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Full Length Research Paper

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

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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 Ib 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

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   1 tggctcagat tgaacgctgg cggcaggcct aacacatgca agtcgagcgg atgaagggag
  61 cttgctcctg gattcagcgg cggacgggtg agtaatgcct aggaatctgc ctggtagtgg
 121 gggataacgt ccggaaacgg gcgctaatac cgcatacgtc ctgagggaga aagtggggga
 181 tcttcggacc tcacgctatc agatgagcct aggtcggatt agctagttgg tggggtaaag
 241 gcctaccaag gcgacgatcc gtaactggtc tgagaggatg atcagtcaca ctggaactga
 301 gacacggtcc agactcctac gggaggcagc agtggggaat attggacaat gggcgaaagc
 361 ctgatccagc catgccgcgt gtgtgaagaa ggtcttcgga ttgtaaagca ctttaagttg
 421 ggaggaaggg cagtaagtta ataccttgct gttttgacgt taccaacaga ataagcaccg
 481 gctaacttcg tgccagcagc cgcggtaata cgaagggtgc aagcgttaat cggaattact
 541 gggcgtaaag cgcgcgtagg tggttcagca agttggatgt gaaatccccg ggctcaacct
 601 gggaactgca tccaaaacta ctgagctaga gtacggtaga gggtggtgga atttcctgtg
 661 tagcggtgaa atgcgtagat ataggaagga acaccagtgg cgaaggcgac cacctggact
 721 gatactgaca ctgaggtgcg aaagcgcggg gagcaaacag gattagatac cctggtagtc
 781 cacgccgtaa acgatgtcga ctagccgttg ggatccttga gatcttagtg gcgcagctaa
 841 cgcgataagt cgaccgcctg gggagtacgg ccgcaaggtt aaaactcaaa tgaattgacg
 901 ggggcccgca caagcggtgg agcatgtggt ttaattcgag caacgcgaag aaccttacct
 961 ggccttgaca tgctgagaac tttccagaga tggattggtg ccttcgggaa ctcagacaca
1021 ggtgctgcat ggctgtcgtc agctcgtgtc ntgagatgtt gggttaagtc ccgtaacgag
1081 cgcaaccctt gtccttagtt accagcacct cgggtgggca ctctaaggag actgccggtg
1141 acaaaccgga ggaaggtggg gatgacgtca agtcatcatg gcccttacgg ccagggctac
1201 acacgtgcta caatggtcgg tacaaagggt tgccaagccg cgaggtggag ctaatcccat
1261 aaaaccgatc gtagtccgga tcgcagtctg caactcgact gcgtgaagtc ggaatcgcta
1321 gtaatcgtga atcagaatgt cacggtgaat acgttcccgg gccttgtaca caccgcccgt
1381 cacaccatgg gagtgggttg ctccagaggt agctagtcta accgcaaggg ggacggttac
1441 cacggagtga ttcatgactg gggtgaagtc gtaacaaggt agccgtaggg gaacctgcg
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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 ^{o}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^{o}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 spectrophometrically 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-SO_4 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^{o}C with 3 ml of 5 mM 1,2-dichloroethane in 50 mM tris SO_4 (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).

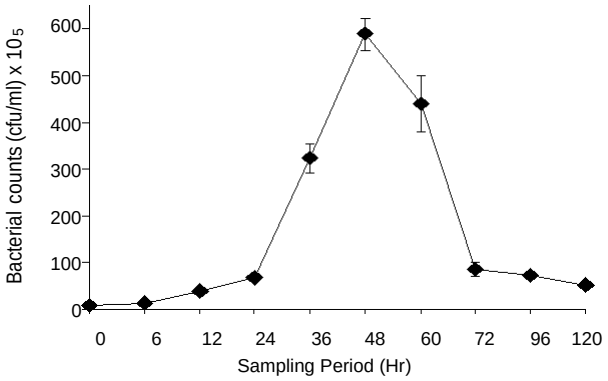

Figure 2. Growth pattern of the bacterial isolate in 1, 2- DCE.

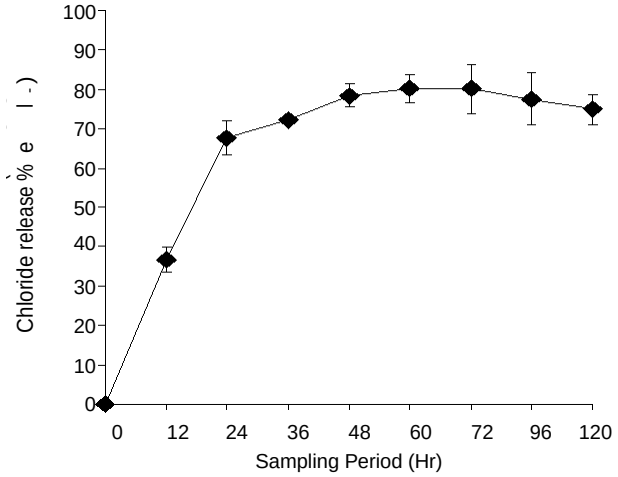

Figure 3. Chloride release during growth of the bacterial isolate in 1, 2 – DCE.

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, dichloro-methane, 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 alkanoic acids, respectively (van der Ploeg et al., 1991). Optimum temperature for the dehalogenase activity against 1, 2 – DCE was observed to be 35^oC (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

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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 *dhl*A 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, Jenssen 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.