Degradation of 2,4-dichlorophenoxyacetic acid by haloalkaliphilic bacteria

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Three 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacterial isolates were obtained from the highly saline and alkaline Alkali Lake site in southwestern Oregon contaminated with 2,4-D production wastes. While similar in most respects, the three isolates differed significantly in 2,4-D degradation rates, with the most active strain, I-18, demonstrating an ability to degrade up to 3000 mg 2,4-D l⁻¹ in 3 d. This strain was well adapted to the extreme environment from which it was isolated, growing optimally on 2,4-D at pH 8.4-9.4 and at sodium ion concentrations of 0.6-1.0 M. According to its optimum salt concentration and pH for growth, this isolate was a moderately halophilic, alkaliphilic bacterium. The 16S RNA gene sequence (303 nt) was identical for all three isolates and most closely resembled those of the moderately halophilic eubacteria of the family Halomonadaceae (91% identity). Biochemical and genetic examination revealed strain I-18 utilizes the same 2,4-D degradation pathway as most of the 2,4-D-degrading bacteria from non-extreme environments. Hybridization data and comparison of the partial sequences of the tfdA gene from the Alkali Lake isolates with those of bacteria from non-extreme environments suggested a common genetic origin of the 2,4-D degradation pathway in the two groups of micro-organisms.

Keywords: Halomonadaceae, 2,4-dichlorophenoxyacetic acid, degradation of chloroaromatic compounds, halophilic bacteria, alkaliphilic bacteria

INTRODUCTION

The critical role of micro-organisms in the degradation of organic pollutants is well known. Although the micro-organisms capable of degradation of organic pollutants and their catabolic pathways have been investigated intensively, information on the microbial degradation of xenobiotics in environments of high salinity and alkalinity is still very limited (Oren et al., 1992). Comparison of analogous catabolic enzymes and pathways of phylogenetically diverse bacteria isolated from extreme and moderate environments should provide information regarding their metabolic diversity. The study of xenobiotic-degrading extremophiles may also help in evaluating their use in bioremediation of contaminated saline and/or alkaline environments.

The 2,4-dichlorophenoxyacetic acid (2,4-D) degradation pathway is one of the best understood, and provides a good model for such investigations. This herbicide has been released into the environment for more than 40 years, and is degraded in soils fairly rapidly. Many bacteria of different genera are able to completely degrade 2,4-D (Don & Pemberton, 1981; Chaudhry & Chapalamadugu 1991; Häggblom, 1992). Degradation of 2,4-D via oxidative cleavage of the ether bond with subsequent chlorophenol hydroxylation followed by the modified ortho-cleavage pathway of chlorocatechols has been demonstrated for most of these isolates. This pathway has been most extensively studied using Alcaligenes eutrophus strain JMP134(pJP4) (Don & Pemberton, 1981, 1985). The enzymes participating in the 2,4-D degradation pathway

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have been purified and characterized, and genes that code for these enzymes (tfdA, tfdB, tfdCDEF) have been sequenced (Ghosal & You, 1988, 1989; Perkins et al., 1984; Johnson et al., 1985). Samples used in this study were taken from soil just above groundwater level across the contaminant plume gradient in April 1993 in cooperation with the State of Oregon Department of Environmental Quality.

**Medium.** The alkaline mineral medium 1 (AMM1), pH 9.5, was prepared by mixing sterile component A (20 g Na$_2$CO$_3$ and 20 g NaHCO$_3$ in 300 ml H$_2$O), component B [40 g NaCl, 3 g KCl, 0.5 g NH$_4$NO$_3$, 0.3 g (NH$_4$)$_2$SO$_4$, 0.4 g MgSO$_4$, 0.3 g NaH$_2$PO$_4$ and trace elements (Imhoff & Trüper, 1977) in 700 ml H$_2$O] and vitamins (Wolin et al., 1963). MgSO$_4$, NaH$_2$PO$_4$ and vitamins were made as 1000 x stock solutions and sterilized separately. Alkaline mineral medium 2, pH 9.4, with decreased carbonate concentration (AMM2) was used in some experiments. It contained 5 g Na$_2$CO$_3$ and 5 g NaHCO$_3$ in component A. AMM agar was prepared by adding 20 g agar to component B before sterilization. Prior to mixing, all solutions were cooled to room temperature or, in the case of AMM agar media, at least to about 45 °C. The carbon sources and their concentrations were 2,4-D (50-3000 mg l$^{-1}$) and yeast extract (50-500 mg l$^{-1}$).

**Enrichment.** Soil samples collected at six points across the contaminant plume gradient were combined. Subsamples (5 g) of the mixture were added to 200 ml AMM1 in Erlemeyer flasks and incubated aerobically at 30 or 37 °C. Cultures were grown statically or on a rotary shaker at 200 r.p.m. 2,4-D (50 mg l$^{-1}$) was used as the only carbon source. In the case of complete disappearance of 2,4-D from enrichment cultures, repeated additions (from two to five) were used to supply microorganisms with growth substrate. The ability to degrade 2,4-D was evaluated by HPLC and by measurement of the $^{14}$C$_{2}$CO$_3$ evolution from $^{14}$C-labelled 2,4-D (see below). Enrichments demonstrating 2,4-D degradation (5 ml) were transferred to fresh AMM1 (50 ml) containing 50 mg yeast extract l$^{-1}$ and 200 mg 2,4-D l$^{-1}$. These enriched samples were plated onto AMM1 agar plates containing 500 mg 2,4-D l$^{-1}$. For purification of isolates, single colonies were selected and transferred onto AMM1 plates containing 500 mg 2,4-D l$^{-1}$. Enrichments were centrifuged and the supernatant was removed. The tubes were inserted into scintillation vials containing on the bottom 1 ml phenethylamine as a trapping agent. Vials were closed with caps and incubated on a rotary shaker at 150 r.p.m. for 3 d. At the end of the incubation period, the microcentrifuge tubes were removed from the scintillation vials and 10 ml of the Bio-Safe II scintillation cocktail was added to the trapping agent. Radioactivity was measured using an LKB 1211 Rackbeta liquid scintillation counter with appropriate correction for quenching.

**Catabolism of $^{14}$C-labelled 2,4-D.** For isotopic experiments, the reaction mixture contained in a 1.5 ml microcentrifuge tube 0-25 ml AMM1, 0-25 ml enrichment cultures and 50 mg l$^{-1}$ ring-labelled 2,4-D (Sigma, specific activity 202 mCi mmol$^{-1}$ (747 MBq mmol$^{-1}$), 0.05 μCi per sample). A control tube contained only labelled 2,4-D and AMM1 without enrichment cultures. The tubes were closed with caps and incubated on a rotary shaker at 150 r.p.m. for 3 d. At the end of the incubation period the microcentrifuge tubes were removed from the scintillation vials and 10 ml of the Bio-Safe II scintillation cocktail was added to the trapping agent. Radioactivity was measured using an LKB 1211 Rackbeta liquid scintillation counter with appropriate correction for quenching.

**High performance liquid chromatography (HPLC).** This was performed with a Hewlett-Packard series 1050 chromatograph equipped with a multiple wavelength detector set at 230 nm. Separation was achieved on a reversed-phase Lichrosorb RP-18 column (Merck) of internal diameter 4 mm and length 250 mm.
The flow rate was 1.5 ml min⁻¹. Compounds were detected at 230 nm. The mobile phase was an aqueous solution of 40% (v/v) methanol and 0.1% (w/v) H₃PO₄.

Utilization of other aromatic compounds as sources of carbon and energy. Growth on aromatic compounds was measured in AMM with benzoic, 3-hydroxy- and 4-hydroxybenzoic acids supplied at 300 mg l⁻¹; 2-chloro-, 3-chloro- and 4-chlorobenzoic acids, and 2-methyl-4-chlorophenoxacyclic acid supplied at 150 mg l⁻¹; and monochlorophenols, 2,4-dichloro-, 2,6-dichloro- and 2,4,6-trichlorophenol, and 2,4,5-trichlorophenoxacyclic acid supplied at 30 mg l⁻¹. Cultures were analysed for increase in OD and removal of the aromatic compound from the medium.

Determination of optimal growth conditions. The specific growth rates under various conditions were analysed during exponential growth by linear regression of the logarithm of OD₆₀₀ with time. Salt dependency of growth was determined in AMM1 and AMM2 containing various concentrations of NaCl (0-1.8 M) in component B. To determine the pH optima for growth, media pH was varied by mixing component B of AMM with various buffer systems: 40 mM HEPES, pH 6.8-7.5; 40 mM Tris, pH 7.1-8.9; and 200 mM Na₂CO₃/NaHCO₃ buffer, pH 8.6-10.6. The pH values reported here are the initial ones, but they varied less than ±0.2 units during growth of the isolate. All media were supplemented with NaCl to keep the sodium ion concentration near 0.8 M.

Fatty acid analysis. The fatty acid methyl ester analysis of lipids was performed by MIDI Laboratories (Newark, DE). Isolate I-18 was precultured on AMM1 agar supplemented with 2 g 2,4-D l⁻¹ and streaked onto plates of tryptic soy agar (TSA) containing 10 g agar l⁻¹ to 513. Four colonies were isolated. Three isolates grew well at 30 °C, but no growth was observed with E. coli. Strains P-3 and 1-17 were grown on AMM1 containing 1 g Casamino acids l⁻¹ these bacteria initially produced small transparent colonies, progressing into yellow colonies with tan pigmentation in the centre and a lobate edge. The isolates grew well at 30 °C, but no growth was observed at 37 °C. All strains were Gram-negative, motile rods. Electron microscopy revealed cells of isolate I-18 to be about 1.7-1.9 μm in length and about 0.6-0.7 μm in diameter (data not shown).

Preparation of cell-free extracts and enzyme assay. Strain I-18 was grown on AMM1 containing 2000 mg 2,4-D l⁻¹ and 50 mg yeast extract l⁻¹ for 36 h, then harvested by centrifugation and washed twice with 50 mM Tris/HCl buffer, pH 8.0 containing 1 mM dithiothreitol and 2 mM MnSO₄. After disruption of the cells by sonication for 2 min at 0 °C, cell-free extracts were separated from whole cells and cell debris by centrifugation at 25000 g for 40 min at 5 °C.

Activities of the modified ortho-cleavage pathway enzymes were measured spectrophotometrically using a Perkin-Elmer Coleman 124 instrument (USA). One unit of enzyme activity was defined as the amount of enzyme that catalysed the formation of 1 pmol product min⁻¹ at room temperature. Activities of chlorocatechol 1,2-dioxygenase (EC 1.13.11.1), chloromuconate cycloisomerase (EC 5.5.1.7) and dienelactone hydrolase (EC 3.1.1.48) were determined as described previously (Dorn & Knackmuss, 1978; Matseva et al., 1994).

Protein concentrations were determined using a Bio-Rad Protein Assay Kit with bovine serum albumin as a standard.

DNA extraction and Southern hybridization. Strain I-18 was cultured with 2,4-D as growth substrate as described above. Strains P-3 and I-17 were grown on AMM1 containing 1 g sodium pyruvate l⁻¹ and 200 mg 2,4-D l⁻¹. The miniprep method of Ausubel et al. (1987) was used to extract total genomic DNA. EcoRI-digested DNA was separated by gel electrophoresis on an 0.8% (w/v) agarose gel and blotted onto Nyblond-N nylon membranes (Amersham). The probes were internal segments of the tfdA, tfdB, tfdC and tfdD genes of A. eutrophus JMP134(pJP4) (Holben et al., 1992). All probes were labelled with digoxigenin dUTP using a DNA labelling kit (Boehringer-Mannheim) according to the manufacturer's instructions. Hybridizations were performed at three different stringencies as described by Fulthorpe et al. (1995).

PCR amplification and sequencing of PCR products. The 16S ribosomal RNA genes were amplified using rD1 and fD1 primers (Weisburg et al., 1991). The tfdA genes were amplified using primers TV1 and TV2 designed by Tatiana Vallaey and Alice Wright and synthesized at the Macromolecular Facility, Michigan State University (Vallaey et al., 1996). The PCR reaction mixtures were prepared according to the manufacturer's protocols (Perkin Elmer Cetus). Thermal cycling was done in a Perkin Elmer 9700 Thermal Cycler using the following conditions: melting at 92 °C for 1 min; cycling 30 cycles at 1 min 90 °C for 1 min 10 s, 55 °C for 30 s and 72 °C for 2 min 10 s; followed by a final extension at 72 °C for 6 min 10 s. Amplified products were purified using a Gene Clean kit (BIO 101). Sequencing was carried out at Michigan State University Sequencing Facility using the Applied Biosystems Model 373A automatic sequencer (Perkin Elmer Cetus) and fluorescently labelled dye termination. The sequencing primer used for 16S was 519R (5'-GTA TTA CAC CAG GTT TGA TCA TAA 3') (Lane et al., 1985). Partial sequences were compared to data in GenBank using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (Altschul et al., 1990) and also the database of the Ribosomal Database Project (Larsen et al., 1993).

Chemicals. Catechol was purchased from Sigma and 4-chlorocatechol from Helix Biotech Corporation. 3-Chlorocatechol and 3,5-dichlorocatechol, and cis-dienelactone were kind gifts from M. Schloemann, Universität Stuttgart, and W. Reineke, Bergische Universität-Gesamthochschule Wuppertal, respectively. Chloromuconic acids were prepared as described by Kuhn et al. (1990) using partially purified chlorocatechol dioxygenase kindly supplied by M. Schloemann and M. Vollmer, Universität Stuttgart.

RESULTS
Characterization of isolates
Three strains able to degrade 2,4-D were isolated from the enrichments and designated P-3, I-17 and I-18. On AMM1 agar supplemented with 1 g yeast extract l⁻¹ and 1 g Casamino acids l⁻¹ these bacteria initially produced small transparent colonies, progressing into yellow colonies with tan pigmentation in the centre and a lobate edge. The isolates grew well at 30 °C, but no growth was observed at 37 °C. All strains were Gram-negative, motile rods. Electron microscopy revealed cells of isolate I-18 to be about 1.7-1.9 μm in length and about 0.6-0.7 μm in diameter (data not shown).

The sequences of approximately 303 nucleotides, corresponding to the E. coli 16S RNA gene sequence from nt 211 to 513, were identical for all three isolates and showed high similarity to 16S RNA sequences of the moderately halophilic eubacteria. The highest identity was found with Halomonas elongata ATCC 33173 (91.4%), Halovibrio variabilis DSM 3051 (91.1%), and Delela halophila DSM 4770 (89.8%), Halomonas meridiana DSM 5425 (89.8%) and Delela marina ATCC 25374 (89.8%).

The major fatty acids of isolate I-18 and their concentration ranges were 10:0 (35.6-45.2%), 12:0 3-OH (28.1-35.8%), 14:0 (19.4-24.0%), 16:0 (84.6-90.6%), 16:1 cis 9 (12.4-15.48%), 17:0 cyclo (49.0-59.0%) and
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Fig. 2. Influence of yeast extract on 2,4-D degradation by strain 1-18. Cells were grown on AMMI, pH 9.5, containing 2,4-D (500 mg L⁻¹) (■) or 2,4-D (500 or 3000 mg L⁻¹) plus yeast extract (50 mg L⁻¹) (●, ○).

Fig. 3. Degradation of 2,4-D by Alkali Lake isolates 1-18 (▲), P-3 (○) and 1-17 (■) in AMMI, pH 9.5, supplemented with 50 mg yeast extract L⁻¹. An unresolved mixture of 18:1 (54:54–56:57 %) and 19:0 cyclo 11-12 (4:41–6:38 %).

Dynamics of 2,4-D degradation

Although the Alkali Lake isolates were able to grow on minimal alkaline medium with 2,4-D as single carbon and energy source, addition of a trace of yeast extract greatly increased the rate of 2,4-D degradation (Fig. 2; data for isolates 1-17 and P-3 not shown). While similar in appearance and colony morphology, the three isolates demonstrated significant differences in ability to metabolize 2,4-D (Fig. 3). The growth range and degradation ability of the most active isolate I-18 were examined in more detail. When supplemented with 50 mg yeast extract L⁻¹, strain I-18 demonstrated an ability to degrade 3000 mg 2,4-D L⁻¹ over a 3 d period. This strain was able to utilize other aromatic compounds including benzoic acid, m-hydroxy- and p-hydroxybenzoic acid, 3-chlorobenzoic acid and, less efficiently, 4-chlorophenol and 2,4-dichlorophenol (data not shown). Growth was not observed with 2-chloro- or 4-chlorobenzoic acid, 2-chloro- or 3-chlorophenol or 2,4,5-trichlorophenoxy-acetic acid.

Salt effects on the growth of strain I-18

Different NaCl optima were observed during growth of isolate I-18 in AMM1 and AMM2 containing different amounts of sodium carbonates (Fig. 4a). When specific growth rates were plotted against total sodium ion concentration, however, almost coincident curves were obtained, indicating the strong growth response to sodium ions and lesser dependence of growth on carbonate or chloride concentration (Fig. 4b). This organism was able to grow over a wide range of salt concentrations on both yeast extract and 2,4-D. However, the use of complex media supported growth over a wider range of NaCl concentrations than did defined media. The optimal sodium ion concentration for growth was the same on both defined and complex media (0.6–1.0 M), and no growth was observed below 0.1 M.

pH effects on the growth of strain I-18

This was studied in media of different pH and a sodium ion concentration of approximately 0.8 M. The bacterium was able to grow on yeast extract over a wide pH range (6.5–10.5) and had a broad pH optimum (8.2–9.6) (Fig. 5). When grown on defined medium with 2,4-D, a more narrow pH range of the growth was found (7.4–9.8) with the optimum between 8.4 and 9.4.

Degradation pathway of isolate I-18

High activities of catechol 1,2-dioxygenase, muconate cycloisomerase and dienelactone hydrolase were detected in cell-free extracts of isolate I-18 grown on 2,4-D (Table 1). Activities of catechol 1,2-dioxygenase and muconate cycloisomerase were higher with chlorinated than with unsubstituted substrates. The data suggest that haloalkaliphilic strain I-18, like the well-studied strain A. eutrophus JMP134(pJP4), uses the modified ortho-cleavage pathway for 2,4-D degradation.

Genetic comparison of the 2,4-D degradation pathway in bacteria from extreme and non-extreme environments

No hybridization of the DNA from the Oregon isolate I-18 with the pJP4 genes tfdA, tfdB, tfdC and tfdD encoding the first four steps of the 2,4-D degradation pathway in A. eutrophus JMP134 was found at high stringency, but positive signals were observed with tfdA, tfdB and tfdD probes at medium stringency and very weak hybridization.
Haloalkaliphilic 2,4-D-degrading bacteria

Fig. 4. Influence of sodium chloride (a) and sodium ion concentration (b) on growth of isolate 1-18 on yeast extract (500 mg l\(^{-1}\); open symbols) and on 2,4-D (500 mg l\(^{-1}\)) plus yeast extract (50 mg l\(^{-1}\)) (filled symbols). Cells were grown in AMM1, pH 9.5, containing 20 g Na\(_2\)CO\(_3\) l\(^{-1}\) and 20 g NaHCO\(_3\) l\(^{-1}\) (○, ●) or in AMM2, pH 9.4, containing 5 g Na\(_2\)CO\(_3\) l\(^{-1}\) and 5 g NaHCO\(_3\) l\(^{-1}\) (▲, ■). Both media were supplemented with different concentrations of sodium chloride.

Fig. 5. Influence of pH on growth of isolate 1-18 on yeast extract (500 mg l\(^{-1}\); open symbols) or on 2,4-D (500 mg l\(^{-1}\)) plus yeast extract (50 mg l\(^{-1}\)) (filled symbols). The pH values of the media were adjusted with the following buffer systems: 40 mM HEPES/NaOH (pH 6.8–7.5) (△), 40 mM Tris/HCl (pH 7.1–8.9) (○, ●), and 200 mM Na\(_2\)CO\(_3\)/NaHCO\(_3\) (pH 8.6–10.6) (□, ■). All media were supplemented with NaCl to keep the sodium ion concentration near 0.8 M.

Table 1. Enzyme activities in cell extracts of 2,4-D-grown cells of isolate 1-18

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Specific activity [mU (mg protein)(^{-1})]</th>
<th>Relative activity (%)(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechol 1,2-dioxygenase</td>
<td>Catechol</td>
<td>110</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3-Chlorocatechol</td>
<td>61</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>4-Chlorocatechol</td>
<td>81</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>3,5-Dichlorocatechol</td>
<td>188</td>
<td>171</td>
</tr>
<tr>
<td>Muconate cycloisomerase</td>
<td>(cis, cis)-Muconate</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2-Chloro-(cis, cis)-muconate</td>
<td>43</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td>3-Chloro-(cis, cis)-muconate</td>
<td>56</td>
<td>329</td>
</tr>
<tr>
<td></td>
<td>2,4-Dichloro-(cis, cis)-muconate</td>
<td>33</td>
<td>194</td>
</tr>
<tr>
<td>Dienelactone hydrolase</td>
<td>(cis)-Dienelactone</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Relative activities of catechol 1,2-dioxygenase and muconate cycloisomerase are expressed as a percentage of the value with catechol and muconate, respectively.

with \(tfdC\) was found at low stringency (Fig. 6). Hybridization patterns for all three Alkali Lake isolates were identical (data for isolates I-17 and P-3 not shown). The sizes of all DNA restriction fragments of these isolates hybridizing with \(tfdA-D\) differed from those of pJP4.

The \(tfdA\) genes from the Alkali Lake isolates were PCR-amplified and the products sequenced. The sequences of approximately 317 nucleotides, corresponding to the \(A.\ eutrophus\) JMP134 \(tfdA\) gene sequence from nt 449–765, were identical for all three isolates. The partial sequences of these isolates showed strong similarity to \(tfdA\) sequences of \(Burkholderia\) sp. strain RASC (formerly \(Pseudomonas\) sp. TFD3) and \(A.\ eutrophus\) JMP134 (94\% and 77\% identity, respectively) (Perkins \textit{et al.}, 1990; Suwa
Halomonadaceae (Halomonas, Deleya and Haloribrio) cannot be resolved on the basis of phylogenetic, chemotaxonomic or phenotypic data and unification of these genera in a single genus has been proposed (Dobson et al., 1993). Until the taxonomy of this group is clarified it is not feasible to assign these bacteria to a species or genus. The close taxonomic relationship of the Alkali Lake isolates and the Halomonadaceae indicated by the 16S RNA sequencing data is consistent with the finding that isolate I-18 is a moderately halophilic bacterium. In addition the major fatty acids of isolate I-18 (16:0, 16:1, 17:0 cyc, 18:1 and 19:0 cyc), representing 90% of the total fatty acids, are the same as for other members of the Halomonadaceae (Franzmann & Tindall, 1990).

The alkaliphilicity of Halomonadaceae strain I-18 is not surprising as it is known that some bacteria of this family are capable of growth at pH 9 and even pH 10 (Del Moral et al., 1988; Vreeland et al., 1980). Recently, it was shown that two groups of alkaliphilic isolates from Kenyan Soda Lakes also belong to the Halomonadaceae (Jones et al., 1994).

The Alkali Lake isolates are the first known aerobic, halophilic bacteria able to use chloroaromatic compounds as sources of carbon and energy. Enzyme activities of strain I-18 suggest that it utilizes the same pathway of 2,4-D degradation as the majority of 2,4-D-degrading bacteria from non-extreme environments (Haggbloom, 1992). Since the contamination of the Alkali Lake site with chloroaromatic compounds is recent, the origin of the 2,4-D degradation pathway in the haloalkaliphilic bacteria is of interest. The DNA from all three Alkali Lake isolates hybridized to pJP4 genes tfdA, tfdB, tfdC and tfdD, responsible for 2,4-D degradation in the well-known 2,4-D-degrading bacterium A. eutrophus JMP134. Moreover, the partial sequences of the tfdA genes from the Alkali Lake isolates were only slightly different from the sequence of the tfdA gene of A. eutrophus JMP134 and identical to that of the 2,4-D-degrading strains R. fermentans B6-9 and TFD31 isolated from non-extreme environments in Ontario and Southern Saskatchewan, respectively (Fulthorpe, 1991; Tonso et al., 1995). These data suggest a common origin of the genes encoding 2,4-D degradation in the haloalkaliphilic bacteria and bacteria from non-extreme environments.

An interesting characteristic of moderately halophilic eubacteria is their mode of osmoregulation. In contrast to the extremely halophilic Archaea that have high intracellular potassium and sodium ion concentrations and enzymes adapted to this environment (Hochstein, 1988), moderately halophilic bacteria can extensively accumulate organic osmolytes called ‘compatible solutes’ which do not interfere with cell metabolism and may even contribute to enzyme stability (Galinski, 1993). It is not clear whether the enzymes of moderately halophilic eubacteria have special adaptive changes to function optimally in an environment of high osmolarity (Kushner & Kamekura, 1988). The data obtained in this investigation indicate that very similar TfdA enzymes can function efficiently in both halophilic and non-halophilic eubacteria. Complete
sequencing of the genes encoding 2,4-D degradation in strain I-18, and study of catabolic gene transfer between halophilic and non-halophilic eubacteria should more firmly establish the compatibility of the enzymes from both groups of micro-organisms and the requirements for engineering xenobiotic-degrading halophilic eubacteria.

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\[ \text{i} \text{fd} \text{A} \text{ genes.} \]

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