Some Factors Affecting Degradation of Organochlorine Pesticides by Bacteria

B. E. LANGLOIS, J. A. COLLINS, and K. G. SIDES
Department of Animal Sciences, Food Science Section
University of Kentucky, Lexington 40506

Abstract
Whole cells of *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Escherichia coli* and *Enterobacter aerogenes* in trypti-
case soy broth degraded DDT into two to eight metabolites. Seven metabolites were
from aerobic growth of the *Bacillus*. Similar metabolites were from anaerobic growth of
*E. coli* and *E. aerogenes* but less than four metabolites were from aerobic growth of
these organisms. Metabolic pathway for the degradation of DDT was similar for the
aforementioned species: DDT $\rightarrow$ DDD $\rightarrow$ DDMU $\rightarrow$ DDMS $\rightarrow$
DDNU $\rightarrow$ (DDOH) $\rightarrow$ DDA $\rightarrow$ DBP or DDT $\rightarrow$ DDE. Whole cells of
*Pseudomonas fluorescens* and *Staphylococcus aureus* were unable to degrade DDT
under aerobic conditions.

None of the species was capable of de-
grading DDT in skim milk or in trypticase soy broth containing 3% whole casein. Deg-
radiation was greatly reduced in trypticase soy broth containing 4.5% lactose or indi-
vidual fractions of α-, β- and γ-casein. None of the species degraded dieldrin and
heptachlor.

Introduction
Recent interest has focused on the mechanisms
by which microorganisms degrade organochlorine
pesticides to less harmful products, since many
of them are not biodegradable and may persist
for a long time in the soil. Most of the pre-
vious work has been with DDT (1,1,1-trichloro-
2,2-bis (p-chlorophenyl)ethane) (2,3,5,7,9,10,13,
14,15). Depending on the animal or microbial
species as well as availability of oxygen, as
many as eight products have been identified
from the degradation of DDT (2,14,15). The
first products formed appear to be DDD (1,1-
dichloro-2,2-bis (p-chlorophenyl)ethane) and
smaller amounts of DDE (1,1-dichloro-2,2-bis (p-
chlorophenyl)ethylene). Wedemeyer (16) found
the pathway for degradation of DDT by *Aero-
bacter aerogenes* to be: DDT $\rightarrow$ DDD $\rightarrow$
DDMU (1-chloro-2,2-bis (p-chlorophenyl)ethyl-
eneferyl) $\rightarrow$ DDMS (1-chloro-2,2-bis (p-chloro-
phenyl)ethane) $\rightarrow$ DDNU (unsym-bis (p-
chlorophenyl)ethylene) $\rightarrow$ DDOH (2,2-bis
(p-chlorophenyl)ethanol $\rightarrow$ DDA (2,2-bis
(p-chlorophenyl)acetic acid $\rightarrow$ DBP (4,4'-
Dichlorobenzophenone) or DDT $\rightarrow$ DDE.
This pathway is similar to the one postulated for
the degradation of DDT by the rat (13).

Degradation of other organochlorine pesti-
cides has not been studied in detail. Several
workers have reported the degradation of
dieldrin (1,2,3,4,10,10-hexachloro-6-6-epoxy-1,4,4-
5,6,7,8,8a-octahydro-1,4-endom- exo- 5,8-dimeth-
anonaphthalene) to aldrin diol (6,7-trans-di-
hydroxydihydroaldrin) (12,16).

Since milk had been found to contain varying
amounts of organochlorine pesticides it seemed
desirable to obtain information on the extent
to which microbial degradation of DDT, dieldrin
and heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7Ta-tetrahydro-4-7-methanoindene) does occur
in milk.

Materials and Methods

Organisms. Strains of *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Escherichia coli*,
*Enterobacter aerogenes*, *Pseudomonas fluores-
cens* and *Staphylococcus aureus* were obtained
from our collection in the department of animal
sciences, food science section.

Culture procedure. The bacteria were rou-
tinely grown in trypticase soy broth at the
optimum temperature for the species. Working
cultures were transferred weekly, whereas the
stock cultures were maintained on nutrient agar
plates stored at 7 C and transferred at 4-month
intervals. The organisms were grown in trypti-
case soy broth for three successive daily trans-
fers, before inoculation of a 15- to 18-hour
culture in flasks containing the test media and
pesticide. Flasks containing only pesticide and
media were run as controls. All flasks were in-
cubated aerobically at optimum temperature

Received for publication July 30, 1970.

1 Published with the approval of the Director of
the Kentucky Agricultural Experiment Station as
journal article no. 70-5-89.

2 Present address, Department of Animal Sci-
ences, University of Arkansas, Fayetteville 72071.
for up to 30 days. Flasks containing E. coli and E. aerogenes also were incubated anaerobi-
cally in a BBL Gas Pak anaerobic jar.

**Test media.** Growth media to determine the ability of the test bacteria to degrade organ-
ochlorine pesticides were: Skimmilk (Difco), Matrix (Galloway-West), and trypticase soy bro-
th (BBL). In addition, lactose, whole casein and α-, β- and γ-caseins were added individually
to trypticase soy broth to give concentrations
normally found in skimmilk. All media were
dispensed into screwcap Erlenmeyer flasks in
amounts of from 50 to 300 ml.

**Preparation of whole casein and casein frac-
tions.** Whole casein was prepared from fresh
skimmilk by acid precipitation (8). The α-, β-
and γ-caseins were prepared from whole casein
by the urea fractionation method of Hipp et al
(8). Purity of whole casein and the casein
fractions was determined by thin layer electro-
phoresis, and the products were stored at 5 C
until used.

**Pesticides.** Degradation of both technical and
purified grades of the following pesticides in
various media was determined: DDT, dieldrin
and heptachlor. Stock solutions of each pesti-
cide were made to contain 15 mg per milliliter
in alcohol. The stock solution was diluted with
alcohol so that final concentrations of 1 to 200 µg
per milliliter of medium could be obtained by
adding 2.0 ml or less to flasks containing 100 to
300 ml of sterile medium. Identification of DDT
degradation products was by the same pro-
cedure as that of Wedemeyer (15). The DDT,
DDD, DDE, DBP and DDA standards used in
this study are commercially available in high
purity. The remainder of the metabolites were
synthesized according to the procedures of
Peterson and Robison (13).

**Extraction of residues and metabolites.** After
incubation the samples were extracted using
both the florisil column cleanup method of
Langlois et al (11) and the method of Wede-
meyer (15), except that residues were redis-
solved in hexane rather than in acetone tri
In addition, some of the skimmilk samples were
extracted by the Soxhlet method of Peterson
and Robison (13). Extracted samples analyzed
by paper chromatography were treated by the
acid cleanup procedure of Peterson and Robi-
son (13) to remove interfering substances.

**Analysis for pesticide residues and metab-
olites.** Extracted solutions were assayed for
residues and metabolites by electron capture
gas chromatography, thin layer chromatography
and paper chromatography. A Perkin-Elmer
Model 811 gas chromatograph with a tritium
electron capture detector was used. Borosilicate
glass columns (20 mm od by 600 mm) packed
with these materials were used for assay: a) 5% DC-11 on 60/80 mesh Gas Chrm Q with
column at 190 C and nitrogen flow of 60 ml
per minute; b) 10% DC-200 on 100/120 mesh
Gas Chrm Q with column at 190 C and nitro-
gen flow at 100 ml per minute; c) 11% (OV-17
+ QF-1) on 80/100 mesh Gas Chrm Q with
column at 190 C and nitrogen flow of 120 ml
per minute.

Eastman chromagram sheets and developing
apparatus were used for thin layer chromato-
graphy (1). The method of Mills (4) and the
solvent systems of Wedemeyer (15) were used
for paper chromatography.

**Results and Discussion**

**Degradation of dieldrin.** None of the species
was able to degrade dieldrin. Our results agree
with those of Chacko et al (5). However,
several investigators have reported microbial
degradation of dieldrin (12,16). Differences in
results might be due to variations in methodology
or to differences in strain of species. Two
of the species reported to be capable of de-
grading dieldrin were isolated from dieldrin-
treated soil, and may have become dieldrin-
tolerant (12); whereas species used in our
study were not subjected to such adaptation.
Wedemeyer (16) isolated dieldrin metabolites
from sonically disrupted cells of *Aerobacter
aerogenes*, whereas liquid medium containing
whole cells was analyzed in our study. These
differences suggest that degradation of dieldrin
is intracellular and metabolites are released
only by lysing the cell.

**Degradation of heptachlor.** As with dieldrin
none of the bacteria studied was able to degrade
heptachlor. In addition, compounds making
up some of the impurities in 73% heptachlor
also were nondegradable by the bacteria studied.
These impurities were heptachlor epoxide,
chlorodane and gamma chlordane.

**Degradation of DDT.** Results from degrada-
tion of DDT by whole cells of test bacteria in
four media under aerobic and anaerobic incuba-
tion are in Table 1. Evidently not all species
are capable of degrading DDT, amount of
degradation is affected by growth medium and
amount of degradation is affected by availability
of oxygen during incubation.

Generally DDD and DDE were detected in
various amounts after two days of incubation
with maximum levels being obtained within
seven days. At least 30 days of incubation
were required to detect all of the metabolites
listed in the table.

Whole cells of *P. fluorescens* and *S. aureus*
<table>
<thead>
<tr>
<th>Species</th>
<th>Medium</th>
<th>Incubation</th>
<th>Metabolites detected after incubation</th>
<th>DDT</th>
<th>DDE</th>
<th>DDD</th>
<th>DDMU</th>
<th>DDMS</th>
<th>DDNU</th>
<th>DDOH</th>
<th>DDA</th>
<th>DEP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TSB</strong></td>
<td></td>
<td></td>
<td>A^e B A</td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anaerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TSB</strong></td>
<td></td>
<td></td>
<td>A B A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>SM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TSB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>TSBC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TSBL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Enterobacteria aerogenes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td><strong>TSB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>SM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td><strong>Bacillus coagulans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudomonas fluorescens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a TSB, trypticase soy broth; SM, skim milk; TSBC, trypticase soy broth + 3% whole casein; TSBL, trypticase soy broth + 4.5% lactose.

^b Products detected by one or all of following: electron capture gas chromatography, thin-layer chromatography and paper chromatography.

^c A, major or only product; B, minor product; C, trace product.
were not capable of degrading DDT, to any great extent, during aerobic incubation in TSB for up to 14 days. Wedemeyer (15) found DDT, DDE, DDD, DDMU and DDNU after DDT was incubated anaerobically with cell-free extracts of *P. fluorescens*. He found less conversion of DDT under aerobic conditions. Whole cells of *P. fluorescens* are unable to degrade DDT aerobically but cell-free extracts are able to convert DDT under both aerobic and anaerobic conditions. Apparently a permeability phenomenon exists with whole cells.

Results with whole cells of *E. coli* and *E. aerogenes* agree with those of other workers (10,15). Wedemeyer (15) found that cell-free extracts of *A. aerogenes* degraded DDT anaerobically to DDD, DDE, DDMU, DDNU, but only to DDD and DDE under aerobic conditions. In our study, anaerobic incubation was for 30 days, and DDMS, DDA and DBP were identified in addition to DDD, DDE, DDMU and DDNU. Generally only DDE and DDD were found after 7 days of incubation.

The *Bacillus* degraded DDT aerobically and faster than *E. coli* and *E. aerogenes*. Most of the metabolites in Table 1 were found in varying amounts after incubation for seven days, with all being readily identified after 30 days. To determine if the *Bacillus* followed the same pathway suggested by Wedemeyer for coliform bacteria, each metabolite in the table was individually incubated with whole cells of *B. subtilis* in TSB. Extracts were analyzed after aerobic incubation for 7 and 30 days. Results indicated that DDT was degraded according to the same metabolic pathway suggested by Wedemeyer (15): DDT → DDD → DDMU → DDMS → DDNU → (DDOH) → DDA → DBP and DDT → DDE.

Amount of DDT degradation by *E. coli*, *E. aerogenes* and the *Bacillus* was affected by the kind of growth media. Since all results for these organisms were similar only those obtained for *E. coli* will be discussed.

Typical chromatograms before and after growth of *Escherichia coli* in trypticase soy broth plus 4.5% lactose containing 100 μg per milliliter of DDT. Symbols: before growth (-----), after growth for 7 days (----).

---

**Fig. 1.** Typical chromatograms obtained before and after 7 days of growth of *Escherichia coli* in trypticase soy broth containing 100 μg per milliliter of DDT. Symbols: before growth (-----), after growth for 7 days (----).

**Fig. 2.** Typical chromatograms obtained before and after 7 days of growth of *Escherichia coli* in trypticase soy broth plus 4.5% lactose containing 100 μg per milliliter of DDT. Symbols: before growth (-----), after growth for 7 days (----).
in a significant increase of DDD or DDE. None of the other metabolites in Table 1 was detected after 30 days of incubation. Reduction in amount of degradation probably was due to fermentation of lactose by bacteria which resulted in lowering pH of the medium. Wedemeyer (15) reported that the amount of metabolites formed was a function of the hydrogen ion concentration. Apparently as the pH is lowered, enzyme systems necessary for degradation are inhibited.

Unlike the two aforementioned broth media, very little degradation of DDT occurred in skim milk or in TSB plus 3% whole casein (Fig. 3). Only small amounts of DDD could be detected after seven days of incubation and did not increase significantly after 30 days. Whole casein prevented heptachlor from inhibiting the growth of S. aureus (6). Apparently casein binds the pesticide and makes it unavailable for degradation by the bacteria. Addition of α-, β- and γ-caseins individually or a mixture of α- and β-caseins to TSB had some effect in reducing degradation; however, the reduction was not as great as that observed for whole casein.

Degradation of DDT in milk cannot be expected to occur, since casein appears to complex DDT and prevent degradation. High concentrations of acids resulting from utilization of sugars, also will prevent degradation of DDT. Degradation probably would occur in suitable liquid products without casein.

References