

SI Figure 1: A phylogenetic tree based on 400 conserved proteins is consistent with the 16S rRNA tree. The phylogenetic tree of the seven *Methylobacterium strains* was built using PhyloPhIAn (38). The conserved proteins were selected based on ubiquity among the entire tree of life. Protein homologs were identified using a threshold of 90% sequence similarity. The most informative residues were extracted from each protein, concatenated, and used as the input for the phylogenetic tree.



SI Figure 2: Non-planktonic strains grown on DCM show a linear relationship between total protein produced and chloride released. DM4 $\Delta dcmA$ +pJM10 (planktonic control), CM4+pJM10, BJ001+pJM10, and *M. radiotolerans*+pJM10 were grown in sealed flasks containing 10 mL of M-PIPES + 5 mM DCM as the sole carbon and energy source. After three days' growth, chloride concentrations were measured in the supernatant. Cells were then pelleted by centrifugation, lysed using a bead beater, and the total protein in the supernatant was measured with a Bradford assay.



SI Figure 3: (A) Transconjugant yield on DCM in pure culture is generally predictive of competitive fitness. The outliers are BJ001, which shows lower fitness than would be predicted based on yield, and *M. radiotolerans*, which shows higher fitness than predicted. (B) Phylogenetic distance is not an effective predictor of competitive fitness. Error bars show one standard deviation, calculated from three biological replicates.



SI Figure 4: (A) All pJM10 transconjugants show DcmA activity in cell suspensions of cultures grown with 3.5 mM succinate and 5 mM DCM. (B) *In vitro* DcmA activity does not predict *in vivo* competitive fitness. The outliers, AM1 and DM4 $\Delta dcmA$, have the lowest and highest specific activities, respectively. However, strains with intermediate fitness cannot be resolved based on DcmA activity.



SI Figure 5: Neither growth rate in media containing high external chloride concentrations nor growth rate in media buffered to low pH are predictive of growth with DCM. (A) The ratio of growth rates when grown with 100 mM NaCl compared to unmodified M-PIPES+succinate does not predict the competitive fitness on DCM. (B) The ratio of growth rates when grown in M-PIPES+succinate at pH 5.9 compared to pH 6.7 is similarly ineffective at predicting competitive fitness on DCM.



SI Figure 6: Calibration curve for the intracellular pH biosensor. A pH-insensitive red fluorescent protein is translationally fused to a pH-sensitive green fluorescent protein. The ratio of green to red fluorescence is indicative of the intracellular pH. For calibration, cells expressing the biosensor construct were incubated in buffer of the indicated pH containing valinomycin and nigericin prior to measurement by flow cytometry. Error bars show one standard deviation calculated from three replicates.



SI Figure 7: The maximum decrease in intracellular pH on addition of 5 mM is not predictive of competitive fitness on DCM. From each of the curves in Figure 4, the lowest intracellular pH measurement was taken as representative of the strain's ability to maintain its intracellular pH under a DCM challenge.