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Role of Carboxydobacteria in Consumption of Atmospheric Carbon Monoxide by Soil

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The carbon monoxide consumption rates of the carboxydobacteria *Pseudomonas* (*Seliberia*) *carboxydohydrogena*, *P. carboxydovorans*, and *P. carboxydoflava* were measured at high (50%) and low (0.5 μl liter^{-1}) mixing ratios of CO in air. CO was only consumed when the bacteria had been grown under CO-autotrophic conditions. As an exception, *P. carboxydoflava* consumed CO also after heterotrophic growth on pyruvate. At low cell densities the CO consumption rates measured at low CO mixing ratios were similar in cell suspensions and in mixtures of bacteria in soil. CO consumption observed in natural soil (loess, eolian sand, chernozem) as well as in suspensions or soil mixtures of carboxydobacteria showed Michaelis-Menten kinetics. The K_m values for CO of the carboxydobacteria (K_m = 465 to 1,110 μl of CO liter^{-1}) were much higher than those of the natural soils (K_m = 5 to 8 μl of CO liter^{-1}). Considering the difference of the K_m values and the observed V_{max} values, carboxydobacteria cannot contribute significantly to the consumption of atmospheric CO.

The carbon monoxide which is present as trace gas in the atmosphere is rapidly decomposed at the soil surface. The global CO consumption by soil accounts for ca. 500 Tg year^{-1} and thus contributes significantly to the atmospheric CO cycle (22). The consumption of atmospheric CO by soil is due to microbial activity (2, 5, 8, 10, 21). It has been assumed that the atmospheric CO may be consumed by the chemolithotrophic carboxydobacteria, which are able to grow aerobically with CO as their sole source of carbon and energy (6, 15, 25). Recently, it was shown that *Pseudomonas carboxydovorans* is able to utilize the low amounts of CO (≦1 μl liter^{-1}) present in the air (5). However, there is also evidence that CO is consumed by eucaryotic soil microorganisms (5, 9). Recently, it was suggested by Bartholomew and Alexander (2) that CO oxidation in soil is not the result of autotrophic metabolism, but of cometabolic oxidation. These authors showed that assimilation of $^{14}CO_2$ by soil was not stimulated when CO was supplied, and that ^{14}CO was mainly oxidized to $^{14}CO_2$ and not incorporated into soil organic matter. Presently, it is unknown which process is responsible for the decomposition of atmospheric CO at the soil surface.

In this paper we studied the role of carboxydobacteria in the CO utilization by soil. Comparison of the kinetic properties of carboxydobacteria and natural soils indicates that the carboxydobacteria are only of minor importance for the consumption of atmospheric CO.

Mixing ratios, given as percent by volume, microliters per liter or nanoliters per liter, are pressure and temperature independent. Therefore, the term mixing ratio is preferred to the term concentration (e.g., micrograms per liter) when speaking about amounts of CO present in the gas phase.

MATERIALS AND METHODS

Bacteria and growth conditions. *Pseudomonas* (*Seliberia*) *carboxydohydrogena* strain Z-1062 (DSM1083) (11, 19, 20, 25), *P. carboxydovorans* strain OM5 (DSM1227) (12) and *P. carboxydoflava* strain Z-1107 (DSM1084) (16, 25) were grown CO autotrophically in mineral medium under the conditions described by Meyer and Schlegel (12). For heterotrophic cultures the mineral medium was supplemented with 0.4% (wt/vol) sodium pyruvate. The bacteria were either grown in 2-liter fluted Erlenmeyer flasks or in a 10-liter fermentor (Biostat; Braun, Melsungen), harvested in the midexponential growth phase, and suspended in 50 mM potassium phosphate buffer (pH 7.0). Protein was determined by the method of Beisenherz et al. (3).

Soils. The soil samples (loess, eolian sand, and chernozem) were taken from the top 10 cm at different locations near Mainz, Germany. The main physical and chemical characteristics of the soils have already been described (24). The soils were passed through a 4-mm mesh screen and stored in polyethylene bottles at 4°C. The CO consumption activity did not change

significantly during the storage time of several weeks. The soil moisture content was determined by weight after drying at 104° for 24 h. Before each experiment the soil moisture content was adjusted to values of 12 to 15% (wt/wt) by adding distilled water. Soil was sterilized by autoclaving at 120°C for 30 min followed by drying under aseptic conditions in air for ≧20 days. Cell suspensions of CO-autotrophically grown *P. carboxydovorans* were added to the soil to give a final soil moisture content of 12 to 15%.

Determination of CO consumption under an atmosphere containing 50% CO. Cell suspensions (2 ml) were placed into 15-ml Warburg vessels. The vessels were closed with serum stoppers and flushed with 100 ml of a gas mixture containing (by volume) 50% CO and 50% air by the technique described by Meyer and Schlegel (13, 14). The flasks were reciprocally shaken at 30°C with 125 rpm. Gas samples (50 μl) were taken through the rubber septum with a gas-tight pressure lock syringe (Hamilton, Bonaduz, Switzerland) and analyzed for CO, O_2, and N_2 with a gas chromatograph with a thermal conductivity detector as described by Meyer and Schlegel (12). The rates of CO and O_2 consumption were determined from the temporal decrease of the CO and O_2 mixing ratios in the gas phase; the N_2 served for calibration (internal standard). This technique allowed the detection of CO consumption rates of ≧0.1 μl h^{-1}. It was ensured that the rate of CO consumption was stoichiometric to the rate of O_2 consumption and was proportional to the amount of cells. The CO consumption rates were also determined by means of Warburg manometry, to allow the direct comparison with other measurements made previously with this technique.

Determination of CO consumption under an atmosphere containing 0.5 μl of CO $liter^{-1}$. Cell suspensions (100 ml) were filled into 550-ml Erlenmeyer flasks and gassed for 15 min with CO-free synthetic air (80% N_2, 20% O_2). The air pressure inside the flasks was adjusted to 130 kPa. CO-containing air was injected into the flasks to give the required CO mixing ratio. The flasks were shaken at 30°C and 150 rpm on a rotatory shaker. The temporal decrease of the CO mixing ratio inside the flasks was determined by taking small air samples (1 to 10 ml) with gas-tight syringes (Glenco, Houston, Tex.). The samples were analyzed in a CO analyzer based on the HgO-to-Hg vapor conversion technique (22, 23). The lower detection limit was 2 nl $liter^{-1}$, allowing the detection of CO consumption rates of ≧1 nl h^{-1} at CO mixing ratios of ≦1 μl $liter^{-1}$. At higher CO mixing ratios the small gas sample had to be diluted with CO-free synthetic air. The CO consumption rates in soil samples or of bacteria mixed into sterile soil were determined by the procedures of Conrad and Seiler (4, 5).

Chemicals and gases. All chemicals were commercially available. The gases were obtained from Messer Griesheim, Düsseldorf, and were of the same purity as those described by Meyer and Schlegel (12–14).

RESULTS

CO consumption by suspensions of carboxydobacteria. *P. (Seliberia) carboxydohydrogena*, *P. carboxydovorans*, and *P. carboxydoflava* were grown under CO-autotrophic conditions as well as under heterotrophic conditions with pyruvate as substrate. Table 1 shows the CO consumption activity of cell suspensions at low CO mixing ratios (0.5 μl $liter^{-1}$) as representative for ambient air conditions and at high CO mixing ratios (50%) as representative for CO-autotrophic culture conditions. At both CO mixing ratios *P. carboxydohydrogena* and *P. carboxydovorans* consumed CO only when they had previously been grown under CO-autotrophic conditions. *P. carboxydoflava*, on the other hand, consumed CO also after heterotrophic growth.

CO consumption as a function of cell density. In addition to the measurements on cell suspensions the CO consumption rates were also measured after mixing the carboxydobacteria into sterile eolian sand. The CO consumption rates were measured as function of the number of cells by using a test atmosphere containing 0.5 μl of CO $liter^{-1}$. A typical result of these measurements is shown in Fig. 1. At less than 3 mg of bacterial protein mixed into 100 ml of buffer or 100 g (dry weight) of sterile soil the CO consumption rates measured in the suspensions were comparable to those measured in the soil mixtures. At cell densities higher than 3 mg of protein, however, the CO consumption rates of the suspensions were no longer proportional to the cell density and became almost constant. The CO consumption rates of the soil-bacteria mixtures, on the other hand, increased with the amount of cells, and even at 40 mg of protein per 100 g (dry wt) of soil, saturation was not reached. This observation may be explained by the enhanced availability of CO to the bacteria when the cells are attached to soil particles as contrasted to being suspended in buffer. Limitation of CO diffusion to the bacteria seems

TABLE 1. *CO consumption by suspensions of carboxydobacteria*

Species	Growth substrate	CO consumption rate (nl h^{-1} mg^{-1} of protein) measured at:		K_m value for CO (μl $liter^{-1}$)
		0.5 μl of CO $liter^{-1}$	50% CO	
P. carboxydohydrogena	CO	959	892,300	465
	Pyruvate	0[a]	0	
P. carboxydovorans	CO	547	609,700	557
	Pyruvate	0	0	
P. carboxydoflava	CO	200	201,600	503
	Pyruvate	148	328,800	1,110

[a] 0, No activity detectable; the detection limits were 1 nl h^{-1} at 0.5 μl of CO $liter^{-1}$ and 0.1 μl h^{-1} at 50% CO.

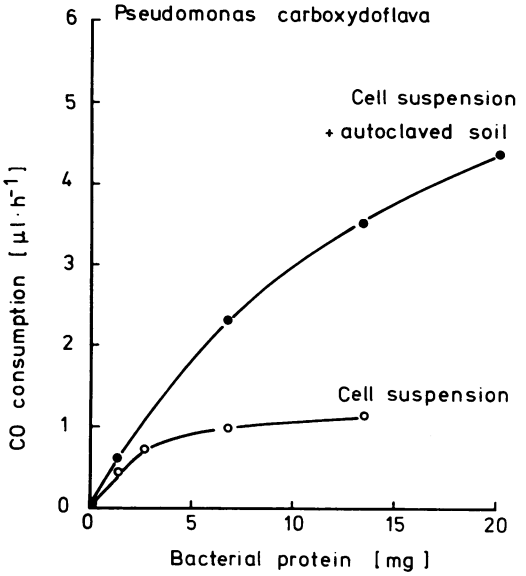

FIG. 1. *CO consumption rates at increasing cell densities of CO-autotrophically grown P. carboxydoflava. The CO consumption rates were measured at a CO mixing ratio of 0.5 μl liter^{-1} in air. The cells were either suspended in 100 ml of phosphate buffer (pH 7.0) or mixed into 100 g (dry weight) of autoclaved eolian sand. The soil moisture content was adjusted to 13 to 14% (wt/wt) by adding water.*

possible particularly at the low CO mixing ratios used. During our experiments we ensured that the CO consumption rates were measured in the proportionality range to cell density.

CO consumption as function of the CO mixing ratio. The CO mixing ratio of the test atmosphere decreased linearly with time when suspensions of carboxydobacteria were incubated at 50% CO, but decreased logarithmically when incubated at 0.5 μl of CO liter^{-1}. This result is consistent with first-order kinetics at low, nonsaturating CO concentrations and zero-order kinetics at high, saturating CO concentrations, indicating that CO consumption by carboxydobacteria follows Michaelis-Menten kinetics. Hence, we calculated the K_m values for CO by using the equation $K_m = S(V_{max} - v)/v$, where v is the CO consumption rate at $S = 0.5$ μl of CO liter^{-1} and V_{max} is the CO consumption rate at S = 50% CO. The calculations give K_m values of 465 to 557 μl of CO liter^{-1} for CO-autotrophically grown cells and of 1,110 μl of CO liter^{-1} for heterotrophically grown *P. carboxydoflava* (Table 1). These values correspond to aqueous CO concentrations of 0.39 to 0.93 μM as determined by using a Bunsen solubility coefficient of 0.019 at 30°C.

CO-autotrophically grown cells of *P. carboxydovorans* were mixed into sterile eolian sand (60 μg of protein per g [dry weight] of soil) and the CO consumption rates were measured at CO mixing ratios up to 200 μl liter^{-1} as the maximum value, which could be determined by using the CO analyzer based on the HgO-to-Hg vapor conversion technique. Saturation of the CO consumption reaction was not reached at 200 μl of CO liter^{-1}. Applying the Lineweaver-Burk plot, we determined a V_{max} of 15.7 μl of CO h^{-1} g^{-1} (dry weight) of soil and a K_m value of 500 μl of CO liter^{-1} (0.42 μM CO in aqueous solution) (Table 2), which is almost identical with the K_m value calculated from the CO consumption rates measured at 0.5 μl of CO liter^{-1} and 50% CO by using suspensions of *P. carboxydovorans* (Table 1).

CO consumption by natural soils. CO consumption rates at increasing CO mixing ratios were also measured by using natural loess, eolian sand, and chernozem, which were not supplemented with carboxydobacteria. In these natural soils the CO consumption reaction was already saturated at CO mixing ratios below 100 μl of CO liter^{-1}. The Lineweaver-Burk plots gave straight lines showing K_m values of 5 to 8 μl of CO liter^{-1} (4 to 7 nM CO in aqueous solution) and V_{max} values of 0.3 to 0.9 μl of CO h^{-1} g^{-1} (dry weight) of soil depending on the type of soil (Table 2).

DISCUSSION

The different strains of carboxydobacteria used in our experiments were able to consume CO even at the low mixing ratios of ambient air (≦1 μl of CO liter^{-1}). Thus, carboxydobacteria indeed possess the capacity for an oligocarbophilic way of life. This is in contrast to hydrogen

TABLE 2. *CO consumption by natural soil and by CO-autotrophically grown P. carboxydovorans mixed into sterile soil*

Soil sample (1 g [dry wt])	V_{max} (nl of CO h^{-1})	K_m (μl of CO liter^{-1})	v at 0.5 μl of CO liter^{-1} (nl of CO h^{-1})
Loess	917	5	111
Chernozem	292	8	37
Eolian sand	323	7	40
Eolian sand plus *P. carboxydovorans*			
60 μg of protein	15,690	500	16
1.2 μg of protein[a]	323	500	0.3
150 μg of protein[a]	39,225	500	40

[a] Values calculated by using the Michaelis-Menten equation and assuming proportionality between CO consumption rate and bacterial protein.

bacteria, which are not able to utilize the trace amounts of hydrogen present in the atmosphere (4). A prerequisite for CO consumption by carboxydobacteria was the previous induction for CO utilization, e.g., by growth on CO as the sole source for carbon and energy. Heterotrophically grown cells could utilize CO neither at high (percent range) nor at low (microliters to nanoliters per liter range) mixing ratios. An exception was *P. carboxydoflava*, which forms the CO-oxidizing enzyme, the key enzyme in the CO metabolism of carboxydobacteria (13, 14), even during heterotrophic growth on pyruvate or other organic substrates (Kieβling, Diplom thesis, University of Göttingen, Göttingen 1980). During heterotrophic growth CO is not consumed by cooxidation (7, 18), but is used as an additional energy source enabling *P. carboxydoflava* to assimilate a surplus of pyruvate.

The K_m values for CO determined in cell suspensions of carboxydobacteria and in cells added to sterile soil were in the range of 500 to 1,000 μl of CO liter^{-1}, corresponding to aqueous CO concentrations of 0.4 to 0.9 μM. These values are significantly lower than the K_m value (53 μM CO) reported for the purified CO:methylene blue oxidoreductase of *P. carboxydovorans* (14), indicating that the affinity of whole cells for CO is higher than that of the purified CO oxidizing enzyme. The higher affinity may be explained by the Bunsen solubility coefficient of CO being higher in organic solvents than in water. Thus, the CO concentration in the lipid fraction of the cytoplasmic membrane, the place where the CO-oxidizing enzyme is probably localized (13), may be higher than in the surrounding aqueous medium. An alternative explanation would be the overestimation of the K_m of the purified enzyme under unphysiological in vitro conditions with methylene blue as electron acceptor.

The K_m values for CO of natural soils were 2 orders of magnitude lower than those observed for carboxydobacteria. With the V_{max} of 323 nl of CO h^{-1} observed in natural eolian sand and with the K_m value of 500 μl of CO liter^{-1} observed in CO-autotrophic *P. carboxydovorans* the CO consumption rate of a mixture of *P. carboxydovorans* in eolian sand is calculated to be 0.3 nl of CO h^{-1} at a CO mixing ratio of 0.5 μl liter^{-1} (Table 2). This value accounts only for <1% of the CO consumption rate observed in natural eolian sand under ambient air conditions (0.5 μl of CO liter^{-1}). On the other hand, if we assume that the soil contains a sufficient number of carboxydobacteria to account for the CO consumption rate observed in natural soil under ambient air conditions, the V_{max} of the soil should be 39,225 nl of CO h^{-1}, which is >100 times higher than was actually observed. Under these conditions the soil must contain 150 μg of protein of carboxydobacteria g^{-1} (dry weight) of soil, equivalent to ≧20% of the total biomass carbon in soil as determined by using a value of ≦750 μg (biomass) of C g^{-1} of soil (17), and equivalent to 10^8 to 10^9 carboxydobacteria g^{-1} of soil as determined by using a value of 0.1 to 1.0 pg of protein per bacterium (1). These figures are unacceptably high values.

Considering all of these arguments we have to conclude that the carboxydobacteria play only a minor role in the consumption of atmospheric CO by the soils studied. Therefore, we have to assume that other microorganisms with a remarkably high affinity for CO are responsible for the decomposition of the atmospheric CO at the soil surface. It is interesting that the CO-consuming microbial communities of surface seawater have a similarly high affinity for CO (K_m = 6 nM CO) as the soil microbial communities (Table 2; K_m = 4 to 7 nM CO) (Conrad and Seiler, unpublished results). However, our results do not allow one to decide whether atmospheric CO is utilized by autotrophic metabolism or by cometabolism by soil microorganisms. Our study also does not allow a conclusive view of the role of carboxydobacteria in the soil environment. Their ecological niche may be seen to be the scavenging of CO at microsites where it arises at high concentrations during breakdown of organic matter.

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