

**INTRODUCTION**

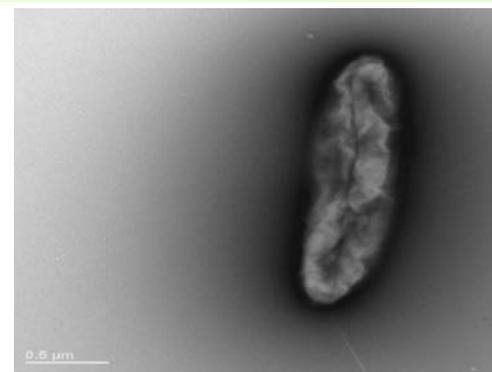
Chlorinated solvents such as chloroform (CF), dichloromethane (DCM) and 1,1,2-trichloroethane (1,1,2-TCA) are common groundwater pollutants owing to extensive industrial production and improper handling and disposal practices. These chlorinated solvents are present at 474, 389 and 131 of the 1319 national priority sites in the USA, respectively. Also, they are in the list of the 126 priority pollutants identified by US EPA. TCM, DCM and 1,1,2-TCA are considered as potential carcinogens. Bioremediation using organochlorine respiring bacteria is a cost effective and sustainable practice for the removal of these pollutants from groundwater.

**OBJECTIVES**

- ◆ Isolate and characterize bacteria for dechlorination of CF
- ◆ Identify other dechlorinating capabilities
- ◆ Identify enzyme (dehalogenase) responsible for dechlorination of CF
- ◆ Scale up and apply cultures for bioremediation of contaminated sites

**FINDINGS**

A novel *Dehalobacter* strain, namely, UNSWDHB (Fig. 1) was isolated from soil obtained from a contaminated site in Sydney, Australia. Its tolerance to TCM (Fig. 3) and ability to dechlorinate other chlorinated solvents was tested. Strain UNSWDHB was able to transform 1,1,2-TCA to 1,2-DCA and vinyl chloride (Fig. 4). From the genome of UNSWDHB, it was found to harbour 19 putative reductive dehalogenases. Using proteomics, a novel reductive dehalogenase (TmrA) responsible for dechlorination of TCM and 1,1,2-TCA was identified and the structure of its operon is as shown in Fig.2.

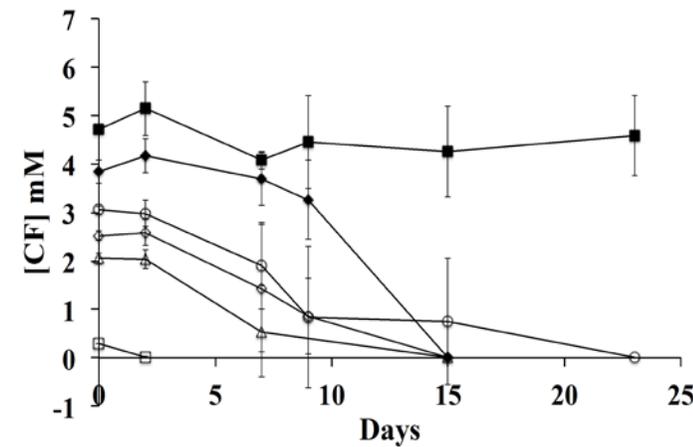


**Fig. 1 :** Transmission electron micrograph of *Dehalobacter sp.* UNSWDHB



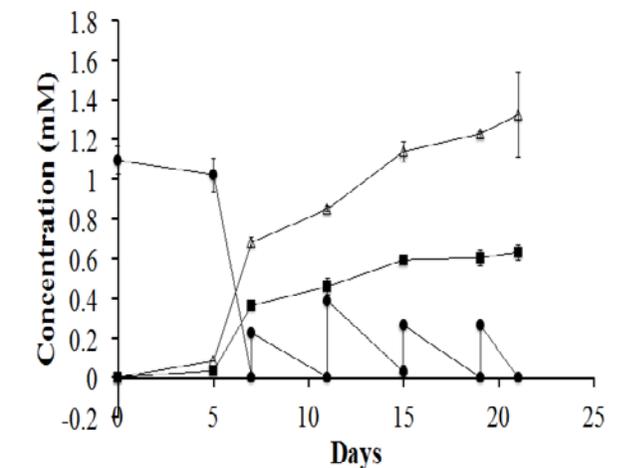
**Fig. 2 :** Gene cluster of reductive dehalogenase identified in UNSWDHB; *tmrA*: catalytic subunit, *tmrC*: transcriptional regulator, *apbE*: thiamine biosynthesis lipoprotein, *CRP*: transcriptional regulator and *tmrT*: chaperone protein. Maroon represents hypothetical genes.

**Tolerance of Dehalobacter sp. UNSWDHB to CF**



**Fig.3 :** The dechlorination of chloroform (CF) by *Dehalobacter sp.* strain UNSWDHB at different CF concentrations (mM) 0.42 (□), 1.68 (△), 2.09(◇), 2.51 (○), 3.35 (u), 4.19 (n). Error bars represent standard deviation (n=3).

**Transformation of 1,1,2-trichloroethane (TCA) by *Dehalobacter sp.* UNSWDHB**



**Fig.4 :** Reductive dechlorination of 1,1,2-TCA (◆) to 1,2-DCA (■) and VC (△) by strain UNSWDHB. Error bars represent standard deviation (n=3)

**IMPACTS**

*Dehalobacter sp.* UNSWDHB is the first strain isolated in Australia able to dechlorinate CF to DCM and 1,1,2-TCA to 1,2-DCA and vinyl chloride. It is able to dechlorinate 0.5 mM and 0.4 mM of CF and/or 1,1,2-TCA per day. Results also showed that UNSWDHB is able to tolerate high concentration of CF, up to 3.34 mM. The identification of the novel reductive dehalogenase (TmrA) in *Dehalobacter sp.* UNSWDHB responsible for dechlorination of CF and 1,1,2-TCA will be a useful tracking tool for the assessment of successful application of UNSWDHB for bioremediation. Lastly, we have successfully scaled up enrichment cultures containing *Dehalobacter sp.* UNSWDHB that has been introduced to a CF contaminated aquifer.

**References**

Lee, M., Low, A., Olivier, Z., Koenig, J., and Manefield, M. (2012) Complete chloroform dechlorination by organochlorine respiration and fermentation. *Environ. Microbiol.* 14: 883-894.

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