Int. J. Environ. Sci. Technol. (2016) 13:571–580
DOI 10.1007/s13762-015-0897-8

ORIGINAL PAPER

Biodegradation of malodorous mercaptans by a novel *Staphylococcus capitis* strain isolated from gas-washing wastewaters of the Tunisian Chemical Group

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Received: 4 April 2015 / Revised: 3 September 2015 / Accepted: 22 September 2015 / Published online: 8 October 2015

Abstract There are increasing concerns over the harmful effects of the phosphate industry on human health and quality of life worldwide, including the Tunisian Chemical Group (GCT) in Sfax, which generates various malodorous gas fractions, such as hydrogen sulfide (H_2S) and fetid mercaptans, causing nuisance to employees and local residents. Accordingly, the present study aimed to investigate the ability of an adapted microbial consortium isolated from the gas-washing wastewaters (GWWs) generated from GCT to degrade hazardous and malodorous mercaptans. A novel mesophilic bacterial strain (SH6), which was noted to display particularly high mercaptan degradation potential, was isolated from the adapted consortium growing on those GWWs and several malodorous mercaptans after enrichment on 1-dodecanethiol. The results from 16 rRNA gene sequencing and identity analysis revealed that the SH6 isolate belonged to *Staphylococcus* genus, with a high sequence similarity to *Staphylococcus capitis* (99.7 %). The SH6 strain was able to completely degrade 1-dodecanethiol, used as the sole carbon and energy source, after 72 h of incubation at 37 °C and 180 rpm. A decrease in the surface tension of cell-free culture supernatants was observed during the oxidation of dodecanethiol, suggesting the production of surface-active compounds. The stain was also able to grow on other mercaptans, such as 1,8-octanedithiol and 2,3-butanedithiol, which further supports its potential candidacy for application in the bioremediation of mercaptan-contaminated sites. Overall, the findings of the present study indicate that the SH6 strain might offer promising opportunities for the development of more adapted, efficient and cost-effective bio-disodoration strategies.

Keywords *Staphylococcus* · Biodegradation · Gas-washing wastewaters · Fetid mercaptans · Phosphate fertilizer plant

Introduction

The phosphate industry has been growing at an unprecedented rate during the last few decades. This increase has been particularly driven by the rising demand for phosphate fertilizers due to the increasing demands for food supplies to nurture a growing world population and, more recently, for biofuels as renewable alternatives to fossil fuels. Although this growing industry is a major contributor to the economy of several Mediterranean countries, including Tunisia, it is associated with obnoxious solid and volatile effluents of concern for workers and the public. The Tunisian Chemical Group (GCT) of Sfax, a public company whose activity is the transformation of phosphate into solid or liquid fertilizers, has often been criticized for releasing unpleasant odors, including mercaptans (RSH) and hydrogen sulfide (H_2S), which causes nuisance to workers and local residents. These volatile organic-sulfuric compounds (VOSCs) pose serious problems to human health and the environment (Shults et al. 1970; Munday 1989; Burgess et al. 2001; Setoguchi et al. 2002). These toxic compounds can become highly disagreeable even at concentrations below their odor perception threshold

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(Tangerman 2009). Moreover, VOSCs are considered as both major sources of fetid odors even in domestic wastewater and as organic micropollutants in the environment (Khoroshko et al. 2006; Sun et al. 2014).

With these concerns in minds, recent research has become increasingly interested in the search for viable strategies to manage and control the malodorous emissions from phosphate processing and manufacturing plants (Mohanrao 1973; Ahmad et al. 2000). The GCT company has, for instance, launched a gas-washing program in its two production units, namely the phosphoric acid (PA) and triple superphosphate (TSP) units, in an attempt to reduce or remove fetid sulfur-containing compounds, such as hydrogen sulfide (H_2S) and mercaptans (RSH), by washing gas with water.

Due to their partial solubility at lower pHs, the chemical removal of fetid sulfur-containing compounds requires large amounts of sodium hydroxide and water (Iliuta and Larachi 2007). Several studies have reported that, during bio-filtration methods, odorous contaminants are adsorbed from the air into the water present in the organic bed [i.e., bioactive layer (biofilm)] where they can be aerobically degraded to various end-products or incorporated into biomass (McNevin and Barford 2000). These findings provide support for the strategy of targeting mercaptans at their liquid state. Accordingly, there has been growing interest in the search for efficient alternative biological strategies to substitute the conventional chemical methods currently employed for the removal of fetid sulfur-containing compounds from phosphate fertilizer wastewaters.

We have previously reported on the isolation of a *Bacillus licheniformis* strain CAN55 from GWWs with promising abilities to oxidize heptanethiol, decanethiol, dodecanethiol and cyclohexylmercaptan as the sole carbon and energy sources at 55 °C (Chebbi et al. 2014). We have also isolated a *Brevibacillus agri* strain CAT37 that was able to degrade only heptanethiol, decanethiol and dodecanethiol at 37 °C (Chebbi et al. 2015). The literature also presents other dimethyl sulfide (DMS) oxidizing strains, including *Thiobacillus* sp. strain MS1 (Sivela and Sundman 1975), *Thiobacillus thioparus* strain TK-m (Kanagawa and Mikami 1989; Treto Fernandez et al. 2013), *Hyphomicrobium* sp. strain VS (Fernández et al. 2013) and *Hyphomicrobium* sp. strain E (Suylen and Kuenen 1986). Another strain, *Pseudomonas* sp. strain WL2, was reported to metabolize ethyl mercaptan (ET) (Zhang et al. 2013). *Paenibacillus polymyxa* strain CZ05 has also been described as a methanethiol-degrader (MT) under high concentration (60 ppm) using the bio-trickling filters (99.5 %) (Wang et al. 2014).

Despite the large flow of data on the biodegradation of *n*-alkanes, little work has for been performed on the use of *n*-alkanethiols as sole carbon and energy sources by bacteria or fungi. The isolation of efficient mercaptan-degrading microorganisms is of great interest for performing efficient and specific biological methods to eliminate mercaptans from industrial emissions and wastewaters. Accordingly, the present study was undertaken to investigate the ability of an adapted microbial consortium isolated from the gas-washing wastewaters (GWWs) generated from GCT to degrade hazardous and malodorous mercaptans. A novel mesophilic bacterial strain, designated as SH6, was isolated from the adapted consortium growing on those GWWs and several malodorous mercaptans after enrichment on 1-dodecanethiol and was noted to display particularly a high mercaptan degradation potential.

Date and location of the research

This work was performed at the Laboratory of Environmental Bioprocesses, Centre of Biotechnology of Sfax, Tunisia, between October 2010 and April 2015.

Materials and methods

Sampling

Samples of gas-washing wastewaters (GWWs) were collected from the Sfax phosphate plant of the Tunisian Chemical Group (GCT), under aseptic conditions, transferred into sterile bottles and kept in the darkness at 4 °C, room temperature and 60 °C.

Chemicals

Mercaptans, including 1-heptanethiol, 1-octanethiol, 1-decanethiol, 1-undecanethiol, 1-dodecanethiol, 1-tetradecanethiol, 1-pentadecanethiol, 2,3-butanedithiol, 1,8-octanedithiol, cyclohexylmercaptan and 2-phenylethanethiol, were purchased from Sigma-Aldrich.

Culture media

The basal medium used in the present study consisted of 0.5 g KH_2PO_4, 0.4 g NH_4Cl, 0.33 g $MgCl_2{\cdot}6\ H_2O$, 0.05 g $CaCl_2$, 2 H_2O, 1 g NaCl and 1 mL trace-element solution (Widdel and Pfennig 1981) per liter of distilled water. The pH was adjusted to 7 with 10 M NaOH solution. The medium was sterilized by autoclaving at 121 °C for 20 min. The Luria–Bertani (LB) medium employed for growing and maintaining the bacterial cultures contained 10 g peptone, 5 g yeast extract and 10 g NaCl per liter of distilled water.

Procedure of enrichments and isolation of mercaptan-degrading bacteria

Due to the low microbial diversity of GWWs, which are characterized by a high temperature and acidic pH, the first aerobic enrichment cultures were performed in the presence of LB medium (50 mL), with the addition of 5 mM glucose and 20 % (v/v) of GWWs used as inoculum at 37 and 55 °C, pH 4 and 7, and 180 rpm. A positive culture (50 mL) containing 30 mL GWWs (a mixture of GWWs from the units of PA and TSP, v/v), yeast extract (0.2 g L^{-1}) and peptone (0.6 g L^{-1}), at pH 7 and 180 rpm was used as inoculum for the screening of aerobic bacterial strains capable of degrading mercaptans. The inoculum was used at 5 % (v/v) to inoculate 50 mL of basal medium containing 3 mM of 1-dodecanethiol as carbon and energy source at 37 °C and 180 rpm. The positive enrichment culture was sub-cultured under the same conditions at least five times. The microbial growth on mercaptan was monitored by measuring OD at 600 nm and microscopic observation. Aliquots (100 µL) of 10^{-1} to 10^{-10} dilutions were taken from the positive enrichment culture on 1-dodecanethiol and plated onto agar basal medium containing 3 mM of 1-dodecanethiol as carbon and energy source, without the addition of yeast extract. The plates were incubated at 37 °C under aerobic conditions for 3 days until colony formation. Seven colonies were picked up and serially diluted in the fresh basal medium containing the same mercaptan. Individual colonies were purified by repeated streaking on mercaptan-agar basal medium. The purity, shape and motility of the isolates were examined using a phase-contrast light microscope (Olympus BX51). Strain SH6 was noted to exhibit maximum growth on dodecanethiol and attractive biodegradation ability in the basal medium without the addition of yeast extract. The SH6 strain was, therefore, selected and maintained for further experimental assays.

Growth of consortium CSH37 on GWWs

The growth of consortium CSH37 was monitored in culture media (50 mL) containing various volumes of GWWs (5, 10, 20, 30 and 36 mL), 0.5 g L^{-1} yeast extract and 1.5 g L^{-1} peptone at 37 °C and 180 rpm. Total carbon removal was examined for cultures containing 30 mL of GWWs incubated for 6 days with consortium CSH37 at 5 % (v/v), pH 7, 37 °C and 180 rpm. The cultures (0 and 6 days) were centrifuged for 20 min at 4000 rpm, and the supernatants were filtered (0.45 µm) and then analyzed.

Characterization of strain SH6

The Gram reaction was determined using a Gram stain Kit (BioMérieux, France) in accordance with the manufacturer's instructions. Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by the measurement of bubble production following the application of 3 % (v/v) hydrogen-peroxide solution. Optimal growth temperature was determined by culture incubation at temperature values ranging between 4 and 65 °C. The inoculation proportion was 5 % (v/v). Various amounts of NaCl (from 0 to 15 %, w/v) were directly weighed in flasks prior to dispersing 50 mL LB medium to determine the NaCl concentration desired for growth. The optimum pH required for growth was studied through adjusting the pH of the medium to a range between 3 and 11 by the addition of 5 M HCl or 10 M NaOH. Optical density at 600 nm was monitored by growth measurements, and data from the mid-log phase growth of the strain were selected for calculating the specific growth rates. A heat resistance test was performed by incubating the cells at 80, 90 and 100 °C for 10 min. The cultures were then cooled quickly to ambient temperature and inoculated into fresh LB media. Growth was recorded after 24 h incubation at 37 °C and 180 rpm. Other phenotypic characteristics were screened by test strips of the API system (BioMérieux, France). The API Staph kit was used as described by Kloos and Wolfshohl (1982).

In order to investigate the mercaptan-degrading ability of the SH6 strain, several substrates, including 1-heptanethiol, 1-octanethiol, 1-decanethiol, 1-undecanethiol, 1-dodecanethiol, 1-tetradecanethiol, 1-pentadecanethiol, 2,3-butanedithiol, 1,8-octanedithiol, cyclohexylmercaptan and 2-phenylethanethiol, were added, at a final concentration of 3 mM, into flasks containing 50 mL basal medium without the addition of yeast extract. An increase in the OD_{600nm} value obtained for substrate-containing cultures, as compared to control flasks lacking substrates, was considered as positive growth.

16S rRNA gene sequence determination and phylogenetic analysis

The DNA was extracted from strain SH6 as described by Redburn and Patel (1993). The 16S rRNA gene of strain SH6 was amplified by PCR using a Stratagene PCR system (Robocycler gradient 96) with GoTaq DNA polymerase (Promega, WI, USA) as previously described elsewhere (Chamkha et al. 2002). The universal primers Fd1 and Rd1 (Fd1, 5′-AGAGTTTGATCCTGGCTCAG-3′; Rd1, 5′-AAGGAGGTGATCCAGCC-3′) (Weisburg et al. 1991) were used to obtain a PCR product of approximately 1.5 kb corresponding to base position 8-1542, based on *Escherichia coli* numbering of the 16S rRNA gene (Winker and Woese 1991). Positions of sequence and alignment ambiguity were omitted, and pairwise evolutionary distances based on 1452 unambiguous nucleotides were calculated using the method of Jukes and Cantor

(Jukes and Cantor 1969). A dendrogram was constructed using the neighbor-joining method (Saitou and Nei 1987) with the Molecular Evolutionary Genetic Analysis (MEGA) program version 6 (Tamura et al. 2013). Confidence in the dendrogram topology was determined using 100-bootstrapped trees (Felsenstein 1985).

Physicochemical analyses and analytical methods

The pH and electrical conductivity (EC) of the GWWs samples were determined using a pH meter model Istek-NeoMet and a conductivimeter model CONSORT C831, respectively. Soluble chemical oxygen demand (COD) was determined according to the method of Knechtel (1978). The five-day biological oxygen demand (BOD_5) was determined by the manometric method using a respirometer (OxiTop® Box) a described by Zayen et al. (2010). Total organic carbon was determined after dry combustion using a TOC analyzer $-V_{CPH}$, SHIMADZU (Gouider et al. 2010). The phosphorus concentration in aqueous media was determined according to the vanado-molybdo-phosphoric acid spectrometry method (Gouider et al. 2010). Total Kjeldahl nitrogen content was determined as described by Kjeldahl (1883), and the hydrogen sulfide concentration was calculated according to Cord-Ruwisch and Widdel (1986). The concentration of metal ions was estimated by an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200; PerkinElmer Instruments LLC) (Hamza and Sayadi 2015).

For gas chromatography (GC) analysis, cultures of SH6 (50 mL) containing 3 mM mercaptan and an abiotic control were extracted three times with dichloromethane (DCM) (Corrêa and Arbilla 2008). The organic fraction was then evaporated to dryness and dissolved in 1 mL of DCM. One microliter of the solution obtained was analyzed by GC using a SHIMADZU GC-17A apparatus equipped with a capillary Supelco column (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm). The carrier gas was helium used at a flow rate of 1 mL min^{-1}. The temperature of the injector was fixed at 250 °C. The temperature was first set at 100 °C for 2 min and then increased to 250 °C at 15 °C min^{-1}.

The surface tension was measured by a Gibertini Tensiometer (TSD 132389, Milan, Italy) as previously described in the literature (Mnif et al. 2011). The values given represent the mean of three replicates (±standard deviation, SD).

Results and discussion

Characteristics of GWWs

The physicochemical parameters of the GWWs collected from GCT are summarized in Table 1. The wastewater samples were characterized by an acidic pH (around 2), high temperature (about 60 °C), low organic matter and high contents of phosphorus, due to the GCT processes which produced phosphoric acid and triple superphosphate. These characteristics can play an important role in impeding microbial growth and activity. The results recorded in terms of COD/BOD_5 values (about 2.8) indicated that the two GWWs samples contained relatively biodegradable organic pollutants (Tebbutt 1997). The literature indicates that wastewaters containing biodegradable components with COD/BOD_5 values ranging between 1 and 3 can be easily treated by biological processes (Tebbutt 1997; Alvarez-Vazquez et al. 2004). In the gas emissions and wastewaters of both GCT production units, the total mercaptan content ranged between 50 and 100 ppm and did not exceed 50 ppm, respectively (Table 1). No hydrogen sulfide (H_2S) content was, however, detected in the wastewater samples. In fact, the acidic character and high temperature of the wastewater samples could presumably have inhibited the solubility of hydrogen sulfide (Millero 1986). The results also revealed that iron was present in comparatively higher concentration than other heavy metals such as copper, nickel and manganese (Table 1). This could presumably be attributed to the use of gypsum, $Ca_3(PO_4)_2$ CaF_2, in the phosphoric acid production process. Overall, the results showed the GWWs samples generated from the phosphoric acid and triple superphosphate units exhibited several similarities. Accordingly, and in order to increase the chances of obtaining a wider range of mercaptan-degrading bacteria, both GWWs samples were equally mixed (v/v) during the enrichment procedures.

Enrichment cultures and isolation of mercaptan-degrading bacteria

Several thermophilic (55 °C) and mesophilic (37 °C) microbial enrichment cultures were performed at pH 4 and pH 7 and monitored at aerobic conditions to adapt the consortia growing on the GWWs samples. The results revealed the absence of microbial growth in the acidic enrichment cultures. Microbial growth remained mostly low at 55 °C and pH 7 °C. The mesophilic enrichment culture showed better microbial growth results, with a bacterial population generally having bacilli and cocci shapes.

The strategy followed in the present study was to increase the volume of the wastewater and decrease the amounts of other nutriments in the culture media so as to ensure the optimal adaptation of microorganisms to those GWWs samples and broaden the chances to screen a wide range of mercaptan-degrading bacteria. Microbial growth was monitored in the presence of various volumes of GWWs by measuring OD_{600nm} at different culture time

Table 1 Physicochemical characteristics of gas-washing wastewaters

Characteristics	Unit of phosphoric acid	Unit of TSP
pH (25 °C)	2.18	1.30
Electrical conductivity (mS cm^{-1})	8.93	39.53
Salinity (g L^{-1})	6.76	33.60
COD (mg L^{-1})	170	140
BOD_5 (mg L^{-1})	60	50
COD/BOD_5	2.83	2.8
TOC		
Total carbon (mg L^{-1})	104.30	77.9
Organic carbon (mg L^{-1})	103.5	76.00
Inorganic carbon (mg L^{-1})	0.80	1.90
TKN (mg L^{-1})	29.40	25.20
Phosphorus (PO_4) (mg L^{-1})	100.66	74.34
Phosphorus pentoxide (P_2O_5)	2.28	1.69
Total mercaptans in wastewaters (ppm)[a]	<50	<50
Total mercaptans in gas emissions (ppm)[a]	50–100	50–100
H_2S (mM)	0	0
Ions and heavy metals (mg L^{-1})		
Sodium (Na)	493.1	480.2
Calcium (Ca)	234	177.2
Magnesium (Mg)	87.6	77.5
Potassium (K)	35.8	33.1
Iron (Fe)	5.213	3.293
Copper (Cu)	0.077	0.221
Nickel (Ni)	0.001	0.006
Manganese (Mn)	0.091	0.038
Lead (Pb)	0	0

TSP triple superphosphate, *TKN* total Kjeldahl nitrogen

[a] Data were obtained from the GCT (Groupe Chimique Tunisien), Sfax, Tunisia

points (Fig. 1). The GWWs were used as carbon and energy sources. The results revealed that growth occurred even in the presence of 36 mL of wastewaters (72 %, v/v). Optimum growth was observed with the addition of 10 mL of wastewaters (20 %, v/v), with an OD_{600nm} value of about 0.6 being recorded after 3 days of incubation at 37 °C. Appropriate growth conditions were particularly observed with the culture medium containing 30 mL GWWs (60 %, v/v), which yielded an OD_{600nm} value of about 0.4 after 3 days of incubation. The latter culture medium, designated as consortium CSH37, was selected and maintained for further investigation (Fig. 1).

The total organic carbon (TOC) was determined for cultures growing on 60 % (v/v) GWWs for 6 days at 37 °C, pH 7 and 180 rpm. After 6 days of incubation, the TOC values decreased by almost 34 % from 2577 to 1691 mg L^{-1}. This could presumably be attributed to the oxidation of organic matter by the CSH37 bacterial

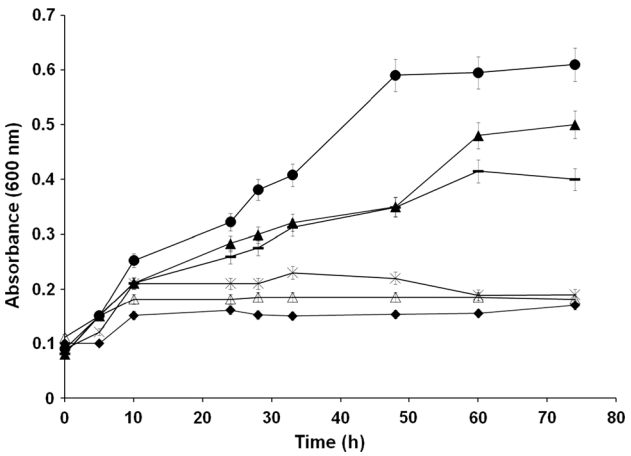

Fig. 1 Growth of the microbial consortium CSH37 in the presence of various amounts of gas-washing wastewaters at 37 °C and 180 rpm; (*filled diamond*) 0 mL; (*times*) 5 mL; (*filled circle*) 10 mL; (*filled triangle*) 20 mL; (*dash*) 30 mL; (*open triangle*) 36 mL

consortium and its potential to grow on the sulfur-containing compounds in those wastewaters.

A stable microbial population was noted to develop after several dilutions and sub-culturing in the basal medium containing 3 mM of dodecanethiol used as the sole carbon and energy source, with the morphological dominance of a non-motile, spherically shaped and non-spore-forming bacterium. This enrichment culture was serially diluted and used to inoculate Petri dishes containing 3 mM dodecanethiol. Seven colonies were picked, and the pure SH6 strain was selected for further characterization on the basis of its capacity to grow on 3 mM of 1-dodecanethiol in liquid and solid media, without yeast extract added.

Characterization of strain SH6

The results revealed that the SH6 strain was aerobic, Gram-positive, coccus, non-motile and non-spore-forming bacterium that occurred individually, in pairs and in tetrads. The findings indicated the occurrence of catalase production, but absence of oxidase formation. Agar colonies formed after overnight culture. They were generally circular, slightly convex, smooth, opaque, glossy and with a diameter of 1–2 mm. Strain SH6 was able to grow over a temperature range of 25–55 °C, with optimum growth at 37 °C. No growth was, however, noted to occur at 4 and 65 °C. The NaCl concentration required for growth was ranged between 0 and 120 g L^{-1}, with an optimum at 10 g L^{-1}. The initial pH range required for growth was pH 5–10, with an optimum at pH 7. No growth occurred at pH 3.3 or 11. The major phenotypic characteristics obtained from the API Staph are presented in Table 2. The 16S rRNA gene sequence of strain SH6, comprising 1452 nucleotides, was determined and deposited in the GenBank nucleotide database under accession number KP455742. Phylogenetic analysis indicated that strain SH6 was most closely related to members of the genus *Staphylococcus*, particularly species of *Staphylococcus capitis* ATCC 27840^T (Bannerman and Kloos 1991), with a sequence similarity of 99.72 % (Fig. 2). The differential phenotypic characteristics of strain SH6 and its closest neighbors in the genus *Staphylococcus* are illustrated in Table 2.

Table 2 Differential phenotypic characteristics of the strain SH6 and other related type strains of the genus *Staphylococcus*

Characteristics	Strain SH6	*Staphylococcus capitis* (Kloos and Schleifer 1975)	*Staphylococcus epidermidis* (Hugh and Ellis 1968)	*Staphylococcus aureus* (La Fuente et al. 1985)
Similarity 16S rRNA with SH6 (%)	100	99.72	99.23	98.34
Gram	+	+	+	+
Oxidase	−	−	v	−
Catalase	+	+	+	+
Non-motile	+	+	+	+
NaCl growth range (g L^{-1})	0–120	0–100	0–75	0–100
Growth at 15 % NaCl	+	+	−	+
Temperature growth range (°C)	25–55	18–45	15–45	10–45
Growth at 15 °C	ND	−	−	+
Growth at 45 °C	+	+	+	+
pH growth range	5–10	ND	ND	4.2–9.3
Urease	−	−	+	+/−
Arginine dihydrolase	+	+	+	+
Assimilation of				
D-Glucose	+	+	+	+
D-Fructose	+	+	+	+
D-Mannose	+	+	+	+
Maltose	+	−	+	+
Lactose	−	−	+	+
D-Trehalose	−	−	−	+
D-Mannitol	−	+/−	−	+
Xylitol	−	−	−	−
Raffinose	−	−	−	−
Xylose	−	−	−	−
Saccharose	+	+	+	+

ND not determined, *v* variable

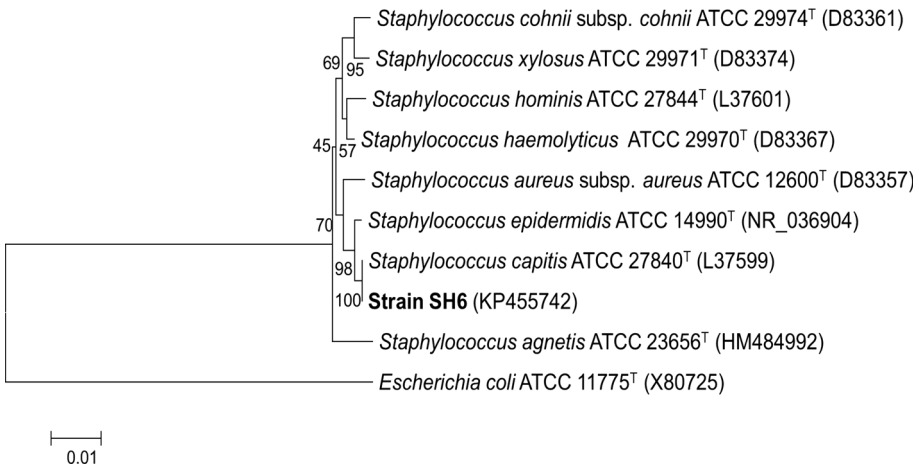

Fig. 2 Phylogenetic tree based on 1452 16S rRNA gene sequence showing the position of strain SH6 among related species of the genus *Staphylococcus*. Genbank accession members are given in *parentheses*. *Escherichia coli* is used as an out group. *Numbers* at the *nodes* indicate the percentages of bootstrap samplings. *Bar* 1 substitution per 100 nucleotides

Biodegradation of dodecanethiol by strain SH6

Strain SH6 was grown aerobically at 37 °C on 3 mM dodecanethiol as the sole carbon and energy source. Aliquots were withdrawn from the culture medium at different time intervals and submitted to GC analysis. The results displayed in Fig. 3 indicate a change in biomass and dodecanethiol content during the cultivation of strain SH6. The dodecanethiol degradation rate showed an increase during the log phase of growth, reaching approximately 82 % after 9 h of incubation (Fig. 3). Dodecanethiol was completely removed after 72 h of incubation (Fig. 3). During the dodecanethiol biodegradation process, and owing to the increased cell density, the OD_{600nm} value increased to approximately 1, thus indicating that the SH6 strain utilized dodecanethiol as a carbon source for growth. The effect of dodecanethiol concentration on the growth of strain SH6 was also studied by measuring the specific growth at a concentration range between 0 and 30 mM (Fig. 4). Strain SH6 was able to grow over a broad range of dodecanethiol concentration, ranging between 1 and 10 mM, with optimum growth being recorded in the presence of 3 mM dodecanethiol (Fig. 4). At 20 mM, the growth was completely inhibited, suggesting the toxicity of this compound (Fig. 4).

Several studies have previously highlighted the promising potential of using *Staphylococcus* spp. in biodegradation and bioremediation processes. The *Staphylococcus auriculans* strain DBF63 was reported for its ability to grow on dibenzofuran ($C_{12}H_8O$) or fluorene ($C_{13}H_{10}$) as the sole carbon and energy source (Monna et al. 1993). The *Staphylococcus* sp. strain PN/Y, isolated from petroleum-contaminated soils, was also described to have the ability to grow on phenanthrene as the sole carbon and energy source (Mallick et al. 2007; Mallick and Dutta 2008). Moreover, *Staphylococcus arlettae* strain VN-11, which was isolated from an activated sludge of a textile industry, was reported to decolorize four different azo dyes under microaerophilic conditions (Elisangela et al. 2009). Likewise, three isolates belonging to *Staphylococcus*

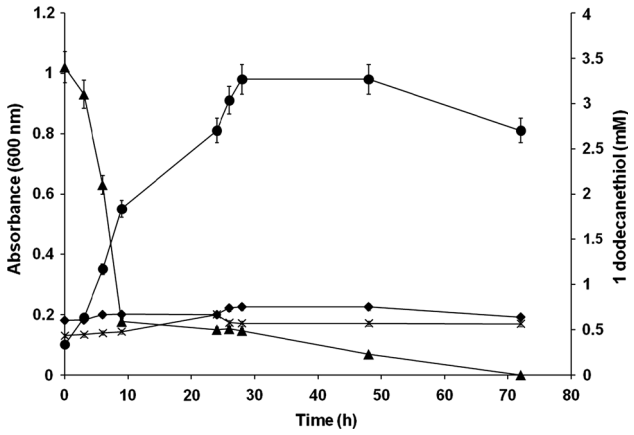

Fig. 3 Aerobic growth of strain SH6 in basal medium containing 3 mM dodecanethiol at 37 °C and 180 rpm. (*Filled circle*) absorbance at 600 nm; (*filled triangle*) concentration of 1-dodecanethiol; (*times*) biological control; (*filled diamond*) abiotic control of 1-dodecanethiol

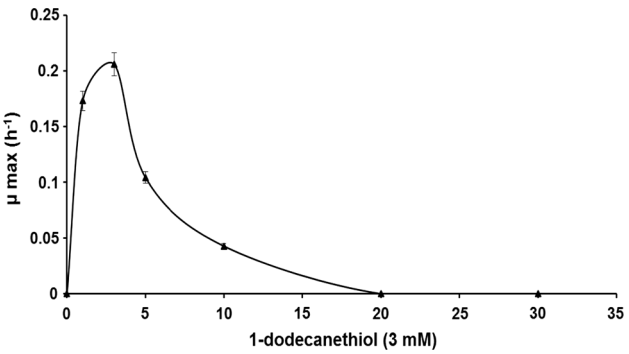

Fig. 4 Effect of 1-dodecanethiol concentration on growth of strain SH6 at 37 °C and 180 rpm

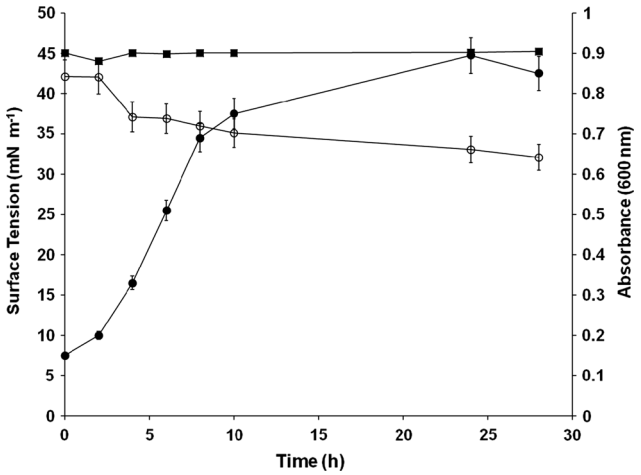

Fig. 5 Surface tension variation during the growth of the strain SH6 on the basal medium containing 3 mM of 1-dodecanethiol at 37 °C and 180 rpm. (*Open circle*) surface tension of the culture SH6; (*filled square*) surface tension of the abiotic control; (*filled circle*) absorbance at 600 nm of culture SH6

saprophyticus, Staphylococcus epidermidis and *Staphylococcus capitis* were identified from selected consortia growing on several polyaromatic hydrocarbons (PAHs) as sole carbon sources (Bidaud and Tran-Minh 1998). Some strains of the genus *Staphylococcus* used linear *n*-alkanes as a sole source of carbon and energy (Walker et al. 1976; Pineda-Flores et al. 2004). In addition, *Staphylococcus* sp. strains 36-GP and 64-GP were identified in a microbial consortium capable of mineralizing asphaltenes (Pineda-Flores et al. 2004). The present study is the first to report on a *Staphylococcus capitis* strain capable of using mercaptan as a substrate for growth in basal medium, which was previously isolated from several hydrocarbon-contaminated sites.

Furthermore, the results revealed that the SH6 isolate was able to reduce the surface tension of the cell-free medium from 42.2 to 32.2 mN m^{-1} during growth on dodecanethiol after 28 h of incubation at 37 °C and 180 rpm (Fig. 5). This suggested that strain SH6 was able to produce biosurfactant(s), thus increasing the solubility and bioavailability of mercaptans. Similarly, *Bacillus licheniformis* strain CAN55 was previously reported to reduce the surface tension during growth on 1-decanethiol and 1-dodecanethiol used as sole carbon and energy sources at 55 °C (Chebbi et al. 2014). These molecules could, therefore, have enhanced the dispersion and biodegradation of mercaptans and facilitated their assimilation through microbial cells. In fact, *Staphylococcus* sp. strain 1E was previously reported to show a relative potential to degrade hydrocarbons and, simultaneously, an interesting ability to produce surface-active compounds (Eddouaouda et al. 2012). The biosurfactant production by a *Staphylococcus hominis* strain, using weathered diesel as a low-cost raw material, was also reported (Mariano et al. 2008). Little work was, however, performed to investigate the biosurfactant(s) produced by the genus of *Staphylococcus* as well as other genera such as *Bacillus and Pseudomonas.* To the authors' knowledge, the present study is the first to report on the secretion of biosurfactants by *Staphylococcus capitis* species growing on mercaptan.

Biodegradative potential on other mercaptans

The ability of strain SH6 to degrade other mercaptans without the addition of yeast extract was also investigated. Growth was monitored by OD_{600nm} measurements and microscopic observations. The SH6 strain was able to grow on 1-dodecanethiol ($C_{12}H_{25}SH$), 1,8-octanedithiol ($C_8H_{16}(SH)_2$) and 2,3-butanedithiol ($C_4H_8(SH)_2$), reaching 1,0.7 and 0.4 after 24 h of incubation at 37 °C and 180 rpm, respectively. The strain was, however, not able to grow on 1-heptanethiol, 1-decanethiol, 1-undecanethiol, 1-tetradecanethiol, 1-pentadecanethiol, cyclohexylmercaptan and 2-phenylethanethiol, indicating affinity with specific mercaptan structures. These results are in agreement with the study performed with *Bacillus licheniformis* strain CAN55, which showed ability to grow only on specific sulfur-containing compounds (Chebbi et al. 2014). In fact, *n*-alkanes biodegradation by bacterial strains was previously reported to depend on molecular weights and structures (Grund et al. 1975). Based on this variability, the investigation of the biodegradation of various mercaptans by a mixture of bacterial strains could expand current knowledge on the range of target substrates. In fact, the targeting of specific chemical structures and use of appropriate microorganisms have previously been reported as fundamental strategies for the biological treatment of malodorous compounds (Rappert and Muller 2005). The enhancement of thiol solubility in aqueous media seems also to offer a promising strategy to obtain higher biodegradation rates. In fact, *n*-alkanes have been described to represent recalcitrant insoluble compounds that are hard to degrade by bacteria or fungi (Haritash and Kaushik 2009; Bisht et al. 2014). Accordingly, further research is needed to investigate the potential association between *n*-alkanes biodegradation and *n*-alkanethiols.

Conclusion

The present study was undertaken to investigate the ability of an adapted microbial consortium isolated from the gas-washing wastewaters (GWWs) generated from GCT to degrade hazardous and malodorous mercaptans. The GWWs samples were characterized by acidic pH and low

biodegradable organic matter. A bacterial consortium (CSH37) showed particular ability to grow on these wastewaters and several mercaptans and was, therefore, selected and maintained for further investigation. A bacterial strain SH6 was isolated from the CSH37 consortium after enrichment on dodecanethiol. This strain was affiliated to *Staphylococcus capitis* based on phenotypic and phylogenetic characteristics. Strain SH6 was able to degrade dodecanethiol used as a sole carbon and energy source. The strain was also noted to induce a reduction in the surface tension of the cell-free culture supernatants, thus suggesting its ability to produce surface-active compounds (biosurfactant(s)). Accordingly, further studies are needed to investigate the nature of these surface-active agents and their effects on mercaptans. Due to its possible human pathogenicity as the great majority of hydrocarbon-degrader genera (e.g., *Pseudomonas* spp.), safety and hazardous investigations are required before large-scale applications in industrial sites. Overall, the results presented in this work could contribute to the current knowledge on the biodegradation of fetid mercaptans generated by phosphate fertilizer plants. They indicate that the SH6 strain and CSH37 consortium could offer promising candidates for use in large-scale applications involving the treatment of specific sulfuric compounds. More research is, however, needed to determine the genes responsible for this microbial biodegradation.

Acknowledgments This work was supported by grants from the Tunisian ministry of higher education and scientific research, the Groupe Chimique Tunisien (GCT) and Research Center of Sfax, Tunisia. The authors would like to express their gratitude Mr. Adel Gargoubi for his technical assistance in CPG analyses and Mrs. Sonia Kchaou for her help in the Test strips of the API system. They would also like to thank Mr. Anouar Smaoui and Mrs. Hanen Ben Salem from the English Language Unit at the Faculty of Science of Sfax, Tunisia, for their valuable proofreading and language polishing services.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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