

## Fungal Transformation of 2,4-Dinitrotoluene and 2,4,6-Trinitrotoluene

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Screening of 190 fungi representing 98 genera showed that the ability to transform 2,4,6-trinitrotoluene was common, whereas transformation of 2,4-dinitrotoluene was rare.

The deleterious effects on plant and animal life of organic nitro compounds in wastes from 2,4,6-trinitrotoluene (TNT) manufacture have long been recognized, and intensive efforts have been made to remove these compounds from wastewaters (11). A number of reports of bacterial transformation of TNT in media supplemented with glucose have appeared (4, 6, 8, 14). In addition, growth of a *Pseudomonas* species on TNT as sole source of carbon and nitrogen has been reported (12). Klausmeier and co-workers (4) also studied the effect of TNT on 24 fungi representing 9 genera in a medium containing glucose. This note describes results from the screening of 190 fungi representing 98 genera for the ability to transform TNT or 2,4-dinitrotoluene (DNT) in shake culture at 29°C in basal medium (9) with 0.5% glucose. The nitroaromatic compound was added at 0 to 5 days after inoculation of the culture medium to give an initial concentration of 100 mg/liter, and the culture filtrate was analyzed at intervals for up to 5 days for TNT and DNT by liquid chromatography (13). Spectrophotometric measurements of TNT (2, 10, 13) and of DNT (3) were unreliable when applied to culture filtrates. Of the 190 organisms, 183 were able to transform TNT under the test conditions. In contrast, only five organisms transformed DNT, and of these, one organism, QM 9651, was far superior to the others in rate of transformation. The low frequency of occurrence of ability to transform DNT was surprising in view of the widespread ability of tissues of animals, plants, and microorganisms to reduce organic nitro compounds (7) and the high frequency with which the same organisms under the same test conditions were able to transform the structurally related TNT.

Transformation of TNT and DNT was effected by washed mycelia of all cultures, and no

activity was found in culture filtrates. An induction period of 2 h preceded uptake of TNT by mycelia, and transformation was complete in 4 h more. After the induction period, and before complete transformation, the presence of TNT in washed mycelia could be shown by extraction with acetone followed by chromatography (13, 14). The transformation products of TNT were identified by thin-layer chromatographic comparison with standards (14) as 4-amino-2,6-dinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and 4,4'-azoxy-2,2',6,6'-tetranitrotoluene. Reduction of nitro groups at positions other than C4 was not observed, in contrast to reports (6, 14) for some bacteria. Studies with labeled TNT (ring-L-[U-<sup>14</sup>C]) gave no evidence for cleavage of the carbon skeleton, as occurs (12) with *Pseudomonas* species.

Similar data were obtained with DNT as substrate. Transformation, preceded by an induction period of about 8 h, was complete in approximately 24 h.

Application of fungi to abatement of pollution from TNT wastewaters appears unpromising in view of their failure to degrade TNT and DNT and the likelihood that the amino transformation products are toxic (1).

### LITERATURE CITED

1. Christensen, H. E., T. T. Luginbyhl, and B. S. Carroll (ed.). 1975. NIOSH Registry of toxic effects of chemical substances. U.S. Department of Health, Education, and Welfare. U.S. Government Printing Office, Washington, D.C.
2. Glover, D. J., and E. G. Kayser. 1968. Quantitative spectrophotometric analysis of polynitroaromatic compounds by reaction with ethylenediamine. *Anal. Chem.* 40:2055-2058.
3. Kaminskii, A. Ya., Z. A. Kozina, and S. S. Gitis. 1966. Determination of 2,4- and 2,6-dinitrotoluenes in their mixture. *Zh. Anal. Khim.* 21:1380-1384.
4. Klausmeier, R. E., J. L. Osmon, and D. R. Walls. 1973. The effect of trinitrotoluene on microorganisms. *Dev. Ind. Microbiol.* 15:309-317.
5. Mandels, M., J. Weber, and R. Parizek. 1971. Enhanced cellulase production by a mutant of *Trichoderma viride*. *Appl. Microbiol.* 21:152-154.
6. McCormick, N. G., F. E. Feeherry, and H. S. Levin-

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- son. 1976. Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. *Appl. Environ. Microbiol.* 31:949-958.
7. Nason, A. 1956. Enzymatic steps in the assimilation of nitrate and nitrite in fungi and green plants, p. 109-136. *In* W. D. McElroy and B. Glass (ed.), *Inorganic nitrogen metabolism*. Johns Hopkins Press, Baltimore.
8. Osmon, J. L., and R. E. Klausmeier. 1972. The microbial degradation of explosives. *Dev. Ind. Microbiol.* 14:247-252.
9. Reese, E. T., and M. Mandels. 1959.  $\beta$ -D-1,3-glucanases in fungi. *Can. J. Microbiol.* 5:173-185.
10. Ruchhoft, C. C., and W. G. Meckler. 1945. Colored TNT derivative and  $\alpha$ -TNT in colored aqueous  $\alpha$ -TNT solutions. *Ind. Eng. Chem.* 17:430-434.
11. Spano, L. A., R. C. Chalk, J. T. Walsh, and C. DiPietro. 1973. Abatement of nitroaromatics in aqueous effluents from TNT production and finishing plants, p. 288-297. *In* E. S. Barrekette (ed.), *Pollution: engineering and scientific solutions*. Plenum Publishing Co., New York.
12. Traxler, R. W., E. Wood, and J. M. Delaney. 1974. Bacterial degradation of  $\alpha$ -TNT. *Dev. Ind. Microbiol.* 16:71-76.
13. Walsh, J. T., R. C. Chalk, and C. Merritt, Jr. 1973. Application of liquid chromatography to pollution abatement studies of munition wastes. *Anal. Chem.* 45:1215-1220.
14. Won, W. D., R. J. Heckly, D. J. Glover, and J. C. Hoffsommer. 1974. Metabolic disposition of 2,4,6-trinitrotoluene. *Appl. Microbiol.* 27:513-516.