

## Genomic insights into the metabolic diversity of the Fe(II)-oxidizer *Sideroxydans* sp. CL21 reveals a mixotrophic lifestyle

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Chemolithoautotrophic, microaerophilic, Fe(II)-oxidizing bacteria (FeOB), including *Sideroxydans* spp., found within the family Gallionellaceae exhibit a wide variety of autotrophic pathways, including Fe(II) oxidation and CO<sub>2</sub> fixation. *Sideroxydans* sp. CL21, an isolate from the slightly acidic, minerotrophic, Fe- and OM-rich Schlöppnerbrunnen fen, was initially characterized as a chemolithoautotroph, primarily due to lack of genomic information. To elucidate the metabolic potential of strain CL21, whole-genome sequencing and further genomic characterization revealed genes encoding homologs of the Fe(II) oxidation genes *mtaAB* and *cyc2*, a lactate permease and lactate dehydrogenase as well as multiple gene clusters encoding NiFe hydrogenases, sulfur oxidation (*sox*) gene homologs, and genes involved motility and biofilm biosynthesis. These findings suggest strain CL21 has an atypical mixotrophic metabolism and is capable of using H<sub>2</sub> or reduced sulfur compounds as alternative electron donors [1,2]. (Pan)genome comparisons between *Sideroxydans* sp. CL21 and *S. lithotrophicus* ES-1 were implemented to estimate the sizes of core (2057 gene clusters) and accessory genomes (1078 versus 700 GCs, respectively) and to identify functional features unique to strain CL21. Genes linked to lactate oxidation, H<sub>2</sub> oxidation, S utilization, motility and biofilm biosynthesis, and various mobile elements were identified in the accessory genome of *Sideroxydans* sp. CL21, providing genomic evidence for metabolic versatility as well as evidence to differentiate strain CL21 from *S. lithotrophicus* ES-1. Next, targeted experiments were conducted to confirm the genome-resolved metabolic potential. Semi-solid and liquid incubations were amended with lactate (0-5mM) and various combinations of alternative electron donors, including H<sub>2</sub> (0-5%), NaS<sub>2</sub>O<sub>3</sub> (0-5mM), Fe<sup>0</sup> (1 g L<sup>-1</sup>), and FeS. All tested electron donors and lactate were utilized by *Sideroxydans* sp. CL21 and 16S rRNA gene copies were 16x higher in incubations with 1 mM lactate plus H<sub>2</sub> and Fe, compared to Fe only. H<sub>2</sub> utilization rates in H<sub>2</sub> plus Fe incubations were 5.3x higher in the presence of lactate, suggesting H<sub>2</sub> may be the preferred electron donor in presence of organic carbon. Our combined genome-resolved and experimental results highlight the mixotrophic lifestyle of *Sideroxydans* sp. CL21, which is especially valuable in OM-, Fe-, and S-rich environments like the Schlöppnerbrunnen fen.

[1]Cooper,et al.(2020),MRA,9:e01444-19

[2]Cooper,et al.(2020),ISME J 14,2675-2690