Engineering bacteria for the degradation of halopropanes

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SUMMARY AND CONCLUDING REMARKS
Bacteria can consume an enormous variety of organic chemicals as carbon and energy source. Exposure to natural organic compounds has provided the selective pressure to drive the evolution of catabolic enzymes and pathways over millions of years, yielding organisms capable of degrading or modifying many unpalatable, yet natural, chemicals. However, over the past 100 years human activities have introduced many xenobiotic halogenated organics into the environment. Most of these compounds contain structural elements to which microorganisms have not been exposed during the course of evolution. Organohalogens are therefore likely to resist biodegradation. Due to their toxicity and persistence, organohalogens pose a serious risk to ecosystems and human health. Nevertheless, nature itself is partially solving the problem of increasing environmental pollution, as illustrated by the existence of microorganisms that have the ability to use synthetic organohalogens as sole carbon and energy source (Chapter 1). Still, many other organohalogens persist in the environment indicating the current inability of the natural microorganisms to deal with all recalcitrant pollutants. Over time, many of these currently recalcitrant chemicals may still begin to disappear from soil and water, due to the further evolution of catabolic activities. But do we really have to wait for the natural evolution of enzymes and metabolic routes, or can we accelerate this process? Genetic analysis of the bacteria involved in degradation of xenobiotics has shown that adaptation to halogenated compounds occurs through a natural genetic engineering process (3, 14, 19). Gene technology offers tools to emulate this process in the laboratory, which could accelerate the development of organisms with new activities. In particular, engineering of bacteria that grow on recalcitrant halogenated compounds for which there is no existing degradative pathway is challenging.

The work described in this thesis was focused on the construction of bacteria for the degradation of 1,2,3-trichloropropane (TCP). TCP is found as a significant groundwater contaminant in various places in Europe and the United States. With estimated half lives under groundwater conditions of up to hundred years, TCP will persist for long periods of time in the groundwater environment (18). Furthermore, TCP is very resistant to natural biodegradation under aerobic conditions. No natural strains have been isolated which are able to metabolize TCP. TCP is also of industrial importance. In the United States TCP is considered as a high volume chemical (30). In addition, it is formed in large amounts as a coproduct during manufacture of the bulk chemical epichlorohydrin (26). The primary question addressed in this work is therefore: can we accelerate biodegradation of TCP and obtain bacterial utilization of TCP by genetic engineering of a designed catabolic pathway? In this chapter I summarize the results and discuss the engineering of a TCP degradative pathway. Furthermore, I will illustrate some new approaches and prospects for improving enzymes and bacteria for environmental applications.

Biodegradation of TCP

For the engineering of a TCP degradative pathway naturally occurring microbial activities are the starting point. Despite extensive efforts, attempts to enrich microbial cultures that can utilize TCP from a variety of inocula were unsuccessful (unpublished
observations). For example, selection of organisms capable of degrading TCP was attempted in small fixed-bed bioreactors. These reactors were considered to provide an optimal system for the selection of natural TCP degraders. First, biofilm formation minimizes washout of cells. Second, due to degradation of secondary carbon sources, substrate gradients are formed along the column, increasing the selective pressure of TCP. Finally, exchange of genetic material is enhanced in biofilm systems. Different mixtures of TCP and secondary carbon sources, such as 1,2-dichloroethane (DCE), 1-chlorobutane (1-CB), 1,2,3-tribromopropane (TBP) and 2,3- and 1,3-dichloropropanol, were added at a concentration of 200 to 500 µM each to maintain the desired degradation capacity. The reactor was inoculated with samples from TCP-contaminated soil and a variety of haloalkane-degrading bacteria, providing a large microbial diversity. Under the applied conditions, no biodegradation of TCP was observed. The applied TCP levels (50 to 100 µM) did not inhibit the desired degradation capacity, since biomass was formed due to the utilization of secondary carbon sources, and complete removal of 2,3- and 1,3-dichloropropanol, DCE, 1-CB was always observed. Furthermore, prolonged selection over more than a year did not result in adaptation of the microbial community to TCP and no significant removal was ever observed. These observations suggest that adaptation to TCP require genetic changes within and/or between microorganisms to improve catabolic activities, which occur very infrequently under these conditions. Nevertheless, microbial activities towards TCP have been described (Chapter 1). Comparative studies on the bacterial degradation of haloalkanes indicated the important role of dehalogenating enzymes in the metabolism of these compounds. Since dehalogenating enzymes are often the first enzymes in a catabolic pathway they usually define the range of substrates that can be metabolized by an organism. The relatively high degree of chlorine substitution of TCP makes this compound resistant to enzymatic degradation, and dehalogenases with sufficient activity may evolve at such a low frequency that they never appeared in the reactors.

Chlorinated compounds with multiple chlorine substituents can often be dehalogenated by aspecific oxygenases due to the spontaneous elimination of a halogen from unstable products that are generated during oxygenation. This oxygenolytic dehalogenation is catalyzed by aspecific oxygenases and plays an important role in the cometabolic degradation of compounds, which are otherwise not mineralized aerobically. The large number of oxygenases in the University of Minnesota Biocatalysis/Biodegradation Database (http://umbbd.ahe.umn.edu/) indicates their importance in xenobiotic degradation. Probably the best-studied monooxygenase for this type of conversions is the soluble methane monooxygenase from Methylosinus trichosporium OB3b. The enzyme is able to oxidize various organic compounds, including haloalkanes. Resting cell suspensions of M. trichosporium OB3b expressing soluble methane monooxygenase (sMMO) were shown to cometabolically convert TCP and a number of other chlorinated propanes (Chapter 2). However, compared to the well-described cometabolic conversion of trichloroethylene, chlorinated propanes are poor substrates. The lowest conversion rate was obtained with TCP. A computational study based on the hydroxylase component of the sMMO suggests that
relatively large substrates, such as chlorinated propanes, probably do not fit in the active site (7). Instead, bulky substrates are bound at positions approximately 14 Å from the active site, near the surface of the hydroxylase protein. It was proposed that a conformational change might be needed for access of large substrates to the active site of sMMO, which may be an explanation for the low conversion rate of TCP (7). Furthermore, we found that degradation of chlorinated propanes was inefficient due to turnover-dependent inactivation of the enzyme. Chlorinated propanols were found as products from chlorinated propane conversion. Conversion of TCP to chlorinated propanols would be a preferable reaction, since chlorinated propanols are easily degradable by various Gram-negative bacteria (Chapter 1). Moreover, sMMO catalyzed oxygen insertion into the carbon-chlorine bond has been suggested to play a role in the conversion 1,2-dichloroethane to 2-chloroethanol. For TCP the initial dehalogenation reaction is most likely due to oxygen insertion into the carbon-hydrogen bond, yielding 2,3-dichloropropionaldehyde and 1,3-dichloroacetone. A small fraction of the latter two products was slowly reduced to the corresponding chlorinated alcohols by cells of *M. trichosporium* OB3b.

Haloalkane dehalogenases were subsequently considered as candidates for the dehalogenation of TCP. These enzymes are able to hydrolyze carbon-chlorine bonds in wide range of haloalkanes (Chapter, 3, 4, (23, 24)). Presently, a substantial amount of mechanistic and structural information is available on the haloalkane dehalogenases from *Xanthobacter autotrophicus* GJ10 (DhlA) (23, 27, 28), *Rhodococcus rhodochrous* NCIMB13064 (DhaA) (16), and *Sphingomonas paucimobilis* UT26 (LinB) (13). Whereas, the latter dehalogenase exhibited no activity on TCP (J. Damborský, pers. commun.), DhlA showed marginal activity on this compound (Chapter 4). In Chapter 4 DhaA was reported to be the first haloalkane dehalogenase with significant activity on TCP. The corresponding *dhaA* gene was found to be widespread within the genus *Rhodococcus*. Moreover, a *Rhodococcus* strain containing the *dhaA* gene was isolated from a TCP-contaminated chemical waste site (21). Nevertheless, the kinetic properties of DhaA on TCP are rather poor with a low turnover number and a high $K_M$. The conversion rate of TCP by DhaA was considerably slower than rates observed with analogous brominated substrates. The poor activity of DhaA on TCP may result from non-productive binding in the enzyme active site. The relatively large enzyme active site may allow TCP to bind in alternative binding modes, which do not always result in dehalogenation. This is supported by docking calculations of wild-type DhaA with TCP, which showed three significantly different binding modes (Chapter 5). TCP binds mainly in a position that is unfavorable for nucleophilic attack of Asp106 on the $C_\alpha$ atom of the substrate. Instead, the $C_\beta$ of TCP appears to be closest to the nucleophilic oxygen of Asp106. Nucleophilic substitution on the $C_\beta$ of TCP would yield 1,3-dichloro-2-propanol. However, this compound has never been observed during dehalogenation of TCP by DhaA.

Kinetic analysis showed that the overall dehalogenation pathway of DhaA consists of four main steps involving substrate binding, formation of an alkyl-enzyme intermediate and simultaneous cleavage of the carbon-bromine bond, hydrolysis of the alkyl-enzyme intermediate, and finally release of the products from the enzyme active site. The overall
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kinetic mechanism of DhaA solved with 1,3-dibromopropane was similar to that of DhlA for 1,2-dichloroethane (Chapter 3). However, whereas in DhlA the rate of halide release represents the slowest step in the catalytic cycle, our results suggest that in DhaA the liberation of 3-bromo-1-propanol is the slowest step during 1,3-dibromopropane conversion. A complete kinetic mechanism of product release has not been determined, however, because the binding steps are not associated with significant changes in intrinsic enzyme fluorescence, as in DhlA.

Engineering a pathway for 1,2,3-trichloropropane metabolism

The strategy of choice to generate a recombinant bacterium able to degrade TCP is summarized in Fig. 1 (Chapter 4). The assembled pathway is obtained by including a DNA segment encoding the haloalkane dehalogenase DhaA of *Rhodococcus* sp. strain m15-3, which affords the conversion of TCP to 2,3-dichloro-1-propanol. The second element was provided by the host organism *Agrobacterium radiobacter* AD1, and encodes the haloalcohol dehalogenase HheC and epoxide hydrolase EchA. HheC dehalogenates 2,3-dichloro-1-propanol via an intramolecular substitution reaction, yielding epichlorohydrin. The latter substrate is subsequently hydrolyzed through the action of epoxide hydrolase EchA. The resulting 3-chloro-1,2-propanediol can be used as growth substrate by *A. radiobacter* AD1. The *dhaA* gene was engineered under control of the *dhlA* promoter, which yielded constitutive expression of DhaA in various Gram-negative bacteria, including *A. radiobacter* AD1 (9). This was necessary to obtain sufficient expression of the *dhaA* gene, since the efficiency of halogenated compound removal is not only influenced by the kinetic properties, but also by the cellular content of the first catabolic enzyme (29). An RSF1010-derived broad host range vector was used to transfer the *dhaA* gene with the *dhlA* promoter to strain AD1. The DhaA phenotype of the recombinant strain was stably inherited.

The strategy described above was used to construct strain AD1(pTB3). The *dhaA* gene was functional and constitutively expressed in strain AD1. Consequently, strain AD1(pTB3) possessed not only the genetic information, but also the functional ability to degrade trihalogenated propanes, including TCP (Fig 2, Chapter 4). Because of the high activity of DhaA on the brominated analogs of TCP, 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane, the recombinant strain was able to rapidly dehalogenate these compounds to the corresponding haloalcohols, which were subsequently utilized by the host strain.
Since TCP is a poor substrate for DhaA, strain AD1(pTB3) converted TCP at a much lower rate, which was not sufficient to sustain bacterial growth.

Fig. 2. Reactions and enzymes involved in the degradation of trihalogenated propanes in *A. radiobacter* AD1(pTB3).

**Directed evolution of haloalkane dehalogenase**

The results presented in *Chapter 4* pinpoint the slow initial dehalogenation of TCP by DhaA as the bottleneck for the utilization of this compound by the recombinant strain. To address this problem we applied directed evolution techniques to the *dhaA* gene to improve the kinetic properties of DhaA for TCP conversion (*Chapter 5*). Directed evolution offers the possibility of doing in the laboratory what nature has not yet accomplished. Evolutionary protein engineering methods are based on the accumulation over multiple generations of beneficial mutations, which are produced randomly and fixed by selective pressure (*Chapter 1*). For successful directed evolution there are a number of requirements (1). First, the desired function should be physically feasible. Second, the function should be evolutionary accessible, in other words, there must exist a mutational pathway to travel from the starting sequence to the product sequence through identifiable variants. Finally, it should be possible to make and screen libraries of mutants complex enough to contain the rare beneficial mutations, required to follow the mutational pathway.

Using directed evolution for improving enzyme function is not a trivial task, but requires a good strategy to maximize the chances of success. Of the known haloalkane dehalogenases, DhaA exhibited the highest activity on TCP and was therefore selected as starting point for directed evolution. The dehalogenase was evolved by random
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Mutagenesis of the \textit{dhaA} gene (Chapter 5). A point mutation rate of 3-4 base substitutions per gene was applied, which will yield a library of DhaA variants containing mainly single amino acid substitutions. Higher error rates generate neutral and deleterious mutations, which might slow down evolutionary progress. Moreover, the number of variants one can generate by introducing point mutations increases dramatically with the number of mutations. While there are about 5,700 possible single mutants of DhaA, there are already $16 \times 10^6$ possible double mutants. Because most mutations have a negative effect, the chances of identifying improved enzymes decreases rapidly with increasing mutation rate. On the other hand, single amino acid changes may often yield only small improvements, which will require a sensitive screen to identify improved variants. Up to $10^{12}$ variants can be tested by selection for growth, while screening, for example in microtiter plates or on agar plates, allows analysis of about $10^6$ different variants.

The most time-consuming part of directed evolution of haloalkane dehalogenase is the screening of mutant libraries for improved variants. An efficient search strategy would be selection for growth on TCP using the recombinant strain AD1(pTB3). However, cloning of mutant \textit{dhaA} libraries into strain AD1 occurs with low efficiency relative to \textit{E. coli}. Selection in strain AD1 is therefore relatively unattractive because of the limited size of the mutant library that can be obtained (third requirement).

Therefore we screened the mutated \textit{dhaA} library in \textit{E. coli} (Chapter 5). The screening consisted of using agar plates containing the indicator mixture eosine-methylene blue and scoring TCP dehalogenation as the development of a red color in the colonies producing active DhaA variants. The development of red colonies is due to the production of HCl during dehalogenation of TCP, which causes mobilization and subsequent accumulation of the indicator dyes into acid-producing colonies. The constitutive expression of the \textit{dhaA} gene in \textit{E. coli} made additional induction step superfluous. The mutant \textit{dhaA} libraries were visually scored for colonies with enhanced red color formation, indicating improved TCP conversion. In this way, two rounds of random mutagenesis and screening of approximately 10,000 transformants per round yielded a double mutant containing the amino acid substitutions Cys176Tyr and Tyr273Phe. The evolved dehalogenase was nearly eight times more efficient in dehalogenating TCP than wild type DhaA. Two other variants of DhaA have been described which also are mutated at position 176. In DhaA\textsubscript{f}, which was isolated from the DBE-utilizing bacterium \textit{Mycobacterium} sp. strain GP1 (20), and a random variant of DhaA obtained in an \textit{in vitro} evolution experiment (8). In both cases the cysteine residue at position 176 was replaced by a phenylalanine. This suggests that for the conversion of small haloalkanes an aromatic side chain is preferred at this position. The phenylalanine at position 273 which is introduced during the selection for improved TCP conversion, was strictly conserved in other haloalkane dehalogenases except for wild-type DhaA which contained a tyrosine at this position (5).

To obtain insight in the mechanism of improvement we performed docking calculations of TCP in the active site of the wild-type and evolved dehalogenase (Chapter 5). The Cys176Tyr substitution appears to cause a two-fold reduction of the active site volume of
the dehalogenase. Whereas the relatively larger active site of the wild-type enzyme provides space for nonproductive binding of TCP, the smaller active site of the mutant DhaA seems to restrict TCP to bind productively to a larger extent. The binding of TCP was further fine tuned by the Tyr273Phe mutation, which causes more subtle changes. The improved binding of TCP in the active site of the mutant DhaA resulted in more efficient catalysis.

**Performance of the engineered pathway**

Using plasmid-encoded catabolic genes, expression with a strong constitutive promoter and directed evolution of the key catabolic enzyme, adaptation to TCP was accelerated, which is apparently a slow process under natural conditions. The resulting recombinant strain AD1(pTB3-M2) was able to utilize TCP for growth, which, to our knowledge, has not been described before. Nevertheless, a number of aspects of the constructed catabolic pathway are clearly not optimal (Chapter 4, 5).

The first and major deficit is the still moderate catalytic effectiveness of DhaA. Despite considerable progress in improving the activity of DhaA on TCP, it is still the rate-limiting step in the metabolism of TCP by the recombinant strain AD1(pTB3-M2). The recombinant strain with the evolved dehalogenase was able to grow on TCP with a doubling time of roughly 90 h. The host strain _A. radiobacter_ strain AD1 grows with a doubling time of about 7 h on 2,3-dichloro-1-propanol, the product of hydrolytic dehalogenation of TCP by DhaA. The conversion rate of TCP by the evolved dehalogenase, therefore, appears to limit the ability of the host bacterium to degrade 2,3-dichloro-1-propanol rapidly enough to sustain a high growth rate. It is likely that DhaA is amenable to further improvement using more rounds of mutagenesis and screening or selection.

The second problem is the enantioselective conversion of 2,3-dichloro-1-propanol by _A. radiobacter_ strain AD1. The host bacterium can only use (R)-2,3-dichloro-1-propanol as sole carbon and energy source, since the responsible enzyme, the haloalcohol dehalogenase HheC, converts only the (R)-enantiomer, while no reaction occurs with the (S)-enantiomer (12). Since DhaA generates both enantiomers of 2,3-dichloro-1-propanol during conversion of TCP, this will limit the amount of substrate that can be funneled through the pathway and utilized for growth. Fortunately, the wild-type and evolved DhaA produce predominantly (R)-2,3-dichloro-1-propanol (≈ 70%) which can be metabolized by strain AD1. Whereas, from a biocatalytic perspective, this enantioselectivity is a highly desirable feature (10), it is undesirable for environmental purposes since halogenated products will accumulate unless they are degraded by other bacteria in a mixed culture.

A third aspect for further improvement is the regulation of gene expression. Whereas expression of the haloalkane dehalogenase is constitutive, expression of the haloalcohol dehalogenase and epoxide hydrolase is inducible by epichlorohydrin. Ideally, expression of the enzymes involved in the degradation of TCP should be coordinately regulated. Although inducible expression is often preferred, strong inducible promoters may hinder rather than favor the expression of genes for the degradation of some carbon sources, since induction of promoter activity is frequently overruled by physiological signals if the metabolic status of
the organism is not suitable (2). Depending on the application constitutive expression of catabolic genes could be a suitable alternative. The 1,2-dichloroethane utilizing bacterium *X. autotrophicus* GJ10, which constitutively produces haloalkane dehalogenase DhlA, has been successfully applied in a full-scale groundwater treatment plant (25).

A final general consideration involves the use of recombinant bacteria in environmental applications. Such applications will likely involve open systems from which engineered organisms can escape. The release of genetically modified organisms into the environment containing plasmid-encoded artificial gene constructs and antibiotic resistance genes has raised questions about the possible effects on microbial ecology and biodiversity. To minimize the lateral spread of the *dhaA* gene with the *dhlA* promoter into the indigenous microbial population, the gene construct should be integrated into the chromosome of the host strain. A number of molecular tools based on mini-transposon vectors have recently been developed to allow stable integration of genes into the chromosome of recipient strains (6). In addition, chromosomal integration may also increase the stability of the cloned genes. Furthermore, antibiotic resistance markers used for selection must be removed or replaced by non-antibiotic markers.

The fact that we can evolve in the laboratory a bacterium that can utilize TCP as growth substrate indicates that it is also possible that such a strain may evolve in nature. However, in spite of extensive attempts, no bacteria have been found that mineralize TCP. This could be due to the relatively recent entry of TCP in the environment (~ 40 years), allowing microorganisms insufficient time to evolve the appropriate pathways. The results described in this thesis may shed some light on the evolutionary potential of the natural microbial population for dealing with TCP. We have shown that the natural evolution of a catabolic pathway for TCP necessitates multiple genetic events and the simultaneous presence of haloalkane- and haloalcohol dehalogenase producing bacteria. The *dhaA* gene was found relatively widespread within the genus *Rhodococcus* (21). Other haloalkane dehalogenase genes have been detected during sequencing of bacterial genomes, but the activity of the corresponding enzymes for TCP may be low. Using 1-chlorobutane as the selective substrate *Rhodococcus* strains harboring the *dhaA* gene can be easily isolated from soil samples from contaminated sites. Moreover, the *dhaA* gene was also found at TCP contaminated sites (21). The problem presented by TCP to these *Rhodococcus* strains is that it does not induce the synthesis of DhaA. This requires changes in the regulation of the expression of the *dhaA* gene. Furthermore, to obtain a productive degradation pathway for TCP the *dhaA* gene must be linked to a 2,3-dichloro-1-propanol catabolic route. This could be achieved by changing an existing pathway of the *Rhodococcus* host to enable degradation of 2,3-dichloro-1-propanol, recruitment of a catabolic pathway for 2,3-dichloro-1-propanol, or the transfer of the *dhaA* gene into 2,3-dichloro-1-propanol degrading bacteria. Database searches indicate that haloalcohol dehalogenase genes do not commonly occur in bacterial genomes. However, even when the basic genetic information for TCP conversion has been assembled the conversion rate of TCP by DhaA must be increased since the activity of the wild-type enzyme is insufficient to support growth. This involves elevation of the expression
level of the \textit{dhaA} gene and/or altering the activity and specificity of DhaA. Thus, the assembly of a productive TCP catabolic pathway requires multiple genetic changes, which occur probably very rarely in nature and even more seldom in a single organism.

**Prospects for engineering**

The recent developments in directed evolution methods and high-throughput screening technologies allow the rapid evolution of improved enzymes and pathways for the degradation of xenobiotics. A number of strategies can be used to improve enzyme function.

Recombination-based methods, such as DNA-shuffling, are most effective, in particularly the recombination of a number of related genes (15). The three most well characterized haloalkane dehalogenases are DhI, DhaA, and LinB. However, the pairwise DNA sequence identity of the genes encoding these enzymes varies between 40 to 60\%, which is not sufficient for current recombination methods. Although there have been strategies developed that do not rely on sequence homology, thus far these methods have not yielded improvement in enzyme function (17). DNA-shuffling is not limited to evolving a single gene, but can also be applied to a whole operon (4). An artificial operon comprising the genes involved in the TCP degradative pathway, \textit{dhaA}, \textit{hheC}, and \textit{echA}, could possibly be evolved for improved TCP conversion. Moreover, this may simultaneously yield variants of HheC with reduced enantioselectivity. Recently, a method was developed to rapidly evolve microbial activities by recursively recombining entire genomes (31). With respect to bioremediation, genome shuffling has the advantage of allowing optimization of multiple properties. In bioremediation enzyme kinetics is not the only issue, but there are also a number of other important properties, such as solvent tolerance, resistance to toxic intermediates or side products, and production of surfactants to enhance bioavailability, which might affect pollutant removal under environmental conditions.

A more rational approach to change substrate specificity of key enzymes would be domain shuffling. This involves combining existing structural and functional elements. This approach has been successfully applied to biphenyl dioxygenase, of which the chimeric enzymes showed broader substrate specificity (11). Haloalkane dehalogenases comprises two domains: an \(\alpha/\beta\)-hydrolase catalytic core, and a helical cap domain which lies on top of the catalytic core. Mutagenesis studies indicated the importance of the cap domain in determining substrate specificity (22). Furthermore, the primary sequence of haloalkane dehalogenases diverges most in the cap domain. Since different haloalkane dehalogenases are evolutionary optimized on different substrates, exchanging of cap domains followed by directed evolution for fine-tuning could lead to enzymes with modified substrate ranges.

**Conclusion**

Until recently, engineering bacteria for improved bioremediation has been successful for a limited number of pollutants. The design of bacteria able to mineralize environmental pollutants for which no degradative pathway is known is therefore a challenging goal. The work described in this thesis was focused on the design of a catabolic pathway for the
environmentally and industrially important chlorinated hydrocarbon 1,2,3-trichloropropene. The development of new catabolic activities depends equally on our understanding of the metabolic pathway involved and on the possibility to improve the activities of the individual enzymes. The major bottleneck in bacterial utilization of TCP is the initial dehalogenation to the corresponding dichloropropanol catalyzed by a haloalkane dehalogenase. The conversion rate of TCP was increased by a high expression level, and directed evolution of haloalkane dehalogenase DhaA. This enabled us to construct a recombinant bacterium that could use 1,2,3-trichloropropene as sole carbon and energy source.

The initial excitement about the ability of genetically engineered microorganisms to transform toxic and persistent man-made pollutants into harmless products should now be translated to the development of commercially viable remediation processes. TCP is an excellent target chemical for this development because of its environmental relevance and the technological feasibility described in this thesis.