# DcaP-Family Porins are Required for Carboxylic Acid Utilization and Infection in *Acinetobacter baumannii*

Hannah R. Noel[1], Nikita R. Kesav[1], Jonathan D. Winkelman[2], Lauren D. Palmer[1]*

[1] Department of Microbiology and Immunology, University of Illinois Chicago, Chicago, IL, USA

[2] Trestle LLC, Milwaukee, WI, USA

*Corresponding author

Lauren D. Palmer

835 S Wolcott Ave

MSB E703

Chicago, IL 60612

ldpalmer@uic.edu

**ABSTRACT**

*Acinetobacter baumannii* is a pathogen of concern and a leading cause of multidrug-resistant healthcare-associated infections. The *A. baumannii* outer membrane is a barrier to antimicrobials and host defenses but must also allow essential nutrients to permeate. Here, we investigate the functional importance of the putative DcaP-family outer membrane porins, which include a proposed vaccine target in *A. baumannii*. All *A. baumannii* genomes surveyed encode multiple DcaP family porins, which we classify in four classes based on protein sequence phylogeny (DcaP1-4). DcaP proteins encoded by species in other genera could not be mapped to these DcaP classes and phylogenetic analysis suggests DcaP1-4 proteins diversified within *Acinetobacter*. Phenotypic array assays and additional experiments show that the DcaP proteins were necessary for growth on multiple di- and tri-carboxylic acids as sole carbon sources, including citric acid and tricarballylic acid. Specifically, DcaP3 was required; however, DcaP1, DcaP2, and DcaP4 were all able to complement the total *dcaP* knockout to varying degrees, suggesting partial functional overlap. Finally, a mutant lacking all DcaP proteins was attenuated in the liver and spleen in a mouse model of bloodstream infection and complemented by expression of DcaP3. However, a Δ*dcaP3* mutant had no defect, demonstrating functional redundancy among DcaP proteins during infection. These findings provide insight on how *A. baumannii* acquires nutrients through the outer membrane barrier and show DcaP proteins are important during infection in specific host niches, validating their potential as a therapeutic target.

35 **INTRODUCTION**

36 *Acinetobacter baumannii* is a pathogen of critical concern and a major cause of
37 healthcare-associated infections. Isolates of *A. baumannii* are often multidrug resistant (MDR)
38 or extensively drug resistant (XDR) and exhibit resistance to first and last line antibiotics, such
39 as meropenem and colistin, respectively (1). The Centers for Disease Control and the World
40 Health Organization have therefore identified *A. baumannii* as an urgent public health threat,
41 calling for new avenues of treatment (2–4). The bacterial cell envelope is the primary barrier
42 from the environment and protects bacteria from environmental stress including antibiotics and
43 host defenses; however, the cell envelope must also allow acquisition of essential nutrients and
44 cofactors (5,6). The outer membrane (OM) of *A. baumannii* is thought to be less permeable
45 compared to other Gram-negative bacteria such as *E. coli*, contributing to intrinsic antibiotic
46 resistance (7). How small molecule nutrients required for growth transit the impermeable OM in
47 *A. baumannii* is incompletely understood.

48 The OM of *A. baumannii* is an asymmetric lipid bilayer where the inner leaflet is
49 composed of glycerophospholipids and the outer leaflet of lipooligosaccharides (LOS) (5,8,9).
50 The OM bilayer is occupied by proteins that serve a variety of functions, including nutrient influx.
51 Nutrients typically pass through the OM via active transport by TonB-dependent transporters or
52 passive diffusion through porins, which are transmembrane channels that allow passage of
53 small molecules (6,10). The primary porin in *A. baumannii* is OmpA, which shows slow
54 molecular transport in liposome swelling assays, contributing to the low permeability barrier of
55 the OM (11–13). However, the primary function of OmpA is thought to be structural, providing a
56 physical tether between the OM and the peptidoglycan cell wall in *A. baumannii* and other
57 species (14,15). *A. baumannii* encodes multiple other porins including CarO, an OM porin that
58 selectively allows influx of ornithine and other basic amino acids (16), and the Occ family porins,
59 responsible for the influx of compounds like benzoate, hydroxycinnamate, and other aromatic
60 molecules (17–19). The protein DcaP is a predicted OM porin widely distributed across

61 *Acinetobacter* species named for its localization within a genetic locus for utilization of
62 dicarboxylic acids (*dca*) (20). Notably, a DcaP family protein is among the most abundant OM
63 proteins in *A. baumannii* during rat and mouse infections (21). Due to this fact, multiple groups
64 have studied a DcaP-like protein, which we define below as DcaP3, as a promising vaccine
65 candidate for protection against *A. baumannii* infection (21–25). However, the biological role of
66 DcaP family proteins remains unclear and their role in dicarboxylic acid utilization has not been
67 experimentally interrogated.
68 First identified in Parke *et al*. as part of the dicarboxylic acid catabolic operon of
69 *Acinetobacter baylyi* ADP1, *dcaP* (putative dicarboxylic acid porin) and DcaP-like proteins were
70 thought to be specific to the Moraxellaceae family (20). The structure for one DcaP-like protein
71 has been solved, presenting as a homo-trimeric 16-stranded beta-barrel protein with an
72 extended periplasmic N-terminus that forms a coiled coil (21). Due to the genetic localization
73 and induction of expression with the dicarboxylic acid adipate, *A. baylyi* DcaP porins were
74 proposed to be nonspecific porins that facilitate the uptake of dicarboxylic acids (20); however, a
75 role for DcaP porins in carboxylic acid catabolism has not been reported. Molecular dynamics
76 and electrophysiology studies determined a DcaP-like protein to have an affinity for anionic
77 compounds as substrates, such as the clinically relevant β-lactamase inhibitor sulbactam (21).
78 In other studies, DcaP-like proteins have been implicated in biofilm formation and mucin
79 catabolism (26,27). Here, we describe four classes of DcaP-family porins in *Acinetobacter* and
80 investigate their role in carbon source utilization in *A. baumannii* ATCC 17978 and AB5075
81 strains.
82
83 **RESULTS**
84 ***Acinetobacter spp. encode multiple DcaP-family porins***
85 The first DcaP was described in *A. baylyi* as part of a dicarboxylic acid catabolic operon
86 (20), and DcaP-like proteins have been identified in various *A. baumannii* clinical isolates and

87 type-strains (21,23). Proteins designated DcaP-like in *A. baumannii* ATCC 17978 are
88 homologous to *A. baylyi* DcaP, sharing ~30-35% amino acid identity by Clustal Omega multiple
89 sequence alignment (28). However, given the abundance of DcaP-family porins within individual
90 *Acinetobacter* genomes, we hypothesized that these proteins could be further classified based
91 on protein sequence. Using DcaP and DcaP-like protein sequences from a set of deduplicated
92 *A. baumannii* genomes, four distinct classes of DcaP-family porins were identified that cluster
93 independently, including the original *A. baylyi* DcaP which we display as DcaP1 (Fig. 1A, S1A).
94 We next determined the distribution of DcaP-family porins across the *Acinetobacter* genus.
95 Using a set of 250 deduplicated *Acinetobacter* genomes, 235 of which were *A. baumannii*,
96 DcaP-family porins were shown to be widely conserved across the genus (Fig. S1B).
97 Additionally, *Acinetobacter* species within the *A. calcoaceticus/baumannii* (*acb*) complex/clade
98 of pathogenic *Acinetobacter* appeared to be enriched for having multiple DcaP-family porins
99 compared to non-pathogenic *Acinetobacter* species (Fig. S1B). Among the *A. baumannii*
100 genomes, over 130 of the strains encode four *dcaP* genes (Fig. 1B). In fact, no *A. baumannii*
101 genome analyzed encoded fewer than two *dcaP* genes (Fig. 1B). The type-strain *A. baumannii*
102 ATCC 17978 used for most studies here encodes *dcaP1*, *dcaP2*, and *dcaP3* encoded in three
103 distinct loci (Fig. S2A). To determine if the *dcaP* genes were equally distributed across the
104 species, the number of each DcaP subfamily were tallied for each genome and organized based
105 on how many total DcaP-family porins were present. The four classes of DcaP-family porins
106 were equally likely to be encoded in a given *A. baumannii* genome (Fig. 1B). For example, in
107 most cases where a genome encodes four DcaP-family porins, it was most likely to have one of
108 each variant. Alternatively, in a genome encoding five DcaP-family porins, DcaP2 was most
109 likely to be duplicated. Finally, *A. baumannii* AB5075 encodes *dcaP1-3* and also encodes *dcaP4*
110 in another distinct locus (Fig. S2A). These data suggest that DcaP-family porins are encoded
111 across the *Acinetobacter* genus, and enriched in pathogenic *Acinetobacter,* including *A.*
112 *baumannii*.

113

114 ***DcaP proteins from other bacterial species are distinct from Acinetobacter DcaP proteins***

115 To investigate the evolutionary history of DcaP-family porins in *Acinetobacter* and
116 determine if DcaP diversified within *Acinetobacter* or across taxa, orthologues in other bacterial
117 taxa were identified through KEGG. Bacterial taxa were selected for further analysis if the DcaP-
118 like protein was encoded in more than half of the genomes for a given genus (29,30). A rooted
119 phylogenomic tree was generated to demonstrate the evolutionary distance between
120 *Acinetobacter* and the bacteria containing a DcaP orthologue (Fig. 2A). Orthologues of DcaP-
121 family porins were found in distantly related organisms, such as *Shewanella spp.* and
122 *Xanthomonas spp.,* albeit generally in fewer numbers (Fig. 2A). These DcaP orthologues could
123 not be further classified into one of the four DcaP classes defined in *A. baumannii*. This
124 suggests that the four DcaP classes of proteins defined here are unique to *Acinetobacter spp*.
125 To better understand the relationship between *Acinetobacter* DcaP-family porins and proteins
126 designated DcaP-like from distantly related species, an unrooted tree of protein sequences was
127 generated. This analysis revealed that DcaP-family porins from AB5075, the included reference
128 *A. baumannii* strain which encodes DcaP1-4, were more similar to each other than to non-
129 *Acinetobacter* DcaP-family porins (Fig. 2B). This suggests that diversification of the four DcaP
130 classes defined here occurred within *Acinetobacter*. In contrast to *A. baumannii*, multiple DcaP-
131 like proteins encoded in other organisms were sequence divergent, such as in
132 *Pseudoxanthomonas daejeonensis*, *Stenotrophomonas spp*., *Shewanella psychrotolerans*, and
133 *Marinobacter spp.* (Fig 2B). Comparison of the species phylogeny and DcaP protein phylogeny
134 suggest there may have been occurrences of horizontal gene transfer in other species outside
135 *Acinetobacter*. Overall, these data suggest that DcaP-like proteins are encoded by species
136 across Pseudomonodota and that DcaP diversification has occurred within the *Acinetobacter*
137 clade.

138

139 ***DcaP family porins are necessary for growth on select di- and tri-carboxylic acids***

140 Next, we hypothesized that the DcaP-family porins are involved in small molecule
141 uptake. One study suggested that DcaP3 may translocate the clinically relevant beta-lactamase
142 inhibitor sulbactam based on electrophysiology and applied field simulation data (21). Many *A.*
143 *baumannii* isolates are intrinsically resistant to the beta-lactam ampicillin due to the expression
144 of beta-lactamases and sulbactam has been shown to resensitize resistant isolates to beta-
145 lactam antibiotics (31,32). We reasoned that if DcaP-family porins facilitated sulbactam entry, a
146 mutant lacking all *dcaP* genes would exclude sulbactam from entering the cell and result in
147 increased resistance to the beta-lactam ampicillin while a wildtype strain would exhibit re-
148 sensitization to ampicillin. We therefore constructed a triple mutant in ATCC 17978 lacking all
149 encoded DcaP proteins, Δ*dcaP1*Δ*dcaP2*Δ*dcaP3* (ΔΔΔ*dcaP1-3*). However, the wildtype and the
150 ΔΔΔ*dcaP1-3* mutant strains exhibited similar susceptibility to ampicillin/sulbactam treatment
151 (Fig. S2). These data suggest that DcaP-family porins are not required for sulbactam activity.

152 We next hypothesized that DcaP-family porins may be important for growth on certain
153 nutrient sources such as dicarboxylic acids as previously suggested (20). The growth of
154 wildtype and ΔΔΔ*dcaP1-3* strains were compared using the high-throughput Biolog PM system
155 to screen for carbon source utilization. Multiple compounds were identified on which ΔΔΔ*dcaP1-*
156 *3* grew more than wildtype and compounds on which wildtype was able to grow but ΔΔΔ*dcaP1-3*
157 had a defect (Fig. 3A-B). The screen suggested ΔΔΔ*dcaP1-3* had increased growth compared
158 to wildtype on amino acids such as glutamic acid and glutaminic acid (Fig. 3A-B); however,
159 validation showed that there was no difference in growth between wildtype and ΔΔΔ*dcaP1-3*
160 (Fig. S3B). Glutamic acid was thus used as a control for subsequent assays to ensure growth of
161 strains in minimal media. Butyric acid also appeared to better support growth of ΔΔΔ*dcaP1-3*
162 and was analyzed in subsequent assays. Many of the compounds on which WT grew better
163 than ΔΔΔ*dcaP1-3* were carboxylic acids, including simple carboxylic acids, hydroxy carboxylic

164 acids, and sugar acids (Fig. 3A-B). These data suggest that DcaP-family porins may allow the
165 influx of carboxylic acid-containing compounds, as previously predicted (20).
166
167 ***DcaP3 is important for growth on multiple carboxylic acid carbon sources***
168 To verify the Biolog results and further determine functional importance of the DcaP
169 classes, growth curves using Δ*dcaP1-3,* Δ*dcaP1*Δ*dcaP2*, Δ*dcaP1*Δ*dcaP3*, and
170 Δ*dcaP2*Δ*dcaP3* and carboxylic acids as sole carbon sources were performed. The strains were
171 also assayed on glutamic acid and succinic acid as controls for growth in minimal media, and
172 butyric acid as a potential carbon source in which Δ*dcaP1-3* grew to higher yield than WT.
173 There were no differences in growth in glutamic acid, and potential minor defects for growth of
174 the Δ*dcaP1-3* strain in succinic acid and butyric acid. This showed that the screen did not
175 identify any conditions where the Δ*dcaP1-3* strain grew more than WT. By contrast, mutants
176 lacking DcaP3 were defective for growth on citric acid, tricarballylic acid, and had a minor defect
177 on mucic acid compared to the WT (Fig. 4A). We were unable to identify conditions in which
178 DcaP1 or DcaP2 were required for growth. Similarly, a single Δ*dcaP3* mutation conferred a
179 defect in growth on citric acid, tricarballylic acid, and mucic acid, whereas single mutants of
180 Δ*dcaP1* or Δ*dcaP2* showed no defect (Fig. 4B).
181 A recent study in *A. baylyi* found that tricarballylic acid metabolism is closely linked with
182 the metabolism of *cis*- and *trans*-aconitic acid (33). The genetic organization of the tricarballylic
183 acid catabolic genes is conserved in *A. baumannii* compared to *A. baylyi* (Fig. S4A). Since the
184 data thus far suggest DcaP3 is important for growth on tricarballylic acid, we hypothesized that
185 DcaP3 may also be important for *cis*- and *trans*-aconitic acid growth. Mutants lacking DcaP3
186 had a defect for growth on both *cis*- and *trans*-aconitic acid (Fig. 4A) and the Δ*dcaP3* single
187 mutant had a defect in growth on both *cis*- and *trans*-aconitic acid compared to wildtype (Fig.
188 4B). To highlight the similarities in the carbon sources in which DcaP3 is important for growth,

189 the chemical structures for each carbon source are outlined in Fig. 4C. Given the lack of
190 identifiable phenotype for Δ*dcaP1* or Δ*dcaP2*, transcript reads from previously published
191 datasets were analyzed to determine if *dcaP1* or *dcaP2* are expressed (34). In *A. baumannii*
192 17978, *dcaP3* was expressed at a high level in untreated lysogeny broth while *dcaP1* and
193 *dcaP2* were not expressed; in *A. baumannii* AB5075, *dcaP3* was similarly highly expressed
194 while *dcaP1*, *dcaP2*, and *dcaP4* were not expressed (Fig. 4D) (34). Similar results were
195 observed in another *A. baumannii* 17978 dataset (35). Taken together, these data suggest that
196 DcaP3 is highly expressed and important for growth using multiple di- and tri-carboxylic acids as
197 sole carbon sources.
198
199 ***DcaP3 is required for growth on di- and tri- carboxylic acids in a recent clinical isolate***
200 To test if DcaP3 is important for growth on di- and tri-carboxylic acids in a more recent
201 clinical isolate, a Δ*dcaP3* mutant was generated in *A. baumannii* AB5075, a member of the
202 widely circulating clonal complex 1 that was isolated in 2008 and also serves as a commonly
203 used reference strain (36). An *A. baumannii* AB5075 mutant lacking *dcaP3* was defective for
204 growth on citric acid, tricarballylic acid, mucic acid, and *cis*- and *trans*-aconitic acid compared to
205 wildtype (Fig. 5). This suggests that the requirement for DcaP3 for growth on select carboxylic
206 acids as sole carbon sources *in vitro* is conserved among *A. baumannii* isolates.
207
208 ***DcaP1-4 can complement ΔΔΔdcaP for growth on some carboxylic acids***
209 Thus far, genetic disruption in *A. baumannii* ATCC 17978 and AB5075 suggested DcaP3 was
210 the primary DcaP important for utilization of certain di- and tri-carboxylic acids. However,
211 published expression analysis suggests only DcaP3 is expressed in laboratory conditions (Fig.
212 4D) (34,35). We hypothesized that individual DcaP proteins may complement the *A. baumannii*
213 17978 Δ*ΔΔdcaP1-3* growth defects if expressed from the *dcaP3* promoter. Therefore,
214 chromosomal mini-Tn*7* (mTn*7*) complementation constructs were constructed expressing *A.*

215 *baumannii* 17978 *dcaP1*, *dcaP2*, or *dcaP3* or *A. baumannii* AB5075 *dcaP4* from the *A.*
216 *baumannii* 17978 *dcaP3* promoter. Consistent with previous observations, the ΔΔΔ*dcaP* mutant
217 was unable to grow using citric acid, tricarballylic acid, or *cis*- and *trans*-aconitic acid and had a
218 slight defect on succinic acid, butyric acid, and mucic acid (Fig. 6). Growth was restored in
219 succinic acid and butyric acid by expressing *dcaP4* (Fig. 6), suggesting DcaP4 can contribute to
220 succinic acid and butyric acid transport. Similarly, growth on citric acid, mucic acid, tricarballylic
221 acid, and trans- and cis-aconitic acid was restored to the ΔΔΔ*dcaP1-3* mutant by expressing
222 *dcaP2, dcaP3*, or *dcaP4* (Fig. 6). This suggests that DcaP2, DcaP3, and DcaP4 have
223 overlapping functions in at least some *A. baumannii* strains and conditions. Expression of *dcaP1*
224 in the ΔΔΔ*dcaP1-3* strain did not restore growth in succinic acid, butyric acid, citric acid, mucic
225 acid, or tricarballylic acid but did restore growth in *trans*- and *cis*-aconitic acid, suggesting a
226 largely non-overlapping functional role for DcaP1.
227
228 ***DcaP3 is sufficient but not required for virulence in the liver and spleen during***
229 ***bloodstream infection of mice***
230 Carbon source utilization can be a critical determinant of pathogenesis for *A. baumannii*
231 and other bacterial species in different host niches and DcaP3 has been proposed as an *A.*
232 *baumannii* vaccine candidate (21–25,37,38). Therefore, we sought to determine if DcaP-family
233 porins are important for *A. baumannii* pathogenesis in a murine model of bloodstream infection.
234 There was no defect of the ΔΔΔ*dcaP1-3* mutant compared to wildtype in lung, kidney, or heart
235 (Fig. 7A). By contrast, there was a defect for the ΔΔΔ*dcaP1-3* mutant compared to wildtype in
236 the spleen and liver (Fig. 7A). The defect in the spleen and liver was reversed by
237 complementing *dcaP3* in the chromosome of the ΔΔΔ*dcaP1-3* mutant, showing DcaP3 is
238 sufficient for proliferation in the liver and spleen (Fig. 7B). However, a single Δ*dcaP3* mutant had
239 no defect during infection (Fig. 7C). This suggests that in the host, DcaP1 and/or DcaP2 are

240 expressed and able to functionally complement the lack of DcaP3 in *A. baumannii* 17978.
241 Together these data show that DcaP-family porins are important in specific host niches during
242 infection.
243
244 **DISCUSSION**
245 The OM protects *A. baumannii* from antimicrobials and other host stresses but must
246 permit influx of nutrients essential for replication. How nutrients pass through the *A. baumannii*
247 OM is not well understood. Here, we designate classes of a family of predicted porins, DcaP1-4,
248 that are widely distributed across *Acinetobacter spp.* and show DcaP-family porins are required
249 for growth on multiple carboxylic acids.
250 DcaP-family porins have been identified in genetic screens for biofilm formation,
251 desiccation, and mucin catabolism, largely referred to as DcaP-like proteins without further
252 distinction (26,27,39–41). We show that *A. baumannii* DcaP-family porins can be grouped into
253 four classes based on protein sequence (Fig. 1A, S1A). DcaP-encoding genes are broadly
254 conserved among *Acinetobacter* spp, and particularly in *A. baumannii* and the *acb* complex (Fig.
255 1B, Fig. S1B). While DcaP-family porins can be found in distantly related organisms (Fig. 2A),
256 the DcaP1-4 classes we define in *A. baumannii* cluster together (Fig. 2B). These findings
257 suggest DcaP1-4 diversified within the *Acinetobacter* clade.
258 Antibiotic resistance in *A. baumannii* is a critical public health problem due to the
259 emergence of XDR isolates. Various porins in *A. baumannii* have been implicated in the uptake
260 of toxic molecules including antibiotics (12,42–44). DcaP3 was proposed to allow entry for the
261 beta-lactamase inhibitor sulbactam (21). Here, we show that a ΔΔΔ*dcaP1-3* mutant displays
262 similar susceptibility to ampicillin/sulbactam as the wildtype *A. baumannii* 17978, suggesting
263 that DcaP-family porins are not required for sulbactam function (Fig. S2). However, additional
264 studies would be required to determine whether sulbactam may enter via DcaP-family porins in
265 addition to other outer membrane porins.

266 The transport of essential nutrients across the cell envelope is incompletely understood
267 in *A. baumannii*. In general, *A. baumannii* utilizes organic acids and amino acids as carbon
268 sources and does not utilize sugars such as glucose (37,45). Accordingly, multiple OM channels
269 have been identified that are important for growth on specific carbon sources including ornithine,
270 arginine, glutamic acid, and benzoic acid (16–19,46). One study in ATCC 19606 found that
271 *dcaP1* (*A1S_1380*) was upregulated in the presence of mucin (26), suggesting that the host
272 environment may affect *dcaP* gene regulation. In a Biolog screen, we identified multiple
273 carboxylic acid carbon sources that supported significantly more growth of the wildtype
274 compared to the ΔΔΔ*dcaP1-3* mutant (Fig. 3), suggesting DcaP-family porins are important for
275 their diffusion through the OM.
276 The data here suggest DcaP3 is responsible for the influx of carboxylic acid carbon
277 sources citric acid, mucic acid, tricarballylic acid, and *cis*- and *trans*- aconitic acid in *A.*
278 *baumannii* ATCC 17978 and AB5075 in the *in vitro* conditions tested, as mutants lacking DcaP3
279 show decreased growth on these substrates (Fig. 4-5). Phylogenomics analysis found three *A.*
280 *baumannii* strains lacking *dcaP3*: *A. baumannii* E-011922, *A. baumannii* VB82, and *A.*
281 *baumannii* RBH2 (Fig. S1B) (47–49). Of these, carbon utilization data were reported for *A.*
282 *baumannii* E-011922, an environmental isolate that retains the ability to grow on citric acid and
283 mucic acid despite not encoding DcaP3 (48). Similarly, *A. baylyi* can grow on citric acid and
284 tricarballylic acid and does not encode DcaP3 but rather DcaP1, DcaP2, and DcaP4 (33). This
285 suggests that in strains that lack DcaP3, other DcaP-family porins are likely expressed at higher
286 abundance and allow transport of these carboxylic acids.
287 Citric acid is an intermediate of the tricarboxylic acid (TCA) cycle, a ubiquitous pathway
288 for energy generation. Studies quantifying citric acid in rat and mouse tissues report it is 0.15-
289 0.3 μmol/g in the liver and 0.27-0.45 μmol/g in the kidney (50–52), suggesting citric acid is
290 abundant in some tissues. Despite this, many bacteria cannot utilize citric acid as a carbon
291 source and how citric acid permeates the OM is unknown (53). Mutants in *P. aeruginosa* OpdH

292 were defective for growth on *cis*-aconitic acid, but not on citric acid, suggesting they encode
293 other citric acid transporters (54,55). A ΔΔΔ*dcaP1-3* mutant displayed a strong defect for growth
294 on citric acid that can be rescued by complementing with *dcaP2*, *dcaP3* or *dcaP4* (Fig. 4A, 4B,
295 Fig. 5). Therefore, encoding multiple porins for OM citric acid passage may be conserved
296 across bacterial genera.

297 Mucic acid, or galactaric acid, is a sugar acid derived from the oxidation of galactose and
298 is unrelated to mucin. Mucic acid is not typically found in the host; but, some antibiotic
299 treatments result in increased mucic acid in the human gut (56–58). However, only a subset of
300 *A. baumannii* strains, including ATCC 17978, encode the D-galactaric acid dehydrogenase
301 required for growth on mucic acid (59). The digestive tract is a reservoir for *A. baumannii*
302 colonization that increases the potential for infection and transmission and *A. baumannii* ATCC
303 17978 can asymptomatically colonize the mouse gut (60–65). Thus, mucic acid may be used as
304 carbon source by some *A. baumannii* in the inflamed gut. Our data suggest that DcaP2, DcaP3
305 or DcaP4 are important for growth on mucic acid.

306 The catabolism of *cis*- and *trans*-aconitic acid has been described in *A. baylyi*, and the
307 catabolism of tricarballylic acid in *A. baylyi* and *Salmonella* (33,66,67). However, the OM porin
308 required for growth on these carbon sources in *Acinetobacter* was unknown. Our data suggest
309 that DcaP2, DcaP3, and DcaP4 porins are important for growth on tricarballylic acid, and that
310 DcaP1 can also support growth on *cis*- and *trans*-aconitic acid.

311 Overall, our data suggests that DcaP3 is the major porin for passive transport of specific
312 carboxylic acid carbon sources in the *in vitro* conditions tested. The requirement for DcaP3 in
313 growth on select di- and tri-carboxylic acids is conserved in the more recent clinical isolate *A.*
314 *baumannii* AB5075, as a Δ*dcaP3* mutant had reduced growth in these conditions (Fig. 6).
315 Published RNA sequencing data show that *dcaP3* is the only *dcaP* gene expressed under
316 laboratory conditions tested, consistent with the defects for the Δ*dcaP3* single mutants in *A.*
317 *baumannii* 17978 and AB5075 strain backgrounds (Fig. 4B, Fig. 5) (34,35).

318 During infection, bacteria must scavenge nutrients from the host to replicate. The *A.*
319 *baumannii* ATCC 17978 ΔΔΔ*dcaP1-3* mutant had a defect compared to wildtype in the mouse
320 liver and spleen that was complemented by expression of *dcaP3* (Fig. 7A-B). However, the
321 Δ*dcaP3* single mutant had no defect (Fig. 7C). Therefore, DcaP3 passive import of carboxylic
322 acid carbon sources is sufficient but not necessary for pathogenesis in the liver and spleen. This
323 suggests the host environment may lead to increased expression of other *dcaP* genes;
324 regulation of *dcaP* expression and the molecular basis for substrate specificity is an area for
325 future study. Additionally, future study will be required to dissect the relevant carboxylic acid
326 carbon sources and DcaP substrates important for *A. baumannii* infection. There is little known
327 about the role for these carboxylic acid carbon sources during bacterial infection. Citric acid has
328 been shown to serve as a signaling molecule for *Staphylococcus aureus* pathogenesis; citric
329 acid was increased in the lung at 4 weeks during *Mycobacterium tuberculosis* infection, and
330 citric acid utilization promoted *Vibrio cholerae* small intestine colonization (68,69). The *Klebsiella*
331 *pneumoniae* citric acid synthase GltA has been shown to be important due to amino acid
332 biosynthesis required in the spleen, liver, and gut, but dispensable in the bloodstream (70).
333 Succinic acid has also been shown to be an important carbon source for *Pseudomonas*
334 *aeruginosa* during infection (71). Alternatively, DcaP-family porins may play another role in
335 pathogenesis. For example, porins in *A. baumannii* have demonstrated roles for the adhesion or
336 invasion of eukaryotic cells (13,72–75). DcaP3 has been proposed as a vaccine candidate due
337 to its high expression in infection models and immunogenicity (21–25). These data suggest that
338 vaccine strategies to functionally block DcaP proteins must block multiple DcaP proteins to
339 affect *A. baumannii* virulence.
340 In summary, we further classify a family of porins previously termed DcaP-like into four
341 classes in *Acinetobacter* spp. This classification allows for the differentiation between DcaP-like
342 proteins and the potential to determine individual functions for DcaP1-4 in *Acinetobacter* spp.
343 DcaP3 was critically important for growth on citric acid, tricarballylic acid, mucic acid, and *cis*-

344 and *trans*-aconitic acid as sole carbon sources. In a murine model of bloodstream infection,
345 DcaP3 was sufficient but not necessary for infection, suggesting functional overlap among DcaP
346 porins in the host. Expressing *dcaP2*, *dcaP3*, or *dcaP4* complemented growth of the *A.*
347 *baumannii* 17978 ΔΔΔ*dcaP1-3* mutant on all carbon sources tested and expression of *dcaP1*
348 complemented growth on *trans*- and *cis*-aconitic acid (Fig. 5). Our data therefore provides a
349 deeper understanding of how *A. baumannii* acquires nutrients including in the host.
350
351
352 **MATERIALS AND METHODS**
353 Bacterial strain construction and growth
354 All bacterial strains and plasmids used in this study are listed in Tables S1 and S2,
355 respectively. The wildtype strain was *A. baumannii* ATCC 17978VU or AB5075 (36,76,77).
356 Oligonucleotides are listed in Table S3 and were purchased from Integrated DNA Technologies
357 (Coralville, IA). Strains were grown in lysogeny broth (LB; Miller) or on LB with 1.5% (wt/vol)
358 agar unless indicated otherwise. Antibiotics were used at the following concentrations:
359 carbenicillin (Carb), 75 mg/L; kanamycin (Kan), 40 mg/L; chloramphenicol, 15 mg/L; hygromycin
360 (Hyg) 300 mg/L. For deletion constructs, 1000 bp of 3' and 5' flanking DNA to the gene of
361 interest was amplified using Q5 High Fidelity 2x Master Mix [New England Biolabs (NEB),
362 Ipswich, MA] from *A. baumannii* ATCC 17978VU or AB5075. The vector pFLP2 was digested
363 with BamHI and KpnI restriction enzymes (NEB, Ipswitch, MA) and gel purified (78). The
364 kanamycin or hygromycin cassette was amplified from pKD4 or pLDP357, respectively (79). A
365 hygromycin-resistant excisable cassette was generated by replacing kanamycin resistance in
366 pKD4 with hygromycin resistance amplified from an *A. baumannii* AB5075 transposon T101
367 mutant (36,77) with XR185/XR186 oligonucleotides and ligation with NEBuilder® HiFi DNA
368 Assembly Master Mix (NEB, Ipswich, MA) to generate pLDP357. Fragments were ligated
369 together using HiFi ligation mix (NEB, Ipswitch, MA), transformed into chemically competent *E.*

370 *coli* DH5α, and plated to selective media. Transformants were confirmed to contain the insert by
371 PCR. To generate the knockout strains, a triparental conjugation using helper *E. coli* strain
372 HB101 containing pRK2013 was performed. Single-crossover merodiploids containing the
373 integrated pFLP2 vector were chosen based on a $Kan^R$ $Carb^R$ or $Hyg^R$ and sucrose-sensitive
374 (10% w/v; $Suc^S$) phenotype. Putative merodiploids displaying were verified by PCR.
375 Merodiploids were then grown on LB agar, resuspended, and plated to LB agar or LB agar with
376 10% sucrose to select for colonies with the second crossover event. Colonies were then
377 screened for a $Kan^R$ $Carb^S$ $Suc^R$ or $Hyg^R$ $Suc^S$ phenotype. Mutants were confirmed via PCR.
378 For chromosomal integration constructs, DNA from ATCC 17978VU or AB5075 was
379 amplified using Q5 High Fidelity 2x Master Mix (NEB, Ipswich, MA). The mTn7 pKNOCK vector
380 was digested with BamHI and KpnI prior to ligation using HiFi ligation mix (NEB, Ipswitch, MA)
381 and transformation into chemically competent *E. coli* DH5α λ*pir116*$^+$ (80). To generate
382 chromosomally integrated mTn7 constructs, a modified four-way parental mating was performed
383 using helper *E. coli* strain HB101 containing pRK2013 and *E. coli* DH5α containing pTNS2 (81).
384 Colonies were screened by PCR for integration into the correct chromosomal locus at the *att*
385 site. All plasmid constructs were verified by whole plasmid sequencing before transforming into
386 *A. baumannii* (Plasmidsaurus, South San Francisco, CA).
387 The *A. baumannii* AB5075 wildtype and Δ*dcaP3*::hyg mutant were verified to have the
388 virulent/opaque (VIR-O) phenotype on 1/2X LB agar plates (82,83).
389
390 Phylogenetic tree construction
391 To identify DcaP orthologs and classify them into hierarchical orthogroups (HOGs),
392 OrthoFinder v2.5.5 was used (84). Two datasets were analyzed: (i) a 253-strain dataset
393 including 235 *A. baumannii* strains deduplicated as previously described (85); (ii) a 28-species
394 dataset that included multiple *Acinetobacter* and a broader range of gammaproteobacteria

395 rooted by one alphaproteobacterial. The protein accession numbers and genome accession
396 numbers are listed in Table S4.
397 Non-Acinetobacter species were selected based on the conservation of DcaP orthologs
398 across the genus or species indicated by KEGG. Specific organisms were selected to portray a
399 representative distribution of DcaP orthologs across bacteria, a group of 22 species. The
400 FastTree v2.1.11-derived (86), manually rooted species tree was used as an input for
401 OrthoFinder HOG inference (84,87), ensuring accurate classification of gene duplication and
402 speciation events. The resulting DcaP classifications were visualized and annotated in iTOL
403 (88). To infer a species phylogeny, a set of 120 universal bacterial marker genes (Bac120) was
404 identified from each proteome using HMMER3 (hmmsearch, v3.3), following the methodology
405 described in Parks *et al*. 2018 (89). The majority of species had only one significant hit for each
406 of the 120 protein HMMs; if a species had more than one hit, the best-scoring hit for that marker
407 was extracted.
408 Each of the 120 marker proteins was independently aligned to its respective HMM profile
409 using hmmalign (HMMER v3.3). Alignments were trimmed to remove poorly aligned regions and
410 concatenated into a single concatenated supermatrix for each species. To improve alignment
411 quality, columns were filtered based on the following criteria: (i) highly gapped regions (>80%
412 gaps) were removed; (ii) completely homogenous columns were excluded to reduce
413 redundancy.
414 Maximum likelihood species trees were inferred from the filtered concatenated alignment
415 using FastTree v2.1.11, with the LG model of amino acid evolution. The resulting unrooted trees
416 were manually rooted and annotated in iTOL using *Brevundimonas subvibrioides* or *E. coli* as
417 outgroups for the smaller and larger datasets.
418
419 <u>DcaP protein phylogeny</u>

420 DcaP homologs were extracted from their respective proteomes by selecting sequences
421 within the DcaP HOG at the root of all *Acinetobacter* species in the 255-species dataset in
422 Orthofinder. To ensure consistent domain identification, sequences were aligned to the Pfam
423 Hidden Markov Model (HMM) PF19577.4, corresponding to the DcaP OM protein family.
424 Alignments were trimmed to remove poorly aligned residues prior to phylogenetic inference. A
425 maximum likelihood phylogenetic tree was constructed using FastTree v2.1.11 with the LG
426 model of amino acid evolution. The inferred clusters were identical to OrthoFinder's DcaP
427 subfamilies, except for a small cluster of DcaP proteins that OrthoFinder grouped with DcaP1,
428 while FastTree grouped the same cluster with DcaP3.
429 To identify putative orthologs of DcaP proteins from the 22 species identified above and
430 *A. baumannii* AB5075, proteomes from genomic assemblies were downloaded from the NCBI
431 Datasets database. Two complementary approaches were employed to verify orthology. First,
432 HMMER v3.4 was used to search each proteome with the Pfam profile HMM PF19577,
433 corresponding to the DcaP protein family. Significant hits were retained as candidate DcaP
434 homologs. To independently assess orthology and reconstruct gene relationships, OrthoFinder
435 v2.5.5 was run on all 23 proteomes, which also produced a rooted gene tree for the DcaP
436 family. The resulting DcaP sequences were realigned to the PF19577 HMM using hmmalign,
437 and poorly aligned or unaligned amino acids were trimmed. A maximum likelihood phylogeny
438 was then inferred using RAxML v8.2.12, under the LG amino acid substitution model.
439 Amino acid sequence alignment and percent identity were determined using the Clustal
440 Omega program (1.2.4) through UniProt and the DcaP-family porin secondary structure was
441 based on PDB entry 6EUS (28,90,91).
442
443
444 <u>Carbon source utilization screen using Biolog plates</u>

445 Plates PM1 and PM2A were obtained from Biolog for carbon source utilization testing. A
446 110 μL aliquot of defined M9 minimal media lacking carbon was dispensed into every well and
447 resuspended. Then, 100 μL was transferred from the Biolog plates into sterile flat-bottom 96-
448 well plates. Overnight bacterial cultures were diluted 100-fold in sterile PBS before 1 μL of
449 diluted culture was inoculated into every well. Plates were incubated at 37°C with shaking and
450 growth was monitored by optical density at 600 nm ($OD_{600}$) in an EPOCH2 or SynergyH1 BioTek
451 plate reader (Winooski, VT). Background absorbance was subtracted before area under the
452 curve calculations for each isolate and each compound were calculated and the difference
453 between wildtype and ΔΔΔ*dcaP1-3* was analyzed. Compounds were included if any isolate of
454 either wildtype or ΔΔΔ*dcaP1-3* reached an $OD_{600}$ of 0.15 or higher.
455
456 Growth curve analyses
457 M9 minimal medium without glucose was supplemented 0.1X Vishniac's trace minerals
458 (92) with the specified carbon source at a concentration to maintain the carbon equivalency of a
459 4-carbon compound at 16.5 mM. Cultures were inoculated with a single bacterial colony and
460 grown in 3 mL LB at 37°C with shaking at 180 rpm for 8-16 hours. Growth curves were
461 performed by diluting LB cultures 100-fold in sterile PBS and inoculating 1 μL diluted culture into
462 99 μL media in a 96-well flat bottom plate. Growth was monitored as described above.
463
464 Murine bloodstream infection
465 Six-week-old female and male ND4 Swiss Webster mice were purchased from
466 Envigo/Harlan Laboratory. Mice were boarded in a temperature-controlled environment with
467 14:10 h light:dark cycles and food and water were provided as needed. Mice were acclimated to
468 the facility for 1 week prior to infection. Mice were anesthetized with isoflurane and inoculated
469 retro-orbitally with approximately $3 \times 10^8$ CFU in a 50 μL bacterial suspension of a 1:1 mixture of

470 *A. baumannii* ATCC 17978VU wildtype and ΔΔΔ*dcaP1-3*::Kn mutant, ΔΔΔ*dcaP1-3*::Kn+*dcaP3*,
471 or Δ*dcaP3*::Kn. For wildtype versus ΔΔΔ*dcaP1-3,* 5 of the mice were infected with wildtype
472 ATCC 17978VU and 5 were infected with ATCC 17978 mTn7(Carb$^R$); for the wildtype versus
473 Δ*dcaP3*, wildtype was ATCC 17978 mTn7(Carb$^R$). All mice infected with the complement strain
474 were infected with ATCC 17978VU. The inoculum dose was determined by serial dilution and
475 plating on selective agar media. Mice were euthanized at 24 h post infection by
476 $CO_2$ asphyxiation, and the organs were excised aseptically. Tissues were homogenized in PBS
477 using a Bullet Blender (Next Advance, Troy, NY), and all samples were serially diluted and
478 plated on LB and LB with carbenicillin or kanamycin selective agar plates for bacterial
479 enumeration. All animal care protocols were approved by the University of Illinois Chicago
480 Institutional Animal Care and Use Committee (IACUC; protocol number 23–119) in accordance
481 with the Animal Care Policies of UIC, the Animal Welfare Act, the National Institutes of Health,
482 and the American Veterinary Medical Association (AVMA). Animals were humanely euthanized
483 consistent with the AVMA guidelines.
484
485 Data reporting, statistical analysis, and figure preparation
486 Each measurement was taken from a distinct biological sample (e.g., bacterial culture
487 from a single colony or an individual mouse). Data processing and statistical analyses were
488 performed using Microsoft Excel 16.98 and GraphPad Prism 10.4.2 or 10.6.1. Statistical tests
489 used are indicated in each figure legend. Figures were prepared in Adobe Illustrator 25.2.3 and
490 30.2.1.
491
492 Author contributions
493 H.R.N. – project conceptualization, methodology, data collection, data analysis, and writing.
494 N.R.K. – data collection, data analysis, and writing. J.D.W – bioinformatics, data analysis, and

writing. L.D.P. – project conceptualization, methodology, data analysis, writing, funding, and resources.

**ACKNOWLEDGMENTS**

We thank the members of the Palmer laboratory for critical reading of the manuscript, Xiaomei Ren for construction of pLDP357, and funding from the National Institutes of Health R01AI189516 to L.D.P. and the Michael Reese Research and Education Foundation Pioneer in Research Award.

## REFERENCES


1. Clark NM, Zhanel GG, Lynch JPI. Emergence of antimicrobial resistance among *Acinetobacter* species: a global threat. Curr Opin Crit Care. 2016 Oct;22(5):491. doi:10.1097/MCC.0000000000000337

2. World Health Organization. No time to wait: securing the future from drug-resistant infections [Report to the Secretary-General of the United Nations]. World Health Organization and Interagency Coordination Group on Antimicrobial Resistance; 2019 Apr. Report No.

3. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol. 2016 Nov;37(11):1288–301. doi:10.1017/ice.2016.174

4. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018 Mar 1;18(3):318–27. doi:10.1016/S1473-3099(17)30753-3

5. Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harb Perspect Biol. 2010 May;2(5):a000414. doi:10.1101/cshperspect.a000414 PubMed PMID: 20452953; PubMed Central PMCID: PMC2857177.

6. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003 Dec;67(4):593–656. doi:10.1128/mmbr.67.4.593-656.2003

7. Geisinger E, Huo W, Hernandez-Bird J, Isberg RR. *Acinetobacter baumannii*: envelope determinants that control drug resistance, virulence, and surface variability. Annu Rev Microbiol. 2019;73(1):481–506. doi:10.1146/annurev-micro-020518-115714 PubMed PMID: 31206345.

8. Kenyon JJ, Hall RM. Variation in the complex carbohydrate biosynthesis loci of *Acinetobacter baumannii* genomes. PLOS ONE. 2013 Apr 16;8(4):e62160. doi:10.1371/journal.pone.0062160

9. Kenyon JJ, Nigro SJ, Hall RM. Variation in the OC locus of *Acinetobacter baumannii* genomes predicts extensive structural diversity in the lipooligosaccharide. PLOS ONE. 2014 Sep 23;9(9):e107833. doi:10.1371/journal.pone.0107833

10. Silale A, Berg B van den. TonB-dependent transport across the bacterial outer membrane. Annu Rev Microbiol. 2023 Sep 15;77(Volume 77, 2023):67–88. doi:10.1146/annurev-micro-032421-111116

11. Sugawara E, Nikaido H. OmpA is the principal nonspecific slow porin of *Acinetobacter baumannii*. J Bacteriol. 2012 Jul 18;194(15):4089–96. doi:10.1128/jb.00435-12

542 12. Iyer R, Moussa SH, Durand-Réville TF, Tommasi R, Miller A. *Acinetobacter baumannii*
543 OmpA Is a selective antibiotic permeant porin. ACS Infect Dis. 2018 Mar 9;4(3):373–81.
544 doi:10.1021/acsinfecdis.7b00168

545 13. Scribano D, Cheri E, Pompilio A, Di Bonaventura G, Belli M, Cristina M, et al. *Acinetobacter*
546 *baumannii* OmpA-like porins: functional characterization of bacterial physiology, antibiotic-
547 resistance, and virulence. Commun Biol. 2024 Aug 6;7(1):1–11. doi:10.1038/s42003-024-
548 06645-0

549 14. Samsudin F, Ortiz-Suarez ML, Piggot TJ, Bond PJ, Khalid S. OmpA: a flexible clamp for
550 bacterial cell wall attachment. Structure. 2016 Dec 6;24(12):2227–35.
551 doi:10.1016/j.str.2016.10.009

552 15. Park JS, Lee WC, Yeo KJ, Ryu KS, Kumarasiri M, Hesek D, et al. Mechanism of anchoring
553 of OmpA protein to the cell wall peptidoglycan of the gram-negative bacterial outer
554 membrane. FASEB J. 2012 Jan;26(1):219. doi:10.1096/fj.11-188425 PubMed PMID:
555 21965596.

556 16. Mussi MA, Relling VM, Limansky AS, Viale AM. CarO, an *Acinetobacter baumannii* outer
557 membrane protein involved in carbapenem resistance, is essential for l-ornithine uptake.
558 FEBS Lett. 2007;581(29):5573–8. doi:10.1016/j.febslet.2007.10.063

559 17. Zahn M, Bhamidimarri SP, Baslé A, Winterhalter M, van den Berg B. Structural insights into
560 outer membrane permeability of *Acinetobacter baumannii*. Structure. 2016 Feb 2;24(2):221–
561 31. doi:10.1016/j.str.2015.12.009

562 18. Clark TJ, Momany C, Neidle EL. The *benPK* operon, proposed to play a role in transport, is
563 part of a regulon for benzoate catabolism in *Acinetobacter* sp. strain ADP1. Microbiology.
564 2002;148(4):1213–23. doi:10.1099/00221287-148-4-1213

565 19. Smith MA, Weaver VB, Young DM, Ornston LN. Genes for chlorogenate and
566 hydroxycinnamate catabolism (hca) are linked to functionally related genes in the *dca-pca-*
567 *qui-pob-hca* chromosomal cluster of *Acinetobacter* sp. strain ADP1. Appl Environ Microbiol.
568 2003 Jan;69(1):524–32. doi:10.1128/AEM.69.1.524-532.2003

569 20. Parke D, Garcia MA, Ornston LN. Cloning and genetic characterization of *dca* genes
570 required for β-oxidation of straight-chain dicarboxylic acids in *Acinetobacter* sp. strain ADP1.
571 Appl Environ Microbiol. 2001 Oct;67(10):4817–27. doi:10.1128/AEM.67.10.4817-4827.2001

572 21. Bhamidimarri SP, Zahn M, Prajapati JD, Schleberger C, Söderholm S, Hoover J, et al. A
573 multidisciplinary approach toward identification of antibiotic scaffolds for *Acinetobacter*
574 *baumannii*. Structure. 2019 Feb 5;27(2):268-280.e6. doi:10.1016/j.str.2018.10.021

575 22. Raoufi Z, Abdollahi S, Armand R. DcaP porin and its epitope-based subunit promise
576 effective vaccines against *Acinetobacter baumannii*; *in-silico* and *in-vivo* approaches. Microb
577 Pathog. 2022 Jan;162:105346. doi:10.1016/j.micpath.2021.105346 PubMed PMID:
578 34864145.

579 23. Fereshteh S, Ajdary S, Sepehr A, Bolourchi N, Barzi SM, Haririzadeh Jouriani F, et al.
580 Immunization with recombinant DcaP-like protein and AbOmpA revealed protections against
581 sepsis infection of multi-drug resistant *Acinetobacter baumannii* ST2Pas in a C57BL/6

582 mouse model. Microb Pathog. 2023 Jan;174:105882. doi:10.1016/j.micpath.2022.105882
583 PubMed PMID: 36403713.

584 24. Khalid K, Irum S, Ullah SR, Andleeb S. In-silico vaccine design based on a novel vaccine
585 candidate against infections caused by *Acinetobacter baumannii*. Int J Pept Res Ther. 2021
586 Dec 2;28(1):16. doi:10.1007/s10989-021-10316-7

587 25. Fereshteh S, Abdoli S, Shahcheraghi F, Ajdary S, Nazari M, Badmasti F. New putative
588 vaccine candidates against *Acinetobacter baumannii* using the reverse vaccinology method.
589 Microb Pathog. 2020 Jun 1;143:104114. doi:10.1016/j.micpath.2020.104114

590 26. Ohneck EJ, Arivett BA, Fiester SE, Wood CR, Metz ML, Simeone GM, et al. Mucin acts as a
591 nutrient source and a signal for the differential expression of genes coding for cellular
592 processes and virulence factors in *Acinetobacter baumannii*. PLoS ONE. 2018 Jan
593 8;13(1):e0190599. doi:10.1371/journal.pone.0190599 PubMed PMID: 29309434; PubMed
594 Central PMCID: PMC5757984.

595 27. Cabral MP, Soares NC, Aranda J, Parreira JR, Rumbo C, Poza M, et al. Proteomic and
596 functional analyses reveal a unique lifestyle for *Acinetobacter baumannii* biofilms and a key
597 role for histidine metabolism. J Proteome Res. 2011 Aug 5;10(8):3399–417.
598 doi:10.1021/pr101299j

599 28. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of
600 high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011
601 Oct 11;7:539. doi:10.1038/msb.2011.75 PubMed PMID: 21988835; PubMed Central PMCID:
602 PMC3261699.

603 29. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res.
604 2000 Jan 1;28(1):27–30. doi:10.1093/nar/28.1.27 PubMed PMID: 10592173; PubMed
605 Central PMCID: PMC102409.

606 30. Kanehisa M, Furumichi M, Sato Y, Matsuura Y, Ishiguro-Watanabe M. KEGG: biological
607 systems database as a model of the real world. Nucleic Acids Res. 2025 Jan
608 6;53(D1):D672–7. doi:10.1093/nar/gkae909 PubMed PMID: 39417505; PubMed Central
609 PMCID: PMC11701520.

610 31. Ji J, Du X, Chen Y, Fu Y, Wang H, Yu Y. In vitro activity of sulbactam in combination with
611 imipenem, meropenem, panipenem or cefoperazone against clinical isolates of
612 *Acinetobacter baumannii*. Int J Antimicrob Agents. 2013 Apr 1;41(4):400–1.
613 doi:10.1016/j.ijantimicag.2012.12.014

614 32. Choi JY, Soo Park Y, Cho CH, Seon Park Y, Shin SY, Song YG, et al. Synergic in-vitro
615 activity of imipenem and sulbactam against *Acinetobacter baumannii*. Clin Microbiol Infect.
616 2004 Dec 1;10(12):1098–101. doi:10.1111/j.1469-0691.2004.00987.x

617 33. Baugh AC, Defalco JB, Duscent-Maitland CV, Tumen-Velasquez MP, Laniohan NS, Figatner
618 K, et al. Regulation of tricarboxylate transport and metabolism in *Acinetobacter baylyi* ADP1.
619 Appl Environ Microbiol. 2024 Jan 30;90(2):e02111-23. doi:10.1128/aem.02111-23

620 34. Rokes AB, Santos-Lopez A, Cooper VS. History shapes regulatory and evolutionary
621 responses to tigecycline in two reference strains of *Acinetobacter baumannii*. Microbiology.

2025 Jun;171(6):001570. doi:10.1099/mic.0.001570 PubMed PMID: 40489210; PubMed Central PMCID: PMC12149411.

35. Kröger C, MacKenzie KD, Alshabib EY, Kirzinger MWB, Suchan DM, Chao TC, et al. The primary transcriptome, small RNAs and regulation of antimicrobial resistance in *Acinetobacter baumannii* ATCC 17978. Nucleic Acids Res. 2018 Oct 12;46(18):9684–98. doi:10.1093/nar/gky603 PubMed PMID: 29986115; PubMed Central PMCID: PMC6182133.

36. Jacobs AC, Thompson MG, Black CC, Kessler JL, Clark LP, McQueary CN, et al. AB5075, a highly virulent isolate of *Acinetobacter baumannii*, as a model strain for the evaluation of pathogenesis and antimicrobial treatments. mBio. 2014 May 27;5(3):e01076-01014. doi:10.1128/mBio.01076-14 PubMed PMID: 24865555; PubMed Central PMCID: PMC4045072.

37. Ren X, Palmer LD. *Acinetobacter* metabolism in infection and antimicrobial resistance. Infect Immun. 2023 May 16;91(6):e00433-22. doi:10.1128/iai.00433-22

38. Barber MF, Fitzgerald JR. Mechanisms of host adaptation by bacterial pathogens. FEMS Microbiol Rev. 2024 Jul 1;48(4):fuae019. doi:10.1093/femsre/fuae019

39. Rumbo-Feal S, Gómez MJ, Gayoso C, Álvarez-Fraga L, Cabral MP, Aransay AM, et al. Whole transcriptome analysis of *Acinetobacter baumannii* assessed by RNA-sequencing reveals different mRNA expression profiles in biofilm compared to planktonic cells. PLoS ONE. 2013 Aug 30;8(8):e72968. doi:10.1371/journal.pone.0072968 PubMed PMID: 24023660; PubMed Central PMCID: PMC3758355.

40. Shin JH, Lee HW, Kim SM, Kim J. Proteomic analysis of *Acinetobacter baumannii* in biofilm and planktonic growth mode. J Microbiol. 2009 Dec 1;47(6):728–35. doi:10.1007/s12275-009-0158-y

41. Gayoso CM, Mateos J, Méndez JA, Fernández-Puente P, Rumbo C, Tomás M, et al. Molecular mechanisms involved in the response to desiccation stress and persistence in *Acinetobacter baumannii*. J Proteome Res. 2014 Feb 7;13(2):460–76. doi:10.1021/pr400603f PubMed PMID: 24299215.

42. Liu J, Chen S, Zhao B, Li G, Ma T. A novel FadL homolog, AltL, mediates transport of long-chain alkanes and fatty acids in *Acinetobacter venetianus* RAG-1. Appl Environ Microbiol. 2022 Sep 28;88(20):e01294-22. doi:10.1128/aem.01294-22

43. Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of β-barrel outer membrane proteins. Antimicrob Agents Chemother. 2005 Apr;49(4):1432–40. doi:10.1128/AAC.49.4.1432-1440.2005 PubMed PMID: 15793123; PubMed Central PMCID: PMC1068641.

44. Catel-Ferreira M, Coadou G, Molle V, Mugnier P, Nordmann P, Siroy A, et al. Structure-function relationships of CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. J Antimicrob Chemother. 2011 Sep;66(9):2053–6. doi:10.1093/jac/dkr267 PubMed PMID: 21705362.

661 45. Juni E. Genetics and physiology of *Acinetobacter*. Annu Rev Microbiol. 1978;32:349–71.
662 doi:10.1146/annurev.mi.32.100178.002025 PubMed PMID: 360969.

663 46. Zahn M, D'Agostino T, Eren E, Baslé A, Ceccarelli M, van den Berg B. Small-molecule
664 transport by CarO, an abundant eight-stranded β-Barrel outer membrane protein from
665 *Acinetobacter baumannii*. J Mol Biol. 2015 Jul 17;427(14):2329–39.
666 doi:10.1016/j.jmb.2015.03.016

667 47. Ambrose SJ, Hamidian M, Hall RM. The extensively antibiotic resistant ST111 *Acinetobacter*
668 *baumannii* isolate RBH2 carries an extensive mobile element complement of plasmids,
669 transposons and insertion sequences. Plasmid. 2023 Sep;128:102707.
670 doi:10.1016/j.plasmid.2023.102707

671 48. Hamidian M, Maharjan RP, Farrugia DN, Delgado NN, Dinh H, Short FL, et al. Genomic and
672 phenotypic analyses of diverse non-clinical *Acinetobacter baumannii* strains reveals strain-
673 specific virulence and resistance capacity. Microb Genomics. 2022 Feb;8(2):000765.
674 doi:10.1099/mgen.0.000765 PubMed PMID: 35166651; PubMed Central PMCID:
675 PMC8942024.

676 49. Prity FT, Tobin LA, Maharajan R, Paulsen IT, Cain AK, Hamidian M. The evolutionary tale of
677 eight novel plasmids in a colistin-resistant environmental *Acinetobacter baumannii* isolate.
678 Microb Genomics. 2023 May;9(5):mgen001010. doi:10.1099/mgen.0.001010 PubMed
679 PMID: 37171842; PubMed Central PMCID: PMC10272872.

680 50. Spencer AF, Lowenstein JM. Citrate content of liver and kidney of rat in various metabolic
681 states and in fluoroacetate poisoning. Biochem J. 1967 May;103(2):342–8.
682 doi:10.1042/bj1030342 PubMed PMID: 6032977; PubMed Central PMCID: PMC1270414.

683 51. Ross KL, Eisenstein RS. Iron deficiency decreases mitochondrial aconitase abundance and
684 citrate concentration without affecting tricarboxylic acid cycle capacity in rat liver. J Nutr.
685 2002 Apr;132(4):643–51. doi:10.1093/jn/132.4.643

686 52. Tanner GA, Tanner JA. Citrate therapy for polycystic kidney disease in rats. Kidney Int. 2000
687 Nov;58(5):1859–69. doi:10.1111/j.1523-1755.2000.00357.x PubMed PMID: 11044205.

688 53. Blount ZD, Borland CZ, Lenski RE. Historical contingency and the evolution of a key
689 innovation in an experimental population of Escherichia coli. Proc Natl Acad Sci U S A. 2008
690 Jun 10;105(23):7899–906. doi:10.1073/pnas.0803151105 PubMed PMID: 18524956;
691 PubMed Central PMCID: PMC2430337.

692 54. Tamber S, Ochs MM, Hancock REW. Role of the novel OprD family of porins in nutrient
693 uptake in *Pseudomonas aeruginosa*. J Bacteriol. 2006 Jan;188(1):45–54.
694 doi:10.1128/JB.188.1.45-54.2006 PubMed PMID: 16352820; PubMed Central PMCID:
695 PMC1317591.

696 55. Tamber S, Maier E, Benz R, Hancock REW. Characterization of OpdH, a *Pseudomonas*
697 *aeruginosa* porin involved in the uptake of tricarboxylates. J Bacteriol. 2007
698 Feb;189(3):929–39. doi:10.1128/JB.01296-06 PubMed PMID: 17114261; PubMed Central
699 PMCID: PMC1797325.

700 56. Hwang I, Park YJ, Kim YR, Kim YN, Ka S, Lee HY, et al. Alteration of gut microbiota by
701 vancomycin and bacitracin improves insulin resistance via glucagon-like peptide 1 in diet-
702 induced obesity. FASEB J. 2015;29(6):2397–411. doi:10.1096/fj.14-265983

703 57. Theriot CM, Koenigsknecht MJ, Carlson PE, Hatton GE, Nelson AM, Li B, et al. Antibiotic-
704 induced shifts in the mouse gut microbiome and metabolome increase susceptibility to
705 *Clostridium difficile* infection. Nat Commun. 2014;5:3114. doi:10.1038/ncomms4114
706 PubMed PMID: 24445449; PubMed Central PMCID: PMC3950275.

707 58. Faber F, Tran L, Byndloss MX, Lopez CA, Velazquez EM, Kerrinnes T, et al. Host-mediated
708 sugar oxidation promotes post-antibiotic pathogen expansion. Nature. 2016 Jun
709 30;534(7609):697–9. doi:10.1038/nature18597 PubMed PMID: 27309805; PubMed Central
710 PMCID: PMC4939260.

711 59. Farrugia DN, Elbourne LDH, Hassan KA, Eijkelkamp BA, Tetu SG, Brown MH, et al. The
712 complete genome and phenome of a community-acquired *Acinetobacter baumannii*. PLOS
713 ONE. 2013 Mar 19;8(3):e58628. doi:10.1371/journal.pone.0058628

714 60. Timsit JF, Garrait V, Misset B, Goldstein FW, Renaud B, Carlet J. The digestive tract is a
715 major site for *Acinetobacter baumannii* colonization in intensive care unit patients. J Infect
716 Dis. 1993 Nov 1;168(5):1336–7. doi:10.1093/infdis/168.5.1336

717 61. Corbella X, Pujol M, Ayats J, Sendra M, Ardanuy C, Domínguez MA, et al. Relevance of
718 digestive tract colonization in the epidemiology of nosocomial infections due to multiresistant
719 *Acinetobacter baumannii*. Clin Infect Dis. 1996 Aug 1;23(2):329–34.
720 doi:10.1093/clinids/23.2.329

721 62. Wong SC, Chen JHK, Chau PH, So SYC, AuYeung CHY, Yuen LLH, et al. Gastrointestinal
722 colonization of carbapenem-resistant *Acinetobacter baumannii*: what is the implication for
723 infection control? Antibiotics. 2022 Sep 22;11(10):1297. doi:10.3390/antibiotics11101297
724 PubMed PMID: 36289955; PubMed Central PMCID: PMC9598245.

725 63. Harris AD, Johnson JK, Pineles L, O'Hara LM, Bonomo RA, Thom KA. Patient-to-patient
726 transmission of *Acinetobacter baumannii* gastrointestinal colonization in the intensive care
727 unit. Antimicrob Agents Chemother. 2019 Jul 25;63(8):10.1128/aac.00392-19.
728 doi:10.1128/aac.00392-19

729 64. Zheng Y, Xu N, Pang J, Han H, Yang H, Qin W, et al. Colonization with extensively drug-
730 resistant *Acinetobacter baumannii* and prognosis in critically ill patients: an observational
731 cohort study. Front Med. 2021;8:667776. doi:10.3389/fmed.2021.667776 PubMed PMID:
732 33996866; PubMed Central PMCID: PMC8119758.

733 65. Ren X, Clark RM, Bansah DA, Varner EN, Tiffany CR, Jaswal K, et al. Amino acid
734 competition shapes *Acinetobacter baumannii* gut carriage. BioRxiv Prepr Serv Biol. 2024
735 Oct 19;2024.10.19.619093. doi:10.1101/2024.10.19.619093 PubMed PMID: 39502362;
736 PubMed Central PMCID: PMC11537318.

737 66. Lewis JA, Stamper LW, Escalante-Semerena JC. Regulation of expression of the
738 tricarballylate utilization operon (*tcuABC*) of *Salmonella enterica*. Res Microbiol. 2009
739 Apr;160(3):179–86. doi:10.1016/j.resmic.2009.01.001 PubMed PMID: 19284970; PubMed
740 Central PMCID: PMC2692759.

741 67. Lewis JA, Horswill AR, Schwem BE, Escalante-Semerena JC. The tricarballylate utilization
742 (*tcuRABC*) genes of *Salmonella enterica* serovar Typhimurium LT2. J Bacteriol. 2004
743 Mar;186(6):1629–37. doi:10.1128/JB.186.6.1629-1637.2004 PubMed PMID: 14996793;
744 PubMed Central PMCID: PMC355976.

745 68. Chen F, Zhao Q, Yang Z, Chen R, Pan H, Wang Y, et al. Citrate serves as a signal molecule
746 to modulate carbon metabolism and iron homeostasis in *Staphylococcus aureus*. PLoS
747 Pathog. 2024 Jul;20(7):e1012425. doi:10.1371/journal.ppat.1012425 PubMed PMID:
748 39078849; PubMed Central PMCID: PMC11315280.

749 69. Liu M, Hao G, Li Z, Zhou Y, Garcia-Sillas R, Li J, et al. CitAB two-component system-
750 regulated citrate utilization contributes to *Vibrio cholerae* competitiveness with the gut
751 microbiota. Payne SM, editor. Infect Immun. 2019 Mar;87(3):e00746-18.
752 doi:10.1128/IAI.00746-18

753 70. Vornhagen J, Sun Y, Breen P, Forsyth V, Zhao L, Mobley HLT, et al. The *Klebsiella*
754 *pneumoniae* citrate synthase gene, *gltA*, influences site specific fitness during infection.
755 PLoS Pathog. 2019 Aug;15(8):e1008010. doi:10.1371/journal.ppat.1008010 PubMed PMID:
756 31449551; PubMed Central PMCID: PMC6730947.

757 71. Riquelme SA, Prince A. *Pseudomonas aeruginosa* consumption of airway metabolites
758 promotes lung infection. Pathogens. 2021 Jul 29;10(8):957.
759 doi:10.3390/pathogens10080957

760 72. Gaddy JA, Tomaras AP, Actis LA. The *Acinetobacter baumannii* 19606 OmpA protein plays a
761 role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with
762 eukaryotic cells. Infect Immun. 2009 Aug;77(8):3150–60. doi:10.1128/IAI.00096-09 PubMed
763 PMID: 19470746; PubMed Central PMCID: PMC2715673.

764 73. Choi CH, Lee JS, Lee YC, Park TI, Lee JC. *Acinetobacter baumannii* invades epithelial cells
765 and outer membrane protein A mediates interactions with epithelial cells. BMC Microbiol.
766 2008 Dec 10;8:216. doi:10.1186/1471-2180-8-216 PubMed PMID: 19068136; PubMed
767 Central PMCID: PMC2615016.

768 74. Choi CH, Hyun SH, Lee JY, Lee JS, Lee YS, Kim SA, et al. *Acinetobacter baumannii* outer
769 membrane protein A targets the nucleus and induces cytotoxicity. Cell Microbiol.
770 2008;10(2):309–19. doi:10.1111/j.1462-5822.2007.01041.x

771 75. Labrador-Herrera G, Pérez-Pulido AJ, Álvarez-Marín R, Casimiro-Soriguer CS, Cebrero-
772 Cangueiro T, Morán-Barrio J, et al. Virulence role of the outer membrane protein CarO in
773 carbapenem-resistant *Acinetobacter baumannii*. Virulence. 2020 Dec;11(1):1727–37.
774 doi:10.1080/21505594.2020.1855912 PubMed PMID: 33300460; PubMed Central PMCID:
775 PMC7733888.

776 76. Wijers CDM, Pham L, Menon S, Boyd KL, Noel HR, Skaar E, et al. Identification of two
777 variants of *Acinetobacter baumannii* 17978 with distinct genotypes and phenotypes. Infect
778 Immun. 2021 Aug 30;IAI0045421. doi:10.1128/IAI.00454-21 PubMed PMID: 34460288.

779 77. Gallagher LA, Ramage E, Weiss EJ, Radey M, Hayden HS, Held KG, et al. Resources for
780 genetic and genomic analysis of emerging pathogen *Acinetobacter baumannii*. J Bacteriol.
781 2015 Jun 15;197(12):2027–35. Located at: 25845845. doi:10.1128/JB.00131-15

78. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. Gene. 1998 May 28;212(1):77–86. doi:10.1016/S0378-1119(98)00130-9

79. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A. 2000 Jun 6;97(12):6640–5. doi:10.1073/pnas.120163297 PubMed PMID: 10829079; PubMed Central PMCID: PMC18686.

80. Carruthers MD, Nicholson PA, Tracy EN, Munson RS Jr. Acinetobacter baumannii utilizes a type VI secretion system for bacterial competition. PLoS One. 2013;8(3):e59388. Located at: 23527179. doi:10.1371/journal.pone.0059388

81. Kumar A, Dalton C, Cortez-Cordova J, Schweizer HP. Mini-Tn7 vectors as genetic tools for single copy gene cloning in *Acinetobacter baumannii*. J Microbiol Methods. 2010 Sep;82(3):296–300. doi:10.1016/j.mimet.2010.07.002 PubMed PMID: 20638421.

82. Tipton KA, Dimitrova D, Rather PN. Phase-variable control of multiple phenotypes in *Acinetobacter baumannii* strain AB5075. J Bacteriol. 2015 Aug 1;197(15):2593–9. doi:10.1128/JB.00188-15 PubMed PMID: 26013481; PubMed Central PMCID: PMC4518826.

83. Chin CY, Tipton KA, Farokhyfar M, Burd EM, Weiss DS, Rather PN. A high-frequency phenotypic switch links bacterial virulence and environmental survival in *Acinetobacter baumannii*. Nat Microbiol. 2018 May;3(5):563–9. doi:10.1038/s41564-018-0151-5 PubMed PMID: 29693659; PubMed Central PMCID: PMC5921939.

84. Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. 2019 Nov 14;20(1):238. doi:10.1186/s13059-019-1832-y

85. Noel HR, Keerthi S, Ren X, Winkelman JD, Troutman JM, Palmer LD. Genetic synergy between *Acinetobacter baumannii* undecaprenyl phosphate biosynthesis and the Mla system impacts cell envelope and antimicrobial resistance. mBio. 2024 Feb 16;15(3):e02804-23. doi:10.1128/mbio.02804-23

86. Price MN, Dehal PS, Arkin AP. FastTree 2 – approximately maximum-likelihood trees for large alignments. PLOS ONE. 2010 Mar 10;5(3):e9490. doi:10.1371/journal.pone.0009490

87. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol. 2015 Aug 6;16(1):157. doi:10.1186/s13059-015-0721-2

88. Letunic I, Bork P. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinforma Oxf Engl. 2007 Jan 1;23(1):127–8. doi:10.1093/bioinformatics/btl529 PubMed PMID: 17050570.

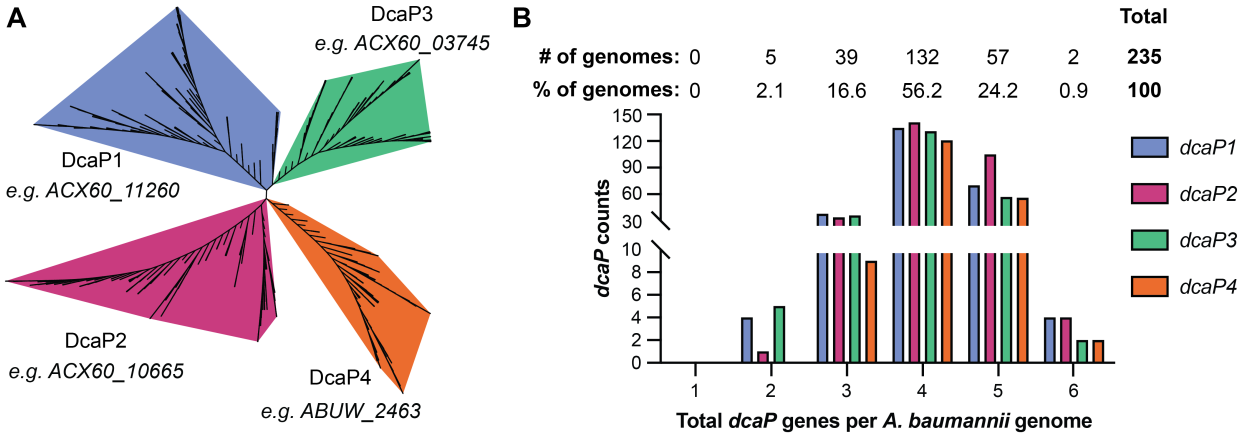
89. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol. 2018 Nov;36(10):996–1004. doi:10.1038/nbt.4229

90. Bhamidimarri SP, Zahn M, Prajapati JD, Schleberger C, Söderholm S, Hoover J, et al. A multidisciplinary approach toward identification of antibiotic scaffolds for *Acinetobacter baumannii*. Structure. 2019 Feb;27(2):268-280.e6. doi:10.1016/j.str.2018.10.021

91. The UniProt Consortium, Bateman A, Martin MJ, Orchard S, Magrane M, Adesina A, et al. UniProt: the Universal Protein Knowledgebase in 2025. Nucleic Acids Res. 2025 Jan 6;53(D1):D609–17. doi:10.1093/nar/gkae1010

92. Vishniac W, Santer M. The thiobacilli. Bacteriol Rev. 1957 Sep;21(3):195–213. doi:10.1128/br.21.3.195-213.1957 PubMed PMID: 13471458; PubMed Central PMCID: PMC180898.

832 **FIGURES**




833
834 **Figure 1. *A. baumannii* DcaP proteins cluster in four classes and are widely distributed**
835 **among *Acinetobacter*. (A)** An unrooted tree of DcaP protein sequences from 255
836 *Acinetobacter* genomes (235 *A. baumannii* genomes). **(B)** Manual counts and distribution of
837 individual DcaP proteins binned by the total number of DcaP proteins in an *A. baumannii*
838 genome.
839

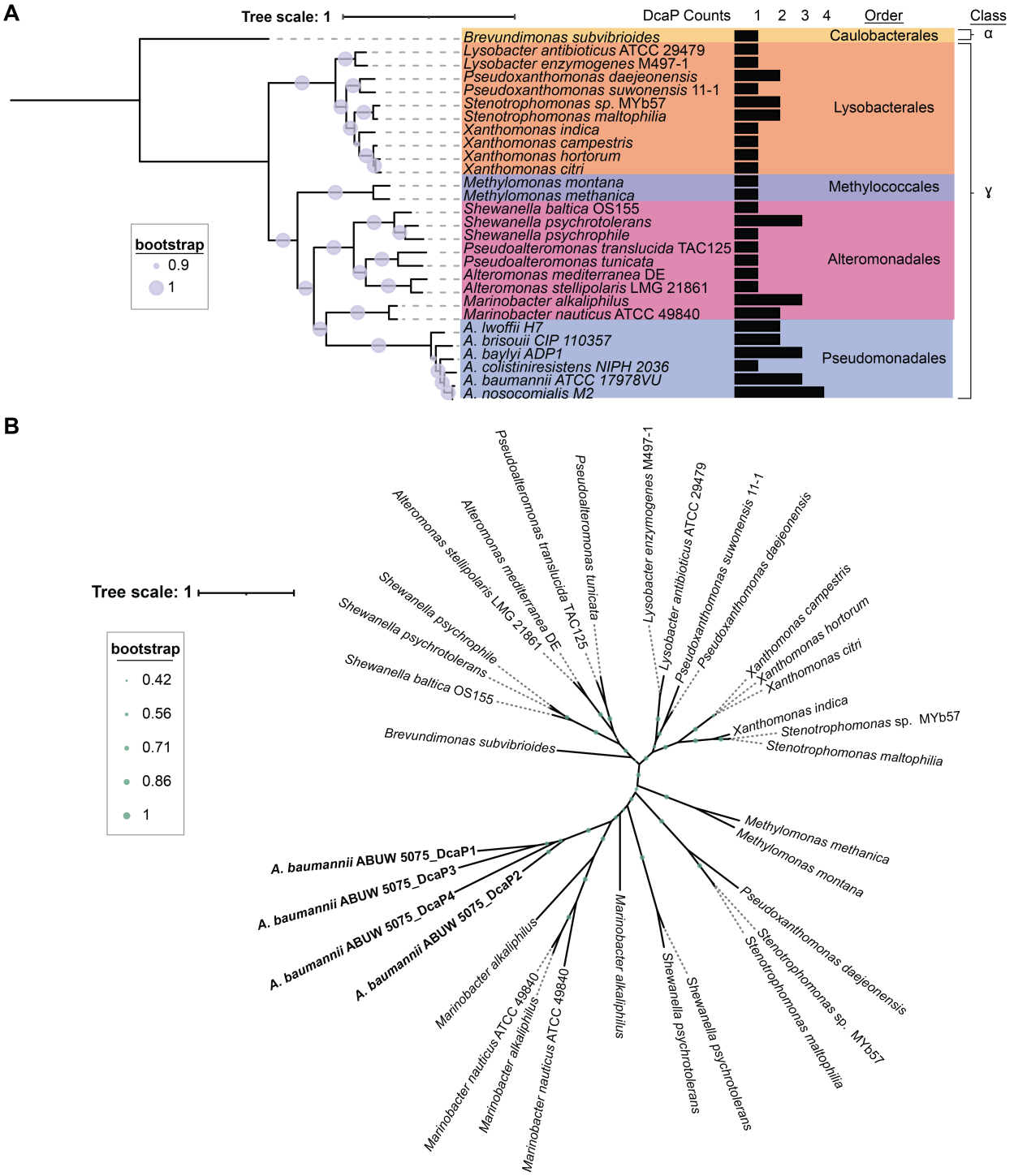



**Figure 2. *A. baumannii* DcaP-family proteins cluster together compared to non-*Acinetobacter* DcaP proteins. (A)** A rooted tree of DcaP containing species outside of *Acinetobacter* and some *Acinetobacter spp.* depicting class, order, and number of encoded DcaP proteins. **(B)** An unrooted tree of DcaP protein sequences from non-*Acinetobacter* species with AB5075 as the *A. baumannii* reference. Tree scale in amino acid substitutions.

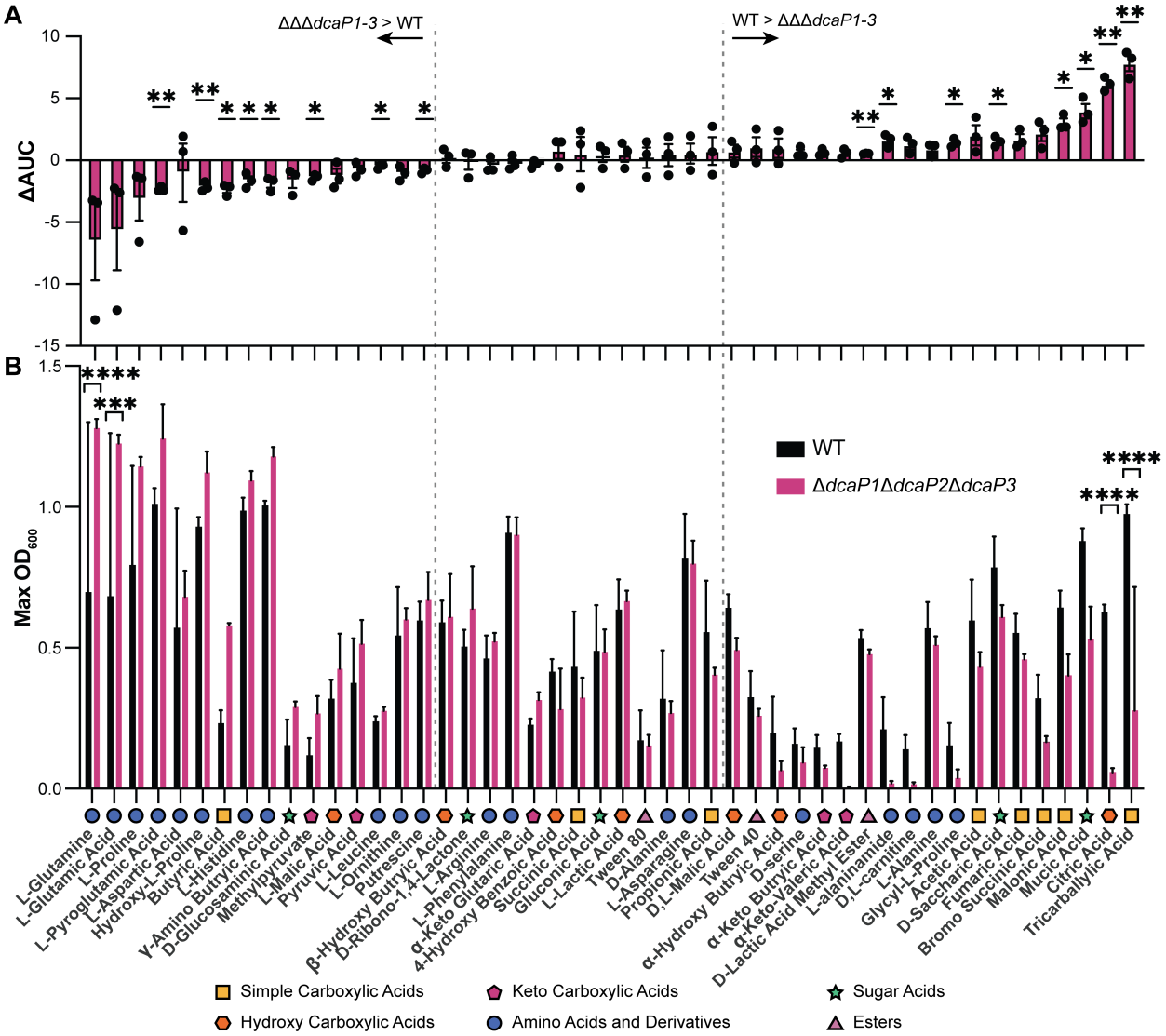



**Figure 3. Wildtype *A. baumannii* grows better than ΔΔΔ*dcaP1-3* mutant on certain di- and tricarboxylic acids as sole carbon sources.** Biolog plates PM1 and PM2a were resuspended in M9 minimal media without a carbon source and inoculated with either WT or the ΔΔΔ*dcaP1-3* mutant. (**A**) Difference in area under the curve (ΔAUC) was calculated for WT – ΔΔΔ*dcaP1-3* mutant. Each point is from one experiment with 1 biological replicate per strain. Significance is by one sample t-test compared to 0. (**B**) Maximum optical density at 600 nm ($OD_{600}$) is shown. Significance is by two-way ANOVA with Sidak's multiple comparisons. Experiments were conducted three times with an n=1 for a total of n=3. Data are mean +/- SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

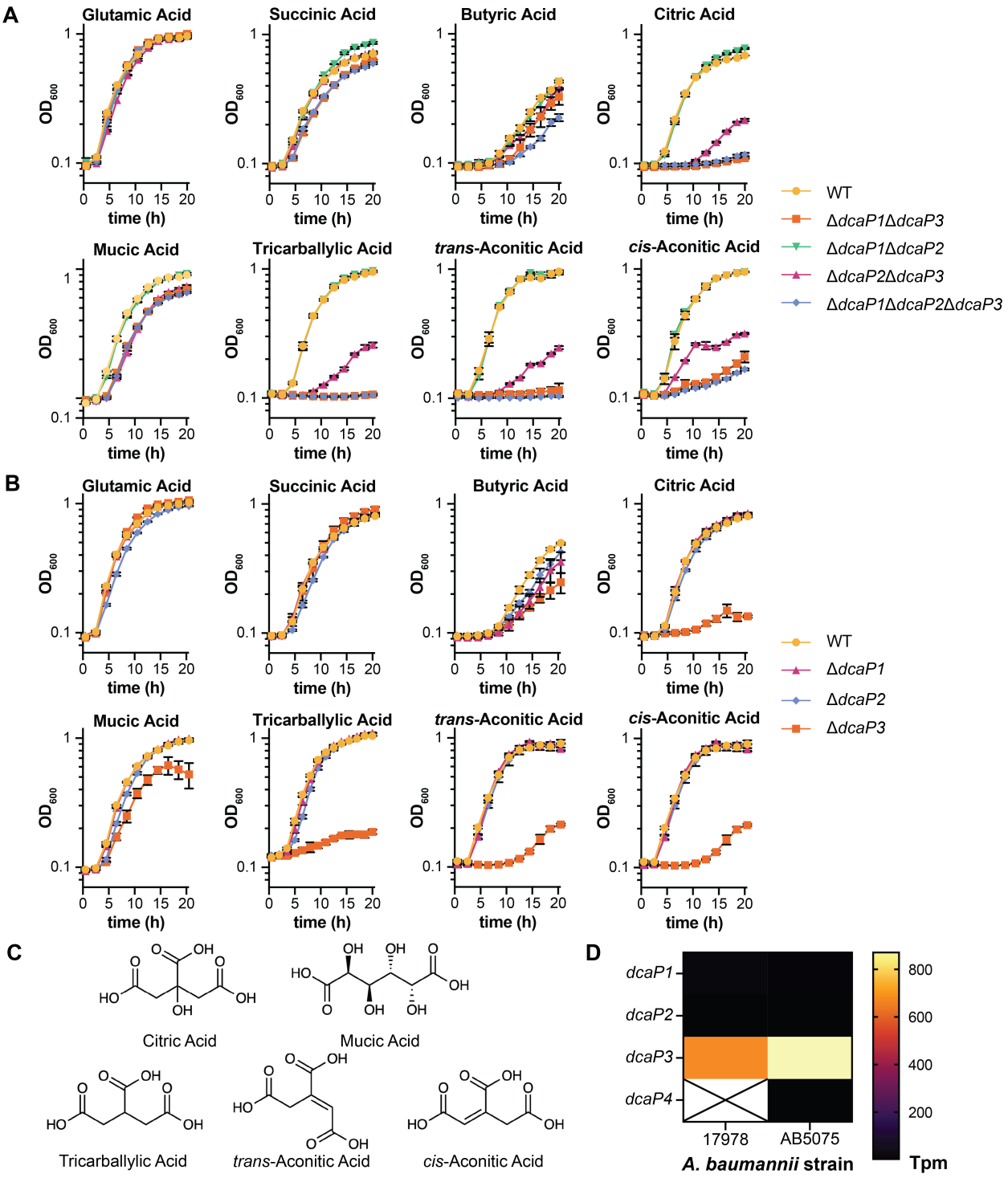



**Figure 4. DcaP3 is important for growth on the carboxylic acid carbon sources citric acid, tricarballylic acid, mucic acid, and *cis*- and *trans*- aconitic acid. (A)** Wildtype, double, and the triple ΔΔΔ*dcaP1-3* mutants were grown in M9 media with the indicated compound as the sole carbon source. Growth was monitored by optical density at 600 nm ($OD_{600}$). **(B)** Wildtype, the ΔΔΔ*dcaP1-3* mutant, and the ΔΔΔ*dcaP1-3* mutant complemented with *dcaP3* were grown on the indicated compound as the sole carbon source. **(C)** The chemical structures of the relevant carbon source compounds. **(D)** Expression of the *dcaP* genes in *A. baumannii* 17978 and

AB5075 from (34). Average transcripts per million (Tpm) reads. (A-B) Data are mean +/- SEM and n=2-3. Experiments were repeated at least twice with similar results.

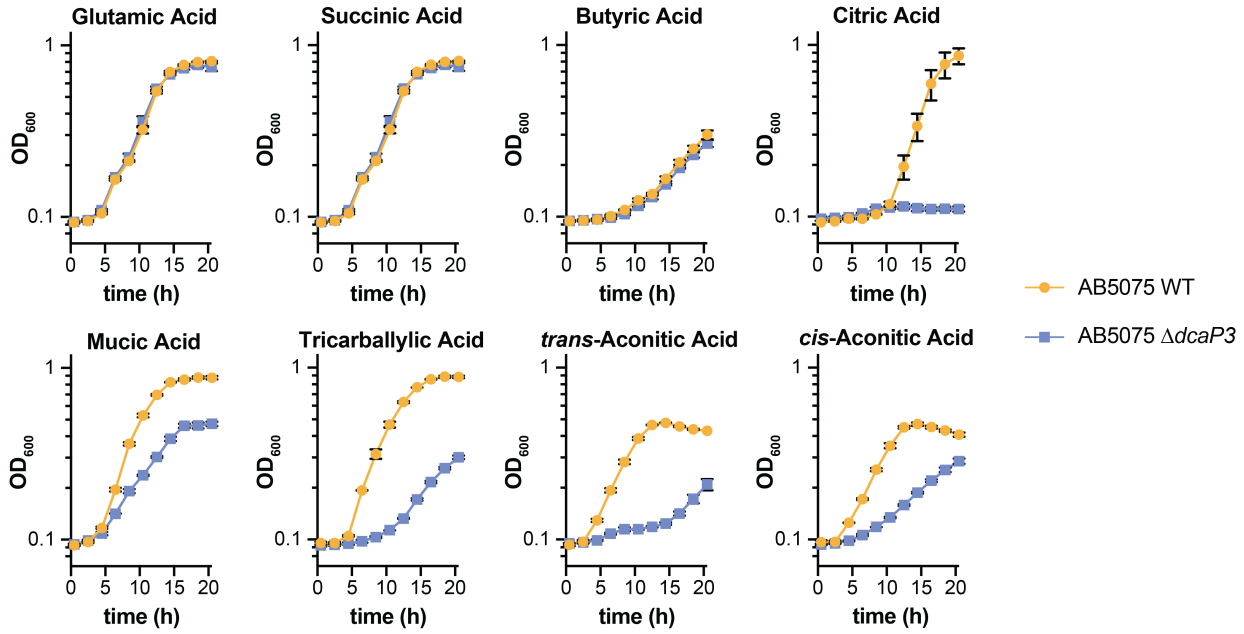



**Figure 5. *A. baumannii* AB5075 Δ*dcaP3* is defective for growth on citric acid, tricarballylic acid, mucic acid, and *cis*- and *trans*-aconitic acid.** *A. baumannii* AB5075 wildtype (WT) and Δ*dcaP3* mutant strains were grown in M9 media with the indicated compounds as the sole carbon source. Growth was monitored by optical density at 600 nm ($OD_{600}$). Data are mean +/- SEM and n=3. Experiments were repeated twice with similar results.

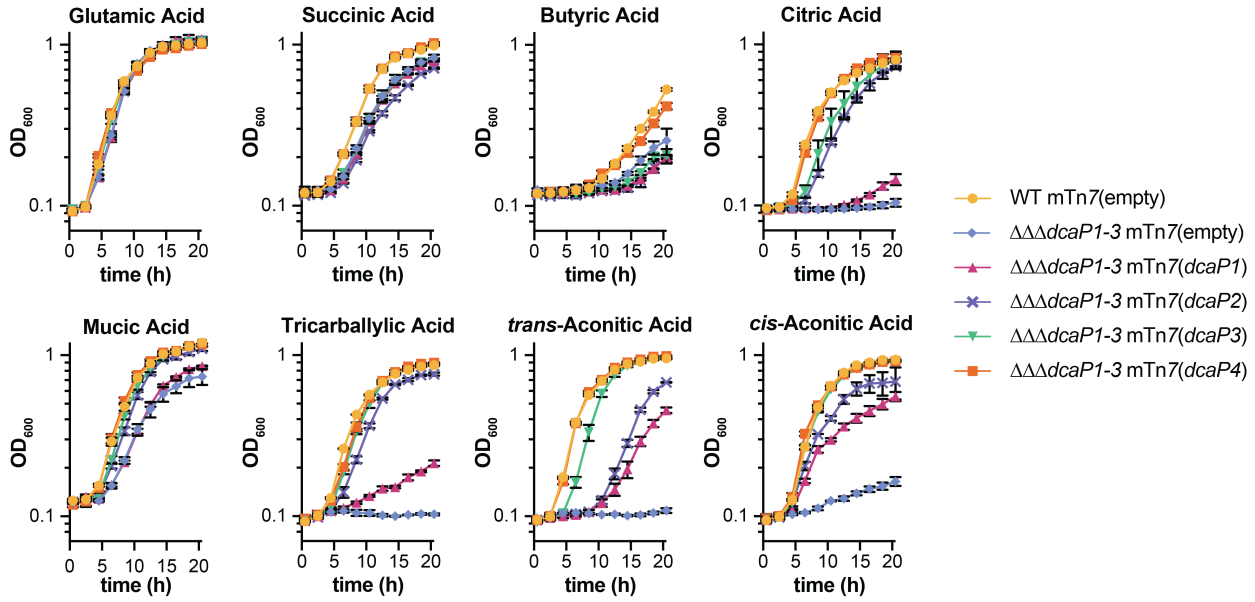



**Figure 6. DcaP1, DcaP2, DcaP3, and DcaP4 can complement growth of *A. baumannii* ATCC 17978 ΔΔΔ*dcaP1-3* with certain sole carbon sources.** Wildtype, the ΔΔΔ*dcaP1-3* mutant, and the ΔΔΔ*dcaP1-3* mutant complemented with *dcaP1, dcaP2, dcaP3,* or *A. baumannii* AB5075 *dcaP4* were grown in M9 media with the indicated compound as the sole carbon source. Growth was monitored by optical density at 600 nm ($OD_{600}$). Data are mean +/- SEM and n=3. Experiments were repeated at least twice with similar results.

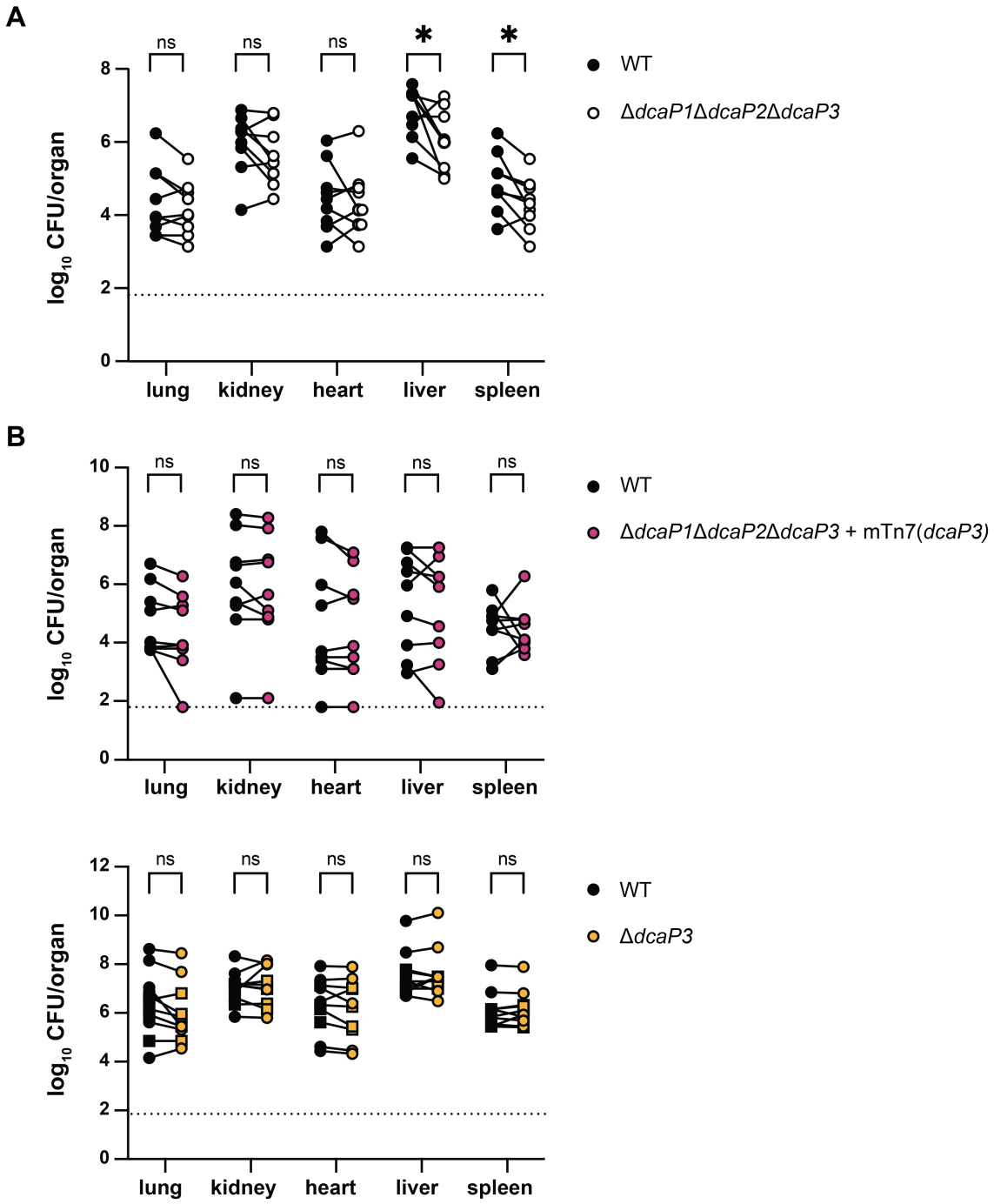



**Figure 7. DcaP3 is important for infecting the liver and spleen in a model of bloodstream infection. (A-C)** Wildtype (WT) and the indicated strains were retro-orbitally inoculated into Swiss Webster mice in a 1:1 ratio. At 24 h post-infection, organs were harvested and bacterial CFU were enumerated. The dotted lines indicate the limit of detection. Each point represents one strain enumerated from one mouse; n=9-10. Circle symbols are female mice and square symbols are male mice. (A-B) Data are combined from two experiments. (C) Data are from one experiment. Statistics are Wilcoxon non-parametric test. *$P < 0.05$.

SI for Noel *et al*., 2026

Supplementary information for

**DcaP-Family Porins are Required for Carboxylic Acid Utilization and Infection in *Acinetobacter baumannii***

Hannah R. Noel[1], Nikita R. Kesav[1], Jonathan D. Winkelman[2], Lauren D. Palmer[1]

[1] Department of Microbiology and Immunology, University of Illinois Chicago, Chicago, IL, USA
[2] Trestle LLC, Milwaukee, WI, USA

Correspondence: ldpalmer@uic.edu

**This PDF includes:**
Figures S1 to S4
Tables S1 to S3
Supplemental methods

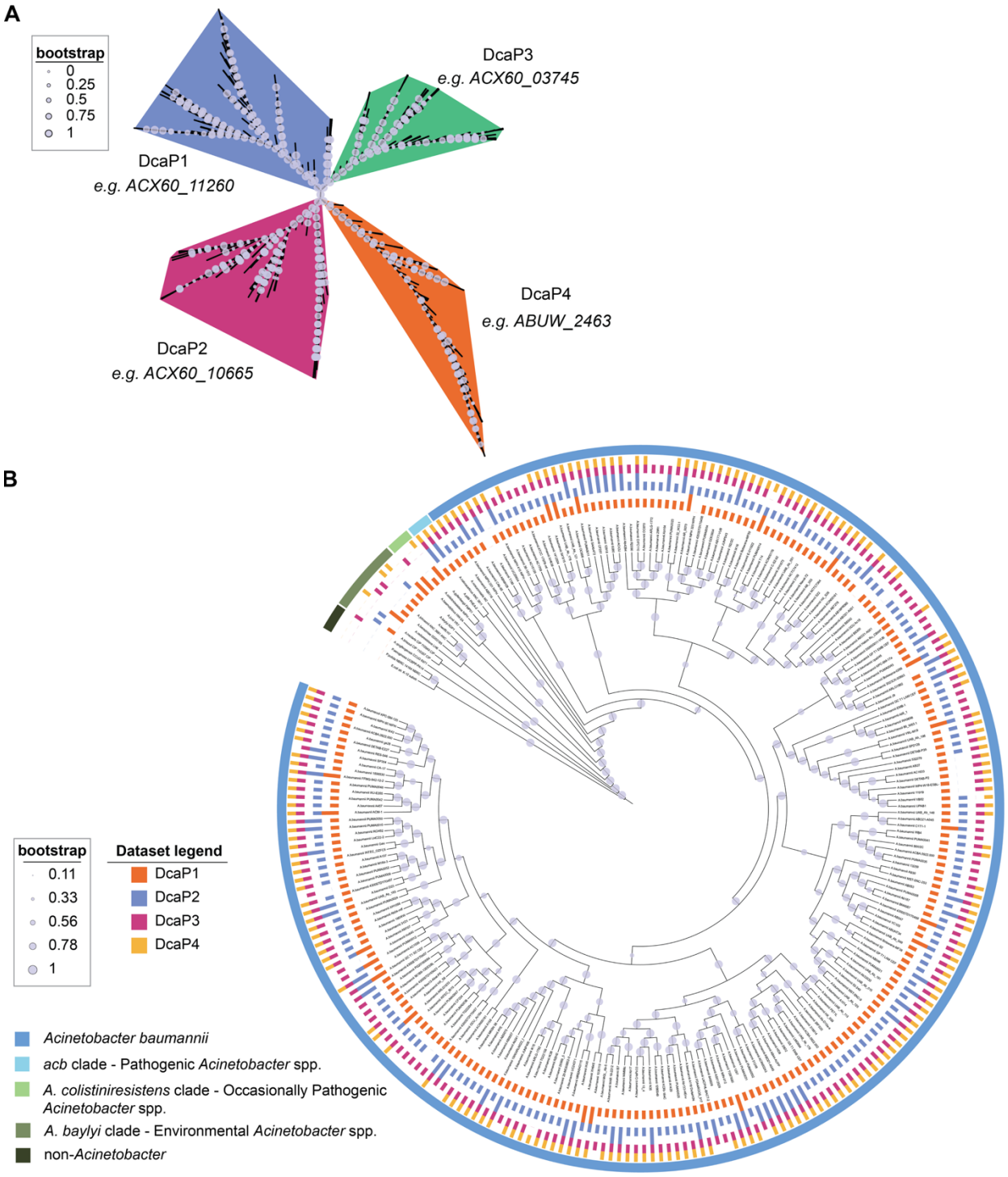
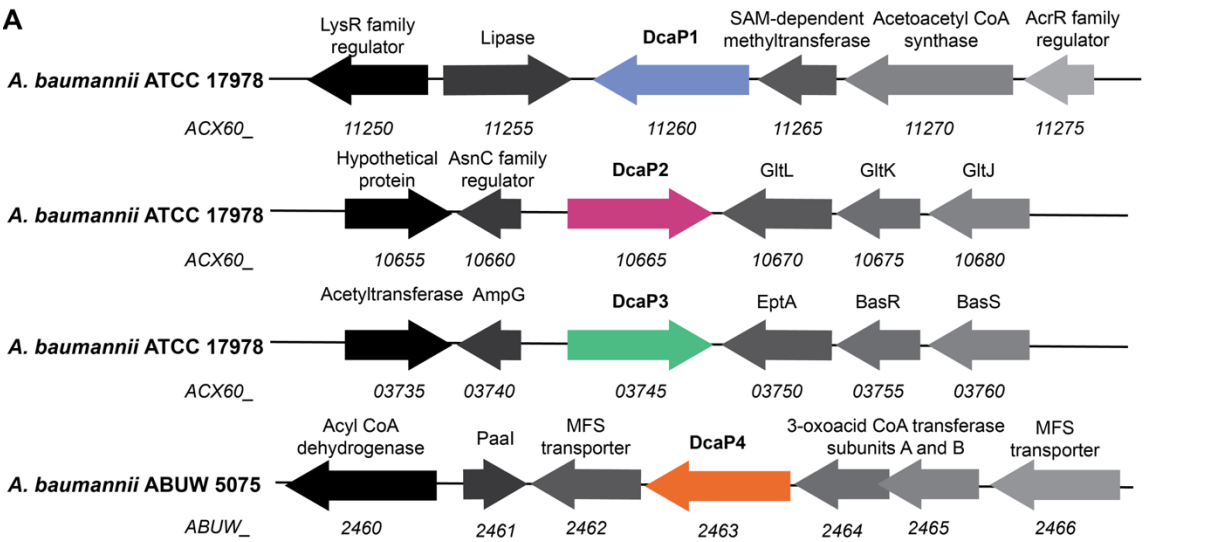



**Figure S1. DcaP proteins are conserved across *Acinetobacter* (A)** *A. baumannii* DcaP proteins clustering with bootstrap values displayed. **(B)** Phylogenetic tree of *Acinetobacter* strains and clades depicting prevalence of individual DcaP proteins. Color coded boxes indicate the presence, absence, or duplication of individual DcaP proteins. Clades within *Acinetobacter* are indicated by the outermost color bar circling the tree. The accession numbers of the data depicted are in Table S4.




**B**

```
                 predicted coiled-coil; α                                β
DcaP4   ----------MKKLILAAACAAASGTIFANSSDTTEQRINLLETELQKLKAELAAQKQTQNNLQVKQVKIEENI--EKTK     68
DcaP3   -MDLFLNRKSFVVKSLAITVTALMMSG-ANAATSDKEEIRKLRQEVEALKALVQEQRQVQQQQQQVQQQQQVQLAEVKAQ     78
DcaP1   MMKIL--NTTVQKSLLAVSVTLLMSS---AFAGQVNPEIEQLRQEIKELRAMLQTNMEAQKSLAQKRV--------EQAA     67
DcaP2   MWGMP--VKNIKTLVLAMSTISLTGVV-MPAHAATDAEVDALRKEVSELKQLVQQLSNQQKAVTVVNTLTVPPTTITASS     77
                .   ** :               . .:  *. *:. *:  :    : *:     .          :
        β                   β       β       β
DcaP4   ATVTEKPVAPSW------VTWTDNVKVYGIARIDAAVDFKSSPDSGGRTTSS-LYRTPFESEKRSNHARSDAMINASRLGV    142
DcaP3   PQPVA---APASPLAGFKSKAGADVNLYGFVRGDANYIIEGADNDFGDVSKS----------DGKTHDKLRATAKTTRLGL    146
DcaP1   PVVTQ---APQS-IQQTLTKSGAEFNLYGYIRADASYQIKGASTMYNNISGVPLENTPE---ESAQKDQLHSTVYVTRFGL    141
DcaP2   PVPQS---KPGW---MQMPDGQTQVKLYGNVRVDATYDFKGSNGSISNGANYPLNKD------DPRQDSLNVSAATTRIGL    146
                *            :.::**  * **   ::.:    .  :                :        .:*:*:
        β       β                                       β       β
DcaP4   YFNSPN--KAVTGNIEADFFDSSNMGTGDGKFRIRHAFFTYKDWTFGQTWSLMSNMETRTEAVDYTQFVGTSYTRTPQIRY    221
DcaP3   DFNTPVGDDKVGGKIEVDFAGST-TD-SNGSLRIRHAYLTYNNWLFGQTTSNFLSN-HAPEMIDFSTNIGGGTKRVPQVRY    224
DcaP1   NFKTPTAIGDVGGKLEMDFFGAA-T---RDQFRIRHAYLTFDKWLIGQTWSTFIAPEYYPETIDAGTFVGSALLRSPLVRY    218
DcaP2   DLTRPTQYGDLTGKIEADFMGGN-GDNGSGTFRVRHAYMSLGKWLAGQTTSPFVNTDTSPETLDFTGAVGTGTTRTVQVRY    226
         :. *     : *::* ** ..       . :*:***:::  .*  *** * :       * :*    :* .  *   :**
            β       β       β       β       β       β       β
DcaP4   DWKIDANNDLKVALEYTGSR--------VSAFPTLTGRYTFKQGPLTALAQ--GFINE-KSTDVATATVKKVSWGAGGSLKY    292
DcaP3   NYKLGPTTQLFVSAEKGDSTTSVTGDSIKYSLPALTAKITQGYAEGRGSASARVLVEN---YKSQLADDDKTGWGVAVGTDF    303
DcaP1   SDNLTANTSFAVSVEDPKYTA-ATDPDNKMRLPALVGRLNHKFANGSM------LSGRSFVAEKRTNNDSEWAWGVGLGGKY    293
DcaP2   TQPINAQQKILVALEGGDVEK-VSNAAGGSRFPALTTRYDFKTADNKGLLQLHGLAHENRVAPKDSSSEEKFGWGVGIGGKY    307
           :    .: *: *               :*:*. :     .          :  .          .: .**.. . .:
        β   β       β               β                       α       β
DcaP4   QLTPQQSVQANYQYIVGDQKFMPYTTQSGLANSTSLNAAGDFSLNEDKTDLVMNKLNSINIGYSYKFNEQWRANLSASMFDY    374
DcaP3   KVSDPLKLFADASYVVGDNSYLYGSNS-------------PYAVDG--NSIEQNEFVAVQVGGTYKILPNLRSTLAYGAQFS    370
DcaP1   QITPDTLLKADYYHVKGDGRFLLWSNS-------------GYAIDDQ-NNMHSNEFDTVSLGITHQFTPQIRSTLGYGYMKA    361
DcaP2   DITPQDSVVANYYHVKGDGRYLSYSNS-------------AYAYDTTTQDINLGEFDSAVIGYQRKWSPILRSTFAIGGIQY    376
        .::    : *:  :: **  ::  :..                   :: :    .:  .:: :  :*   :     *:.:. .
                β       β   β   β
DcaP4   DDDTAYAKLN---PDANKRLTDYAANVFYSPIEQMDIGMEYHQGKREVFDGRTADVSRVNFVSMYKF          438
DcaP3   DDGTDYARLN---ASANEKVQQAWINFIYTPVKPIDLGVEYVNGKRDTFDGKSYKDNRVGLMAKYSF          434
DcaP1   KDDNRFAELQRTSTTQNKQLWQGWVNVLYNPYKPITLGIEYVYGERETFDNRNGIDNRFNMIASYDF          428
DcaP2   KDNSVYANSNLTNTSYNKEIYNALVNLIWNPVKNIDLGAEYTYGQRETFAGDKGDYSRINLLAKYNF          443
        .*.. :*. :      *:.: :   *.::.* : : :* **  *:*:.* . .   .*..::: *.*
```

**Figure S2. *dcaP* genomic neighborhoods and DcaP protein alignments. (A)** The genomic loci of *dcaP1-4* are depicted. **(B)** CLUSTAL Protein alignment of DcaP proteins from ABUW 5075 (1). Secondary structure modeled after PDB entry 6EUS (2).

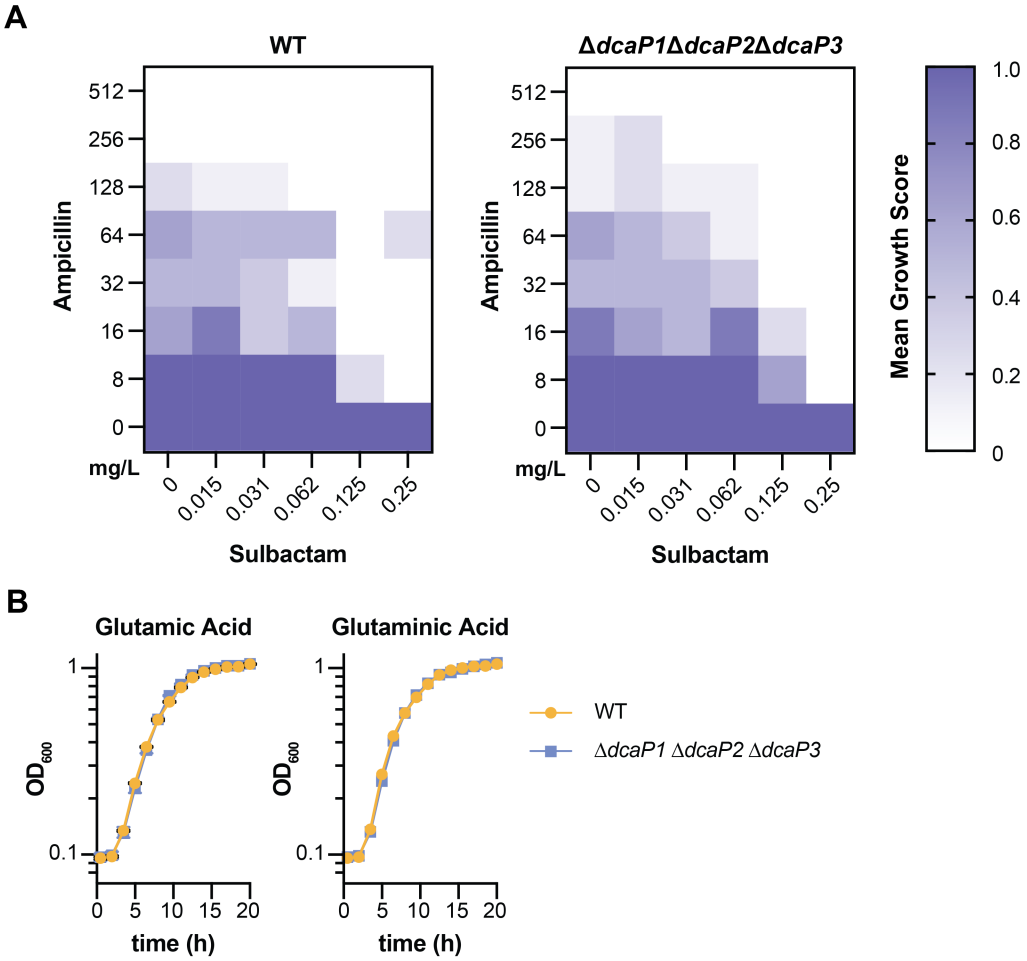



**Figure S3. Checkerboard assay of ampicillin and sulbactam for wild-type and triple DcaP mutant and growth on glutamic acid and glutaminic acid.**
**(A)** A 96-well plate was filled with stocks of LB with ampicillin and sulbactam and serially diluted to obtain 100 μL of unique concentrations for each compound in each well before inoculation with 1 μL of indicated bacterial strain. Growth was scored as 1, strong turbidity; 0.5, mild turbidity; 0, little to no turbidity. Experiment was repeated 3 times with an n=2 for a total n=6. Means are shown. **(B)** Wildtype and the triple Δ*ΔΔdcaP1-3* mutant were grown in M9 media with the indicated compound as the sole carbon source. Growth was monitored by optical density at 600 nm ($OD_{600}$). Data are mean +/- standard error of the mean (SEM). N=3 and data are representative of at least 2 independent experiments.

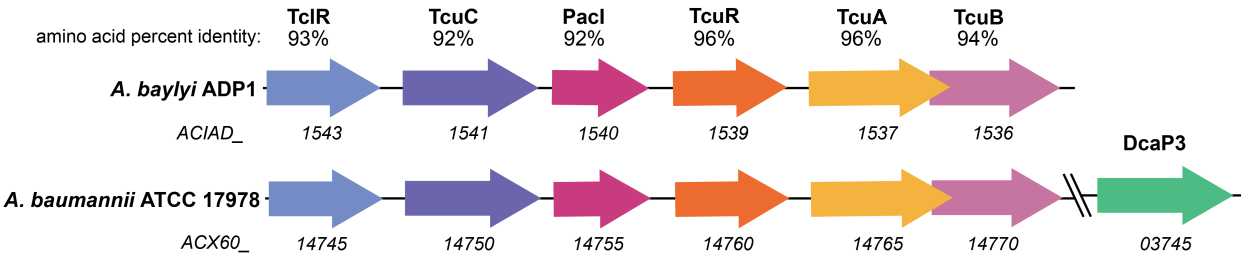



**Figure S4. The genetic organization of the *tcu* metabolic locus in *Acinetobacter baylyi* ADP1 and *A. baumannii* ATCC 17978.** Amino acid identity was determined by CLUSTAL Protein alignment using UniProt (1, 3).

**Table S1. Strains**

| Strain | Source | Identifier |
|---|---|---|
| *A. baumannii* ATCC 17978VU (WT) | ATCC | LP486 |
| *A. baumannii* ATCC 17978VU *att*::mTn*7*(empty); Carb$^{R}$ | Ren and Clark *et al.*, 2025 (4) | LP526 |
| *A. baumannii* ATCC 17978VU Δ*dcaP1::kan* (*ACX60_11260*) | This manuscript | LP1191 |
| *A. baumannii* ATCC 17978VU Δ*dcaP2::kan* (*ACX60_10665*) | This manuscript | LP1190 |
| *A. baumannii* ATCC 17978VU Δ*dcaP3::kan* (*ACX60_03745*) | This manuscript | LP1189 |
| *A. baumannii* ATCC 17978 Δ*dcaP1*Δ*dcaP2*::kan | This manuscript | LP1253 |
| *A. baumannii* ATCC 17978VU Δ*dcaP1* Δ*dcaP3* Δ*dcaP2*::kan | This manuscript | LP1308 |
| *A. baumannii* ATCC 17978VU Δ*dcaP3*Δ*dcaP1*::kan | This manuscript | LP1302 |
| *A. baumannii* ATCC 17978VU Δ*dcaP3*Δ*dcaP2*::kan | This manuscript | LP1227 |
| *A. baumannii* ATCC 17978VU Δ*dcaP1* Δ*dcaP3* Δ*dcaP2*::kan *att*::mTn*7*(*dcaP3p-dcaP3*) | This manuscript | LP1310 |
| *A. baumannii* ATCC 17978VU Δ*dcaP1* Δ*dcaP3* Δ*dcaP2*::kan *att*::mTn*7*(*dcaP3p-dcaP4(ABUW_2463*)) | This manuscript | LP1314 |
| *A. baumannii* AB5075 | Colin Manoil (University of Washington) (5, 6) | LP345 |
| *A. baumannii* AB5075 Δ*dcaP3*::hyg (*ABUW_RS04045*) | This manuscript | LP1315 |
| *A. baumannii* ATCC 17978VU Δ*dcaP1* Δ*dcaP3* Δ*dcaP2*::kan *att*::mTn*7*(*dcaP3p-dcaP1*) | This manuscript | LP1368 |
| *A. baumannii* ATCC 17978VU Δ*dcaP1* Δ*dcaP3* Δ*dcaP2*::kan *att*::mTn*7*(*dcaP3p-dcaP1*) | This manuscript | LP1369 |

**Table S2. Plasmids**

| Use or Phenotype | Source | Name | Identifier |
|---|---|---|---|
| Kan$^{R}$; mobilization helper plasmid | Figurski and Helinski, 1979 (7) | pRK2013 | |
| Carb$^{R}$; plasmid encoding mTn7 transposition pathway | Choi *et al.*, 2005 (8) | pTNS2 | |
| Carb$^{R}$; allelic exchange vector with sucrose sensitivity | Hoang *et al.*, 1998 (9) | pFLP2 | |
| Contains an FRT-flanked kanamycin resistance gene | Datsenko and Wanner, 2000 (10) | pKD4 | |
| Contains a mTn7 with carbenicillin resistance gene | Carruthers *et al.*, 2013 (11) and Alexeyev, 1999 (12) | pKNOCK | |
| Carb$^{R}$; construct to complement *dcaP3* | This manuscript | pKNOCK-*dcaP3* | pLDP343 |
| Carb$^{R}$; express the AB5075 DcaP4 in ATCC 17978 | This manuscript | pKNOCK-*dcaP4* | pLDP359 |
| Carb$^{R}$ Kan$^{R}$; deletion construct for DcaP1 | This manuscript | pFLP2-*dcaP1* | pFLP2-11260 |
| Carb$^{R}$ Kan$^{R}$; deletion construct for DcaP2 | This manuscript | pFLP2-*dcaP2* | pFLP2-10665 |
| Carb$^{R}$ Kan$^{R}$; deletion construct for DcaP3 | This manuscript | pFLP2-*dcaP3* | pFLP2-03745 |
| pKD4 with hygromycin$^{R}$ replacing kanamycin$^{R}$ cassette | This manuscript | pKD4-Hyg | pLDP357 |
| Carb$^{R}$ Hyg$^{R}$; deletion construct for DcaP3 in AB5075 | This manuscript | pFLP2-5075*dcaP3* | pLDP360 |

| | | | |
|---|---|---|---|
| Carb$^{R}$; construct to express *dcaP1* | This manuscript | pKNOCK-*dcaP1* | pLDP401 |
| Carb$^{R}$; construct to express *dcaP2* | This manuscript | pKNOCK-*dcaP2* | pLDP402 |

**Table S3. Oligonucleotides**
All oligonucleotides were purchased from IDT (Coralville, IA)

| Sequence | Identifier | Name |
|---|---|---|
| tgaacggcaggtatatgtgatggg | LP52 | pFLP2 fwd |
| ccatgattacgaattcgagc | LP54 | pFLP2 rev |
| gtgtaggctggagctgcttc | LP154 | pKD4-FRTfrag_F |
| catatgaatatcctccttagttcctattc | LP155 | pKD4-FRTfrag_R |
| ttcctttagcagcccttg | LP181 | FRT_Frag_102_R |
| gatctcatgctggagttc | LP183 | FRT_Frag_1384_F |
| cttctctcaaaataaggaacttcg | HN355 | 03745 up up fwd |
| caatccaagtcacttggtaac | HN356 | 03745 dn dn rev |
| aaaaggatcgatcctctagaggatcacatcaccatgcttggg | HN357 | 03745 up fwd |
| acttcgaagcagctccagcctacaccaaagactcctaaaaatatattttatgc | HN358 | 03745 up rev |
| aggaactaaggaggatattcatatgtaacgatttttttagctaaaaatagaaaag | HN359 | 03745 dn fwd |
| atgattacgaattcgagctcggtacagttgaaaatatcttgtttagaaagc | HN360 | 03745 dn rev |
| aaaaggatcgatcctctagaggatccgttgtatttaaaattttcatcatg | HN285 | pFLP-11260-up-fwd |
| attctctagaaagtataggaacttctcaaaagctcggtcaaag | HN286 | pFLP-11260-up-rev |
| aggaactaaggaggatattcatatgtcccgtttatttttctggtca | HN287 | pFLP-11260-dn-fwd |
| atgattacgaattcgagctcggtacttcaacatgatagcgagttatg | HN288 | pFLP-11260-dn-rev |
| gcaaagaacacatttcacac | HN318 | 11260 up up rev |
| ccagagcaagtatctaatcagac | HN319 | 11260 dn dn fwd |
| ctggtgcagaagatattgaag | HN351 | 10665_dcaP_up up fwd |
| cagacttcttaggtcgtgc | HN352 | 10665 dcaP dn dn rev |
| aaaaggatcgatcctctagaggatccaatgctgaagtagtgatgag | HN353 | pFLP2-10665-up fwd |
| acttcgaagcagctccagcctacactggatataaaagatttccaatatgtgc | HN354 | pFLP2-10665-up rev |
| aggaactaaggaggatattcatatgaatccggttaaaaatattgatttagg | HN363 | pFLP2-10665-dn-fwd |
| atgattacgaattcgagctcggtacggtgtatttctgtagctcgt | HN364 | pFLP2-10665-dn-rev |
| tcatgcatgagctcactagtggatcggttctgtaaaccaaaaagt | HN377 | pKNOCK-03745_fwd |
| ggcctgcaaggccttcgcgaggtacttagaaactatatttagccattaaac | HN378 | pKNOCK-03745_rev |
| tcatgcatgagctcactagtggatcgattggatattagcctaaagtct | HN400 | dcaP3 promoter fwd |
| ctgcagctaaaattaattttttcatcaaagactcctaaaaatatattttatgc | HN401 | dcaP3 promoter rev |
| taaaatatatttttaggagtctttgatgaaaaaattaattttagctgcag | HN402 | dcaP4 fwd |
| ggcctgcaaggccttcgcgaggtacttagaatttatacatcgatacaaag | HN403 | dcaP4 rev |
| aacttcggaataggaacttcggaaatgtgcgcggaacccctatttgtttattttt | XR185 | hyg-pKD-F |
| ttcgaaccccagagtcccgctcaggcgccgggggcggtgtccggc | XR186 | hyg-pKD-R |
| aaaaggatcgatcctctagaggatcacatcaccatgcttgg | HN385 | 5075 dcaP3 up fwd |
| acttcgaagcagctccagcctacaccaaagactcctaaaaatatattttatg | HN386 | 5075 dcaP3 up rev |
| aggaactaaggaggatattcatatggaacgatttttagctaaaaatagaaaag | HN387 | 5075 dcaP3 dn fwd |
| atgattacgaattcgagctcggtacaaaagttgaaaatatcttgtttagaaag | HN388 | 5075 dcaP3 dn rev |
| gaacttcgttcttggttaatc | HN389 | 5075 dcaP3 up up fwd |
| ctttacaacccgagttattatc | HN390 | 5075 dcaP3 dn dn rev |

| | | |
|---|---|---|
| tcatgcatgagctcactagtggatcgattggatattagcctaaagtctaa | NK5 | pKNOCK-dcaP3p-dcap2 FWD |
| tatttttcacaggcattccccacatcaaagactcctaaaaatatattttatgc | NK6 | pKNOCK-dcaP3p-dcap2 REV |
| taaaatatatttttaggagtctttgatgtggggaatgcctgtgaaaaata | NK7 | pKNOCK-dcaP3p-dcap2 FWD |
| ggcctgcaaggccttcgcgaggtacttagaaattatattttgctaataaatttatgc gt | NK8 | pKNOCK-dcaP3p-dcap2 REV |
| ccgttgtatttaaaattttcatcatcaaagactcctaaaaatatattttatgc | NK10 | pKNOCK-dcaP3p-dcaP1 REV |
| cataaaatatatttttaggagtctttgatgatgaaaattttaaatacaacggt | NK11 | pKNOCK-dcaP3p-dcaP1 FWD |
| ggcctgcaaggccttcgcgaggtacttaaaaatcataactcgctatcatg | NK12 | pKNOCK-dcaP3p-dcaP1 REV |

**Supplementary Methods**

Checkerboard assay

In a 96-well plate, each well was filled with 100 µl LB. Stock solutions of ampicillin (100 mg/mL) and sulbactam (30 mg/mL) were diluted into LB for a final concentration of 1024 µg/mL and 32 µg/mL, respectively. An additional solution of 2048 µg/mL ampicillin was prepared. 100 µl aliquots of 1024 µg/mL ampicillin were placed in row A, wells 1-11, and 2024 µg/mL ampicillin in well 12. 2-fold serial dilutions were performed beginning in row A and ending in row H, discarding the remaining 100 µl. 100 µl aliquots of 32 µg/mL sulbactam were placed in every well in column 12, and 2-fold serial dilutions were performed beginning in column 12 and ending in column 2, discarding the remaining 100 µl. The maximum concentration for each antibiotic was therefore 512 µg/mL ampicillin and 16 µg/mL sulbactam. Then, 1 µl overnight culture of *A. baumannii* ATCC 17978 wild-type or the ΔΔΔ*dcaP* mutant was inoculated into each well before incubation at 37°C with shaking at 180 rpm for 8-16 hours. The following day, plates were scored for growth or no growth phenotypes. Wells with strong optical turbidity were assigned a value of '1' and wells with mild turbidity or mild turbidity with clumped cells were assigned a value of '0.5'. Wells with no visual growth or fully clumped cells were assigned a value of '0.'

**Supplementary References**


1. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539.

2. Bhamidimarri SP, Zahn M, Prajapati JD, Schleberger C, Söderholm S, Hoover J, West J, Kleinekathöfer U, Bumann D, Winterhalter M, Van Den Berg B. 2019. A multidisciplinary approach toward identification of antibiotic scaffolds for *Acinetobacter baumannii*. Structure 27:268-280.e6.

3. The UniProt Consortium, Bateman A, Martin M-J, Orchard S, Magrane M, Adesina A, Ahmad S, Bowler-Barnett EH, Bye-A-Jee H, Carpentier D, Denny P, Fan J, Garmiri P, Gonzales LJDC, Hussein A, Ignatchenko A, Insana G, Ishtiaq R, Joshi V, Jyothi D, Kandasaamy S, Lock A, Luciani A, Luo J, Lussi Y, Marin JSM, Raposo P, Rice DL, Santos R, Speretta E, Stephenson J, Totoo P, Tyagi N, Urakova N, Vasudev P, Warner K, Wijerathne S, Yu CW-H, Zaru R, Bridge AJ, Aimo L, Argoud-Puy G, Auchincloss AH, Axelsen KB, Bansal P, Baratin D, Batista Neto TM, Blatter M-C, Bolleman JT, Boutet E, Breuza L, Gil BC, Casals-Casas C, Echioukh KC, Coudert E, Cuche B, De Castro E, Estreicher A, Famiglietti ML, Feuermann M, Gasteiger E, Gaudet P, Gehant S, Gerritsen V, Gos A, Gruaz N, Hulo C, Hyka-Nouspikel N, Jungo F, Kerhornou A, Mercier PL, Lieberherr D, Masson P, Morgat A, Paesano S, Pedruzzi I, Pilbout S, Pourcel L, Poux S, Pozzato M, Pruess M, Redaschi N, Rivoire C, Sigrist CJA, Sonesson K, Sundaram S, Sveshnikova A, Wu CH, Arighi CN, Chen C, Chen Y, Huang H, Laiho K, Lehvaslaiho M, McGarvey P, Natale DA, Ross K, Vinayaka CR, Wang Y, Zhang J. 2025. UniProt: the Universal Protein Knowledgebase in 2025. Nucleic Acids Research 53:D609–D617.

4. Ren X, Clark RM, Bansah DA, Varner EN, Tiffany CR, Jaswal K, Geary JH, Todd OA, Winkelman JD, Friedman ES, Jarrett RN, Zemel BS, Wu GD, Zackular JP, DePas WH, Behnsen J, Palmer LD. 2025. Amino acid competition shapes *Acinetobacter baumannii* gut carriage. Cell Host Microbe 33:1396-1411.e9.

5. Jacobs AC, Thompson MG, Black CC, Kessler JL, Clark LP, McQueary CN, Gancz HY, Corey BW, Moon JK, Si Y, Owen MT, Hallock JD, Kwak YI, Summers A, Li CZ, Rasko DA, Penwell WF, Honnold CL, Wise MC, Waterman PE, Lesho EP, Stewart RL, Actis LA, Palys TJ, Craft DW, Zurawski DV. 2014. AB5075, a highly virulent isolate of *Acinetobacter baumannii*, as a model strain for the evaluation of pathogenesis and antimicrobial treatments. mBio 5:e01076-14.

6. Gallagher LA, Ramage E, Weiss EJ, Radey M, Hayden HS, Held KG, Huse HK, Zurawski DV, Brittnacher MJ, Manoil C. 2015. Resources for genetic and genomic analysis of emerging pathogen *Acinetobacter baumannii*. J Bacteriol 197:2027–35.

7. Figurski DH, Helinski DR. 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc Natl Acad Sci U S A 76:1648–1652.

8. Choi K-H, Gaynor JB, White KG, Lopez C, Bosio CM, Karkhoff-Schweizer RR, Schweizer HP. 2005. A Tn7-based broad-range bacterial cloning and expression system. Nat Methods 2:443–448.

9. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. Gene 212:77–86.

10. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A 97:6640–6645.

11. Carruthers MD, Nicholson PA, Tracy EN, Jr RSM. 2013. *Acinetobacter baumannii* utilizes a type VI secretion system for bacterial competition. PLOS ONE 8:e59388.

12. Alexeyev MF. 1999. The pKNOCK series of broad-host-range mobilizable suicide vectors for gene knockout and targeted DNA insertion into the chromosome of gram-negative bacteria. Biotechniques 26:824–826, 828.