Chapter 6
The Genus *Dehalococcoides*

Stephen H. Zinder

Abstract *Dehalococcoides*, now called *Dehalococcoides mccartyi*, was first discovered in an enrichment culture from sewage sludge that reductively dechlorinated the groundwater pollutants tetrachloroethene (PCE) and trichloroethene (TCE) to vinyl chloride (VC) and ethene, in contrast to other organohalide-respiring bacteria that dechlorinated PCE and TCE only as far as dichloroethenes (DCEs). The first isolate, strain 195, was a tiny disk-shaped bacterium in the phylum *Chloroflexi* that had an S-layer protein subunit cell wall lacking peptidoglycan. It was a strict anaerobe using only H_2 as the electron donor and organohalides as respiratory electron acceptors. Other *D. mccartyi* strains are similar and use a variety of halogenated aliphatic and aromatic compounds as electron acceptors. The genomes of *D. mccartyi* are highly streamlined, varying from 1.34 to 1.5 MB, yet contain 10–36 different copies of *rdhAB* operons predicted to encode reductive dehalogenases (RDases), most with adjacent genes predicted to encode transcriptional regulators, indicating that organochloride respiration is a highly evolved and regulated process in *D. mccartyi*. The presence of *D. mccartyi* at chloroethene-contaminated groundwater sites appears necessary for dechlorination of PCE and TCE past DCEs, and molecular tests for *D. mccartyi* and its associated *rdhAB* genes have become part of contaminated site characterization. Moreover, *D. mccartyi*-containing cultures have been commercially developed for bioaugmentation of those sites to abet dechlorination to ethene, especially cultures that contain *D. mccartyi* strains that can efficiently convert VC to nontoxic ethene in a respiratory process, like strains BAV1 and VS. This tiny unusual bacterium is now considered to be an important player in the restoration of chloroethene-contaminated sites.

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Abbreviations

1,2-DCA	1,2-dichloroethane
1,2-DCP	1,2-dichloropropane
3-Cl-4-OHPA	3-chloro-4-hydroxyphenylacetic acid
AQDS	Anthraquinone-2,6-disulfonate
BP	Bromophenol
Bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
CD	Carbon dichloride
CF	Chloroform
CP	Chlorophenol
CT	Carbon tetrachloride
Cysteate	Alanine-3-sulfonate
DBP	Dibromophenol
DCA	Dichloroethane
DCHQ	Dichlorohydroquinone
DCP	Dichlorophenol
DMSO	Dimethyl sulfoxide
HCB	Hexachlorobenzene
Ioxynil	3,5-diiodo-4-hydroxybenzonitrile
Isethionate	2-hydroxyethanesulfonate
OHRB	Organohalide-respiring bacteria
PCE	Tetrachloroethene
PCP	Pentachlorophenol
RDase	Reductive dehalogenase
rdh	Reductive dehalogenase homologous genes
TCA	Trichloroethane
TCE	Trichloroethene
TCHQ	2,3,5,6-tetrachlorohydroquinone
TCMP	2,3,5,6-tetrachloro-4-methoxyphenol
TCP	Trichlorophenol
TeCP	Tetrachlorophenol
VC	Vinyl chloride

6.1 Discovery

I will begin this chapter with a more personal history of the discovery and isolation of *Dehalococcoides*, which took over 7 years and required some paradigm shifts in my and others thinking along the way, followed by more general descriptions of members of this fascinating genus.

6.1.1 In the Beginning

The discovery of *Dehalococcoides* began with my colleague, James Gossett, in the School of Civil and Environmental Engineering at Cornell. During a sabbatical in 1984–1985 at the USAF Armstrong Laboratory at Tyndall Air Force Base in Florida, USA, he became interested in chlorinated solvents, which were groundwater contaminants at numerous military bases. While there, he developed a simple protocol to determine the Henry's law constants for these solvents (Gossett 1987), information important in designing air stripping processes used to remove solvents from groundwater in pump and treat systems, the predominant remediation process used at that time.

Having done research on anaerobic digestion, Gossett also became interested in the reductive dechlorination of these solvents by anaerobes. At that time, reductive dechlorination was considered to be a slow cometabolic process carried out by reduced transition metal containing cofactors like vitamin B_{12}, coenzyme F_{430}, or hemes found in anaerobes like methanogens, acetogens, or sulfate-reducing bacteria (Fathepure and Boyd 1988; Gantzer and Wackett 1991; Wood et al. 1968). Moreover, reductive dechlorination of chloroethenes apparently stopped at vinyl chloride (VC), a known human carcinogen, so it was not considered to be a viable remediation process. Gossett's first tries at obtaining significant activity from anaerobic digestor sludge in Florida were unsuccessful.

6.1.2 Success in Ithaca

Upon his return to Cornell, Gossett had a graduate student David Freedman and started some enrichments using sludge from the Ithaca sewage treatment plant. Two types of enrichments showed activity, those with dichloromethane (Freedman and Gossett 1991), which will not be discussed here, and those with tetrachloroethene (PCE). According to the operator of the sewage plant (Gleason, personal communication), at the time of Freedman's enrichment, the anaerobic digestors were having problems with chlorinated solvents, with dry cleaners, industry, and Cornell University as potential sources, so the presence of *Dehalococcoides* in those digestors is not unreasonable. This aged sewage treatment plant was decommissioned in 1988, and its buildings now serve as a science museum.

A laboratory-scale anaerobic digestor was established by Freedman, and serum bottle microcosms containing 100 ml of the sludge were derived from it. Twenty-five PCE doses of 3–4.5 μmol/L were added to the microcosms over 55 days, and organic matter in the sludge served as the electron donor. After about 15 days, DCEs began to accumulate, followed by VC at 30 days, and after 50 days, ethene was detected. When PCE feeding was stopped, VC was slowly converted stoichiometrically to ethene. Freedman and Gossett were able to detect ethene because they were using a gas chromatograph with a flame ionization detector (FID),

which is sensitive to essentially all organic compounds, while previous researchers had used electron capture detectors or electrolytic detectors, which are highly sensitive to organochlorides, but do not detect non-chlorinated compounds like ethene. Thus, if ethene was produced in previous studies, it would have been missed.

Material from these microcosms was transferred into a mineral growth medium amended with 50 mg/L yeast extract as a nutrient, and either glucose or methanol as an electron donor, and with multiple doses of ~4 μM PCE. Cultures with either electron donor converted PCE doses to VC with little buildup of TCE or DCEs, but only the methanol amended ones converted VC to ethene. Thus, began the use of methanol as an electron donor for reductive dechlorination of chloroethenes, a substrate used often in subsequent studies of chloroethene degradation (Duhamel et al. 2002; Rossetti et al. 2003). The cultures could be transferred multiple times, showing the reaction was sustainable.

Since finding PCE conversion to ethene was novel, Freedman and Gossett wanted to confirm its origin and identification. They demonstrated that label from ^{14}C-PCE could be found in VC and ethene fractions, verifying that PCE was indeed the precursor of ethene, and used the Cornell gas-chromatograph mass-spectrometry facility to indisputably identify ethene as the product. Despite these measures, the manuscript describing these findings initially had a difficult time in review because of disbelief of some of the reviewers, including the belief that an FID was unsuitable for measuring chloroethenes (Gossett, personal communication).

I served on Freedman's thesis PhD committee representing microbiology and became interested in culturing organisms potentially responsible for PCE dechlorination. Since a PCE-reducing methanogen conformed to the conventional wisdom at that time, we isolated a methanol-utilizing methanogen from a high dilution from the culture. However, it did not dechlorinate PCE to any extent, and I suspected that another type of organism was involved in dechlorination. Because the cultures were being fed micromolar amounts of PCE but were converting millimolar amounts of methanol to methane, I was concerned that the dechlorinators would be a tiny minority in the culture, and finding them would be like looking for a "needle in a haystack."

6.1.3 Ramping the Culture up

This concern became less salient when graduate student Thomas DiStefano took over the study in Gossett's lab and started increasing the PCE doses added to the cultures. Surprisingly, the more PCE that was added, the better the cultures performed (DiStefano et al. 1991) until the dose reached 0.55 mmol/L, approaching the solubility limit of PCE. These cultures produced little or no methane, presumably because of inhibition by chloroethenes and ethene, yet dechlorination continued unabated, with the PCE dose usually converted to ca. 20 % VC

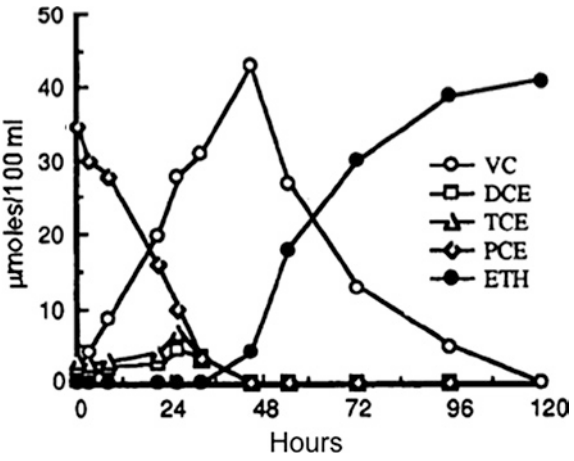

Fig. 6.1 Time course for conversion of PCE to ethene by an acetogenic methanol-PCE enrichment culture. Slow equilibration of added PCE caused its underestimation in early time points (from Tandoi et al. 1994 with permission)

and 80 % ethene within 2–3 days and nearly completely to ethene by about five days (Fig. 6.1a). Indeed, dechlorination was now consuming nearly one third of the electron equivalents added to the culture, with the remainder going to acetogenesis, instead of methanogenesis, from methanol. A methanol-utilizing *Peptostreptococcus*-like acetogen that resembled one of the main morphotypes in the culture was isolated and did not significantly dechlorinate PCE (Tandoi and Zinder, unpublished). Subsequent studies showed that H_2 rather than methanol was the direct electron donor for dechlorination (DiStefano et al. 1992).

At this point we hypothesized that rather than a cometabolic process carried out by methanogens or other anaerobes, reductive dechlorination of chloroethenes in these cultures was a respiratory process, based on the following information: (1) the high rates of and high proportion of metabolism devoted to reductive dechlorination, much higher than those described in cometabolic cultures; (2) the continuation of those rates despite the inhibition of methanogenesis by high PCE doses; (3) the low dechlorination activity of methanogenic and acetogenic isolates from the cultures; (4) the ability to transfer the activity to new cultures in which the rate of dechlorination increased over time, indicative of growth; (5) the favorable thermodynamics of reductive dehalogenation (Vogel et al. 1987); (6) the precedent of organochloride respiration by *Desulfomonile tiedjei* using chlorobenzoates (DeWeerd et al. 1990; Suflita et al. 1982).

However, this hypothesis contradicted the prevailing paradigm at the time for reductive dechlorination of chloroethenes, and an early grant Gossett and I submitted making the case for anaerobic respiration was rejected with reviewers saying that our arguments were specious and we simply did not understand the cometabolic nature of the process, and that the proposed studies would only add incrementally to the already large body of knowledge about reductive dechlorination of chloroethenes. Fortunately, the research was funded by the US Air Force Armstrong Laboratories at Tyndall AFB under the aegis of Cathy Vogel.

6.1.4 Microbiology Begins

Valter Tandoi, a microbiologist working at a water research institute in Rome, Italy came to my lab in 1991 as a visiting scientist just as DiStefano was finishing his research, and we began more concerted microbiological studies. He successfully transferred the methanol-PCE culture into a growth medium with nitrilotriacetate, the chelating agent we used in our medium for methanogens (Zinder et al. 1987), so that that the medium remained clear, instead of black with metal sulfide precipitates, facilitating microscopic observation. Tandoi examined dechlorination kinetics by the culture, demonstrating that VC and *trans*-DCE dechlorination followed first-order kinetics while the other steps occurred at similar rates and were essentially zero order (linear) (Tandoi et al. 1994). These kinetics were considerably different from those described for transition metal cofactors like corrinoids or F_{430} where each successive step was an order of magnitude slower (Gantzer and Wackett 1991).

Even in clear medium, the only organism I could see using phase-contrast microscopy that was present in numbers sufficient to account for the dechlorination activity in the culture was the *Peptostreptococcus*-like organism which we had already isolated and did not detect reductive dechlorination. However, there always seemed to be large numbers of tiny irregular particles of "junk" undergoing Brownian motion in the cultures, but they did not resemble any microbes that I had ever seen and I considered them an unusual precipitate. Tandoi collaborated with members of the lab of my colleague Bill Ghiorse, and used Bill's newly acquired scanning confocal microscope and acridine orange staining to demonstrate that the junk actually contained DNA and was in reality tiny irregular coccoid cells (Fig. 6.2).

Tandoi's last experiment before returning to Rome was to initiate tenfold serial dilutions of the methanol/PCE culture into basal medium with electron donors and

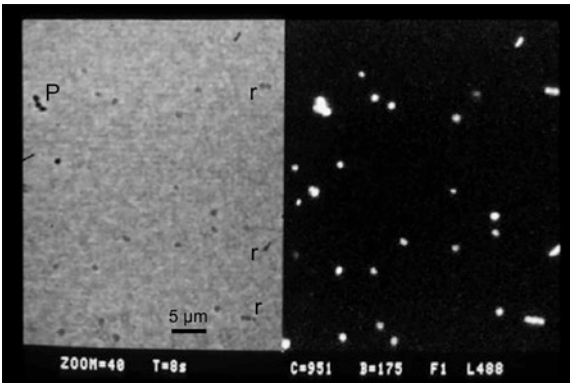

Fig. 6.2 *Left panel* phase-contrast micrograph from an acetogenic enrichment culture. We used an agar slide causing a rough background and the *D. mccartyi* cells to lie down flat making them barely phase-dark. *Right panel* fluorescence micrograph showing acridine orange fluorescence of the tiny barely visible cells. P, *Peptostreptococcus*-like organism (short chain); r, rods

acceptors designed to enumerate various physiological groups by the most probable number technique. Methanol and acetate-utilizing methanogens were not present in any dilutions, H_2–CO_2 using methanogens and acetogens were estimated at 10^4/ml, H_2-utilizing sulfate reducers at 10^6/ml, and fermentative heterotrophs and methanol-utilizing acetogens were estimated at 10^7/ml. Most importantly, in medium with H_2 as the electron donor and PCE as the electron acceptor we obtained growth and dechlorination in some of the 10^{-6} dilutions. These tubes did not appear to contain any sulfate reducers, but still contained fermentative heterotrophs that grow on the 0.2 g/L of yeast extract that we added to the culture, and methanol-utilizing acetogens. The heterotrophs remained in transfers of this culture but the methanol-utilizing acetogen could not use H_2–CO_2 and was lost on subsequent transfer.

Thus our reward from these studies was a PCE-dechlorinating culture free of other H_2 utilizing organisms such as methanogens, acetogens, or sulfate reducers. It contained those tiny organisms growing on H_2 and PCE and some rod-shaped heterotrophs, and was therefore more highly enriched. At around this time in 1992, I received by mail a PhD thesis published by Christof Holliger at Wageningen University that described his studies on reductive dechlorination and included a chapter on his pioneering studies of *Dehalobacter restrictus*, which dechlorinated PCE to *cis*-DCE (cDCE) using H_2 as the electron donor (Holliger et al. 1993, 1998). This demonstration that chloroethenes could indeed be used as electron acceptors did not surprise me. What did surprise me was that despite the long lists of electron donors and acceptors tried, *D. restrictus* could only use H_2 and PCE/TCE as its electron donor/acceptor pair. It seemed incredible to me that an organism would be so specialized in using compounds that had only been added to the environment in the past few decades.

Subsequently, several other organisms that reductively dechlorinated PCE/TCE as far as cDCE were described, including *Sulfurospirillum* (*Dehalospirillum*) *dehalogenans* (Scholz-Muramatsu et al. 1995), *Desulfitobacterium* sp. PCE and *D. hafniense* (Christianssen and Ahring 1996; Gerritse et al. 1996), *Desulfuromonas chloroethenica* (Krumholz 1997), and *Geobacter lovleyi* (Sung et al. 2006a), organisms covered in other chapters in this volume. Unlike *D. restrictus,* they could use other electron donors besides H_2 and electron acceptors besides chloroethenes. They were all members of the commonly cultivated phyla *Proteobacteria* and *Firmicutes*. Clearly the organisms in our cultures were different since they produced VC and ETH and had an unusual morphology.

6.2 Isolation and Characterization

6.2.1 Optimizing Growth

Once we had a highly enriched culture, our goal was to isolate organisms responsible for the dechlorination. Initial attempts using agar roll tubes failed, so our best chance was dilutions into liquid medium. This method involves a “numbers

game," where you need somewhere near an order of magnitude greater numbers of the desired organism versus contaminants so that by the laws of probability dictated by the Poisson distribution, some of the highest dilution tubes will contain only the desired organism, hopefully a single cell. Thus, we had to optimize the medium to maximize the numbers of the dechlorinators versus other organisms.

At this time, a new graduate student, Xavier Maymó-Gatell, came to the laboratory from Barcelona, Spain and began nutritional studies on the culture (Maymó-Gatell et al. 1995) so that we could grow them in a better defined medium. He found that he could obtain PCE dechlorination to VC at rates that increased over time, indicative of growth, in medium amended with H_2 as the electron donor, 25 % v/v filter-sterilized centrifuged anaerobic digestor sludge supernatant (a source of organic nutrients we sometimes used in culturing methanogens, similar to the use of rumen fluid to culture rumen anaerobes (Hungate 1969), a vitamin solution, and 2 mM sodium acetate, instead of yeast extract. Eliminating yeast extract was a major step forward in reducing contamination since it supported growth of a "zoo" of fermentative heterotrophs, whereas organisms that could catabolically use acetate, like methanogens or sulfate reducers, were no longer present in the culture.

Once yeast extract was omitted from the medium, Maymó-Gatell found that the culture would not transfer if we omitted the vitamin solution we routinely added to the medium. By preparing vitamin stocks, each one missing one of the ten vitamins in the solution, he narrowed it down to vitamin B_{12}, which was required in amounts 50-fold higher than that by organisms that used it for biosynthetic purposes. This high requirement suggested to us that it was used instead for catabolic purposes as a prosthetic group in dechlorinating enzymes, based on vitamin B_{12}'s in vitro dechlorination activity (Gantzer and Wackett 1991). The minimum concentration for optimal dechlorination was 0.05 mg/L, identical with that subsequently determined for the pure cultures of strains 195 and BAV1 (He et al. 2007).

An interesting parallel set of experiments occurred in the Gossett lab during these studies. Graduate student Donna Fennell was switching a PCE-dechlorinating bioreactor from methanol to butyrate as the electron donor with the idea that butyrate would poise H_2 low (Schink 1997), so that dechlorination, which is more thermodynamically favorable than methanogenesis, would be more competitive. The bioreactor initially performed well, producing more ethene than VC on Day 20 (Fennell et al. 1997), but over time the VC to ethene ratio increased until VC was the only product detected, and then by Day 100 TCE and a few days later, PCE were the only chloroethenes detected—the bioreactor had gone into failure.

Fennell, stopping by our lab, learned that the enrichment required vitamins, took a bottle of our stock solution back with her, and began adding it on Day 125. Within days, the bioreactor recovered and was making VC and ethene. After we learned that large amounts of vitamin B_{12} were needed, she upped the B_{12} dose and performance improved to the point that the product was nearly all ethene. This bioreactor, dubbed Donna II, is studied in the laboratory of Ruth Richardson to this day (Rowe et al. 2012). Our interpretation of this phenomenon is that

corrinoid proteins are more abundant in methanol-utilizing methanogens and acetogens, where they are integral parts of catabolic pathways, than in butyrate oxidizing syntrophs and hydrogenotrophic methanogens, and some portion of those corrinoids was excreted/leaked into the medium for use by dechlorinators. It was gratifying that our culture studies could have such a direct effect on bioreactor studies, which beautifully demonstrated that the dechlorinators were more competitive with methanogens when the H_2 concentration was poised low (Fennell et al. 1997).

6.2.2 Purification and Isolation

In the next stage of isolation, we took advantage of the surprising finding that the dechlorinators were resistant to bacterial peptidoglycan synthesis inhibitors such as 100 mg/L vancomycin (DiStefano et al. 1991) or up to 3 g/L ampicillin (Maymó-Gatell et al. 1997). Transfer of the culture into medium supplemented with acetate, a high B_{12} vitamin solution, and 25 % sewage sludge supernatant (ABSS) and containing either of these inhibitors led to growth and PCE dechlorination, and microscopically, the culture consisted of tiny coccoid organisms. However, the culture would not transfer a second time, suggesting that a sufficient amount of a limiting nutrient, perhaps provided by contaminating bacteria, was carried over with the first 2 % v/v inoculum but became too dilute on subsequent transfer. We tried various amendments known to support growth of anaerobes and culture supernatants and extracts, including branched chain fatty acids, horse serum, and extracts of *Escherichia coli* and *Clostridium pasteurianum* and obtained the best results with a filter-sterilized cell-free extract from a mixed dechlorinating culture, and for many years afterward we would obtain material from the "Donna II" butyrate-PCE culture and prepare extracts from the centrifuged pellet. As described presently, it was subsequently found that strain 195 can actually grow in defined medium.

Using this medium, we obtained growth in a 10^{-7} dilution of the culture, and this culture appeared microscopically pure (rod-shaped contaminants are easily detected when present), and contaminants were not detected in various growth media. We deemed the culture pure and later tests, including examination of its genome sequence, showed the culture was clonal. We named it "*Dehalococcoides ethenogenes*" strain 195 with the genus name signifying that it dehalogenated (like *Dehalobacter*) and was coccoid rather than a nearly spherical coccus, the species epithet signifying that it produced ethene, and the strain name signifying that the isolated culture was first obtained in January 1995. As described presently, it and all other *Dehalococcoides* strains have been named *Dehalococcoides mccartyi* (Löffler et al. 2013).

While discussing isolation it should be mentioned that while we were unable to obtain colonies of *D. mccartyi* in roll tubes, Adrian et al., in their studies on strain CBDB1 (Adrian et al. 2000), obtained colonies from diluted cultures in

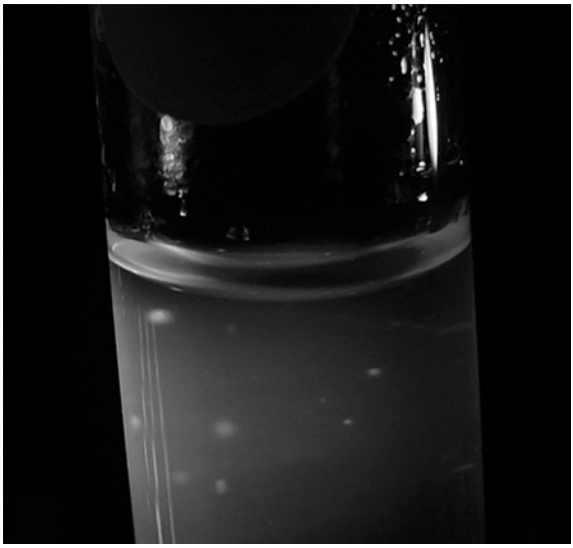

Fig. 6.3 Agar shake tube showing colonies of *D. mccartyi* strain 195. The tube is 18 mm in diameter

low-melting agarose shake tubes, as we subsequently found in collaboration with Adrian, does strain 195 (Fig. 6.3). These colonies are much more likely to be clonal populations than are dilutions into liquid medium. Another approach for purifying cultures takes advantage of the small size of *D. mccartyi*. Cultures are passed through a 0.45 μm membrane filter that retains nearly all other organisms (LaRoe et al. 2014). It is possible that other small organisms and mycoplasmas also pass through these membranes, so further purification may be needed. An extensive description of techniques for culture and isolation of *D. mccartyi* and other dehalogenators is given by Löffler et al. (Löffler et al. 2005).

6.2.3 Initial Characterization: Physiology, Morphology/Ultrastructure, and Phylogeny

Our first publication on *D. mccartyi* strain 195 (Maymó-Gatell et al. 1997) described its physiology, including that it did not use any electron donor we tested other than H_2 nor any of the common electron acceptors such as oxygen, nitrate, or sulfate, a degree of specialization similar to *D. restrictus* (Holliger et al. 1998). A 100 μmol/L dose of PCE added to an active culture was stoichiometrically converted to VC within 3 h, with little accumulation of TCE or DCEs as intermediates. VC was then slowly dechlorinated to ethene in about 600 h following first-order kinetics. It was later shown that dechlorination of VC or *trans*-DCE did not support growth in strain 195 making those steps cometabolic (Maymó-Gatell et al. 1999, 2001). Doubling times near one day were found for cultures converting PCE to VC. Since then, strain 195 has been shown to use some chlorophenols

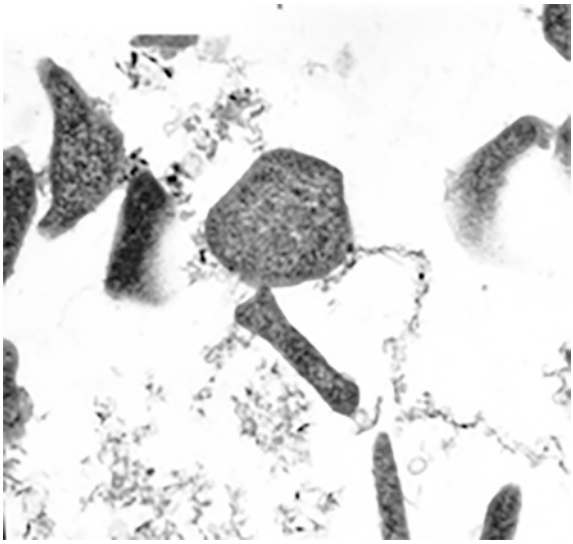

Fig. 6.4 Low power thin-section electron micrograph of *D. mccartyi* strain 195 showing disk-shaped morphology of the cells. The central cell is approximately 0.5 μm in diameter

(Adrian et al. 2007a) and highly chlorinated chlorobenzenes (Fennell et al. 2004) metabolically, and dechlorinate some chloronaphthalenes and PCBs although it is not certain whether these reactions are growth supporting (Fennell et al. 2004).

Thin-section electron micrographs of strain 195 showed an unusual cell structure. Cells appeared 0.1–0.5 μm in diameter, and it was not realized until lower power micrographs like Fig. 6.4 were examined after publication that the cells are actually curved disks 0.4–0.5 μm in diameter and 0.1–0.2 μm in height. The biovolume of these cells is estimated as 0.02 μm^3, roughly 30-fold smaller than a typical *E. coli* cell, and about twice that of *Pelagibacter ubique* in the SAR 11 cluster (Rappe et al. 2002), considered the smallest bacterium known. The small size allows a high surface area to volume ratio, useful for uptake of scarce substrates. More unusual was the cell wall structure, which resembled the S-layer protein subunit cell walls of *Archaea* (Albers and Meyer 2011), and others have also seen S-layer cell wall structures in electron micrographs of *D. mccartyi* strains, including strain CBDB1 which had a 14 nm repeating structure (Adrian et al. 2000; Löffler et al. 2013). A peptidoglycan layer was not visible in the thin sections nor was it detected using a fluorescent lectin stain for N-acetylglucosamine (Maymó-Gatell et al. 1997), and subsequent genomic studies (Seshadri et al. 2005) demonstrated the absence of peptidoglycan synthesis genes. The lack of a peptidoglycan layer readily explained strain 195's resistance to vancomycin and ampicillin. All other strains of *D. mccartyi* are also tiny disk-shaped cocci with S-layer cell walls (Löffler et al. 2013).

The cell wall structure, resistance to antibiotics, and hydrogen-based metabolism in strain 195 made us wonder whether the organism belonged to the *Archaea* rather than the *Bacteria*, and we began sequencing its 16S rRNA gene. We obtained a sequence of ~200 bp using the manual sequencing gel methods

available at the time. As we got a more complete sequence it was clear that *Dehalococcoides* was in the *Bacteria*, a finding corroborated by its sensitivity to tetracycline. However, we were unable to place it in any of the known phyla (even when we included *Chloroflexus* in the analysis). Different sequence sets and algorithms caused shifts in its position on the tree. Soon after publication of the paper both Phil Hugenholtz, then at UC Boulder and Floyd Dewhirst at the Forsyth Dental Institute ran it against their more extensive databases that included uncultured sequences, and it was pulled into the *Chloroflexi* by sequences like that of SAR202. Also, a subsequent discussion with researchers at DuPont revealed that a sequence from a contaminated site in Victoria Texas, that had been discarded as a likely chimera, was over 98 % identical with that of strain 195, and eventually *D. mccartyi* strain VS was cultured from that site (Cupples et al. 2003; Müller et al. 2004). This was our first inkling that our organism from a sewage digestor might be important at contaminated groundwater sites.

6.3 Diversity and Phylogeny

6.3.1 Isolation of Other D. mccartyi Strains

Since the isolation of strain 195, several other strains of *D. mccartyi* have been isolated, and I will discuss the first three followed a briefer discussion of others as well as summarize their descriptions in Table 6.1. In 2000 Adrian et al. (Adrian et al. 2000) described *D. mccartyi* strain CBDB1 that dechlorinated chlorobenzenes with three or more chlorines (Jayachandran et al. 2003). It was shown to dechlorinate other chloroaromatics including dioxins (Bunge et al. 2003), chlorophenols including pentachlorophenol (Adrian et al. 2007a), and PCBs (Adrian et al. 2009). It also debrominates bromobenzenes to benzene (Wagner et al. 2012). Although originally reported not to use chloroethenes (Adrian et al. 2000), strain CBDB1 was subsequently shown to grow dechlorinating PCE and TCE to a 3.4/1 mixture of *trans*-DCE and *cis*-DCE (Marco-Urrea et al. 2011a), an ability it shares with strain *D. mccartyi* MB (Cheng and He 2009).

A major shortcoming of *D. mccartyi* strain 195 for use in remediation of chloroethenes is that, despite its original species name "*ethenogenes*," it produces large amounts of VC that is then only slowly cometabolized to ethene. Strains that use VC for organochloride respiration and growth are much more desirable and two such strains were described in 2003.

D. mccartyi strain BAV1 was cultured from the chloroethene-contaminated Bachman Road site in Michigan, USA (He et al. 2003a). It was enriched with pyruvate followed by H_2 with VC, and isolated using ampicillin and multiple 10^{-7} dilutions (He et al. 2003b). Besides VC, strain BAV1 could also use all three DCE isomers, 1,2-dichloroethane (DCA, dechlorinated to ethene) and vinyl bromide.

D. mccartyi strain VS was enriched on VC (Rosner et al. 1997) from a benzoate/TCE culture derived from a contaminated site in Victoria, Texas.

Table 6.1 *Dehalococcoides mccartyi* strains in pure culture

Strain	Representative substrates for OHR → products	Group	Identified RDase genes	References
195	PCE, TCE, cDCE, 1,1DCE → VC (ETH)[a] 1,2-DCA → ETH 2,3-DCPh → 3-CPh HCB → 1,2,4,5 TeCB, 1,3,5-TCB	Cornell	*pceAB, tceAB*	Adrian et al. (2007a), Fennell et al. (2004), Maymó-Gatell et al. (1997, 2001)
CBDB1	HCB → 1,4-DCB, 1,3-DCB, 1,3,5-DCB Dioxins PCBs[b] PeCPh → TCPhs + 2-CPh + 3-CPh Bromobenzenes → benzene PCE, TCE → tDCE	Pinellas	*cbrAB, "pceAB"*	Adrian et al. (2000, 2007a, 2009), Bunge et al. (2003), Jayachandran et al. (2003), Wagner et al. (2012)
BAV1	DCEs, VC → ETH VBr → ETH	Pinellas	*bvcAB*	He et al. (2003a, b)
VS	TCE, cDCE, 1,1-DCE, VC → ETH	Victoria	*vcrAB*	Cupples et al. (2003), Müller et al. (2004)
FL2	TCE, cDCE, tDCE → VC (ETH)[a]	Pinellas	*tceAB*	He et al. (2005)
GT	TCE, cDCE, 1,1-DCE → ETH	Pinellas	*vcrAB*	Sung et al. (2006b)
MB	PCE, TCE → tDCE	Cornell	*mbrA*	Cheng and He (2009)
ANAS1	TCE, cDCE, 1,1-DCE, → VC (ETH)	Cornell	*tceA*	Lee et al. (2011)
ANAS2	TCE, cDCE, 1,1-DCE, VC → ETH	Cornell	*vcrA*	Lee et al. (2011)
11a	TCE, DCEs, VC → ETH 1,2-DCA → ETH	Pinellas	*vcrA*	Lee et al. (2013)
11a5	TCE, cDCE, 1,1-DCE, tDCE → VC	Pinellas	*tceA*	Lee et al. (2013)
DCMB	Dioxins 1,2,3-TCB → 1,2-DCB	Pinellas		Pöritz et al. (2013)
JNA	PCBs (flanked meta)	Pinellas		LaRoe et al. (2014)
CG1	PCBs (meta > para) PCE → TCE	Victoria	*pcbAB1*	Wang et al. (2014)
CG4	PCBs (para > meta) PCE → tDCE	Cornell	*pcbAB4*	Wang et al. (2014)
CG5	PCBs (meta + para) PCE → cDCE + tDCE	Pinellas	*pcbAB5*	Wang et al. (2014)

Abbreviations *A* ethane; *B* benzene; *Ph* phenol; *VBr* vinyl bromide; *PCBs* polychlorinated biphenyls; *Pe* penta; *Te* tetra

[a]VC → ethene cometabolic

[b]Para and meta Cls removed from PCBs; growth using PCBs not demonstrated but likely

Table 6.2 Selected enriched cultures containing *D. mccartyi*

Culture	Description and reactions	References
Donna II	Bioreactor inoculated from an Ithaca, NY, USA sewage plant-fed butyrate that converts PCE to VC and ethene and contains a *D. mccartyi* essentially identical with strain 195	Fennell et al. (1997), Rowe et al. (2012)
RC & KS	Highly enriched cultures from the presumably pristine Red Cedar River in MI, USA and the hydrocarbon contaminated King Salmon River in AK, USA. Both use H_2 for converting 1,2-dichloropropane to propene by a dihaloelimination process	Löffler et al. (1997), Padilla-Crespo et al. (2014)
KB-1	Commercial culture from a TCE-contaminated site in Ontario, Canada used to treat chloroethenes. Contains multiple Pinellas group *D. mccartyi* strains, some using PCE and others converting VC to ethene using VcrA or BvcA	Duhamel et al. (2002, 2004), Waller et al. (2005)
ANAS	Lactate-fed reactor, inoculated from the Alameda Naval Air Station, CA, USA. Converts TCE to ethene and contains the two *D. mccartyi* strains described in Table 6.1	Johnson et al. (2005), Lee et al. (2011)
SDC-9	Commercial culture fed lactate and yeast extract and converting PCE to ethene. Contains at least two *D. mccartyi* strains	Schaefer et al. (2009)
BTF08	Highly enriched culture from Bitterfeld, Germany converting PCE to ethene. Genome sequence shows it contains homologues of PceA, TceA, and VcrA	Pöritz et al. (2013)

H_2-dependent growth of a *D. mccartyi*-like organism on VC was demonstrated using the quantitative polymerase chain reaction (qPCR) and *D. mccartyi*-specific 16S rRNA gene primers (Cupples et al. 2003). It also used cDCE and 1,1-DCE (Müller et al. 2004).

After these initial strains, several more have been isolated and are listed in Table 6.1, whereas some *D. mccartyi*-containing mixed cultures that have been intensively studied are listed in Table 6.2. Strain FL2 was isolated from dechlorinating microcosms constructed using sediments from the presumably pristine Red Cedar River in Michigan, USA. It used TCE following a pattern resembling that of strain 195 in which VC accumulated and was only slowly cometabolized to ethene, but it could not use PCE. Other *D. mccartyi* strains have been isolated that use chloroethenes, dioxins, and PCBs as electron acceptors (Table 6.1). While strain JNA was isolated directly on PCBs (LaRoe et al. 2014), a long and arduous task since PCBs are nearly insoluble and are used very slowly, strains CG1, CG4, and CG5 were isolated by shifting PCB enrichment cultures to the more readily used PCE, which was converted to TCE or DCEs, and demonstrating that the resulting isolates could still use PCBs (Wang et al. 2014). Metagenomic sequencing was used to verify that the *D. mccartyi* strains enriched on PCE were the same as those using PCBs.

All *D. mccartyi* strains described to date resemble strain 195 in that they are small disk-shaped organisms that have been found to use only H_2 as an electron donor and organohalides as electron acceptors for growth.

6.3.2 *Dehalococcoides mccartyi* Phylogeny

Strains 195, VS, and CBDB1/BAV1 are the founding members of three closely related clades of *D. mccartyi*, Cornell, Victoria, and Pinellas, described by Hendrickson et al. (Hendrickson et al. 2002) in their PCR studies of the distribution of *D. mccartyi* 16S rRNA genes in samples from numerous contaminated sites, naming them after where the first sequences were detected (in the latter case, Pinellas, FL, USA). These three clades have remained intact as many more sequences from cultured and uncultured *D. mccartyi* have accumulated in the databases (Fig. 6.5). Overall *D. mccartyi* strains form a tight cluster. For example, full-length 16S rRNA genes from strains 195 and CBDB1 derived from genome sequences are 98.9 % identical.

Because the *D. mccartyi* strains had such closely related 16S rRNA genes as well as considerable homology and synteny of housekeeping genes, and because the dehalogenating phenotype did not correlate with 16S rRNA sequence (see below) a group of researchers working on *D. mccartyi* decided to put all *Dehalococcoides* strains into a single species, *D. mccartyi* (Löffler et al. 2013), named after the pioneering environmental engineer Perry McCarty. Descriptions of isolated *D. mccartyi* strains and their substrates are found in Table 6.1.

Until recently, the next closest relatives to *D. mccartyi*, even including environmental sequences, were *Dehalogenimonas* (Moe et al. 2009), which uses polychlorinated aliphatic alkanes, and its relatives (Kittelmann and Friedrich 2008),

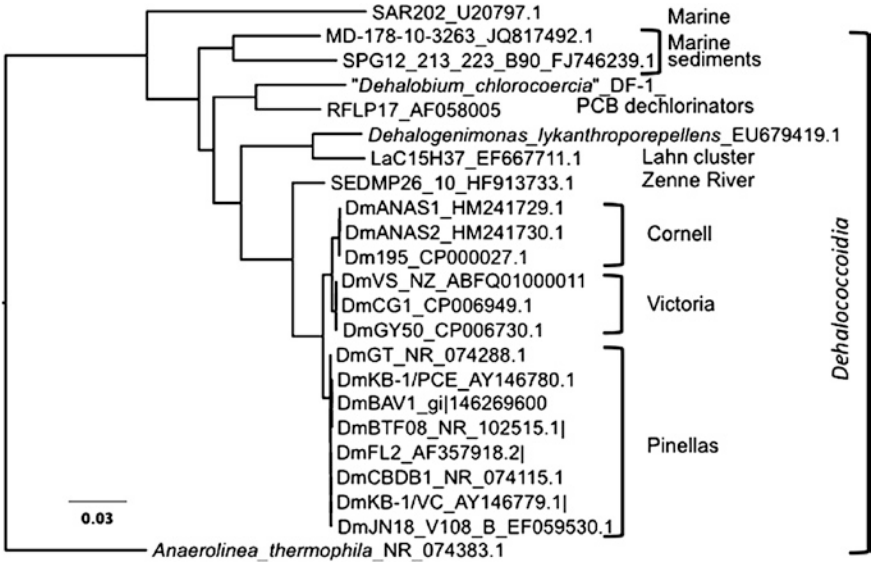

Fig. 6.5 Neighbor joining 16S rRNA phylogenetic tree for some *Dehalococcoides mccartyi* (Dm) strains and relatives along with their accession numbers. Most of the sequences used are full or near full length

at about 90 % 16S rRNA gene identity. In contrast to the tree in Fig. 6.5, most other bacterial phylogenic trees are more bush-like, with a continuum of related species. This suggests that the ancestors of *D. mccartyi* passed through a bottleneck some time in the past. However, a sequence from the chloroethene and chloroethane contaminated Zenne River in Belgium (Hamonts et al. 2014), otherwise dominated by the *D. mccartyi* Pinellas group, does have an intermediate position, with about 96 % identity with the *D. mccartyi* sequences (Fig. 6.3). Nothing is presently known about this "missing link."

More distantly related to *D. mccartyi* are the PCB dechlorinators cultured from Baltimore Harbor "*Dehalobium chlorocoercia*," which uses double-flanked chlorines, and RFLP17, which uses ortho chlorines. There is also a distinct and diverse clade of 16S rRNA sequences that are often numerous in surveys of marine sediments (Durbin and Teske 2011; Inagaki et al. 2006) that is more distantly related to *D. mccartyi*. The entire group of organisms described here forms the class *Dehalococcoidia* (Löffler et al. 2013), formerly called "*Dehalococoidetes*," a term more appropriate for a phylum than a class. While it is likely that nearly all the organisms encompassed by *D. mccartyi* and *Dehalobium* are OHRB, RDase genes were not found in two recent single-cell genomes sequences from the marine sediments cluster (Wasmund et al. 2014; Kaster et al. 2014). The genome sequences were estimated to be 61–85 % complete, so it is possible that the RDase genes were in the missed parts, but if the cluster is found not to contain OHRB, a determination should be made whether they should represent an order in the *Dehalococcoidia* distinct from the OHRB, or possibly given their own class designation.

6.4 Genomics and Physiology

6.4.1 Streamlined Genomes

Because *D. mccartyi* are fastidious anaerobes that grow slowly to low densities, they are poor candidates for study using standard biochemical and genetic techniques that require large amounts of cell material and growth as colonies on Petri dishes. Fortunately, the genomic age was dawning as the first *D. mccartyi* strains were isolated. The genome sequence of strain 195 (Seshadri et al. 2005) set the pattern that is followed by the other strains (Table 6.3). *D. mccartyi* genomes are among the smallest found in free-living organisms, 1.34–1.5 MB, less than one third the size of the *E. coli* genome. The previously mentioned *Pelagibacter ubique* has a genome size of 1.31 Mb (Giovannoni et al. 2005), and it is possible that these tiny cells cannot harbor more DNA. There were only single copies of most housekeeping genes in the *D. mccartyi* strain 195 genome, and genes for some common bacterial functions like peptidoglycan synthesis, motility, and many environmental adaptations, were absent in these stripped-down genomes, nor were there any recognizable genes for using electron acceptors other than organohalides or for most electron donors, in agreement with physiological studies.

Table 6.3 *D. mccartyi* genomes

Strain	Size (MB)	Predicted ORFs	Predicted RDase genes	Accession #	References
195	1.47	1591	17	CP000027	Seshadri et al. (2005)
CBDB1	1.39	1458	32	AJ965256	Kube et al. (2005)
BAV1	1.34	1371	11	CP000688	McMurdie et al. (2009)
VS	1.41	1447	36	CP001827	McMurdie et al. (2009)
GT	1.36	1417	20	CP001924	Unpublished
DCMB5	1.43	1477	23	CP004079	Pöritz et al. (2013)
BTF08*	1.45	1529	20	CP004080	Pöritz et al. (2013)
CG1	1.49	1557	35	CP006949	Wang et al. (2014)
CG4	1.38	1421	15	CP006950	Wang et al. (2014)
CG5	1.36	1413	28	CP006951	Wang et al. (2014)

6.4.2 Multiple RDases

Despite genome streamlining, *D. mccartyi* genomes contained 11–36 sets of *rdhAB* operons predicted to encode RDases (McMurdie et al. 2009; Seshadri et al. 2005). The large number of RDases encoded in their otherwise streamlined genomes shows that *D. mccartyi* strains are highly evolved to use organohalides as electron acceptors. Moreover, the RDase genes often have adjacent genes encoding two-component or MarR transcription regulators, indicating that they are part of a highly regulated metabolic network. The large number and phylogenetic depth of the RDase genes (Hug et al. 2013; McMurdie et al. 2009) indicate that they are ancient, probably dating back at least to the "Great Oxidation Event" over two billion years ago, since nearly all of the enzymes producing organohalides use oxygen or peroxides as reactants (Gribble 2010).

The few *D. mccartyi* RDases that have had functions assigned to them are shown in Table 6.4. PCE RDase and TCE RDase were isolated from a mixed culture containing *D. mccartyi* 195 (Magnuson et al. 1998), which grew to higher yields than the pure culture, using "brute force" biochemical purification techniques. The amino acid sequences of peptides from the proteins were used to design degenerate primers to clone and sequence the RDase genes (Magnuson et al. 2000), an approach originally used to obtain the sequence of the PCE RDase in *Sulfurospirillum multivorans* (Neumann et al. 1998). A similar approach was used to obtain the VC-reducing VcrA gene sequence from strain VS (Müller et al. 2004). Other studies have used transcriptomic (Krajmalnik-Brown et al. 2004) or proteomic techniques to infer RDase function. A useful technique to identify RDases is preparative native electrophoresis (Adrian et al. 2007b), or blue native polyacrylamide electrophoresis (BN-PAGE) which separates native proteins on the basis of size. Various fractions can be assayed for activity, and active bands can

Table 6.4 Characterized reductive dehalogenases found in *D. mccartyi* strains

Name	Reactions	Molecular wt.*	Accession #	References
TceA	TCE, cDCE, 1,1-DCE → VC 1,2-DCA → ETH + trace VC	62,025	YP_180831	Magnuson et al. (2000)
PceA	PCE → TCE 2,3-DCPh → 3-CPh	55,155	YP_181066.1	Magnuson et al. (2000)
BvcA	DCEs, VC → ETH	57,274	YP_001214307	Krajmalnik-Brown et al. (2004)
VcrA	TCE, cDCE, 1,1-DCE, VC → ETH	57,403	YP_003330719	Müller et al. (2004), Parthasarathy et al. (2015)
CbrA	1,2,3,4-TeCB → 1,2,4-TCB	54,141	CAI82345	Adrian et al. (2007b)
DcpA	1,2-DCP → propene	53,904	JX826287	Padilla-Crespo et al. (2014)

*Molecular weights are for the entire proteins before cleavage by the TAT system

be sent for proteomic identification (Tang et al. 2013). Much more detail about RDases can be obtained elsewhere in this volume.

Not only does each *D. mccartyi* strain harbor a large number of RDase genes, their distribution in those strains is disparate (Table 6.1). Each isolate described thus far has a unique complement of RDase genes with only partial overlap with other strains. This became apparent when the genome sequence of *D. mccartyi* strain CBD1 (Kube et al. 2005), which has 32 predicted RdhAB clusters, was compared with that of strain 195 (Seshadri et al. 2005). which had 17. Most of the RDase genes were found in "high-plasticity" regions near the origin of replication in these two organisms, a pattern followed by other *D. mccartyi* strains (McMurdie et al. 2009). It appears that rapid RDase gene exchange among *D. mccartyi* strains occurs in these regions (McMurdie et al. 2009, 2011). For example, strains 195 and FL2 are in the Cornell and Pinellas clades respectively, yet they contain *tceA* genes that are 99.4 % identical at the nucleotide level. In fact, the *tceA* genes from these two strains plus those amplified from several mixed cultures from various sites in North America were all greater than 96.3 % identical, and the intergenic spacer region and the *tceB* genes were 100 % identical (Krajmalnik-Brown et al. 2007). Another example is that the *vcrAB* genes can be found in all three *D. mccartyi* clades (Table 6.1). The recently described 1,2-dichloropropane (DCP) RDase in *D. mccartyi* RC (Padilla-Crespo et al. 2014) is nearly identical with that from the distantly related *Dehalogenimonas lykanthroporepellens* (Fig. 6.3), indicating that horizontal gene exchange can involve other genera. Thus, while finding *D. mccartyi* 16S rRNA genes at a contaminated site can be considered presumptive evidence for the presence of a desired dechlorinating reaction, RDase genes are considered more specific biomarkers, especially *tceA*, *bvcA*, and *vcrA* in the case of chloroethenes (Ritalahti et al. 2006; Behrens et al. 2008; Holmes et al. 2006).

6.4.3 Electron Transport

To carry out a cycle of reductive dechlorination, the Co^{3+} in the corrinoid cofactors in RDases must be reduced back to Co^{1+}. While electrons with $E^{\circ\prime}$ values near −150 mV can reduce Co^{+3} to Co^{+2}, much lower reducing potentials, −500 mV or lower, are needed to reduce Co^{+2} to Co^{+1} (Schumacher et al. 1997) (Fig. 6.5). The ultimate source of these electrons is H_2, but the path that they travel from H_2 to the RDases in *D. mccartyi* is mysterious.

The *D. mccartyi* genome is predicted to encode five distinct hydrogenase complexes, showing that it is highly adapted to use this "simple" electron donor. Of these potential hydrogenases, the one annotated as a periplasmic Ni–Fe uptake (Hup) hydrogenase is found in highest amounts in transcriptomic and proteomic studies of strain 195 (Morris et al. 2006; Rahm and Richardson 2008) (Fig. 6.6) and is considered to be responsible for catabolic H_2 uptake. Detected at lower levels was a cytoplasmic two-subunit complex annotated as Vhu and one with a membrane bound and three cytoplasmic subunits annotated as Hym, an iron hydrogenase. Closer examination of the Hym complex (Mansfeldt et al. 2014) shows that it resembles electron bifurcating hydrogenases that take electrons from H_2 and transfer them to both the low potential ferredoxin and the higher potential NAD^+ (Schut and Adams 2009). While one could imagine this complex generating both the low potential and high potential electrons needed for the RDase, it is found in much lower levels than the Hup hydrogenase, and possibly provides electrons needed for biosynthesis. The energy coupled hydrogenase (Ech) and one annotated as Hyc both contain several membrane bound subunits predicted to pump protons, and may be involved in reverse electron transport using a proton motive force to produce low potential electrons needed for biosynthesis when H_2 concentrations are very low.

A complex annotated as a formate dehydrogenase (FDH) is highly expressed, with its large subunit often visible in one dimensional polyacrylamide gels of *D. mccartyi* strain 195 extracts (Morris et al. 2006). No *D. mccartyi* has ever been found to use formate, and no FDH activity was detected in extracts from *D.*

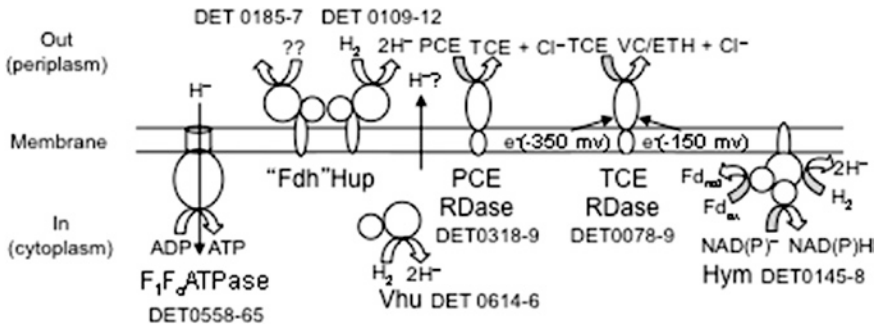

Fig. 6.6 Cartoon depicting predicted electron transport and energy conserving proteins with high coverage in proteomic studies of *D. mccartyi* strain 195 (Morris et al. 2006, 2007), their locus numbers and proposed functions

mccartyi strain 195 (Morris et al. 2006). Phylogenetically, the large subunit clusters with bona fide FDHs, but an examination of its predicted amino acid sequence showed that instead of a cysteine or selenocysteine residue considered to be essential for catalysis, the cognate amino acid in the *D. mccartyi* enzyme was predicted to be serine, which has very different chemical properties. Proteomics verified that this amino acid was indeed serine (Morris et al. 2007). Thus, the function of this highly expressed protein is unknown. Its transcription pattern under a variety of conditions was recently shown to correlate to that for the Hup hydrogenase (Mansfeldt et al. 2014), and it was noticed that both the Hup and the "FDH" complexes were missing subunits that the other possessed, suggesting that they form a hydrogenase "supercomplex," especially since FDHs can have hydrogenase activity (Soboh et al. 2011).

No physiological electron donor has been shown to supply electrons to *D. mccartyi* RDases, and viologen dyes are the only known donors that work at all (Jayachandran et al. 2004; Magnuson et al. 1998; Nijenhuis and Zinder 2005). There are some interesting differences between *D. mccartyi* and the well-characterized *S. multivorans* system (Miller et al. 1997). Similar to *D. restrictus* PCE RDase (Schumacher et al. 1997), either methyl viologen (MV, $E^{\circ\prime} = -440$ mV) or the weaker reducing agent benzyl viologen (BV, $E^{\circ\prime} = -360$ mV) can support reductive dehalogenation (Jayachandran et al. 2004; Nijenhuis and Zinder 2005), whereas only MV supports dehalogenation by the *S. multivorans* PCE RDase. Also, addition of the protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), which collapses proton gradients, inhibited dechlorination in intact *S. multivorans* cells, suggesting a need for an energized membrane to carry out reverse electron transport, CCCP did not inhibit dechlorination in *D. mccartyi* cells (Jayachandran et al. 2004; Nijenhuis and Zinder 2005) or those of *D. restrictus* (Schumacher and Holliger 1996). *D. restrictus* uses menaquinone as an electron donor, and recent genomic evidence (Goris et al. 2014) implicates quinones in *S. multivorans* electron transport. Despite an early report of the presence of ubiquinone in *D. mccartyi*, all recent genomic and biochemical evidence (Schipp et al. 2013) indicates quinones are not present in *D. mccartyi*.

Thus, our understanding is poor regarding the mechanism of electron transport from H_2 to the RDases in *D. mccartyi*, and how that electron transport is coupled to generation of a proton motive force, considerably worse than in the quinone-coupled systems in *D. restrictus* and *S. multivorans*. Energy conservation via a proton motive force in *D. mccartyi* is supported by the findings that subunits of an F_1F_o ATPase in strain 195 are readily detected in proteomic surveys (Morris et al. 2006) and transcript levels of ATPase encoding genes correlate with respiration rates (Mansfeldt et al. 2014; Rahm and Richardson 2008). It should be noted that a gene predicted to encode a proton pumping pyrophosphatase (DET0766) resides in the *D. mccartyi* 195 genome, and peptides of this protein were detected in a proteomic survey (Morris et al. 2006), suggesting a role for pyrophosphate-coupled energy conservation. Also present in *D. mccartyi* genomes are genes predicted to encode an NADH dehydrogenase complex (Complex I, DET0923-33) that lacks an NADH receiver domain and presumably does not use a quinone electron acceptor, and a molybdopterin oxidoreductase distantly related to a tetrathionate reductase (DET0101-3) (Seshadri et al. 2005).

6.4.4 Nutrition

Since *D. mccartyi* strain 195 apparently required complex nutrients, it was hoped that its genome sequence (Seshadri et al. 2005) would provide some insight into which biosynthetic pathways were missing. In accordance with nutritional studies demonstrating a vitamin B_{12} requirement (He et al. 2007; Maymó-Gatell et al. 1995), genes involved in corrin ring synthesis were not found, whereas those encoding enzymes involved in corrinoid transport and salvage, including remodeling of the benzimidazole lower ligand, were present (Seshadri et al. 2005; Yan et al. 2013). Cells of *D. mccartyi* strain 195 provided with corrinoids with no lower ligand or the incorrect one are able to synthesize functional cofactors when provided with exogenous benzimidazoles (Yi et al. 2012), which have been detected in microbial habitats (Crofts et al. 2014). Interestingly, there is a tandem duplication of a 34 gene segment (DET0640-74 and DET0675-707) in the strain 195 genome that contains the genes for corrinoid transport, some corrinoid salvage genes, and some Wood-Ljungdahl pathway genes (Seshadri et al. 2005). This tandem duplication has not been found in other *D. mccartyi* genomes (McMurdie et al. 2009), and we speculated (Seshadri et al. 2005) that this duplication resulted from a period of corrinoid starvation in the early stages of enrichment. It is curious that *D. mccartyi* strains outsource corrin ring synthesis to other organisms, since they need corrins in large amounts to produce the RDases that are essential to their metabolism.

Only a few biosynthetic pathways seemed to be absent in the *D. mccartyi* strain 195 genome. For example there are no biotin or thiamin biosynthetic genes, and some pathways appeared incomplete, such as the methionine synthesis pathway. However, most biosynthetic pathways appeared intact. Moreover, the genome sequence of *D. mccartyi* strain CBDB1 was released soon after (Kube et al. 2005), and despite the fact that this strain could be grown in a defined medium with no organic nutrient additions except acetate and vitamins, the genome contained essentially identical biosynthetic genes. Indeed, it appears that all *D. mccartyi* strains can be grown in simple mineral defined medium in which the only organic amendments are MOPS buffer, acetate, and vitamins (Löffler et al. 2013).

An important contribution to our understanding of biosynthesis in *D. mccartyi* came from the studies of incorporation of positionally labeled ^{13}C-acetate and $^{13}CO_2$ into amino acids and peptides (Marco-Urrea et al. 2012; Tang et al. 2009) which demonstrated biosynthesis of all 17 of the readily analyzed amino acids, including methionine. In *D. mccartyi* genome sequences, there was no gene annotated as encoding citrate synthase, yet this enzyme is essential for glutamate synthesis. Positional labeling of glutamate indicated that a *re*type citrate synthase was used (Tang et al. 2009) in strain 195. That enzyme was demonstrated in strain CBDB1 (Marco-Urrea et al. 2011b) and is encoded by CbdbA1708, originally annotated as a homocitrate synthase.

A particularly interesting scenario applies to acetyl-CoA metabolism. While the genomic and biosynthetic studies showed that acetate is used as a carbon source as had been expected, it was noted early on (Seshadri et al. 2005) that *D. mccartyi* had an incomplete Wood-Ljungdahl pathway for acetyl-CoA biosynthesis/degradation,

missing methylene-tetrahydrofolate reductase, and the carbon monoxide dehydrogenase (CODH) subunit of the CODH/acetyl-CoA decarbonylase-synthase (ACDS) complex, a curious finding.

It was recently demonstrated that the methyl group of methionine is derived from the methyl group of acetate (Zhuang et al. 2014) in a manner explained by splitting of acetyl-CoA by ACDS into a methyl group transferred to tetrahydrofolate, CoA, and CO. Since there is no CODH to oxidize CO to CO_2, it is released into the growth medium, where it was demonstrated to accumulate to levels as high as 0.1 % a level that can completely inhibit growth. Thus, in pure culture *D. mccartyi* eventually poisons itself. It had been known that the presence of a *Desulfovibrio* strain allowed much better growth of *D. mccartyi*, and it was found that the *Desulfovibrio* could use CO bringing its concentration down by an order of magnitude. These results may explain the common finding that *D. mccartyi* can grow to much higher densities in bioreactors than in pure cultures, and often the purer a culture becomes the worse *D. mccartyi* grows. In natural systems *D. mccartyi* can outsource CO utilization, common in many anaerobes, much as it outsources corrinoid biosynthesis, in line with it's having a stripped-down genome. A theory on the strategy of gene loss by an organism when other organisms are providing "public goods" is called the Black Queen hypothesis (Morris et al. 2012) with examples of *Prochlorococcus* and *Pelagibacter ubique*, both marine organisms with stripped-down genomes that outsource various functions, such as detoxification of radical oxygen species, to other organisms.

Strain 195, alone of all *D. mccartyi* with annotated genomes, also has a set of genes encoding the nitrogenase enzyme complex (*nif*, DET1151-8) and an associated Mo transporter (DET1159-61). The *nif* genes are in the family typically found in anaerobes, and are most closely related to those found in deltaproteobacterial sulfate reducers (Lee et al. 2009). The mol% G + C of the *nif* genes was 49–54, somewhat higher than the genomic average (49 %), suggesting a fairly recent genetic transfer. When grown in medium limited for fixed nitrogen, incorporation of $^{15}N_2$ was detected, but little growth occurred (Lee et al. 2009). Diazotrophic growth may occur under conditions more natural than in a batch culture, which may contain inhibitory levels of H_2 or CO.

6.5 Habitat and Ecology

The natural ecology of *D. mccartyi* is poorly understood. It has been cultured from ostensibly pristine sites as well as contaminated ones. As mentioned previously, there are thousands of natural organohalide compounds in terrestrial and marine habitats (Gribble 2010) so there can be a general rationale for the presence of *D. mccartyi* and other dehalogenators in anaerobic niches in these habitats. A more specific example comes from recent studies (Krzmarzick et al. 2012) in which soil humus was treated with chloroperoxidase, an enzyme commonly used by fungi for lignin degradation, which caused higher amounts of carbon-bonded chlorine in the humus.

Adding this treated humus to soils stimulated the growth of *Chloroflexi* related to *D. mccartyi*, believed to be dehalogenating, although not of *D. mccartyi* itself.

While *D. mccartyi* has been found worldwide, there is evidence that it is not present at all PCE- or TCE-contaminated sites, where its absence leads to "DCE stall" since organisms that dechlorinate as far as DCE are considered ubiquitous, whereas *D. mccartyi*, needed for full dechlorination to ethene, is not. Early results with PCR supported this hypothesis (Hendrickson et al. 2002) as have bioaugmentation studies (Ellis et al. 2000; Major et al. 2002) in which addition of *D. mccartyi*-containing cultures allowed more complete dechlorination in microcosms and at contaminated sites. PCR tests for *D. mccartyi* and other dehalogenators are now often part of contaminated site characterization, and *D. mccartyi*-containing cultures for bioaugmentation at contaminated sites have been commercially developed, a topic covered elsewhere in this volume.

The sporadic distribution of *D. mccartyi* at contaminated sites contradicts the maxim in microbial ecology attributed to Bejierinck and Baas-Becking (de Wit and Bouvier 2006) that "everything is everywhere and the environment selects." A recent finding suggests that *D. mccartyi* may be more cosmopolitan than originally thought (Delgado et al. 2014). Microcosms constructed from pristine garden soil and mangrove sediments using lactate or methanol as electron donors showed DCE stall, but when material was transferred to growth medium, *D. mccartyi* that carried out complete dechlorination to ethene grew, and carried out complete dechlorination when added back to the microcosms. The authors suggested that *D. mccartyi*, present in low numbers, was not competitive with methanogens and other hydrogenotrophs in the microcosms, but was competitive after a round of enrichment, a finding that needs to be verified in other samples.

6.6 Conclusions

The isolation of *D. mccartyi* is an example of how examining an applied problem, chloroethene contamination of groundwater, can lead to some very interesting and important fundamental science, a tradition in microbiology since Pasteur studied "diseases" of wine and beer. Had humankind avoided polluting groundwater with organohalides, the *Dehalococcoidia* would most likely be yet another small branch on the great tree of 16S rRNA sequences from uncultured organisms. It was also serendipitous that Jim Gossett, an environmental engineer with a good appreciation of microbiology, and myself, with a strong interest in chemical transformations carried out by microorganisms and a knack for culturing difficult anaerobes, were at the same institution and were friendly. However, it should be pointed out that several other groups were studying the microbiology of reductive dechlorination of chloroethenes in the early 1990s, and discovery of *Dehalococcoides* would barely have been delayed had we not begun our studies.

D. mccartyi is one of the most unusual bacteria ever described. It has a cell wall resembling those of *Archaea* and is among the smallest bacteria known with

one of the smallest genomes found in a free-living bacterium. That it can use only H_2 as an electron donor and organohalides as electron acceptors makes it one of the most metabolically specialized organisms known, comparable to some methanogens that only use H_2–CO_2. Despite their streamlined genomes, *D. mccartyi* strains contain 10–36 distinct sets of *rdhAB* genes, most with adjacent transcriptional regulator genes, suggesting sophisticated regulation in this "one trick pony." We know the substrates for only a small fraction of the RDases in *D. mccartyi* and other organohalide reducing bacteria, and discerning the functions of the vast majority of RDases is a major unsolved problem.

D. mccartyi grows so poorly in pure culture that often turbidity is not visible, yet it often thrives in anaerobic groundwater habitats, driving the turnover of tons of organohalides worldwide. It has provided a fascinating glimpse into how organisms evolve in response to human activity, in this case the anthropogenic release of large amounts of organohalides. We still have much to learn about this tiny but powerful organism.

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