

TITLE: Diversity in CO₂-Concentrating Mechanisms among Chemolithoautotrophs from the Genera *Hydrogenovibrio*, *Thiomicrospira*, and *Thiomicrothrix*, Ubiquitous in Sulfidic Habitats Worldwide

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ABSTRACT:

Members of the genera *Hydrogenovibrio*, *Thiomicrospira*, and *Thiomicrothrix* fix carbon at hydrothermal vents, coastal sediments, hypersaline lakes, and other sulfidic habitats. The genome sequences of these ubiquitous and prolific chemolithoautotrophs suggest a surprising diversity of mechanisms for the uptake and fixation of dissolved inorganic carbon (DIC); these mechanisms are verified here. Carboxysomes are apparent in the transmission electron micrographs of most of these organisms but are lacking in *Thiomicrothrix* sp. strain Milos-T2 and *Thiomicrothrix* *arctica*, and the inability of *Thiomicrothrix* sp. strain Milos-T2 to grow under low-DIC conditions is consistent with the absence of carboxysome loci in its genome. For the remaining organisms, genes encoding potential DIC transporters from four evolutionarily distinct families (Tcr_0853 and Tcr_0854, Chr, SbtA, and SulP) are located downstream of carboxysome loci. Transporter genes collocated with carboxysome loci, as well as some homologs located elsewhere on the chromosomes, had elevated transcript levels under low-DIC conditions, as assayed by reverse transcription-quantitative PCR (qRT-PCR). DIC uptake was measurable via silicone oil centrifugation when a representative of each of the four types of transporter was expressed in *Escherichia coli*. The expression of these genes in the carbonic anhydrase-deficient *E. coli* strain EDCM636 enabled it to grow under low-DIC conditions, a result consistent with DIC transport by these proteins. The results from this study expand the range of DIC transporters within the SbtA and SulP transporter families, verify DIC uptake by transporters encoded by Tcr_0853 and Tcr_0854 and their homologs, and introduce DIC as a potential substrate for transporters from the Chr family. **IMPORTANCE** Autotrophic organisms take up and fix DIC, introducing carbon into the biological portion of the global carbon cycle. The mechanisms for DIC uptake and fixation by autotrophic Bacteria and Archaea are likely to be diverse but have been well characterized only for "Cyanobacteria." Based on genome sequences, members of the genera *Hydrogenovibrio*, *Thiomicrospira*, and *Thiomicrothrix* have a variety of mechanisms for DIC uptake and fixation. We verified that most of these organisms are capable of growing under low-DIC conditions, when they upregulate carboxysome loci and transporter genes collocated with these loci on their chromosomes. When these genes, which fall into four evolutionarily independent families of transporters, are expressed in *E. coli*, DIC transport is detected. This expansion in known DIC transporters across four families, from organisms from a variety of environments, provides insight into the ecophysiology of autotrophs, as well as a toolkit for engineering microorganisms for carbon-neutral biochemistries of industrial importance.

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