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Trichloroethylene cometabolic degradation by *Rhodococcus* sp. L4 induced with plant essential oils

Oramas Suttinun · Rudolf Müller · Ekawan Luepromchai

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Abstract Cometabolic degradation of TCE by toluene-degrading bacteria has the potential for being a cost-effective bioremediation technology. However, the application of toluene may pose environmental problems. In this study, several plant essential oils and their components were examined as alternative inducer for TCE cometabolic degradation in a toluene-degrading bacterium, *Rhodococcus* sp. L4. Using the initial TCE concentration of 80 μM, lemon and lemongrass oil-grown cells were capable of 20 ± 6% and 27 ± 8% TCE degradation, which were lower than that of toluene-grown cells (57 ± 5%). The ability of TCE degradation increased to 36 ± 6% when the bacterium was induced with cumin oil. The induction of TCE-degrading enzymes was suggested to be due to the presence of citral, cumin aldehyde, cumene, and limonene in these essential oils. In particular, the efficiency of cumin aldehyde and cumene as inducers for TCE cometabolic degradation was similar to toluene. TCE transformation capacities (T_c) for these induced cells were between 9.4 and 15.1 μg of TCE mg cells^{-1}, which were similar to the known toluene, phenol, propane or ammonia degraders. Since these plant essential oils are abundant and considered non-toxic to humans, they may be applied to stimulate TCE degradation in the environment.

Keywords Trichloroethylene · Cometabolic degradation · Plant essential oils · Toluene-degrading bacteria · *Rhodococcus*

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Introduction

Contamination of soil and groundwater with chlorinated solvents, especially trichloroethylene (TCE) has become an important problem because of its toxicity and persistence. Under aerobic condition, TCE biodegradation mainly occurs through cometabolic degradation, in which TCE-degrading bacteria utilize methane, ammonia, propane, phenol, toluene or cumene as growth substrate (Alvarez-Cohen and Speitel 2001). Consequently, bioremediation trials

have been conducted by injecting methane (Moran and Hickey 1997; Ohlen et al. 2005), phenol (Lee 2003; Chen et al. 2004), and toluene (Hopkins and McCarty 1995; Kuo et al. 2004) to promote the growth of TCE-degrading bacteria in contaminated soil and groundwater. Toluene-degrading bacteria are the largest group of aerobic TCE degraders and widely distributed in the environments (Morono et al. 2006). However, the application of toluene may pose potential regulatory problems, thus alternative compounds for the induction of toluene/TCE-degrading enzymes are required.

Singer et al. (2003) suggested that the introduction of plant secondary metabolites would be acceptable for enhancing bioremediation of subsurface soil and aquifers since these compounds are effective at low concentrations and are considered non-toxic. The most studied metabolites are components of plant essential oils, which have been reported to induce polychlorinated biphenyls (PCB) degradation by several bacteria. For example, carvone and limonene, the principal components of spearmint and lemon oil are able to induce PCB biotransformation in *Arthrobacter* sp. B1B, *Alcaligenes eutrophus*, and *Pseudomonas stutzeri* (Gilbert and Crowley 1997; Koh et al. 2000; Tandlich et al. 2001). In addition, the application of plant materials rich in essential oil such as orange peels, ivy leaves, pine needles or eucalyptus leaves to contaminated soil was reported to stimulate the growth of biphenyl utilizers and simultaneously induce Aroclor 1242 and 1248 degradation (Hernandez et al. 1997; Dzantor and Woolston 2001). The induction ability of these essential oil components is probably due to the similarity between their chemical structures and the pollutants (Crowley et al. 2001; Singer et al. 2003).

In this study, plant essential oils and their components were tested for the ability to induce TCE degradation in *Rhodococcus* sp. L4, a toluene-degrading bacterium isolated in Thailand. The utilization of plant essential oils as a substrate and/or inducer for TCE biodegradation has not been studied before. However, four essential oil components including cumene, limonene, carvone and pinene were previously tested for induction of TCE degradation in *Rhodococcus gordoniae* P3 (Suttinun et al. 2004). Cumene was more effective than other essential oil components. Similarly, *Rhodococcus erythropolis* BD2 utilized cumene as a substrate for TCE cometabolism (Dabrock et al. 1992, 1994). However, this compound can not be applied to the environment due to its potential toxicity (http://toxnet.nlm.nih.gov/).

To find alternative plant-derived inducers, we tested another strain of *Rhodococcus*. Bacteria of this genus are interesting since they can degrade a wide variety of recalcitrant compounds and have therefore gained considerable interest for use in bioremediation of contaminated sites (Larkin et al. 2005). The strain L4 was able to cometabolize TCE after growth on toluene. To replace toluene, *Rhodococcus* sp. L4 were grown in mineral media containing a selected plant essential oil with/without glucose before conducting the TCE degradation assay. The ability of plant essential oils to induce TCE degradation was further evaluated with their purified oil components. The results confirmed that the components of plant essential oils were responsible for promoting TCE degradation. This study opens the prospect of applying plant essential oils, which are relatively safe and inexpensive, to stimulate TCE-degrading bacteria for TCE bioremediation.

Materials and methods

Chemicals

Plant essential oils including lemon oil, lemongrass oil, spearmint oil and pine oil were purchased from an aroma shop (Aromatherapy Hydration, Bangkok). Seeds of cumin (*Cuminum cyminum* L.) were obtained in one batch from its distributor (Nguan Soon, Bangkok). There was approximately 1.4–2.8% essential oil in cumin seeds (Beis et al. 2000; Jalali-Heravi et al. 2007). These essential oils contain several oil components that have previously been shown to be effective in stimulating xenobiotic degradation (Crowley et al. 2001). TCE (99.5%), toluene (99.5%) and eight essential oils components (cumene (99.0%), R-(+)-limonene (96.0%), cumin aldehyde (4-isopropyl benzaldehyde) (90.0%), (cis + trans) citral (95.0%), (+)-α-pinene (97.0%), (+)-carvone (99.0%), γ-terpinene (95.0%) and *p*-cymene (99.0%)) were obtained commercially from Merck, USA. TCE stock solutions were prepared by dissolving aliquots of TCE in N,N-dimethylformamide (Merck, USA) to obtain the desired concentration. All other chemicals were obtained from Fluka, Switzerland.

Bacterial strain, culture maintenance and growth conditions

Rhodococcus sp. L4 is deposited at the Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR) and given the accession number TISTR 1542. The partial 16S rRNA gene sequences of *Rhodococcus* sp. L4 is available in GenBank under the accession number EF527237. The bacterium used in this study was maintained by culturing on mineral salts (MS) agar incubated in a glass box equilibrated with toluene vapour at room temperature. MS medium is a chloride-free minimal medium prepared according to Focht (1994). The preparation of pre-inoculum was carried out by growing *Rhodococcus* sp. L4 in 100 ml MS medium that was supplied with toluene in the vapour phase. The culture was incubated overnight at room temperature with shaking at 200 rpm.

Inoculum preparation for TCE biodegradation test when toluene or plant essential oil was used as sole carbon source for *Rhodococcus* sp. L4

Rhodococcus sp. L4 was cultured by transferring 10 ml of pre-inoculum into a 250-ml Erlenmeyer flask containing 100 ml of MS medium. The optical density (OD) at 600 nm was about 0.06–0.08 at the beginning. The growth substrate was supplied as a vapour by adding 200 µl of toluene or plant essential oil to a sterilized Eppendorf tube fixed at the top of the flask. The vapor phase of cumin oil was achieved by placing 15 g of cumin seeds on a sterilized cheesecloth hung at the top of the flask. The cultures were incubated on an orbital shaker at 200 rpm, room temperature, for 24 h. Then, the cells were harvested by centrifugation at 2,516× *g* for 10 min. Cells were washed twice and resuspended in MS medium to a OD_{600} of 2.0 before they were used as inoculum in the TCE biodegradation test as described below.

Inoculum preparation for TCE biodegradation test when plant essential oil or its component was used as inducer for *Rhodococcus* sp. L4

The tested compound was applied at a low concentration when used as inducer for *Rhodococcus* sp. L4. Ten milliliter pre-inoculum was transferred to 100 ml MS medium containing 20 mM glucose as carbon and energy sources. The optical density (OD) at 600 nm was about 0.06–0.08 at the beginning. Stock solutions of each essential oil component i.e. cumene, limonene, cumin aldehyde and citral prepared in N,N-dimethylformamide were added to the culture to give a final nominal concentration of 80–400 µM. These concentrations showed no toxic effects on the bacteria and did not exceed their solubility. The optimum concentrations of each essential oil component, 400 µM citral, 80 µM cumin aldehyde or 200 µM limonene/cumene were applied as inducer for *Rhodococcus* sp. L4 for TCE degradation and dechlorination assay. For cumin oil, 10 g of cumin seeds was put on sterilized cheesecloth and hanged at the top of the flask. The culture was incubated overnight and harvested by centrifugation at 2,516× *g* for 10 min. Cells were washed twice with MS medium and resuspended in 10 ml MS medium containing either essential oil components or cumin seeds as described above. The repeated addition of these compounds was used to ensure adequate enzyme induction. After 1 h, the induced-cells were harvested, washed, and resuspended in MS medium to a final OD_{600} of 2.0 before they were used as inoculum in the TCE biodegradation test as described below.

TCE biodegradation assay

TCE biodegradation experiments were carried out in liquid culture by a method adapted from Nelson et al. (1987) and Luu et al. (1995). Generally, the experiments contained resting cell suspensions and TCE in a 22-ml headspace vial sealed with a Teflon-lined silicone septum and aluminum crimp cap. TCE stock solution was introduced to the vials containing 1 ml MS medium to give a final concentration of 160 µM before shaking at 200 rpm at room temperature for at least 2 h to achieve the equilibrium between gas and aqueous phase. The concentrations of TCE were calculated as if TCE would be completely dissolved in the aqueous phase. Biodegradation experiment was initiated by adding 1 ml inoculum with a sterile microsyringe into the vials. The final nominal concentration of TCE was equal to 80 µM. The vials were incubated at 200 rpm at room temperature. The final density of bacteria cells was 1.0 OD

(0.4 mg dry cells ml^{-1}). Cells dry weight was determined by measuring the mass difference between preweighed dry crucible with 10 ml of mineral salts (MS) medium added and those with 10 ml of bacterial culture added after both sets were incubated overnight at 103–105°C. Oxygen was provided by leaving 90% of air headspace in the tested vial only at the beginning of study to minimize the loss of TCE via volatilization. Two sets of triplicate samples were made at each time point for measuring TCE and chloride ion concentrations separately. The reactions were stopped by adding one drop of 10 M H_2SO_4 to the vials before analysis. The control sets consisted of heat-killed cells and non-induced cells containing TCE, which represented abiotic loss and degradation by non-induced bacteria, respectively.

Protein synthesis inhibition test

Chloramphenicol, a protein synthesis inhibitor was used to confirm that the enzymes involved in TCE biodegradation in *Rhodococcus* sp. L4 were not constitutively produced. At first, non-induced inoculum was prepared by growing cells on glucose–MS medium. The cells were harvested, washed, and resuspended in MS medium to a final density of 2.0 OD_{600}. Then, chloramphenicol was added to the inoculum to a final concentration of 310 μM (100 μg ml^{-1}). The suspension was shaken for 1 h before the assay was performed. Another set of samples containing glucose-grown cells in the absence of chloramphenicol was used as control. Then, 1 ml of the inoculum was mixed with 1 ml MS media containing 400 μM toluene and 160 μM TCE. During 25 h incubation, the amount of toluene and TCE was measured at intervals.

Quantitative analysis of toluene, TCE and chloride ions

The amount of toluene and TCE was analyzed by the PerkinElmer TurboMatrix Automated Headspace Sampler with the Clarus 500 gas chromatography equipped with a flame ionization detector (GC-FID) and a HP-5 (5% phenyl methyl siloxane) fused-silica capillary column (30 m × 0.32 mm ID; thickness, 0.25 μm). Sample vials were heated to 93°C for 30 min for equilibration. The head-space pressure was 20 psi. The gas chromatography conditions were as follows: injector temperature 150°C, detector temperature 250°C, initial column temperature 40°C (1.80 min) then, programmed at 40–55°C at a rate of 45°C min^{-1}, and 55–135°C at a rate of 10°C min^{-1}. The carrier gas (nitrogen) flow rate was 14 ml min^{-1}. The retention time of TCE and toluene were 1.78 and 2.05 min, respectively. External standard quantitative calibrations were performed for the analysis of toluene and TCE concentrations.

The concentrations of chloride ions generated from TCE dechlorination were monitored by an ion-sensitive chloride combination electrode (model 94-17B, Thermo Electron Formerly Orion Research, Inc., USA). A 1,000 ppm sodium chloride solution was used for calibration. Ionic strength adjustor, $NaNO_3$ was added at 2% (v/v) before measuring the calibration standards and samples in a stirred beaker.

Results and discussion

TCE cometabolic degradation activity of toluene-grown *Rhodococcus* sp. L4

The resting cells of toluene-grown *Rhodococcus* sp. L4 were able to cometabolize TCE effectively. About 50 ± 5% of the initial TCE remained after 4-h incubation (Fig. 1a, b). The TCE was dechlorinated as seen from the gradual increase of chloride ions in this experiment. On the other hand, only 1–8% TCE was lost and nearly no chloride ions were generated by killed-cells and glucose-grown cells (Fig. 1a, b). Thus, TCE removal by abiotic processes and non-induced bacteria were considered to be insignificant in this study.

After 8-h incubation, about 45 μM TCE was degraded by toluene-grown cells, while there were 120 μM chloride ions produced. The molar ratio of TCE degraded to chloride ions was nearly 1:3, suggesting that the degraded TCE molecules were completely dechlorinated by toluene-grown *Rhodococcus* sp. L4.

The TCE transformation capacity (T_c) and the maximum specific rate (k_c) of TCE cometabolic degradation (Alvarez-Cohen and McCarty 1991) were 15.1 ± 1.04 μg TCE mg $cells^{-1}$ and 0.14 ± 0.001 mg TCE mg $cells^{-1}$ day^{-1} for toluene-grown

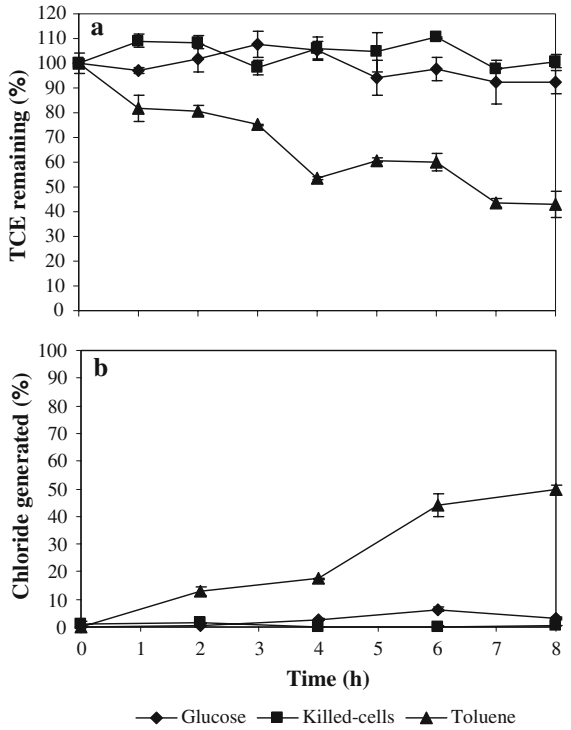

Fig. 1 Time course of TCE degradation (**a**) and chloride formation (**b**) by killed-cells, glucose- and toluene-grown cells. All data are the average of triplicate samples with the standard deviations (error bars) given

Rhodococcus sp. L4 cells (Table 2). With these results our toluene-grown cells exhibit similar activities compared to other known TCE-degrading bacteria. There were a number of reports, which showed that the T_c and k_c values by toluene-degrading bacteria were 5.2–8.5 μg TCE mg cells^{-1} and 0.17–1.3 mg TCE mg cells^{-1} day^{-1}, respectively (Heald and Jenkins 1994; Landa et al. 1994; Chang and Alvarez-Cohen 1996; Arcangeli and Arvin 1997; Kelly et al. 2000).

To confirm that *Rhodococcus* sp. L4 required enzyme induction before TCE degradation, chloramphenicol was added to the glucose-grown cells to inhibit protein synthesis. In the presence of chloramphenicol the cells could not degrade TCE or toluene (Fig. 2). In experiments without chloramphenicol the TCE decreased only after toluene was degraded. These results indicate that the enzymes involved in TCE biodegradation were not constitutively produced. Moreover, the presence of toluene was necessary for the induction of enzymes involved in TCE degradation of *Rhodococcus* sp. L4.

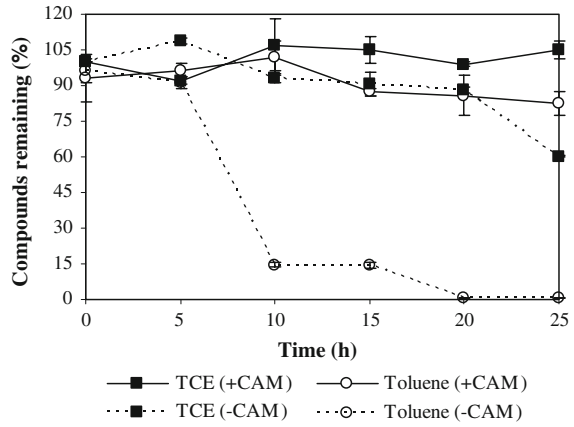

Fig. 2 TCE and toluene degradation by glucose-grown cells with and without chloramphenicol (+/−CAM). All data are the average of triplicate samples with the standard deviations (error bars) given

Effects of plant essential oils on growth of *Rhodococcus* sp. L4 and TCE cometabolic degradation

The toxicity of toluene prevents its use in contaminated sites. Therefore, we examined the efficiency of plant essential oils as its alternative. Five essential oils including lemon, lemongrass, cumin, pine, and spearmint oils were selected since they contain some oil components that have been reported as inducers for xenobiotic-degrading enzymes in bacteria (Table 1). *Rhodococcus* sp. L4 was able to utilize lemon oil as sole carbon source but with a slightly slower growth than with glucose or toluene (Table 1). The growth level of bacteria grown on lemongrass oil or cumin oil was about 60% and 20% compared to growth on glucose or toluene. No growth was detected when spearmint oil and pine oil were used as carbon source. The antibacterial activities of various plant essential oils have long been recognized in particular for the application as food preservatives (Burt 2004). High concentrations of spearmint oil were toxic to *Arthrobacter* sp. B1B, a PCB-degrading bacterium and resulted in cell lysis (Gilbert and Crowley 1997).

Lemon and lemongrass oil-grown cells were able to degrade 20 ± 6% and 27 ± 8% of 80 μM TCE after 8-h incubation, respectively (Table 1). The extent of TCE degradation was higher than with glucose-grown cells. The results suggested that some components in lemon and lemongrass oils induced

Table 1 Growth and TCE degradation of *Rhodococcus* sp. L4 after utilizing different compounds as sole carbon source

Substrate	Essential oil components (%)[a]	Growth[b]	TCE degradation[c] (%)
Plant essential oils			
Lemon oil	Limonene (31.67) and citral (2.35)	++++	20.4 ± 6.3
Lemongrass oil	Limonene (36.72) and citral (36.17)	+++	26.6 ± 7.8
Cumin oil	Terpinene (24.07), pinene (21.85), cymene (19.56), cumin aldehyde (16.10) and cumene (1.31)	+	ND
Pine oil	Pinene (64.11) and limonene (10.51)	−	ND
Spearmint oil	Carvone (55.10)	−	ND
Control			
Glucose	None	+++++	7.8 ± 4.7
Toluene	None	+++++	57.1 ± 5.2

ND, not determined; In these cases, there were not enough bacteria to perform TCE biodegradation assay

[a] The concentrations of essential oil components were determined by GC analysis with the standard compounds as references. Only the major oil components or the one that has been reported as inducer for xenobiotic-degrading bacteria are shown

[b] −, no growth; +, growth, with the turbidity in cell culture increasing from + to +++++

[c] TCE degradation was determined after 8-h incubation by comparing with the initial TCE concentration. Values given are average of three samples ± standard deviation

TCE-degrading enzymes in *Rhodococcus* sp. L4. Nevertheless, toluene was the most effective substrate for TCE cometabolic degradation by this bacterium. This is probably due to the fact, that lemon and lemongrass oil contained many oil components and some of them might inhibit the production of TCE-degrading enzymes or might be toxic to the cells. To prevent the toxic effects of mixed oil components, four purified oil components, limonene, citral, cumin aldehyde, and cumene were selected for further study and their concentrations as inducer for *Rhodococcus* sp. L4 were optimized.

Efficiency of plant essential oil components as inducers for TCE cometabolic degradation

Limonene, citral, and cumin aldehyde were selected for further experiments because they are the major components of lemon, lemongrass, and cumin oils. In addition, our preliminary study showed that these oil components could induce TCE degradation in *Rhodococcus* sp. L4. Pinene did not have TCE inducing activity, while terpinene and cymene had much lower activity than that of cumin aldehyde. Although, cumene is a trace component in cumin oil, it was included here because several cumene enriched bacteria can degrade TCE (Dabrock et al. 1992, 1994; Pflugmacher et al. 1996; Morono et al. 2004, 2006). TCE degradation assay was carried out by using resting cells of *Rhodococcus* sp. L4, that had been grown in glucose–MS media containing the selected oil components. All of the induced cells were able to degrade TCE. Citral- limonene- and cumene-induced bacteria showed a similar trend in TCE degradation. There was a slight difference in TCE degradation when the oil concentrations were increased from 80 to 400 μM (Fig. 3a–d). Cumin aldehyde-induced cells showed lower TCE degradation when the oil concentrations were higher than 80 μM. The high concentration of cumin aldehyde was probably toxic to the cells. This may be the reason why *Rhodococcus* sp. L4 did not grow well on cumin oil (Table 1).

In the following experiment, either 400 μM citral, 80 μM cumin aldehyde or 200 μM limonene/cumene was applied as inducer for *Rhodococcus* sp. L4 before conducting TCE biodegradation assay. These concentrations were selected since they had the highest efficiency on inducing TCE cometabolic degradation (Fig. 3a–d). A significant reduction of TCE along with the production of chloride ion was observed after incubating the resting cells of induced *Rhodococcus* sp. L4 with TCE (Fig. 4a, b). TCE degradation and dechlorination were different when the cells were induced with different oil components. Cumene-induced cells showed the highest TCE degradation.

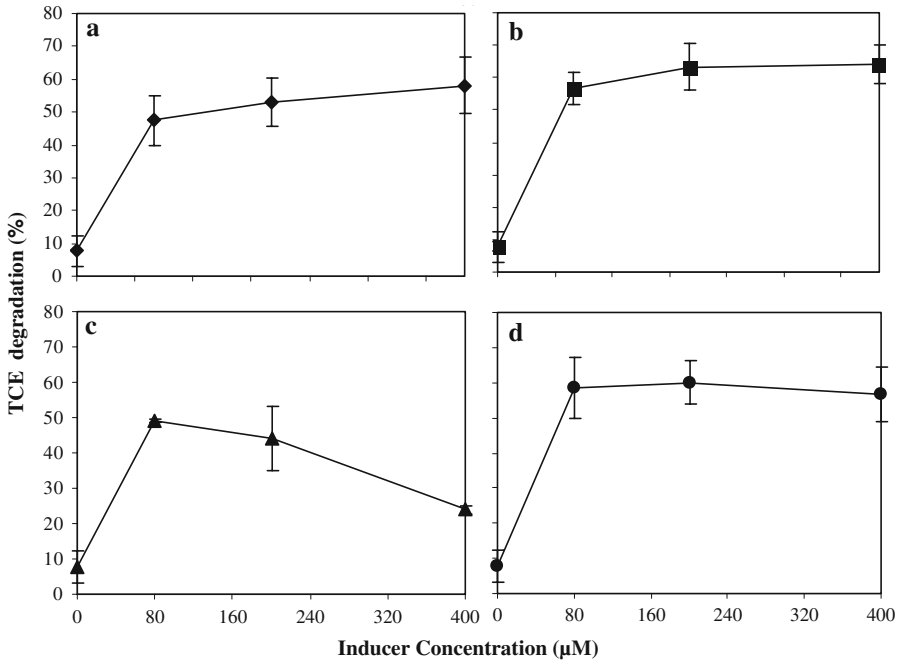

Fig. 3 TCE degradation after 24-h incubation using cell suspensions of *Rhodococcus* sp. L4 after induction with citral (**a**), limonene (**b**), cumin aldehyde (**c**) and cumene (**d**) at various concentrations. TCE degradation was calculated by comparison with the efficiency of glucose-grown cells in the same period

About 53 ± 3% of the initial 80 μM TCE was degraded after 8-h incubation (Fig. 4a, b). The efficiency of cumene-induced cells was comparable to toluene-grown cells (Table 1). Limonene-, citral- and cumin aldehyde-induced cells degraded 36 ± 4%, 40 ± 9% and 49 ± 7% of TCE, respectively.

The variation in TCE degradation activity may be explained by the variation of the amounts of inducible enzymes or different types of enzymes may be produced from each inducer. TCE degradation can be initiated by several monooxygenases and dioxygenases, which have relaxed substrate ranges (Arp et al. 2001). The efficiency of cumene on inducing TCE degradation is suggested to be due to the bulky isopropyl residue adjacent to the double bond on benzene ring, which may mimic the two chlorines in the TCE molecule (Dabrock et al. 1992). This structure is also present in cumin aldehyde, potentially explaining why cumin-aldehyde induced cells contributed to a higher TCE degradation than citral- and limonene-induced cells (Fig. 4). The presence of many types of monooxygenase and dioxygenases in Rhodococci is common (Larkin et al. 2005). Moreover, the ability to induce multiple aromatic oxygenase genes in *Rhodococcus* sp. T104 has been reported for limonene as well as for other plant terpenes (Kim et al. 2003). Consequently, it is possible that *Rhodococcus* sp. L4 might produce other TCE-degrading oxygenases when induced with citral and limonene.

Cumin seeds as alternative inducer for *Rhodococcus* sp. L4 to degrade and dechlorinate TCE

Cumin is commonly used as spice or constituent of household medicines in Asia. Cumin volatile oil and its main component, cumin aldehyde, have antimicrobial activities especially against fungi and yeasts (Shetty et al. 1994). In this study, *Rhodococcus* sp. L4 could not utilize cumin oil for growth. However, cumin oil contained several oil components including cumin aldehyde and cumene that were effective for induction of TCE-degrading enzymes (Fig. 4). The high concentration of essential oils in cumin seeds makes them an interesting candidate for use as alternative inducer for *Rhodococcus* bacteria to degrade TCE. Bacteria induced by cumin oil could degrade TCE from 80 to 52 μM, which corresponded to 36 ± 6% TCE degradation (Fig. 5). The partial TCE degradation correlated to the generation of chloride to the medium. The molar ratio of TCE degraded to chloride ions generated was approximately 1:3.

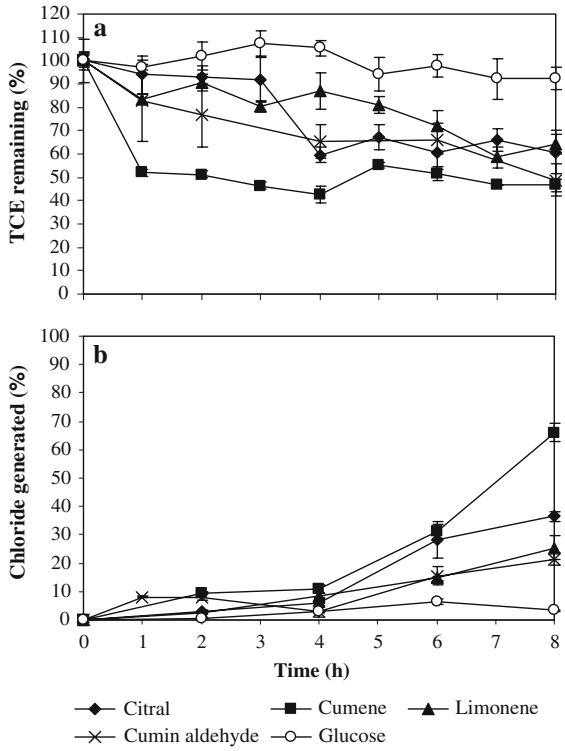

Fig. 4 Time course of TCE degradation (**a**) and chloride formation (**b**) by *Rhodococcus* sp. L4 after induced with various essential oil components. The bacteria were grown in glucose–MS media containing citral, limonene, cumin aldehyde or cumene. Control is the bacteria grown on glucose–MS media without essential oil components. All data are the average of triplicate samples with the standard deviations (error bars) given

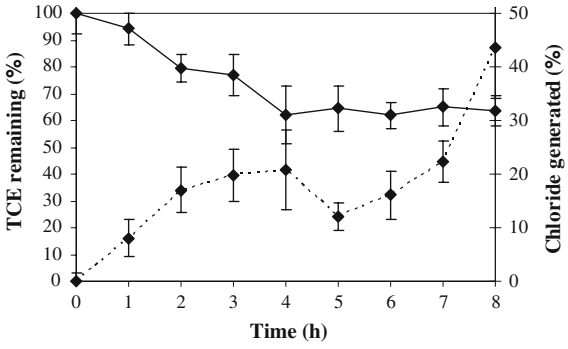

Fig. 5 Time course of TCE degradation (full line) and chloride formation (dash line) by *Rhodococcus* sp. L4 induced with essential oil of cumin seeds. All data are the average of triplicate samples with the standard deviations (error bars)

The TCE-degrading efficiency of cumin oil-induced cells was higher than lemon- and lemongrass oil-grown cells (Table 1). The results suggested that cumin aldehyde and cumene, which are only present in cumin oil, enhanced TCE-degrading enzyme induction. Cumin oil-induced cells had lower TCE-degrading capability than cells induced with the purified oil components (Fig. 4). This was probably due to the slow release of essential oil from the seeds or the presence of other components in cumin oil. The application of cumin seeds for TCE biodegradation and bioremediation would be preferred because they are abundant in many countries and are considered non-toxic to humans. However, plant varieties, harvesting period and planting conditions have been shown to affect the quantities of cumin essential oils as well as the types of oil components (Beis et al. 2000; El-Sawi and Mohamed 2002; Jalali-Heravi et al. 2007). This would lead to the variation in TCE-degrading enzyme induction by different batches of the seeds.

Kinetics of TCE degradation by *Rhodococcus* sp. L4

TCE transformation capacity, T_c, defined as mass of TCE degraded prior to inactivation per mass of biomass added (μg of TCE mg cells^{-1}) and maximum specific rate of TCE degradation, k_c (mg TCE mg cells^{-1} day^{-1}), of *Rhodococcus* sp. L4 were calculated as described by Alvarez-Cohen and McCarty (1991) (Table 2). Glucose-grown cells showed the lowest T_c and k_c values. Comparing between induced cells, we could divide the T_c values into two groups. One group including citral-, limonene- and cumin oil-induced cells had a moderate T_c ranging from 9.4 to 10.7 μg of TCE mg cells^{-1}. The second group consisted of cumene- and cumin aldehyde-induced cells, which showed the higher T_c values of 14.2–15.1 μg of TCE mg cells^{-1}. These values were similar to toluene-grown cells. Neglecting the lag phase, the range of k_c for all induced cells was between 0.09 and 0.3 mg TCE mg cells^{-1} day^{-1} with the following sequence: cumene > citral > cumin aldehyde > cumin oil > limonene. There was no direct correlation between T_c and k_c values, since T_c is a measure for the amount of TCE that can be transformed by a given culture prior to inactivation while the maximum specific rate of TCE degradation is a measure for the maximum enzyme efficiency (Chu and Alvarez-Cohen 1998).

With the exception of methanotrophs, the range of T_c and k_c values of all essential oil component-

Table 2 TCE transformation capacity and rate by *Rhodococcus* sp. L4 compared with other bacteria

Microorganism	Substrate	Transformation capacity, T_c (μg TCE mg cells^{-1})	Maximum specific degradation rate, k_c (mg TCE mg cells^{-1} day^{-1})
Rhodococcus sp. L4 (this study)	Glucose	2.03 ± 0.06	0.009 ± 0.007
	Toluene	15.06 ± 1.04	0.142 ± 0.001
	Cumene[a]	15.11 ± 0.71	0.303 ± 0.007
	Cumin aldehyde[a]	14.17 ± 0.69	0.110 ± 0.005
	Citral[a]	10.68 ± 0.63	0.203 ± 0.030
	Limonene[a]	9.44 ± 0.81	0.060 ± 0.005
	Cumin oil[a]	9.72 ± 0.59	0.094 ± 0.004
M. trichosporium OB3b (Fitch et al. 1996; Oldenhuis et al. 1991)	Methane; Formate	150; 290	21; 55
P. putida BH (Futamata et al. 2001)	Phenol	NA	0.06
P. putida BCRC 14349 (Chen et al. 2007)	Phenol	NA	0.408 (mg TCE mg VSS^{-1} day^{-1})
P. putida (Heald and Jenkins 1994)	Toluene	5.20	NA
P. cepacia G4 (Folsom et al. 1990; Landa et al. 1994)	Toluene; Phenol	34; NA	1.5; 0.94
M. Vaccae JOB5 (Wackett et al. 1989)	Propane	NA	0.057
Mixed culture (Chang and Alvarez-Cohen 1995)	Propane	6.50	0.45
N. Europaea (Ely et al. 1997)	Ammonia	8.00	1.00

NA, not available

[a] The compound was used as inducer for *Rhodococcus* sp. L4

induced cells were similar to other toluene, phenol, propane or ammonia degraders, which have the T_c and k_c ranges between 5.2–34.0 μg of TCE mg cells^{-1} and 0.057–1.5 mg TCE mg cells^{-1} day^{-1}, respectively (Table 2). Although these kinetic parameters are specific for the tested bacterial strain, the values are also depended on the test conditions. We did not add substrate and/or inducing compound during TCE biodegradation experiments; thus the bacteria might lose their activity during TCE degradation and could not produce new enzymes. Other possibilities that explain low values of kinetic coefficients in our study might be the relatively high concentration of initial TCE. The published studies conducted TCE cometabolic degradation with initial concentrations between 0.038 and 60 μM (0.05–8 mg l^{-1}) (Folsom et al. 1990; Hopkins and McCarty 1995; Fitch et al. 1996; Chen et al. 2007). Chen et al. (2007) suggested that the suppression of TCE degradation at higher TCE concentration (i.e. 15–150 μM) is probably due to the limitation of oxygenase enzymes. The toxicity resulted from high TCE concentration and the inactivation of oxygenases.

Conclusions

Essential oils from cumin, lemon, and lemongrass were found to induce *Rhodococcus* sp. L4, an isolate generally grown on toluene to degrade TCE. Lemon and lemongrass oil could be used as sole carbon source for the bacteria; however cumin oil should only be used in small amounts as an inducer. The induction of TCE-degrading enzymes was suggested to be due to the presence of citral, cumin aldehyde, cumene, and limonene in the essential oils. *Rhodococcus* sp. L4 induced with either of these oil components effectively cometabolized and dechlorinated TCE. Consequently, cumin, lemon, and lemongrass oils or plant materials rich in these essential oils such as cumin seeds are suggested as alternatives to toluene to induce TCE-degrading enzymes. The application of these plant essential oils will result in a low cost and environmentally friendly approach for TCE bioremediation.

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