Check for updates

Biodegradation of alicyclic amines by a newly isolated hypersaline tolerant strain *Paenarthrobacter* sp. TYUT067

Kaiqing Feng, Jiandong Zhang, Lili Gao and Qi Tian

ABSTRACT

Alicyclic amines are widely used in several types of industries, and considerable attention has been devoted to possible environmental pollution by alicyclic amines in hypersaline industrial wastewater. In this study, a new hypersaline tolerant bacterial TYUT067 capable of growing in liquid basal salt medium with cyclohexylamine (CHAM) as the sole carbon source and energy source, was isolated from soil, and discovered with highly efficient CHAM degrading ability. The strain TYUT067 was identified as *Paenarthrobacter* sp. based on 16S rDNA gene sequence, and its degradation characteristic was examined. The results revealed that the isolated TYUT067 could grow well under pH range of 6.5–10.0, temperature from 20 °C to 30 °C. For degradation of 60 mM of cyclohexylamine, 100% degradation could be finished within 120 h. The TYUT067 could degrade 10 mM CHAM under hypersaline conditions (3–5% NaCl, w/v), revealed the hypersaline tolerance of TYUT067. Different type of amines was also tested with TYUT067, the degradations of >90% were achieved toward several alicyclic amines. The current results suggested that TYUT067 was a potential species could be efficiently used for the degradation of alicyclic amines and might be applicable to a hypersaline wastewater treatment system for the removal of alicyclic amines.

Key words | alicyclic amine, bacteria, biodegradation, cyclohexylamine, hypersaline wastewater

Kaiqing Feng
Lili Gao (corresponding author)
College of Environmental Science and Engineering,
Taiyuan University of Technology,
Taiyuan, Shanxi 030024,
China
E-mail: *gaolili@tyut.edu.cn*

Jiandong Zhang
Department of Biological and Pharmaceutical Engineering,
Taiyuan University of Technology,
Taiyuan, Shanxi 030024,
China

Qi Tian
College of Civil Engineering,
Taiyuan University of Technology,
Taiyuan, Shanxi 030024,
China

HIGHLIGHTS

- A new cyclohexylamine degradation bacterial strain TYUT067 was isolated from soil environment.
- The isolated TYUT067 is an alkali-tolerant strain and mesophilic microorganisms.
- The isolated TYUT067 can degrade CHAM under hypersaline environment.
- The isolated TYUT067 can degrade various alicyclic amines.

INTRODUCTION

Alicyclic amines are nonaromatic amines that have a cyclic alkyl moiety attached to the nitrogen atom. They are widely used as precursors or reagents for manufacturing several industrial chemicals, such as dyes, pesticides, rubber and foods (Cavender 2001; Lawrence 2004). Owing to their broad usage, alicyclic amines can reach the environment directly from industrial effluents or indirectly from pesticide degradation. The industrial wastewater with alicyclic amines will lead to high level of nitrogen and may become poisonous to environment and human health. Among the alicyclic amines, cyclohexylamine is widely used in the synthesis of antiseptic and insecticide, and considerable attention has been attracted to its potential toxicity toward human beings and aquatic ecosystems (Price *et al.* 1970; Petersen *et al.* 1972; Kroes *et al.* 1977).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Licence (CC BY-NC-ND 4.0), which permits copying and redistribution for non-commercial purposes with no derivatives, provided the original work is properly cited (http://creativecommons.org/licenses/by-nc-nd/4.0/).

doi: 10.2166/wst.2021.130

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

Considering the great harm of amines to environment and human health in wastewater, various methods have been developed to degrade amines in wastewater. Conventional methods such as electrochemical degradation (Slein & Sansone 1980), chemical oxidation (Casero *et al.* 1997), photocatalytic oxidation (Maldotti *et al.* 1994) and thermal desorption–degradation of cyclohexylamine (Breen *et al.* 2000) were used for degrading amines. Recently, photocatalytic hydrogen production under visible light with simultaneous organic amine degradation was also reported (Wu *et al.* 2004; Peng *et al.* 2014; Yang *et al.* 2019). However, these methods usually have many limitations such as huge investments, complex processes and secondary pollution. Comparing the conventional physicochemical methods, biodegradation as a promising method with the advantages of low cost, practical feasibility, and eco-friendly, has become a hot spot worldwide in the field of wastewater treatment (Wei *et al.* 2003; Chanthamalee *et al.* 2013). In the past few decades, biodegradation have been widely used in the degradation of organic amines in wastewater. In 1979, Tokieda *et al.* reported anaerobic deamination of CHAM by the intestinal microorganisms of rabbits (Tokieda *et al.* 1979). In 1980, Calamari *et al.* reported the short-term toxicity of several aliphatic and aromatic amines on three species of organisms (*Selenastrum capricornutum*, *Daphnia* and *Salmo gairdneri*); simultaneously, biodegradation of these amines including the CHAM was investigated with three different inoculums (Calamari *et al.* 1980). In 1999, a pure culture of gram-positive bacterium (*Brevibacterium oxydans* IH-35A) with highly efficient CHAM degrading ability was isolated from soil, the CHAM degradation pathway in this strain was proposed as the following route: CHAM was first deaminated to cyclohexanone (CHnone) by the cyclohexylamine oxidase, CHnone was then oxidized to 6-hexanolactone, which was hydrolyzed to 6-hydroxyhexanoate and further converted into adipate (Iwaki *et al.* 1999a). Later, a novel cyclohexylamine oxidase (CHAO) from *Brevibacterium oxydans* IH-35A responsible for the initial step of the degradation of CHAM to cyclohexanone was purified and characterized (Iwaki *et al.* 1999b; Mirza *et al.* 2013). In the last 10 years, two gram-negative bacteria *Pseudomonas plecoglossicida* NyZ12 (Shen *et al.* 2008) and *Acinetobacter* sp. YT-02 (Yan *et al.* 2018) capable of utilizing CHAM as the sole source of carbon and nitrogen, have been isolated from soil and the sludge in wastewater treatment pool, respectively. Their genomes were sequenced, open reading frames (ORFs) predicted to encode CHAM degradation were identified by bioinformatics analysis, the genes encoding cyclohexylamine oxidase and the gene cluster responsible for cyclohexanol and cyclohexanone degradation were proposed (Li *et al.* 2015; Yan *et al.* 2017).

Besides the alicyclic amines in industrial wastewaters, the high salinity is also a component of many industrial wastewaters. It is estimated that 5% of industrial wastewater is saline or hypersaline (Borgne *et al.* 2008). The treatment of this kind of wastewater poses a serious challenge, since the high ionic strength of wastewater can disrupt most of the bacteria present in activated sludge systems, blocking the degradation of organic pollutants. Halotolerant microorganisms are promising in bio-treatment of hypersaline industrial wastewater because their metabolism requires sodium chloride (Ventosa & Nieto 1995). At present, successful examples for biodegradation of aromatic acids, alkanes, chlorinated hydrocarbons, PAHs and phenol under the condition of high salinity have been documented (Margesin & Schinner 2001; Deng *et al.* 2017). However, only one document was reported for aerobic biodegradation of amines by activated sludge in industrial saline wastewaters with 3–7% NaCl (w/v) (Campo *et al.* 2011), while no pure cultures of hypersaline tolerant bacterium were reported for biodegradation of alicyclic amines. Then, isolation of halotolerant microorganisms for alicyclic amines degradation is highly required in industrial wastewater treatment.

In this study, a new hypersaline tolerant CHAM degradation strain was isolated for the first time from the soil environment. The strain was identified by 16SrDNA sequencing. The factors affecting the strain growth and CHAM degradation, including the pH, temperature, salinity, and the concentration of CHAM were examined. The degradation of different types of amines was also investigated with the isolated strain, and excellent degradation effect on alicyclic amines was observed.

MATERIALS AND METHODS

Chemicals

Aniline, benzylamine, phenethylamine, CHAM, cycloheptylamine, alpha-methylbenzylamine, trans-2-aminocyclohexanol, N-methyl-cyclohexylamine, cyclohexanol, cyclohexanone, ϵ-caprolactone and adipate were from Aladdin (Shanghai, China). All other chemicals and organic solvents were commercially available.

Enrichment and isolation of CHAM degradation bacteria

Soil samples were collected from the lawn and garden of Taiyuan University of Technology (TYUT) (Taiyuan,

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

Shanxi province, China), where the insecticide may be sprayed for vegetation protection. CHAM are widely used as precursors or reagents for manufacturing insecticide, and the CHAM can reach the environment indirectly from insecticide degradation. Then, the soil from these places may contain the efficient CHAM degrading bacteria. Samples were collected in sterile 50-mL polyethylene tube, and stored at 4 °C for further use. Modified basal salt medium (MBSM) (pH 7.0) was utilized in the enrichment culture of CHAM-degrading bacteria from soil, which contained 10–20 mM CHAM-HCl as the sole source of carbon and energy, and was composed of (g/L): NH_4NO_3 1.0; KH_2PO_4 0.5; $K_2HPO_4{\cdot}3H_2O$ 5.24; $MgSO_4{\cdot}7H_2O$ 0.2; 2.0 mL of 1% of $CaCl_2$; 200 μL of 1% $FeCl_3$; 5.0 mL of 1% yeast extract and 2.0 mL of trace elements solution. A 5.0 g of the soil sample was inoculated into the 50.0 mL of the CHAM-supplemented MBSM (10 mM CHAM-HCl). The cultures were incubated at 30 °C and 150 rpm. After 7 days, aliquots of the cultures (1 mL) were transferred to fresh CHAM containing MBSM (20 mM CHAM-HCl, 1% (w/v) NaCl). After five consecutive enrichment cultures, 1 mL of bacterial culture broth was serially diluted and 0.1 mL plated on CHAM containing agar medium, further incubated for 24–48 h at 30 °C for isolation of pure cultures. Single colonies grow on the agar medium were selected as the candidate CHAM-degrading strains, and their ability to grow in liquid medium with 20 mM CHAM as the sole source of carbon and energy were tested.

Strain identification

Single colonies grow on the CHAM containing agar medium were selected as the candidate CHAM-degrading strains. These colonies were then tested for their ability to grow in liquid MBSM with 20 mM CHAM as the sole source of carbon and energy. An isolate showing the maximum growth rate and the highest CHAM degradation was selected for further studies. The morphological properties (such as colony characteristics, shape of cells and gram reactivity) of the isolate were initially examined. The phylogenetic analysis of 16S rDNA gene was further performed for identification of the isolate. The genome DNA of selected strain was extracted using QIAGEN DNA extraction kit according to the manufacturer's instructions. The part of the 16S rDNA gene from the isolated strain was amplified by polymerase chain reaction (PCR) using a pair of forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse (5′-GGTTACCTTGTTACGACTT-3′) primers. The PCR amplification was performed in 30 μL reaction mixture containing 15 μL 2× Taq PCR Master Mix, 12 μL ddH_2O, 1.0 μL each primer and 1.0 μL template genome DNA. The PCR amplification procedure were as follows: initial denaturation for 95 °C for 5 min, followed by 30 cycles of 94 °C for 40 s, 55 °C for 40 s and 72 °C for 1.0 min, and final extension at 72 °C for 10 min. The amplified products were purified by QIA Quick PCR Purification Kit (QIAGEN) and the sequencing was carried out at Sangon Biotech Co., Ltd (Shanghai, China). The 16S rDNA sequence was analyzed using the National Center for Biotechnology Information (NCBI) BLASTN programs (http://www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree was constructed by neighbor-joining method with MEGA 7.0.

Effect of pH and temperature

The effect of pH on microbial degradation of cyclohexylamine was determined by incubating (temperature at 30 °C) the pure bacterial culture in MBSM (50 mL) at pH values range from 4.0 to 10.0, 20 mM CHAM-HCl and 1% (w/v) NaCl were contained in the medium. The effect of temperature on microbial degradation of cyclohexylamine was evaluated by incubating the pure bacterial culture in MBSM (pH 7.0, 50 mL) at the temperature from 20 to 45 °C, 20 mM CHAM-HCl and 1% (w/v) NaCl were included in the medium. After 48 h of incubation, a 0.5 mL aliquot of the suspensions were taken and centrifuged and basified (pH > 10.0) by adding NaOH (10 μL, 10 N), then the samples were saturated with NaCl and extracted with 0.5 mL ethyl acetate containing 20 mM of n-dodecane as an internal standard. The organic phase was dried over sodium sulfate and subjected to GC analysis. The growth of isolate was measured by monitoring optical density (OD) at 600 nm using a spectrophotometer. The assays were performed in triplicate.

Degradation of different concentration of CHAM

The effect of different concentration of CHAM on the growth of microbial and degradation of CHAM were evaluated by incubating the pure bacterial culture in MBSM (pH 7.0, 50 mL) containing 10.0–60.0 mM CHAM-HCl and 1% (w/v) NaCl at 30 °C. The flasks were shaken on the rotary shaker at 200 rpm for 48 h. Then, a 0.5 mL aliquot of the suspensions was taken and the samples were extracted as described above. The growth of isolate was measured as described above. The assays were performed in triplicate.

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

Effect of different concentration of NaCl on cyclohexylamine degradation

The effect of different concentration of NaCl on the growth of microbial and degradation of cyclohexylamine were evaluated by incubating the pure bacterial culture in MBSM (pH 7.0, 50 mL) containing 10 mM CHAM-HCl and 1–7% (w/v) NaCl at 30 °C. The flasks were shaken on the rotary shaker at 200 rpm for 72 h. At interval times, a 0.5 mL aliquot of the suspensions was taken and the samples were extracted as described above. The growth of isolate was measured as described above. The assays were performed in triplicate.

Degradation of different type of carbon sources

The effect of different type of carbon sources on the growth of microbial were evaluated by incubating the pure bacterial culture in MBSM (pH 7.0, 50 mL) containing 10.0 mM carbon sources (aniline, benzylamine, phenethylamine, cycloheptylamine, cyclohexylamine, alpha-methybenzylamine, trans-2-aminocyclohexanol, N-methyl-cyclohexylamine, cyclohexanol, cyclohexanone, ϵ-caprolactone, adipate) and 5% (w/v) NaCl, respectively. The flasks were shaken on the rotary shaker at 200 rpm for 72 h. Then, a 0.5 mL aliquot of the suspensions was taken and the samples were extracted as described above. The growth of isolate was measured as described above. The assays were performed in triplicate.

Assay method

The concentrations of amines, cyclohexanol, cyclohexanone and ϵ-caprolactone were quantified using gas chromatograph (GC-14C, Shimadzu, Japan) with a flame ionization detector (FID) and an HP-5 column (30 m × 0.320 mm × 0.25 mm; Agilent Technologies, Inc.). The details of instrumental conditions were as follows: injection temperature, 250 °C; detector temperature, 250 °C; column temperature, 120 °C.

RESULTS AND DISCUSSION

Enrichment and isolation of the CHAM-utilizing bacteria

Initially, 200 bacterial strains grown on the MBSM agar medium containing 20 mM CHAM-HCl and 1% NaCl were isolated. The potential bacterial isolates were selected according to the cell density (OD_{600}) of the strain and degradation rate of CHAM. The results showed that few selected pure cultures grew well (cell density (OD_{600}) > 2.0) in MBSM containing 10 mM CHAM-HCl and 1% NaCl, while a great number of the isolates grew less than 1.0 (OD_{600}). After the first-round screening, 45 isolates with more than 50% of CHAM degradation were kept. In the second-round screening, 45 isolated strains were separately incubated in CHAM (20 mM) containing MBSM (1% NaCl included) for 24 h, the cell growth (OD_{600}) and degradation of CHAM were examined, respectively. At last, seven bacterial strains with fastest growing and highly efficient CHAM degradation (>60%) were selected for further studies. As shown in Figure 1, strain TYUT067, which showed the highest degradation rates (100%) of CHAM and fastest growing in MBSM (OD_{600} 3.1) among the isolated strains, was selected as the best candidate for further tests.

Identification of isolated strain

The strain TYUT067 showed as light yellow, opaque, smooth and moist on the surface of the cells, when it was growing on the MBSM agar medium (Figure 2(a)). The cells of strain TYUT067 was observed as gram positive and rod shaped by using an optical microscope. Based on the 16S rDNA sequencing and taxonomic analyses, strain TYUT067 was confirmed to belong to the genus *Paenarthrobacter* sp., showed 99.72% identity to *Arthrobacter* sp. TS-15 and *Paenarthrobacter* sp. EB58, respectively. *Arthrobacter* sp. TS-15 is a gram-positive soil bacterium with the ability to degrade ephedrine. This strain is closely related to *Arthrobacter aurescens* (Shanati & Ansorge-Schumacher 2020). Recently, these kinds of strains (*Arthrobacter aurescens*) were reclassified as *Paenarthrobacter aurescens* (Busse 2016). Therefore, strains TYUT067 was designated as *Paenarthrobacter* sp. TYUT067 (GenBank Accession No. MW320521) (Figure 2(b)). To our best knowledge, *Paenarthrobacter* sp. is one of the most studied degraders

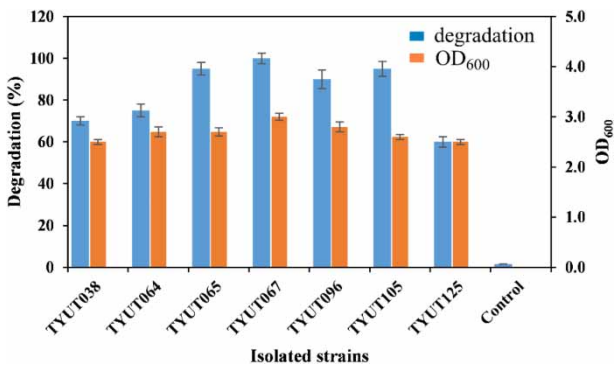

Figure 1 | The isolated strains for degradation of CHAM.

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

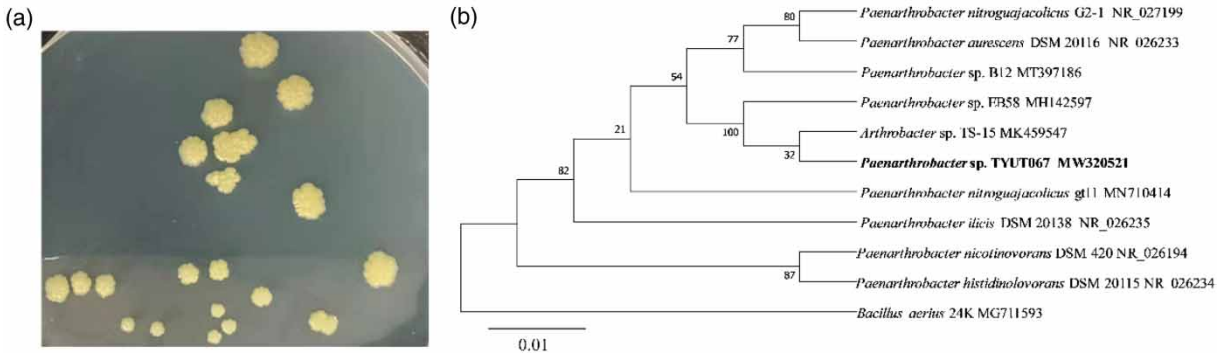

Figure 2 | The TYUT067 grown on the MBSM agar plate (a) and the phylogenetic dendrogram based on the 16S rDNA gene sequence (b). Neighbor-joining tree based on nearly complete 16S rDNA gene sequences showing the phylogenetic position of strains among closely related taxa. Numbers after the names of organisms are accession numbers of published sequences. Bootstrap values were based on 1,000 replicates. *Bacillus aerius* 24 K was used as the outgroup.

of herbicide (Deutch *et al.* 2018), nicotine (Mihăşan *et al.* 2018) and iprodione (Yang *et al.* 2018), while no relevant documents were reported on the degradation of CHAM with the strain of *Paenarthrobacter* sp.

Effect of temperature and pH on CHAM degradation

The optimum pH of TYUT067 for the degradation of CHAM was determined by incubation of the strain from pH 4.0 to 10.0. As shown in Figure 3(a), TYUT067 showed the highest degradation of CHAM (>99%) at pH 7.0–9.0, and the cells grown very well at these pH range, the highest OD_{600} can reach to 3.0 at pH 7.0. When the pH was below 7.0 or above 9.0, the degradation of CHAM was evidently decreased, and the TYUT067 stop growing at the pH below 6.0. The effect of temperature on the degradation of CHAM was examined at the temperatures from 20 to 45 °C. As shown in the Figure 3(b), TYUT-067 grow well in the MBSM medium at the temperatures from 20 to 35 °C, with the CHAM degradation of 55–100% at 24 h cultivation. When the TYUT067 was incubated at the temperature above 35 °C, the cells were growing very slowly and stopped growing at 45 °C. The optimum temperature for the CHAM degradation and TYUT-067 growing was determined at 30 °C, with the degradation of 100% and cell density (OD_{600}) of 3.0, respectively. These results revealed that the isolated TYUT067 was an alkali-tolerant strain and mesophilic microorganisms. The genus *Paenarthrobacter* are widely distributed in soils from various environments all over the world. However, alkaliphilic or alkali-tolerant *Paenarthrobacter* have rarely been reported, and the strain TYUT067 may be particularly suitable for bioremediation under certain environmental stresses.

Growth curve and degradation of CHAM

The growth curve of TYUT067 and the degradation of CHAM were then analyzed by inoculating TYUT067 in MBSM medium with 10 mM CHAM-HCl and 1% NaCl. As shown in Figure 4, the TYUT067 grew very slowly at the lag phase. After 6 h, the TYUT067 entered into exponential growth phase, and the highest cell density (OD_{600}) of TYUT067 could reach to 2.1 after cultivation for 26 h; after that, the cells entered into stationary phase. At the

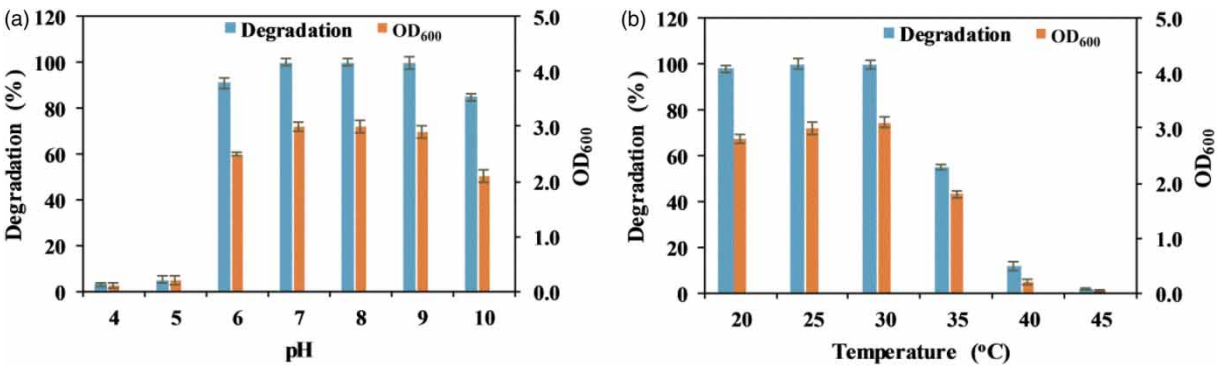

Figure 3 | Effect of pH (a) and temperature (b) on the cell growth and CHAM degradation.

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

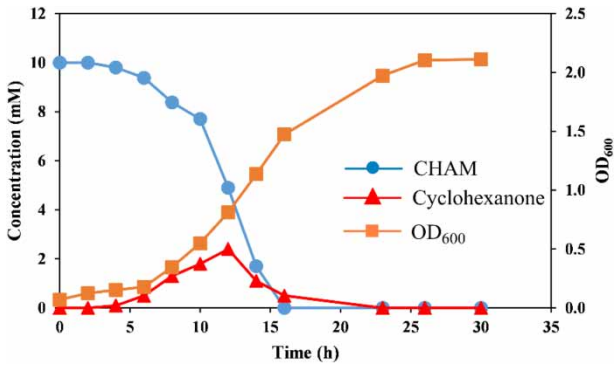

Figure 4 | The time curve of CHAM degradation and growth of TYUT067.

same time, 100% degradation of CHAM could be achieved. During the CHAM degradation, cyclohexanone was evidently detected in the growth process. The concentration of cyclohexanone (CHnone) intermediate product was increased at the first 12 h cultivation, then started to decrease, and completely degraded after 24 h cultivation. The accumulation of intermediate CHnone in the CHAM degradation process suggested that the biodegradation pathway of CHAM in this strain involves an initial deamination step. This phenomenon is similar to the previous reported strains (such as *Brevibacterium oxydans* IH-35A and *Pseudomonas plecoglossicida* NyZ12) which can degrade CHAM (Iwaki *et al.* 1999a; Shen *et al.* 2008). In addition, a trace amount of cyclohexanol was detected in the CHAM degradation process, and it was then metabolized transiently. We speculated that there may be an alcohol dehydrogenase (or ketoreductase) in the TYUT067 which is involved in the reduction of accumulated CHnone.

Degradation of different concentration of CHAM

Degradation of different concentration of CHAM was examined by inoculating TYUT067 in MSBM with CHAM-HCl from 10 to 60 mM for 48 h. As shown in Figure 5(a), the degradation of CHAM was gradually decreased (from 100% to 38%) with the increased concentration of CHAM (from 10 to 60 mM). For degradation of 10 and 20 mM CHAM, 100% degradation of CHAM could be finished within 48 h, with the OD_{600} of 2.1 and 3.0, respectively. For degradation of 30 mM CHAM, 80% degradation of CHAM could be finished after 48 h cultivation, and trace amount of cyclohexanone was observed. Further prolonging the cultivation time to 72 h, 100% degradation of CHAM could be finished. For 40 and 60 mM CHAM, the degradations of CHAM were decreased to 50 and 35% after 48 h cultivation, respectively, while 120 h cultivation time were needed for consumption of such high concentration (60 mM) of CHAM completely (Figure 5(b)).

Effect of different concentration of NaCl on CHAM degradation

The treatment of hypersaline wastewaters represents a challenge since the high salt concentrations can change the morphology of strain and decrease their metabolism of some carbon compounds. However, limited information was documented on amines biodegradation in hypersaline industrial effluents. Then the effect of different concentrations of NaCl on the degradation of CHAM by strain TYUT067 was investigated. As shown in Figure 6, with 1% (w/v) NaCl included in the medium, the degradation of CHAM could reach to 100% after 16 h cultivation, and the cell density (OD_{600}) of TYUT067 could reach to 2.1 after 26 h cultivation. With 3% NaCl included in the medium, a slow degradation of CHAM was evidently observed, and 100% degradation of CHAM could be finished after 36 h cultivation. With 5% NaCl added, 100% degradation of CHAM could be finished after 72 h cultivation. Further increasing the NaCl to 7%, the TYUT067 stop growing,

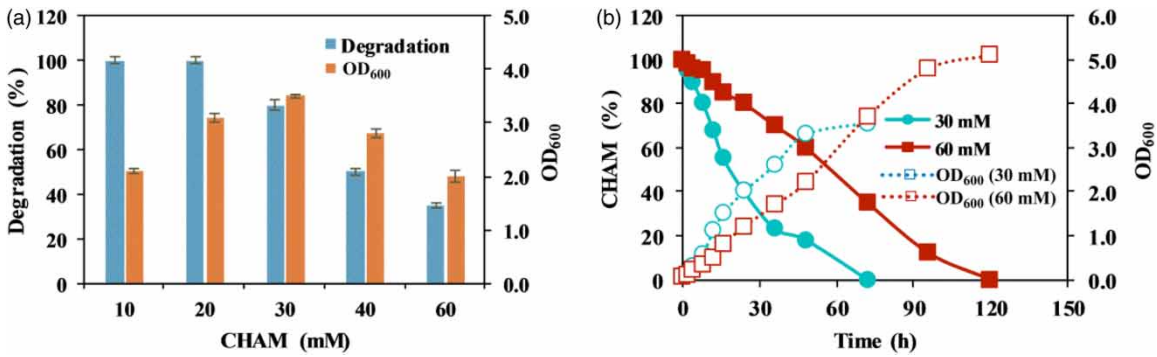

Figure 5 | Effect of different concentration of CHAM on degradation (a) and the time curve for degradation of 30 and 60 mM CHAM (b).

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

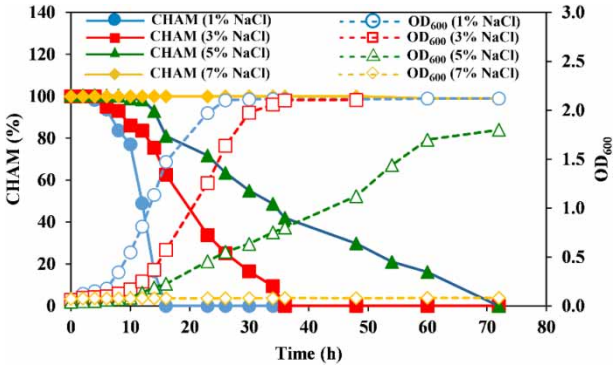

Figure 6 | Effect of different concentrations of NaCl on CHAM degradation.

and trace amount of CHAM was degraded (<2%). As we know, waters containing dissolved salts (mainly NaCl) >1% (w/v) and >3.5% (w/v) are called saline water and hypersaline water (Lefebvre & Moletta 2006; Gayathri & Namasivayam 2010). The strain TYUT067 could grow in CHAM containing MSBM with high salt concentration (5% NaCl), indicated that the TYUT067 was a hypersaline tolerant bacterial which can be utilized to degrade CHAM in hypersaline wastewater.

Degradation of different substrates by TYUT067

The ability of TYUT067 to grow on various substrates was tested in liquid MBSM containing the carbon sources at a concentration of 10 mM. As shown in the Figure 7, TYUT067 grew very well in alicyclic amines containing MBSM, such as CHAM, cycloheptylamine, N-methycyclohexylamine, *trans*-2-aminocyclohexanol, with the degradation of 80–100% after 72 h cultivation. With aniline, benzylamine, and phenethylamine as sole carbon sources, TYUT067 grew very slowly and trace amounts of amines (<10%) were degraded. TYUT067 stop growing at the initial growth stage with adipate as the sole carbon source and energy source, which indicated that the TYUT067 could not utilize it for growing. As best as we know, assimilation of adipate by *Paenarthrobacter aurescens* is usually very weak (Busse 2016). Interestingly, TYUT067 grew very well on cyclohexanol, cyclohexanone and ϵ-caprolactone, and complete degradation of these substrates could be finished within three days. These results are consistent with the previous reported strains *Pseudomonas plecoglossicida* NyZ12 (Yan *et al.* 2017) and *Acinetobacter* sp. YT-02 (Yan *et al.* 2018), which can utilize cyclohexanol, cyclohexanone and ϵ-caprolactone as the sole carbon sources and energy sources. Then, a possible CHAM degradation pathway of TYUT067 was deduced as follows (Figure 8): CHAM was first deaminated to CHnone by the cyclohexylamine oxidase, CHnone was oxidized to 6-hexanolactone by the CHnone monooxygenase, 6-hexanolactone was then hydrolyzed to ring-cleaved product 6-hydroxyhexanoate by a hydrolase. Considering the TYUT067 cannot use adipate as the sole source of carbon and energy for growing, we speculate that there may be a novel degradation pathway for cyclohexylamine included in TYUT067. The cyclohexanol could be degraded by TYUT067, and CHnone was also detected in the cyclohexanol degradation process, suggesting that CHnone was a metabolic intermediate of cyclohexanol, we speculated that there may be a cyclohexanol dehydrogenase (or cyclohexanone reductase) was included in the cyclohexanol degradation pathway responsible for oxidation of cyclohexanol to cyclohexanone. Further analysis of the genome sequence information and identification of metabolites could provide the insight into metabolic mechanism of CHAM in TYUT067.

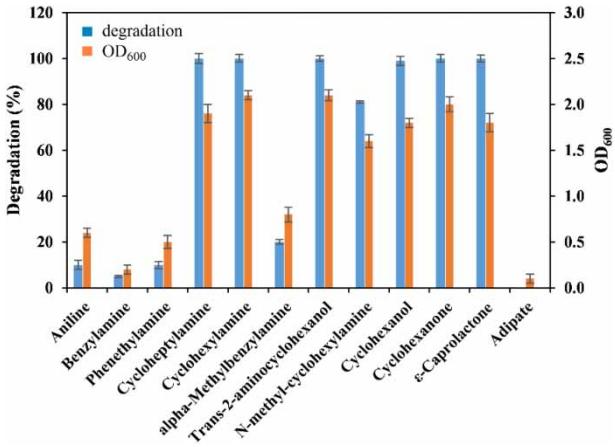

Figure 7 | Degradation of different substrates.

CONCLUSIONS

In conclusion, a new halotolerant strain TYUT067 was isolated from the soil environment with highly efficient cyclohexylamine degradation ability. The strain was confirmed as belonging to the genus *Paenarthrobacter* sp. by 16SrDNA sequencing and 16SrDNA similarity analysis. The strain TYUT067 was effective under pH range of 6.5–10.0, and temperature from 20 °C to 35 °C, revealed that the isolated TYUT067 was an alkali-tolerant strain and mesophilic microorganisms. The TYUT067 can degrade CHAM under hypersaline conditions (3–5% NaCl), and showed that TYUT067 is a hypersaline tolerant microorganism. Degradation of different types of amines was conducted

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

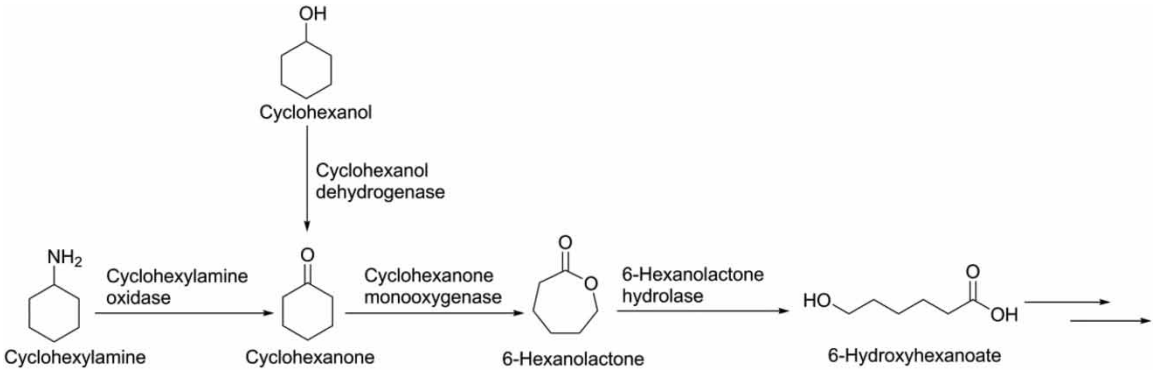

Figure 8 | The proposed pathway for cyclohexylamine and cyclohexanol degradation in TYUT067.

with the isolated TYUT067, and excellent degradation effect was observed toward alicyclic amines. To our best knowledge, this is the first report on pure culture of hypersaline tolerant microorganism for alicyclic amine degradation. With the characteristic of fast growth, alkali resistance, hypersaline tolerant and highly efficient alicyclic amines degradation ability, the isolated TYUT067 may be a potential candidate to be used for the removal of alicyclic amines in hypersaline industrial wastewater treatment system. Further investigations on the degradation pathway of CHAM and cloning of corresponding enzyme genes from TYUT067 responsible for CHAM degradation are currently underway.

ACKNOWLEDGEMENTS

This work was financially supported by the Key Research and Development (R&D) Project of Shanxi Province (201803D31050).

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Borgne, S. L., Paniaqua, D. & Vazquez-Duhalt, R. 2008 Biodegradation of organic pollutants by halophilic bacteria and archaea. *J. Mol. Microbiol. Biotechnol.* **15**, 74–92.

Breen, C., Forsyth, J., Yarwood, J. & Hughes, T. 2000 Thermal desorption–degradation of cyclohexylamine over Ni^{2+}- and Al^{3+}-exchanged bentonite studied using evolved gas analysis (TG-EGA) and diffuse reflectance spectroscopy (DRIFTS). *Phys. Chem. Chem. Phys.* **2**, 3887–3892.

Busse, H. J. 2016 Review of the taxonomy of the genus *Arthrobacter*, emendation of the genus *Arthrobacter sensu lato*, proposal to reclassify selected species of the genus *Arthrobacter* in the novel genera *Glutamicibacter* gen. nov., *Paeniglutamicibacter* gen. nov., *Pseudoglutamicibacter* gen. nov., *Paenarthrobacter* gen. nov. and *Pseudarthrobacter* gen. nov., and emended description of *Arthrobacter roseus*. *Int. J. Syst. Evol. Microbiol.* **66** (1), 9–37.

Calamari, D., Da Gasso, R., Galassi, S., Provini, A. & Vighi, M. 1980 Biodegradation and toxicity of selected amines on aquatic organisms. *Chemosphere* **9**, 753–762.

Campo, P., Platten III., W., Suidan, M. T., Chai, Y. & Davis, J. W. 2011 Aerobic biodegradation of amines in industrial saline wastewaters. *Chemosphere* **85**, 1199–1203.

Casero, I., Sicilia, D., Rubio, S. & Perez-Bendito, D. 1997 Chemical degradation of aromatic amines by Fenton's reagent. *Water Res.* **31**, 1985–1995.

Cavender, F. L. 2001 Aliphatic and alicyclic amines. In: *Patty's Toxicology*, 5th edn, vol. 4 (E. Bingham, B. Cohrssen & C. H. Powell, eds). John Wiley & Sons, Inc., New York, NY, USA, pp. 706–711.

Chanthamalee, J., Wongchitphimon, T. & Luepromchai, E. 2013 Treatment of oily bilge water from small fishing vessels by PUF-immobilized *Gordonia* sp. JC11. *Water Air Soil Pollut.* **224**, 1601.

Deng, T., Wang, H. & Yang, K. 2017 Phenol biodegradation by isolated *Citrobacter* strain under hypersaline conditions. *Water Sci. Technol.* **77**, 504–510.

Deutch, C. E., Bui, A. P. & Ho, T. 2018 Growth of *Paenarthrobacter aurescens* strain TC1 on atrazine and isopropylamine during osmotic stress. *Ann. Microbiol.* **68**, 569–577.

Gayathri, K. V. & Namasivayam, V. 2010 Enrichment of phenol degrading moderately halophilic bacterial consortium from saline environment. *J. Biorem. Biodegrad.* **1**, 104.

Iwaki, H., Shimizu, M., Tokuyama, T. & Hasegawa, Y. 1999a Biodegradation of cyclohexylamine by *Brevibacterium oxydans* IH-35A. *Appl. Environ. Microbiol.* **65**, 2232–2234.

Iwaki, H., Shimizu, M., Tokuyama, T. & Hasegawa, Y. 1999b Purification and characterization of a novel cyclohexylamine

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest

oxidase from the cyclohexylamine-degrading *Brevibacterium oxydans* IH-35A. *J. Biosci. Bioeng.* **88**, 264–268.

Kroes, R., Peters, P. W., Berkvens, J. M., Verschuuren, H. G., de Vries, Th. & Va Esch, G. J. 1977 Long term toxicity and reproduction study (including a teratogenicity study) with cyclamate, saccharin and cyclohexylamine. *Toxicology* **8**, 285–300.

Lawrence, S. A. 2004 *Amines: Synthesis, Properties and Applications*. Cambridge University Press, Cambridge, UK.

Lefebvre, O. & Moletta, R. 2006 Treatment of organic pollution in industrial saline wastewater: a literature review. *Water Res.* **40**, 3671–3682.

Li, X., Li, C. Z., Mao, L. Q., Yan, D. Z. & Zhou, N. Y. 2015 Complete genome sequence of the cyclohexylamine-degrading *Pseudomonas plecoglossicida* nyz12. *J. Biotechnol.* **199**, 29–30.

Maldotti, A., Amadelli, R., Varani, G., Tollari, S. & Porta, F. 1994 Photocatalytic processes with polyoxotungstates: oxidation of cyclohexylamine. *Inorg. Chem.* **33**, 2968–2973.

Margesin, R. & Schinner, F. 2001 Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl. Microbiol. Biotechnol.* **56**, 650–663.

Mihăşan, M., Babii, C., Aslebagh, R., Channaveerappa, D., Dupree, E. & Darie, C. C. 2018 Proteomics based analysis of the nicotine catabolism in *Paenarthrobacter nicotinovorans* pAO1. *Sci. Rep.* **8**, 1–9.

Mirza, I. A., Burk, D. L., Xiong, B., Iwaki, H., Hasegawa, Y., Grosse, S., Peter, C. K. L. & Berghuis, A. M. 2013 Structural analysis of a novel cyclohexylamine oxidase from *Brevibacterium oxydans* IH-35A. *PLoS One* **8**, e60072.

Peng, S., Ding, M., Yi, T. & Li, Y. 2014 Photocatalytic hydrogen evolution in the presence of pollutant methylamines over Pt/ZnIn2S4 under visible light irradiation. *J. Mol. Catal.* **5**, 466–473.

Petersen, K. W., Legator, M. S. & Figge, F. H. J. 1972 Dominant-lethal effects of cyclohexylamine in C57Bl/Fe mice. *Mutat. Res.* **14**, 126–129.

Price, J. M., Biava, C. G., Oser, B. L., Vogin, E. E., Steinfeld, J. & Ley, H. L. 1970 Bladder tumors in rats fed cyclohexylamine or high goses of a mixture of cyclamate and saccharin. *Science* **167**, 1131–1132.

Shanati, T. & Ansorge-Schumacher, M. B. 2020 Biodegradation of ephedrine isomers by *Arthrobacter* sp. strain TS-15: discovery of novel ephedrine and pseudoephedrine dehydrogenases. *Appl. Environ. Microbiol.* **86** (6), e02487–19.

Shen, Y., Yan, D. Z., Chi, X. Q., Yang, Y. Y., Leak, D. J. & Zhou, N. Y. 2008 Degradation of cyclohexylamine by a new isolate of *Pseudomonas plecoglossicida*. *World J. Microbiol. Biotechnol.* **24**, 1623–1625.

Slein, M. W. & Sansone, E. B. 1980 *Degradation of Chemical Carcinogens*. Van Nostrand Reinhold, New York, NY, USA.

Tokieda, T., Niimura, T., Yamaha, T., Hasegawa, T. & Suzuki, T. 1979 Anaerobic deamination of cyclohexylamine by intestinal microorganism in rabbits. *Agric. Biol. Chem.* **43**, 25–32.

Ventosa, A. & Nieto, J. J. 1995 Biotechnological applications and potentialities of halophilic microorganisms. *World J. Microbiol. Biotechnol.* **11**, 85–94.

Wei, Y., Van Houten, R. T., Borger, A. R., Eikelboom, D. H. & Fan, Y. 2003 Minimization of excess sludge production for biological wastewater treatment. *Water Res.* **37**, 4453–4467.

Wu, Y., Lv, G. & Li, B. 2004 Hydrogen production by Pt/TiO_2 anaerobic photocatalytic reforming degradation of aqueous monoethanolamine. *Acta. Phys.-Chim. Sin.* **20**, 755–758.

Yan, D. Z., Li, X., Li, C. Z., Mao, L. Q., Chi, X. Q. & Zhou, N. Y. 2017 Genome-wide identification and characterization of genes encoding cyclohexylamine degradation in a novel cyclohexylamine-degrading bacterial strain of *Pseudomonas plecoglossicida* nyz12. *J. Biotechnol.* **251**, 166–173.

Yan, D. Z., Gan, Y. T., Zhou, H., Liu, J. & Li, X. 2018 Draft genome sequence of cyclohexylamine-degrading strain *Acinetobacter* sp. YT-02 isolated. *Curr. Microbiol.* **75**, 284–287.

Yang, Z., Jiang, W., Wang, X., Cheng, T., Zhang, D., Wang, H., Qiu, J., Cao, L., Wang, X. & Hong, Q. 2018 An amidase gene, ipaH, is responsible for the initial step in the iprodione degradation pathway of *Paenarthrobacter* sp. Strain YJN-5. *Appl. Environ. Microbiol.* **84**, e01150–18.

Yang, R., Song, K., He, J., Fan, Y. & Zhu, R. 2019 Photocatalytic hydrogen production by RGO/$ZnIn_2S_4$ under visible light with simultaneous organic amine degradation. *ACS Omega* **4**, 11135–11140.

First received 11 January 2021; accepted in revised form 25 March 2021. Available online 2 April 2021

Downloaded from http://iwaponline.com/wst/article-pdf/83/9/2160/888464/wst083092160.pdf
by guest