

1 **Supplemental Figures**

2

3 **Fig. S1. Fungi from identified bacterial-fungal pairings are sensitive to PCA.** A) *Lecythophora* and
4 *Aspergillus* species grown on PDA, PDA supplemented with 300 μ M PCA, and finally in the presence of
5 PCA along with co-isolated bacterial partners, a *Paraburkholderia* species and a *Luteibacter* species,
6 respectively. Plates were grown for 48 hrs at 30 °C. B) Phenazine production is required to inhibit growth
7 of the *Aspergillus* species. *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* inhibit fungal
8 growth, but the *::phzD* and *::phzB* strains, respectively, fail to inhibit fungal growth after growing on
9 PDA for 48 hrs at 30 °C.

10 **Fig. S2. Bacterial aggregates accumulate along the ridge of co-colonies treated with PCA.** A band of
11 bacteria can be seen in both treated and control colonies between the inner and outer zones. In the PCA
12 treated colony, this band is thicker with more well formed aggregates, and is representative of the region
13 larger aggregates are visible to the eye during co-colony growth on PCA. Samples were cleared using the
14 MiPACT protocol, and images were captured on a Nikon Ti2 Eclipse. Red represents the signal produced
15 by eubacterial HCR probes coupled to Alexa fluor 647 nm..

16 **Fig. S3. *P. edwinii* does not degrade PCA in liquid culture.** *P. edwinii* was grown in potato dextrose
17 broth shaking at 30 °C spiked with and without 300 μ M PCA, and with PCA and its fungal partner. PCA
18 concentration was measured every 24 hours for three days. A control lacking *P. edwinii* was included to
19 confirm PCA did not degrade over this time period. Quantification occurred by measuring absorbance at
20 365 nm. Error bars represent standard deviation of four biological replicates.

21

22 **Fig. S4. Morphology of WT and Δ *hrcA* *P. edwinii* and properties of the *Aspergillus* isolate with and
23 without PCA challenge.** A) Comparison of the *Aspergillus* isolate grown alone (left, top), with WT *P.*
24 *edwinii* (center), and with the Δ *hrcA* mutant (bottom). Note large aggregates in the Δ *hrcA* mutant despite
25 the absence of PCA B) WT (left) the the Δ *hrcA* mutant (right) grown in the absence (top) and presence
26 (center) of PCA, and next to its partner fungus when challenged with PCA (bottom). The WT strain
27 shows a thickening of the colony in response to PCA, as well as an increase sheen. A similar thickening
28 and sheen is visible for the Δ *hrcA* mutant in all conditions. C) The *Aspergillus* isolate produces an
29 oxidizing environment when growing on potato dextrose agar in the absence of PCA, but generates a
30 more reducing environment during challenge with 300 μ M PCA. Error bars represent the standard
31 deviation of 3 measurements at each depth. All colonies were grown for 48 hours at 30 °C.

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34 **Supplemental Tables**

35 **Supplementary Table 1.** List of transposon mutants found in *P. edwinii* that alter its ability to protect its
36 partner fungus, including phenotype (left), closest homologue of *P. edwinii* gene each transposon hit was
37 found in based on NCBI BLAST using arbitrary PCR on the mutant strains, and any apparent homologue
38 the gene encodes.

39

40 **Supplementary Table 2.** List of strains and plasmids used in this study.

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43 **Supplemental References**

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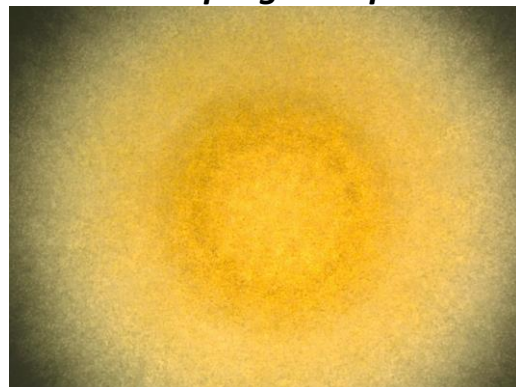
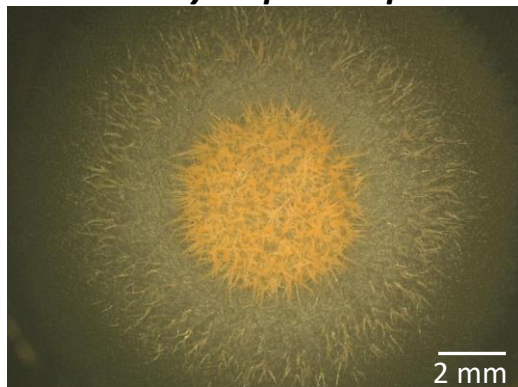
Figure S1

A

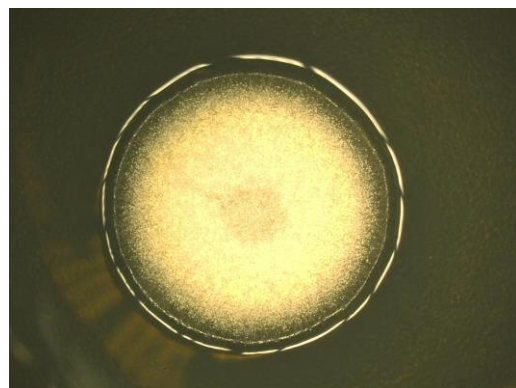
Lecythophora sp.

Aspergillus sp.

Fungus (-) PCA



Fungus (+) PCA



Co-colony (+) PCA

+ *Paraburkholderia* sp.



+ *Luteibacter* sp.



B

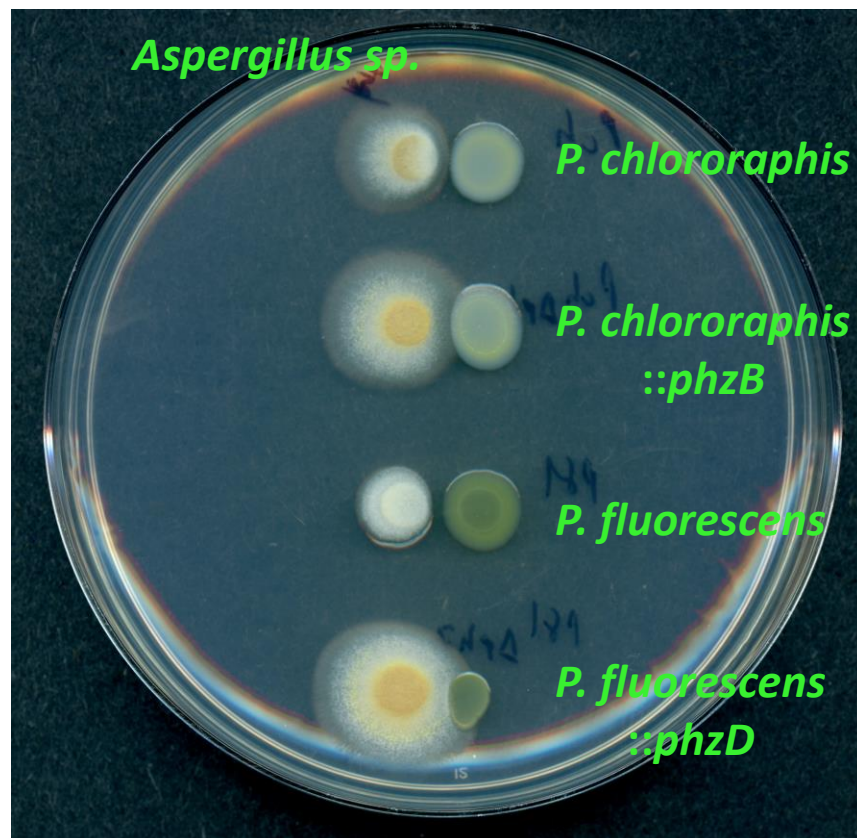


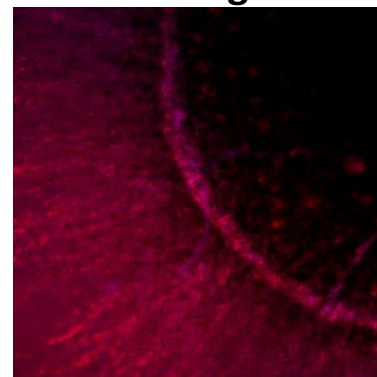
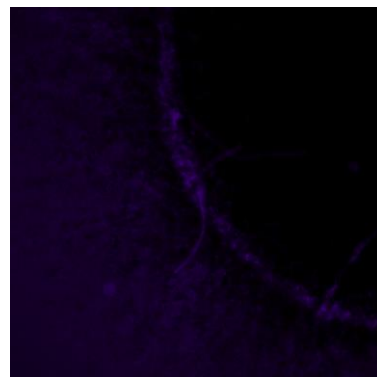
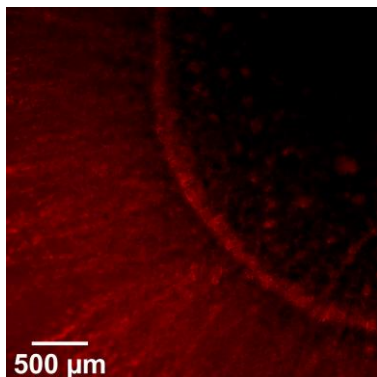
Figure S2

Eubacteria HCR

DAPI

Merge

(-) PCA



(+) PCA

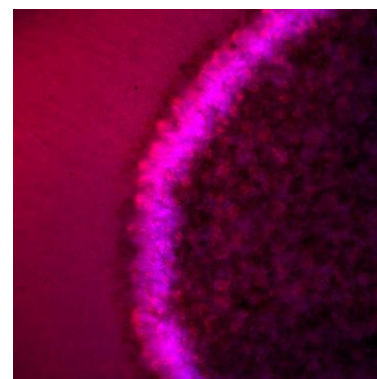
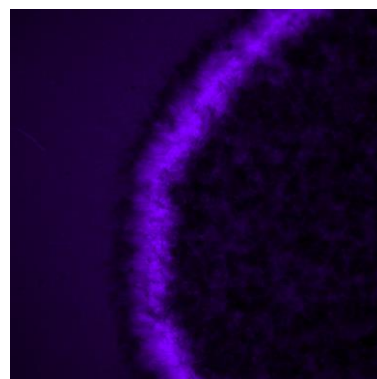
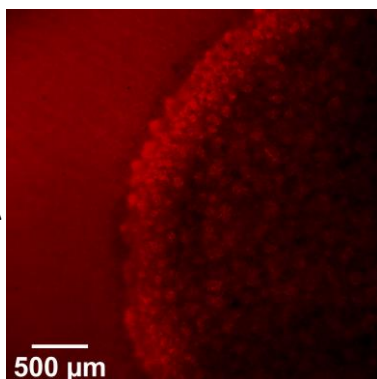


Figure S3

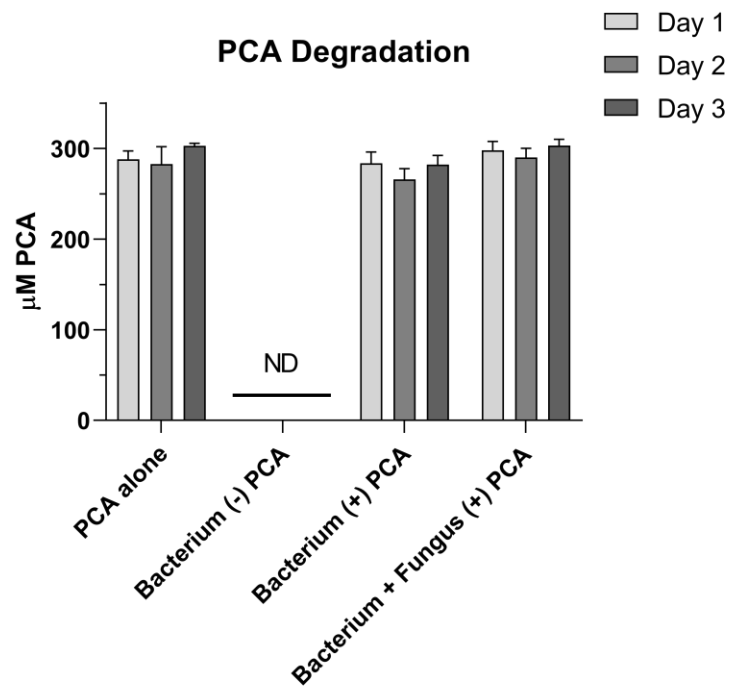
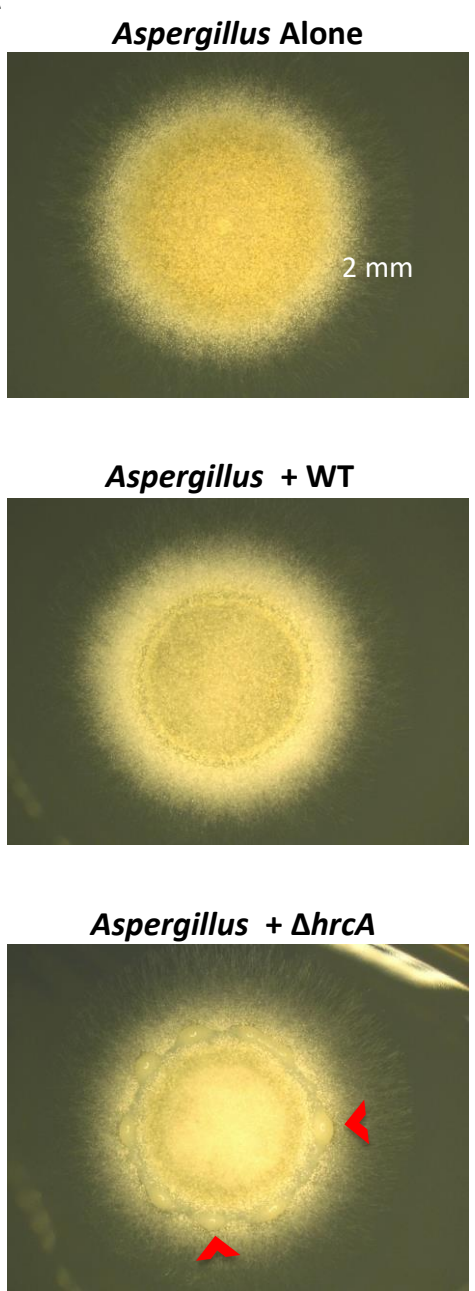
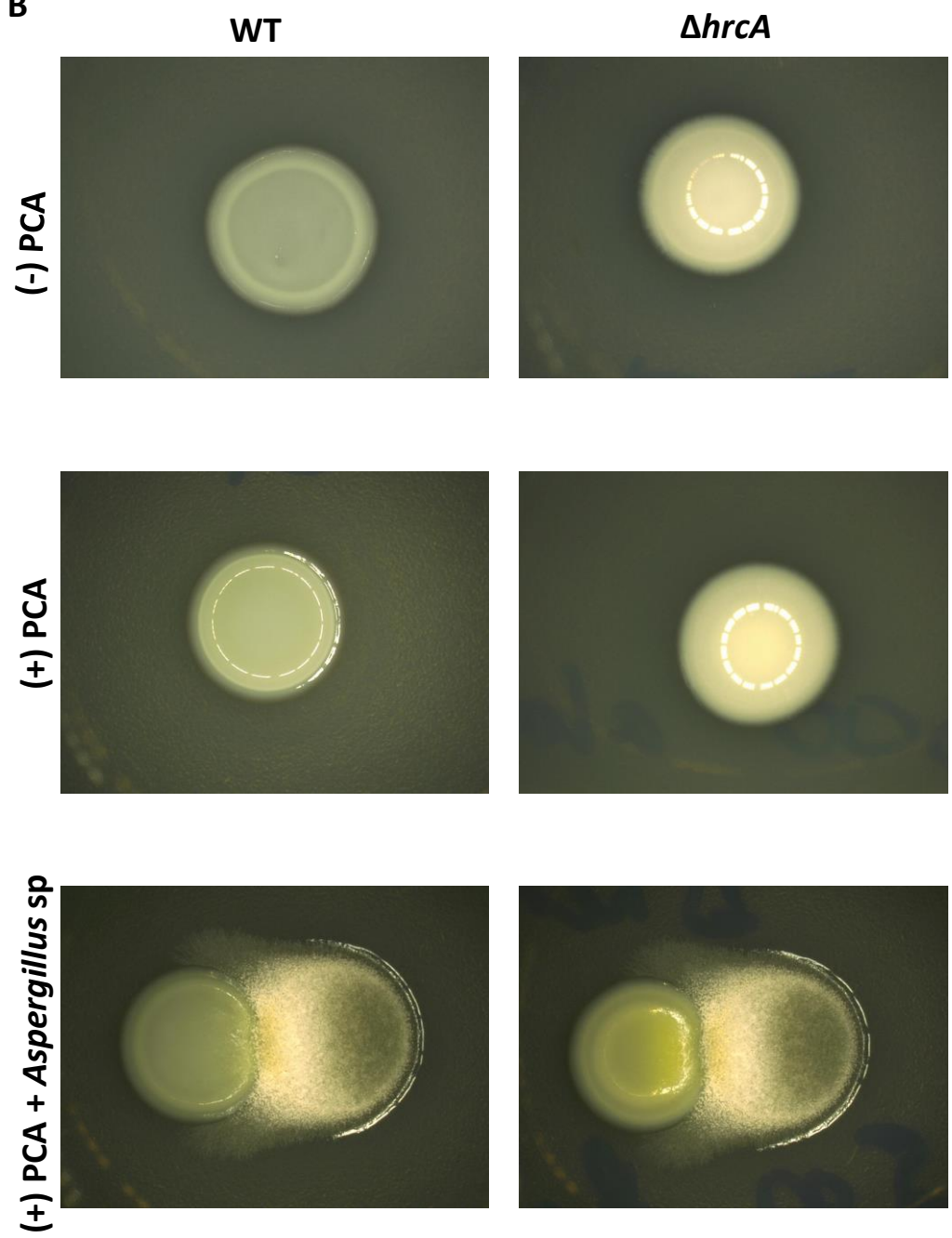


Figure S4

A



B



C

