Acetonitrile degradation under haloalkaline conditions by *Natronocella acetinitrilica* gen. nov., sp. nov.

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Nitriles are important environmental compounds, both as natural products and industrial pollutants. Until now, there have been no data on the possibility of microbial nitrile degradation at high pH/salt conditions. Acetonitrile (CH3CN) is the simplest organic nitrile. Here, evidence is provided of microbial utilization of acetonitrile as a carbon, energy and nitrogen source at extremely high pH and moderate salinity. Positive enrichment cultures with acetonitrile at pH 10 and salt content equivalent to 0.6 M total Na+ were obtained from mixed sediment samples from soda lakes, but not from soda soils. Purification of these cultures resulted in the isolation of two bacterial strains capable of growth with acetonitrile as sole carbon, energy and nitrogen source under haloalkaline conditions. Apart from acetonitrile, the bacteria also grew with propionitrile. Nitrile hydrolysis to acetamide was identified as the rate-limiting step of acetonitrile degradation via the nitrile hydratase/amidase pathway. The new bacteria belonged to moderately salt-tolerant obligate alkaliphiles with optimum growth at pH 10 and 0.5 M total Na+. The cells were yellow-coloured due to a high concentration of carotenoids dominated by zeaxanthin. Phylogenetic analysis placed the isolates into a new lineage within the family *Ectothiorhodospiraceae* in the *Gammaproteobacteria*. On the basis of unique phenotypic properties and their separate phylogenetic position, the new bacteria are placed into a new genus and species for which the name *Natronocella acetinitrilica* gen. nov., sp. nov is proposed.

**INTRODUCTION**

Compounds containing a C=N group belong to the wide family of nitriles. These are mostly organic molecules of variable complexity with only two inorganic species (cyanide and thiocyanate) included. Most of the nitriles are industrially produced as intermediates and building blocks in organic synthesis and as organic solvents. There are also a few examples of naturally occurring nitriles, such as linamarin and dhurrin, formed by cyanogenic plants from cyanide (Vetter, 2000). In addition, simple aliphatic nitriles, such as isobutyronitrile, can be produced during anaerobic degradation of proteins (Harper & Gibbs, 1979).

The nitrile bond is chemically very stable and most of the nitriles are hydrophobic and toxic compounds. Therefore, the environmental role of its enzymic degradation is very important. Currently, two different mechanisms, resulting in enzymic conversion of nitriles to corresponding carboxylic acids, are known. The group of nitrile hydratases hydrolyses a wide range of mostly aliphatic and arylaliphatic nitriles into acids and ammonium (Kobayashi & Shimizu, 1998). In case of amidase deficiency, a consortium with an amidase-producing partner can be very efficient in complete nitrile degradation as has recently been shown for acetonitrile degradation in a binary culture of Gram-positive bacteria (Kohyama et al., 2006). Alternatively, the enzyme family of nitrilases directly converts mostly aromatic nitriles into acids and ammonium.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains ANL 1 and ANL 6-2T are EF103127 and EF103128, respectively.
in a single step (Kobayashi & Shimizu, 2000; Podar et al., 2006). The micro-organisms possessing these enzymes are valuable biocatalysts and can be used either in organic synthesis or in environmental biotechnology (Banerjee et al., 2002; Håkansson et al., 2005; Manolov et al., 2005; Kohyama et al., 2006).

The obvious advantages of enzymic nitrile degradation have stimulated active screening for producers both directly, by a traditional microbiological approach (Layh et al., 1997) and indirectly, using molecular screening of environmental DNA and whole-genome sequences (Vetter, 2000). Currently, many strains, mostly bacterial but also several fungal, are characterized as active producers of nitrile-hydrolysing enzymes. The most active group among them, producing extremely active nitrile hydratases and nitrilases, belongs to the genus Rhodococcus (Bunch, 1998; Kobayashi & Shimizu, 1998). So far, all known nitrile-degrading micro-organisms are neutrophilic, i.e. growing optimally at neutral pH values, and one of them, Bacillus pallidus, is thermo-

phlic (Almatawah et al., 1999).

Until now, there has been no evidence of the possibility of nitrile biodegradation at high pH/salt conditions. Micro-organisms, mainly prokaryotes, which grow optimally at a pH above 9 and up to 11 in soda/NaCl brines of variable concentrations are called haloalkaliphiles and can be found in such natural habitats as soda lakes and soda solonchak soils. This ability is widely distributed among different phylogenetic lineages of prokaryotes and almost all physiological groups are represented (Jones et al., 1998; Sorokin & Kuenen, 2005; Zavarzin et al., 1999). There are some data on biodegradation of inorganic nitriles, such as cyanide (N≡C\(^{-}\)) (Luque-Almagro et al., 2005) and thiocyanate (N≡C-S\(^{-}\)) (Sorokin et al., 2001) at high pH. However, the enzymes involved are different from the nitrile-degrading nitrile hydratase and nitrilase, and organic nitriles cannot be degraded by these bacteria. This prompted us to look at the potential for nitrile degradation in haloalkaliphilic microbial communities. Acetonitrile (CH\(_3\)-C≡N) was chosen as a start substrate, because it is the simplest organic nitrile, widely used as solvent and an important environmental pollutant. The results indicate the presence of a specialized group of previously unknown haloalkaliphilic bacteria capable of growing with acetonitrile as sole substrate.

**METHODS**

**Samples.** Three combined sediment samples from Kulunda Steppe (Altai, Russia; 10 subsamples), Wadi Natrun (Egypt; 8 subsamples), north-east Mongolian (6 samples) soda lakes and one sample from soda solonchak soils (Kulunda Steppe, 10 subsamples) were used as the inoculum to enrich for acetonitrile-degrading haloalkaliphiles. The lake properties were described previously (Sorokin & Kuenen, 2005). The pH of the water extract from the solonchak samples varied from 9.5 to 10.8, total alkalinity ranged from 0.05 to 1.2 mol kg\(^{-1}\) and total salt content from 3 to 20% (w/w). The dominant ions in both habitats were Na\(^+\), Cl\(^-\), HCO\(_3\)-/CO\(_3\)\(^{2-}\), and SO\(_4\)\(^{2-}\).

**Medium composition and enrichment strategy.** A mineral medium based on sodium carbonate buffer at pH 10 and 0.6 M total Na\(^+\) was used for enrichments and pure culture studies (g l\(^{-1}\)): Na\(_2\)CO\(_3\), 22; NaHCO\(_3\), 8; NaCl, 6; K\(_2\)HPO\(_4\), 0.5. The pH of this medium was stable even after prolonged incubation. After sterilization, the medium was supplemented with 1 ml trace metal solution l\(^{-1}\) (Pfenng & Lippert, 1966), 1 mM MgSO\(_4\) and 1 mg filter-sterilized vitamin B\(_12\), \(1^{-}\). Enrichment cultures were established in 100 ml serum bottles closed with rubber stoppers containing 20 ml medium and 1 ml sediment samples or 1 g soil. Acetonitrile (Merck) was added to a final concentration of 10 mM from a 5 M filter-sterilized stock solution. The cultures were incubated statically at 28\(^\circ\)C and were periodically checked for ammonia production. When the ammonia concentration reached 5 mM, the culture was taken off into a new medium at 1:10 dilution. After four successful 1:10 transfers, it was serially diluted up to \(10^{-11}\). The culture from a maximal positive dilution was plated onto solid medium, initially containing 20 mM acetonitrile. The plates were incubated in closed jars for 30 days. Separate colonies were placed into liquid medium with acetonitrile in 30 ml serum bottles with 5 ml liquid and closed with rubber septa. Positive cultures were plated again to check for purity.

Growth experiments with pure cultures were performed in 250 ml closed serum bottles with 50 ml liquid on a rotary shaker at 100 r.p.m. and 30\(^\circ\)C. Substrates were used at 10–20 mM concentration. Growth was monitored by optical density and the degradation of nitriles was followed by ammonium production. When compounds other than nitriles were used as substrates, 5 mM NH\(_4\)Cl was added as the N source. Anaerobic growth with acetonitrile or acetate was studied in 100 ml serum bottles filled with 80 ml medium, containing 10 mM substrate and either 10 mM nitrate or 5 mM nitrite. The cultures were made anoxic by several cycles of evacuation/flushing with argon. Large-scale cultivation with acetonitrile was performed in 20 l closed bottles with 4 l medium. pH profiling of growth was done according to Sorokin (2005). The salt dependence of growth was investigated in a range of sodium carbonate-based media containing 0.2–4.0 M total Na\(^+\) at pH 10.

**Experiments with washed cells and cell-free extract.** To determine the metabolic activity of the pure cultures with various substrates and the influence of pH and salt concentration on the activity of acetonitrile degradation, cells grown at pH 10 and 0.6 M Na\(^+\) were harvested, washed and resuspended in 0.5 M sodium bicarbonate, pH 8.2, at a cell density of 20 mg protein ml\(^{-1}\). This concentrated suspension could be kept on ice for at least a week without substantial loss in activity. To obtain a cell-free extract, the same cell suspension was treated by sonication, followed by centrifugation. Activity tests with washed cells and cell extract were carried out in 2.2 ml Eppendorf tubes with 2 ml reaction mixture. The pH and salt influence on activity in cell preparations was examined in the same buffers as used in growth experiments, except that potassium phosphate was replaced by 50 mM KCl.

**Analytical procedures.** Protein concentration was measured by the Lowry method. Ammonium concentration was determined by the phenol-hypochlorite method, according to Weatherburn (1967). Nitrite was analysed qualitatively using the Merckquant Nitrite Test (Merck) and quantitatively according to Gries-Romijn-van Eck (1966). The concentration of acetonitrile, acetamide and acetic acid in culture supernatant was determined by GC after extraction with dichloromethane (5:95). The analysis was carried out on a Varian Star 3400 CX system with a Varian Chroompack CP-Wax 52 CB column (50 m \(\times\) 0.32 mm, o.d. = 0.70 mm, d.f. = 2.0) with a temperature gradient of 50–250\(^\circ\)C. The detection limit of the analysis was around 1 mM. Acrylonitrile, acrylamide and acrylic acid were...
detected by HPLC using a Merck Chromolith SpeedROD RP-18e (50–4.6 mm) with the eluent containing MilliQ water (98.94 %, v/v), acetonitrile (1 %, v/v) and acetic acid (0.06 %, v/v). The flow rate was 1 ml min⁻¹ and the column temperature was 21 °C. The compounds were detected using a Shimadzu SPD-6A UV spectrophotometric detector with a wavelength of 230 nm. Pigments were extracted from freeze-dried cells with acetone/methanol (7:2, v/v) and analysed by an HPLC system equipped with a μBondapak C18 column (8 × 100 mm, RCM-type; Waters) with methanol as the eluent (Takaichi & Shimada, 1992). The circular dichroism spectrum was measured using a J-820 spectropolarimeter (JASCO) in diethyl ether/2-pentanone/ethanol (5:5:2, by vol.) at room temperature. The relative molecular masses were measured using an FD-MS: M-2500 double-focusing gas chromatograph-mass spectrometer (Hitachi) equipped with a field-desorption apparatus. The 1H-NMR (500 MHz) spectra in CDCl₃ at 24 °C were measured using the UNITY INOVA-500 system (Varian). Membrane fatty acids were extracted from the freeze-dried cells with methanol/chloroform and analysed by GC-MS as described by Zhilina et al. (1997).

Phase-contrast micrographs were obtained using a Zeiss Axiosplan Imaging 2 microscope. For electron microscopy, cells were fixed with glutaraldehyde (final conc 3 %, v/v) and positively contrasted with 1 % (w/v) uranyl acetate. For thin sectioning, the cells were fixed in 1 % (w/v) OsO₄ and 0.5 % NaCl for 3 h at room temperature, washed and stained overnight with 1 % (w/v) uranyl acetate, dehydrated in ethanol series and embedded in Epon resin. Thin sections were stained with 1 % (w/v) lead citrate. The isolation of the DNA and subsequent determination of G+C content and DNA–DNA hybridization were performed by the thermal denaturation/renaturation technique (Marmur, 1961; De Ley et al., 1970).

Genomic DNA for phylogenetic analysis was extracted from cells by using the UltraClean Soil DNA Extraction Kit (MolBio Laboratories), following the manufacturer’s instructions. 16S rRNA genes were amplified using general bacterial primers. The PCR products were purified from low-melting-point agarose using the Wizard PCR-Prep kit (Promega), according to the manufacturer’s instructions. Sequencing was performed using the Big Dye Terminator v.3.1 sequencing reaction kit on an ABI 3730 DNA automatic sequencer (Applied Biosystems). The sequences were first compared with those stored in GenBank using the BLAST algorithm and aligned using CLUSTALW. Phylogenetic trees were constructed with four different algorithms using the TREECONW program package (van de Peer & de Wachter, 1994).

**RESULTS**

**Enrichment and isolation of pure cultures of acetonitrile-utilizing haloalkaliphiles**

An enrichment from soda soils failed, but in two out of three enrichments from soda lakes (Kulunda Steppe and Mongolia) some ammonia formation was observed after more than a month of incubation. After several 1:100 transfers, the growth gradually intensified to a visible turbidity. The culture from the Kulunda lakes was highly aggregated in contrast to the homogeneous Mongolian enrichment. In serial dilutions, growth was observed until 10⁻⁷ and it never resulted in pure cultures. Therefore, final isolation was performed on solid medium with acetonitrile. Purification on solid medium also took a very long time, since the fast-growing and numerically dominant colonies belonged to satellite heterotrophs unable to grow when transferred into liquid medium with acetonitrile. However, when after a month of incubation large yellow-orange colonies appeared, it became clear that they were responsible for the acetonitrile degradation. Their separation from the satellites took several cycles of transfer to liquid medium-plating. Eventually, two pure cultures, both yellow-coloured, were obtained, strain ANL 1 from the Kulunda lakes enrichment and strain ANL 6-2T from the Mongolian lakes enrichment.

**Phenotypic and taxonomic characteristics**

In both isolates the cells were rod-shaped and of variable length, motile by a single polar or subpolar flagellum and with a typical Gram-negative cell wall structure and extended periplasm (Fig. 1). Strain ANL 1 formed aggregates during growth with acetonitrile. The biomass of both strains was yellow-orange. HPLC analysis of the crude pigment extract from strain ANL 6-2T showed five carotenoid peaks. The major carotenoid was identified as (3R,3′R)-zeaxanthin based on the retention time on HPLC, the absorption spectrum (Takaichi & Shimada, 1992), the circular dichroism spectrum, the relative molecular mass of 564 and the ¹H-NMR spectrum, which were compatible with those of authentic (3R,3′R)-zeaxanthin. Four minor components were identified as adonixanthin, phoenicoxanthin, β-cryptoxanthin and β-carotene based on the retention times on HPLC, the absorption spectrum and the relative molecular masses. The component ratio was 79 % zeaxanthin, 9 % adonixanthin, 4 % phoenicoxanthin, 4 % β-cryptoxanthin and 4 % β-carotene. Fatty acid analysis of the membrane lipids showed a composition typical for moderately salt-tolerant bacteria with an absolute domination of 18:1ω7 (66 % of total) and 16:0 as a secondary dominant (13 % of total).

The G+C content in the genomic DNA of ANL 1 and ANL 6-2T was 50.6 and 51.5 mol% (Tm), respectively. According to the DNA–DNA hybridization results, the two isolates might belong to separate gene species (DNA similarity level 55 %). However, their phenotypic similarity and almost identical total protein profiles (data not shown) indicated that the new strains belong to the same species. Phylogenetic analysis based on 16S rRNA gene sequencing placed the bacteria into the Gammaproteobacteria as a separate lineage within the family Ectothiorhodospiraceae (Fig. 2). The level of sequence homology between the strains was 98.7 % and with the closest cultured relatives (Alkalilimnicola-Alkalilimnus group), 95 %.

**Growth characteristics of the pure cultures**

Both strains could grow with acetonitrile as sole source of carbon, energy and nitrogen. Ammonia accumulated in the medium but with different dynamics for the two strains. In strain ANL 6-2T it was parallel to biomass growth, while in strain ANL 1 ammonia production was much faster but biomass yield was lower (Fig. 3a), indicating an imbalance between nitrile catabolism and biomass growth. The GC
analysis of possible intermediates in the ANL 6-2T culture growing on acetonitrile was inefficient, i.e. neither acetonitrile nor acetic acid was found in the culture supernatant, probably due to a fast consumption and a low detection limit. However, GC analysis proved that acetonitrile consumption was parallel to biomass production (Fig. 3b). Maximal experimentally measured growth rates for strains ANL 1 and ANL 6-2T with acetonitrile were 0.038 and 0.056 h\(^{-1}\), respectively. Growth with acetate and ammonium was approximately two times faster. From the other tested aliphatic nitriles (propio-, butyro-, isobutyro, valero- and acrylo-), only propionitrile could be used as sole substrate supporting growth with a maximum growth rate (ANL 6-2T) of 0.02 h\(^{-1}\). Ammonia was toxic to the bacteria (growing with acetate as substrate) with a \(K_{50}\) of 10 mM, thus it was a limiting and selective factor for alkaliphilic nitrile degradation, similar to what was found in the case of thiocyanate-utilizing alkaliphiles (Sorokin et al., 2001).

Limited potential to grow anaerobically with nitrate as the electron acceptor was observed in both strains either with acetate or acetonitrile as the electron donor. Nitrate was only reduced to nitrite, and growth was inhibited when the nitrite concentration reached 3 mM.

Both strains grew optimally with acetonitrile within an alkaline pH range, although there was a slight difference in optimal pH values (Fig. 4a). According to these data, the new isolates can be classified as obligate alkaliphiles. When inoculated from the medium containing 0.6 M total Na\(^+\), pH 10, both strains were able to grow with acetonitrile at sodium carbonate/bicarbonate concentrations up to 3.0 M Na\(^+\) with an optimum at 0.5 M (Fig. 4b). Furthermore, after adaptation of the cultures at 3 M Na\(^+\), they also started to grow in saturated soda brines (4 M total Na\(^+\)), which qualified the new bacteria as extremely salt-tolerant natronophiles (Sorokin & Kuenen, 2005).
Metabolic activity of washed cells and in cell-free extract

Washed cells of ANL strains grown with acetonitrile or propionitrile produced ammonium. When other substrates were used for growth, the specific activity of acetonitrile conversion dropped significantly, indicating the inducible nature of the nitrile-hydrolysing system. Apart from acetonitrile and propionitrile, the nitrile-grown cells also had substantial activity with acrylonitrile and low activity with butyronitrile and valeronitrile (Table 1). Cell disruption by sonication completely inactivated the nitrile-hydrolysing activity, but the amidase remained active even at pH values above 11. The amidase activity was observed by HPLC analysis (not shown).

A substantial difference was observed in the pH response between whole-cell acetonitrile hydrolysis and amide activity in cell-free extract (Fig. 5). The amide activity in strain ANL 6-2T is an apparently intracellular enzyme with an optimal pH of about 7.5, while overall whole-cell acetonitrile hydrolysis was observed by HPLC analysis (data not shown).

DISCUSSION

The results of this study have demonstrated for the first time the presence of nitrile-degrading potential in haloalkaliphilic bacteria living in soda lakes. The fact that these bacteria were able to grow with aceto- or propionitrile as sole carbon, energy, and nitrogen sources could be natural substrates in soda lake habitats. It was found that these microorganisms had substantial activity with acrylonitrile and low activity with propionitrile, indicating that these alkaliphiles use the nitrile hydratase/amidase pathway. This pathway was also evident from experiments with acrylonitrile, where the formation of acrylamide and acrylate was observed by HPLC analysis (not shown).

The results indicated that most probably, despite the high pH tolerance and its general alkaliphilic phenotype, nitrile hydrolysis in the cell-free extract remained active even at pH values above 11. Unfortunately, because of inactivation after cell disruption, no conclusion can be made about the pH preference of the nitrile hydratase in these alkaliphiles.
alkalitolerant. The latter is most probably a result of intracellular localization of the enzymes, as the cytoplasmic pH in alkaliphilic bacteria grown at an external pH of 10 stays around pH 8. At least, this must be true for acetamidase, which was almost completely inactivated at pH 10 – a pH value optimal for the growth of intact cells. Although such results are disappointing as far as our expectations of finding unusual nitrile-hydrolysing enzymes are concerned, on the other hand, whole-cell biocatalysis is still a major way of using nitrile-degrading micro-organisms in industry because of the unstable nature of nitrile-hydrolysing enzymes. In this respect, the new alkaliphilic bacteria still have at least 2 pH units (pH 9–11) advantage over all known whole-cell nitrile-degraders.

Activity tests with whole cells and cell-free extract demonstrated that, similar to neutrophilic bacteria, the new alkaliphiles also use the nitrile hydratase/amidase pathway, but in this case the nitrile hydratase is the rate-limiting step. While the presence of the second enzyme (a nitrilase) cannot be completely excluded, it is not very likely, since the bacteria in this study could only degrade a few simple aliphatic nitriles, and nitrilases usually specialize on aromatic or arylaliphatic nitriles (Banerjee et al., 2002). Furthermore, amidase activity in nitrilases, if present at all, is very low (Kobayashi et al., 2002). However, further specialized enzymological and molecular biological studies are required.

Phylogenetic analysis demonstrated that the new isolates belong to a previously unknown lineage within the family Ectothiorhodospiraceae, consisting mostly of phototrophic and lithotrophic (halo)alkaliphilic bacteria. The new bacteria, however, are obligate heterotrophs. Another difference is the presence of a high concentration of ketocarotenoids, which have not been found previously within the Ectothiorhodospiraceae (Takaichi, 1999). Based on the unique phenotype and separate phylogenetic position, these alkaliphilic acetanilide-utilizing isolates are proposed to be accommodated into a new genus and species for which the name Natronocella acetinitrilica gen. nov., sp. nov. is proposed.
**Table 1.** Substrate specificity of nitrile-hydrolysing system in washed cells of strain ANL 6-2T at pH 10

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activity of NH₃ formation [nmol min⁻¹ (mg protein)⁻¹]</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>91</td>
</tr>
<tr>
<td>Propionitrile</td>
<td>30</td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>85</td>
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<tr>
<td>Butyronitrile</td>
<td>12</td>
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<tr>
<td>Isobutyronitrile</td>
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<td>Valeronitrile</td>
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<td>Phenylacetonitrile</td>
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<tr>
<td>CN⁻, NCS⁻</td>
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</tr>
<tr>
<td>Acetamide</td>
<td>680</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>270</td>
</tr>
<tr>
<td>Isobutyroamide</td>
<td>204</td>
</tr>
<tr>
<td>Formamide</td>
<td>60</td>
</tr>
</tbody>
</table>

1, Cells grown on acetonitrile; 2, cells grown on propionitrile; 3, cells, grown on acetate; 4, cells grown on yeast extract. ND, Not determined.

**Description of Natronocella gen. nov.**

*Natronocella* (Na.tron.o.cel’la Gr. n. natron, soda; L. fem. n. cella, a cell; N.L. fem. n. Natronocella a cell that can tolerate soda)

Gram-negative bacteria with rod-shaped, yellow-pigmented cells. Dominant membrane fatty acids are C₁₈:₁ω₇ and C₁₆:₀. High-salt-tolerant and obligately alkaliphilic. Obligately heterotrophic. Can use aliphatic nitriles as carbon and energy source. Member of the family *Ectothiorhodospiraceae*, *Gammaproteobacteria*. Habitat is soda lakes. Type species is *Natronocella acetinitrilica*.

**Description of Natronocella acetinitrilica sp. nov.**

*Natronocella acetinitrilica* (ace.ti.ni.tri-li.ca N.L. adj. acetinitrilica pertaining to the ability to utilize acetonitrile)

Cells are rods, 1.5–4.0 × 0.4–0.5 μm, motile with a single polar or subpolar flagellum. Yellow-coloured due to the presence of ketocarotenoids, dominated by zeaxanthin. Utilizes acetonitrile and propionitrile as carbon, energy and nitrogen source via nitrile hydratase/amidase enzyme system. Obligately alkaliphilic with a pH range for growth from 8 to 10.5 (optimum at 9.5–9.8). Can grow in saturated soda brines containing up to 4 M total Na⁺ (natronophile) with an optimum at 0.6 M. G+C content of the DNA is 50.6–51.5 mol%. Includes two strains isolated from south-west Siberia (ANL 1=NCCB 100101=UNIQEM U235) and north-east Mongolia (ANL 6-2T=NCCB 100123T=UNIQEM U236T) soda lakes. Type strain is ANL 6-2T. 16S rRNA gene sequences of strains ANL 1 and ANL 6-2T have been deposited in GenBank under the accession numbers EF103127 and EF103128, respectively.

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