Physiological and Metabolic Effects of Carbon Monoxide Oxidation in the Model Marine Bacterioplankton Ruegeria pomeroyi DSS-3

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Ruegeria pomeroyi expresses carbon monoxide (CO) dehydrogenase and oxidizes CO; however, CO has no effect on growth. Nuclear magnetic resonance (NMR) spectra showed that CO has no effect on cellular metabolite profiles. These data support ecosystem models proposing that, even though bacterioplankton CO oxidation is biogeochemically significant, it has an insignificant effect on bacterioplankton productivity.

Aerobic chemolithoautotrophic utilization of carbon monoxide (CO) by carboxidotrophic bacteria has been studied for some time (1). Carboxidotrophs use CO oxidoreductase, commonly referred to as CO dehydrogenase (CODH), to convert CO to carbon dioxide (CO$_2$). With the energy conserved from CO oxidation, carboxidotrophs assimilate the CO$_2$ produced from CO, typically via the Calvin Benson Bassham cycle, with the key enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) (2).

Primarily as a result of genome sequencing, a relatively new group of CO-utilizing bacteria have been identified and termed the “carboxydovores” (2). In contrast to carboxidotrophs, carboxydovores appear unable to use CO as a source of carbon for growth. Typical elevated CO concentrations used to grow carboxidotrophs appear to inhibit growth of some carboxydovores, and many lack the metabolic capacity to assimilate CO$_2$ (3). Carboxydovores are probably chemolithoheterotrophs, using CO as a supplementary energy source but requiring organic carbon for growth (2, 3).

Ruegeria pomeroyi DSS-3 is one of the group of carboxydovores first identified by King (3). The R. pomeroyi genome contains two different CO oxidation (cox) gene operons and lacks RuBisCO (4). Even though R. pomeroyi has been shown to oxidize CO (3–5), the effects of CO oxidation on R. pomeroyi, or any other CO-oxidizing marine Roseobacter clade strain, remain uncharacterized. The aim of this study is to establish a baseline understanding of CO physiology and metabolism in the model marine bacterioplanckton R. pomeroyi.

R. pomeroyi was grown in marine ammonium mineral salt medium (MAMS) modified from that described in reference 6 by adding NaCl (20 g per liter) and was supplemented with glucose. CO was added to flask headspaces (0.1%), and flasks were incubated with shaking (150 rpm) at 30°C. CO uptake was monitored using an electrochemical CO meter (detection limit, 0.010 h$^{-1}$) and 0.114 ± 0.012 h$^{-1}$, showing that CO had no significant effect during exponential growth. Throughout the experiment, R. pomeroyi depleted the headspace concentration of CO, indicating that CO oxidation occurs independently of growth phase. No effect of CO was detected when cells became glucose starved during stationary phase (Fig. 1). CO also had no effect on growth rate and growth yield on R. pomeroyi grown in MAMS medium with 10-fold less glucose (data not shown).

Based on the annotated genome, R. pomeroyi glucose catabolism is possible through both the Embden-Meyerhof-Parnas (EMP) glycolysis pathway and the Entner-Doudoroff pathway. EMP glycolysis when combined with the Krebs cycle and oxidative phosphorylation has a potential yield of 38 ATP molecules per glucose molecule (7). Assuming that one CO molecule yields one ATP molecule (8), the potential relative energy gain from CO under the experimental conditions here is <1% of the potential energy gain from glucose. Under natural seawater glucose concentrations, ranging from 1 μM to 10 mM (9), and CO concentrations of ≤15 nM in coastal waters and ≤3 nM in oligotrophic waters (10), CO still contributes only <1% toward the theoretical energy budget of the cell.

Previous studies on CO oxidation in heterotrophically grown facultative carboxidotrophs have used higher CO concentrations than those used in this study, typically 10 to 90% (11–14). Comparative experimentation is not possible, because R. pomeroyi is inhibited at headspace CO concentrations of >1% (3). Inhibition caused by high CO concentrations has been shown in a range of bacterial species and is probably caused by damage to the electron transport chain (12). Hydrogenophaga pseudoflavus when grown heterotrophically in the presence of CO (10%) had an increased growth yield but similar growth rate compared to those of cells grown in the absence of CO (13). Similar mixotrophic growth with high CO has also been shown in other facultative carboxidotrophs (14). However, some facultative carboxidotrophs which grown heterotrophically do not oxidize CO (11, 15).

Strains of the closely related Stappia spp. and Labrenzia aggregata have the potential to utilize CO as an energy source and as a carbon source, because some harbor the RuBisCO gene eebL (16). Stappia and L. aggregata grown heterotrophically also rapidly oxidize CO at the same concentrations as those used in this study.
Alkalilimnicola ehrlichii, a facultative arsenite-oxidizing chemoheterotroph isolated from Mono Lake, when grown heterotrophically can also oxidize CO at concentrations similar to those used in this study and also did not show any evidence of increased growth from CO (17).

In order to determine CODH activity, exponential-phase cells were harvested and proteins were extracted using BugBuster master mix (Novagen) by following the manufacturer’s instructions. Cell debris was removed by centrifugation, and the protein concentration in the resulting supernatant was determined (18). Native polyacrylamide gel electrophoresis was used for in-gel activity staining of CODH and was conducted as previously described (19).

Glucose-grown cells and glucose-grown cells plus CO showed similar CODH activities, indicating that CODH enzyme expression and activity was the same under both conditions (Fig. 2). CODH enzyme activity was also present in H. pseudoflava grown heterotrophically using glucose in the presence and absence of CO (13). Proteome analysis of R. pomeroyi incubated in seawater collected from a range of sources (e.g., marina, beach) indicates, however, that CODH expression can be variable (20).

Metabolomics can provide an accurate snapshot of the global physiological and metabolic state of bacterial cells. Exponential-phase cells (15 ml) were harvested by filtration onto ashed GF/F filters (Whatman, United Kingdom) and washed twice with 15 ml of 350 mM NaCl (30°C) before metabolism was quenched using liquid nitrogen, and after which cells were immediately stored at −80°C. Metabolites were extracted and profiled with one-dimensional 1H-nuclear magnetic resonance spectroscopy using established protocols (I. Lidbury, R. Davidson, U. Sommer, M. R. Viant, M. Cunliffe, submitted for publication). Comparison of NMR spectra from R. pomeroyi grown with and without CO showed that there was no significant difference in the profile of cellular metabolites, indicating that CO had no effect on metabolism (Fig. 3).

Bacterioplankton oxidation is the primary CO sink in the marine environment, consuming up to 86% of the CO produced and minimizing the amount of CO that escapes to the atmosphere (21). cox genes are abundant in marine metagenomes, indicating that CO oxidation capability is widely distributed in the bacterioplankton (4, 8, 22). Moran and Miller (8) modeled the potential effects of CO-based lithoheterotrophy on bacterioplankton productivity along the Mid- and South Atlantic bights. The model indicated that only a minor fraction (maximum of 0.2%) of bacterioplankton productivity is attributed to CO oxidation. These data support the conclusion that bacterioplankton have the capacity to rapidly oxidize CO; however, CO has a negligible effect on bacterioplankton production.

ACKNOWLEDGMENTS

This work was supported by the Marine Biological Association of the United Kingdom. NMR spectroscopy was conducted at the Natural En-
environment Research Council Biomolecular Analysis Facility Birmingham and was supported by the grant Developing Metabolomics to Study Energy Metabolism in Marine Bacteria (NBAF467).

REFERENCES