Surface reactivity of the iron and manganese-oxidizing bacterium *Leptothrix cholodnii* SP-6

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Abstract

Surfaces of prokaryotic cells play a significant role in the adsorption of metals from aqueous solution and the formation of authigenic minerals (Konhauser 2006). Although most studies focus on the cell wall, it is known that many bacteria synthesise an extracellular layer of polysaccharides and proteins, including what are known as sheaths. It has been shown that the cyanobacterium *Calothrix* sp. produces as sheath which is neutrally charged at circumneutral pH values, and it was hypothesized that such a sheath might allow the cyanobacterium to survive in geothermal settings with high silicification rates (Phoenix et al. 2002). Specifically, the dominance of hydroxyl sites on *Calothrix*'s sheath surface facilitates hydrogen bonding with aqueous silica species, inducing the precipitation of amorphous silica on the sheath and thus protecting the underlying cell (Phoenix et al. 2002). *Leptothrix cholodnii* is a sheathed, iron and manganese-oxidizing bacterium that frequently inhabits minerals seeps, where Fe^{2+} and Mn^{2+} discharge into oxygenated surface waters (Spring et al. 1996). As a result, the sheath becomes encrusted with Fe(III) and Mn(IV) oxyhydroxides while the underlying cells are protected from mineralization (Emerson and Ghiorse 1992, Emerson et al. 2010). However, unlike *Calothrix, Leptothrix*'s sheath composition suggests that it might behave differently at circumneutral pH (Emerson and Ghiorse 1993). To investigate the surface reactivity of *Leptothrix*'s sheath and cell wall we analyzed isolated sheaths, sheathless cells, and intact filaments of *L. cholodnii* SP-6. We studied these components using potentiometric titration, zeta-potential, Cd-adsorption, and Fourier transform infrared (FTIR) spectroscopy to elucidate changes in surface charge between the cell wall and sheath. For the isolated sheaths and intact filaments, titration data were fit using a two-site protonation model, resulting in the following pKa values: 6.05 (±0.29) and 9.34 (±0.11); and 7.77 (±0.17) and 10.50 (±0.20), respectively. For the sheathless cells, the best fit was obtained by using a three-site protonation model, resulting in the following pKa values: 5.40 (±0.59), 8.11 (±1.64) and 10.73 (±0.45). Total proton-active site concentrations were lower in isolated sheaths compared to intact filaments. Additionally, at circumneutral pH, net negative

charge was lower for sheathless cells compared to intact filaments and isolated sheaths (Fig. 1). This information agrees with the Cd adsorption behaviour found for the three materials (Fig. 2). Thus, our preliminary results suggest that *Leptothrix*'s sheath is less reactive than the intact filaments at circumneutral pH, leading us to hypothesize that the outermost layer would sequester relatively lower amounts of cations, including Mn^{2+}, from solution and potentially would protect the underlaying cell from deleterious mineralization. In addition to that, the less reactive sheath's surface would also contribute to cell attachment, which is important for a species commonly found in streams (Phoenix et al. 2002, Emerson et al. 2010).

Keywords

acid-base properties, manganese-oxidizing bacteria, biomineralization

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Conflicts of interest

The authors have declared that no competing interests exist.

References

- Emerson D, Ghiorse W (1992) Isolation, Cultural Maintenance, and Taxonomy of a Sheath-Forming Strain of *Leptothrix discophora* and Characterization of Manganese-Oxidizing Activity Associated with the Sheath. Applied and Environmental Microbiology 58 (12): 4001-4010. https://doi.org/10.1128/aem.58.12.4001-4010.1992
- Emerson D, Ghiorse WC (1993) Ultrastructure and chemical composition of the sheath of *Leptothrix discophora* SP-6. Journal of Bacteriology 175 (24): 7808-7818. https://doi.org/10.1128/jb.175.24.7808-7818.1993
- Emerson D, Fleming E, McBeth J (2010) Iron-Oxidizing Bacteria: An Environmental and Genomic Perspective. Annual Review of Microbiology 64 (1): 561-583. https://doi.org/10.1146/annurev.micro.112408.134208
- Konhauser K (2006) Introduction to geomicrobiology. Wiley-Blackwell, 440 pp. [ISBN 978-0-632-05454-1]

- Phoenix VR, Martinez RE, Konhauser KO, Ferris FG (2002) Characterization and Implications of the Cell Surface Reactivity of *Calothrix* sp. Strain KC97. Applied and Environmental Microbiology 68 (10): 4827-4834. https://doi.org/10.1128/aem.68.10.4827-4834.2002
- Spring S, Kampfer P, Ludwig W, Schleifer K (1996) Polyphasic Characterization of the Genus *Leptothrix*: New Descriptions of *Leptothrix mobilis* sp. nov. and *Leptothrix discophora* sp. nov. nom. rev. and Emended Description of *Leptothrix cholodnii* emend. Systematic and Applied Microbiology 19 (4): 634-643. https://doi.org/10.1016/s0723-2020(96)80036-1

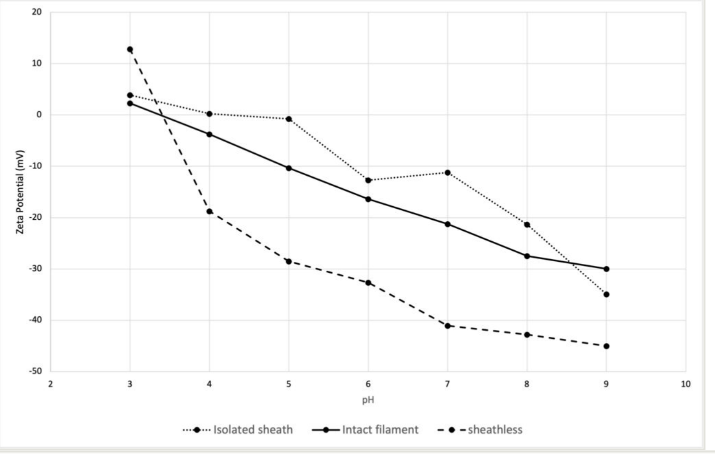

Figure 1.

Zeta potential measurements carried out on 0.2 g/L suspensions of *L. cholodnii* SP-6 intact filaments, sheathless cells and isolated sheathsin 0.01 M $NaNO_3$ as a function of pH.

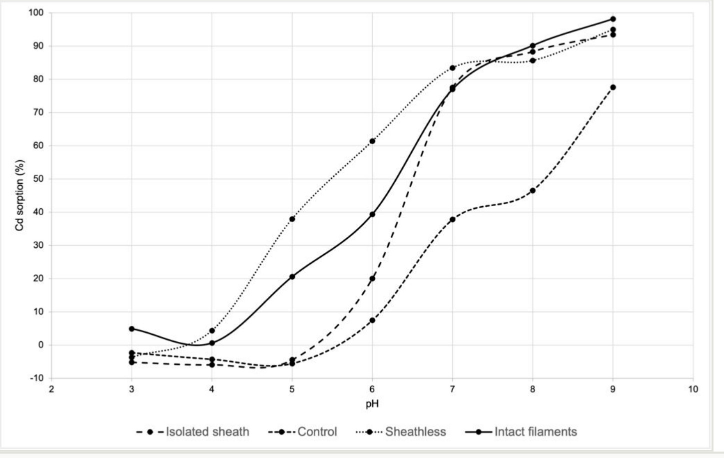

Figure 2.

Cd adsorption behaviour onto 1 g/L *L. cholodnii* SP-6 intact filaments, sheathless cell and isolated sheaths in 0.01 $NaNO_3$. The initial Cd concentration was 1 ppm.