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Study of *Shewanella oneidensis* MR-1 for metal bioremediation in marine environments

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Introduction

For many years, sea has received a large amount of metal waste from the continental coast and rivers, which cannot be degraded by through biological, chemical, or physical means to an innocuous by-product. Bioavailable metals exert toxicity in cells, Thus, metal-contaminated oceans pose serious ecological and health risks (Maier et al., 2009).

Shewanella oneidensis MR-1 is a Gram-negative proteobacteria, which is predominantly found in deep sea anaerobic habitats, but can also reside in soil and sedentary habitats. It is able to use a wide range of terminal electron acceptors during anaerobic respiration as Fe(III), Mn(III) and (IV), Cr(VI), U(VI), As(V) or V(V) (Yin et al., 2011) and precipitate them extracellularly. Due to its dissimilatory metal reducing activity *S. oneidensis* MR-1 has been a target of marine bioremediation research. The aim of this bibliographic review is to study what are the characteristics that make *Shewanella oneidensis* MR-1 a very useful microorganism for ocean bioremediation.

Metal reducing pathway

In absence of O_2, *S. oneidensis* MR-1 can use metals as terminal electron acceptors through by the metal-reducing pathway (Mtr pathway), with machinery for transferring electrons from the inner-membrane to surface metals. Cytochrome CymA is the entry point of the Mtr pathway. It is found in the periplasm, anchored in the inner-membrane. CymA is a quinol dehydrogenase, oxidizes quinol to quinone and transfers the released electrons to MtrA in the outer-membrane. Cytochrome MtrA is attached to MtrB, a porin-like protein anchored in the outer-membrane. The MtrAB complex facilitates the electron transfer across the outer-membrane to the terminal reductases MtrC and OmcA (Shi et al., 2012).

Metal localization

S. oneidensis MR-1 has two distinct mechanisms for metal location. It can respond to soluble forms of the oxidized metals, usin “energy taxis”, to detect energetically favorable environments. Moreover, it can respond chemotactically to reduced forms of these metals in the absence of electron acceptors (Bencharit et al., 2005).

Metal precipitation

Cytochromes MtrC and OmcA have a determining role in metal reduction. Strains *ΔmtrC* or/and *ΔomcA* have lower metal reduction rates than the wild type (Figure 1). Although the double mutant still reduces metals, the precipitates were found in the cytoplasm instead of in the EPS (Figure 2) interfering with normal *Shewanella* cellular functions (Belchik et al., 2011).

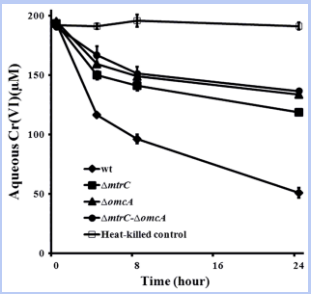

Figure 1. Cr(VI) reduction

Cr(VI) reduction kinetics of S. oneidensis MR-1 wild type and the mutants without MtrC and/or OmcA (Belchik et al., 2011).

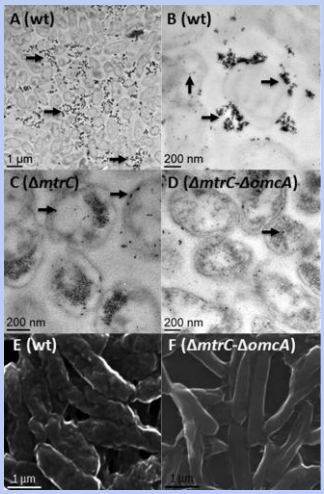

Figure 2. Cr(III) precipitation

Cellular locations of Cr(III) precipitates in S. oneidensis MR-1 wild type and the mutants without MtrC and/or OmcA. (Aand B) TEM images of wild type. (C) TEM image of ΔmtrC. (D) TEM image of double mutant. (E) SEM image of wild type. (F) SEM image of double mutant (Belchik et al., 2011).

Metal reduction kinetics

Until now, no studies have analysed *S. oneidensis* MR-1 ability to reduce metals in the sea. Lall et al. did a *in vitro* study in 2007, measuring the reduction rates of Fe(III), Co(III), U(VI), Cr(VI) and Tc(VII) (Figure 4). The results showed that Fe and Co were reduced in a few minutes while reducing U, Cr and Tc needed hours.

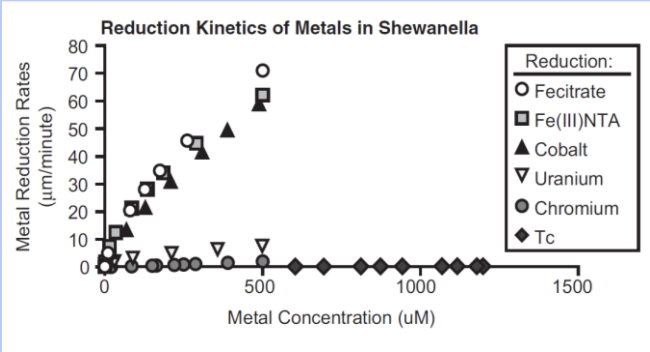

Figure 4. Metal reduction kinetics

Reduction kinetics of metals in S. oneidensis MR-1 (Lall et al., 2007).

Nanowires

S. oneidensis MR-1 has extracellular electrically conductive nanowires (Figure 3) which may facilitate extracellular electron transport. Mutants deficient in genes *mtrC* and *omcA* produce appendages that are morphologically consistent with bacterial nanowires, but they were found to be nonconductive (El-Naggar et al., 2010).

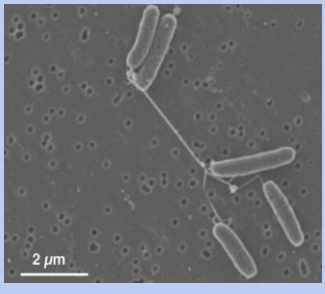

Figure 3. Nanowires

SEM image of wild type S. oneidensis MR-1 (Gorby et al., 2006).

Conclusions

The characterization of Mtr pathway in *S. oneidensis* MR-1 has improved the understanding of the molecular mechanisms by bacteria reduce metals. The finding that the elimination of MtrC and OmcA reduces the extracellular precipitates demonstrate that these genes have a key role in the extracellular metal reduction as a terminal reductases.

Under conditions of electron acceptor limitations, as would be in the deep sea, *S. oneidensis* MR-1 makes nanowires to facilitate the extracellular electron transport. This and its ability of localize metals using "energy taxis" and chemotaxis makes this specie may gain a competitive advantage over other dissimilatory metal-reducing bacteria in the colonization of mineral surfaces in the ocean.

Even though there are studies *in vitro* that demonstrate that *S. oneidensis* MR-1 is able to reduce different species of metals, and there are experiments that calculate the kinetics of reduction of these different elements, it would be necessary to study *Shewanella oneidensis* MR-1 *in situ*, to determine the optimal conditions of this specie to reduce metals, and be able to use it to bioremediate the ocean.

Bibliography

Belchik SM, Kennedy DW, Dohnalkova AC, Wang Yuanmin, Sevinc PC, Wu H, Lin Y, Lu HP, Fredrickson JK and Shi L. *Extracellular Reduction of Hexavalent Chromium by Cytochromes MtrC and OmcA of Shewanella oneidensis MR-1*. Applied and environmental microbiology. 2011; 77: 4035-4041.

Bencharit S and Ward MJ. *Chemotactic Responses to Metals and Anaerobic Electron Acceptors in Shewanella oneidensis MR-1*. Journal of Bacteriology. 2005; 187: 5049-5053.

El-Naggar MY, Wagner G, Leung KM, Yuzvinsky TD, Southam G, Yang J, Lau WM, Nealson KH and Gorby YA. Electrical transport along bacterial nanowires from Shewanella oneidensis MR-1. PNAS. 2010; 107: 18127-18131.

Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedt DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH and Fredrickson JK. *Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms*. PNAS. 2006; 103: 11358-11363.

Lall R and Mitchell J. *Metal reduction kinetics in Shewanella*. Bioinformatics. 2007; 23: 2754-2759.

Maier RM, Pepper IL and Gerba CP. *Environmental Microbiology*. 2nd ed. Amsterdam: Academic Press. 2009.

Shi L, Rosso KM, Clarke TA, Richardson DJ, Zachara JM and Fredrickson JK. *Molecular underpinnings of Fe(III) oxide reduction by Shewanella oneidensis MR-1*. Frontiers in Microbiology. 2012; 3.

Yin J and Gao H. *Stress Responses of Shewanella*. International Journal of Microbiology. 2011.