

## Rare earth elements and bacterial C1 metabolic system

MINAMISAWA, Kiwamu<sup>1\*</sup>; SEKI, Kenjiro<sup>1</sup>; ZHIHUA, Bao<sup>1</sup>; SUGAWARA, Masayuki<sup>1</sup>; SHINODA, Ryo<sup>1</sup>; TANI, Akio<sup>2</sup>; MASUDA, Sachiko<sup>2</sup>; MITSUI, Ryoji<sup>3</sup>

<sup>1</sup>Graduate School of Life Sciences, Tohoku University, <sup>2</sup>Institute of Plant Science and Resources, Okayama University, <sup>3</sup>Department of Biochemistry, Faculty of Science, Okayama University of Science

The rare earth elements (REEs) include lanthanum (La) and cerium (Ce) as light REEs. La and Ce are very low abundant in the universe, but relatively higher abundant in the earth crust including soils. Recently, it has been reported that the product of *xoxF* gene in *Methylobacterium extorquens* AM1 is La- and Ce-dependent methanol dehydrogenase (MDH) (Nakagawa et al. 2012), which is different from classical Ca-dependent MDH encoded by *mxoF* gene. Although bradyrhizobia are ubiquitous bacterial in the environments, they are often associated with legume and non-legume plants. In the present work, we examine the effects of La and Ce on C1 metabolism (methanol oxidation) of these bradyrhizobia. We used six strains of *Bradyrhizobium oligotrophicum* S58, *Bradyrhizobium* sp. BTAi1, *Bradyrhizobium* sp. ORS278, *Bradyrhizobium* sp. RP5, *Bradyrhizobium* sp. RP7, and *Bradyrhizobium* sp. WD16. The former three strains formed root nodules of an aquatic legume plant (*Aeschynomene indica*), while the latter three strains are endophytes in paddy rice roots. They are also able to survive in oligotrophic environments such as soils (Okubo et al. 2013). BLASTN search were conducted on the genomes of six strains by the DNA sequences of *xoxF* and *mxoF* gene in *M. extorquens* AM1. As a result, the former three strains of the aquatic legume plant (*A. indica* symbionts) have *xoxF* gene that presumably encodes La- and Ce-dependent methanol dehydrogenase (MDH), while the latter three strains of rice endophytes have both *xoxF* and *mxoF* gene. Culture experiments supported these results: The cell growth of *B. oligotrophicum* S58, *Bradyrhizobium* sp. BTAi1 and *Bradyrhizobium* sp. ORS278 (*A. indica* symbionts) was enhanced by La or Ce in HM medium containing methanol as a sole carbon source. They utilized methanol in the medium. On the other hand, the growth enhancement of bradyrhizobial rice endophytes (*Bradyrhizobium* sp. RP5, *Bradyrhizobium* sp. RP7, and *Bradyrhizobium* sp. WD16) by La or Ce additions were not observed in the same culture condition, probably because the existence of classical Ca-dependent MDH encoded by *mxoF* gene. We constructed two types of *xoxF* mutants, *xoxF::omega* and *delta xoxF* of *B. oligotrophicum* S58 by using omega cassette and sac markerless system, respectively. In the presence of La or Ce in HM medium supplemented with methanol, the growth of *xoxF::omega* mutant decreased as compared with that of wild-type strain of *B. oligotrophicum* S58. On the other hand, the growth of *delta xoxF* mutant increased as compared with that of wild-type strain S58. This apparent discrepancy indicates two suggestions in methanol catabolism in *B. oligotrophicum* S58. Firstly, the polar effect of omega cassette probably induced the repression of gene for formaldehyde catabolism, which located on downstream of the *xoxF* gene in *xoxF::omega* mutant. Secondly, there are other *xoxF* genes for La- or Ce- dependent MDHs. Indeed, we found redundant *xoxF* gene candidates on the genome of *B. oligotrophicum* S58 by extensive survey. Finally, we want to discuss the geobiological significance of light REEs in environmental bacteria.

Keywords: Rare earth elements, Bacteria, C1 compound metabolism, *Bradyrhizobium*, Methanol, Methanol dehydrogenase