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論　文　名	「Bioremediation of water by removing manganese using *Saccharomyces cerevisiae* and isolation of high manganese-accumulating strain」
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論文要旨

Many countries in Asia, such as Bangladesh, India, and Vietnam, where water for human use, including drinking water, comes from surface-water and under-ground sources, are facing issues of contamination with heavy metals such as arsenic, manganese, and iron that adversely affect the health of millions of people. In these countries, contaminated water is not only used for drinking but also broadly used for irrigation of rice, especially during the dry season. Irrigation has led to the accumulation of heavy metals in paddy soil, and has consequently caused an increase in metal levels in rice grains. The widespread contamination of paddy field soils and increased heavy metal content in the grains occur because of extensive industrial and metal mining areas that cause heavy metal pollution in these countries. The most common inorganic pollutants in groundwater are iron and manganese. Manganese (Mn) concentration must be lower than 0.05 mg L^{-1} as a secondary contaminant according to the United States Environmental Protection Agency (EPA) (1979); however, a level of 0.02 mg L^{-1} is more appropriate to ensure consumer safety and minimize the potential for water discoloration.

Although Mn is an essential element for living organisms, it becomes toxic when it presents at high concentrations in cells.

Although conventional methods for removing metal ions from aqueous solution, such as chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, and adsorption on activated carbon, have been studied in detail, these methods are not efficient for treating large amounts of water or wastewater containing heavy metals at low concentrations. Therefore, an alternative technique is needed, which is safe, efficient, and economical. In addition to the advantage of low operating costs, a biological method is an ideal candidate for the treatment of high volume of water containing low concentration of metal ions. Such a method may be able to decrease the concentration of heavy metal ions in solution from the ppt to the ppb level.

Although numerous studies from different disciplines have demonstrated that bio-sorption/bioaccumulation is a useful alternative to conventional systems for heavy metal removal from aqueous solutions, further research is still needed to develop the bio-sorption/bioaccumulation processes and apply them to the treatment of water and wastewater sources. In recent decades, there has been increasing interest in using yeast and specific components of yeast cells as bio-sorbent materials. These applications have been based on new scientific evidence that suggests a role of yeast and yeast-derived products in modern systems. One of special interests is the use of *Saccharomyces cerevisiae* as biomaterial. Use of *S. cerevisiae* as a model system in studies of the mechanism of its stress response to metals, as well as other stresses, has provided an important framework that can be used to gain a deeper understanding of the mechanisms of metal toxicity and detoxification in higher eukaryotes. It has also aided us in the expansion of potential resources for biomaterials. Using yeasts is not only economical but also malleable to genetic, non-pathogenic, and morphological manipulations that result in better raw biomaterials. Although there are some reports of heavy metal bioaccumulation by *S. cerevisiae,* these studies do not primarily focused on Mn. Thus, more research is needed to determine bioaccumulation of Mn by the growing cells.

Screening of suitable microbial strains, which are more efficient and adaptable to environmental stresses, is important to enhance the applicability of bioaccumulation of Mn using growing microorganisms as a biomaterial. There is also little information regarding the screening of new strains that may have a greater ability to accumulate Mn and be more applicable for water treatment using growing cells.

In the framework of this thesis, I would like to focus on bioremediation, especially bioaccumulation of Mn by *Saccharomyces cerevisiae* cells, attracting continuing attention for developing satisfactory solution for the water decontamination in recent years. In this study, a promising strain of yeast with significant Mn accumulation has been isolated for real water/wastewater treatment using living cells. The thesis consists of 4 Chapters as follows:

In Chapter 1, I provided some overviews of Mn contaminant information, types of biomaterial using for bioremediation, methods for treatment of heavy metals in water/wastewater. Bioremediation using yeasts as biosorbent (dead cells/treated cells) or as bio-accumulator (living/alive cells) were also reviewed. Furthermore, purposes of this thesis were also addressed.

In Chapter 2, Mn accumulation of *Saccharomyces cerevisiae* BY4741 was examined and screening of high Mn-accumulating of variants was performed from the *Saccharomyces cerevisiae* strain. Based on the ability of Mn accumulation, a variant having a capacity of four to five fold Mn accumulation than the parental strain was selected, suggesting that this variant may become a promising tool for Mn removal from water.

In Chapter 3, the function of Mn(II) ion in scavenging reactive oxygen species (ROS) within yeast cells was examined. It was shown that Mn(II) ion could act as a ROS scavenger to protect yeast cells against the ROS caused by ^{60}Co-gamma irradiation and other ROS sources such as hydrogen peroxide, menadione by using the yeast cells grown in nutrient medium supplemented with 0.5 mM of Mn(II). On the contrary, the isolated high Mn-accumulating variant showed lower activity of catalase and superoxide dismutase and lower content of trehalose, and was sensitive to ROS caused by ^{60}Co-gamma irradiation and other ROS sources such as hydrogen peroxide and menadione. These results suggest a new trend of Mn(II) function to scavenge ROS collaborating with other ROS scavenging functions in yeast cells.

In Chapter 4, the main results of this thesis and perspectives of future research were summarized.

審査結果の要旨

本論文は古くから発酵、醸造に用いられ、容易に入手可能で安全性が確立されている酵母 *Saccharomyces cerevisiae* を水のマンガン汚染除去に利用することを目的とし、マンガン吸収特性を解析し、高マンガン吸収酵母変異株の分離を行った。さらにこれらの株の放射線感受性について検討し、以下の成果を得ている。

(1) *Saccharomyces cerevisiae* の標準株 BY4741 株を用いてマンガン（Mn）の吸収特性を調べた結果、Mn(II)添加培地中では 5 mM 以上の濃度で顕著な増殖阻害が見られた一方、添加濃度の増加に応じて Mn を細胞内により多く吸収することが見出された。また細胞を Mn 水溶液に懸濁した非増殖の細胞、及び放射線照射や加熱により不活化した細胞には Mn は吸着しなかったことから、*Saccharomyces cerevisiae* への Mn 吸収能は細胞の増殖に伴って発揮されると 結論した。

(2) より高度な Mn 吸収能を持つ *Saccharomyces cerevisiae* の変異株を得るために、高濃度の Mn 存在下でも十分な増殖能が保たれる生存株をスクリーニングしたところ、15 mM の Mn(II)存在下でも Mn 非存在下と同等の増殖能を有し、なおかつ標準株 BY4741 よりも 5 倍以上の Mn(II)蓄積能を持つ変異株 IM3 の分離に成功した。また本株は親株同様、温度 30℃～37℃、及び pH4～9 の範囲で有効な Mn 蓄積能を示すことを確認した。

(3) 放射線抵抗成菌が顕著な Mn 蓄積能を示すことがすでに報告されているので、本研究によって得られた高 Mn 吸収株 IM3 についても同様の特性が見られるかどうか検討した。*Saccharomyces cerevisiae* 標準株 BY4741 と高 Mn 吸収分離株 IM3 に ^{60}Co ガンマ線を照射したところ、IM3 の方が顕著に放射線感受性であることが判明した。次に増殖能が維持される Mn(II)の濃度範囲で Mn を吸収さ

せた ^{60}Co ガンマ線を照射したところ、株の種類にかかわらず 0.5 mM の Mn(II)存在下では Mn(II)非存在下に比べて 1.5 倍程度の放射線抵抗性の増大が見られた。しかし、BY4741 と IM3 との放射線感受性の差には有意な変化が見られなかった。両者の放射線照射前後の活性酸素量を調べたところ、Mn(II)無添加、1 mM 添加の場合はガンマ線照射後、IM3 の活性酸素量が標準株よりも 3 倍程度高く、活性酸素消去酵素であるカタラーゼ、スーパーオキシドディスムターゼの活性も低下していた。一方、放射線抵抗性の増大が見られた 0.5 mM の Mn(II)添加においては活性酸素量の顕著な減少が見られた。以上の結果から BY4741、IM3 ともに一定量以上蓄積された Mn は放射線抵抗性には寄与せず、既報の放射線抵抗性菌とは異なる機構で細胞にストレスを与えている新規な可能性が示唆された。

以上の研究結果は微生物を用いた水質浄化技術の発展の端緒となり得る有用な知見を与えており、また微生物の放射線抵抗性機構についても新たな知見が得られており、今後の研究の発展に貢献するところ大である。また、申請者が自立して研究活動を行うに必要な能力と学識を有することを証したものである。