

Full Length Research Paper

Dechlorination of 1,2-dichloroethane by *Pseudomonas aeruginosa* OK1 isolated from a waste dumpsite in Nigeria

A. I. Okoh^{1*}, A. O. Olaniran^{1,2} and P. Golyshin³

¹Department of Microbiology, Obafemi Awolowo University, Ile - Ife, Nigeria.

²Department of Microbiology, University of Durban-Westville, Durban, South Africa.

³Molecular microbial ecology lab, German Research Center for Biotechnology, Braunschweig, Germany.

Accepted 9 September 2004

As part of our attempt at isolating and stocking some indigenous microbial species, we isolated a bacterium from a waste dumpsite with appreciable dechlorination activity. 16S rDNA profiling revealed the isolate to be a strain of *Pseudomonas aeruginosa* and the sequence has been deposited in the NCBI nucleotide sequence database (accession number AJ550306). The bacterium utilized 0.1% (v/v) 1, 2-dichloroethane (1, 2-DCE) as sole source of carbon and attained peak cell density of 6.0×10^7 cfu/ml in 48 h. It also has a proportionate increase in chloride release during this period resulting in the release of 80% free Cl⁻. The bacterium also had dehalogenase activities against other chlorinated organics such as monochloroacetic acid, trichloroacetic acid, dichloromethane, trichloromethane and tetrachloromethane at pH 7.5 and 9.0. Optimum temperature for dehalogenase activity against 1, 2-DCE was 35°C.

Key words: Dechlorination, 16S rDNA, bioremediation, *Pseudomonas aeruginosa* OK1.

INTRODUCTION

1, 2-dichloroethane (1,2 - DCE), is an environmentally important compound with a production volume in excess of 12 billion lb per year, a volume larger than that of any other industrial halogenated chemical (Janssen et al., 1989). They are widely used as chemical intermediates and as solvents in a variety of industrial processes (Leisinger and Brunner, 1986; Vogel et al., 1987). They are common contaminants of soil and ground waters owing to improper disposal practices or accidental spills (McCarty, 1997) causing serious environmental and human health problems as a result of their persistence and toxicity (Squillace et al., 1999). Hence they have been classified as one of the priority pollutants by the US

Environmental Protection Agency.

The ability of microorganisms to detoxify halogenated aliphatic chlorinated hydrocarbons is of great importance. 1, 2 - DCE has been reported to be degraded via 2-chloroethanol, 2-chloroacetaldehyde, and chloroacetic acid to glycolate with dehalogenation step being catalysed by two hydrolytic dehalogenases (Janssen et al., 1987).

In our previous study (Olaniran et al., 2001), we isolated a bacterium with appreciable dehalogenation potential. In this paper, we describe the nucleotide sequence of the 16SrRNA genes of the bacterium as well as its dechlorination of 1, 2-DCE by axenic culture of the bacterium in liquid system.

This is part of our effort at developing an active indigenous bacterial consortium that could be of

*Corresponding author. E-Mail: aokoh98@yahoo.com

```

1  tggctcagat  tgaacgctgg  cggcaggcct  aacacatgca  agtcgagcgg  atgaagggag
61  cttgctcctg  gattcagcgg  cggacgggtg  agtaatgcct  aggaatctgc  ctggtagtgg
121  gggataacgt  cgggaaacgg  gcgctaatac  cgcatacgtc  ctgagggaga  aagtggggga
181  tcttcggacc  tcacgctatc  agatgagcct  aggtcggatt  agctagttag  tggggtaaag
241  gctaccaag  ggcagcgtcc  gtaactggtc  tgagaggatg  atcagtcaca  ctggaactga
301  gacacgggcc  agactcctac  gggaggcagc  agtggggaat  attggacaat  gggcgaaagc
361  ctgatccagc  catgccgcgt  gtgtgaagaa  ggtcttcgga  ttgtaaagca  ctttaagtgt
421  ggaggaaggg  cagtaagtta  ataccttgct  gttttgacgt  taccaacaga  ataagcaccg
481  gctaacttcg  tgccagcagc  cgcggtaata  cgaaggggtg  aagcgttaat  cgggaattact
541  gggcgtaaag  cgcgcgtagg  tggttcagca  agttggatgt  gaaatccccg  ggctcaacct
601  gggaaactgca  tccaaaacta  ctgagctaga  gtacggtaga  ggggtgggga  atttcctgtg
661  tagcggtgaa  atgcgtagat  ataggaagga  acaccagtgg  cgaaggcgac  cacctggact
721  gatactgaca  ctgaggtgcg  aaagcgcggg  gagcaaacag  gattagatac  cctggtagtc
781  cacgccgtaa  acgatgtcga  ctagccgttg  ggatccttga  gatcttagtg  ggcagctaa
841  cgcgataagt  cgaccgcctg  gggagtacgg  ccgcaagggt  aaaactcaaa  tgaattgacg
901  ggggcccgca  caagcggtag  agcatgtggt  ttaattcgag  caacgcgaag  aaccttacct
961  ggccttgaca  tgctgagaac  ttccagaga  tggattgggt  ccttcgggaa  cctgacaca
1021  ggtgctgcat  ggctgtcgtc  agctcgtgct  ntgagatggt  gggttaagtc  ccgtaacgag
1081  gcgaaccctt  gtocctagtt  accagcaoct  cgggtgggca  ctctaaggag  actgccggtg
1141  acaaaccgga  ggaaggtggg  gatgacgtca  agtcatcatg  gcccttacgg  ccagggctac
1201  acacgtgcta  caatggtcgg  taaaaaggg  tgccaagccg  cgaggtggag  ctaatcccat
1261  aaaaccgatc  gtagtccgga  tcgcagtctg  caactcgact  gcgtgaagtc  ggaatcgcta
1321  gtaatcgtga  atcagaatgt  cacggtgaat  acgttcccgg  gccttgata  caccgccggt
1381  cacaccatgg  gagtgggttg  ctccagaggt  agctagtcta  accgcaaggg  ggacggttac
1441  cacggagtga  ttcatgactg  gggtagaagtc  gtaacaaggt  agccgtaggg  gaacctgocg

```

Figure 1. Nucleotide sequence of *P. aeruginosa* OK1 (accession number: AJ550306).

relevance in the bioremediation of halogenated hydrocarbons polluted systems in Nigeria.

MATERIALS AND METHODS

Isolation, maintenance and identification of bacterial strain

Isolation and maintenance of the bacterial strain was done as earlier reported (Olaniran et al., 2001) except that BHM was supplemented with 0.1% (w/v) yeast extract for the batch culture experiments used in the enzyme assay. Carbon source was filter-sterilized to prevent thermal dechlorination and added at 0.1% (v/v) in a 1 litre flask incubated aerobically at 35 °C on an orbit shaker at 150 rpm. Identification of the bacterial strain was done using molecular techniques that exploited the nucleotide sequences of its 16S rRNA gene. Amplification of the 16S rRNA gene was done as described by Wilson (1987) using the 16F27 and 16R1492 primers (Lane, 1991). The amplified product (1.5 kb) was purified and sequenced using an automated DNA sequencer (Perkin-Elmer, Applied Biosystems, version 377), and the nucleotide sequences were analysed as described elsewhere (Pearson and Lipman, 1988).

Screening for dehalogenase activities, growth and chloride release assay

The pure bacterial isolate was initially screened for dehalogenase activity as described by Slater et al. (1992), and dehalogenase activity was further confirmed by the method of Yu and Welander (1995). The growth pattern of the bacterial isolate in 1,2 - DCE was monitored by cultivating the standardized (OD_{600nm} 0.1) suspension of the isolate in 100 ml of the defined growth medium containing 1,2 - DCE at a final substrate concentration of 0.1% (v/v) at 35°C

and 150 rpm. Total bacterial counts were carried out at each sampling time using standard spread plate technique (Seeley and Vandemark, 1981). Chloride release was monitored spectrophotometrically at 460 nm with mercury thiocyanate and ferric ammonium sulfate as described by Bergman and Sanik (1957) and modified by Coleman et al. (2002).

Enzyme assay and protein determination

Crude extracts were prepared from cells grown to the late-exponential phase (Janssen et al., 1987). The cells were harvested by centrifugation for 20 min at 11000 g, washed once with 10 mM Tris-SO₄ buffer (pH 7.5), and suspended in the same buffer. After sonication, unbroken cells and debris were removed by centrifugation for 30 min at 12,000 g. Enzyme assays were done within 6 h after preparation of extracts to prevent loss of activity. Dehalogenase assays were carried out by incubating 0.1 ml of crude extract or an adequate dilution thereof at 35°C with 3 ml of 5 mM 1,2-dichloroethane in 50 mM tris SO₄ (pH 7.5) and glycine-NaOH (pH 9). Chloride liberation was followed spectrophotometrically as described earlier. Protein concentrations were determined using the Bio-Rad Bradford protein determination kit with bovine serum albumin (fraction V; Sigma) as protein standard. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of chloride per hour. The effect of temperature on enzyme activity was determined by incubating the crude extract at different temperature conditions in water baths.

RESULTS AND DISCUSSION

The PCR amplification of the 16S rRNA gene of the

bacterial isolate yielded the expected DNA band of 1.5 kb. Analysis of the full nucleotide sequence of the 16S rDNA revealed the bacterium to be a strain of *Pseudomonas aeruginosa* and the nucleotide sequence has since been deposited in the NCBI nucleotide sequence database. The bacterium utilized 1, 2- DCE for growth after an initial lag period of about 6 h and attained peak cell density of about 600×10^5 cfu/ml in 48 h before declining, probably due to accumulation of toxic metabolites which became harmful to the bacterium (Figure 2). This growth pattern was corroborated by the pattern of Cl^- release which increased proportional to increase in cell density and peaked between 48 and 60 h with approximately 80% free Cl^- released (Figure 3). These activities support the report of many studies regarding the versatility of the *Pseudomonas* genus (Okoh, 2003; Olaniran et al., 2001; Zaitsev and Karasevich, 1985).

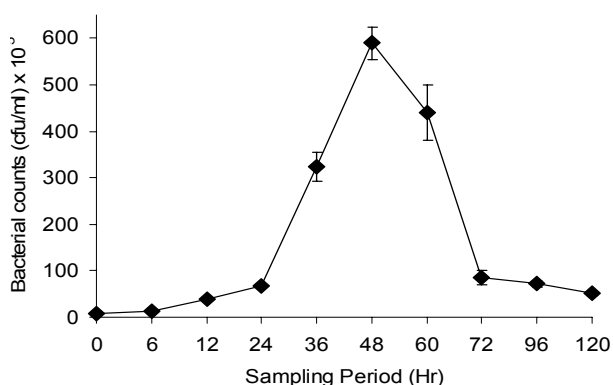


Figure 2. Growth pattern of the bacterial isolate in 1, 2- DCE.

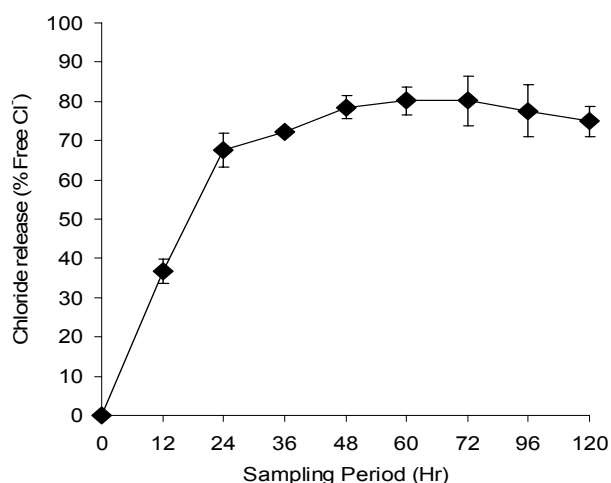


Figure 3. Chloride release during growth of the bacterial isolate in 1, 2 – DCE.

The dehalogenation potential of the bacterium was further corroborated by the dehalogenase activities of the crude extract of the bacterium against five other chlorinated organics. The enzymatic activities of the cell-free extract is an important advantage over the use of live organisms against the backdrop of public anxieties on the effect of releasing live organisms into the environment as reported elsewhere (Olaniran et al., 2001). These activities appeared to be influenced by pH (Table 1), such that dehalogenase activities against monochloroacetic acid, trichloroacetic acid were higher under pH 9.0 than pH 7.5, while pH 7.5 favoured dehalogenation of 1, 2 – dichloroethane, dichloromethane, trichloromethane and tetrachloromethane. These pH regimes fall within the range reported by Slater et al. (1992), and further confirmed the different pH optima of 7.5 and 9.0 previously reported for dehalogenase activities against chlorinated alkanes and chlorinated alkanic acids, respectively (van der Ploeg et al., 1991). Optimum temperature for the dehalogenase activity against 1, 2 – DCE was observed to be 35°C (Figure 4) and falls within the range established by Cookson (1995).

This study further confirms the immense potential of *P. aeruginosa* OK1 for use in the bioremediation of hydrocarbons and halogenated hydrocarbons polluted systems as have been severally reported for other pollutants in our previous studies (Okoh et al. 2003; Okoh et al. 2002; Olaniran et al. 2001). The optimization of the process conditions, especially the application of cell-free extracts for bioremediation purpose is the subject of our on-going study.

Table 1. Dehalogenase activities of crude extracts of the bacterial isolate prepared from 1, 2 – DCE grown cells towards various substrates.

Substrate	Dehalogenase activity (U/mg protein)	
	Substrate	Dehalogenase activity (U/mg protein)
1, 2 - dichloroethane	16.1 ± 0.4	9.5 ± 0.4
Dichloromethane	10.4 ± 0.3	6.6 ± 0.4
Trichloromethane	11.1 ± 0.1	7.1 ± 0.5
Tetrachloromethane	10.1 ± 0.2	5.7 ± 0.5
Monochloroacetic acid	5.8 ± 0.3	19.6 ± 0.5
Trichloroacetic acid	4.6 ± 0.4	14.9 ± 0.3

ACKNOWLEDGEMENTS

The first author is grateful to the UNESCO/Biotechnology Action Council for the fellowship to the Molecular microbial ecology laboratory of the German Research

Center for Biotechnology (GBF), Braunschweig, Germany, where some aspects of this work was done, and also to Professor Don Cowan of University of the Western Cape, Bellville, South Africa for the use of his laboratory facilities for the preparation of this manuscript. The second author thanks the technical crew of the Department of Microbiology, University of Durban-Westville, South Africa for their assistance.

REFERENCES

- Bergmann JC, Sanik J (1957). Determination of trace amounts of chlorine in naphtha. *Anal. Chem.* 29: 241 - 243.
- Coleman NV, Mattes TE, Gossett JM, Spain JC (2002). Biodegradation of cis-Dichloroethene as the sole carbon source by a β -proteobacterium. *Appl. Envir. Microbiol.*, 68(6): 2726 - 2730.
- Cookson Jr. JT (1995). Bioremediation engineering design and application. McGraw-Hill, New York.
- Janssen DB, Keuning S, Witholt B (1987). Involvement of a quinoprotein alcohol dehydrogenase and an NAD-dependent aldehyde dehydrogenase in 2-chloroethanol metabolism in *Xanthobacter autotrophicus* GJ10. *J. Gen. Microbiol.* 133: 85 - 92.
- Janssen DB, Jan Van Der Ploeg FP, Kazemier B, Terpstra P, Witholt B (1989). Cloning of 1, 2 - Dichloroethane Degradation genes of *Xanthobacter autotrophicus* GJ10 and Expression and Sequencing of the *dhIA* Gene. *J. Bact.*, 171(12): 6791 - 6799.
- Lane DJ (1991). 16S/23S sequencing. In: Stackebrandt, E. and Goodfellow, M (eds). *Nucleic acid technologies in bacterial systematics*. John Wiley and Sons, Chichester, 115 - 175.
- Leisinger T, Brunner W (1986). Poorly degradable substances. In: H. J. Rehm and G. Reed (ed.), *Biotechnology*, vol. 8, p. 475-513. VCH Publishers, New York.
- McCarty L (1997). Breathing with chlorinated solvents. *Science*, 276: 1521 - 1522.
- Olaniran AO, Babalola GO, Okoh AI (2001). Aerobic dehalogenation potentials of four bacterial species isolated from soil and sewage sludge. *Chemosphere*, 45: 45 - 50.
- Okoh IA, Ajisebutu S, Babalola GO, Trejo - Hernandez MR (2002). "Biodegradation of Mexican heavy crude oil (*Maya*) by *Pseudomonas aeruginosa*". *J. Trop. Biosc.*, Vol. 2(1): 12 - 24.
- Okoh AI (2003). Biodegradation of Bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. *Afr. J. Biotech.*, 2(5): 104 - 108.
- Pearson WR, Lipman DJ (1988). Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci.* 85: 2444 - 2448.
- Seeley HW, Vandemark PJ (1981). *Microbes in action. A Laboratory Manual of Microbiology*, third ed., W. H. Freeman and Company, U.S.A.
- Slater JH, Weightman AL, Weightman AJ (1992). Microbial dehalogenation of trichloroacetic acid. *World J. Microbiol. Biotechnol.*, 8: 512 - 518.
- Squillace PJ, Moran MJ, Lapham WW, Price CV, Clawges RM, Zogorski JS (1999). Volatile organic compounds in untreated ambient groundwater of the United States, 1985 - 1995. *Environ. Sci. Technol.* 33: 4176 - 4187.
- Van der Ploeg, vanHall J, Janssen DB (1991). Characterization of the haloacid dehalogenase from *Xanthomonas autotrophicus* GJ10 and sequencing of the *dh1B* gene. *J. Bacteriol.*, 173: 7925 - 7933.
- Vogel TM, Criddle CS, McCarty PL (1987). Transformation of halogenated aliphatic compounds. *Environ. Sci. Technol.* 21: 722-736.
- Wilson K (1987). Preparation of genomic DNA from bacteria. In: *Current Protocols in Molecular Biology*. Ausubel FM, Brent R., Kingston RE, Moore DD, Seidman J G, Smith AJ, Struhl K (editors), Wiley publishers, New York, USA, pp. 2.4.1 - 2.4.2.
- Yu P, Welander T (1995). Growth of an aerobic bacterium with TCA as sole source of energy and carbon. *J. Appl. Microbiol. Biotechnol.* 42: 769 - 774.
- Zaitsev GM, Karasevich YN (1985). Preparative metabolism of 4-chlorobenzoic and 2, 4-dichlorobenzoic acids in *Corynebacterium sepedonicum*. *Mikrobiologiya*, 54: 356 - 359.