Studies on the effects of cadmium and zinc stress on Rhodobacter capsulatus B10

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In this study, I investigated the effects of cadmium and zinc on growth, morphology and protein expressions in *Rhodobacter capsulatus* B10. Particular attention was focused on change of morphology using a scanning electron microscope with an energy dispersive X-ray spectrometer and Scanning Transmission electron microscope-energy dispersive X-ray spectroscopy (STEM-EDX). I furthermore analyzed cadmium and zinc-induced protein expressions using two-dimensional polyacrylamide gel electrophoresis, and identified some cadmium and zinc-binding proteins using metal-binding affinity column chromatography.

Both cadmium and zinc caused inhibition for the growth of *R. capsulatus* B10. 0.05mM and 0.1mM $CdCl_2$ scarcely inhibited the growth of *R. capsulatus* B10, but 0.15 mM $CdCl_2$ caused inhibition for the bacterial growth. On the other hand, 0.6mM and 1.2mM $ZnCl_2$ did not inhibit the bacterial growth but 1.5mM $ZnCl_2$ caused growth inhibition.

R. capsulatus B10 changed the morphology in response to cadmium and zinc stress. The cells cultivated in the presence of 0.15 mM $CdCl_2$ had filamentous shapes. Furthermore, EDAX analyses indicated that a significant amount of cadmium was taken up by the filamentous cells, while the phosphorus content decreased in the cadmium-treated cells. The cells cultivated with 1.5mM $ZnCl_2$ also had filamentous shapes. Interestingly, small granules attached to the filamentous cells in the presence of zinc. EDAX analyses indicated that the granules contain high amounts of zinc, and phosphorus.

I found that some proteins are induced under cadmium (0.15 mM $CdCl_2$) stress. GroEL2 and DnaK were highly induced in the cadmium-treated cells. This suggest that the bacterium may produce these proteins to overcome changes that involve protein denaturation induced by cadmium. S-adenosylmethionine synthetase was also induced under cadmium stress. This enzyme is required for cell growth and

division. Ribosomal protein S1, aspartate aminotransferase and phosphoglycerate kinase were induced under cadmium stress. It has been known that cadmium causes damage to cells primarily by the generation of reactive oxygen species, which causes single-strand DNA damage, and disrupts the synthesis of nucleic acids and proteins. Therefore, *R. capsulatus* B10 may respond to exposure to cadmium by the induction of proteins that are required for protein synthesis, such as ribosomal protein S1, and energy generation, such as aspartate aminotransferase and phosphoglycerate kinase.

Under zinc stress, GroEL2, DnaK, S-adenosylmethionine synthetase, Ribosomal protein S1, aspartate aminotransferase and phage shock protein A were highly induced in *R.capsulatus* B10. The increased synthesis of S-adenosylmethionine synthetase suggests that zinc-treated cells may experience decrease in S-adenosylmethionine pool. This decrease led to inhibition in methylation of a cell division protein leading to inhibition of cell division and cause cell filamentation.

I could identify six cadmium-binding proteins by metal-binding affinity column chromatography. Those are 2-methylcitrate dehydratase, periplasmic phosphate-binding protein precursor, IMP dehydrogenase/GMP reductase, inositol monophosphatase, lytic murein transglycosylase and periplasmic chaperone protein. When cadmium binds to periplasmic phosphate-binding protein precursor, the phosphate uptake seems to be inhibited because EDAX analysis indicates that phosphorus level decreased in the cadmium-treated cells. Lytic murein transglycosylase functions as space makers to allow the insertion of new peptidoglycan material into the cell wall during growth and as pore makers in the peptidoglycan layer to allow transport of DNA and proteins across the cell wall. Cadmium inhibits the growth, and produces the filamentous cells. Therefore, it seems likely that cadmium specifically binds the lytic murein transglycosylase, resulting in the inhibition of the cell division.

I could identify three zinc-binding proteins, ketose-bisphosphate aldolase, phosphoribulokinase and ribosome recycling factor. Ketose-bisphosphate aldolase is a glycolytic enzyme that catalyzes the reversible aldol cleavage or condensation of fructose-1,6-bisphosphate into dihydroxyacetone-phosphate and glyceraldehyde 3-phosphate. It requires a divalent metal ion, generally zinc, for their activity. Phosphoribulokinase catalyses the ATP-dependent phosphorylation of ribulose-5-phosphate to ribulose-1,5-phosphate, a key step in the pentose phosphate pathway. The later is thus crucial to maintain a balanced intracellular oxidation-reduction potential (or redox poise) under photoheterotrophic growth conditions. Ribosome recycling factor which dissociates ribosomes from mRNA after termination of

translation, and is essential for bacterial growth. Although no papers concerning metal-binding properties of phosphoribulokinase and ribosome recycling factor have been published, the inhibition of growth might be caused by binding of zinc to phosphoribulokinase and ribosome recycling factor. It should be studied in the future.

学位論文審査結果の要旨

５．審査結果の要旨（600～650字）

バイオレメデーションは、石油、有機溶剤、重金属等による汚染を微生物によって修復する技術で、安価でしかも効率よく無毒化する方法として期待されている。これまでに、光合成細菌を用いたバイオレメデーション技術開発が報告されてきたが、CdやZn等の重金属が光合成細菌の生育にどの様な影響を及ぼすか、タンパク質レベルで研究した報告はない。

本論文は、光合成細菌 *Rhodobacter capsulatus* B10を用いて、本細菌のCd(Zn)誘導タンパク質やCd(Zn)結合タンパク質を２次元電気泳動により同定し、これらのタンパク質の機能とCd(Zn)による生育阻害や形態変化との関連を明らかにしたものである。例えば、本細菌を0.15mM$CdCl_2$(1.5mM$ZnCl_2$)存在下で培養すると、GroELやDnaKなどの一般的なストレス応答タンパク質が大量発現するが、リボゾームタンパク質であるS1も発現誘導されることを見いだした。さらに、S1は温度ストレスでは誘導されず、重金属濃度依存的に発現誘導される重金属ストレス特異的なタンパク質であることを明らかにした。一方、本細菌の主要なCd結合タンパク質は細胞壁の合成に関わるlytic murein transglycosylaseであることを見いだし、Cdストレスにより細胞が伸長することの原因が本酵素のCdによる阻害であるを明らかにした。

以上、本研究により、光合成細菌 *Rhodobacter capsulatus* B10のCdやZnによる重金属ストレスに関して、多くのことを明らかにし、それらの知見は当該分野の研究発展に大いに寄与するものである。従って、審査委員会は、本論文が博士論文として妥当であると判断した。