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## ***Tindallia californiensis* sp. nov., a new anaerobic, haloalkaliphilic, spore-forming acetogen isolated from Mono Lake in California**

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**Abstract** A novel extremely haloalkaliphilic, strictly anaerobic, acetogenic bacterium strain APO was isolated from sediments of the athalassic, meromictic, alkaline Mono Lake in California. The Gram-positive, spore-forming, slightly curved rods with sizes 0.55–0.7×1.7–3.0 µm were motile by a single laterally attached flagellum. Strain APO was mesophilic (range 10–48 °C, optimum of 37 °C); halophilic (NaCl range 1–20% (w/v) with optimum of 3–5% (w/v), and alkaliophilic (pH range 8.0–10.5, optimum 9.5). The novel isolate required sodium ions in the medium. Strain APO was an organotroph with a fermentative type of metabolism and used the substrates peptone,

bacto-tryptone, casamino acid, yeast extract, L-serine, L-lysine, L-histidine, L-arginine, and pyruvate. The new isolate performed the Stickland reaction with the following amino acid pairs: proline + alanine, glycine + alanine, and tryptophan + valine. The main end product of growth was acetate. High activity of CO dehydrogenase and hydrogenase indicated the presence of a homoacetogenic, non-cycling acetyl-CoA pathway. Strain APO was resistant to kanamycin but sensitive to chloramphenicol, tetracycline, and gentamycin. The G+C content of the genomic DNA was 44.4 mol% (by HPLC method). The sequence of the 16S rRNA gene of strain APO possessed 98.2% similarity with the sequence from *Tindallia magadiensis* Z-7934, but the DNA-DNA hybridization value between these organisms was only 55%. On the basis of these physiological and molecular properties, strain APO is proposed to be a novel species of the genus *Tindallia* with the name *Tindallia californiensis* sp. nov., (type strain APO = ATCC BAA-393 = DSM 14871).

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### **Introduction**

The wide application and importance of extremophilic microorganisms have increased interest in the study of biodiversity in ecosystems with extreme physicochemical parameters. Athalassic, hypersaline, alkaline soda lakes are examples of such ecosystems that occur on all continents of Earth (Pikuta et al. 2003b). Cyanobacterial communities in salt and soda lakes have been well studied (Grant and Tindall 1986; Jones et al. 1998; Zavarzin et al. 1999). The normal functioning of a cyanobacterial community depends upon the equal balance of producing and decomposing links in the

trophic chain. The main producers of organic matter in soda lakes are algae (diatoms, cyanobacteria) and alkaliphilic, anoxygenic purple bacteria. In soda lakes the decomposition of organic matter, as in marine and fresh water anaerobic sediments, is performed by primary anaerobes (cellulolytics, saccharolytics, and proteolytics) and by secondary anaerobes (methanogens, sulfate reducers, and homoacetogens). In the last 10 years many new alkaliphiles from soda lakes have been described, representing new genera and families phylogenetically distant from known freshwater and marine species. Three new acetogenic anaerobes have been isolated from Lake Magadi (Kenya), all of which are taxonomically distant from *Clostridium* and classified into new genera: *Natroniella*, *Tindallia*, and *Natronoincola* (Zhilina et al. 1996, 1998; Kevbrin et al. 1998). Recent investigations have been focused on the study of the biodiversity of the meromictic, athalassic, alkaline Mono Lake in northern California. Several new anaerobic haloalkaliphilic cultures have been isolated and characterized (Pikuta and Hoover 2001; Hoover et al. 2003; Pikuta et al. 2003a).

In the present paper we describe a new haloalkaliphilic, proteolytic, spore-forming acetogen isolated from Mono Lake that is capable of growth at pH 10 with 20% NaCl.

## Materials and methods

### Source of the organism, media, isolation, and growth conditions

Columns (10 cm thick) of black mud sediments with a strong smell of sulfide were collected near the south shore of Mono Lake in California on 15 August 2000. The mud columns were sampled by anaerobic technique from shallow water (pH  $9.9 \pm 0.02$ , temperature  $21.6 \pm 0.1$  °C, salinity 7%) and hermetically sealed in sterile glass vessels with Teflon screw caps. The samples were maintained at 4 °C during transportation and stored at 2 °C in the Astrobiology Laboratory of the NASA Marshall Space Flight Center.

To grow enrichment cultures, 0.5 g of sediment material was injected into standard Hungate tubes containing a medium and were incubated at 35 °C for 4 days. Pure cultures were obtained by the dilution method on a medium with peptone. Growth of colonies was obtained by the "roll-tube" method on a 3% (w/v) agar medium, where carbonates were added separately after autoclaving. For cultivation of the new isolate, a modified medium (Zhilina et al. 1997) was used. It contained ( $l^{-1}$ ): NaCl, 30 g;  $Na_2CO_3$ , 2.76 g;  $NaHCO_3$ , 24.0 g; KCl, 0.2 g;  $K_2HPO_4$ , 0.2 g;  $MgCl_2 \cdot 6H_2O$ , 0.1 g;  $NH_4Cl$ , 1.0 g;  $Na_2S \cdot 9H_2O$ , 0.4 g; resazurin, 0.001 g; yeast extract, 0.5 g; peptone, 5.0 g; vitamin solution (Wolin et al. 1963), 2 ml; and trace mineral solution (Whitman et al. 1982) 1 ml. The final pH was adjusted to 10.0 with 6 N NaOH. High-purity nitrogen was used for the gas phase. All experiments were performed at a temperature of 35 °C, and the substrates were added at a concentration of 5 g  $l^{-1}$ . Dependence upon the  $CO_3^{2-}$  ion was checked on a medium in which  $Na_2CO_3$  and  $NaHCO_3$  were replaced with equimolar amounts of NaCl, and the pH was buffered with 50 mM serine (pH 9.2). Dependence upon  $Na^+$  ions was tested by substituting K salts for the Na salts in the medium. Dependence upon  $Cl^-$  ions was tested by replacing the Cl salts with half-molar equivalents of the sulfate salts.

Bacterial growth was measured by direct cell counting under a phase-contrast microscope (Fisher Micromaster) or by the increase in optical density at 595 nm (Genesis 5; Spectronic Instruments, USA).

### Analytical procedures

The end products of peptone fermentation in the liquid phase were determined by HPLC. Separation was performed on an Aminex HPX-87H (BioRad) column with 5 mM  $H_2SO_4$  as the mobile phase. The gases were measured using a gas chromatograph 3700 (Varian) equipped with a Porapak Q column and a TCD detector. Nitrogen was used as the carrier gas. Catalase activity was assayed by adding 3%  $H_2O_2$  to the liquid culture (Gerhardt et al. 1981). Proteins were determined by the BCA Protein Assay Kit (PIERCE, USA).

### Electron microscopy

Transmission electron microscopy was carried out using a JEOL TEM 100 CX II operating at 80 kV. Cells of the clean culture were centrifuged and re-suspended in a small volume of 0.2 M  $NaH_2PO_4 \cdot H_2O$  (pH 7.3) buffer. A drop of liquid containing the cells was placed on a Formvar-covered, 300-mesh grid and negatively stained with 2% aqueous (w/v) uranyl acetate. The grid was placed at 30-degree tilt, and excess liquid was drawn off using sterile filter paper touched to the edge of the staining droplet after a 30-s exposure. The sample was covered with a sterile Petri dish and allowed to air dry before viewing.

### Antibiotic susceptibility

The concentrations used for testing antibiotic resistance were kanamycin, gentamycin, tetracycline (250  $\mu g ml^{-1}$ ), and chloramphenicol (125  $\mu g ml^{-1}$ ).

### Determination of enzyme activities

Activities of hydrogenase and -dehydrogenase were measured in cell extracts.

Cells were harvested by centrifugation for 20 min at 17,000 g from 400 ml culture in the exponential phase of growth. The pellet was re-suspended in 10 ml Tris HCl-buffer (p 9.5) with 10 mM sodium formate and 25 mM sodium thioglycolate. Cells were disrupted by lysozyme, 5 mg/ml, at anaerobic conditions under nitrogen gas; DNase, 1  $\mu g/ml$  was added, and the mixture was incubated at 37 ° for 24 h. Cell debris and undisrupted cells were removed by centrifugation at 17,000 g for 20 min.

$CO$  dehydrogenase and hydrogenase activities were measured at 600 nm with a model "300 Bio UV-visible Spectrophotometer" (Varian, USA) in anaerobic conditions (Pusheva et al. 1989). The cuvettes were flushed for 2 min with oxygen-free  $CO$  or  $H_2$  gases and filled with 2.3 ml of the reaction mixture, which contained 50 mM Tris-1 buffer (p 9.5), 2 mM dithionite, 3.5 mM dithiotreitol, and 2 mM benzyl viologen. The measurements of the reaction were then carried out for 8 min at 35 °C in the hermetically sealed cuvettes. The reaction was initiated by the injection of 0.1 ml of cell extract, and the specific activity was expressed as  $\mu mol$  of benzyl viologen reduced per minute per  $mg$  of protein.

### Fatty acids analysis

Fatty acid methyl esters (FAMES) were extracted from fresh biomass and identified following the procedure recommended by Microbial Identification System (MIDI, Sherlock Microbial Identification System Version 4.0, MIS Operating Manual March 2001, Newark, Del.) The cells were harvested from a 48-h broth culture grown in strain APO medium, as described above, at 37 °C. The extraction included sonication, methylation, solvent extraction (transferring of FAMES from an aqueous phase to an

organic phase), and a base wash. The cell extracts were then subjected to analysis by gas chromatography (MIDI, Sherlock Microbial Identification System Version 4.0, MIS Operating Manual March 2001).

#### Genome characterization

The G+C content of the genomic DNA of strain APO was measured by the HPLC method as previously described (Mesbah et al. 1989), except that an Alltima C18 column (250×4.6 mm, 5- $\mu$ m particle size [Alltech, Deerfield, Ill.]) and 8% (v/v) methanol were used. The results reported are the mean of two determinations for each of two degradations of the DNA.

#### 16S rRNA gene sequence analysis

Genomic DNA was extracted by a phenol/chloroform method followed by ethanol precipitation. The 16S rRNA gene was selectively amplified with the following primers: 5'-AGAGTTT-GATCCTGGCTCAG-3' (forward) and 5'-TACGGCTACCTT-GTTACGAC-3' (reverse). PCR was performed with 30 pmol of each primer in a 50  $\mu$ l volume, using 1.2 U Pfu polymerase (Promega, USA) in the provided buffer and 10% DMSO. The thermal cycling profile was as follows: 5 min at 95 °C initial denaturation, followed by 10 cycles of 2 min denaturation at 95 °C, 45 s annealing at 56 °C and 3 min extension at 73 °C, then 20 additional cycles with annealing at 50 °C (other parameters same as the first 10 cycles). The final extension step was for 15 min at 73 °C. The amplified fragment was extracted from a 1.5% agarose gel using the Qiaquick extraction kit (Qiagen, USA), and then subcloned using the Zero Blunt TOPO PCR Cloning kit (Invitrogen, USA). Eight clones were sequenced in both directions using the dye terminator AmpliTaq FS cycle sequencing kit (Applied Biosystems, USA) with both vector primers and 16S internal primers.

The sequence of strain APO was aligned with 18 closely related sequences found in GenBank after a Blast search (Altschul et al. 1990). The alignment was performed by the PileUp program from the GCG Wisconsin package (USA). Pairwise distances were computed with Mega version 2.0 (Kumar et al. 2001) using the Jukes-Cantor (1969) model. An unrooted phylogenetic tree was constructed with the same Mega program using the neighbor-joining method (Saitou and Nei 1987).

#### DNA melting temperatures

Purified genomic DNA (250  $\mu$ g) from strain APO and *Tindallia magadiensis* = *Tindallia magadii* strain Z-7934 (Kevbrin et al. 1998; Validation List 1999) were sonicated with a Sonic Dismembrator 50 (Fisher Scientific) and DNA fragments between 600 bp and 800 bp were generated. The sonicated DNA was treated with DNase-free RNase A (Ambion) and S1 nuclease (USB Biochemical) (Ausubel et al. 1987) to remove residual RNA and single-stranded DNA, respectively. The concentration of the DNA was determined from the optical density readings at 260 nm. The ratio between 260 nm and 280 nm was  $1.9 \pm 0.02$ , which indicated that the DNA was highly purified. The DNA (80  $\mu$ g) in 1× SSC (Ausubel et al. 1987) was denatured by increasing the temperature from 24 °C to 100 °C at a rate of 1 °C per minute, and the absorbance at 260 nm wavelength was recorded using a "UV 160 Spectrophotometer" (Shimadzu, Japan). Melting temperatures ( $T_m$ ) of DNA from these microorganisms were calculated following the procedures described by De Ley et al. (1970). These experiments were conducted in triplicate.

#### DNA-DNA hybridization

Purified, sonicated genomic DNA (85  $\mu$ g) from strain APO and *T. magadiensis* Z-7934 in 4× SSC buffer (pH 7.0) and 25% (v/v) deionized formamide was denatured by raising the temperature to 100 °C. The samples were then cooled to 5 °C above their respective melting temperatures and kept for 3 min. Then the temperature was rapidly (2 min) lowered to the reassociation temperature, and the optical density at 270 nm was recorded at 5-s intervals for a total of 20 min (De Ley et al. 1970; Johnson 1985). The initial reassociation kinetics was determined by linear regression analysis. The experiment was conducted in triplicate. The percent homology of the DNA from these two microorganisms was calculated using the equation described by De Ley et al. (1970). All statistical analyses were performed using Microsoft Excel software.

#### Genome size

The total genome sizes of strain APO and *T. magadiensis* Z-7934 were determined based on the DNA reassociation kinetics and following the equation described by Gillis et al. (1970).

## Results

### Colonies

The colonies of the new isolate were round with flat smooth edges, matt-yellowish in color, lens shaped in deep agar, and 1.0–2.2 mm in diameter after 4–7 days. Cells from one colony were chosen for further characterization and were designated strain APO (= ATCC BAA-393 = DSM 14871). The purity of the culture was indicated by the absence of growth on medium at pH 7 and by microscopic examination.

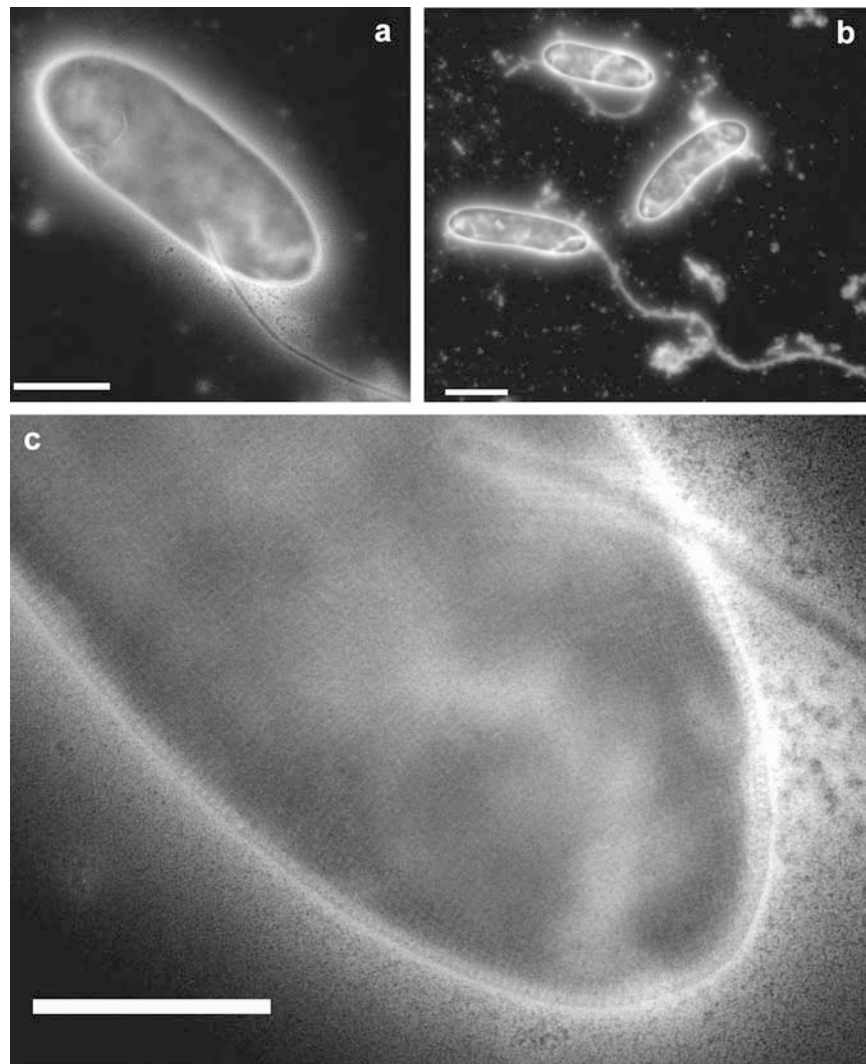
### Morphology

Cells of strain APO are highly motile curved rods with rounded ends and a single laterally attached flagellum (Fig. 1a). Cells of strain APO had sizes of 0.55–0.7  $\mu$ m in diameter and 1.7–3.0  $\mu$ m in length. Cells occurred singly, in pairs, or as short, irregular curved chains. Multiplication was by binary fission. Gram-stained cells of strain APO exhibited the blue color typical for Gram-positive cell walls. Strain APO formed spores following a nutrient downshift (from a rich peptone medium to a defined pyruvate medium). Brilliant green, oval-shaped spores were observed after malachite green staining; the location of spores was in the center or subpolar area without swelling of the sporangium.

### Growth parameters and metabolism

The new isolate grew at 10–48 °C, with optimum growth at 37 °C. Growth was absent at 5 °C and at 50 °C. The doubling time at 37 °C and pH 9.5 was 7 h. The dependence of growth upon pH is shown in Fig. 2a. Strain APO was an obligate alkaliphile and did not grow

**Fig. 1a–c** Transmission electron microscope image of strain APO: **a** laterally attached flagellum (*scale bar* 0.5  $\mu\text{m}$ ), **b** shape of cells is straight or slightly curved rods (*scale bar* 1.0  $\mu\text{m}$ ), **c** tetragonal structure of outer S-layer (*scale bar* 0.2  $\mu\text{m}$ )



at pH 7.0–7.5. Strain APO required  $\text{Na}^+$  ions for growth, and no growth occurred in medium in which all the  $\text{Na}^+$ -containing salts were substituted with  $\text{K}^+$  salts. The optimum concentration and range of NaCl for growth are shown in Fig. 2b. No growth was observed at 1% or at 23% (w/v) NaCl. Strain APO grew well without morphological changes on serine-buffered (pH 9.5) medium (an alternative for carbonate buffer). This demonstrated that strain APO is not dependent upon  $\text{CO}_3^{2-}$  ions. The new isolate grew only under strictly anaerobic conditions and had a catalase-negative reaction. Strain APO was an organotroph with a fermentative type of metabolism. It grew on peptone, bacto-tryptone, casamino acids, yeast extract, L-serine, L-lysine, L-histidine, L-arginine, and pyruvate as substrates. No growth was detected on glucose, fructose, mannose, starch, acetate, citrate, propionate, butyrate, formate, lactate, methanol, ethanol, glycerin, betaine, trimethylamine, L-isoleucine, L-phenylalanine, L-leucine, D-leucine, D-tryptophan, D-methionine, L-valine, L-methionine, L-aspartic acid, L-tyrosine, L-cysteine,

L-proline, L-asparagine, L-glutamine, L-threonine, L-cystine, D-threonine, L-alanine, glycine, D-histidine, or trans-L-hydroxy-L-proline. The main end product of growth with peptone was 21 mM acetate, and minor products were 1 mM lactate, 3.8 mM propionate, and 1.4 mM ethanol. In the gas phase, a trace (0.5%) of hydrogen was detected. Strain APO was capable of respiration by the Stickland reaction on the following amino acid pairs: L-proline + L-alanine, glycine + L-alanine, L-tryptophan + L-valine, and glycine + L-leucine. Strain APO did not grow on L-proline + L-valine, L-proline + L-isoleucine, L-proline + L-leucine, glycine + L-valine, or glycine + L-isoleucine. The new isolate required yeast extract for growth.

#### Antibiotic susceptibility

Strain APO was resistant to kanamycin (growth without morphological changes) but sensitive to tetracycline, gentamycin, and chloramphenicol.

## Enzyme activities

Hydrogenase and CO dehydrogenase activities in the cell extract at p 9.5 (which is optimal for growth of strain APO) were  $0.32 \pm 0.003 \mu\text{mol}/\text{min}$  per mg and

$0.33 \pm 0.003 \mu\text{mol}/\text{min}$  per mg, respectively (mean  $\pm$  SD,  $n = 3$ ).

## Fatty acids analysis

The major fatty acid methyl esters were  $C_{16:1 \text{ cis } 7}$  4.47%,  $C_{16:1 \text{ cis } 9}$  35.44%,  $C_{16:0}$  13.50%, dimethyl acetate  $C_{16:0}$  15.32%, and  $C_{18:0}$  10.05%.

## Genome analysis

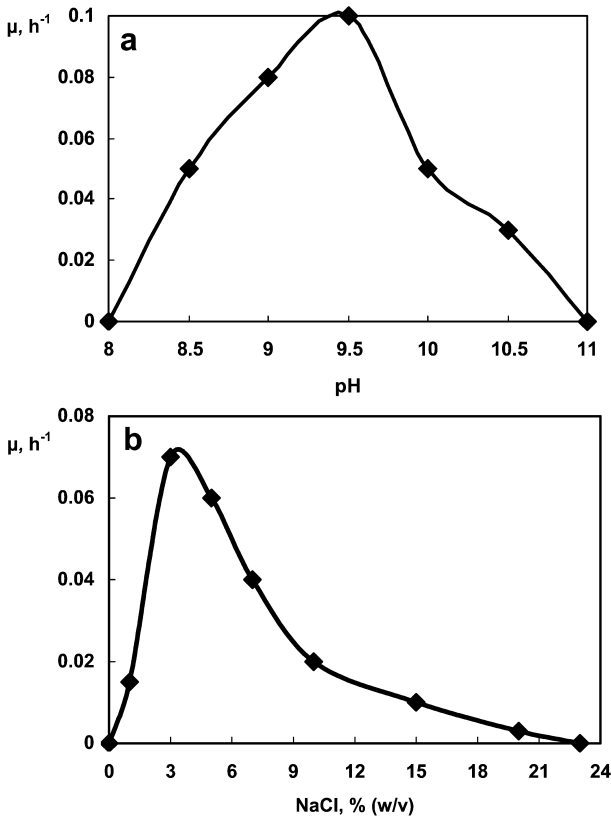
The G + C content of the genomic DNA of strain APO was  $44.4 \pm 0.2 \text{ mol}\%$  (mean  $\pm$  SD,  $n = 4$ ).

## 16S rRNA gene sequence analysis

A sequence covering 1,481 nucleotides of the 16S rRNA gene of strain APO was obtained, corresponding to positions 28–1,515 of the *Escherichia coli* 16S rRNA gene sequence. The G + C content of this sequence was 56.58 mol%. The sequence was compared with all sequences presently available in the GenBank database and appeared to be most similar (98.2%) to the sequence from *Tindallia magadiensis* Z-7934. This organism belongs to the cluster of low mol% G + C clostridia. The phylogenetic dendrogram shows the relationships between strain APO and the closely related described species (Fig. 3).

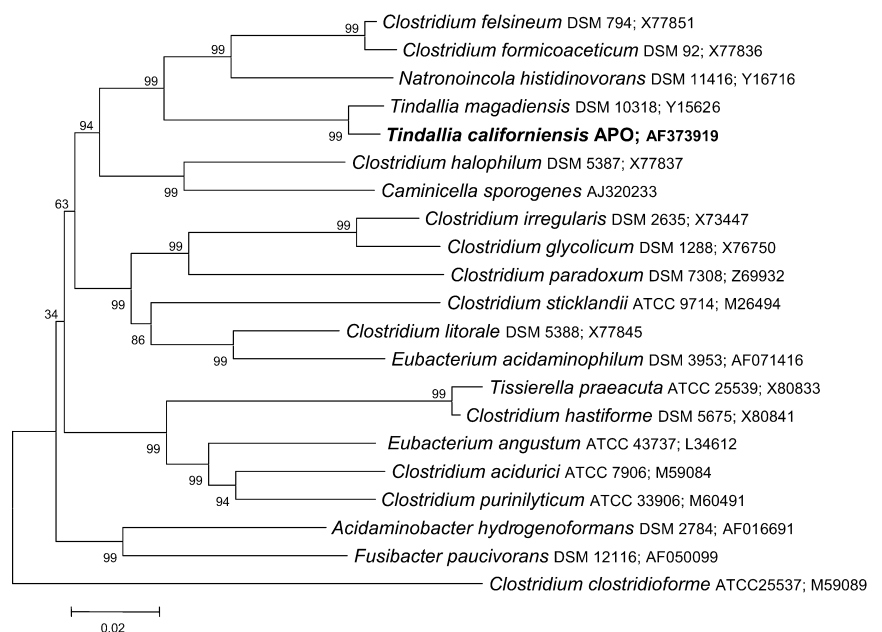
## DNA melting temperatures

The  $T_m$  of the genomic DNA of strain APO was  $86 \pm 2 \text{ }^\circ\text{C}$  (mean  $\pm$  SD,  $n = 3$ ) in  $1\times$  SSC, whereas it was  $81 \pm 2 \text{ }^\circ\text{C}$  (mean  $\pm$  SD,  $n = 3$ ) for *T. magadiensis* Z-7934.



**Fig. 2** Effect of **a** pH (at optimal NaCl concentration and temperature) and **b** NaCl (at optimal pH and temperature) on growth of *Tindallia californiensis* APO

**Fig. 3** Unrooted phylogenetic tree showing the position of *T. californiensis* APO within the radiation of the low G + C subphylum of the Gram-positive bacteria. The scale bar corresponds to 2 substitutions per 100 nucleotides



## DNA-DNA hybridization

The regression analysis during the first 1.9 min of DNA hybridization between strain APO and *T. magadiensis* Z-7934 exhibited a decrease of  $0.00886 \pm 0.000206$  ( $r^2 = 0.99$ ) OD units per min. Based on the reassociation kinetics and the equation described by Gillis et al. (1970), these results indicated that DNA-DNA hybridization established 55% homology between the genomes of strain APO and *T. magadiensis* Z-7934.

## Genome size

The genome size for strain APO was  $1.02 \times 10^9$  daltons and  $1.14 \times 10^9$  daltons for *T. magadiensis* Z-7934, as calculated using the equation described by Gillis et al. (1970).

## Discussion

The basic function of the new isolate in the haloalkaliphilic anaerobic bacterial community is the degradation of organic matter via the proteolytic pathway. This organism performs the role of a primary anaerobe producing the low molecular organic products, which are used by secondary anaerobes in the trophic ecological

chain. The non-cycling acetyl-CoA pathway is characteristic of most acetogenic bacteria. The key enzymes of this pathway (hydrogenase and carbon monoxide dehydrogenase) were studied for strain APO. The detection of a high concentration of acetate, which is the main end product of strain APO, and high activities of hydrogenase and CO dehydrogenase indicate the functioning of the homoacetic pathway (acetyl-coenzyme A-CO-dehydrogenase pathway) in the metabolism of the new isolate. The study of enzymes of the homoacetic pathway for the closest phylogenetically related species, *Tindallia magadiensis* Z-7934, also showed high activity for both CO dehydrogenase and hydrogenase ( $1.0 \mu\text{mol}/\text{min}$  per mg and  $0.36 \mu\text{mol}/\text{min}$  per mg, respectively; unpublished data). Besides fermentation, the new isolate is able to receive energy by respiration according to the Stickland reaction; this characteristic of the new isolate is also different from *T. magadiensis* Z-7934. Strain APO grows on a narrower spectrum of amino acid pairs, whereas *T. magadiensis* Z-7934 is capable of growth on the following electron donor-acceptor pairs: proline + valine, proline + alanine, proline + isoleucine, proline + leucine, glycine + valine, glycine + histidine, glycine + alanine, glycine + isoleucine, and glycine + leucine. The differences between strain APO and *Tindallia magadiensis* strain Z-7934 are shown in Table 1. Both strains were isolated from athalassic, epicontinental, hypersaline, alkaline lakes formed separately on the African and

**Table 1** Comparative features of *Tindallia californiensis* APO and *Tindallia magadiensis* Z-7934. (+): weak growth; +: good growth; -: no growth

Features:	<i>T. californiensis</i> strain APO (present work)	<i>T. magadiensis</i> strain Z-7934 (Kevbrin et al. 1998)
Cell sizes ( $\mu\text{m}$ )	0.55–0.7 × 1.7–3.0	0.5–0.6 × 1.2–2.5
Motility	+	-/+ <sup>a</sup>
Spores	+	-
Temperature ( $^{\circ}\text{C}$ ) range (optimum)	10–48 (37)	19–47 (37)
pH range (optimum)	8.0–10.5 (9.5)	7.5–10.5 (8.5)
NaCl (%) range (optimum)	1–20 (3)	1–10 (3–6) <sup>b</sup>
Substrates:		
L-Serine	+	(+)
L-Lysine	+	ND
L-Histidine	+	(+)
L-Arginine	+	+
L-Threonine	-	(+)
Glycine	-	(+)
L-Glutamine	-	(+)
L-Aspartate	-	(+)
Pyruvate	+	+
Citrate	-	+
End products:		
H <sub>2</sub>	+	+
Formate	+	+
Acetate	+	+
Propionate	+	+
Lactate	+	+
Ethanol	+	-
Iso-valerate	ND	-
G + C (mol%)	44.4	37.6
T <sub>m</sub> ( $^{\circ}\text{C}$ )	86	81
Genome size (Da)	$1.02 \times 10^9$	$1.14 \times 10^9$

<sup>a</sup> Motility was observed in the culture from DSM 10318 (= Z-7934), but in the original article, strain Z-7934 was described as non-motile

<sup>b</sup> Range of NaCl concentration is our data

North American continents. Lake Magadi in Kenya, equatorial Africa, with more evaporite climatic conditions (except during the rainy season) contains a cyanobacterial community similar to that of the North American soda lake, Mono Lake. More details of the geological and ecological characteristics of Mono Lake have been described (Hoover et al. 2003; Pikuta et al. 2003b).

The main morphological differences between strain APO and *T. magadiensis* Z-7934 are spore formation and motility of strain APO by a single laterally attached flagellum. Flagella with this position rarely occur. Similar flagellar attachments occur in *Syntrophomonas wolfei* (DSM 2245A), with laterally inserted flagella on the concave side (McInerney et al. 1982, emended in Lowitz et al. 1989), and *Dethiosulfovibrio marinus* WS100 (Surkov et al. 2001), which have several flagella located on the concave side of the cells. There are also differences in the physiology of these strains: strain APO has optimum pH 9.5, but the optimum pH of Z-7934 is 8.5. Strain APO is also distinguished from *T. magadiensis* Z-7934 because strain APO is not capable of growth on L-threonine, glycine, L-glutamine, L-aspartate, or citrate. The G+C content of the new isolate is also higher than that of *T. magadiensis* Z-7934 by 6.8 mol%, and the  $T_m$  of the genomic DNA for strain APO was 5 °C higher than that of *T. magadiensis* Z-7934. The genome size of strain APO was 0.12 daltons less than that of strain Z-7934. DNA-DNA hybridization of strain APO genomic DNA with *T. magadiensis* Z-7934 was 55%.

On the basis of phenotypic and genotypic characteristics (Gram-positive cell wall, fermentative type of metabolism, 16S rDNA sequence, and DNA-DNA hybridization), we conclude that strain APO represents a new species of the genus *Tindallia*. The name *Tindallia californiensis* was suggested for this organism according to the geographical location of Mono Lake.

#### Description of *Tindallia californiensis* sp. nov.

*Tindallia californiensis* (ca.li.for.nien'sis. N.L. fem. adj. *californiensis*, pertaining to California, the region where Mono Lake is located, from which this organism was isolated).

Motile, slightly curved rods with single laterally attached flagellum; cell sizes are between 0.55 and 0.7 µm wide and 1.7–3.0 µm long. Gram-positive structure of cell wall. Spore-forming (central or subpolar location) without swelling of sporangium.

Haloalkaliphilic: the pH range for growth is 8.0–10.5, with optimum growth at pH 9.5. Growth is dependent upon Na<sup>+</sup> but not CO<sub>3</sub><sup>2-</sup> ions. The range of NaCl is > 1–20% (w/v), with optimum growth at 3–5% (w/v). Mesophilic: the temperature range for growth is 10–48 °C, with the optimum being 37 °C.

Strictly anaerobic, catalase negative, and does not reduce sulfate. Organotroph, using peptone, bacto-tryptone, casamino acids, yeast extract, L-serine, L-lysine,

L-histidine, L-arginine, and pyruvate as substrates. Capable of growth by performing the Stickland reaction on the following amino acid pairs: proline + alanine, glycine + alanine, tryptophan + valine, and glycine + L-leucine. Main end product is acetate; minor end products are propionate, lactate, and ethanol. Requires yeast extract for growth. Resistant to kanamycin but sensitive to tetracycline, gentamycin, and chloramphenicol.

G+C content of genomic DNA is 44.4 mol% (by HPLC method).

Habitat: isolated from the mud sediments of the hypersaline, meromictic, alkaline Mono Lake in California, North America.

Type strain: APO. Deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSM 14871, and with American Type Culture Collection, ATCC BAA-393.

The GenBank accession number for the 16S rDNA sequence of strain APO is AF 373919.

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