ELSEVIER

Journal of Biotechnology 95 (2002) 171–179

JOURNAL OF Biotechnology

www.elsevier.com/locate/jbiotec

Screening and characterization of aldehyde dehydrogenase gene from *Halomonas salina* strain AS11

Thanya Sripo [a], Amornrat Phongdara [a,*], Chokchai Wanapu [b], Allan B. Caplan [c]

[a] *Department of Biochemistry, Faculty of Science, Prince of Songkla University, Hatyai, Songkla 90112, Thailand*
[b] *School of Biotechnology, Institute of Agriculture Technology, Suranaree University of Technology, 111 University Avenue, Maung, Nakon Ratchasrima 30000, Thailand*
[c] *Department of Microbiology, Molecular Biology and Biochemistry, College of Agriculture, University of Idaho, Moscow, ID 83844-3052, USA*

Received 9 August 2001; received in revised form 2 January 2002; accepted 14 January 2002

Abstract

A population survey was made of moderately halophilic bacteria in prawn pond sediment in the Songkla region of Thailand. Twenty-two isolated halophilic bacteria capable of growing on modified ATCC culture medium 1270 for halobacterium were then assayed for aldehyde dehydrogenase (ALDH) activity which might be involved in the metabolism of xenobiotic compounds. One isolate, designated AS11, was selected based on its high amount of ALDH activity. This organism can grow at sodium chloride concentrations ranging from 2.5 to 25%, although optimum growth occurs at 5% NaCl. Phenotypic and phylogenetic studies indicated that AS11 was an isolate of *Halomonas salina*. The *aldh* gene coding for this enzyme was then cloned. The open reading frame of the *aldh* gene was 1521-bp long and coded for a protein of 506 amino acid residues with a calculated molecular mass of 55 kDa. The *aldh* gene product proved to be 76% identical to the NAD-dependent acetaldehyde dehydrogenase gene from *Pseudomonase aeruginosa*.

Keywords: Halobacterium; *Halomonas*; Aldehyde dehydrogenase

1. Introduction

The growth of the shrimp aquaculture industry in Thailand has benefited the social and economic well being of numerous individuals but also had a significant environmental impact on many parts of the country. This industry has led to the destruction of wetlands and a deterioration of water-quality as a result of siltation, eutrophication, oxygen depletion, and the release of toxic sulfides, ammonia, and xenobiotics compounds from therapeutic and wastewater treatment chemicals (Dierberg and Kiattisimkul, 1996). The biotransformation of a large number of drugs and other xenobiotics generates aldehyde as intermediates or as products resulting from oxidative deaminations

* Corresponding author. Tel.: +66-74-288384; fax: +66-74-446656.
E-mail address: pamornra@ratree.psu.ac.th (A. Phongdara).

0168-1656/02/$ - see front matter
PII: S0168-1656(02)00006-8

and dealkylations, as well as the oxidation of primary alcohol (Lindahl, 1992). Aldehydes frequently are highly reactive, and many have significant biological effects, including cytotoxicity, mutagenicity, genotoxicity and carcinogenicity, on organisms exposed to them. However, the majority of aldehydes are encountered as physiologically derived intermediates in the metabolism of other compounds. Endogenous sources include aldehydes arising from the metabolism of amino acids, biogenic amines, carbohydrates, vitamins, steroids and lipids. A variety of enzymes have evolved to metabolize aldehydes to less reactive forms. The most effective pathway for aldehyde metabolism is their oxidation to carboxylic acids by aldehyde dehydrogenases (ALDHs). Regardless of their specificity, these NAD/NADP-dependent enzymes share common structural and functional features that catalyze the oxidation of a broad spectrum of aliphatic and aromatic aldehydes (Hempel et al., 1993).

The use of bacterial remediation treatments is becoming more commonplace in the aquaculture industry in the belief that the growth of selected microbial species will help maintain low ammonia concentrations, reduce organic matter concentrations and improve the quality of sediment accumulated in the pond (Funge-Smith and Briggs, 1998). Several research groups have isolated novel microorganisms from soil, wastewater, and culture collections that have proven capable of catabolizing some of the more exotic compounds introduced into the environment by today's agricultural practices. The aerobic bacterium V2, for example, can grow on vinyl acetate using an enzymatic pathway that involves vinyl acetate esterase, ALDH and alcohol dehydrogenase (Nieder et al., 1990). In a similar way, *Pseudomonas fluorescens* transforms environmentally persistent compounds including volatile chlorinated aliphatic hydrocarbons into non-toxic, non-chlorinated components (Vandenbergh and Kunka, 1988).

The ponds and run-offs of shrimp-farming enterprises present a particularly difficult challenge to bioremediative programs. Most of the organisms that have proven effective in soil cannot grow well in the saline environments of these ponds. On the other hand, these ponds are the natural home of halophilic bacteria that are very well adapted to live in environments with high salinities. They are easy to grow and have simple nutritional requirements (Larsen, 1962; Ventosa, 1994). Moreover, many of them produce compounds of industrial interest including enzymes, polymers and osmoprotectants.

We have isolated a halophilic microorganism from prawn pond sediment. It is a gram negative bacterium that produces high levels of ALDH. This report shows that this gene was part of a cluster encoding three proteins, ALDH, alcohol dehydrogenase and a possible regulatory protein. This bacterium may prove to be useful in future programs to accelerate the breakdown of toxic metabolites produced by the prawn industry.

2. Materials and methods

2.1. Isolation and characterization of strain AS11

Ten grams of prawn pond sediment were collected from a farm in Songkla, Thailand, and mixed with sterile 0.85% NaCl solution. The dilutions of sediment extract were spread onto agar plates containing 0.3% beef extract, 0.5% peptone and 10% sodium chloride solidified with 1.5% (w/v) agar (Difco laboratories Detroit, Mich.). The plates were incubated at 30 °C overnight. Several colonies with different morphological characteristics were picked and streaked out several times on the same medium to obtain purified colonies.

Taxonomical characterization of one isolated strain was conducted according to Bergey's Manual of Systematic Bacteriology (Brinley-Morgan et al., 1984) and on the basis of substrate utilization (Biolog GN microplate identification system, Biolog, Hayward, Calif.). For the phylogenetic analysis of the 16S rRNA sequences, total DNA was extracted from the isolated strains according to the method of Ausubel et al. (1987). DNA fragments encoding 16S rRNA were amplified from the extracted

total DNA by PCR and sequenced according to the method described by Edwards et al. (1989). Phylogenetic analysis of the 16S rRNA sequences was performed as described by Maidak et al. (1994).

2.2. Culture conditions and preparation of crude cell extracts

The bacterial strain AS11 was used in this study. For the production of enzymes, cells were grown in modified ATCC culture medium 1270 containing 5% NaCl, 1.6% $MgCl_2$, 2.4% $MgSO_4$, 0.1% $CaCl_2$, 0.5% KCl, 0.02% $NaHCO_3$, 0.05% NaBr and 0.5% yeast extract, pH adjusted to 7.3. After 24 h of continuous shaking at 30 °C, the cells were harvested by centrifugation at $5000 \times g$ for 10 min. Suspensions of washed bacteria in 50 mM phosphate buffer pH 7.0 were disrupted by ultrasonication. The resulting crude bacterial extract was centrifuged at $12\,000 \times g$ for 20 min to remove unbroken cells and cell debris.

2.3. Enzyme assays

Enzyme activity was determined spectrophotometrically (model Ultrospec III, Pharmacia) at 30 °C by monitoring the reduction of NADH at 340 nm. The enzyme activity was determined by incubation of the cellular extract with 10 mM of each substrate (propionalaldehyde, acetaldehyde, betaine aldehyde, and ethanol (Sigma)) at 30 °C (von Tigerstrom and Razzell, 1968). One unit is defined as the activity that catalyzes the formation of 1 μmol of product (NADH) per min.

Protein concentrations were determined as described by Lowry et al. (1951), with bovine serum albumin as the standard.

The molecular masses of denatured ALDHs were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). Phosphorylase b (molecular mass 94 kDa), Albumin (67 kDa), Ovalbumin (43 kDa), Carbonic anhydrase (30 kDa), and Trysin inhibitor (20 kDa) were used as marker proteins. The gels were strained with Coomassie brilliant blue.

2.4. Synthesis of the probes for aldehyde dehydrogenase

A pair of degenerate oligonucleotide primers was synthesized based on conserved sequences (ELGGKS; 5′-TCG-AAC-TGG-GYG-GHA-A-3′ and EEIFGP; 5′-GGC-CCG-AAG-ATY-TCY-TC-3′) common to ALDH genes of several bacteria. The PCR reaction was performed on AS11 DNA with a DNA thermal cycler (model *Touch-Down* Hybaid, UK) for 35 cycles at 92 °C for 90 s, 60 °C for 90 s and 72 °C for 2 min. Each reaction was performed in a 50 μl volume containing 200 μM dNTPs; 10 × PCR buffer (15 mM $MgCl_2$, 50 mM KCl and 100 mM Tris–HCl pH 8.3); 50 pmol each of the PCR primers; 50–100 ng of AS11 DNA; and 1 U Taq DNA polymerase (Promega). The PCR-amplified fragment was purified by agarose gel electrophoresis and cloned into pGEM-T easy (Promega). The amino acid sequence deduced from the nucleotide sequence of the inserted fragment was consistent with that of an ALDH gene. The fragment was then labeled with digoxigenin (Boehringer Mannheim) and used as a probe for cloning.

2.5. Cloning of the aldehyde dehydrogenase gene

Chromosomal DNA from bacteria strain AS11 was digested using several restriction enzymes and analyzed by Southern hybridization (Sambrook et al., 1989) using a probe prepared from the PCR-amplified *aldh* gene. Based on these results, a sublibrary of *Bam*HI fragments of approximated 10 kb was prepared from the AS11 genome and cloned into the *Bam*HI site of LambdaGEM-11 (Promega). The ligated mixture was packaged and transfected into the host *Escherichia coli* LE392. The library was screened with the *aldh* probe, leading to the identification of a positive clone. The phage containing the 10 kb insert was digested with various enzymes and reprobed. A 7.5 kb *Pst*I-restricted fragment was isolated from an agarose gel and cloned into pSPORTI (Gibco BRL). Three *Bgl*II/*Pst*I fragments were subsequently subcloned into pSPORTI to obtain the nucleotide sequence of the genomic fragment.

2.6. Nucleotide sequencing

Nucleotide sequencing was accomplished using the dideoxynucleotide chain termination method (Sanger et al., 1977) with an automated sequencer (model 373A, Applied Biosystems, USA). The sequences of both DNA strands were determined. Computer analysis was performed using the DNASIS program (version 2.0, Hitachi, USA). The deduced amino acid sequences of the open reading frames were compared with databases using the BLAST program.

2.7. Nucleotide sequence accession number

The nucleotide sequence of the *aldh* and partial 16S rRNA gene has been submitted to the GenBank nucleotide sequence database under accession number AF284553 and AF301407, respectively.

3. Results

3.1. Isolation of halophilic bacteria

Sediment from a prawn pond with a salinity level of 35 ppm was collected. It was judged that high organic content in the sediment should harbor a diverse population of bacteria. Thirty-six strains were purified as described in Section 2. After 24 h incubation and followed by several rounds of isolation of a single colony, 36 isolates were obtained. They were selected under the primary condition of high salinity, therefore were classified as halophiles. The microbes were screened for ALDH activity. Twenty-two from thirty-six of the isolated halophilic bacteria showed ALDH activity on modified ATCC culture medium 1270 for halobacterium. Under unoptimized conditions, four of them (isolates AS2, AS3, AS11 and AS14) produced high levels of activity (4.3–4.6 U mg^{-1} protein). The strain AS11 was chosen for continued study because of its high growth rate and ability to grow even in 25% NaCl.

3.2. Characterization of isolate strain AS11

The cells of strain AS11 were short gram negative, non-motile rod that were both oxidase and catalase-positive. This strain was able to grow on arabinose, glucose, fructose, maltose, galactose, cellobiose, trehalose, sucrose and gluconate but not on mannitol and lactose as sole carbon sources in minimal media. It produced creamy white circular colonies on nutrient media. The bacteria requires at least 2.5% NaCl for growth in both minimal and rich medium. No growth was obtained at 0.5% and the growth was optimum at 5% NaCl (Fig. 1) in a medium using sodium chloride as the sole salt. These data together with those obtained by Biolog GN microplate identification system indicated that AS11 belonged to the genus *Halomonas*.

By means of PCR amplification, a nearly complete 16S rRNA sequence corresponding to *Pseudomonas aeruginosa* 16S rRNA positions 207 through 1320 was determined for AS11. The

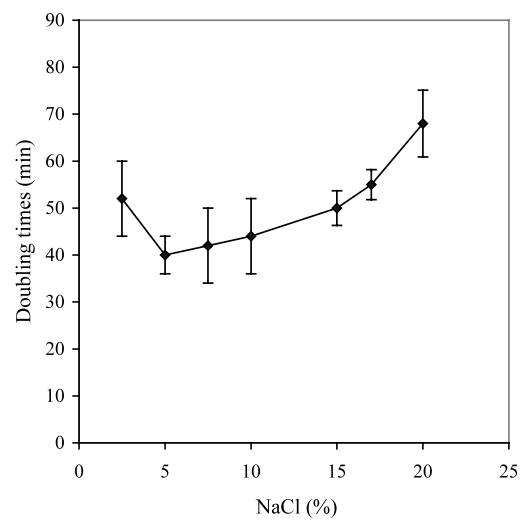

Fig. 1. Effect of sodium chloride concentration on the doubling times of *H. salina* AS11. The cells were grown in rich medium at 30 °C. Doubling times were determined after inoculation of fresh medium with cells from a preculture grown on the same sodium chloride concentration. Each point represents the mean number from triplicate samples.

Table 1
Specific activity of ALDH and alcohol dehydrogenase in crude extracts of AS11

Medium	OD 600 nm	Activity (U mg^{-1} protein)	
		ALDH	Alcohol dehydrogenase
A[a]	1.235	0	0
B[b]	0.958	10.46	51.70
B[b]+0.2% PEG	0.980	8.46	168.6
B[b]+3% ethanol	0.548	10.85	179.3
B[b]+0.2% glucose	1.214	5.98	126.89
B[b]+5% propionol	0.931	15.18	141.1

Cells were grown on medium at 30 °C.
[a] Rich medium.
[b] Modified ATCC culture medium 1270.

16S rRNA sequence analysis of AS11 showed 99% identity to the rRNA sequence of *Halomonas salina* (ATCC 49509 accession no. AJ243447), 96% identity to the rRNA sequence of *Deleya salina* (EMBL accession no. X87217) which is a synonym for *H. salina*, and 89% identity to the rRNA sequence of *P. aeruginosa* (EMBL accession no. Z76651). A phylogenetic tree based on this information shows the relationships of strain AS11 to *H. salina* and several other species. On the basis of these data, the isolated strain is referred to as *H. salina* strain AS11.

3.3. Growth of *H. salina* strain AS11 and the production of enzymes

In addition to extreme tolerance to salt, AS11 also proved able to catabolize a number of simple alcohols. Although it grew best using glucose as a sole carbon source, it could also utilize isopropanol, ethanol, or polyethylene glycol (data not shown). On the other hand, it could not utilize methanol as a sole carbon source.

No ALDH activity was detected in AS11 grown in rich medium to either early or late stationary phase. On the other hand, ALDH and alcohol dehydrogenase were produced in a minimal medium (modified ATCC culture medium 1270) supplemented with different alcohols (Table 1). This experiment was repeated with cells grown to the early stationary phase; similar levels of activity were obtained.

3.4. Cloning of the aldehyde dehydrogenase gene cluster from Halomonas salina strain AS11

In order to screen for genes involved in the metabolism of xenobiotic compounds. Two degenerate DNA primers were synthesized based on the conserved ELGGKS and EEIFGP peptide motifs of *aldh* sequences from *Vibrio cholerae* (P23240), *P. putida* (U24215), *P. aeruginosa* (AF068264) (Schobert and Goerisch, 1999), *E. coli* (S47809), *Alteromonase* sp. (AB009654), and *Alcaligenase eutrophus* (P46368) (Priefert et al., 1992). Within the *P. aeruginosa* AcDH sequence, these peptides are located at amino acids 262–268 and 401–406, respectively (Hidalgo et al., 1991).

Genomic DNA from *H. salina* strain AS11 was subjected to PCR amplification with the designed primers to obtain a fragment from the ALDH gene. The amino acid sequence deduced from the nucleotide sequence was indeed 73.0% identical to that of the ALDH of *Alteromonas* sp. In order to construct a genomic library enriched for this gene, Southern blot analysis of the genomic DNA from AS11 was carried out using the cloned PCR fragment as a probe. The region of a *Bam*HI digest of genomic DNA was then isolated from an agarose gel and cloned into the Lambda-GEM11 vector. The enriched sublibrary of 5.34×10^5 plaque-forming units was screened and ten positive clones, each containing a 10.0 kb fragment, were isolated. The cloned fragment was then digested with *Bgl*II and *Pst*I and subcloned into pSPORTI to produce three plasmids, pPGP1, pPGP8 and pPB1. DNA from these plasmids was sequenced. Three open reading frames, ORF 1–3, were detected (Fig. 2). The nucleotide sequence of ORF1 from PB1 fragment was completed, while only partial sequences were obtained from the 5′ and 3′ ends from PGP1 and PGP8 fragments.

3.5. Analysis of the nucleotide sequence

The region of the AS11 genome contained three ORFs. The gene organization was similar to that obtained from other organisms. The 3227 bp sequence region (from PB1, and PGP8 fragments) span one large reading frame of 1521 bp or 506-amino acids. The ORF1 was preceded by a putative Shine–Dalgano sequence seven nucleotides upstream of the translational start codon. The deduced amino acid sequence of the ORF1 sequence exhibited a high degree of similarity to ALDHs of other bacteria. The gene has an average G + C content of 66.7 mol%, which was in the upper end of the range of the values reported for the genomic DNA of *Halomonas* (52–68 mol%) by Dobson et al. (1993).

A second open reading frame (ORF2) of 705 bp was identified upstream to the ORF1 but oriented in the opposite direction on the complementary strand, and was not preceded by a Shine–Dalgano sequence. The encoded protein was similar to the glycerol regulatory protein (M60805) from *P. aeruginosa* (44% identical amino acid), and the putative two-component system transcriptional respond regulator TcsR (U33883) from *Bradyrhizobium japonicum* (36%).

Analysis of the DNA sequence downstream of the *aldh* gene found a partial reading frame that was in the same orientation as *aldh*. The deduced amino acid sequence (122 amino acids) of this partial reading frame displayed between 55.0 and 58.0% similarity to the *N*-termini of a group of alcohol dehydrogenases including those encoded by *Sinorhizobium moliloti* ADHA gene (AF031940), *Bacillus stearothermophilus* ADH-HT gene (Z27089), and *Streptomyces coelicolor* putative alcohol dehydrogenase (AL109989).

3.6. Expression of ALDH in E. coli

To check the biological activity of the ORF1 gene product, we examined its expression in *E. coli*. The 1.5 kbp *Hin*dIII/*Kpn*I fragment of PB1 was cloned into a QE40 expression vector (QIAGEN), resulting in plasmid pQA 802, with the

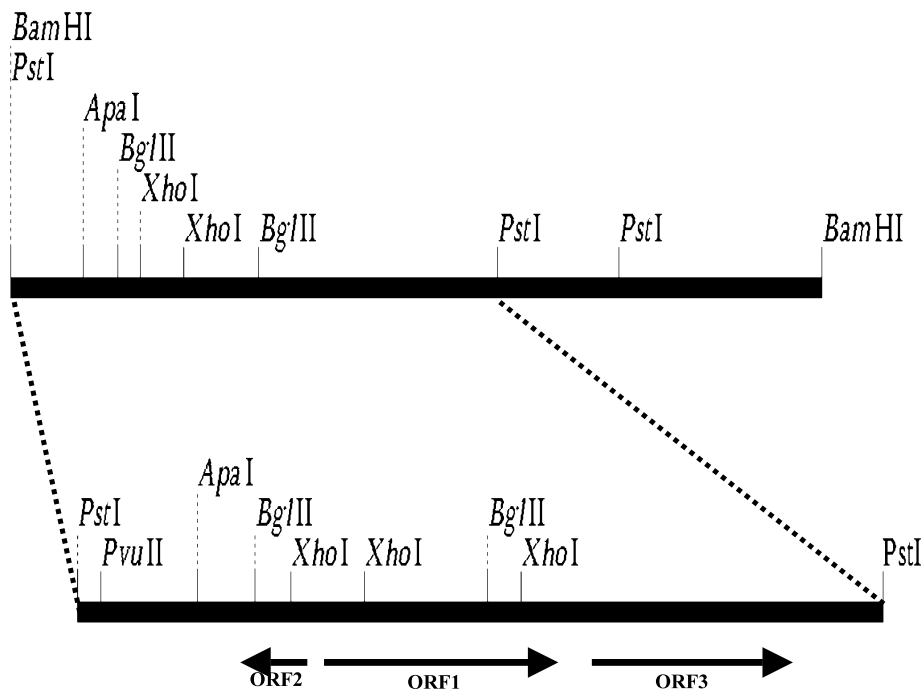

Fig. 2. Physical and genetic map of 10.0 kb DNA fragment containing the cluster gene in *H. salina* chromosome. The arrows shown directly below the physical map denote the open reading frames and the direction of transcription.

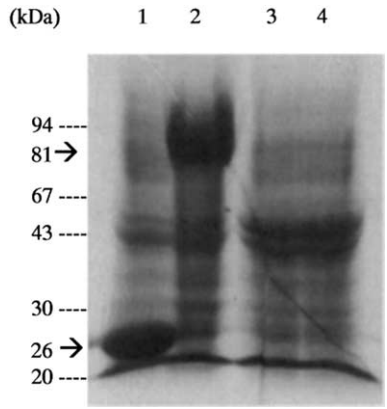

Fig. 3. Production of the ALDH protein in recombinant *E. coli*. Crude extracts prepared from different cultures were analyzed by SDS-PAGE. Lanes; 1 and 2 *E. coli* XL-1 Blue harboring pQE40 and pQA802 grown on LB with 5 μM IPTG, 3 and 4 *E. coli* XL-1 Blue harboring pQE40 and pQA802 grown on LB.

aldh gene located downstream from the T5 promoter. Expression of ALDH was stimulated by treatment with 5 μM isopropyl-β-D-thiogalactopyranoside (IPTG). An overnight culture of *E. coli* harbouing pQA802 produced 2.437 U ALDH activity mg^{-1} protein while enzyme levels in bacteria containing pQE40 were undetectable. Total proteins extracted from each of these two cultures were run on an SDS-PAGE to identify the plasmid-encoded protein. This SDS-PAGE analysis revealed that bacteria producing ALDH activity produced an extra protein of approximately 81 kDa that most likely corresponds to the 26 kDa 6 × His-DHFR tag fused to the expected 55 kDa ALDH gene product (Fig. 3).

4. Discussion

We have begun to assess the ability of microorganisms isolated from saline environment to some of the toxic by-products of prawn aquaculture. Our first approach has been to isolate some of the bacteria that live in this highly saline environment and characterize the breadth of their metabolic capabilities. The isolate AS11 showed the carbohydrate utilization pattern, Gram reaction, colony and cell morphology characteristic of previously published data for several *Halomonas* species (Ventosa et al., 1998). Studies based on 16S rRNA sequence analysis provided additional proof of the phylogenetic position of the strain AS11 to *H. salina*.

Up to now, the capability to degrade toxic compounds has been mainly observed among members of *Pseudomonas* (van Ginkel et al., 1992; Vandenbergh and Kunka, 1988), *Ancylobactor* (van den Wijngaard et al., 1992, 1993) and *Xanthobacter* (Janssen et al., 1985; Tardif et al., 1991; van den Ploeg et al., 1994) groups, and has not been described for halophilic bacteria. The *H. salina* strain AS11 was found to produce ALDH and alcohol dehydrogenase on production media containing isopropanol or ethanol. Since the expression of ALDH is specifically induced only during growth on these substrates, this enzyme seems to be responsible for the oxidation of aldehyde to the corresponding carboxylic acid in both catabolic pathways. The structural gene of *aldh* was localized and sequenced. The gene is clustered with two genes that encode an alcohol dehydrogenase gene (*adh*), and possibly a transcriptional regulatory protein. The regulatory and *adh* gene lie upstream and downstream of *aldh*, respectively. Although we have not identified the promoter or messenger for these genes, it is possible that the *aldh* reading frame and that the immediate downstream reading frame, *adh*, are cotranscribed with the *aldh* as part of the same operon.

Translation of the *aldh* DNA sequence yields a protein of 506 amino acid residues with a calculated molecular mass of 54 919.31 Da. The deduced amino acid sequence of the identified *aldh* gene shows all characteristics common to dehydrogenase including the motif VELGGKSP beginning at amino acid position 261, and FENGGEVCTCPS at amino acid position 294, that are essential for the activity of this family of protein (Von Bahr-Lindstrom et al., 1985). This protein also has the sequence GYGAEAG at amino acid position 218–224 that resembles the G-X-G-X_3-G motif believed to be involved in NAD^+ binding site in ALDHs catalyzing irreversible reactions (Hidalgo et al., 1991). By comparison, enzymes such as alcohol dehydrogenase that catalyze reversible reactions have the alternative motif, G-X-G-X_2-G (Scrutton et al., 1990).

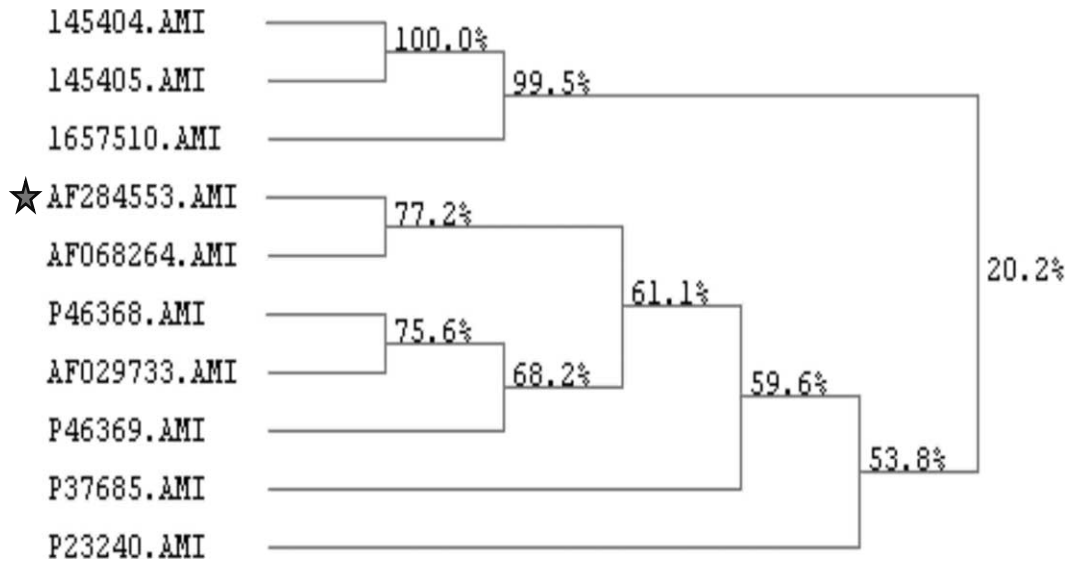

Fig. 4. Phylogenetic relationship of bacteria AS11 putative *aldh* gene product and representative bacterial ALDH. 145404, 145405, 1657510: *E. coli* betaine ALDH, AF284553: *H. salina* AS11 ALDH, AF068264: *P. aeruginosa* NAD^+ dependent ALDH, P46368: *A. eutrophus* ALDH, AF029733: *X. autotrophicus* chloroacetaldehyde dehydrogenase, P46369: *Rhodococcus erythropolis* EPTC-inducible ALDH, P37685: *E. coli* lactaldehyde dehydrogenase and P23240: *V. cholerae* ALDH.

We have also confirmed the activity of the putative *aldh* by expressing it in *E. coli* and assaying for its activity.

Comparison of the amino acid sequence of ALDH with those of proteins collected in the NCBI database showed extended homologies with ALDHs from other sources. The phylogenetic relationship of deduced amino acid sequences of AS11 and other bacterial ALDH gene product is shown in Fig. 4.

Studies in the future will focus on the identification of the promoter region, and isolation and characterization of the regulatory protein(s), which is required for transcription of *aldh* and *adh*. The knowledge will contribute to the understanding of controlled gene expression in halophile *Halomonas* sp.

Acknowledgements

This work was supported by Graduate Research and Education Consortium grant GREC-40-01-007 from National Science and Technology Development Agency, Thailand.

References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K., 1987. Current Protocols in Molecular Biology. Wiley, New York.

Brinley-Morgan, W.J., Ley, D.J., Dye, D.W., Larsen, H., Vincent, J.M., Whittenbury, R., 1984. Gram negative aerobic rods and cocci. In: Krieg, N.R. (Ed.), Bergey's Manual of Systematic Bacteriology, vol. 1, ninth ed. The Williams & Wilkins Co, Baltimore, MD, pp. 140–407.

Dierberg, F.E., Kiattisimkul, W., 1996. Issues, impacts, and implications of shrimp aquaculture in Thailand. Environ. Management 20, 649–666.

Dobson, S.J., McMeekin, T.A., Franzmann, P.D., 1993. Phylogenetic relationships between some members of the genera *Deleya*, *Halomonas*, and *Halovibrio*. Int. J. Syst. Bacteriol. 43, 665–673.

Edwards, U., Rogall, T., Blocker, H., Emde, M., Bottger, E.C., 1989. Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. 17, 7843–7853.

Funge-Smith, S.J., Briggs, M.R.P., 1998. Nutrient budgets in intensive shrimp ponds: implications for sustainability. Aquaculture 164, 117–133.

Hempel, J., Nicholas, H., Lindahl, R., 1993. Aldehyde dehydrogenase: widespread structural and functional diversity within a shared framework. Prot. Sci. 2, 1890–1900.

Hidalgo, E., Chen, Y., Lin, E.C.C., Aguilar, J., 1991.

Molecular cloning and DNA sequencing of the *Escherichia coli* K-12 ald gene encoding aldehyde dehydrogenase. J. Bacteriol. 173, 6118–6123.

Janssen, D.B., Scheper, A., Dijkhuizen, L., Witholt, B., 1985. Degradation of halogenated aliphatic compounds by *Xanthobactor autotrophicus* GJ10. Appl. Environ. Microbiol. 49, 673–677.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227, 680–685.

Larsen, H., 1962. Halophilism. In: Gunsalus, I.C., Stanier, R.Y. (Eds.), The Bacteria, vol. IV. Academic Press, London, pp. 297–342.

Lindahl, R., 1992. Aldehyde dehydrogenases and their role in carcinogenesis. Crit. Rev. Biochem. Mol. Biol. 27, 283–335.

Lowry, O.H., Rosbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Maidak, B.L., Larsen, N., McCaughey, M.J., Overbeek, R., Olsen, G.J., Fogal, K., Blandy, J., Woese, C.R., 1994. The ribosomal database project. Nucleic Acids Res. 22, 3485–3487.

Nieder, M., Sunarko, B., Meyer, O., 1990. Degradation of vinyl acetate by soil, sewage, sludge, and the newly isolated aerobic bacteria V2. Appl. Environ. Microbiol. 56, 3023–3025.

Priefert, H., Kruger, N., Jendrossek, D., Schmidt, B., Steinbuchel, A., 1992. Identification and molecular characterization of the gene coding for acetaldehyde dehydrogenase II (acoD) of *Alcaligenes eutrophus*. J. Bacteriol. 174, 899–907.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, second ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463–5467.

Schobert, M., Goerisch, H., 1999. Cytochrome c550 is an essential component of the quinoprotein ethanol oxidation system in *Pseudomonas aeruginosa*: cloning and sequencing of the gene encoding cytochrome c550 and an adjacent acetaldehyde dehydronase. Microbiol. 145, 471–481.

Scrutton, N.S., Berry, A., Perham, R.N., 1990. Redesign of the coenzyme specificity of a dehydrogenase by protein engineering. Nature (London) 343, 38–43.

Tardif, G., Greer, C.W., Labbe, D., Lau, P.C.K., 1991. Involvement of a large plasmid in the degradation of 1,2-dichloroethane by *Xanthobacter autotrophicus*. Appl. Environ. Microbiol. 57, 1853–1857.

van den Ploeg, J., Smidt, M.P., Lamda, A.S., Janssen, D.B., 1994. Identification of chloroacetaldehyde dehydrogenase involved in 1,2-dichloroethane degradation. Appl. Environ. Microbiol. 60, 1599–1605.

van den Wijngaard, A.J., van der Kamp, K.W.H.J., vander Ploeg, J., Pries, F., Kazemier, B., Jenssen, D.B., 1992. Degradation of 1,2-dichloroethane by *Ancylobacter aquaticus* and other facultative methylotrophs. Appl. Environ. Microbiol. 58, 976–983.

van den Wijngaard, A.J., Prins, J., Smal, A.J.A.C., Jenssen, D.B., 1993. Degradation of 2-chloroethylvinylether by *Ancylobacter aquaticus* AD25 and AD27. Appl. Environ. Microbiol. 59, 2777–2783.

van Ginkel, C.G., van Dijk, J.B., Kroon, A.G.M., 1992. Metabolism of hexadecyltrimethylammonium chloride in *Pseudomonas* strain B1. Appl. Environ. Microbiol. 58, 3083–3087.

Vandenbergh, P.A., Kunka, B.S., 1988. Metabolism of volatile chlorinated aliphatic hydrocarbons by *Pseudomonas fluorescens*. Appl. Environ. Microbiol. 54, 2578–2579.

Ventosa, A., 1994. Taxonomy and phylogeny of moderately halophilic bacteria. In: Priest, F.G. (Ed.), Bacterial Diversity and Systematics. Plenum Press, New York, NY, pp. 231–242.

Ventosa, A., Nieto, J.J., Oren, A., 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev. 62, 504–544.

Von Bahr-Lindstrom, H., Jeck, R., Woenckhaus, C., Sohn, S., Hempel, J., Jornvall, H., 1985. Characterization of the coenzyme binding site of liver aldehyde dehydrogenase: differential reactivity of coenzyme analogues. Biochemistry 24, 5847–5851.

von Tigerstrom, R.G., Razzell, W.E., 1968. Aldehyde dehydrogenase: Purification and properties of the enzyme from *Pseudomonas aeruginosa*. J. Biol. Chem. 243, 2691–2702.