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Biodegradation of Halogenated Organic Compounds

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INTRODUCTION

Man-made chemicals used as refrigerants, fire retardants, paints, solvents, and herbicides and pesticides cause considerable environmental pollution and human health problems as a result of their persistence, toxicity, and transformation into hazardous metabolites. Many environmentally important xenobiotics, introduced for industrial use, are halogenated, and halogenation often is implicated as a reason for persistence (102). Halogenated organics are used as herbicides, plastics, solvents, and degreasers. Chlorinated compounds are the most extensively studied because of the highly publicized problems associated with 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), other pesticides, and numerous industrial solvents (113). Hence, chlorinated compounds serve as the basis for most of the information available on the biotransformations of synthetic compounds. Several excellent reviews have been published (5, 6, 53, 60, 70, 83, 84, 86, 91, 100, 106, 108, 122, 134, 154, 162, 163, 181); two relatively recent ones (28, 126) deal with various aspects of the environmental fate of xenobiotics.

This article is an overview of the current understanding of the physiological and genetic basis of biodegradation of halogenated compounds, specifically the chlorinated hydrocarbons, by isolated aerobic and anaerobic microorganisms. Most of the information available on the biodegradation of chlorinated compounds is on oxidative degradation, since aerobic culture techniques are relatively simple compared with anaerobic culture methods. Also, aerobic processes are considered the most efficient and generally applicable. Recently, anaerobic microbial communities have shown the ability to degrade a variety of groundwater pollutants, including chlorinated aromatic compounds and trichloroethylene (TCE) (17, 18, 28, 37, 38, 54, 64, 82, 89, 94, 95, 101, 116, 126, 142, 145, 147, 156–158, 162, 166, 174). However, most of these bacteria have not been characterized or genetically analyzed for their biochemical properties.

For convenience, the chlorinated hydrocarbons degraded by microorganisms (bacteria and fungi) are grouped into three classes: (i) aliphatic, (ii) polycyclic, and (iii) aromatic. Some of the chlorinated hydrocarbons and the microorganisms capable of degrading them are listed in Tables 1 to 3. These tables list chlorinated compounds that are degraded by some soil and aquatic microorganisms. They also illustrate the wide variety of microorganisms that participate in environmentally significant biodegradation reactions. An understanding of the biochemistry and genetics of the degradation of chlorinated hydrocarbons will allow characterization of the appropriate genes necessary to construct improved strains with enhanced degradation ability. Most of the biodegradations of chlorinated hydrocarbons are plasmid mediated, such as the 3-chlorobenzoate (3CBA)-degrading plasmid pAC25 (23) and the 2,4-dichlorophenoxyacetate (2,4-D)-degrading plasmids pJP4 and pRC10 (27, 32). The role of plasmids in the biodegradation of these compounds and the construction of new strains with novel biodegradative pathways will be discussed.

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TABLE 1. Biodegradation of chlorinated aliphatic compounds

Compound	Microorganism	Plasmid[a]	Reference
2-Monochloropropionic acid	*Pseudomonas* sp.	–	56
2-Monochloroacetic acid	*Alcaligenes* sp.	pUU204 (53 kb)	
1,2-dichloroethane	Anaerobes	–	17
	Xanthobacter autotrophicus (GJ10)	–	66
	Methane-utilizing bacteria	–	184
	P. fluorescens	–	171
1,1,1-TCE	Anaerobes	–	17
	Methanotrophs		174
Perchloroethane	*Methanosarcina* sp.	–	38
1-Chlorobutane	Aerobes and anaerobes	–	183
1,2-Dichloropropane	*P. fluorescens*	–	171
1,3-Dichloropropane	Aerobes	–	184
1,9-Dichlorononane	Anaerobes	–	184
TCE	Anaerobes	–	17
	Anaerobes	–	82
	Aerobes	–	179
	Aerobes	–	103
	Methane-utilizing bacteria	–	40
	P. putida	–	104
	Methane-oxidizing bacteria	–	89
	P. fluorescens	–	171
3-Chloro-4-hydroxybenzaldehyde	Anaerobes	–	101
Chlorolignin	*Sporotrichum pulverulentum*	–	36
Monochloro-, bromo-, iodoalkanes	*Arthrobacter* sp.	–	138

[a] –, No plasmid was detected.

HALOGENATED ALIPHATIC HYDROCARBONS

Halogenated aliphatic compounds are prevalent groundwater contaminants and are significant components of hazardous wastes and landfill leachates. Many hazardous halogenated aliphatic compounds released from industrial, commercial, and agricultural sources are chlorinated or brominated alkanes and alkenes that contain one to three carbon atoms, such as halogenated alkanoic acids (HAA), haloalkanes, TCE, trichloroethane (TCA), and ethylene dibromide (EDB). Chlorinated ethanes and ethers have been commonly used as refrigerants in manufacturing, as solvents in the dry-cleaning (metal and plastic) and lacquer industries, and in semiconductor manufacturing. Their apparent hazard to human health has prompted investigations concerning their fate in subsurface waters and in the soil environment (17, 38, 40, 56–58, 66, 82, 89, 90, 103, 104, 107, 171, 174, 179). Although abiotic transformations can be significant within the time scales commonly associated with groundwater movement, the biotic processes typically proceed much faster, provided that there are sufficient substrates, nutrients, and microbial populations to mediate such transformations.

The transformations of some chlorinated aliphatic compounds by soil and aquatic microorganisms are listed in Table 1. Vogel and McCarty (174) reported the degradation of TCA under abiotic and biotic conditions. Abiotic degradation of TCA can result in a mixture of 1,1-dichloroethylene and acetic acid, whereas the biotransformation of TCA results in 1,1-dichloroethane and chloroethane.

The degradation of halogenated alkanes such as 1-chlorobutane, 1,3-dichloropropane, and 1,9-dichlorononane has been studied by Yokota et al. (184). Several bacterial strains including methane-utilizing bacteria capable of utilizing haloalkanes were isolated. The microbial dehalogenation of haloalkanes by these strains is mediated by oxygenase and hydrolase. Scholtz et al. (138) reported a soil isolate, *Arthrobacter* sp. strain HA1, that can utilize at least 18 1-chloro, 1-bromo-, and 1-iodoalkanes, but not 1-fluoroalkane, as the sole source of carbon and energy. There is no information concerning the metabolic pathway or the genetic basis of degradation of these monohalogenated alkanes.

With the exception of 2-monochloropropionic acid and monochloroacetic acid (haloalkanoic acids), detailed studies of the biochemical and genetic basis for the biodegradations of chlorinated aliphatic compounds are lacking. Although detailed genetic information on these microorganisms is not available, the degradation of HAA and TCE serves as a good example of recent studies on the microbial metabolism of chlorinated aliphatic hydrocarbons.

TABLE 2. Biodegradation of chlorinated polycyclic compounds

Compound	Microorganism	Plasmid[a]	Reference
DDT	Mixed cultures	−	155
	Phanerochaete chrysosporium	−	21
Atrazine	*Pseudomonas* sp.	−	14
4-Chlorobiphenyl	*Acinetobacter* sp.	pKF1	44
	Achromobacter sp.	16 and 72 kb	93
	Bacillus brevis	−	
	Alcaligenes sp.	+	134
	Pseudomonas sp.	−	159
	Acinetobacter sp.	pSS50 (35 kb)	144
	Alcaligenes sp.	pSS50 (35 kb)	144
	A. eutrophus H850	−	13
	Pseudomonas sp.	−	12
	P. cruciviae	−	161
1,4-Dichlorobiphenyl	*K. pneumoniae*	pAC21	25
3-Chloro-4-hydroxybiphenyl	*P. cruciviae*	−	161
1- and 2-chloronaphthalene	*Pseudomonas* sp.	−	98
PCB	*Aspergillus niger*	−	31
	P. cruciviae	−	161

[a] +, Existence of plasmid(s); −, no plasmid was detected.

Halogenated Alkanoic Acids

A number of soil microorganisms which synthesize dehalogenase are capable of utilizing HAA (56–58). Hardman et al. (56) examined four *Pseudomonas* and two *Alcaligenes* species capable of growth on 2-monochloropropionic acid and monochloroacetic acid. They found that all isolates contained a single plasmid with a molecular size of 53 kb (pUU204) or more. Curing of the four *Pseudomonas* species with ethidium bromide resulted in the concomitant loss of the plasmids and dehalogenase activities and demonstrated that the gene was plasmid encoded. However, attempts to transfer the plasmid either to new host strains or to cured strains of the donor strain failed. These strains can express up to four different dehalogenases; this represents one of the first demonstrations of isoenzyme gene multiplication on plasmids (57). Unlike the chlorinated phenoxyacetate plasmids (pJP1 to pJP6), the HAA plasmids exhibit considerably variety when the restriction endonuclease patterns are compared (32). This observation indicates a high degree of gene mobility, not only between strains but also between plasmids.

Trichloroethylene

Chlorinated ethenes such as vinyl chloride, TCE, and tetrachloroethylene (perchloroethylene) have been frequently detected in drinking-water aquifers (90). TCE is one of the major industrial solvents used for degreasing and cleaning metals and electronic components. These compounds are persistent in the environment and are transported rapidly in groundwater. Their presence in the drinking-water aquifers is of public concern because of their toxicity and/or carcinogenicity (67, 100). Identification of conditions which favor the biodegradation of chlorinated ethenes could contribute significantly to efforts to restore contaminated aquifers.

Currently, there is much concern regarding the microbial metabolism of TCE. Bouwer and McCarty (17) demonstrated that under anaerobic conditions in the laboratory, TCE and perchloroethylene could be degraded during vigorous methanogenesis supported by growth on acetate. Similar transformations were obtained by Parsons et al. (107), who found that perchloroethylene was reductively dechlorinated to TCE, dichloroethylene, and vinyl chloride in Florida muck and subsurface water microcosms. Whether TCE is similarly biotransformed was not investigated but is implicated by their study. Kleopfer et al. (82) demonstrated that reductive dechlorination of TCE to 1,2-dichloroethylene occurred in soil. However, the anaerobic degradation of TCE was very slow.

TCE can be metabolized under aerobic conditions as well. The report by Wilson and Wilson (179) on aerobic TCE metabolism showed that TCE was mineralized when soil microflora were exposed to natural gas in air, implicating methanotrophs in the degradation of TCE (167). Similar results were obtained by Fogel et al. (40). They used a liquid medium containing methane-utilizing bacteria and showed that TCE was converted into CO_2. The observation that biodegradation of TCE was inhibited by acetylene, a specific inhibitor of methane oxidation by methanotrophs, supported the hypothesis that a methanotroph was responsible for the TCE biodegradation. Under methanogenic conditions both tetrachloroethylene and TCE wee converted to ethylene (41).

Recently an aerobic, methane-oxidizing bacterium that degrades TCE in pure culture was isolated. TCE biodegradation by this bacterium appeared to be a cometabolic process (89). Another aerobic microorganism degraded TCE in the presence of phenol (104). The studies of Nelson et al. (103, 104) implicate a toluene dioxygenase in TCE metabolism. This result has been supported by studies showing that recombinant *Escherichia coli* containing toluene dioxygenase genes degraded TCE (180, 191).

TABLE 3. Biodegradation of chlorinated aromatic compounds

Compound	Microorganism	Plasmid[a]	Reference
1,2DCB	*Pseudomonas* sp.	−	55
1,3DCB	*Alcaligenes* sp.	−	30
1,4DCB	*Alcaligenes* sp.	−	139
	Pseudomonas sp.	−	148
	P. putida	+	105
	Alcaligenes sp.	−	
2,6-Dichlorotoluene	*P. cepacia* HCV	+	172
2CBA	*P. cepacia*	−	188
3CBA	*A. eutrophus*	pJP4	22
	Acinetobacter calcoaceticus	−	188
	Pseudomonas sp. strain B13	pAC27	33
	P. putida	pAC27 (110 kb)	51
	Flavobacterium sp.	pRC10 (45 kb)	27
4CBA	*Arthrobacter* sp.	−	91
	Pseudomonas sp. strain CBS3	−	132
	A. denitrificans NTB-1	−	169
	Corynebacterium sepedonicum	−	189
3,5DCBA	*Pseudomonas* sp.	−	61
	Pseudomonas sp.	pAC31	23
	Anaerobes	−	64
2,4DCBA	*Corynebacterium sepedonicum*	−	189
	A. denitrificans NTB-1	−	168
4-Chloro-2-nitrophenol	*Pseudomonas* sp.	from pJP4	20
2,4,5-T	*P. cepacia* AC1100	−	71
4-Chlorophenol	*Pseudomonas* sp.	−	137
	Alcaligenes sp.	+	
2-, 3-, and 4-chloro- and 2,4-dichlorophenol	Anaerobes	−	18
	Alcaligenes sp.	−	140
PCP	*Arthrobacter* sp.	−	149
		−	120
	Aerobes	−	97
	P. cepacia AC1100	−	71
	Anaerobes	−	54
	Flavobacterium sp.	80 to 100 kb	28
	Anaerobes	−	94
	Flavobacterium sp.	+	130
	Rhodococcus chlorophenolicus	−	8
	Flavobacterium sp.	−	19
	Anaerobes	−	95
	Flavobacterium sp.	−	152
3-, 4-, and 5-Chlorosalicylate	*Pseudomonas* sp.	+	129
3,4-Dichloroaniline	*P. putida*	−	185
2-, 3-, and 4-Chloroaniline	*Pseudomonas* sp.	+	87
4-Chlorophenylacetate	*Pseudomonas* sp. strain CBS3	−	81
	Pseudomonas sp. strain CBS3	−	92
α-Hexachlorocyclohexane	Aerobes	−	10
2,4-D	*A. eutrophus*	+	110
	A. eutrophus	pJP1-6	109
	Pseudomonas sp.	−	79

Continued on following page

TABLE 3—*Continued*

Compound	Microorganism	Plasmid[a]	Reference
	Pseudomonas sp.	+	115
	A. eutrophus	pJP1-6	32
	Pseudomonas sp.	+	117
	Aspergillus niger	−	141
	Aerobes	−	178
	A. eutrophus	pJP4 (80 kb)	33
	Alcaligenes sp.	pEML159	7
	Pseudomonas sp.	−	
	Anaerobes	−	94
	Aerobes	−	146
	Arthrobacter sp.	−	131
	Flavobacterium sp.	pRC10	27
MCPA	*Pseudomonas* sp.	−	79
	A. eutrophus	pJP4	32
	Flavobacterium sp.	pRC10	27
2,4,5-T	*P. cepacia*	−	78
	Pseudomonas sp.	−	70
	Anaerobes	−	158
	Anaerobes	−	94
	P. cepacia	+	52
Chlorotoluene	*P. cepacia*	+	116

[a] +, Existence of plasmid(s); −, no plasmid was detected.

The ability to degrade TCE may be a general characteristic of methanotrophic bacteria since several researchers have observed biodegradation of TCE in methane-enriched mixed cultures from a variety of environments (40, 89, 104, 179). These reactions may prove to be important as a potential treatment of TCE-contaminated sites via stimulation of methanotrophic bacteria. However, information on the genetic analysis of these TCE-utilizing microorganisms, especially the methanotrophs, is not available. TCE has also shown to be metabolized by aromatic compound-degrading (59, 103, 145) and ammonia-oxidizing (9, 164) bacteria. The metabolic pathway for the aerobic and anaerobic degradation of TCE is shown in Fig. 1.

Ethylene Dibromide (EDB)

EDB is a brominated hydrocarbon that has been used as a soil fumigant and in anti-knock gasoline. It has been widely investigated over the last few decades because of numerous environmental contamination problems caused by its use. Pignatello (119) has reported the microbial degradation of EDB in aquatic environments. However, no information is available on the microorganisms and their genetic traits involved in EDB transformation.

In conclusion, the fate of chlorinated aliphatic hydrocarbons in the environment is dependent on their particular chemical properties. Potential chemical and biological transformations occurring under given environmental conditions are controlled by the number and position(s) of chlorinated substituents. A general trend with regard to the degradation of chlorinated aliphatic hydrocarbons is that the more chlorinated the aliphatic compound, the higher the relative rate of reduction, and the less chlorinated the compound, the higher the rate of oxidation (173). Although the chemical structures of the compounds and abiotic reactions play a role in the removal of certain chlorinated hydrocarbons, biodegradation by microorganisms is of primary importance. An understanding of the pathways and products of degradation of chlorinated aliphatic hydrocarbons by these microorganisms can be useful in evaluating contamination patterns and in selecting the most appropriate remediation procedures. Genetic and molecular information on the biodegradation of these compounds by soil and aquatic microorganisms allows the construction, by recombinant DNA technology, of microorganisms which have better degradative capabilities than do natural populations of microorganisms.

CHLORINATED POLYCYCLIC HYDROCARBONS

Polycyclic aromatic hydrocarbons occur as natural constituents and combustion products of fossil fuels and are widespread environmental contaminants (63). There are several reports concerning the biodegradation of chlorinated polycyclic hydrocarbons (12–14, 21, 22, 44, 85, 93, 98, 134, 143, 144, 155, 159, 161). Of these, DDT polychlorinated biphenyls (PCBs), and *p*-chlorobiphenyls (p-CBs) are of great interest because of their widespread occurrence in the environment. Although DDT is a persistent environmental pollutant, it does appear to undergo slow degradation. Bumpus and Aust (21) isolated a lignin-degrading fungus, *Phanerochaete chrysosporium*, which is capable of utilizing DDT as a nitrogen source. The major metabolic pathway of DDT by this fungus is an oxidation and dechlorination process; ultimately DDT is degraded into carbon dioxide. Other polycyclic chlorinated compounds such as PCBs and p-CBs are used as examples of the microbial degradation of chlorinated polycyclic hydrocarbons.

p-Chlorobiphenyls

It has been reported that p-CBs and PCBs are subject to biodegradation (12, 13, 22, 44, 93, 134, 144, 159, 161). Furukawa and Chakrabarty (44) isolated an *Acinetobacter* sp. capable of utilizing p-CB. They showed that the degradation of this compound is encoded on an 82-kb plasmid (pKF1). Since then, several p-CB-degrading plasmids, such

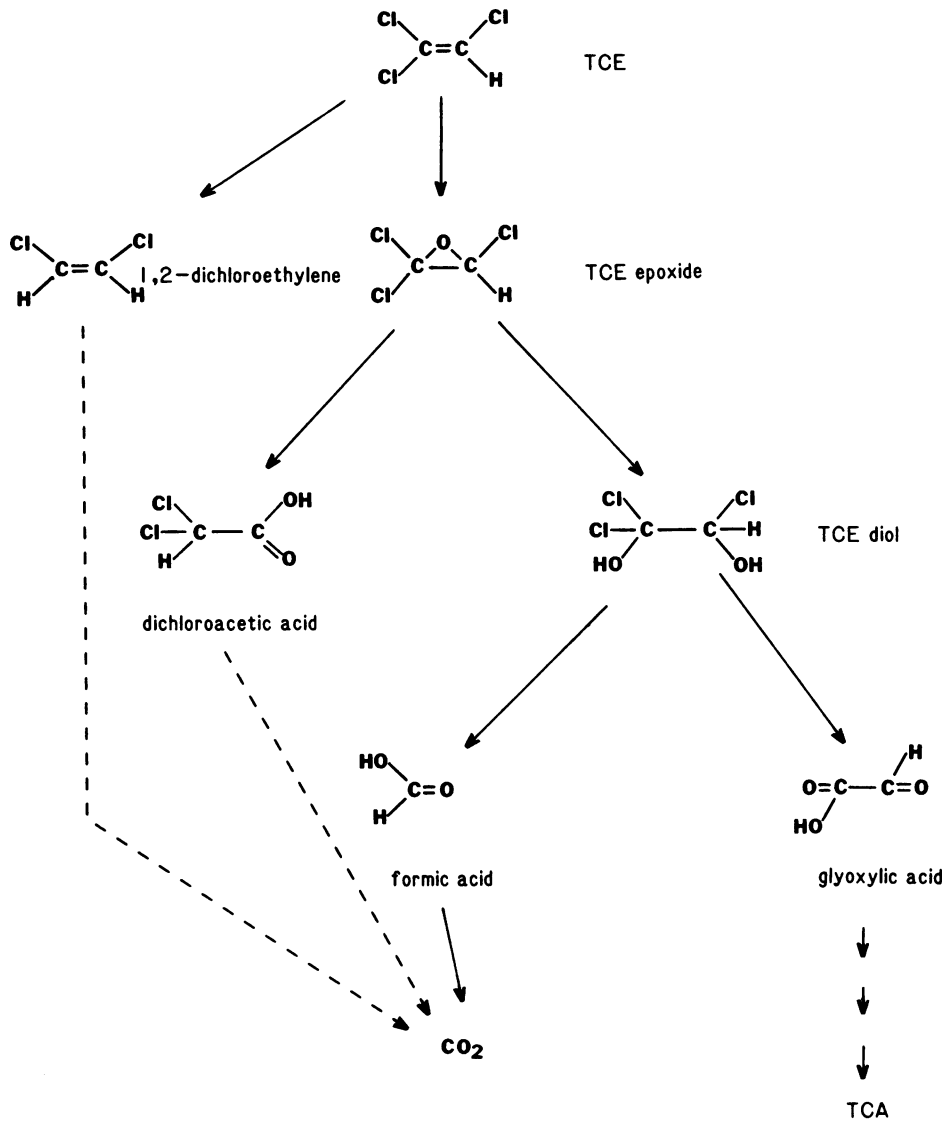

FIG. 1. Proposed pathways for the microbial degradation of TCE.

as pSS50 (53 kb) from *Alcaligenes* sp., have been isolated (93, 134, 144); however, there is limited information about these plasmids.

Another chlorobiphenyl-degrading plasmid carries genes that code for the enzymes necessary to catabolize p-CB (22). This plasmid, pAC21, is about 65 MDa in size and was isolated from strains of *Klebsiella pneumoniae*. Plasmid pAC21 was cured with mitomycin C. Since it is a conjugative plasmid, it was then transferred into *E. coli* by conjugation. Although it is unstable in *E. coli*, it allows a low level of expression of p-CB degradation in transconjugants. Masse et al. (93) reported two isolates, an *Achromobacter* sp. and a *Bacillus brevis* strain, which were able to utilize p-CB. Both strains generated the same metabolites, with 4-chlorobenzoate (4CBA) as the major metabolic product. A large (72-kb) and a smaller (16-kb) plasmid were isolated from *Achromobacter* sp.; however, the role of these plasmids in the degradation of p-CB is unknown.

Since 4CBA is the major product of the degradation of p-CB, Sylvestre et al. (159) demonstrated a more rapid and complete degradation of p-CB by a two-membered bacterial culture. One strain was able to grow on p-CB and transform it into 4CBA, and the other strain degraded 4CBA. Similarly, complete degradation of 4,4-dichlorobiphenyl by the coculture of two *Acinetobacter* strains, which separately degraded biphenyl and 4CBA, was reported by Adriaens et al. (1). The metabolic pathway for the bacterial degradation of p-CBs is shown in Fig. 2. The p-CB is converted into 4CBA before the dehalogenation occurs.

Barton and Crawford (12) isolated a *Pseudomonas* sp. that is capable of utilizing p-CB as the sole carbon and energy sources. The metabolic degradation of p-CB by this bacterium is via a different route, and 4-chloroacetophenone may be an end product. No genetic information about this *Pseudomonas* sp. is available.

Polychlorinated Biphenyls

PCB has 209 possible isomers in theory, but about 100 exist in commercial formulations. These formulations are

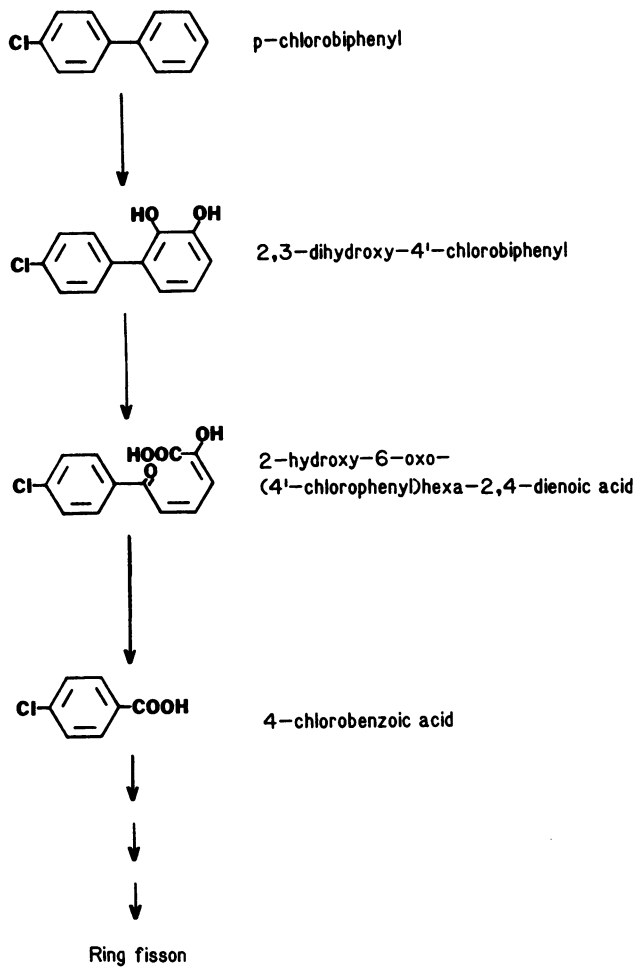

FIG. 2. Proposed pathways for the microbial degradation of p-CB.

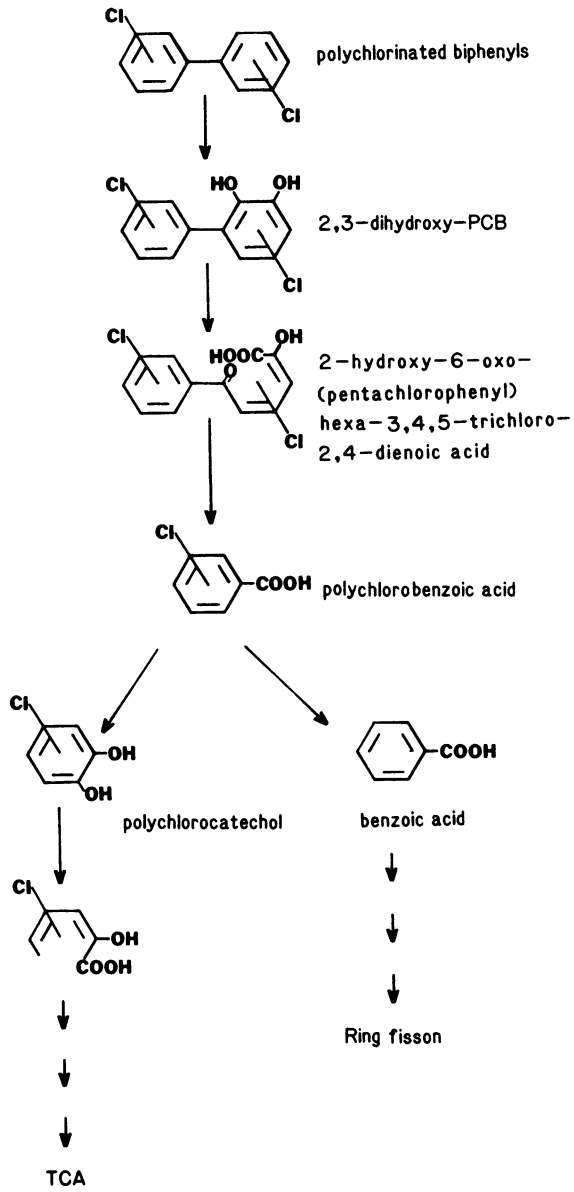

FIG. 3. Proposed pathways for the microbial degradation of PCBs.

nearly water insoluble, nonpolar, lipid soluble, and inert. They are ideal fluids for use in electronic equipment and are useful constituents of insulators, lubricating oils, herbicides, medicines, and antimicrobial agents. Because of their toxicity to animals and humans (67, 100), their use has been prohibited. However, the environment is still burdened as a result of their past use. PCBs with higher chlorination tend to be more environmentally persistent. Several reports showed the effect of chlorine substitution on the biodegradability of various PCB isomers (48). Both aerobic and facultative anaerobic bacteria capable of utilizing PCBs have been isolated from the environment (2, 11, 74, 161, 182). Takase et al. (161) isolated a *Pseudomonas cruciviae* strain that could grow on more than 10 biphenyl-related compounds including p-CB. They demonstrated that biphenyl ether was degraded through an *ortho* cleavage pathway and that biphenyl was degraded through a *meta* cleavage pathway. Several reports showed cometabolism of various PCBs by biphenyl-degrading bacteria (2, 11, 15, 16, 45, 46). Dmochewitz et al. (31) reported an *Aspergillus niger* that was capable of utilizing mixtures of PCBs, and Eaton (35) showed mineralization of PCBs by a ligninolytic fungus.

Physiologic and genetic studies have shown that the genes for the degradation of PCBs may be plasmid encoded (44, 144) or present on the chromosome (43, 47, 74–76, 121, 124). Studies are now focused on the organization, cloning, and characterization of the genes encoding PCB degradation (62, 76). Like the genes involved in the degradation of halogenated aromatic compounds, the genes encoding PCB catabolism are clustered in an operon, *bphABCD*.

The degradation of PCB is through ring cleavage and dehalogenation. Figure 3 illustrates a complete aerobic and anaerobic pathway for PCB degradation. This pathway is based on the detected accumulated metabolites and on the known pathway for biphenyl.

Other Polycyclic Compounds

Several naphthalene-degrading *Pseudomonas* spp. were able to metabolize monochloronaphthalene, but the chloronaphthalene did not support growth (98). This is one example of cometabolism. The cometabolism of monochloronaph-

thalene is dependent on the normal enzymes of naphthalene metabolism, which are induced during the cometabolism.

Two to six plasmids ranging in molecular mass from 8 to 300 MDa were isolated from strains of soil bacteria that are shown to degrade chloridazon (5-amino-4-chloro-2-phenyl-3-pyridazinone) and an analog, antipyrine (2,3-dimethyl-1-phenylpyrazolone) (85). Detailed studies of the genetic basis of biodegradation of these compounds are not presently available. At least 19 different strains were isolated with the capacity to grow on these compounds, indicating the transfer of p-CB- and PCB-degrading plasmids in natural mixed populations.

CHLORINATED AROMATIC COMPOUNDS

Chlorinated aromatic compounds are major environmental pollutants because they are often released in substantial quantities, are toxic and resistant to degradation, and accumulate in sediment and biota. Although some compounds are degraded only slowly by soil and aquatic microorganisms, others are metabolized relatively quickly. There have been many studies on the ability of soil and aquatic microorganisms to dissimilate chlorinated aromatic hydrocarbons such as chlorotoluene (116, 118), chlorobenzenes (30, 55, 105, 125, 139, 148), chlorobenzoates (22–25, 51, 61, 64, 69, 77, 91, 126, 130, 141, 142, 160, 168, 169, 177, 187–189), chlorophenols (8, 18–20, 28, 54, 65, 95, 97, 99, 120, 130, 137, 139, 149–152, 170), chloroacetamide (133), 4-chlorophenylacetate (81, 92), and chlorophenoxyacetates (27, 32–34, 42, 70, 71, 77–79, 96, 110, 114, 115, 117, 146, 153, 158) (Table 3).

Microorganisms are challenged to develop new pathways by altering their own preexisting genetic information as a result of either mutation(s) in single structural and/or regulatory genes or perhaps recruitment of single silent genes when they encounter the foreign compounds (123). However, one should recognize that it may take microorganisms a long time to acquire the ability to degrade all the new synthetic chemicals introduced into the environment by modern technology. If a synthetic chemical is biodegradable in a reasonable time frame, that compound is unlikely to pose a threat to public health. Nevertheless, although microorganisms can adapt to remove many toxic substances, the great variety of xenobiotics used today may disrupt the balance of the ecosystem. In the future it will be necessary to develop microbial systems that can speed the evolution of degradative traits. The strategy to develop an accelerated evolution of pathways is the rationale for the restructuring of existing pathways by using techniques of genetic manipulation, including recombinant DNA techniques (123, 127), or by continuous culture in a chemostat (44). The advantage of experimental evolution of pathways is that laboratory selection conditions can be custom designed for each biotransformation. In this fashion the evolutionary process can be considerably accelerated (127). In the following section, the degradation of some aromatic compounds will be discussed and the genetic basis of biodegradation of 2,4-D will be used as a model system to study the construction of strains with broader biodegradative potential.

Pentachlorophenol

The chlorinated phenols used as wood preservatives, herbicides, fungicides, and general biocides are a large group of toxic xenobiotics that are serious environmental pollutants. In 1983, worldwide production of pentachlorophenol (PCP) was estimated at 5×10^7 kg (29). The toxicity of chlorinated phenols tends to increase with their degree of chlorination, and because few microorganisms can decompose them, the more highly chlorinated phenols tend to accumulate in the environment. Microorganisms such as *Arthrobacter* sp. (149), *Pseudomonas cepacia* (71), and *Flavobacterium* sp. (19, 28, 130, 150–152) can degrade some, but not all, of the chlorinated phenols. The *Flavobacterium* sp. isolated from PCP-contaminated dump sites was characterized in extensive studies (19, 130, 151, 152, 165). An 80- to 100-kb plasmid was found in the PCP-degrading *Flavobacterium* sp. However, analysis the role of this plasmid in PCP catabolism and its genetic makeup is not complete. The initial steps in the catabolism of PCP by the *Flavobacterium* sp. are conversion of PCP to tetrachloro-*p*-hydroquinone and then to trichloroquinone and dichlorohydroquinone (150). Under anaerobic conditions, however, the PCP is degraded into tri-, di-, and monochlorophenol. The benzene ring is then broken to produce methane and carbon dioxide (Fig. 4). Recently, cell extracts from an *Arthrobacter* sp. have been shown to dehalogenate PCP (136).

Reductive dechlorination of PCP has been observed in flooded soils. Early studies (65, 99) suggest reductive dechlorination as a degradative pathway for PCP. The fate of PCP in anaerobic sewage sludges has also been studied (54). Actinomycetes and fungal organisms have also been found to metabolize PCP (8, 96). However, little is known about the microorganisms which are responsible for the anaerobic degradation of PCP. Attempts have been made to degrade PCP in contaminated water by using immobilized cells (106). More research is needed to develop feasible bioreclamation methods.

Dichlorobenzene

The extensive use of chlorobenzenes as solvents, fumigants, and intermediates in the production of pesticides and dyes has led to their widespread release into the environment. There is little information about the role of microorganisms in the elimination of such halogenated compounds. Recently, bacteria such as *Alcaligenes* spp. and *Pseudomonas* spp. that utilize chlorobenzene (122, 125), 1,2-dichlorobenzene (o-DCB) (55), 1,3-dichlorobenzene (m-DCB) (30), and 1,4-dichlorobenzene (p-DCB) (105, 138, 148) as the sole source of carbon have been isolated or constructed. However, the genetic basis of degradation of chlorobenzenes in these microorganisms has not been described. Dechlorination of chlorobenzenes has been shown to occur under anaerobic conditions as well. Sewage sludge completely transformed 190 μM hexachlorobenzene to DCB (39). Further mineralization of dichlorobenzene may require anaerobic conditions. The metabolic pathway for the biodegradation of o-DCB (148), m-DCB (30), and p-DCB (105) proposes that they all form a common intermediate, dichlorocatechol, and then the benzene ring is broken (Fig. 5).

Chlorobenzoates

The degradation of chlorobenzoates by soil microorganisms and the plasmids involved have been studied (23, 27, 34, 52, 61, 64, 91, 122–126, 132, 168, 169, 187–189). A metabolic pathway for degradation of 3CBA by *Pseudomonas* sp. strain B13 is shown (Fig. 6) and the enzymes responsible for each step have been identified (123). Zaitsev and Baskunov (187) reported a similar scheme for the metabolism of 3CBA by *Acinetobacter calcoaceticus*. Chatterjee and Chakrabarty (23) reported that plasmid pAC25

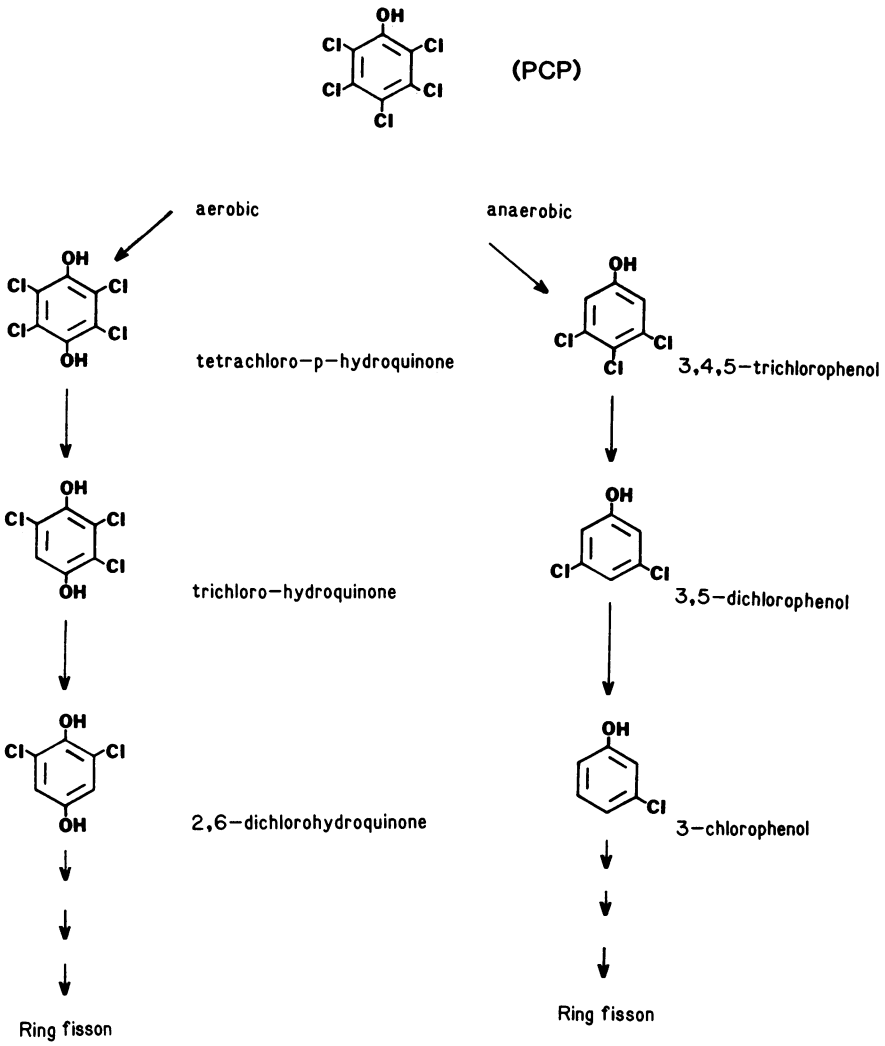

FIG. 4. Proposed pathways for the microbial degradation of PCP.

(and, later, pAC27 and pAC31) from *P. putida* specifies the biodegradation of 3CBA. They rearranged pAC25 and a portion of TOL plasmids and enabled the host cells to utilize 4CBA and 3,5-dichlorobenzoate (3,5DCBA). The metabolic pathways for the degradation of these two compounds by the constructed strains are shown in Fig. 7. Reineke and Knackmuss (124) reported the isolation of *Pseudomonas* sp. strain B13, which capable of utilizing 3CBA; a plasmid, pWR1, was isolated from this strain. Plasmids pAC25 and pWR1 were found to be closely related by hybridization studies (23). The plasmid from *Pseudomonas* sp. strain B13 (pWR1) is 6 kb shorter than pAC25. The pathway for the degradation of the other chlorobenzoates, such as 4CBA and 2,4DCBA, by *Corynebacterium sepedonicum* is also shown in Fig. 7 (189). The same scheme was obtained for *Alcaligenes denitrificans* NTB-1 (168).

Chlorobenzoates can also be biodegraded under reductive conditions. However, little information is available concerning the microorganisms capable of reductive degradation of chlorobenzoates or the role of plasmids in the utilization of these compounds.

Several chlorophenoxyacetate-degradative plasmids not only code for the degradation of phenoxyacetates, but also code for the degradation of chlorobenzoates (27, 32). As expected, these plasmids (pJP4 and pRC10) show genetic homology with pAC25 (pAC27) and pWR1 (27, 49–51).

Chlorophenoxyacetates

Chlorinated derivatives of phenoxyacetates, such as 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), have been released into the environment as herbicides over the past 40 years. Unlike many of the recalcitrant synthetic compounds, 2,4-D is rapidly degraded by soil microorganisms (27, 32, 79, 94, 110, 115, 140, 146, 178). Those that have been most thoroughly studied are strains identified as *Alcaligenes eutrophus* (32). In these strains the genes which coded for the 2,4-D degradation (*tfd*) are plasmid borne, and plasmid pJP4 from *A. eutrophus* JMP134 is one of the most extensively investigated (33, 34, 49, 50, 51, 163). It is an 80-kb, broad-host-range, P1 incompatibility group plasmid; several restriction maps for this plasmid, and similar plasmids, have been published (33, 34, 52). Plasmid pJP4 carries genes essential for the degradation of 3CBA and expression of mercury resistance, as well as the degradation of 2,4-D (32). Several genes coding for the 2,4-D metabolic pathway have

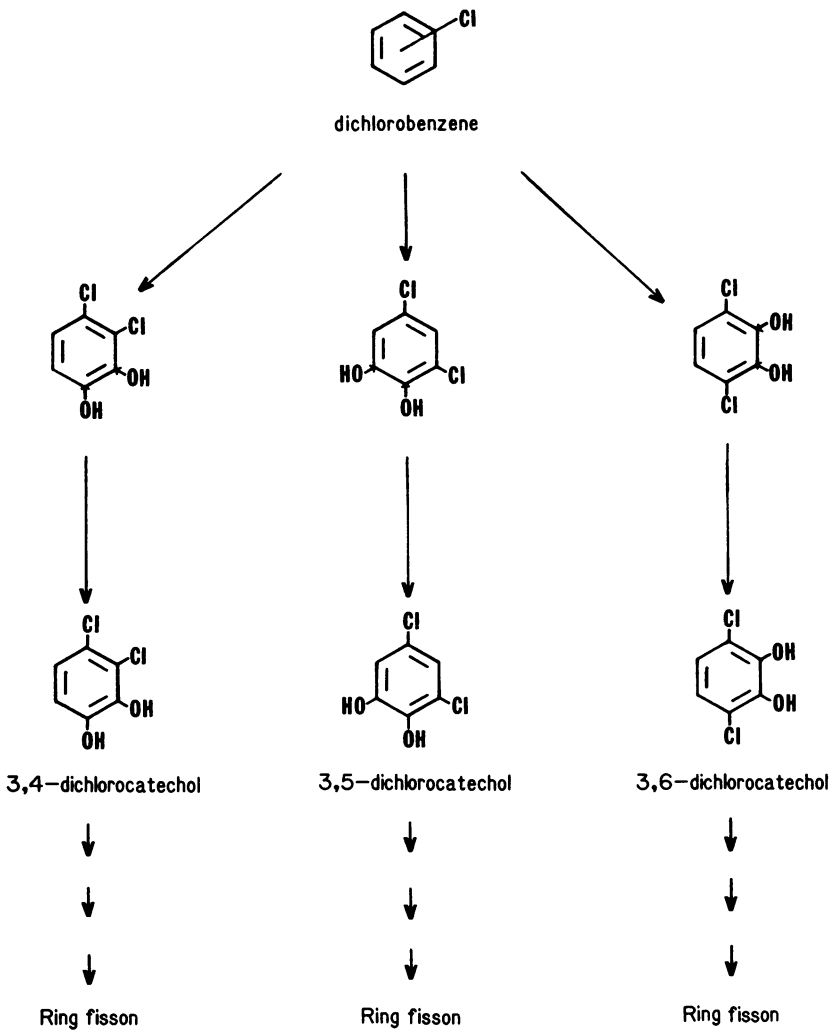

FIG. 5. Proposed pathways for the microbial degradation of DCB.

been mapped by transposon mutagenesis (34). Don and Pemberton (33) reported that a plasmid containing the *Eco*RI B fragment transferred into a cured strain of *A. eutrophus* JMP134 (JMP228) enabled the strain to grow on 2,4-D. Amy et al. (7) cloned a presumptive 2,4-D monooxygenase (encoded by *tfdA*) by demonstrating the release of radiolabeled CO_2 from culture grown with 2,4-D labeled on the alkyl side chain. Recently, the *tfdA* gene has been cloned as a 2-kb subfragment of the *Hin*dIII B fragment of pJP4 (153).

Several other microorganisms, such as *Acinetobacter*, *Arthrobacter*, *Corynebacterium*, *Flavobacterium*, and *Pseudomonas* spp., have also been shown to degrade 2,4-D and related phenoxyacetates. Unlike the situation for *Alcaligenes* spp., little information is available on the mechanism of 2,4-D degradation by these microorganisms. Furthermore, the occurrence and nature of the 2,4-D-degradative plasmids in these microorganisms are not known.

Chaudhry and Huang (27) isolated a new 2,4-D-degradative plasmid, pRC10, from a *Flavobacterium* sp. This plasmid shows considerable differences in size and restriction patterns from pJP4. It is a 45-kb plasmid, carries genes essential for the degradation of 3CBA and 2-methyl-4-chlorophenoxyacetate (MCPA), imparts resistance to mercury, and encodes the utilization of 2,4-D. Comparison with plasmid pJP4 showed strong homology with the regions containing 2,4-D-degradative genes; the first two genes responsible for the 2,4-D degradation, *tfdA* and *tfdB*, have been cloned as a subfragment of the *Eco*RI A fragment of pRC10. Expression of pRC10 in *P. putida* (Nld^r) and *A. eutrophus* JMP228 showed the cloned fragment coding for *tfdA* and *tfdB*. However, the expression of pRC10 in *E. coli* conferred on the cells only the resistance to mercury. Chaudhry and Huang (27) also proposed the first few steps of degradation of 2,4-D (Fig. 8) and indicated the genes coding for the enzymes.

More recent studies have focused on the organization, cloning, and characterization of the *tfd* genes, particularly on the plasmid pJP4 (23, 27, 33, 34, 60, 111, 112, 114, 151). Like TOL and NAH plasmids (135, 186), the expression of *tfd* genes also has been found to be regulated by regulatory elements. It has been shown that *tfdR* regulates the expression of *tfdA* and the operon *tfdCDEF* but not *tfdB* (68). The promoters for *tfdA* and the operon are homologous.

The degradation of another commonly used herbicide, 2,4,5-T, has been less extensively investigated, and most of the information concerning the 2,4,5-T degradation has been gathered by using reductive (anaerobic) sediments (52, 70, 78, 94, 158). As expected, degradation is slow and the

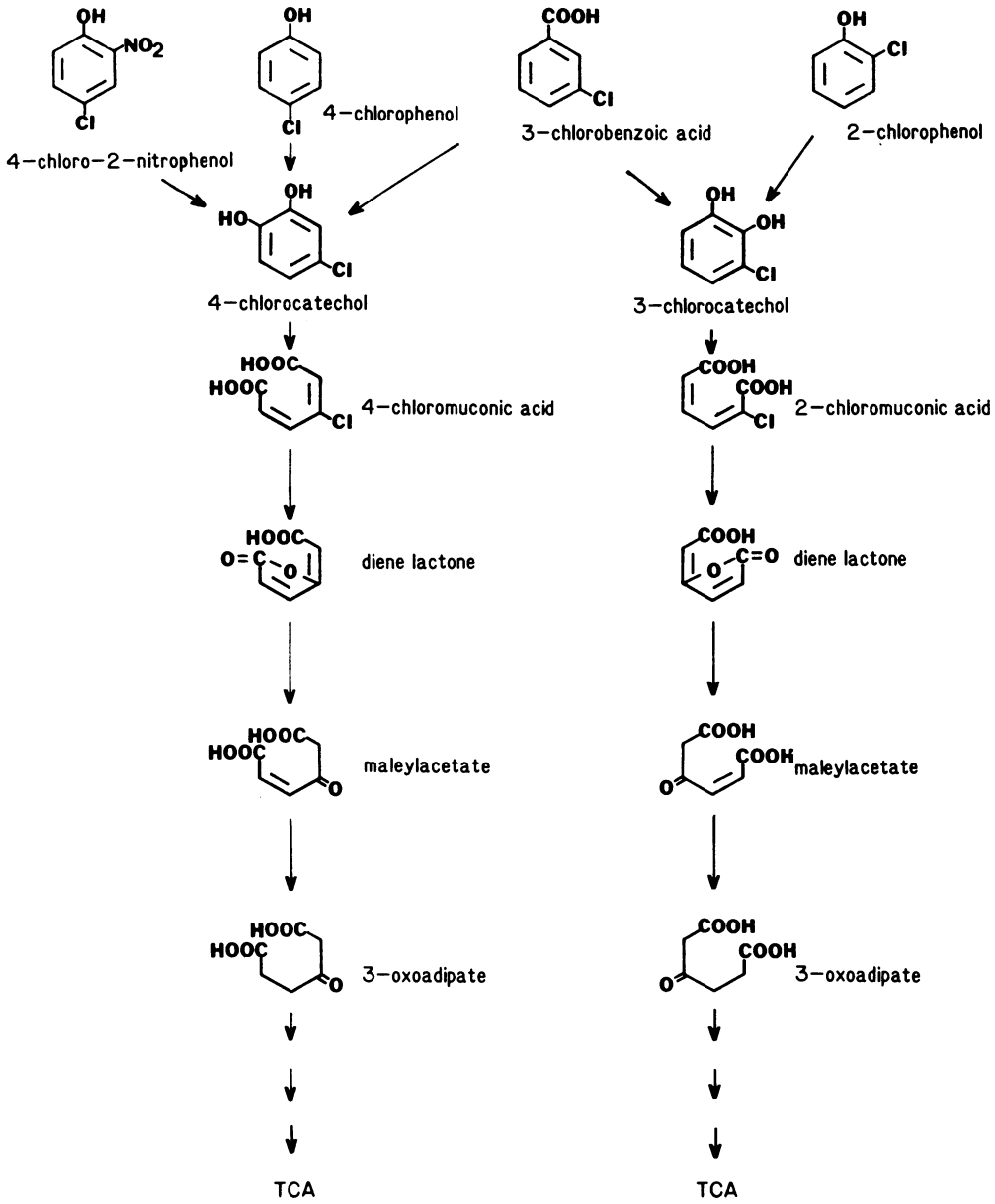

FIG. 6. Proposed pathways for the microbial degradation of 3CBA and chlorophenols.

metabolic pathway is not quite established. Little is known about the role of plasmids, if present, in the degradation of this compound. Ghosal et al. (52) reported the isolation of a plasmid from *P. cepacia* AC1100; however, genetic information about this plasmid is not available. The proposed metabolic pathway for the degradation of 2,4,5-T is shown in Fig. 9. 2,4,5-T is converted into trichlorophenol and chlorocatechol before ring fission occurs.

Chlorotoluene

Pierce et al. (116) reported that *P. cepacia* and several other *Pseudomonas* species were capable of utilizing mono- and dichlorinated toluenes as the sole source of carbon and energy. These strains contained plasmids of about 72 MDa which coded for chlorotoluene degradation. Restriction analysis shows that these plasmids, although of similar size, are not identical. By using the cloning vectors, the chlorotoluene-degradative plasmids have been successfully cloned into *E. coli*. However, information on the expression of the cloned fragments in *E. coli* is unavailable, as is the genetic map of these plasmids (118).

4-Chlorophenylacetate

Pseudomonas sp. strain CBS3 is able to utilize 2-chloroacetate, 4CBA, and 4-chlorophenylacetate (81, 92). In the degradation of 4-chlorophenylacetate, 3,4-dihydroxyphenylacetate is the major intermediate compound that is further metabolized via the *meta*-cleavage pathway. This pathway is different from that of 2,4-D degradation as 2,4-D first loses its acetate group and changes into 2,4-dichlorophenol. The metabolic pathway of 4-chlorophenylacetate is shown as the elimination of halogen occurring before the ring fission.

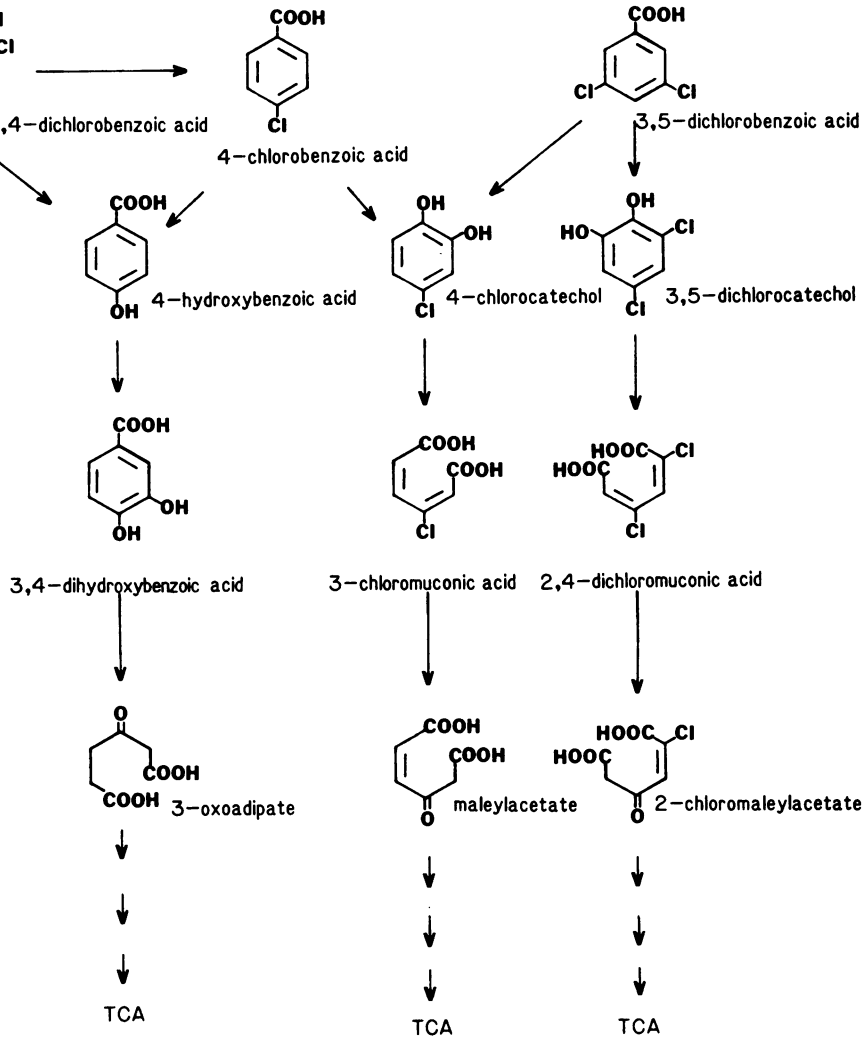

FIG. 7. Proposed pathways for the microbial degradation of 4CBA and DCBAs.

Other Halogenated Aromatic Compounds

Chaudhry and Cortez (26) reported microbial degradation of bromacil by a *Pseudomonas* sp. This bacterium harbors two plasmids of approximately 60 and 100 kb in size. These plasmids code for the resistance of ampicillin; however, it is not clear whether the plasmid DNA is responsible for the degradation of this compound. Hexabromobenzene has been shown to be debrominated in river and estuary sediments (175), but the microbial role in the transformation of hexabromobenzene is not clear.

GENETIC STUDIES ON THE BIODEGRADATION OF CHLORINATED AROMATIC COMPOUNDS

When microorganisms encounter a new organic chemical in their environment, they may obtain the new catabolic genes needed for degradation of that compound from other microorganisms through conjugational or transformational events or they may modify existing genes through mutational processes. Even though the chlorinated hydrocarbons have been synthesized and released into the environment only during the past few decades, microorganisms in nature apparently have developed the ability to degrade some chlorinated compounds. The genes for the degradation of these compounds are often plasmid associated. The compounds known to be degradable by naturally occurring microorganisms carrying plasmids are listed in Table 4.

As mentioned above, the HAA plasmid (pUU204) responsible for the degradation of HAA is quite different from the phenoxyacetate plasmids (pJP4, pRC10) in the sense of diversity (56). However, there are several independently isolated chlorobenzoate-degradative plasmids (pAC25, pAC27, and pWR1) that show genetic homology (24). The same occurs in the case of 2,4-D-degradative plasmids (pJP4, pEML159, and pRC10) (7, 14, 27). Also, there appears to be a common evolutionary emergence of the chlorobenzoate- and 2,4-D-degradative pathways encoded by plasmids pAC25, pAC27, and pWR1 for chlorobenzoate and pJP4, pEML159, and pRC10 for 2,4-D, as indicated from the hybridization studies (7, 24, 27, 51).

Unlike 3CBA and 2,4-D, the plasmids involved in the degradation of other chlorinated hydrocarbons have not been well investigated. Kawasaki et al. (73) reported the isolation of haloacetate-degrading plasmids pUO1 and pUO11 from *Moraxella* sp. strain B. pUO1 and pUO11 are about 43 and 40 MDa, respectively, and also code for resistance to mercury; however, there is no detailed genetic information about these plasmids.

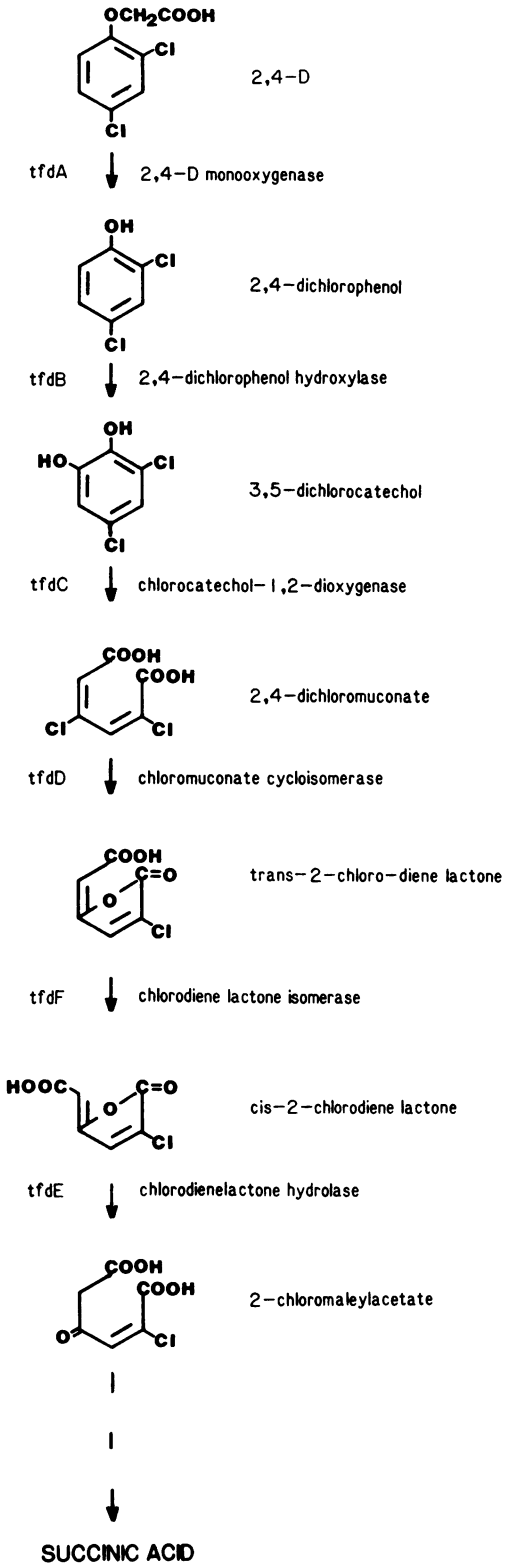

FIG. 8. Proposed pathways for the microbial degradation of 2,4-D. Reprinted from reference 27 with permission.

Pierce et al. isolated several chlorotoluene-degrading plasmids which are similar in size (72 MDa) but have different restriction patterns (116, 118). The plasmids have been cloned, but the analysis and expression of these clones have not been performed.

The plasmids which code for PCP, 2,4,5-T, chlorobiphenyl, chloroaniline, 4-chlorophenylacetate, and chlorosalicylate degradation are not well understood either. Relatively little information is available concerning the abundance and stability of the bacteria or the plasmid-encoded genes within the microflora. There is also little information on the incompatibility grouping of the isolated plasmids relative to other plasmids.

Recently, several investigators have shown increased interest in the study of plasmids encoding 2,4-D degradation (13, 27, 60, 68, 111, 153). This has led to characterization of at least two of these plasmids, pJP4 and pRC10, in detail. Several of the *tfd* genes on these plasmids have been mapped, cloned, and sequenced. These studies showed that *tfd* genes are organized into a large operon, *tfdCDEF*, separated from *tfdA* and *tfdB* (68, 111, 112). Another gene, *tfdR*, is involved in the regulation of the operon and of *tfdA*, the first gene of the pathway (Fig. 8). The mechanism of regulation of *tfdB* is not known. The *tfdD* and *tfdE* products appeared to be 63 and 53% identical (on the basis of amino acids) to those functionally similar enzymes encoded by *clcB* and *clcD*, respectively, from pAC27 of *P. putida* (112). These studies and hybridization studies (3, 4, 49–51, 53) suggest that the plasmids encoding 2,4-D and 3CBA degradation arose from a common ancestor. Thus, it is not surprising that the promoters for the *tfdCDEF* operon, *tfdA*, and *clcABD* have similar conserved-operator regions as observed by nucleotide sequencing (112, 128). It should be interesting to determine how *tfdB* is regulated. In addition, analysis and comparison of a relatively simple 2,4-D plasmid, pRC10 (27), should help us to understand its evolutionary relationship to pJP4 and pAC27. pRC10 appeared to have features in common with, but not identical to, those of pJP4 and pAC27.

Another area of interest in which substantial progress has been made in recent years is the study of genes involved in the degradation of chlorobiphenyls. These genes also have been organized as a cluster forming a chromosomal operon, *bphABCD*, in strains of *P. putida* (47, 74–76). However, the independently cloned operon from two different strains of *P. putida* shows some striking dissimilarities in that *bphC* from *P. putida* OU83 (75, 76) does not hybridize with the TOL and NAH plasmids (exhibiting genes for a similar function), whereas *bphC* from *P. putida* KF715 (80) does. It should be interesting to determine the divergence between the isofunctional genes evolved in two different bacteria.

Understanding the genetic basis of the biodegradation of chlorinated hydrocarbons and using genetic engineering techniques may allow construction of an improved strain with enhanced degradative ability and broader biodegradative potential. Examples of genetic manipulation to construct a bacterial strain with novel abilities are described in the following section.

GENETIC MANIPULATION TO CONSTRUCT STRAINS WITH BROADER BIODEGRADATION POTENTIAL

Several microorganisms are reported to utilize chlorinated aromatic compounds such as 3CBA and 2,4-D. However, the specificity of the oxidation of such substrates by individual microorganisms appears to be strict. Bacterial strains

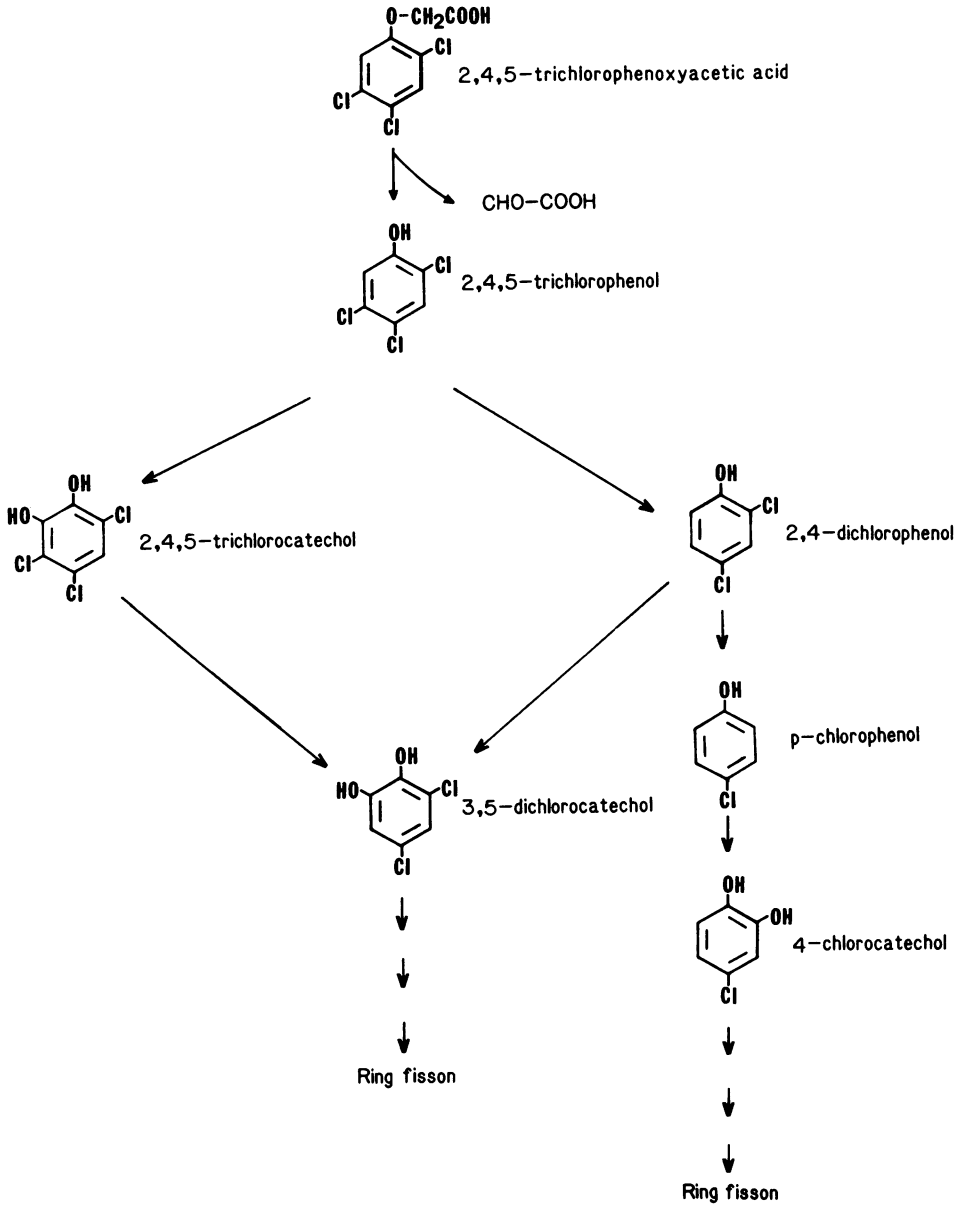

FIG. 9. Proposed pathways for the microbial degradation of 2,4,5-T.

that can metabolize 3CBA and 4-chlorocatechol, such as *Pseudomonas* sp. strain B13, cannot oxidize 4CBA because of the stringent specificity of the 3CBA oxygenase, which cannot act on 4CBA. Reineke and Knackmuss (124) reported that the TOL plasmid has a broad-substrate-specific benzoate oxygenase which allows conversion of 4CBA to 4-chlorocatechol. Once the TOL-mediated conversion of 4CBA to 4-chlorocatechol is accomplished, the metabolite can be rapidly oxidized by *Pseudomonas* sp. strain B13.

Chatterjee and Chakrabarty (23) grew the 3CBA-degrading *Pseudomonas* sp. strain B13 cells harboring the plasmid pAC25 in a chemostat in the presence of the cells harboring the TOL plasmid and found that the host cells were able to utilize 4CBA. Plasmid pAC27 isolated from such cells showed deletion of an 11-kb *Eco*RI fragment from the pAC25 plasmid; a portion of the TOL plasmid (about 41.5 kb) was also found to be transposed onto the chromosome of such cells. The acquisition of the 41.5-kb fragment of TOL which codes for the toluate 1,2-dioxygenase activity (*xylD*) by *Pseudomonas* sp. strain B13 enables such cells to utilize 4CBA and also facilitates the isolation of mutants capable of utilizing 3,5DCBA. Further enrichment of TOL^+ and $pAC27^+$ cells on 3,5DCBA would produce cells capable of slowly utilizing 3,5DCBA as a source of carbon and energy. Isolation of plasmids from such cells demonstrates the presence of a 72-kb plasmid (pAC29) in addition to pAC27. Restriction hybridization studies showed that pAC29 is derived primarily by duplication of a segment of pAC27 and a fragment from TOL. Genetic rearrangement in plasmids can extend the substrate range of 3CBA-degrading *Pseudomonas* spp. to include 4CBA and 3,5DCBA degradation.

Weightman et al. (176) used the same rationale as described above to construct a chlorosalicylate-utilizing organism and chloronaphthalene-degrading organisms. A *Hin*dIII-

TABLE 4. Plasmids encoding degradation of chlorinated hydrocarbons

Plasmid[a]	Compound	Molecular size[b]	Reference
pUU204	2-Monochloropropionic acid	53 kb	56
pKF1	4-Chlorobiphenyl	82 kb	44
*	4-Chlorobiphenyl	16 and 72 kb	93
*	4-Chlorobiphenyl	50 MDa	134
pSS50	4-Chlorobiphenyl	53 kb	144
pAC21	1,4-Dichlorobiphenyl	65 MDa	25
*	1,4DCB	?	105
pJP4	3CBA 2,4-D and MCPA	80 kb	33
pAC27	3CBA	110 kb	23
pAC31	3,5DCBA	105 kb	23
pRC10	3CBA 2,4-D and MCPA	45 kb	27
pEML159	2,4-D	?	7
*	2,4-D	50–150 MDa	115
*	PCP	80–100 kb	28
*	3-, 4-, and 5-Chlorosalicylate	?	129
*	2-, 3-, and 4-Chloroaniline	?	87
*	2,4,5-T	?	52
*	Chlorotoluene	72 MDa	116
*	Chloridazon	?	85
pUO1	Fluoroacetate	44 MDa	72
pUO11	Fluoroacetate	40 MDa	

[a] *, No designation.
[b] ?, Size unknown.

digested fragment of the NAH7 plasmid that contains a gene coding for a broad-specificity salicylate hydroxylase (*nahG*) from a *Pseudomonas* sp. was introduced into a strain capable of catabolizing chlorocatechols. The resulting cells, containing the *nahG* gene, were able to mineralize 3-chloro, 4-chloro, and 5-chlorosalicylates. The plasmid (pPL300-1) isolated from such cells showed deletion of a 1.6-kb fragment. As is the case with the expansion of the 3CBA pathway to include 4CBA, the expansion of the same pathway to include chlorosalicylates required no genetic change other than acquisition of the gene (*nahG*) encoding the enzyme to be recruited.

The chloronaphthalene-degrading organisms were obtained by simultaneous multiple genetic changes. The 2-methylnaphthalene-degrading *Pseudomonas* sp. strain SB104 was shown to carry an 80-kb plasmid (pSB104) which specifies a pathway similar to that of NAH7 for the degradation of naphthalene via salicylate, catechol, and a *meta* cleavage pathway. Plasmid pSB104 is similar, although not identical, to NAH7, and its catabolic pathway exhibits minor differences from that of NAH7 in terms of regulation of the pathway and the specificities of certain enzymes. An insertion mutant derivative of pSB104 was obtained by transposon mutagenesis in its catechol 2,3-dioxygenase gene (i.e., a mutant blocked in the *meta* cleavage pathway). This mutant was conjugated into *Pseudomonas* sp. strain B13, and the transconjugants grew on 2-chloronaphthalene and released chloride.

Another example of genetic rearrangement (deletion-fusion followed by genetic duplication) is the modification of plasmid pJP4 (80 kb) to generate plasmid pYG2 (93 kb), which allows *P. putida* cells to grow on 3CBA (51). This modification occurs spontaneously when pJP4 is transferred to *P. putida*, selecting for growth on 3CBA. The expression of the pJP4 3CBA genes in *P. putida* requires deletion of a segment of about 15 kb from pJP4, followed by duplication of a 25-kb segment as an inverted repeat.

TABLE 5. Growth of engineered *A. eutrophus* and *Flavobacterium* sp. strains in the presence of different substrates

Species	Genotype	Growth on[a]:					
		TFD	PAA	4CB	CPH	SA	CSA
Flavobacterium sp.	Hg^r *tfd*$^+$ (pRC10)	+	+	−	+	−	−
A. eutrophus	Hg^r *tfd*$^+$ (pJP4)	+	+	−	+	−	−
A. eutrophus	Cured	−	−	−	−	−	−
A. eutrophus	Hg^r *tfd*$^+$ (pRC10)	+	+	−	+	−	−
A. eutrophus	Hg^r *tfd*$^+$ (pRC301)	+	+	−	+	−	−
A. eutrophus	Hg^r *tfd*$^+$ *nahG*	+	+	−	+	+	+
A. eutrophus	Hg^r *tfd*$^+$ *xylD*	+	+	+	+	−	−

[a] Abbreviation: TFD, 2,4-D; PAA, phenoxyacetic acid; 4CB, 4-chlorobenzoate; CPH, chlorophenol; SA, salicylate; CSA, chlorosalicylate.

Attempts to construct a strain capable of degrading both 2,4-D and 2,4,5-T through the transfer of plasmids pJP2 and pJP4, which specify 2,4-D degradation, to the 2,4,5-T degrader *P. cepacia* DC102 have resulted in a new strain which is able to utilize both substrates as carbon and energy sources (51). Analysis of plasmids isolated from such cells harboring pJP2 showed the presence of intact pJP2 as well as unmodified pDC102. However, in all exconjugants harboring pJP4, an approximately 10-kb fragment of pJP4 apparently has been lost. This may be due to the interaction between incoming pJP4 and the resident plasmid in the recipient.

Several attempts to clone the *Pseudomonas* genes encoding 2,4-D degradation were unsuccessful (52). Initially, *tfdA*, which codes for the 2,4-D monooxygenase, was identified and cloned (27, 153). The 2-kb *Bam*HI-*Sal*I fragment of pJP4 containing *tfdA* was transferred into *Pseudomonas* sp. strain B13 and enabled these cells to utilize phenoxyacetate and 4-chlorophenoxyacetate. A similar result is obtained by cloning a 1.6-kb subfragment of the *Eco*RI A fragment of pRC10 into *P. putida* (27). To determine whether pRC10 can be expressed in *A. eutrophus*, pRC10 was cloned into *E. coli* and the resulting conjugants were mated with a cured strain of *A. eutrophus* (JMP228). The transconjugants harboring pRC10 were able to grow in minimal medium containing 2,4-D. More recently, *xylD*, *xylL*, and *nahG* in *A. eutrophus* and *P. putida* harboring pRC10 were investigated. These genes conferred on the host the ability to utilize several new compounds as the sole source of carbon (Table 5). Similar studies with other hosts may allow the development of microorganisms capable of degrading a wide variety of toxic chemicals with greater efficiency.

Recent studies on cloning and characterization of the degradative genes should help in developing bacterial strains which can attack persistent pollutants more aggressively. Since genes encoding catabolic pathways are often highly clustered and plasmid borne, these clusters can be cloned; if they are readily expressed in new hosts, microorganisms with a broader substrate range can be developed. For example, transfer of *bphABCD* into CBA-, chlorobenzene-, and 2,4-D-degrading bacteria may enable them to degrade some PCBs. Alternatively, the cloned genes have been used to expand the degradation ability of microorganisms (136, 180, 183, 190, 191). Table 5 shows the ability of genetically engineered strains of *A. eutrophus* to metabolize several chlorinated compounds. Similar examples of construction of new metabolic pathways in microorganisms have been re-

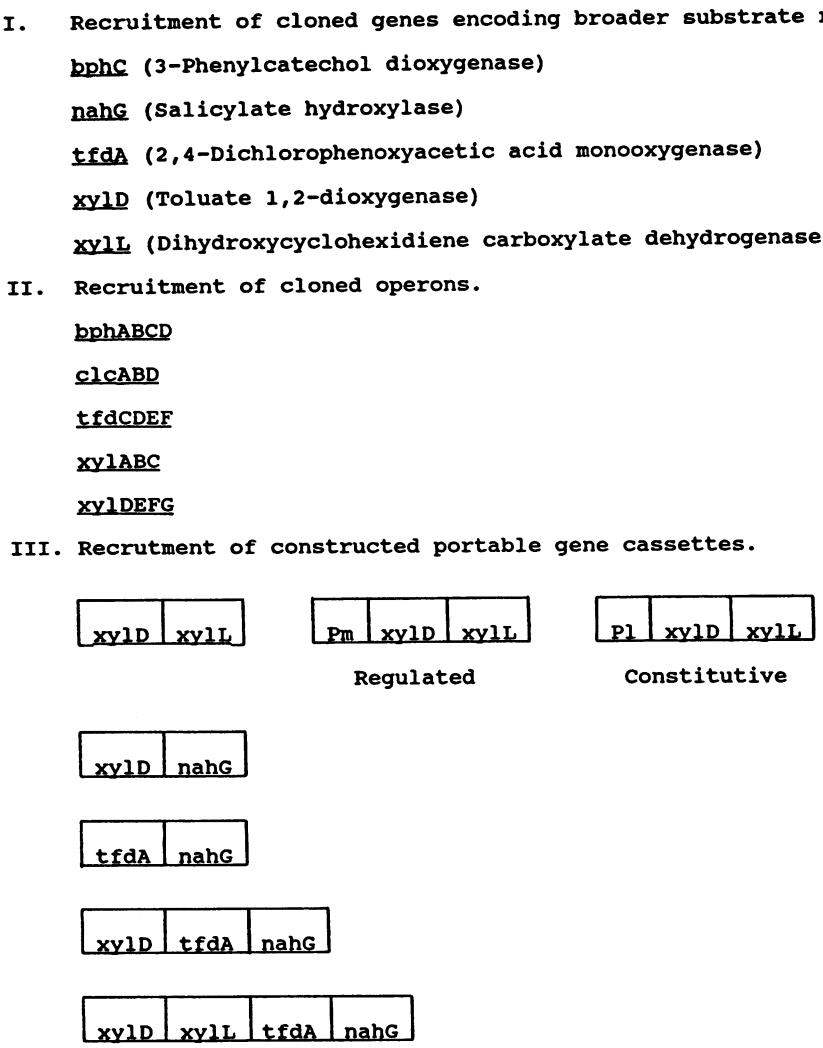

FIG. 10. Strategies for the construction of recombinant strains.

ported (136, 183, 190). Aside from cloning individual genes or gene clusters (88, 127, 136) for expanding the substrate range of microorganisms, portable catabolic cassettes (Fig. 10) may be constructed that can be placed in desired microorganisms potentially useful for cleaning toxic wastes.

Numerous microbial isolates have been reported to be very good at degrading organic compounds under laboratory conditions but fail to execute the task of decontamination under field conditions. This is because these microorganisms usually are isolated by enrichment on a single compound and so do not degrade mixtures of organic compounds, a situation that they face in the real world. To use microorganisms for remediation purposes, it may be necessary to provide them with extra catabolic abilities. This may help them to be effective in situations such as industrial and municipal wastes or when several compounds are contaminating the environment. The strategies outlined in Fig. 10 could be used to achieve these goals.

CONCLUSION

Xenobiotic compounds have been used extensively in agriculture as herbicides and insecticides and in the manufacturing industry as solvents and degreasers. Public concern about the possible hazardous effects of these chemicals on humans and their environment has focused largely on a few classes of compounds. Of these compounds, chlorinated hydrocarbons are the most publicized. Many of the chlorinated compounds are highly toxic, and because they are often recalcitrant or insoluble, they escape degradation. However, the microorganisms exposed to these synthetic chemicals have evolved the ability to utilize some of them. Bacteria of several different genera have been shown to degrade chlorinated hydrocarbons. Most of the xenobiotic-degrading microorganisms harbor plasmids which code for the catabolic genes. By understanding the biochemistry and genetics of plasmid-borne degradation and by using the recombinant DNA techniques, it is possible to characterize the appropriate genes and transfer them to construct improved strains with enhanced ability for degradation of several toxic compounds.

One of the objectives of genetic engineering of toxic chemical-degrading microorganisms is to develop so-called "superbugs," capable of detoxifying or decontaminating the toxic chemicals in the natural environment. To establish the potential applications of the recombinant strains in the

environment, the strains must be stable members of the indigenous microflora and the recruitment of catabolic enzymes and gene regulators with appropriate effector specificities (by natural gene transfer or by laboratory manipulation) to generate new hybrid pathways for chlorinated compounds must not significantly alter the host or the natural ecosystem.

Although the risk of releasing recombinant organisms is not known, the prospects for the construction of catabolic pathways to effect mineralization and detoxification of halogenated compounds are encouraging. However, the utility of constructed organisms in dealing with problems related to environmental pollution in nature has yet to be tested. Obviously, the most effective means of avoiding further pollution lies in restricting the use of recalcitrant compounds and replacing them with nonrecalcitrant alternatives, as well as reducing and decontaminating the toxic waste at the site of generation. Genetically engineered microorganisms could be useful in decontaminating waste in contained environmental situations such as biodegradations reactors, dump sites, and waterworks systems.

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