Conversion of DDT to DDD by Pathogenic and Saprophytic Bacteria Associated with Plants

Abstract. *Of 27 microorganisms examined, 23 plant pathogenic and saprophytic bacterial species were found to convert* p,p′ *DDT to* p,p′ *DDD under anaerobic conditions. The range of conversion of DDT (10 micrograms per milliliter) during an incubation of 14 days was from a trace to over 5 micrograms per milliliter, with the majority of the bacteria showing the greatest activity during the final 7-day period. There is evidence that metabolites of DDT other than DDD are also produced.*

The recalcitrance of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] to microbial degradation is well documented (*1*). However, investigators have reported the presence of DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] in water, soil, vegetables, rumen fluid, and animal samples from areas where only DDT treatment has been used (*2*, *3*). The reductive dechlorination of DDT to DDD by mixed populations of microorganisms is implied in these reports. The work of Miskus *et al.* (*3*), in which DDT was found to be converted to DDD in samples of sealed lake water (7 days) and in stagnating rumen fluid (0 to 24 hours), suggests that conversion is enhanced under conditions forming anaerobiosis. Direct evidence for the potentiation of dechlorination of DDT by microorganisms under anaerobic conditions has been presented by Stenersen (*4*) and Wedemeyer (*5*). Each noted that the efficacy of degradation of DDT by bacteria is inversely proportional to the availability of oxygen in the environment. The extent of the literature dealing with the dechlorination of DDT to DDD or other metabolites by pure cultures of single microbial species is limited (*4–6*) with only one account referring to soil isolates (*7*).

We attempted to ascertain the efficacy of a wide spectrum of pathogenic and saprophytic bacteria associated with plants in degrading *p,p′* DDT. The bacterial species were regenerated from the lyophilized state, grown aerobically in brain-heart infusion medium (*8*) agitated with a water shaker or anaerobically in thioglycolate medium (*8*). Pure *p,p′* DDT (*8*) (100 μg) was introduced in ethanol to 10 ml of bacterial culture containing 10^6 bacterial cells. Both aerobic and anaerobic samples were incubated at 28°C and examined after 7 and 14 days.

Table 1. Degradation of *p,p′* DDT (100 μg of DDT per 10 ml of culture fluid) to *p,p′* DDD by bacteria.

Bacterial species	Concentration of DDD (μg)		
	Aerobic (14 days)	Anaerobic (7 days)	Anaerobic (14 days)
Control, media	None	None	None
Achromobacter sp.	None	11.4	44.7
Aerobacter aerogenes	None	19.3	11.4
Agrobacterium tumefaciens	None	11.7	30.9
Azotobacter sp.*	None		
Bacillus cereus	Trace†	Trace	14.3
Bacillus cereus mycoides	Trace	Trace	20.4
Bacillus subtilis	Trace	Trace	34.6
Clostridium pasteurianum	S.A.‡	18.2	27.4
Clostridium sporogenes	S.A.‡	None	None
Corynebacterium michiganense	None	None	Trace
Erwinia amylovora	None	Trace	28.1
Erwinia ananas	None	21.9	42.9
Erwinia carotovora	Trace	10.0	45.3
Erwinia chrysanthemi	None	10.0	32.4
Erwinia sp.	None	19.6	50.1
Kurthia zopfii	None	14.5	27.0
Pseudomonas fluorescens	None	8.4	31.9
Pseudomonas glycinea	None	13.9	48.8
Pseudomonas marginalis	None	Trace	7.8
Pseudomonas mors-prunorum	None	6.7	32.6
Pseudomonas syringae	None	16.0	40.8
Pseudomonas tabaci	None	Trace	23.2
Sarcina lutea	None	None	None
Xanthomonas pruni	None	Trace	5.7
Xanthomonas stewartii	None	5.4	54.4
Xanthomonas uredovorus	None	30.1	48.6
Xanthomonas vesicatoria	None	Trace	8.1

* Grown only aerobically. † 1 to 2 μg. ‡ Strict anaerobe.

Extractions of bacterial cells were made with a mixture of acetone and petroleum ether [modified after Mills *et al.* (*9*)]. The petroleum ether extract was brought to a final volume of 100 ml and dried immediately with anhydrous Na_2SO_4. With a 10-μl Hamilton syringe for the extraction, we assayed 5-μl samples for metabolites with a Barber-Colman Model Series 5000 gas chromatograph equipped with a tritium ionization detector, an 8300 recorder, and disk chart integrator Model 205. The detector was operated at 220°C with a current of 25 volts d-c, the flash temperature at 230° to 240°C, and column temperature at 188° to 190°C. The carrier gas was purified nitrogen with a flow rate of 88 ml per minute with initial pressure of 1.3 atm (gauge). The column, a glass U-tube 180 cm by 4 mm, was packed with equal weights of QF 1 (5 percent by weight) on acid-washed Chromosorb W (60 to 80 mesh) and Dow 11 (5 percent by weight) on acid-washed Chromosorb W. The Chromosorb W coated with QF 1 was placed in the direction of the carrier gas flow, and the Dow 11 was placed on the detector side.

For further identification and verification of DDT and its metabolites, thin-layer chromatography (TLC) was used. Eastman chromatographic sheets (Type K 301 R) coated with silica gel and activated at 100°C for 30 minutes were used. Two-dimensional chromatograms were made with hexane and a mixture of hexane and carbon tetrachloride (7:3 by volume). The dried sheets were sprayed with fresh 0.5 percent Rhodamine B in ethanol and then with 10 percent aqueous Na_2CO_3 and examined under ultraviolet light.

In evaluating 27 bacterial species for their ability to degrade *p,p′* DDT, we found the occurrence of this degradation aerobically to be essentially nil, with traces of DDD in a few culture extracts. Significantly, however, the dechlorination of DDT to DDD was found to be widespread among bacteria when grown under anaerobic conditions (Table 1). In general, the conversion of DDT to DDD occurred most actively during the second 7-day period of incubation under anaerobic conditions. For example, eight species which revealed only trace amounts after 7 days had produced from 5.7 to 34 μg after 14 days. On the other hand, *Xanthomonas uredovorus* and three other species produced more DDD during the initial 7-day incubation period. The strictly aerobic spe-

cies, *Sarcina lutea*, grown either aerobically or anaerobically (surface growth only) produced no detectable metabolites of DDT.

We noted that strict anaerobes may be variable with regard to DDT degradation. Of two species of *Clostridium* studied, *Cl. sporogenes* showed no detectable activity, while *Cl. pasteurianum*, an anaerobic nitrogen fixer, converted nearly 30 μg of DDT to DDD in 14 days. Another nitrogen-fixing species, the aerobe *Azotobacter* sp., produced no discernible metabolites of DDT. The degradation pattern of *Aerobacter aerogenes* seems to indicate a secondary conversion of DDD to other unknown metabolites in view of the fact that the 20 μg of DDD detected after an incubation of 7 days had diminished to 12 μg after 14 days.

The pattern of degradation in some organisms (*Erwinia* sp., *E. ananas*, and *X. uredovorus*) seemed to parallel the rate of growth. On the other hand, *X. stewartii, Pseudomonas tabaci*, and *Bacillus subtilis*, as well as others, produced only traces of DDD after 7 days, despite heavy growth, and yet converted appreciable amounts of DDT to DDD during the final 7 days.

Recovery of DDT from the controls, which were run repeatedly, exceeded 95 percent. Of significance, however, is the fact that, where degradation occurred, we were unable to account for approximately 10 percent of the insecticide as either DDT, DDD, or DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] after incubation for 14 days. In these instances, gas-liquid chromatography (GLC) peaks of unknown origin, particularly with *E. chrysanthemi, Pseudomonas syringae, X. uredovorus*, and *Aerobacter aerogenes*, were regularly observed. These suggest the degradation of DDT by bacteria to a number of metabolites still unidentified. The report of Barker and Morrison (*10*) on the degradation of DDD by *Proteus vulgaris* seems to support this contention.

The retention times (GLC) and the R_F values (TLC) of the standards and culture extractions of DDT and DDD were identical. We were unable to detect any autodegradation in control samples or as the result of column conversion (GLC) (*11*). Quantitation was based on integration counts compared to standards with the disk chart integrator.

Our results indicate that a broad range of plant pathogenic and saprophytic bacteria have the capacity to convert *p,p'* DDT to *p,p'* DDD under anaerobic conditions in vitro and that this phenomenon is more ubiquitous than had previously been reported.

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Addiction Liability of Albino Rats: Breeding for Quantitative Differences in Morphine Drinking

Abstract. *Selective breeding produced two strains of rats that differ in their susceptibility to morphine addiction. Inbreeding the more susceptible rats in an unselected population produced susceptible offspring; inbreeding resistant rats produced resistant offspring. Further selection and inbreeding increased the strain difference in the F_2 and F_3 generations. The F_3 generation also differed in their susceptibility to alcohol addiction.*

A procedure, based on operant conditioning techniques and the known physiological effects of opiates, has been devised which can cause a relatively lasting change in the behavior of rats toward morphine. Weeks after their last dose of morphine, rats continue to show opiate-directed behavior (*1–3*).

The procedure used in this study was developed from an analysis of human drug addiction. If one accepts two propositions—(i) that opiate addiction is *not* something unique, but merely the repetition of a response, and (ii) that responses are repeated because they are followed by reinforcers—then it seems reasonable to believe opiates may be reinforcers.

Opiates appear to share at least one important characteristic with many other reinforcers: they reduce drives. Drives are tensions which goad a person into activity. But an opiate makes the subject inactive: after a "fix," an addict enters a stuporous, semi-dozing state. The drives produced by pain, hunger, anxiety, and sex are all simultaneously reduced by opiates (*4*); this property suggests that the opiates should be powerful reinforcers.

When the effects of an opiate wear off, the initially suppressed drives return. Actually, these drives rebound with increased vigor because they are augmented by the body's counteradaptations (*3–5*). Their return is experienced as tensions which soon increase to the point of distress. Human addicts, faced with approaching withdrawal, energetically seek drugs; this, with other evidence, indicates that the addicts are in a high-drive state (*3*).

It follows from this analysis that opiates would have a greater reinforcing or behavior-changing effect if taken when the subject is in the withdrawal stage because the opiates would have greater drive-reducing potential under these conditions. This deduction has been experimentally verified (*3*).

The procedure used in this experiment operantly conditions the response of drinking a normally refused morphine solution. The degree of behavioral change is quantified by choice tests in which individually caged rats are offered two calibrated 100-ml drinking tubes (Kimble No. 44875), one containing tap water, and the other a morphine solution (0.5 mg morphine per milliliter of water). Inexperienced rats prefer water and drink only 1 to 2 ml of the morphine solution in 24 hours. But during the cyclical reinforcing stage of treatment, rats drink increasingly more morphine solution on interposed choice tests. This change in