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Microbial degradation of 2,4,6-trinitrotoluene (TNT)

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The study on 2,4,6-trinitrotoluene (TNT) degradation by some white rot fungi isolated in Korea was carried out to examine their capability to metabolize TNT. When 50 mg/L of TNT was added into a nutrient-rich medium at the beginning of the incubation, four of the fungal strains completely removed TNT during several days of incubation and showed higher removal rates than that of *Phanerochaete chrysosporium* used as a comparative control. In the culture of *Irpex lacteus*, up to 200 mg/L of TNT was removed within 12 hours after adding it to a 5-day old culture, and the removal rates were higher than those in N-limited minimal medium. Interestingly *I. lacteus* can metabolize TNT with two different initial transformation pathways. In one metabolic pathway of TNT a nitro group was removed from the aromatic ring of TNT. Formation of hydride-Meisenheimer complex of TNT (H^--TNT), which is an important intermediate colored dark red, was confirmed with LC/MS and LC/photodiodearray detector. 2,4-Dintrotoluene and nitrite which might be produced from the denitration of H^--TNT were detected in fungal culture and supported this transformation pathway. In the other TNT pathway, nitro groups in TNT were successively reduced to amine groups via hydroxylamines. The initial formation of hydroxylamino-dinitrotoluenes (2- and 4- OHAmDNT) was observed, and followed by their successive transformation to aminodinitrotoluenes (2- and 4-AmDNT). Transformation of TNT to AmDNTs via OHAmDNTs was fast, but the next step was slow. The activity of a membrane-associated aromatic nitroreductase was detected in the cell-free extract of *I. lacteus*. Among inducers and cofactors $FeSO_4$ and Tween 80 enhanced the degradation of TNT and its metabolites by *I. lacteus*. The mineralization of [U-^{14}C]TNT by *I. lacteus* was also tested in static and shaken cultures. The mineralization rate of TNT in static culture was higher than that in shaken culture, and addition of Tween 80 enhanced the mineralization of TNT in static culture. The addition of carbon and nitrogen after depletion of C and N slightly stimulated mineralization of TNT in static culture.

Several TNT-degrading bacteria were isolated from activated sludge used as a inoculum for second reactor in two step bench-scale continuous stirred tank reactors. A strain which showed the highest TNT-degrading activity was identified as *Enterobacter cancerogenus*. TNT was completely disappeared within 6 hours of further incubation when 100 mg/L of TNT was added into 1-day pregrown *Enterobacter* culture. OHAmDNTs, AmDNTs, and diaminonitrotoluenes (DAmNTs) were detected as main TNT metabolites in three different carbon sources (glucose, fructose, and sucrose). The transformation rates of TNT were similar with all three carbon sources, but the transformation rates of OHAmDNTs and AmDNTs were higher with fructose. Among three nitrogen sources [NH_4NO_3, $(NH_4)_2SO_4$, and $NaNO_2$], the addition of sodium nitrite showed the highest transformation rates of TNT and its metabolites than those of other two N sources. The aerobic and anaerobic TNT degradation by *Enterobacter* sp. were also compared. In addition to the main metabolites described above, various unknown metabolites were produced in anaerobic degradation. The transformation of TNT to OHAmDNTs was fast in anaerobic culture than that in aerobic culture, but the transformation of OHAmDNTs to next metabolites was very slow. The aromatic nitroreductase involved in the nitro group reduction of TNT was par-

tially purified. In enzymatic reactions, OHAmDNTs, AmDNTs, and DAmNTs were produced. Finally, *Enterobacter* sp. was tested for its ability to mineralize TNT. The mineralization rate of *Enterobacter* sp. was much lower than those of white rot fungi reported, but was higher than those of other bacteria reported.