

# Microscopic and physiological studies of the phototrophic Fe(II)-oxidizing *Chlorobium ferrooxidans* sp. KoFox in co-culture with *Geospirillum* sp. strain KoFum

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The green sulfur bacterium *Chlorobium ferrooxidans* strain KoFox was isolated as a phototrophic Fe(II)-oxidizer in co-culture with *Geospirillum* sp. strain KoFum. Previous studies revealed that in this co-culture cells of strain KoFox remained largely free of iron minerals during Fe(II) oxidation, while cell surfaces of strain KoFum were observed to exhibit a thin crust of globular Fe particles [1]. This suggests that in this co-culture it is the non-Fe(II)-oxidizing partner and not the Fe(II)-oxidizer that will leave a trace in the rock record as a microfossil. However, as silica was present in Archaean oceans in which anoxygenic photoferrotrophs thrived, its presence might have influenced encrustation in these cultures and our interpretations.

Therefore, in this study we analyzed the encrustation process in the co-culture in order to answer the following questions: (1) How do the two strains associate with formed Fe(III) oxyhydroxide minerals? (2) How does encrustation evolve in the course of Fe(II) oxidation? (3) Do we still see the same encrustation pattern in the presence of silica?

To this end, we conducted electron microscopic studies on the co-culture under different growth conditions, including low light (<50 lux) vs. normal light (>500 lux) conditions, presence of dissolved silica (2 mM), and the addition of fumarate (5 mM), which was used as an organic substrate by strain KoFum. FESEM and TEM imaging did not reveal a distinct encrustation pattern for any of the strains. Cells of both KoFox and KoFum were associated with minerals, but cell surfaces remained mostly free of iron particles and no encrustation could be detected. Silica did not influence this encrustation pattern, but it did stimulate Fe(II) oxidation, potentially by lowering Fe(II) toxicity to the cells, while the addition of fumarate increased cell numbers of strain KoFum.

[1] Schädler *et al.* (2009) *Geomicrobiol J* **26**(2): 93-103