SOM_Table 5: CO_2 fixation pathway in *Kuenenia stuttgartiensis*

The presence of all genes involved in the acetyl CoA pathway for CO_2 fixation in the genome assembly of *Kuenenia stuttgartiensis*, and the very depleted delta-^{13}C values of ladderane lipids triggered the experimental investigation of key enzymes activities in CO_2 fixation in crude extracts of this anammox bacterium.

Enzymes assay conditions. *K. stuttgartiensis* cells were washed twice under anaerobic conditions with 50 mM anaerobic Tris-HCl pH 7.8, 1 mM DTT and 2 mM $MgCl_2$ and suspended anaerobically inside a glove box (95% N_2, 5% H_2) in 4 ml buffer. The suspensison was passed through a French pressure cell at 4 °C. When necessary, unbroken cells and debris were removed by centrifugation in gas-tight tubes at 40,000 x g. The supernatant was used as cell extract and contained about 12 mg protein per ml. Assays were carried out in anaerobic quartz cuvettes as described elsewhere (1-4) using enzymes or extracts of the appropriate reference organisms as controls (4).

Enzyme activities. Strictly anaerobically prepared cell extracts of *Kuenenia stuttgartiensis* were used to determine the activities of the key enzymes of the four known CO_2-fixation pathways (**SOM Table 5**). However, the extracts showed activities less than 1 nmol per min per mg protein while the enzyme potentially involved in providing reducing equivalents to the CO_2 fixation pathway, nitrate::nitrite oxidoreductase (see Fig. 1B), showed an activity of 42 nmol per min per mg protein, indicating that cell extract preparations did not compromise the observed activities. Only when broken cells were immediately assayed without further centrifugation could a methyl- or benzyl-viologen dependent CO dehydrogenase (COdh) and formate dehydrogenase (Fdh) activity of 3-4 nmol per min per mg protein be observed. Within 2 min after preparation the COdh or Fdh activity decreased below the detection level, indicating very labile or oxygen-sensitive enzymes. Reductive activation with ATP was not possible. Furthermore the COdh activity was strongly inhibited by CO concentrations above 2%, making future investigation very difficult.

SOM Table 5. Enzyme activities in cell extracts of *Kuenenia stuttgartiensis*

Enzyme	***K. stuttgartiensis* specific activity (nmol/min/mg protein)**
CO dehydrogenase*	3
Formate dehydrogenase*	4
Hydrogenase	<1
ATP Citrate (pro-3S)-lyase	<1
Ribulose bisphosphate carboxylase	<1
3-OH propionate dehydrogenase	<1
Nitrate::nitrite oxidoreductase (nitrate reductase)	42

* Only measurable when broken cells were assayed within 1 min after passage through a French pressure cell.

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