学 位 論 文 要 旨

Chemolithotrophic growth of fungi including *Fusarium solani* on elemental sulfur
Fusarium solani を含む菌類の単体硫黄を用いた化学合成無機栄養的生育

環境資源共生科学専攻 環境保全学大講座
徐 海博

Chemolithotrophic sulfur oxidation, one of the major reactions of global sulfur cycle, is thought to be an ancient metabolic process carried out exclusively in prokaryotes. Some literatures exist concerning oxidation of elemental sulfur (S^0) and thiosulfate by fungi in the presence of organic compound. Previous study on *Fusarium solani* strain THIF01, harboring an endobacterium, showed that it could grow chemolithotrophically using S^0 as a sole energy source. However, chemolithotrophic sulfur oxidation by endobacteria-free fungi has not been unequivocally documented. Given that mixotrophic sulfur oxidation by fungi has been found existing in diverse niches including nutrient-limiting environments, it is reasonable to doubt about the “fact” that eukaryotes are not capable of chemolithotrophic sulfur oxidation. Therefore the aims of this study are to investigate the distribution of chemolithotrophic sulfur-oxidizing fungi and the physiological characteristics of sulfur oxidation by fungi.

Our group has found that 13 named fungal strains from culture collections oxidized S^0 on an organic compounds-free medium. In this study, seven of these fungal strains, forming larger area of clearing zones on S^0-containing plates, were screened for absence of endobacteria and ability of chemolithotrohpic sulfur oxidation. No 16S rRNA gene was amplified from the genomic DNA, indicating that these seven fungal strains did not harbored endobacteria. All of the strains grew in an S^0-containing mineral-salts submerged medium. *F. solani* f.sp. *pisi* NBRC9425 exhibited the intensest sulfur oxidation because it formed the largest clearing zone on agarose-solidified medium and the culture pH showed the most dramatic decrease in mineral-salts liquid medium.

Activity of sulfur-oxidation of strain NBRC9425 was examined after the fungus was cultured in S^0-containing mineral-salts medium for 15 days. Neither culture filtrate nor the concentrate of filtrate incubated with sulfur substrate such as GSSG/GS_nG, S^0, $S_2O_3^{2-}$, and SO_3^{2-} exhibited any activity of sulfur oxidation, indicating sulfur oxidation occurred intracellularly. However, cell-free extract also did not show any activity of sulfur oxidation. It is considered that the integrity of the fungal cell was vital to maintain the activity of sulfur oxidation, and the cell disruption when preparing cell-free extract caused loss of the activity.

The proteomic profiling in response to chemolithotrophic growth condition on sulfur and heterotrophic growth condition on maltose was analyzed on two dimensional electrophoresis (2-DE) gels. In this study, optimization of the conditions of protein extraction and isoelectronic focusing was performed. 2D display of soluble proteins of strain NBRC9425 grown on the different energy sources suggested greatly distinct metabolism. This study paves way for comparative proteomic study of identifying proteins participating in fungal sulfur-oxidation.

Physiological characteristics of chemolithotrophic fungal growth on reduced inorganic sulfur compounds were investigated. Chlamydospores collected from carbon-free medium served as the inoculum. When grown in mineral-salts medium containing S^0, strain NBRC9425 produced thiosulfate, and the produced thiosulfate decreased after 20 days cultivation. According to the decrease of thiosulfate, sulfate was produced, and the concentration was kept increasing during a period of 50 days. Neither tetrathionate nor sulfite was detected in the culture. These phenomena suggest that S^0 was oxidized to thiosulfate, and then to sulfate eventually. When grown with thiosulfate in mineral-salts medium, strain NBRC9425 also produced sulfate, but the concentration of sulfate decreased from the 5th day. Notably, sulfate production and biomass yield were greatly enhanced when thiosulfate was supplemented into S^0 medium.

In order to study the effects of organic compounds on the sulfur oxidation by fungi, different concentrations of yeast extract (0–200 mg L^{-1}) were added into S^0-containing medium. As the supplemented yeast extract increased, thiosulfate and sulfate production and fungal biomass increased accordingly. However, the values of sulfur compounds equivalent, the quotient of sulfate/thiosulfate divided by ergosterol, showed that the highest sulfur oxidation ability was achieved at 15 mg L^{-1} yeast extract. Fungal hypha were observed to attach onto the S^0 particles when growing with less than 100 mg L^{-1} yeast extract, but no such association was evident when incubated with 200 mg L^{-1} or more of yeast extract. Although high concentrations of organic compounds shifted the metabolism from chemolithotroph to chemoorganotroph, the fungus oxidized more S^0 because of higher yield of biomass.

Therefore, it was proved that chemolithotrophic sulfur oxidation by fungi occurs abundantly and fungi exhibited distinct physiological properties of chemolithotrophic sulfur oxidation from that of prokaryotes.