Cometabolic degradation of trichloroethylene by *Burkholderia cepacia* G4 with poplar leaf homogenate

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**Abstract:** Trichloroethylene (TCE), a chlorinated organic solvent, is one of the most common and widespread groundwater contaminants worldwide. Among the group of TCE-degrading aerobic bacteria, *Burkholderia cepacia* G4 is the best-known representative. This strain requires the addition of specific substrates, including toluene, phenol, and benzene, to induce the enzymes to degrade TCE. However, the substrates are toxic and introducing them into the soil can result in secondary contamination. In this study, poplar leaf homogenate containing natural phenolic compounds was tested for the ability to induce the growth of and TCE degradation by *B. cepacia* G4. The results showed that the G4 strain could grow and degrade TCE well with the addition of phytochemicals. The poplar leaf homogenate also functioned as an inducer of the toluene-ortho-monoxygenase (TOM) gene in *B. cepacia* G4.

**Key words:** bioremediation, *Burkholderia*, phytochemical, poplar, trichloroethylene.

**Mots-clés:** biorestauration, *Burkholderia*, substance phytochimique, peuplier, trichloroéthylène.

**Introduction**

Trichloroethylene (TCE), a suspected human carcinogen, is one of the most widely used chlorinated organic solvents and is used as a cleaning agent for many commercial, military, and industrial applications (Wartenberg and Gilbert 2014). Due to this widespread use, in conjunction with improper handling and leakage, TCE has become one of the most frequently detected volatile organic chemicals in groundwater in the United States (Moran et al. 2006; Sercu et al. 2013).

Bioremediation of TCE-contaminated sites has received increasing attention as an alternative to physical and chemical engineering treatments (Snyder et al. 2000; Kato and Davis 2006; Soda et al. 2006; Hosoda et al. 2012), since the microorganisms can transform TCE to less-toxic compounds (Lee et al. 1998; Watanabe et al. 2002; Humphries et al. 2005; Chomsurin et al. 2008).

Several classes of microorganisms have been shown to degrade TCE by a cometabolic oxidation process where the bacteria require the addition of specific substrates, such as toluene, phenol, and benzene, to induce the expression of enzymes required for the degradation of TCE (Landa et al. 1994; Reij et al. 1995; Lee et al. 2006; Kim et al. 2010). Many studies reported that *Burkholderia cepacia* G4 can aerobically degrade TCE when grown on aromatic compounds as carbon and energy sources (Nelson et al. 1986; Mars et al. 1996; Snyder et al. 2000). But these compounds are expensive to apply and environmentally hazardous.

Due to the drawbacks described above, researchers have engineered microbes that can degrade TCE without the addition of any toxic inducer (Shields and Reagin 1992; Matin et al. 1995). Engineered bacteria, however, may have limitations in field applications. Also, public concerns over the safety of engineered bacteria and its impact to the environment can make field-scale applications difficult.

As a result, phytochemicals have been attracting much interest as natural alternatives to synthetic toxic compounds (Wu-Yuan et al. 1988; Sato et al. 1996; Sinha et al. 2009). Plants synthesize a wide variety of natural phenolic compounds (Kesselmeier and Staudt 1999; Dudonné et al. 2011). Many studies have shown that plant phenolic compounds induce catabolic genes in microorganisms as well as genes required to form active associations with plants (Peters and Verma 1990). Although some plants contain compounds capable of inhibiting microbial growth (de Souza et al. 2005; Mukherjee et al. 2007), the plant essential oils and their components can be used as alternative inducers for TCE cometabolic degradation in the toluene-degrading bacterium *Rhodococcus* sp. strain L4 (Suttinun et al. 2009, 2010). However, producing these essential oils is very costly because of complex manufacturing processes and requires a lot of raw plant material.

In this study, the poplar leaf homogenate, a mixture of natural phenolic compounds, was tested for the ability to induce growth of and TCE degradation by *B. cepacia* G4, since the genus *Populus* is widely distributed and fast-growing and already used in remediation projects (Dudonné et al. 2011). *Burkholderia cepacia* G4 is the best-known representative of the group of toluene-oxidizing, TCE-degrading aerobic bacteria (Shields et al. 1991; Mars et al. 1996; Snyder et al. 2000). The ability of poplar leaf homogenate to
induce expression of the gene encoding the enzyme toluene-ortho-monoxygenase (TOM) was also evaluated with real-time quantitative reverse transcriptase polymerase chain reaction (qRT–PCR).

Poplar leaves were collected from hybrid poplar *Populus tremula × Populus alba* N717 1-B4 (INRA). Poplar leaf homogenate was made with 5 g of fresh poplar leaves and 30 mL of deionized water. The leaves were ground using a mortar and pestle and passed through a 150 μm screen, then sterilized by filtration with a 0.2 μm syringe filter. Growth experiments of *B. cepacia* G4 with poplar leaf homogenate were conducted to test whether strain G4 could grow in medium containing phytochemicals. Some studies reported that plant-derived compounds (phytochemicals), including aromatic compounds, have been used as antimicrobial agents to inhibit the growth of bacteria (Nascimento et al. 2000; Simes et al. 2009; Jayaraman et al. 2010). G4 strain was grown in 25 mL of M9 minimal medium without glucose, containing 500 μL of poplar leaf homogenate. The components of M9 minimal medium (per litre) were 6 g of Na₂HPO₄ (anhydrous), 3 g of KH₂PO₄, 0.5 g of NaCl, 1 g of NH₄Cl, 1 mmol/L MgSO₄, 0.1 mmol/L CaCl₂, pH to 7.4 (Sambrook et al. 1989). The cultures were grown in 125 mL Erlenmeyer flasks and shaken for 2 days at 200 r/min and 30 °C. The growth was monitored using a Biochrom WPA Biowave spectrophotometer by measuring the optical density (OD) at 660 nm. *Burkholderia cepacia* G4 without poplar leaf homogenate was used as a negative control.

The TCE biodegradation assay with poplar leaf homogenate experiments were conducted in M9 minimal liquid culture. *Burkholderia cepacia* G4 was grown overnight in Luria–Bertani medium. The cells were harvested and washed 3 times with deionized water to remove residual culture medium and glucose and then adjusted to an OD₆₆₀ of 0.1 in M9 minimal medium without glucose. A 500 μL volume of poplar leaf homogenate was added to 5 mL of M9 minimal medium in the 25 mL serum bottles. A 50 μL volume of TCE-saturated water solution was added to a TCE concentration of 10 mg/L. The serum bottles were sealed immediately using aluminum crimp caps with Teflon-lined septa. The experiments were performed in triplicate, with 3 serum bottles containing TCE with G4 cells without poplar leaf homogenate in M9 minimal medium as a negative control. Also, 3 serum bottles containing TCE and toluene with G4 cells were tested as a positive control. Medium from each of the 9 serum bottles was sampled every 4 h. The cultures were shaken at 150 r/min and 30 °C for 5 days. For TCE quantification, 1 mL of headspace gas was taken by piercing the septum with a gas-tight syringe and was injected through the top ports. Headspace gas was analyzed with a Clarus 500 gas chromatograph equipped with an electron capture detector and a PTE-5 (5% phenyl methyl siloxane) fused-silica capillary column (30 m × 0.32 mm ID; thickness, 0.32 μm). The gas chromatography conditions were as follows: injector temperature 150 °C, detector temperature 250 °C, initial column temperature 40 °C (1.80 min), programmed at 40–55 °C at a rate of 45 °C/min, and 55–135 °C at a rate of 10 °C/min. The carrier gas (hydrogen) flow rate was 14 mL/min.

To test the expression levels of the targeted *tom* gene of *B. cepacia* G4, total RNA was prepared after 5 h of TCE exposure by using an RNeasy Protect Bacteria Mini kit (Qiagen) according to the manufacturer’s instructions. Once the RNA extraction was complete, samples were stored at –80 °C for further processing. The quality of each RNA sample was assessed using either the RNA 6000 Pico or Nano LabChip kit with a 2100 Bioanalyzer (Agilent Technologies). Approximately 20 ng of total RNA from each sample were used for labeling and hybridization. Approximately 2 μg of total RNA was reverse transcribed into cDNA using the BioRad iScript kit (Bio-Rad, Hercules, California) according to the manufacturer’s instructions. Primers of the *tom* gene were designed based on existing DNA sequences (Table 1). The selected *tom* gene was compared with an internal reference gene (16S rRNA) that served to normalize expression levels and to give unitless, realistic values of candidate gene expression.

![Fig. 1. Growth curves of *Burkholderia cepacia* G4 with poplar leaf homogenate (■) and without poplar leaf homogenate (○) in M9 minimal medium without glucose. The experiments were performed in triplicate; error bars represent standard deviation.](image-url)
or poplar leaf homogenate, and the ratio of TCE degradation was nearly the same in both samples for the first 20 h. The degradation of TCE in B. cepacia G4 with toluene stabilized after 20 h, with nearly 40% of TCE was degraded. However, B. cepacia G4 with poplar leaf homogenate continued to degrade TCE with a total of 80% of the TCE removed in 2 days. In the absence of poplar leaf homogenate and toluene, TCE was not removed. This result indicates that the poplar leaf homogenate can function as a cosubstrate like phenol and toluene to degrade TCE.

To further support the induced TCE degradation by B. cepacia G4 with poplar leaf homogenate and to confirm the expression of the tom gene, qRT–PCR was carried out. The results of the qRT–PCR analysis are presented in Fig. 3. Assuming equal annealing and amplification properties of the PCR primers, the relative amounts of individual mRNAs were normalized to the expression of 16S rRNA. The result showed that the expression levels of tom genes with poplar leaf homogenate were 1.8 times higher than that of the control. Although there was a strong trend in the data, the effect of poplar leaf homogenate was not statistically significant (p = 0.062) due to the variability in the expression data. Further experiments to identify the precise timing of tom gene induction or further replications could probably reduce the variability in the data. But all of these results are also supported by a recent study revealing that phenolic compounds derived from plants act as specific substrates or signaling molecules for microorganisms in the soil (Badri et al. 2013).

To the best of our knowledge, this work represents the first report on degradation of TCE using microorganisms with poplar leaf homogenate as the TOM gene inducers and energy sources. Natural plant homogenate has the potential to be used as a substitute for toxic substrates to induce cometabolic degradation of TCE under aerobic conditions. More research is needed to characterize the unknown phytochemicals in poplar leaf homogenate. This study opens the prospect of applying plant homogenates, which are naturally safe and inexpensive phytochemicals, to stimulate TCE-degrading bacteria for TCE bioremediation in the environment.

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References


