Bidirectional redox cycling of phenazine-1-carboxylic acid by *Citrobacter portucalensis* MBL drives increased nitrate reduction

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Running Head: *C. portucalensis* MBL links PCA and nitrate redox cycles

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ABSTRACT

Phenazines are secreted metabolites that microbes use in diverse ways, from quorum sensing to antimicrobial warfare to energy conservation. Phenazines are able to contribute to these activities due to their redox activity. The physiological consequences of cellular phenazine reduction have been extensively studied, but the counterpart phenazine oxidation has been largely overlooked. Phenazine-1-carboxylic acid (PCA) is common in the environment and readily reduced by its producers. Here, we describe its anaerobic oxidation by *Citrobacter portucalensis* strain MBL, which was isolated from topsoil in Falmouth, MA, and which does not produce phenazines itself. This activity depends on the availability of a suitable terminal electron acceptor, specifically nitrate or fumarate. When *C. portucalensis* MBL is provided reduced PCA and either nitrate or fumarate, it continuously oxidizes the PCA. We compared this terminal electron acceptor-dependent PCA-oxidizing activity of *C. portucalensis* MBL to that of several other γ-proteobacteria with varying capacities to respire nitrate and/or fumarate. We found that PCA oxidation by these strains in a fumarate- or nitrate-dependent manner is decoupled from growth and correlated with their possession of the fumarate or periplasmic nitrate reductases, respectively. We infer that bacterial PCA oxidation is widespread and genetically determined. Notably, reduced PCA enhances the rate of nitrate reduction to nitrite by *C. portucalensis* MBL beyond the stoichiometric prediction, which we attribute to *C. portucalensis* MBL's ability to also reduce oxidized PCA, thereby catalyzing a complete PCA redox cycle. This bidirectionality highlights the versatility of PCA as a biological redox agent.

IMPORTANCE Phenazines are increasingly appreciated for their roles in structuring microbial communities. These tricyclic aromatic molecules have been found to regulate gene expression,

38 be toxic, promote antibiotic tolerance, and promote survival under oxygen starvation. In all of
39 these contexts, however, phenazines are studied as electron acceptors. Even if their utility arises
40 primarily from being readily reduced, they would need to be oxidized in order to be recycled.
41 While oxygen and ferric iron can oxidize phenazines abiotically, biotic oxidation of phenazines
42 has not been studied previously. We observed bacteria that readily oxidize phenazine-1-
43 carboxylic acid (PCA) in a nitrate-dependent fashion, concomitantly increasing the rate of nitrate
44 reduction to nitrite. Because nitrate is a prevalent terminal electron acceptor in diverse anoxic
45 environments, including soils, and phenazine-producers are widespread, this observation of
46 linked phenazine and nitrogen redox cycling suggests an underappreciated role for redox-active
47 secreted metabolites in the environment.
48
49 **OBSERVATION**
50 Physiological studies of phenazines have focused on cellular reduction of these secreted
51 molecules for over 120 years. Reduction of phenazines by bacteria was first proposed in the 19th
52 century as an indicator for the presence of enteric bacteria in water supplies (1). Several decades
53 later, pyocyanin, one of the phenazines produced by *Pseudomonas aeruginosa*, was described as
54 an "accessory respiratory pigment" that increased the rate of oxygen consumption by
55 *Staphylococcus*, *Pneumococcus*, and erythrocytes by shuttling electrons from the cells to oxygen
56 (2). Once it became apparent that phenazines can have cytotoxic effects for cells that do not
57 produce them, they were characterized as antimicrobial compounds that destructively abstract
58 electrons from the transport chain (3). It was then discovered that phenazine reduction can
59 greatly benefit *P. aeruginosa* by: 1) regulating gene expression in *P. aeruginosa* during quorum
60 sensing by oxidizing a transcription factor; 2) acting as alternative terminal electron acceptors to

61 promote anoxic survival; and 3) facilitating iron acquisition (4–8). These reports paint a complex
62 picture of the multifarious effects phenazines can have, but in each case the conceptual model
63 ends with the cell reducing the phenazine. Reduced phenazines can be re-oxidized by inorganic
64 electron acceptors like oxygen and ferric iron, and this abiotic process has been invoked to
65 explain redox cycling of phenazines in biofilms (9, 10). However, when these electron acceptors
66 are unavailable, biotic oxidation of reduced phenazines could close the redox cycle by
67 regenerating oxidized phenazines. This process has not been shown to exist for secreted redox-
68 active metabolites.
69
70 In parallel to these physiological studies, phenazines have been used as generic electron shuttles
71 in bioelectrochemical reactor research, selected according to their chemical properties and
72 suitability for a given application (11, 12). Electrochemically reduced neutral red (NR), a
73 phenazine, has been successfully used as an electron donor to cells, chosen for its standard
74 midpoint potential (very near to that of NADH/NAD$^+$, $E^{o\prime}_{\frac{1}{2}} = -320$ mV vs. NHE) and
75 hydrophobicity (13–15). Anaerobic NR oxidation in BERs has been coupled to the reduction of
76 several terminal electron acceptors, including nitrate and fumarate (16). A limitation of these
77 studies is that NR is not found in nature. Therefore, despite NR oxidation being useful in
78 regulating electrosynthesis, the existence of natural bacterially driven phenazine oxidation
79 remains unexplored.
80
81 PCA is one of the mostly widely synthesized phenazines in the microbial world, from which
82 other phenazines are derived (17, 18). PCA is known to be reduced by its producers, driving
83 current generation in bioelectrochemical systems, in which it is re-oxidized by the anode (5, 19).

84 These facts make PCA a fitting candidate for microbial oxidation during anaerobic metabolism.
85 In previous work, we enriched for PCA oxidizers from topsoil by incubating them with reduced
86 PCA, acetate (a non-fermentable carbon source), and nitrate as the only terminal electron
87 acceptor, and successfully isolated the PCA-oxidizing *Citrobacter portucalensis* MBL (20).
88
89 ***C. portucalensis* MBL oxidizes PCA in a nitrate- and fumarate-dependent manner.** We did
90 not observe reduced PCA (PCA_{red}/PCA_{ox} $E^{o\prime}_{\frac{1}{2}} = -116$ mV vs. NHE (9)) oxidation in the
91 absence of a terminal electron acceptor in both the abiotic and biotic regimes (Fig 1A, top left).
92 When 10 mM fumarate (fumarate/succinate $E^{o\prime}_{\frac{1}{2}} = +33$ mV vs NHE (15)) or nitrate (NO_3^-/NO_2^-
93 $E^{o\prime}_{\frac{1}{2}} = +433$ mV vs NHE (15)) were added (right panels), *C. portucalensis* MBL readily
94 oxidized PCA_{red}. Neither fumarate nor nitrate oxidize PCA abiotically (grey curves). During both
95 dissimilatory and assimilatory nitrate reduction, nitrate is first reduced to nitrite (21). Nitrite
96 (NO_2^-/NO $E^{o\prime}_{\frac{1}{2}} = +350$ mV vs NHE (15)) is a more reactive nitrogen oxide than nitrate.
97 Accordingly, we observed slow abiotic oxidation of PCA_{red} by nitrite occurs (Fig 1A, bottom left
98 in grey).
99
100 When *C. portucalensis* MBL is incubated with PCA_{red} and nitrate or fumarate, PCA_{red} oxidation
101 proceeds much faster than it does abiotically by nitrite. Furthermore, when *C. portucalensis*
102 MBL is incubated with PCA_{red} and nitrite, there is no observable PCA_{red} oxidation. We interpret
103 this to mean that *C. portucalensis* catalyzes the oxidation of PCA_{red} when an appropriate terminal
104 electron acceptor is available but reduces oxidized PCA (PCA_{ox}) when such an electron acceptor
105 is absent. We observed PCA_{ox} reduction when the cells started with 200 μM PCA_{ox}, but the rate

106 of reduction decreased according to the provided terminal electron acceptor (Fig 1B). Adding
107 fumarate (Fig. 1B, top right) decreased the rate of PCA_{ox} reduction by *C. portucalensis* MBL by
108 2.38 ± 0.12 μM/hr; adding nitrate caused reduction to be non-detectable; and the presence of
109 nitrite, which abiotically oxidized PCA_{red}, caused a slight dampening in the reduction rate by
110 1.25 ± 0.09 μM/hr (Fig. 1C and Supp. Fig. 4). The asymptotic steady state that *C. portucalensis*
111 MBL approaches with fumarate in Fig. 1A and B indicates that the oxidation and reduction of
112 PCA proceed simultaneously. This is further supported by the fact that PCA_{ox} reduction by *C.*
113 *portucalensis* MBL in the presence of fumarate is significantly slower than in the absence of a
114 terminal electron acceptor (Fig. 1C).
115
116 **Comparative study of nitrate- and fumarate-dependent PCA oxidation by several γ-**
117 **proteobacteria.** We assayed whether other γ-proteobacteria can also oxidize PCA in a terminal
118 electron acceptor-dependent manner (Fig. 1). Using the same assay as above, *E. coli MG1655*, *P.*
119 *aeruginosa* UCBPP-PA14 Δ*phz**, *P. chlororaphis phzB::TnluxAB*, and *P. aureofaciens*
120 *phzB::lacZ* (all of which cannot synthesize PCA either naturally or due to the specified
121 mutations) were incubated with PCA and 10 mM nitrate, nitrite, or fumarate. With the exception
122 of *P. chlororaphis* and *aureofaciens*, all strains exhibited PCA oxidation with nitrate, with *C.*
123 *portucalensis* MBL being the fastest with an initial rate of -25.23 ± 0.84 μM PCA_{red}/hr (Fig. 1C).
124 The assayed *P. aureofaciens* and *chlororaphis* oxidized PCA_{red} with nitrite faster than the abiotic
125 control: -2.75 ± 0.80 and -3.10 ± 0.58, respectively, vs. -1.48 ± 0.29 μM PCA_{red}/hr abiotically
126 (Fig. 1C). Only *C. portucalensis* MBL obviously oxidized PCA in the presence of fumarate. *E.*
127 *coli*, like *C. portucalensis* MBL, reduces PCA_{ox} more slowly with fumarate than without it,
128 indicating that it can also oxidize PCA_{red} with fumarate (Fig 1C, light blue), even if not apparent

129 in Fig. 1A. When the reduction rate by cells was not determined (N.D.), we interpret that the
130 concurrent oxidation rate overwhelmed the reduction rate. None of the strains exhibited
131 significant growth in these assays (Supp. Figs. 1 and 2).
132
133 **The effect of PCA oxidation on the initial rate of nitrate reduction by *C. portucalensis***
134 **MBL.** In Fig 1A it is clear that *C. portucalensis* MBL completes its oxidation of ~200 μM
135 PCA_{red} within 10 hours when nitrate is available. We repeated this experiment in anaerobic
136 culture tubes inoculated with *C. portucalensis* MBL, 10 mM NO_3^-, and either 200 μM PCA_{red},
137 200 μM PCA_{ox}, or no PCA and measured nitrate and nitrite concentrations over time via ion
138 chromatography. We observed that PCA_{red} oxidation significantly increased the rate of nitrate
139 reduction to nitrite (Fig 2A). Nitrate consumption was stoichiometrically matched by nitrite
140 production. We did not observe the production of any other nitrogen oxides or ammonium (data
141 not shown). The no-PCA control did not show any nitrate reduction over the first eight hours (3
142 ± 55 μM/hr; 95% confidence interval reported for all rate measurements, calculated from a linear
143 regression of the data from the first two timepoints for each curve in Fig. 2A). During this time,
144 131 ± 49 μM/hr nitrate was reduced in the PCA_{red} condition. In contrast, the PCA_{ox} control
145 exhibited only a small increase in nitrate reduction (35 ± 35 μM/hr). To verify that nitrate was
146 reduced to nitrite, we tracked nitrite's production. We observed that over the first eight hours the
147 no-PCA control produced nitrite at the rate of 22 ± 3 μM/hr, the PCA_{ox} control at 58 ± 2 μM/hr,
148 and the PCA_{red} condition at 147 ± 44 μM/hr of nitrite over the first eight hours. Thus, we
149 estimate the effect of PCA_{red} vs PCA_{ox} to be 96 ± 60 μM/hr of increased nitrate reduction or 89 ±
150 44 μM/hr of increased nitrite production. The increase in nitrate reduction due to PCA_{red} was not
151 stoichiometric: PCA redox and nitrate reduction are both two-electron processes (9, 21), and a

152 process without a redox cycle would predict that oxidizing PCA_{red} at a rate of 25 μM/hr (Fig. 1C)
153 would reduce at most 25 μM/hr nitrate to nitrite. However, the lowest range of the confidence
154 intervals suggests that at least an additional 36 μM/hr nitrate was reduced by the cells when
155 PCA_{red} was provided, implying that the cells are redox-cycling the PCA.
156
157 **Conclusions.** The effect of PCA oxidation by *C. portucalensis* MBL on its rate of nitrate
158 reduction was not stoichiometric (Fig 2A). This is consistent with two explanations: 1) a prior
159 report argues that neutral red (a synthetic phenazine) oxidation affects electrosynthesis during
160 anaerobic respiration primarily by changing gene regulation via menaquinone reduction (16), and
161 so it is plausible that PCA oxidation may increase transcription of a rate-limiting factor in the
162 electron transport chain to nitrate; 2) we observed that a PCA redox cycle by *C. portucalensis*
163 MBL is possible (Fig 1, most evident in the case of fumarate, top right panels in A and B, where
164 oxidation and reduction proceed simultaneously in the steady state); it is likely that PCA redox
165 cycling may also directly stimulate nitrate reduction. We cannot determine whether reduced PCA
166 (PCA_{red}) serves as an effective electron donor to the cells' metabolism from our above
167 observations. Regardless, nitrate reduction driven by PCA redox-cycling has environmental
168 ramifications far beyond the cell.
169
170 Despite all being capable of nitrate reduction, not all the γ-proteobacteria that we tested were
171 capable of oxidizing PCA_{red} with nitrate (Fig. 1C), suggesting a genetic basis for this process.
172 The only strains that did not readily oxidize PCA_{red} with nitrate were *P. chlororaphis* and
173 *aureofaciens*, and, unlike the other species, they do not possess the periplasmic nitrate reductase
174 operon *nap*. Curiously, it has been previously noted that wildtype *P. aeruginosa* UCBPP-PA14

175 oxidizes another phenazine, pyocyanin, in the presence of nitrate, while a *napA* transposon
176 knockout strain does not (22). We propose that this operon may be necessary for rapid PCA_{red}
177 oxidation and are developing genetic tools in *C. portucalensis* MBL to test this idea directly.
178 Nitrate-dependent PCA_{red} oxidation is likely to be common in anoxic environments, such as
179 soils, as it is not species-specific and occurs readily. If the outsized effect of PCA_{red} oxidation on
180 nitrate reduction that we observe generalizes to other species, the production and reduction of
181 phenazines by organisms like *Pseudomonads* likely affects the rate of nitrate consumption in
182 their environs, adding another function to the phenazine arsenal (18). We propose that cells may
183 catalyze a PCA redox cycle whenever they have internal stores of reducing equivalents and a
184 usable terminal electron acceptor (Fig 2B). While both intracellular (e.g., sulfur) and
185 extracellular (e.g., humics) bacterial redox cycles have been described (23, 24), to our knowledge
186 this has not been appreciated for secreted metabolites, such as phenazines. Our observation
187 implies that these molecules may act as "electron buffers", enabling cells to reduce and oxidize
188 them according to whether they are lacking a terminal electron acceptor or an electron donor,
189 respectively, and in so doing significantly impact other biogeochemical cycles.
190

191 **MATERIALS AND METHODS**

192 **Strains and media.** *Citrobacter portucalensis* MBL was isolated in our previous work (20). In
193 the comparative PCA oxidation and reduction experiments, we used strains of γ-proteobacteria
194 that cannot synthesize phenazines, either natively or due to mutations. We used *E. coli* MG1655,
195 *P. aeruginosa* UCBPP-PA14 Δ*phz**, *P. chlororaphis phzB::TnLuxAB*, and *P. aureofaciens*
196 *phzB::lacZ*. The wildtype *Pseudomonads* can synthesize PCA, but the used mutants cannot. All
197 strains were grown and incubated under the same conditions. The basal medium for the

198 experiments contained 20 mM potassium phosphate buffer (final pH 7), 1 mM sodium sulfate, 10
199 mM ammonium chloride, 1x SL-10 trace elements, 1x freshwater salt solution (17.1 mM sodium
200 chloride, 1.97 mM magnesium chloride, 0.68 mM calcium chloride, and 6.71 mM potassium
201 chloride), and 1x 13-vitamin solution (10 μM MOPS pH 7.2, 0.1 μg/mL riboflavin, 0.03 μg/mL
202 biotin, 0.1 μg/mL thiamine HCl, 0.1 μg/mL L-ascorbic acid, 0.1 μg/mL d-Ca-pantothenate, 0.1
203 μg/mL folic acid, 0.1 μg/mL nicotinic acid, 0.1 μg/mL 4-aminobenzoic acid, 0.1 μg/mL
204 pyridoxine HCl, 0.1 μg/mL lipoic acid, 0.1 μg/mL NAD, 0.1 μg/mL thiamine pyrophosphate,
205 and 0.01 μg/mL cyanocobalamin). Depending on the experimental condition, as indicated in the
206 figure legends, a terminal electron acceptor would be added (10 mM of fumarate, nitrate, or
207 nitrite) or omitted. For oxidized PCA, a 10 mM stock in 20 mM NaOH was prepared. For
208 reduced PCA, an 800 μM stock in the basal medium was reduced by electrolysis. Both stocks
209 were diluted into plates to a final target concentration of 200 μM.
210
211 **Cell preparation.** All cell incubations and experiments were performed at 30 C. Cells were
212 preserved in 35% glycerol stocks at -80 C. Two days prior to the experiments, frozen cells for
213 each strain assayed were struck out on lysogeny broth (LB) agar plates and incubated overnight.
214 The evening prior to the experiment, a patch from the streaks was inoculated into liquid LB in a
215 respective culture tube and incubated slanted, shaking at 250 rpm, overnight. The morning of the
216 experiment, 1 mL of each cell culture was washed three times into the basal medium by spinning
217 for two minutes at 6000 x g, aspirating the supernatant, and gently resuspending with a pipette.
218 The OD_{600} of each washed culture was measured. The cultures were brought into a Coy glove
219 box, where they were washed three times into the same basal medium that had been made

220 anoxic, following the same procedure as above. After being left to stand for 1-2 hours, the cells
221 were inoculated into the different experimental conditions at a target starting OD_{600} of 0.1.
222
223 **Measurement of PCA redox and nitrogen oxide concentrations**. All PCA redox
224 measurements were performed in a Coy chamber using a BioTek Synergy 5 plate reader.
225 Reduced PCA concentration was measured by fluorescence (excitation 360 nm and emission 528
226 nm) (25). In these cultures, 1 mM acetate was provided. Plates were incubated shaking on the
227 "medium" setting. For nitrogen oxide concentration measurements, *C. portucalensis* MBL cells
228 were prepared as above, but incubated in culture tubes in the Coy chamber to allow for sampling.
229 In these cultures, no acetate was provided. The tubes were kept at 30 C, but not shaking. Samples
230 were filtered through a 0.2 µm cellulose-acetate spin filter and stored at -80 C prior to analysis.
231 Nitrate and nitrite concentrations were measured by ion chromatography using a Dionex ICS-
232 2000 instrument.
233
234 **Data analysis.** Initial redox rates in the conditions with supplied nitrite did not include the first
235 1.5 hours of the assay because that period showed a systematic artifact due to fluorescence
236 quenching (Fig. 1A, lower left panel). The linear fits were calculated over the first five hours
237 after the first detectable PCA_{red} measurement, which was determined to be appropriate based on
238 scanning for R^2 values over increasing time frames (Supp. Figs. 4 and 5). 95% confidence
239 intervals are calculated as the estimated value ± 1.96 * (standard error). When comparing rates,
240 the reported error is the geometric mean of the intervals for the two measurements. All plots
241 were generated using Bokeh and the legends and titles were adjusted using Inkscape. All the raw

data and the Jupyter notebook used for their analysis are available at https://github.com/ltsypin/Cportucalensis_observation.

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320
321

322 **Main text figure legends**

323 **Figure 1. Oxidation and reduction of PCA by bacteria provided different terminal electron**
324 **acceptors (TEAs).** (A-B) The light circles correspond to three independent biological replicates
325 and the dark lines to their respective means. Cells only oxidize PCA when an appropriate TEA is
326 available. Fumarate, nitrite, or nitrate (top right, bottom left, and bottom right panels,
327 respectively) stimulate different strains to oxidize PCA. When no TEA is provided (top left
328 panels), no strains oxidize PCA and all reduce it at different rates (enumerated in C). With
329 nitrate, only *P. chlororaphis* and *aureofaciens* (blue and umber curves) appear to not oxidize
330 PCA but reduce it. Nitrite abiotically oxidizes PCA (grey curve, lower left in A), but *P.*
331 *aureofaciens and chlororaphis* catalyze an even faster biological oxidation. In contrast, the
332 *Enterics* (yellow and orange curves) reduce PCA faster than the abiotic reaction. Given fumarate,
333 *C. portucalensis* MBL (orange) clearly oxidizes PCA, and the *Pseudomonads* reduce it. (C) This
334 table reports the estimated initial rates of oxidation or reduction according to a linear fit over the
335 first five hours (Supp. Figs. 3 and 4). This timeframe was determined by tracking the R^2 for the
336 linear fit over increasing time windows (Supp. Figs. 5 and 6). N.D. signifies rates that were not
337 detectable. We can only observe the relative magnitude of the rates of oxidation and reduction in
338 a given condition. However, whether the oxidation of PCA overpowers its reduction depends on
339 both the provided TEA and is associated with the presence of the periplasmic nitrate reductase
340 (NAP) or fumarate reductase (FRD). PCA reduction is calculated as a positive rate (shades of
341 blue); PCA oxidation is calculated as a negative rate (shades of red).

342

343 **Figure 2. PCA oxidation by *C. portucalensis* MBL increases its initial rate of nitrate**
344 **reduction.** (A) Either 200 μM reduced PCA (PCA_{red}), 200 μM oxidized PCA (PCA_{ox}), or no

345 PCA was added to each condition. Ion chromatography shows that over the 10 hours that the
346 cells are oxidizing PCA_{red} (Fig. 1A), their initial rate of nitrate reduction is substantially
347 increased. The nitrate is stoichiometrically reduced to nitrite. Error bars are 95% confidence
348 intervals around the mean of three independent biological replicates. When not visible, they are
349 smaller than the circles (nitrate) or squares (nitrite) denoting the measurements. (B) Any cell that
350 has internal stores of reducing equivalents and an appropriate terminal electron acceptor (TEA_1)
351 may catalyze an internal PCA redox cycle (bolded arrows). We observe this for *E. coli* and *C.*
352 *portucalensis* MBL with nitrate or fumarate, *P. aeruginosa* with nitrate, and *P. aureofaciens* and
353 *chlororaphis* with nitrite. In addition to this cellularly-catalyzed reaction, the product of TEA_1's
354 reduction (TEA_2) may abiotically oxidize PCA, which we observe when nitrate is reduced to
355 nitrite (Fig. 1A, bottom left).
356
357

358 **Supplementary Figure Legends**

359 **Supplementary Figure 1.** OD_{600} measurements for all strains in all conditions during the
360 oxidation assays (Fig. 1A). Light circles represent all measurements from three independent
361 biological replicates corresponding to the PCA_{red} measurements in Figure 1A. The solid lines are
362 the means of the replicates. The curves are colored by condition (i.e., which, if any, terminal
363 electron acceptor was added). While there are some OD_{600} trends in the first five hours, there is
364 no evidence of growth that would indicate a doubling. In the abiotic graph, all the data points
365 overlie each other.

366

367 **Supplementary Figure 2.** OD_{600} measurements for all strains in all conditions during the
368 reduction assays (Fig. 1B). Light circles represent all measurements from three independent
369 biological replicates corresponding to the PCA_{red} measurements in Figure 1B. The solid lines are
370 the means of the replicates. The curves are colored by condition (i.e., which, if any, terminal
371 electron acceptor was added). While there are some OD_{600} trends in the first five hours, there is
372 no evidence of growth that would indicate a doubling. In the abiotic graph, all the data points
373 overlie each other.

374

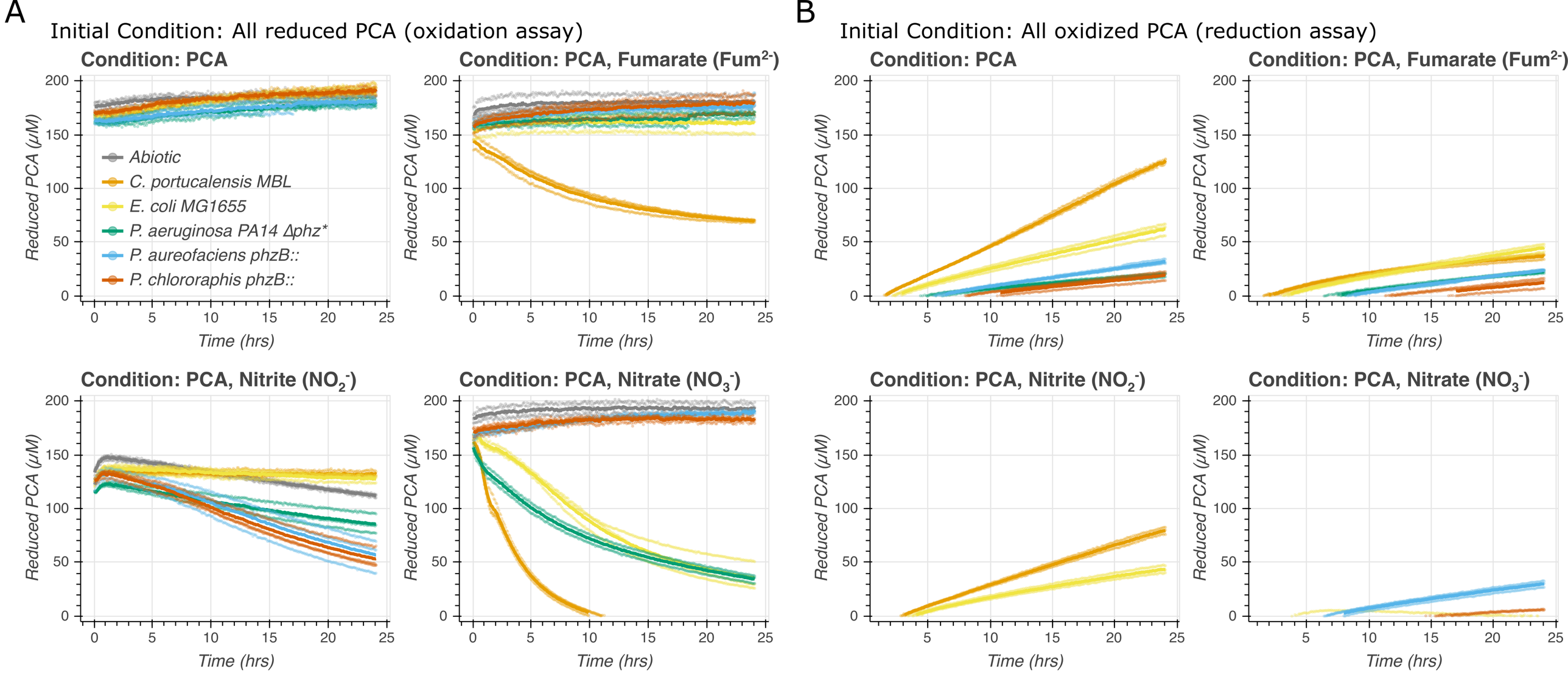
375 **Supplementary Figure 3.** Oxidation assay results grouped by strain rather than by condition.
376 This is a different representation of the same data as in Fig. 1A, including the linear regressions
377 (dashed lines) that generated the initial rate estimates as reported in Fig. 1Cα.

378

379 **Supplementary Figure 4.** Reduction assay results grouped by strain rather than by condition.
380 This is a different representation of the same data as in Fig. 1B, including the linear regressions

381 (dashed lines) that generated the initial rate estimates as reported in Fig. 1Cβ. In the abiotic
382 conditions, no reduction was detected (N.D.).
383
384 **Supplementary Figure 5.** Evaluation of the appropriate window for performing the linear
385 regressions to estimate initial redox rates in the oxidation assays (Fig. 1Cα). Starting from the
386 first valid PCA_{red} measurement in each condition, a linear regression was performed over
387 increasing lengths of time and the corresponding R^2 value was plotted. A time window of five
388 hours (red line) was selected as a systematic compromise for all conditions.
389
390 **Supplementary Figure 6.** Evaluation of the appropriate window for performing the linear
391 regressions to estimate initial redox rates in the reduction assays (Fig. 1Cβ). Starting from the
392 first valid PCA_{red} measurement in each condition, a linear regression was performed over
393 increasing lengths of time and the corresponding R^2 value was plotted. A time window of five
394 hours (red line) was selected as a systematic compromise for all conditions.

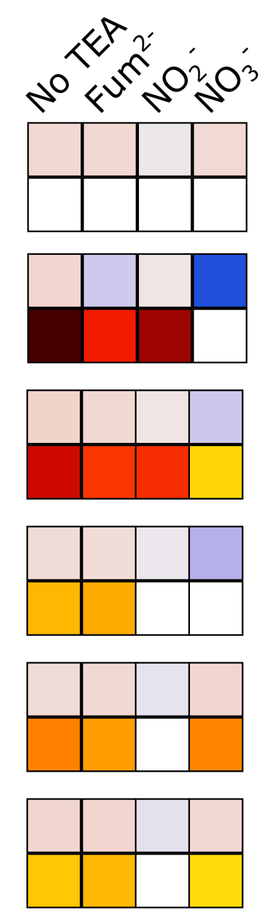
A



C

Initial redox rates (μM PCA_{red}/hr) ± 95% confidence interval:
(α) oxidation assay; (β) reduction assay

Strain	FRD?	NAP?	PCA alone	PCA with Fum^{2-}	PCA with NO_2^-	PCA with NO_3^-
Abiotic	-	-	(α) 1.36 ± 0.32 (β) N.D.	(α) 1.41 ± 0.63 (β) N.D.	(α) -1.48 ± 0.29 (β) N.D.	(α) 1.26 ± 0.45 (β) N.D.
C. portucalensis MBL	+	+	(α) 1.45 ± 0.15 (β) 5.30 ± 0.05	(α) -6.49 ± 0.50 (β) 2.92 ± 0.11	(α) -0.41 ± 0.39 (β) 4.05 ± 0.07	(α) -25.23 ± 0.84 (β) N.D.
E. coli MG1655	+	+	(α) 1.87 ± 0.21 (β) 3.42 ± 0.16	(α) 1.43 ± 0.57 (β) 2.70 ± 0.12	(α) -0.50 ± 0.60 (β) 2.78 ± 0.13	(α) -6.70 ± 0.27 (β) 0.80 ± 0.15
P. aeruginosa PA14 *Δphz**	-	+	(α) 0.93 ± 0.32 (β) 1.24 ± 0.13	(α) 0.80 ± 0.34 (β) 1.44 ± 0.13	(α) -1.84 ± 0.42 (β) N.D.	(α) -10.09 ± 0.42 (β) N.D.
P. aureofaciens phzB::	-	-	(α) 1.02 ± 0.23 (β) 1.97 ± 0.08	(α) 1.41 ± 0.26 (β) 1.63 ± 0.09	(α) -2.75 ± 0.80 (β) N.D.	(α) 1.49 ± 0.21 (β) 1.91 ± 0.18
P. chlororaphis phzB::	-	-	(α) 1.52 ± 0.16 (β) 1.02 ± 0.19	(α) 1.79 ± 0.50 (β) 1.22 ± 0.07	(α) -3.10 ± 0.58 (β) N.D.	(α) 1.33 ± 0.35 (β) 0.71 ± 0.09



Color maps for initial redox rates (μM PCA_{red}/hr)

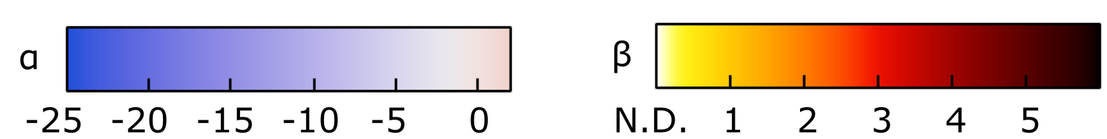


Figure 1. Oxidation and reduction of PCA by bacteria provided different terminal electron acceptors (TEAs). (A-B) The light circles correspond to three independent biological replicates and the dark lines to their respective means. Cells only oxidize PCA when an appropriate TEA is available. Fumarate, nitrite, or nitrate (top right, bottom left, and bottom right panels, respectively) stimulate different strains to oxidize PCA. When no TEA is provided (top left panels), no strains oxidize PCA and all reduce it at different rates (enumerated in C). With nitrate, only P. chlororaphis and aureofaciens (blue and umber curves) appear to not oxidize PCA but reduce it. Nitrite abiotically oxidizes PCA (grey curve, lower left in A), but P. aureofaciens and chlororaphis catalyze an even faster biological oxidation. In contrast, the Enterics (yellow and orange curves) reduce PCA faster than the abiotic reaction. Given fumarate, C. portucalensis MBL (orange) clearly oxidizes PCA, and the Pseudomonads reduce it. (C) This table reports the estimated initial rates of oxidation or reduction according to a linear fit over the first five hours (Supp. Figs. 3 and 4). This timeframe was determined by tracking the R^2 for the linear fit over increasing time windows (Supp. Figs. 5 and 6). N.D. signifies rates that were not detectable. We can only observe the relative magnitude of the rates of oxidation and reduction in a given condition. However, whether the oxidation of PCA overpowers its reduction depends on both the provided TEA and is associated with the presence of the periplasmic nitrate reductase (NAP) or fumarate reductase (FRD). PCA reduction is calculated as a positive rate (shades of blue); PCA oxidation is calculated as a negative rate (shades of red).

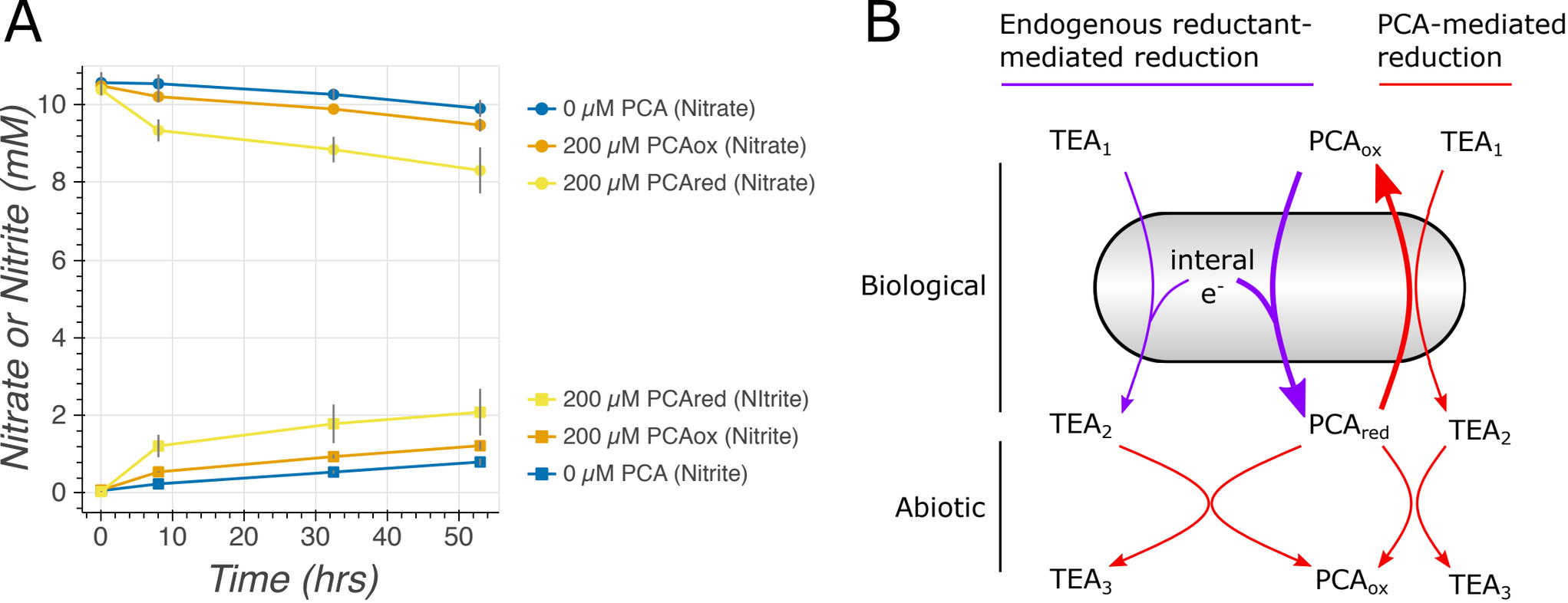


Figure 2. PCA oxidation by C. portucalensis MBL increases its initial rate of nitrate reduction. (A) Either 200 μM reduced PCA (PCA_{red}), 200 μM oxidized PCA (PCA_{ox}), or no PCA was added to each condition. Ion chromatography shows that over the 10 hours that the cells are oxidizing PCAred (Fig. 1A), their initial rate of nitrate reduction is substantially increased. The nitrate is stoichiometrically reduced to nitrite. Error bars are 95% confidence intervals around the mean of three independent biological replicates. When not visible, they are smaller than the circles (nitrate) or squares (nitrite) denoting the measurements. (B) Any cell that has internal stores of reducing equivalents and an appropriate terminal electron acceptor (TEA_1) may catalyze an internal PCA redox cycle (bolded arrows). We observe this for E. coli and C. portucalensis MBL with nitrate or fumarate, P. aeruginosa with nitrate, and P. aureofaciens and chlororaphis with nitrite. In addition to this cellularly-catalyzed reaction, the product of TEA_1's reduction (TEA_2) may abiotically oxidize PCA, which we observe when nitrate is reduced to nitrite (Fig. 1A, bottom left).