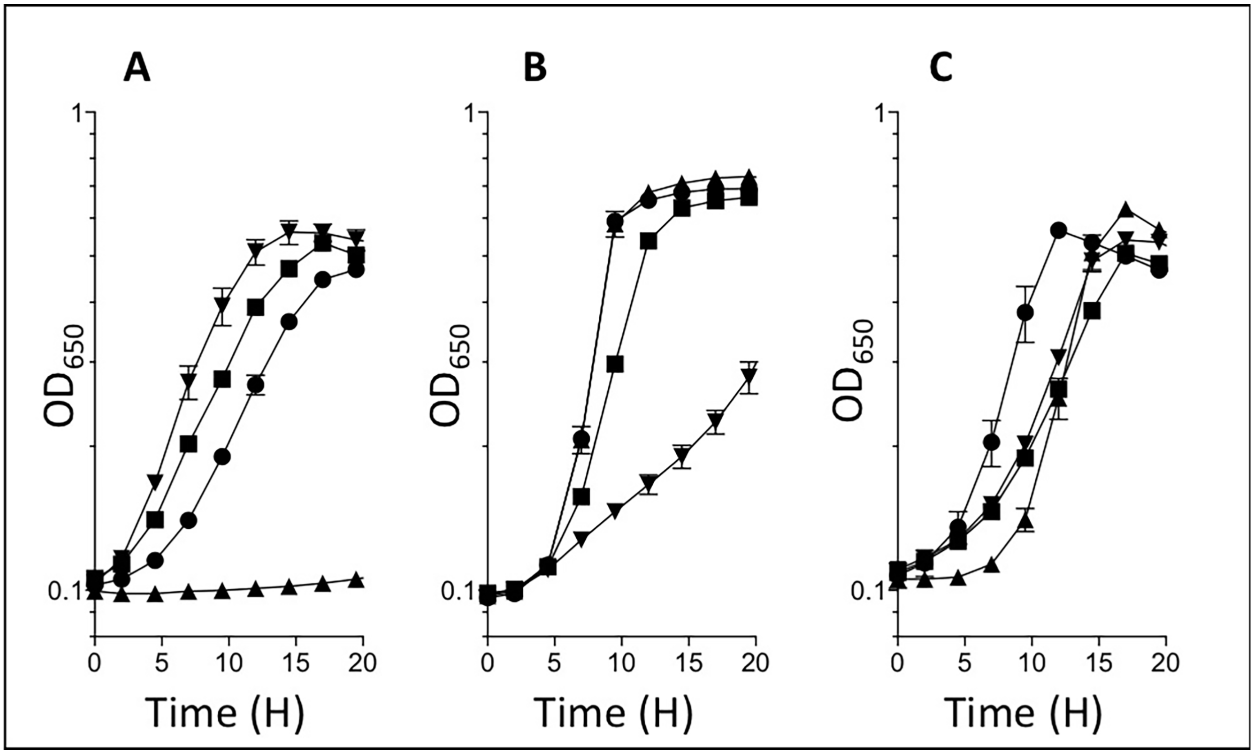


Figure S2: Sensitivity to serine differs among organisms lacking RidA. All organisms were grown in 20 mM glycerol +/- the addition of 3 mM serine and 0.2% arabinose (to induce *ilvA219* in *E. coli* chromosomal construct). The addition of 3 mM serine caused varying growth defects, suggesting 2AA sensitivity (from serine) differs in each organism. Historically, in minimal medium, 5 mM serine has been used to cause a growth defect in *S. enterica ridA* and *E. coli ilvA219* Δ*ridA* Δ*tdcF* mutants (1). These data show, in minimal medium with glycerol, 3 mM serine completely inhibits growth of a *P. aeruginosa* $_{PA}$*ridA*:Tn mutant (A), partially inhibits growth of the *S. enterica ridA*::MudJ mutant (B), and does not inhibit growth of the *E. coli ilvA219* Δ*ridA* Δ*tdcF* mutant (C).

A) *P. aeruginosa* $_{PA}$*ridA*:Tn mutants: circles--DM15943 (pEmpty vector) glycerol only, squares--DM15944 (pPA5339) glycerol only, triangles--DM15943 glycerol/serine, inverted triangles--DM15944 glycerol/serine

B) *S. enterica:* circle--wildtype in glycerol, squares--*ridA*::MudJ in glycerol, triangles--wildtype in glycerol/serine, inverted triangles--*ridA*::MudJ in glycerol/serine.

C) *E. coli*: circles--DM14931 (ilvA219) in glycerol, squares—DM15055 (*ilvA219* Δ*ridA* Δ*tdcF*), triangles-- DM14931in glycerol/serine, inverted triangles—DM15055 in glycerol/serine.

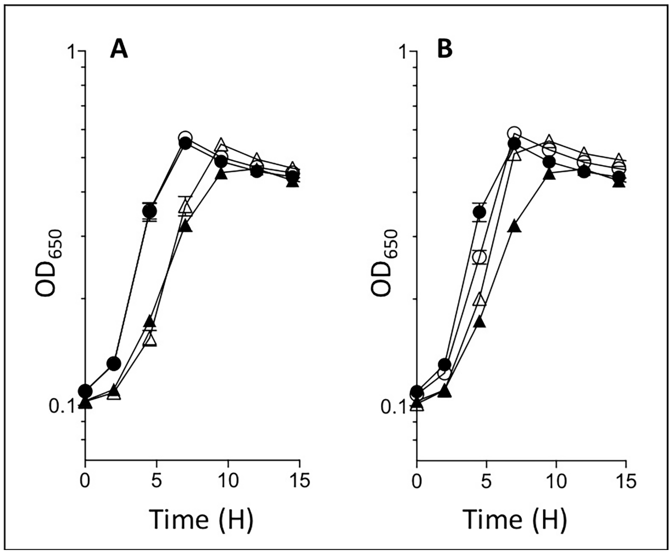


Figure S3: Growth of a $_{PA}$*ridA*::Tn mutant is improved by threonine but not glycine.

A) Growth in minimal medium with 11 mM glucose (closed symbols), PAO1/WT (circles), PW9994 $_{PA}$*ridA*::Tn mutant (triangles), and with exogenous 1 mM glycine (open symbols). C) Growth in minimal medium with 11 mM glucose (closed symbols), PAO1/WT (circles), PW9994 $_{PA}$*ridA*::Tn mutant (triangles), and with exogenous 1 mM threonine (open symbols). The error bars represent standard errors of the mean from three biological replicates.

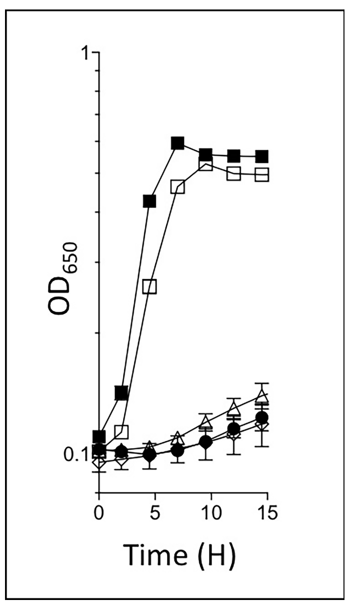


Figure S4: Growth of a $_{PA}$*ridA*::Tn mutant is restored by $_{SE}$*ridA* or $_{PA}$*ridA in trans*.

Expression of $_{SE}$*ridA* or $_{PA}$*ridA in trans,* DM16328 (squares) or DM15944 (open square), respectively, supported growth of the $_{PA}$*ridA*::Tn mutant in minimal medium with 11 mM glucose and 5 mM serine. The empty vector control in DM15943 (circles), or expression of Rid family members, PA5083 in DM16443 (open diamonds), or PA3123 in DM16327 (open triangles) did not support growth of the $_{PA}$*ridA*::Tn mutant. The error bars represent standard errors of the mean from three biological replicates.

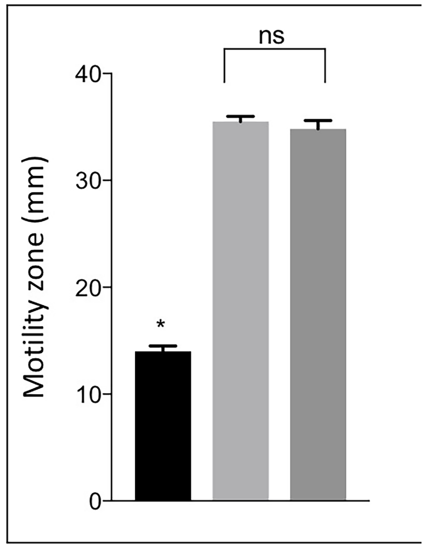


Figure S5: Motility of PAO1 and the complemented ***PAridA*****::Tn mutant are similar.** Diameter of motility was measured after 24 hours in minimal NCE glucose medium. There is a significant difference in motility diameter of DM15943 (black bar) as compared to DM15944 or PAO1. There is no significant difference between the motility of DM15944 (*PAridA*::Tn/PAridA) and PAO1. Significance was determined by Students test and a P values <0.005.

Literature Cited.

1. Borchert AJ, Downs DM. 2017. The Response to 2-Aminoacrylate Differs in Escherichia coli and Salmonella enterica, despite Shared Metabolic Components. J Bacteriol 199.