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Dear Editor,

Please find our uploaded manuscript, "DABs: a new class of inorganic carbon pumps found throughout prokaryotic phyla," which we are submitting for publication as an Article. The manuscript contains six main text figures and includes an additional Supplementary Information document. This is an updated manuscript of a previously submitted work that has been modified per a discussion with Cláudio Nunes-Alves.

Organisms that fix CO₂ using the enzyme Rubisco (e.g. plants, cyanobacteria, etc.) are highly constrained by this enzyme's intrinsic deficiencies of slow and mistake-prone activity. As a consequence, many such organisms use CO₂ Concentrating Mechanisms (CCMs) that improve Rubisco activity by increasing the local concentration of CO₂. It is widely believed in the photosynthesis research community that components of bacterial CCMs could be used to improve plant productivity (Long et al. 2015 Cell). **Although older genetic screens identified several CCM components (e.g. Price et al. 1989 Plant Phys.), surprisingly no quantitative, systematic search for CCM genes has been carried out.** As a result, the complete 'parts list' of bacterial CCMs remains unknown. This is particularly true for inorganic carbon (C_i) transporters. Few have been characterized, fewer are mechanistically understood in significant detail, and none are currently capable of being functionally expressed in a plant, which is a major goal of the above efforts.

Here we describe the first systematic screen for bacterial CCM genes using modern genetic techniques. We perform a barcoded genome-scale transposon mutagenesis screen on the model chemoautotroph *Halothiobacillus neapolitanus*. This comprehensive approach enables us to identify both essential genes and CCM genes in *H. neapolitanus*, producing the first essential gene set for a bacterial chemotroph and the first exhaustive documentation of CCM components.

Exhaustive documentation of CCM components is an important starting point for reconstituting the CCM in heterologous hosts (i.e. in plants). **Moreover, our screen highlighted an intriguing gene cluster that we show encodes a highly novel C_i transporter.** We functionally and biochemically characterized this transporter (termed DAB for DABs Accumulate Bicarbonate). Specifically, we demonstrate heterologous DAB function in *E. coli*, show that it is likely an active transporter, and purify it to show biochemically that it contains a zinc-binding active site similar to a carbonic anhydrase enzyme. **This represents arguably the most thorough biochemical characterization of any C_i transporter yet, sets the stage for detailed structure-function studies, and also clearly defines a transport activity that can be used in engineered CCMs.**

Interestingly, we also find that DAB genes appear throughout prokaryotic phyla. This includes bacteria and archaea known to fix CO_2 and also many heterotrophs that do not. Surprisingly, many of the latter includes known pathogens including *V. cholerae*, *B. anthracis*, and *L. pneumophila*. In order to demonstrate the significance of this observation to the broader audience of microbiologists, **we have also isolated the genes for *V. cholera* and *B. anthracis* and demonstrated that these variants are functional C_i pumps.** We thus suspect that DABs support bacterial growth or persistence through other, as-yet unknown mechanisms, in non- CO_2 -fixing bacteria and that this demonstration will be of interest to many microbiologists.

Altogether, our work combines state-of-the-art techniques in bacterial genetics and protein biochemistry to interrogate an environmentally-critical pathway of CO_2 fixation. Our quantitative, genome-wide screen led us to a mechanistic analysis of a novel C_i transporter which holds great promise for future bioengineering, for biochemical study of C_i transport, and as a source of insight into the carbon metabolism of microbes broadly. We therefore believe our results will be of great interest to a wide range of scientists at the intersections of microbiology with biochemistry, pathogenesis, and plant biology. We look forward to hearing your feedback regarding our work.

Regards,

A handwritten signature in black ink, appearing to read 'D. F. Savage'.

David Savage