1339 メタロチオネイン遺伝子を保持した大腸菌による重金属の回収

(宮崎大・農・生物資源、*阪大・工・応生) ○加藤智朗、吉田直人
石川貴靖、小川喜八郎、室岡義勝*

【目的】本研究は金属結合タンパク質であるメタロチオネイン遺伝子を保持した大腸菌を用い、汚染環境からの重金属回収を目標として、培地中からの重金属回収実験を試みた。

【方法及び結果】β-ガラクトシダーゼとヒトメタロチオネイン-Ⅱ融合タンパク質を発現する大腸菌を、重金属を添加した培地にて生育させると、カドミウムや亜鉛に対して耐性の増大が確認された。菌体が培地より金属を取り込み、蓄積するかどうかを培地内金属濃度や、菌体に含まれる金属量を計測することにより調べた。数種類の金属について、金属濃度の減少が見られ、特にカドミウムについてはコントロールでは平均 9.91 %の濃度減少が見られたのに対し、メタロチオネイン遺伝子保有株では 21.69 %の減少が確認された。また、カドミウムの局在を調べると、細胞内と細胞膜にはほぼ同量のカドミウムが存在した。

Uptake of Heavy-metal ions by *E. coli* harboring methallothionein gene.

○Tomoaki Kato, Naoto Yoshida, Takanobu Ishikawa, Kihachiro Ogawa, and Yoshikatsu Murooka* (Dept. Biol. Resouce Sci., Miyazaki Univ., *Dept. Biotechnol., Osaka Univ.)

【Keywords】 methallothionein-Ⅱ, heavy metal, bioremediation, cadmium

1340 Microbial Degradation of Nickel Protoporphyrin Disodium

Gina Rio Dedeles, Ayumi Abe, Yoko Sumita, Katsuichi Saito, Atsushi Yokota and Fusao Tomita (Division of Applied Bioscience, Graduate School of Agriculture)

[Purpose] A soil isolate, designated as the YA-1 strain was screened for its ability to degrade nickel protoporphyrin disodium (NiPPDS). This was identified as *Pseudomonas azelaica*, a gram-negative aerobic rod.

[Methods and Results] The screening medium was prepared with 0.1g/L of NiPPDS. The YA-1 cell was incubated into tubes of this screening medium at 27^0 C for 7 days. The results showed that YA-1 cells have the ability to degrade NiPPDS. The degradation potential of YA-1 cell was analyzed by adjusting the pH to 2 with 1 N HCl, extraction of ethyl acetate and measured spectrophotometrically at an absorbance of 398 nm. To determine the efficiency of this organism in the degradation of NiPPDS, the cell was examined in three cycles at an interval of two days incubation period. The rate of NiPPDS degradation appeared to decrease at every cycle. During the first cycle, 57% of NiPPDS was degraded, in the second cycle, 55% and in the last cycle was 13%. These results revealed that the cell was stable only up to the second cycle to degrade the NiPPDS. Then the reaction mechanism of the enzyme involved in this process was investigated. The reaction mixture was composed of Tris-HCl buffer (pH 7.0), NiPPDS in methanol solution and the crude extract as the enzyme source. The results obtained attests the enzymatic action of YA-1 to degrade the NiPPDS. This NiPPDS-degrading enzyme of YA-1 was purified 6 fold from crude-extract with a recovery of 25% by means of ammonium sulfate precipitation, chromatography with DEAE Toyopearl 650 M and CM Toyopearl 650 M. Further characterization of the enzyme is presently undergoing investigation.

[Key Words] *Pseudomonas*, degrading ability, nickel-protoporphyrin disodium