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ORIGINAL ARTICLE

Monitoring the degradation capability of novel haloalkaliphilic tributyltin chloride (TBTCl) resistant bacteria from butyltin-polluted site

Hamdy A. Hassan[a,b,*], **Somya E. Dawah**[c], **Mostafa M. El-Sheekh**[c]

[a] *Department of Environmental Biotechnology, Genetic Engineering and Biotechnology Research, Institute, University of Sadat City, Sadat City, Egypt*
[b] *Department of Biological Science, Faculty of Science and Humanity Studies at Al-Quwayiyah, Shaqra University, Al-Quwayiyah 11971, Saudi Arabia*
[c] *Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt*

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KEYWORDS
Tributyltin;
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Abstract Tributyltin (TBT) is recognized as a major environmental problem at a global scale. Haloalkaliphilic tributyltin (TBT)-degrading bacteria may be a key factor in the remediation of TBT polluted sites. In this work, three haloalkaliphilic bacteria strains were isolated from a TBT-contaminated site in the Mediterranean Sea. After analysis of the 16S rRNA gene sequences the isolates were identified as *Sphingobium* sp. HS1, *Stenotrophomonas chelatiphaga* HS2 and *Rhizobium borbori* HS5. The optimal growth conditions for biodegradation of TBT by the three strains were pH 9 and 7% (w/v) salt concentration. *S. chelatiphaga* HS2 was the most effective TBT degrader and has the ability to transform most TBT into dibutyltin and monobutyltin (DBT and MBT). A gene was amplified from strain HS2 and identified as TBTB-permease-like, that encodes an ArsB-permease. A reverse transcription polymerase chain reaction analysis in the HS2 strain confirmed that the TBTB-permease-like gene contributes to TBT resistance. The three novel haloalkaliphilic TBT degraders have never been reported previously.

PALABRAS CLAVE
Tributiltina;
Biodegradación;
Haloalcalifílico;
ArsB permeasa

Monitorización de la capacidad de degradación de las nuevas bacterias haloalcalifílicas resistentes a cloruro de tributiltina (TBTCl) en una ubicación contaminada por butiltina

Resumen Se considera a la tributiltina (TBT) como un problema medioambiental serio a escala global. Las bacterias haloalcalifílicas degradadoras de TBT pueden constituir un factor clave

* Corresponding author.
E-mail address: hamdy.hassan@gebri.usc.edu.eg (H.A. Hassan).

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para remediar áreas contaminadas con dicho xenobiótico. En este estudio se aislaron 3 cepas de bacterias haloalcalifílicas procedentes de un sitio contaminado con TBT en el mar Mediterráneo. Tras analizar las secuencias del gen de 16S del ARNr, se identificaron los aislados como *Sphingobium* sp. HS1, *Stenotrophomonas chelatiphaga* HS2 y *Rhizobium borbori* HS5. Las condiciones de crecimiento óptimas para la biodegradación de TBT por parte de las 3 cepas fueron pH 9 y 7% (p/v) de concentración de sal. *S. chelatiphaga* HS2 fue el degradador de TBT más efectivo, con capacidad de transformar la mayor parte de ese compuesto en dibutiltina y monobutiltina (DBT y MBT). Se amplificó un gen de la cepa HS2, que fue identificado como tipo TBTB-permeasa, que codifica para una ArsB permeasa. Un análisis de la cepa HS2 por reacción en cadena de la polimerasa con transcriptasa inversa (RT PCR) confirmó que el gen TBTB-permeasa contribuye a la resistencia al TBT. Estos 3 nuevos degradadores haloalcalifílicos de TBT no habían sido reportados previamente.

Introduction

Tributyltin (TBT) is an organotin compound commonly used as fungicide, bactericide, pesticide, wood preservative, polyvinyl chloride (PVC) stabilizer and component of antifouling paints, which are used to coat structures exposed to aquatic environments, including ships, pleasure boats, buoys, pilings, sea walls, oil rigs, cables, and water intake pipes[12]. TBT repels or kills harassing organisms such as barnacles[21].

TBT can be highly toxic to many aquatic and terrestrial eukaryotic or prokaryotic organisms and causes immune system suppression and endocrine disruption in humans[11]. Generally, tributyltins (TBT) are more toxic than dibutyltin (DBT) and monobutyltin (MBT)[13]. Although the industries containing TBT have been banned by the International Maritime Organization[22], some countries continued to use it as a biocide and wood preservative component. On the other hand, DBT and MBT have been found in drinking water[29].

Although TBT is toxic to both gram positive and gram negative bacteria collected from sediment, gram positive bacteria are generally more sensitive to TBT[5]. Bacterial TBT resistance can be achieved either by sequential removal of butyl groups from the tin atom, leading to the formation of DBT and MBT or pumping out TBT through a multidrug efflux pump[24] or bioaccumulation of TBT into the bacterial cell[15]. TBT can be degraded by gram negative or gram positive bacteria[5,11], whereas metabolic pathways of TBT by the reported bacteria have not been well studied and still requires elucidation of TBT degradation[28].

Haloalkaliphilic organisms are interesting from two points of view, fundamentally research and biotechnology[23]. Haloalkaliphilic degrading bacteria is an interesting class of extremophilic organisms that possess special adaptation strategies that make them interesting for the remediation of polluted sites[19,32]. Moderate halophiles have the ability for exciting and promising applications making them the most potential candidates compared with other extremophiles[18,27].

To our knowledge no haloalkaliphilic bacteria from the Mediterranean Sea have been previously isolated and reported to exhibit resistance or to possess TBT degradation ability.

The objective of this study is to isolate and characterize haloalkaliphilic bacteria strains from the Mediterranean Sea, Abu Qir- coastline, Egypt, that have the ability to degrade TBT into DBT and MBT.

Materials and methods

Isolation and identification of haloalkaliphilic bacteria degrading TBT

Water and sediment samples contaminated with TBT were collected from the Mediterranean Sea, Abu Qir-coastline, Egypt. Samples were taken with pH 9 and salinity 32% (w/v) and placed in sterile bottles swabbed with 75% (v/v) ethanol, and stored at 4 °C until they were used. Primary enrichment cultures were prepared in 100-ml bottles by adding 1 ml of water sample to 19 ml of mineral medium (MM)[9] containing 3% NaCl (w/v) and pH 9 (adjusted by adding Na_2CO_3) with TBT (125 μM) as a sole source of carbon and energy. The enrichment cultures were incubated at 30 °C; after one month 10% (v/v) of the culture was transferred to fresh MM containing 5% NaCl (w/v) and cultured for a further month and this step was repeated on 7% NaCl (w/v). Dilution of the cultures was spread onto MM agar plates supplemented with TBT and incubated for 7 days, the colonies observed were purified on MM agar plates with TBT. Three purified isolates (HS1, HS2 and HS5) were selected and identified by 16S rRNA. Three primers were used in the amplification of 16S rDNA, which included: Bact 27f (5′-AGAGTTTGATC(A/C)TGGCTCAG-3′), Bact 1492r (5′-TACGG(C/T)ACCTTGTTACGACTT-3′), and Bact 1098r (5′-AAGGGTTGCGCTCGTTGCG-3′)[2]. Theoretically, amplification with Bact 27f -1492r should yield 1505 bp whereas amplification with Bact27f -1098r should yield 1108 bp from the 16S rRNA. Amplifications with these two primer sets were used to obtain the nearly full-length sequence (1492 bp) of the 16S rDNA of the isolate. PCR amplification was performed in a total volume of 5 μl in a Touch Screen Thermal Cycler PCR Model: A100/A200 (Hangzhou LongGene Scientific Instruments Co., Ltd). The PCR product was purified by Gene JET™

Gel Extraction kit (Thermo Scientific) and sequenced in both directions. The determined 16S rRNA gene nucleotide sequences were entered for BLAST search into the NCBI website (http://www.ncbi.nlm.nih.gov/blast/), and aligned using Clustal W implemented in MEGA software version 3, 1. The phylogenetic tree was constructed using Phylogeny.Fr[8].

TBT sensitivity

HS1, HS2 and HS5 samples were spread on the surface of MM agar plates containing 125 μM TBT. Plates were incubated at 30 °C in the dark to avoid photodegradation of TBT. The isolates that have the ability to grow on 125 μM TBT were successively transferred to MM plates containing increasingly higher TBT concentrations (0–3 mM). Successive transferring to a new media occurred as long as growth was observed and the isolates were scored as resistant if they could grow along the gradient plates.

Antibiotic susceptibility

Antibiotics to be tested were selected according to the Clinical Laboratory Standard Institute (CLSI)[4] guidelines, using the following antibiotic susceptibility discs supplied by Oxoid (Wade Rd, Basingstoke, Hampshire RG24 8PW, United Kingdom): Ampicillin 10 μg/disc, ampicillin–sulbactam 10/10 μg/disc, amoxicillin 10 μg/disc, amoxicillin–clavulanic acid 30 μg/disc, erythromycin 15 μg/disc, azithromycin 15 μg/disc, levofloxacin 5 μg/disc, ciprofloxacin 5 μg/disc, tetracycline10 μg/disc, tigecycline 15 μg/disc, clindamycin 2 μg/disc, rifampicin 30 μg/disc, septrin 25 μg/disc, linezolid 30 μg/disc, vancomycin 30 μg/disc, oxacillin 1 μg/disc, gentamicin 10 μg/disc, and amikacin 30 μg/disc.

Effect of pH and salinity on TBT degrading ability

Bacterial pure cultures from HS1, HS2 and HS5 were grown at 30 °C with shaking (150 rpm) in MM containing 2 mM of TBT until late exponential growth phase. Cells were harvested by centrifugation, washed twice in sterile MM and resuspended to an $OD_{600\,nm}$ of 5. The effect of salt on TBT biodegradation was determined at various concentrations of 0–12% NaCl (w/v) by the methods described above. The effect of pH on TBT biodegradation was determined by using 50 mM $NaHCO_3$ (pH 7.0–9.0), 50 mM Na_2CO_3 (pH 9–11). CFU/ml was monitored in sterile TBT-containing medium, and the residual TBT concentration of each compound from TBT separately was measured at regular intervals over an incubation period[18,20].

Growth curve and TBT analysis

To quantify growth rate and substrate disappearance, *Sphingobium* sp. HS1, *Stenotrophomonas chelatiphaga* HS2 and *Rhizobium borbori* HS5 were grown as described above and cultures were harvested during late exponential growth phase by centrifugation at 7000 rpm for 10 min. Cells were washed twice with 50 mM phosphate buffer (pH 7) and resuspended in liquid MM to give an $OD_{600\,nm}$ of 0.1. Degradation of TBT was tested in sterilized glass tubes containing 2 ml cell suspension ($OD_{600\,nm}$ = 0.1) and 2 mM of TBT as sole carbon source. The test tubes were incubated at 150 rpm and 30 °C. For estimation of the colony forming units (CFU), aliquots were serially diluted, 100 μl aliquots were plated on solid LB medium and the CFUs were counted after 2 days of incubation at 30 °C. Uninoculated tubes and tubes without substrate served as controls. For the TBT analysis, the sample vial was purged with nitrogen gas to achieve an inert atmosphere chamber to exclude oxygen and water. A 90 μl solution of 0.8 M *n*-hexylmagnesium bromide was added and derivatized for 30 min. The reaction was stopped by adding 1 ml of 2 M HCl and the solution was set aside for 30 min. The organic phase was separated by *n*-hexane and moisture was removed with anhydrous ammonium sulfate, 1 μl was injected into gas chromatography (Agilent 7890A GC system) with a flame ionization detector (FID). GC analyses were performed under flow of nitrogen (2 ml/min) on an HP-5 column (30 m length, 0.25 mm I.D., and 0.25 μM film thickness). The oven temperature was increased from 80 °C to 320 °C by a rate of 3 °C/min. TBT and DBT, purchased from Sigma Aldrich, (Germany) were injected into GC-FID used as standards according to their retention time and peak area.

Amplification of TBT degrading gene

Genomic DNA was extracted from strains HS1, HS2 and HS5 grown on MM + Na_2CO_3 (pH 10) and salinity 7% NaCl (w/v) in the presence of TBT as a sole carbon source. The isolated DNA was screened for the presence of *tbt* gene using specific primers designed based on the Av27-sugE gene, which was involved in TBT degradation by *Aeromonas molluscorum* Av27 (5′-ATGCCCTGGATATTGCTGCTC-3′ and 5′-GGGTGAAACCTTGGGTGTATTTG-3′)[7]. To validate that the correct PCR fragments had been amplified, the suspected band was purified, ligated into pGEM®-T Easy Vector system 1 (Promega) and cloned into *Escherichia coli* DH5α prepared as competent cells by transform aid bacterial transformation Kit and its protocol (Thermo Scientific). The transformed cells were plated on Luria-Bertani (LB) agar plates containing ampicillin (50 μg/ml) and the plate was incubated at 37 °C overnight. Positive colony plasmids were isolated using the GeneJET Plasmid Miniprep Kit (Thermo Scientific) and the obtained gene was amplified by PCR using the M13f (5′-AGCGGATAACAATTTCACACAGGA-3′) and M13r (5′-CATTTTGCTGCCGGTCA-3′). The purified DNA was sequenced and the nucleotide sequences determined in this study were compared with existing sequences in GenBank by performing a BLASTn and BLASTp search.

Extraction of mRNA, cDNA synthesis, and RT-PCR

For gene expression studies, *S. chelatiphaga* HS2 was grown on TBT (2 mM) as the sole carbon source. To assess constitutive expression, the strain was grown in parallel on fructose (2 mM). Cultures were harvested during exponential growth by centrifugation. Total RNA was isolated from 3 ml of TBT or fructose-grown cells. Harvested cells were resuspended in 100 μl water and immediately processed as previously described[34]. RNA was subsequently purified using the RNeasy kit (Qiagen), and 2 μl of the eluted RNA (60 μl) was separated in 1% (w/v) agarose

gels and stained with ethidium bromide. cDNA was synthesized from 1 μl of total RNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) from RNA templates. The kit uses RevertAid Reverse Transcriptase (RT), a recombinant M-MuLV RT which maintains activity at 42–50 °C. RiboLock RNase Inhibitor supplied with the kit effectively protects RNA templates from degradation. The reverse transcription reaction mixtures were serially diluted (3.2-fold) with nuclease-free water (Qiagen), and 1 μl of each dilution was subjected to amplification by PCR using the primer set TBTRNAF (5′-GATTCTTTGTGACGCGCCTT-3′) and TBTRNAR (5′-TCCCCATGTTATCCTCTGCC-3′) to amplify the TBTB permease gene fragment. Amplification products were separated on 1% (w/v) agarose gels and stained with ethidium bromide. Product bands were purified from agarose gels using a GeneJET PCR Purification Kit (Thermo Scientific) and sequenced to verify their identity.

Nucleotide sequence accession number

The 16S rRNA sequence for the reported strains in this study *Sphingobium* sp. HS1, *S. chelatiphaga* HS2 and *R. borbori* HS5, were deposited in the GenBank database under accession numbers KR780648, KR780649 and KR780650, respectively and the one corresponding to the TBTB permease under accession number KT590040.

Results

Haloalkaliphilic bacteria isolation and identification

In this study three moderately haloalkaliphilic bacteria capable of growing on TBT as a sole source of carbon and energy were isolated from alkaline and saline water samples. The isolates could grow at pH 7.5–10, with optimum growth at pH 9 and 1.5–10% NaCl (w/v), with optimum growth at 7% NaCl (w/v). The three isolates could not grow at pH lower than 7 or salinity lower than 1.5% (w/v). 16S rRNA sequence results suggested that the isolates were phylogenetically most closely related to *Sphingobium yanoikuyae* with 97% similarity, *S. chelatiphaga* strain LPM-5 with 100% similarity and *R. borbori* strain DN316 with 99% similarity. The isolates were then classified as *Sphingobium* sp. HS1, *S. chelatiphaga* HS2 and *R. borbori* HS5, designated as HS1, HS2 and HS5, respectively and submitted to Genbank under accession numbers KR780648, KR780649 and KR780650.

TBT sensitivity and degradation by the three identified haloalkaliphilic bacterial strains

TBT and its degradation products (DBT and MBT) were measured during the incubation of strains HS1, HS2 and HS5 for 40 h in MM containing TBT concentrations (0–3 mM), 7% NaCl (w/v) and pH 9. The negative control (the same media without inoculation) did not show TBT degradation. The results showed that strains HS1 and HS5 can grow at TBT concentrations (up to 1 mM) while HS2 strain can grow at high TBT concentrations (up to 3 mM) and its best growth was attained at 2 mM TBT. Moreover, strains HS1, HS2 and HS5 showed a decrease in TBT percentage and generation of DBT and MBT as degradation products, indicating that these bacterial strains have the ability to reduce TBT concentration. No degradation occurred at NaCl concentrations above 10% (w/v), even after a higher incubation time, indicating that biodegradation inversely correlated with salinity at higher salt concentrations (data not shown).

In strain HS1 about 41% of the original tin remained as TBT after 40 h of incubation, 30% was converted into DBT and 20% into MBT and the remaining amount of TBT was converted into inorganic tin. In the case of strain HS5, the mean percentage of TBT that remained was almost 50%, after 40 h of incubation, 25% was converted into DBT and 15% into MBT. According to the above results, strain HS1 and HS5 can be classified as moderate TBT degraders. Strain HS2 degraded more than 90% of TBT, converting 50% into DBT and 35% into MBT. The degradation proceeded according to the postulated scheme (Fig. 1).

Finally, strain HS2 showed the highest TBT degradation capability, exceeding HS1 and HS5 abilities (Fig. 2). Phylogenetic analysis of 16S rRNA gene for *S. chelatiphaga* HS2 (Fig. 3) clearly shows 100% similarity with *S. chelatiphaga* LPM-5 and 93% similarity with *Stenotrophomonas maltophilia* KB2.

The TBT degradation profile over 40 h of incubation at 30 °C, pH 9 and salinity 7% (w/v) for strain HS2 is shown in Figure 2B. Between 10 and 30 h a decrease in TBT concentration was observed, which was consistent with the rise in bacterial growth. Between 30 and 50 h, the degradation rate declined along with the available nutrients, achieving more than 95% of degradation within 50 h. The degradation of TBT contrasted with the generation of DBT and MBT as intermediate products. DBT concentration increased during incubation, reaching around 50% of the original TBT concentration (2 mM), and MBT was detected with 35% during this period of incubation. The culture medium showed the brown, yellow and white colors that coincided with the postulated products that could be inorganic tin in the form of insoluble compounds (data not shown).

Antibiotic susceptibility of *S. chelatiphaga* HS2

Antibiotic susceptibility tests of strain HS2 showed that it was resistant to ampicillin, amoxicillin-clavulanic acid 30 μg/disc as β-lactams antibiotics; however, it was susceptible to all other antibiotics tested.

Detection and expression of a gene involved in tributyltin (TBT) resistance in *S. chelatiphaga* HS2

In the present study, a gene was amplified using specific primers designed based on the TBT gene resistant sequence from TBT degrading bacterium *A. molluscorum* Av27[7]. The obtained fragment was cloned in *E. coli* DH5α using pGEM® -T Easy Vector. This clone contained pGEM® -T Easy vector with an inserted fragment of ~725 bp, whose deduced amino acid sequence has high homology (93% homology) with an arylsulfatase from *S. maltophilia* and (87% homology) with an arsenic efflux pump membrane protein from *Xanthomonas oryzea* and was identified as TBTB-permease (Fig. 4).

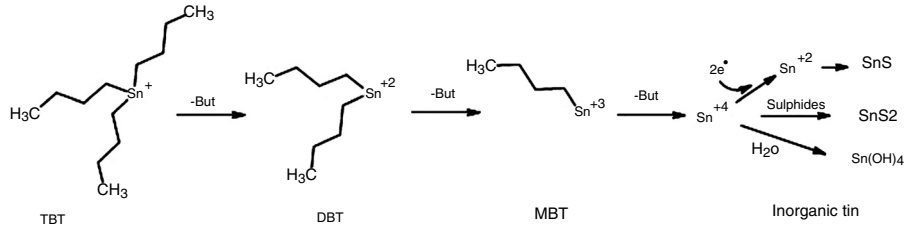

Figure 1 Proposed TBT debutylation pathway based on this study and other studies[26,35].

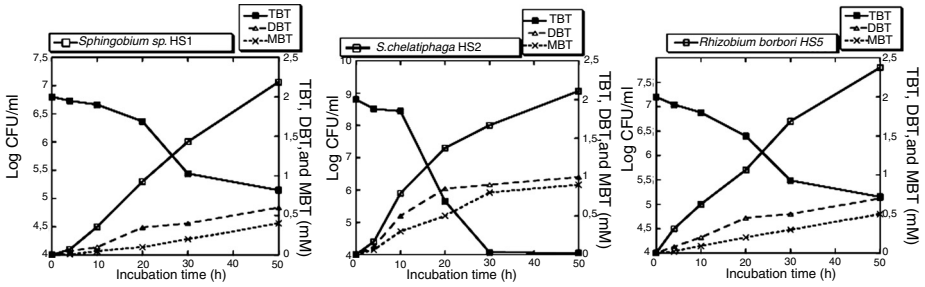

Figure 2 Growth of *Sphingobium* sp. HS1, *Stenotrophomonas chelatiphaga* HS2 and *Rhizobium borbori* HS5 on 2 mM TBT as a carbon source. Growth was monitored by following colony-forming units (CFU), TBT depletion and formation of DBT and MBT were assessed by GC.

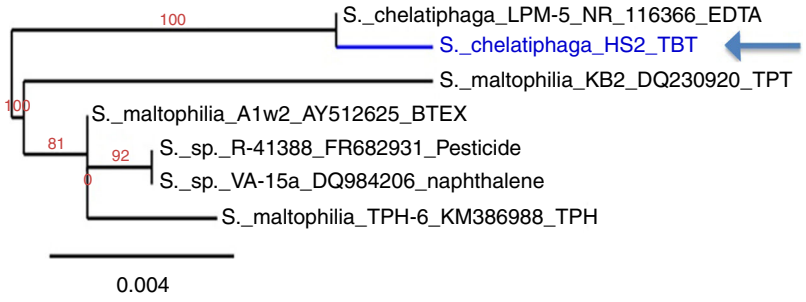

Figure 3 Phylogenetic tree based on 16S rRNA gene sequences showing the relationship with *Stenotrophomonas chelatiphaga*. HS2 strain (accession number KR780649) pointed by an arrow to other reported *Stenotrophomonas* strains. The strain followed by the accession numbers and degraded substrate; petroleum hydrocarbon (petroleum H), benzene (BTEX), toluene, ethylbenzene and xylenes, triphenyltin (TPT), ethylenediaminetetra acetic acid (EDTA), and tributyltin (TBT) degraders.

To verify whether the TBTB permease gene was expressed in response to TBT, RT-PCR experiments were performed with total RNA extracted from *S. chelatiphaga* HS2 growing on TBT and fructose. RT-PCR amplification products of the expected 400-bp size were observed with 3 pg of RNA extracted from the culture grown on TBT (Fig. 5B), whereas no product was detected with RNA extracted from the culture grown on fructose (Fig. 5A), indicating that gene transcripts are induced at least 70-fold and also specifically induced in the presence of TBT. In addition, no amplification products were observed in controls devoid of reverse transcriptase or template cDNA (Fig. 5B lane C1′ and lane C2′). Sequencing of the approximately 400-bp product confirmed that it was identical to the corresponding TBTB permease gene fragment from genomic DNA of *S. chelatiphaga* HS2. These results showed direct evidence that the TBTB-permease gene product from *S. chelatiphaga* HS2 is definitely involved in TBT resistance.

Discussion

Tributyltin (TBT) is considered a recalcitrant compound used as a biocide in the antifouling paints of boats and ship hulls, and then released slowly to the aquatic ecosystem in considerable amounts with toxic effect to a large number of aquatic organisms[3]. Despite the toxic effect of TBT on eukaryotic or prokaryotic organisms[1], some microorganisms are resistant to TBT[35]. TBT degradation by the three strains decreased as salinity increased; furthermore, no degradation occurred at salinity greater than 10% NaCl (w/v) even with a long incubation time. This observation is consistent with those noted in extremely halotolerant bacteria[33]. Although much of the research on TBT degradation in terrestrial and marine environments has been studied extensively under oxic conditions[7,24], little is known about TBT degradation in hypersaline environments. Bioremediation of polluted hypersaline wastewaters and other

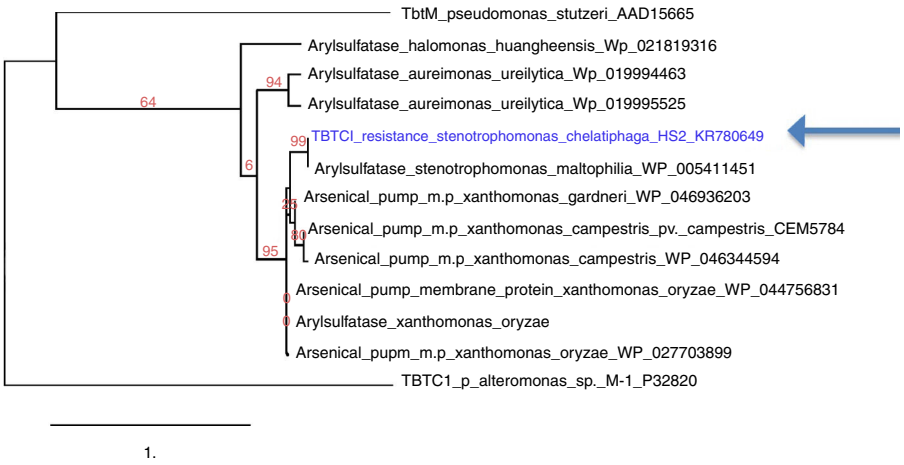

Figure 4 Phylogenetic tree showing the relatedness on the basis of TBTB permease. The dendrogram was calculated using Phylogeny.fr based on protein sequence alignments. TBTB permease proteins from *Stenotrophomonas chelatiphaga* HS2 are pointed by an arrow. The scale bar corresponds to an estimated evolutionary distance of 1 amino acid substitution per site.

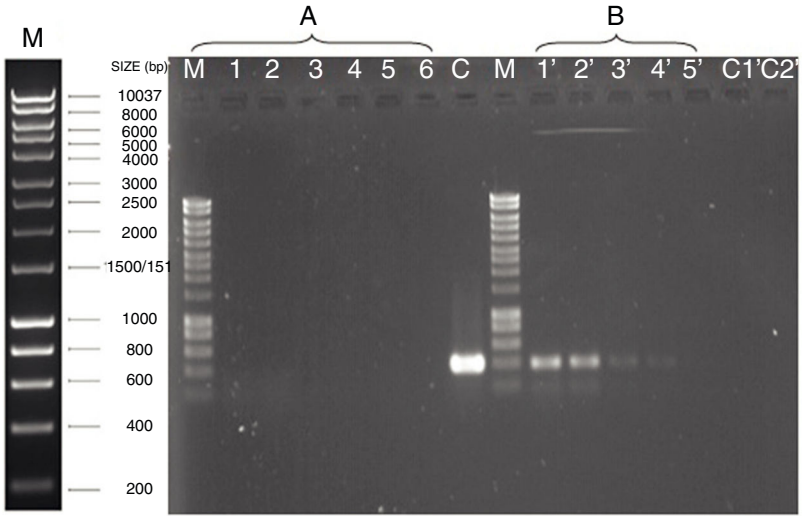

Figure 5 RT-PCR amplification of TBTB permease mRNA from *S. chelatiphaga* HS2 grown on fructose (A) or TBT (B). M lanes contain the molecular size marker Hyperladder 1 (Bioline). cDNA generated from 1 μg template RNA was serially diluted (3.2-fold) with nuclease-free water, and 1 μl of each dilution was subjected to amplification by PCR (lanes 1 to 6) for cDNA from fructose and (lanes 1′ to 5′) for cDNA from TBT. Negative controls included undiluted RT-PCR mixtures devoid of reverse transcriptase (lane C1′) or template cDNA (lane C2′). PCR mixtures containing 1 ng of genomic DNA as a template were used as positive control (lane C) in the same experiment.

environments with nonhalophilic microorganisms is difficult because salt inhibits their growth and the degradation of organic compounds[30].

The HS2 strain had higher ability to degrade TBT than HS1 and HS5 and showed 99% similarity with *S. chelatiphaga* LPM-5, the most closely related organism, which is an aerobic EDTA-degrading bacterium[25] and 93% with *S. maltophilia* KB2, which is a triphenyltin-degrading bacterium[16]. The HS2 strain has the ability to use TBT as carbon source in a mineral salt medium with 7% salinity and pH 9. Furthermore, TBT degraders *A. molluscorum* Av27 and *Aeromonas veronii* Av27, had also the capacity to use TBT as carbon source in a mineral salt medium[5] and showed optimum growth with salinity 4% (w/v) and pH 9[6].

Antibiotic test results confirm that the HS2 strain does not exhibit potential risk of toxicity and could be used for TBT bioremediation. The resistance of common antibiotics could be toxic and affect human health[14]. Moreover, the rapid appearance of antibiotic resistance among environmental bacteria as a result of horizontal gene transfer through plasmids, transposons and integrons is a known process[17].

To our knowledge, this is the first reported halophilic and alkaliphilic bacteria from the Mediterranean Sea that has the ability to degrade TBT under both halophilic and alkaliphilic conditions. The results suggested that *S. chelatiphaga* HS2 degraded TBT into DBT and MBT as less toxic compounds and this haloalkaliphilic strain could be applied in the remediation of TBT contaminants or natural attenuation

of extreme alkaline and hypersaline TBT contaminated sites.

TBT degraders received great attention, especially genes, pathways, and mechanisms of their degradation; however, until now the cellular and molecular mechanisms involved in TBT resistance are not clear and they are supposed to be different either among bacterial genera or even the same species or strain[7,24]. Furthermore, it is not known whether haloalkaliphilic bacteria degrade TBT using novel genes and pathways compared to those used by non haloalkaliphiles. HS2 harbor the TBTB-permease gene which is a single, non-redundant, protein sequence, where anion permease like ArsB has been shown to export arsenate and antimonite in eubacteria and archaea. A typical ArsB permease contains 8–13 transmembrane helices and can function as a chemiosmotic transporter or as a channel-forming subunit of an ATP-driven anion pump. ArsB proteins belong to the ArsB/NhaD superfamily of permeases that translocate sodium, arsenate, sulfate, and organic anions across biological membranes[31]. Most metal resistance systems included tin accomplished by ATPase or a single polypeptide like (ArsB) efflux systems[10]. These systems could explain the ability of TBT resistant bacteria, either by formation of DBT and MBT as a result of removing butyl groups from the tin atom[5], or pumping out TBT through a multidrug efflux pump[24] or TBT bioaccumulation in the bacterial cells[11].

This work presented the important role of TBT-degrading bacteria isolated from the Mediterranean Sea at a TBT-contaminated site, where three halophilic and alkaliphilic bacterial strains *Sphingobium* sp. HS1, *S. chelatiphaga* HS2 and *R. borbori* HS5 were isolated, having the ability to use TBT as a carbon source in minimal media with 7% (w/v) salinity and pH 9.

S. chelatiphaga HS2 was the most efficient TBT degrader and had the ability to transform most of the TBT into DBT and MBT. In TBT-resistant bacterium *S. chelatiphaga* HS2, a new gene was identified as TBTB-permease, which had 93% similarity with aryl sulfatase in *S. maltophilia* and 87% similarity with arsenic efflux pump membrane protein in *X. oryzea*. The TBTB-permease gene was only expressed in the presence of TBT. The three novel haloalkaliphilic strains and their TBT degradation ability have never been reported previously. Moreover, HS2 strain is an effective candidate bacterium for TBT bioremediation in halophilic and alkaliphilic TBT contaminated sites.

Conflict of interest

The authors declare that they have no conflicts of interest.

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