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Utilization of arylaliphatic nitriles by haloalkaliphilic *Halomonas nitrilicus* sp. nov. isolated from soda soils

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Abstract An enrichment culture from saline soda soils, using acetate as carbon and energy source and 2-phenylpropionitrile as nitrogen source (PPN) at pH 10, resulted in the isolation of strain ANL-αCH3. The strain was identified as a representative of the genus *Halomonas* in the Gammaproteobacteria. The bacterium was capable of PPN utilization as a nitrogen source only, while phenylacetonitrile (PAN) served both as carbon, energy and nitrogen source. This capacity was not described previously for any other haloalkaliphilic bacteria. Apart from the nitriles mentioned above, resting cells of ANL-αCH3 also hydrolyzed mandelonitrile, benzonitrile, acrylonitrile, and phenylglycinonitrile, presumably using nitrilase pathway. Neither nitrile hydratase nor amidase activity was detected. The isolate showed a capacity to grow with benzoate and salicylate as carbon and energy source and demonstrated the ability to completely mineralize PAN. These clearly indicated a potential to catabolize aromatic compounds. On the basis of unique phenotype and distinct phylogeny, strain ANL-αCH3 is proposed as a novel species of the genus *Halomonas*—*Halomonas nitrilicus* sp. nov.

Keywords Nitriles · Phenylpropionitrile (PPN) · Phenylacetonitrile (PAN) · Haloalkaliphilic · *Halomonas* · Nitrilase

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Introduction

Nitriles are mostly synthetic compounds containing a C≡N functionality, although a few examples exist of naturally occurring nitriles formed by cyanogenic plants from cyanide (Vetter 2000) or during anaerobic degradation of amino acids by bacteria (Harper and Gibbs 1979). Nitrile compounds are widely used as intermediates and building blocks in organic synthesis. For example, the enzymatic hydrolysis of racemic nitriles into enantiomerically pure α-substituted carboxylic acids is of great industrial interest. Nitriles can be used as precursors in the synthesis of α-hydroxycarboxylic acids (Breuer et al. 2004) and amino acids, which are pharmaceutical intermediates and important resolution agents in organic resolution processes (Gröger 2001; Ravichandran et al. 1998). Most nitriles are toxic and difficult to degrade. Nevertheless, the potential for enzymatic hydrolysis of the nitrile bond is widely spread among bacteria and fungi (Banerjee et al. 2002). Two different enzymatic systems are known to convert nitriles into the corresponding carboxylic acids. The metalloenzyme nitrile hydratase catalyzes the hydration of a wide range of aliphatic, arylaliphatic, and aromatic nitriles into the corresponding amides, which are then further converted into carboxylic acids and ammonium by amidases (Kobayashi and Shimizu 1998). Nitrilases hydrolyze nitriles, mostly

aromatic, directly into acids and ammonium in a single step (Kobayashi and Shimizu 2000). The microorganisms possessing these enzymes are valuable biocatalysts and can be used either in (enantioselective) organic synthesis or in environmental biotechnology (Layh et al. 1997; Bunch 1998; Håkansson et al. 2005).

Until now, all known nitrile-degrading microorganisms included only neutrophilic species, i.e., growing optimally at neutral pH values. However, a recent survey of naturally occurring haloalkaline habitats, such as soda lakes and soda soils, demonstrated the possibility of biodegradation of simple aliphatic nitriles at extremely alkaline conditions. The compounds, such as aceto-, propio-, butyro-, iso-butyro-, and valero-nitriles, were utilized as carbon, energy, and nitrogen source by a novel haloalkaliphilic Gammaproteobacterium, an actinobacterium and bacilli (Sorokin et al. 2007a, b). The potential for enzymatic nitrile hydrolysis at highly alkaline conditions might have certain advantages, particularly when cyanide is involved in the reaction process. For example, a well-known chemical Strecker reaction could be coupled with enzymatic α-aminonitrile hydrolysis to (enantioselectively) produce α-aminoamides and/or α-aminoacids (Duthaler 1994).

In this paper, the biodegradation of a variety of arylaliphatic nitriles is demonstrated under extremely haloalkaline conditions by a novel species of the genus *Halomonas* isolated from soda soils. The capacity to hydrolyze nitrile compounds has never been demonstrated previously for this metabolically diverse group of halo (alkali)philic Gammaproteobacteria.

Materials and methods

Source of inoculum

A mixed sediment sample from Kulunda Steppe (southwestern Siberia, Altai, Russia) soda lakes and a mix sample from soda solonchak soils (Kulunda Steppe and northeastern Mongolia) were used as the inoculum for the enrichments. The pH and salinity of the lake brines were within the range of 9.5–11.05 and 50–400 g L^{-1}, respectively. The pH of water extract from the solonchak soil samples varied from 9.5 to 10.8 and the total salt content—from 30 to 200 g kg^{-1}.

Medium composition and growth conditions

A mineral medium based on sodium carbonate buffer (pH 10, 0.6 M total Na^+) was used for enrichments and pure culture study (g L^{-1}): Na_2CO_3, 22; $NaHCO_3$, 8; NaCl, 6; K_2HPO_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} (Pfennig and Lippert 1966), 1 mM $MgSO_4$, and 0.1 mg L^{-1} of filter-sterilized vitamin B_{12}. The enrichment was performed in 100-mL serum bottles closed with grey rubber septa (to prevent loss of substrate) containing 20 mL medium and 1 cm^3 sediment or soil mixture. Nitriles were added directly from the pure source into the medium (2–4 mM) and the bottles were immediately sealed to prevent the substrate loss. It is necessary to stress that the nitriles used in the growth experiments and pH profiling (phenylpropionitrile (PPN) and phenylacetonitrile (PAN)) were chemically stable up to pH 11 as was demonstrated in abiotic controls. In case of solid medium (solidifying agent was Noble agar, Difco), PPN was added after cooling the medium down to 50 °C to prevent excessive loss of substrate and the plates were incubated in closed jars for 30 days. Liquid cultures were incubated on a rotary shaker at 150 rpm and 28 °C and were periodically checked for growth and ammonia formation. The positive cultures were serially diluted and the culture obtained from a maximal positive dilution was plated onto the solid medium. Separate colonies were placed into liquid medium with PPN and acetate in 30-mL serum bottles closed with rubber septa and with 5 mL liquid. Positive cultures were plated again to check for the purity.

Growth experiments with pure cultures were performed in 1 L closed serum bottles with 100 mL liquid on a rotary shaker at 150 rpm and 30 °C. Substrates were used at 2–20 mM concentration. Growth was monitored by optical density and the degradation of nitriles was followed by ammonium production, disappearance of substrate, and formation of intermediates. The pH profiling of growth was performed in the medium containing 0.6 M total Na^+ either as NaCl (pH 6.5–8.0) or $NaHCO_3$/Na_2CO_3 (pH 8.5–11.0). The salt dependence of growth was investigated in a range of sodium carbonate-based media containing 0.1–4.0 M total Na^+ at pH 10. The substrate utilization spectrum was investigated in standard medium at pH 10 using NH_4Cl (4 mM) as the nitrogen source.

Experiments with washed cells and cell-free extract

To determine the spectrum of nitrile degradation and the influence of pH and salt concentration on the nitrilase activity, the cells grown at pH 10 and 0.6 M Na^+ with PPN+acetate or with PAN alone were harvested, washed and resuspended in 0.5 M sodium carbonate buffer, pH 9 at a cell density of 20–25 mg protein mL^{-1}. Routine activity check was performed by measuring ammonia formation over time at pH 10 and 0.6 M total Na^+. Activity and time course measurements with the determination of nitrile consumption and amide and acid

production were carried out in 2-mL Eppendorf tubes shaken on a ThermoTWISTER comfort shaker (QUAN-TIFOIL Instruments) at a cell density of 0.1–0.6 mg protein mL^{-1} in either 20 mM citrate buffer pH 5.5, 0.1 M phosphate buffer pH 7 or 20 mM carbonate buffer pH 8.4 and at 5 or 25 °C. Reactions were initiated by the addition of nitriles (10 mM) in the presence of 4 mM internal standard (veratrol). The final reaction volume was 1.0 mL. Regular samples were taken at regular time intervals and quenched in HCl-acidified mobile phase solution. After centrifugation of the denatured protein, the supernatant was injected directly on the high performance liquid chromatograph (HPLC). Samples for straight-phase HPLC measurements were extracted with ethyl acetate and then dried over Na_2SO_4 before injection.

For the fed-batch experiments, 150 μL of *Halomonas* sp. cell suspension (0.87 mg) was washed and resuspended in 0.1 M phosphate buffer pH 7 to a final volume of 1 mL in a 1.5-mL Eppendorf tube. Also, 12 μL of pure PAN (89 mM) or 16 μL (100 mM) *rac*-phenylglycinonitrile was added to start the reaction. Additional substrate was added only after full conversion was achieved. The reaction was carried out at 25 °C and samples were taken periodically.

In order to test the stability of the whole cell biocatalyst, resting cells were suspended in sodium carbonate buffer of pH 10 (20–25 mg protein mL^{-1}) and subsequently stored on ice. During time the activity of the cells was determined by incubating a sample of the cell suspension in 20 mM citrate buffer of pH 5.5 with 10 mM PAN and 2.5 mM of internal standard (veratrol) at 25 °C.

Cell-free extract was obtained either by using an IKS Lab Equipment Constant Cell Disruption System at 2 kbar at room temperature or by osmotic shock after lysozyme treatment (1 mg mL^{-1}). The cell debris and unbroken cells were removed by centrifugation at 30,000 ×*g* for 5 min.

Analytical procedures

Nitrile, amide, and carboxylic acid concentrations during the reaction were determined by HPLC. The conversions of mandelonitrile, PPN, benzonitrile, and PAN were followed on an Alliance Waters 2695 Separation Module equipped with a Waters 2487 Dual *λ* UV Absorbance Detector using a 4.6×50 mm Merck Chromolith SpeedROD RP-18e reversed-phase column and H_2O:ACN (90:10)+0.1% (*v/v*) trifluoroacetic acid (TFA) as eluent (1 mL min^{-1}). Wavelength of the detector was set at 215 nm.

The enantiomeric purity of the hydrolysis products of *rac*-mandelonitrile and *rac*-PPN was determined on an HPLC system equipped with a Waters 515 pump and a Waters 486 Tunable Absorbance Detector, which was set at 215 nm. Separation was achieved using a straight-phase 4.6×250 mm Chiralpak AD-H column with hexane: isopropanol 80:20 (*rac*-mandelonitrile) and 90:10 (*rac*-PPN)+0.1% (*v/v*) TFA as eluents (0.5 mL min^{-1}). The conversion of *rac*-phenylglycinonitrile and the enantio-purities of the corresponding acids were determined using the same HPLC module as above. Separation was obtained using a Daicel Crownpak CR(+) column and H_2O+0.1% (*v/v*) $HClO_4$ as eluent (0.5 mL min^{-1}). Conversions of acrylonitrile and 3-cyanopyridine were followed on an HPLC system with a Waters 590 programmable HPLC pump and a Shimadzu SPD-10A VP UV–VIS detector. Separation was achieved using three 4.6×50 mm Merck Chromolith™ SpeedROD RP-18e columns in series for acrylonitrile and a single column for 3-cyanopyridine. Water+0.1% (*v/v*) TFA (acrylonitrile) or acetic acid (3-cyanopyridine) was used as eluent. The UV detector was set at 210 nm.

Ammonium and cell protein concentrations were detected spectrophotometrically according to Weatherburn (1967) and Lowry et al. (1951).

The isolation of the DNA and subsequent determination of the G+C content were performed by the thermal denaturation/reassociation technique (Marmur 1961; Marmur and Doty 1962). Genomic DNA for phylogenetic analysis was extracted from the cells using the Ultra-Clean Soil DNA Extraction Kit (MolBio Laboratories, USA) following the manufacturer's instructions. The 16S rRNA genes were amplified using general bacterial primers. The PCR products were purified from low-melting agarose using the Wizard PCR-Prep kit (Promega, USA) 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, Inc., USA). The sequences were first compared with those stored in GenBank using the BLAST algorithm. The sequences were aligned with those from the GenBank using CLUSTALW. Phylogenetic trees were constructed with four different algorithms using the TREECONW program package (van de Peer and de Wachter 1994).

Results

Enrichment and isolation of a PPN-utilizing haloalkaliphile

No growth or ammonia formation was observed upon incubation of soda soil and soda lake sediments at pH 10 with PPN as the only substrate. However, when it was used as a nitrogen source in the presence of acetate as carbon and energy source, a positive enrichment culture was obtained from soda soils. The culture was dominated
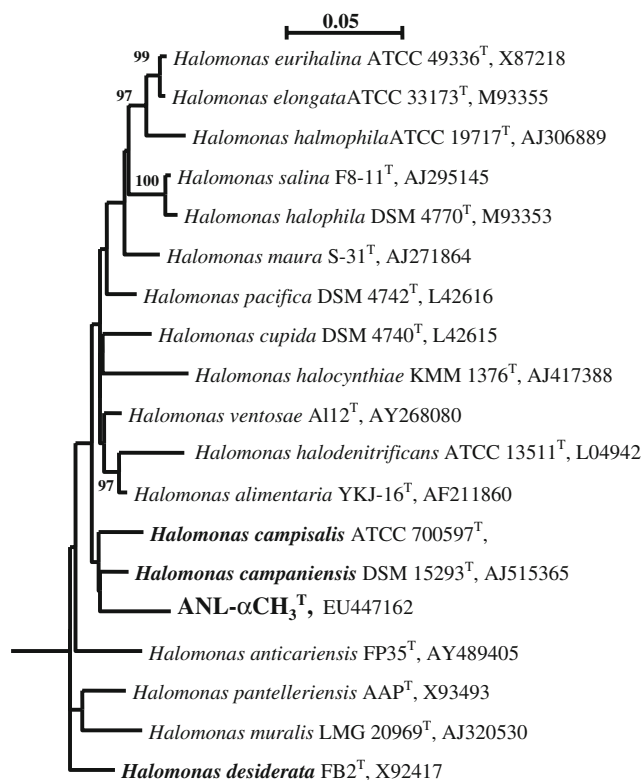
by fat nonmotile rods filled with refractive inclusions (Supplementary Fig. 1). A pure culture, designated strain ANL-αCH3, was isolated from a single colony and proved to be able to utilize PPN as nitrogen source. Phylogenetic analysis (Fig. 1) placed the isolate into the genus *Halomonas* in the Gammaproteobacteria with haloalkaliphilic species *H. campisalis* and *H. campaniensis* as the closest relatives (97% sequence similarity). The genus *Halomonas* accommodates a wide range of species characterized by high halo- and alkali-tolerance but the ability to utilize nitriles has never been demonstrated in this physiologically versatile group.

Growth of strain ANL-αCH3 with arylaliphatic nitriles at pH 10

The bacterium was not able to grow with PPN alone. When the latter was supplied at concentrations 0.5– 4 mM in presence of 20 mM acetate, the growth occurred with the maximum specific growth rate of

Fig. 1 Phylogenetic tree based on 16S rRNA gene sequencing, showing the position of strain ANL-αCH3 within the genus *Halomonas*. Tree topography and evolutionary distances are given by the neighbor-joining method with Jukes & Cantor distances. Numbers at the nodes indicate the percentage of bootstrap values for the clade in 1,000 replications. Only values above 90% are shown. Haloalkaliphilic species are shown in *bold*

$0.12 \ h^{-1}$, usually after a 20–30-h lag phase. Ammonium was not detected in the culture supernatant.

In contrast, PAN supported the growth without an additional carbon and energy source and the final growth yield was proportional to the initial PAN concentration up to 5 mM. Higher PAN concentrations inhibited growth of the bacterium. During the growth, PAN was rapidly consumed releasing nearly stoichiometric amounts of ammonia during the early logarithmic growth phase with only a limited intermediate accumulation of phenylacetic acid (PAA) (Fig. 2). The biomass production continued after complete PAN disappearance, signifying the formation of an essential intermediate. HPLC analysis, however, did not show any formation of phenylacetamide. High growth yield (biomass on 2 mM PAN was equivalent to the yield on 10 mM acetate) indicated complete utilization of PAN. The latter indicates an ability to utilize the aromatic ring, which is not an ordinary property of the genus *Halomonas*. The capacity of the bacterium to metabolize the aromatic ring was confirmed in growth experiments with benzoic and salicylic acids. The culture, pre-grown on PAN, started to grow with benzoate (up to 5 mM) and salicylate (up to 3 mM) after a 1-day lag phase. The growth resulted in the accumulation of a brownish soluble compound in both cases, which is in contrast to the PAN culture (Supplementary Fig. 2). The same effect was observed in sterile control alkaline medium when it was incubated with catechol. This suggests that the oxidation of benzoic and salicylic acids was going through a diquinol with partial spontaneous side oxidation of the latter at highly alkaline conditions. In contrast, utilization of PAN either did not result in substantial accumulation of autooxidizable intermediate(s) or proceeded through a different ring hydroxylation pathway. Phenol (1–2 mM)

Fig. 2 Growth dynamic of strain ANL-αCH3 at pH 10 with PAN as substrate. *Open circles*, biomass growth; *triangles*, ammonia formation; *closed circles*, PAN; *crosses*, phenylacetic acid (*PAA*)

did not support growth of the novel bacterium, in contrast to *H. campisalis*.

### Influence of pH on growth and nitrile hydrolysis

One of the main purposes of this work was to investigate a possibility of bio-conversion of arylaliphatic nitriles at extremely haloalkaline conditions. The pH profile for growth with PPN as the nitrogen source indicated a moderate alkaliphilic nature of the novel isolate (Fig. 3). On the other hand, resting washed cells of strain ANL-αCH3, pre-grown at pH 10 with PPN and acetate, were able to actively hydrolyze nitriles within a much broader pH range from pH 5 and at least up to pH 11 with an optimum within the alkaline region (Fig. 3).

Catalytic properties of *Halomonas* ANL-αCH3

Resting cell incubation tests with 2-phenylpropionamide, phenylacetamide, and *rac*-mandelamide did not result in acid formation even after 20 h of incubation. As was mentioned before, intermediate amide formation was also not observed during growth of the organism on PAN and

PPN. These observations indicate that ANL-αCH₃ degrades aromatic nitriles through the nitrilase pathway.

Nine different aliphatic and aromatic nitriles with straight and branched side chains were tested for conversion by using washed cells of strain ANL-αCH₃ pre-grown with PPN. The influence of different α-substitutes (and enantiomers) on the reaction rate was also investigated (Table 1). As expected, the highest conversion rate was obtained with PAN, since it was utilized as carbon source. The nitrilase proved to be strongly (R)-selective. The experimental results with (R)- and (S)-mandelonitrile showed that the (R)-enantiomer is converted 20 times faster than the (S)-one (Fig. 4a). The strong but not absolute (R)-selectivity of ANL-αCH3 was confirmed in kinetic resolution tests with other chiral nitriles, such as phenylglycinonitrile and 2-phenylpropio-nitrile (Fig. 4b and c). An interesting example of application of the nitrilase from ANL-αCH3 strain is in situ dynamic kinetic resolution of (R, S)-mandelonitrile (Fig. 4d). In the process, the unwanted enantiomer was racemized via the nitrile decomposition and chemical hydrocyanation pathway according to the following scheme:
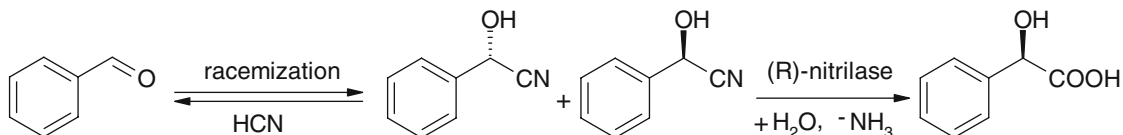
The fast racemization assured a constant supply of the desired (R)-enantiomer of cyanohydrin, which was then fully converted by the nitrilase into (R)-mandelic (+95% ee) acid in 2 h.
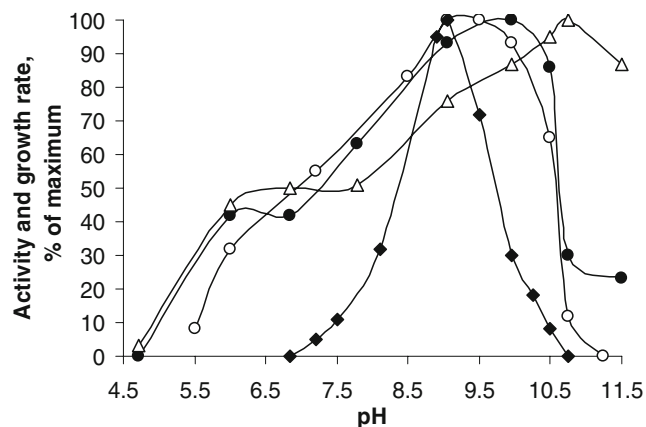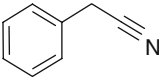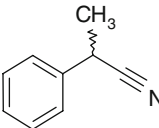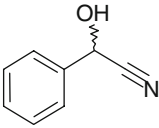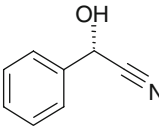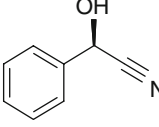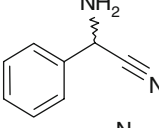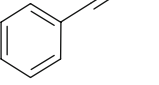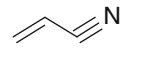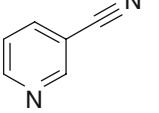

Fig. 3 Influence of pH on growth of strain ANL-αCH3 with PPN and acetate (*diamonds*) and on activity of nitrile hydrolysis (ammonia formation rate) by washed cells (PPN, *closed circles*; PAN, *open circles*; acrylonitrile, *triangles*)

A fed-batch experiment with PAN demonstrated that the *Halomonas* cells could convert 0.8 M of the nitrile into crystalline PAA. The substrate was added in nine equal portions. Every subsequent portion reached full conversion slower than the previous one (first portion was converted in <3.5 h and the eighth portion was fully converted in ~27 h). The cells eventually stopped working after converting a part of the last, ninth, portion. In a similar experiment with phenylglycinonitrile, at least 0.2 M was converted into the corresponding *rac*-phenylglycine (Fig. 4a) and the cells would have probably converted more since there were no signs of inhibition by the final product.

A 3-month storage test on ice demonstrated that the cells, when resuspended in sodium carbonate buffer of pH 10, are highly stable. No change in activity was observed during this time of storage.

During cell disruption experiments, the cell-free preparations obtained by using osmotic shock resulted in better activity retention compared to disruption using rapid decompression. Moreover, 85% of the activity was recovered in the extract when using anaerobic conditions, and 31% when using aerobic conditions during the osmotic

Table 1 Nitrilase activity (carboxylic acid formation from nitrile) in resting cells of strain ANL-αCH3 (grown with PAN at pH 10)

| Nitrile compound	Structure of nitrile	Nitrilase activity, nmol (mg protein min)$^{-1}$	pH of the test
PAN		770	7.0
PPN		50	7.0
(R, S)-mandelonitrile		180 26 180	5.5 5.5 (5°C) 7.0
(R)-mandelonitrile		2.5	5.5 (5°C)
(S)-mandelonitrile		50	5.5 (5°C)
Phenylglycinonitrile		460	8.4
Benzonitrile		0.11	7.0
Acrylonitrile		210	7.0
Nicotinonitrile		1.7	7.0

shock. In contrast, rapid decompression resulted in only 8% activity recovery.

Discussion

This is the first report in literature of a haloalkaliphilic bacterium capable of utilizing arylaliphatic nitriles. The bacterium can hydrolyze several arylaliphatic nitriles at a pH as high as 10–10.5 and salt concentrations of up to 3.5 M total Na$^+$. Most interesting is the capacity of the novel *Halomonas* isolate to grow exclusively with PAN. In fact, the potential to use phenol and its derivative carbonic acids at haloalkaline conditions has so far been shown only for a few *Halomonas* strains and only one of them is haloalkaliphilic: the immediate relative of strain ANL-αCH3 *H. campisalis* (Alva and Peyton 2003). But, in contrast to the latter, our isolate did not utilize phenol (1–5 mM). The exact pathway of the PAN and PPN degradation in the novel strain needs special investigation.

Fig. 4 Biocatalytic properties of washed cells of strain ANL-αCH3. **a** Hydrolysis of *rac*-phenylglycinonitrile with *Halomonas* sp. resting cells at pH 8.4 and 25 °C (*diamonds*, 2-phenylglycinonitrile; *squares*, phenylglycine; *circles*, ee (*R*)-phenylglycine). **b** Hydrolysis of *rac*-mandelonitrile at pH 5.5 and 5 °C (*triangles*, benzaldehyde; *diamonds*, mandelic acid; *squares*, mandelonitrile; *circles*, ee (*R*)-mandelic acid). **c** Hydrolysis of *rac*-2-phenylpropionitrile at pH 7 and 25 °C (*diamonds*, 2-phenylpropionitrile; *squares*, 2-phenylpropionic acid; *circles*, ee (*R*)-phenylpropionic acid). **d** One pot chemo-enzymatic dynamic kinetic resolution synthesis of (*R*)-mandelic acid at pH 7 (*squares*, *rac*-mandelonitrile; *diamonds*, mandelic acid; *triangles*, benzaldehyde; *circles*, ee (*R*)-mandelic acid)
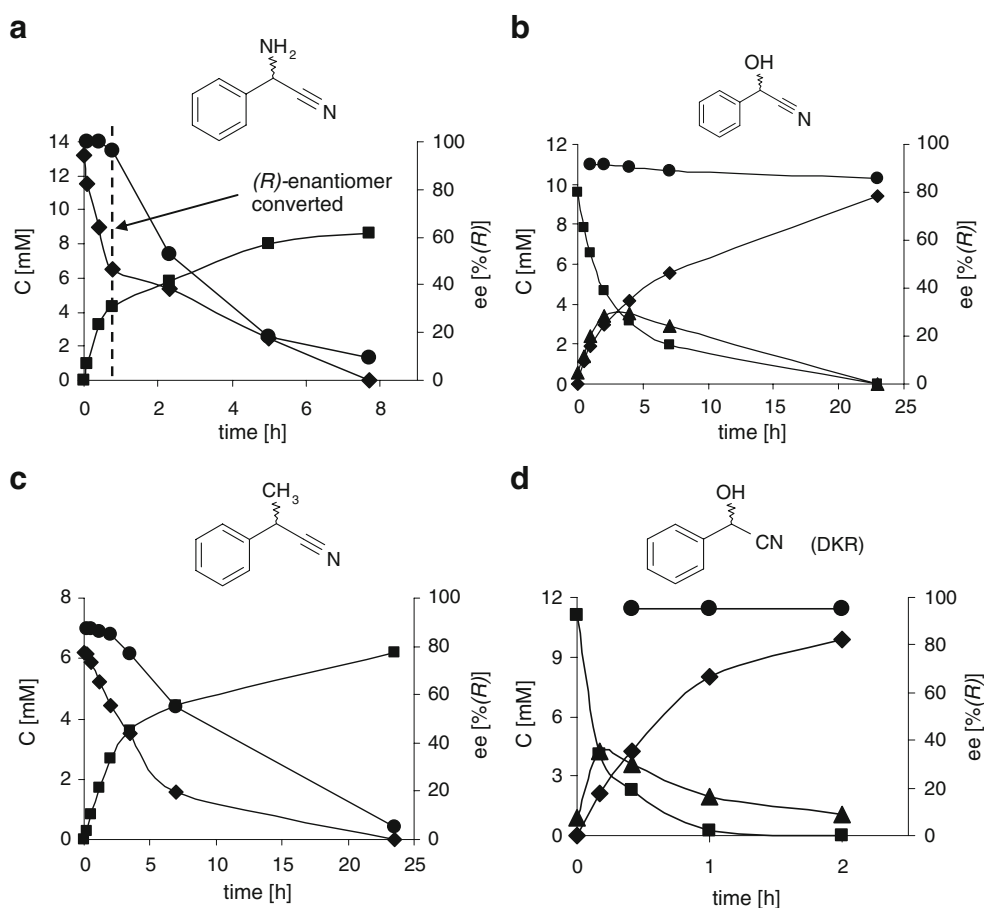
The ability of the isolate to grow with benzoic and salicylic acids suggests a potential for the complete aromatic ring degradation. Absence of PAA accumulation in the culture supernatant during growth on PAN and a certain disbalance between PAN hydrolysis and PAA accumulation by washed cells indicated rapid utilization of the acid. However, while it is easy to explain for the growing culture, the reason for PAA deficiency in washed cell experiments is not clear. Our first hypothesis was that the washed cells could have rapidly oxidized the PAA formed from PAN. However, special anoxic test showed the same disbalance, while anoxic incubation with PAA demonstrated no consumption. Perhaps, the cells might contain some of the PAA formed during intracellular PAN hydrolysis. While PAN is clearly a xenobiotic, PAA is a natural compound with plant hormone activity which occurrence is related to phenylalanine metabolism. Many bacteria can utilize this compound by activation with CoA followed by ring hydroxylation (Navarro-Llorens et al. 2008). In any case, the exact mechanism of PAA utilization in alkaliphilic *Halomonas* strain needs further detailed analysis.

Assuming its *R*-enantioselectivity, the nitrilase from ANL-αCH3 might be useful in the (dynamic) kinetic

resolution of phenylglycinonitrile (see Fig. 4). The hydrolysis product, (*R*)-phenylglycine, is an intermediate in the synthesis of β-lactam antibiotics (Mueller and Huebner 2003).

The differences in anaerobic and aerobic cell disruption experiments revealed a negative influence of oxygen on the enzyme. The activity loss, caused by exposure to oxygen, might be caused by oxidation of the catalytic cysteine present in the ANL-αCH3 nitrilase. This catalytic cysteine is present in all known nitrilases (Mateo et al. 2006). The stability of the free enzyme is an important issue. The nitrilase is unstable outside of the cell environment. Unlike the whole cells, cell-free extracts from both disruption experiments quickly (after several days) lost most of their initial activity. The activity loss is not caused by proteases and it can be partially improved by adding glycerol to the storage medium.

In conclusion, the data presented indicate the possibility of biodegradation and utilization of a broad range of arylaliphatic nitriles at a pH range between 5.5 and 11 by a novel haloalkaliphilic isolate, strain ANL-αCH3, presumably through nitrilase system. Assuming unique phenotypic properties and distinct phylogeny, strain ANL-αCH3 is proposed as a new species *Halomonas nitrilicus* sp. nov.

Description of *Halomonas nitrilicus* sp. nov.

(*ni.tri'li.cus* N.L. n. *nitrilum* nitrile, nitrile group; N.L. adj. *nitrilicus* nitrile-utilizing)

Nonmotile, Gram-negative rods, 0.5×1.5–3 μm. Utilizes phenylpropionitrile as nitrogen source and phenylacetonitrile as carbon, energy, and nitrogen source. Able to hydrolyze a range of arylaliphatic and aliphatic nitriles using the nitrilase system. Also utilizes as carbon and energy source the following substrates: acetate, propionate, butyrate, pyruvate, fumarate, succinate, citrate, malate, lactate, ethanol, glucose, maltose, sucrose, cellobiose, galactose, raffinose, trehalose, glycerol, mannitol, sorbitol, xylose, and glycogen. Degrades and utilizes as sole growth substrates benzoate and salicylate. Cannot grow anaerobically with nitrate, nitrite, or N_2O. Moderately alkaliphilic with a pH range for growth between 7.5 and 10.5 with an optimum at pH 9 and extremely salt tolerant, growing at salt concentrations from 0.1 to 3.5 M total Na^+ with an optimum at 0.3–0.5 M. Optimum growth temperature is 32 °C. The G + C content in the genomic DNA is 62.5 mol% (Tm). The type strain is ANL-αCH3^T (NCCB 100121^T=UNIQEM U241^T) is isolated from soda soils in south-eastern Siberia. The nearest cultured relative is *Halomonas campisalis*. The GenBank accession number of the 16S-rRNA gene sequence of the type strain is EU447162.

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