Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.933038

53 (2015) 3244–3252
March

Taylor & Francis
Taylor & Francis Group

Challenging tin toxicity by a novel strain isolated from freshwaters

Semih Cerit[a], Fadime Yilmaz[b], Bulent Icgen[b,*]

[a]*Department of Biology, Kırıkkale University, Kırıkkale 71450, Turkey*
[b]*Department of Environmental Engineering, Middle East Technical University, Ankara 06800, Turkey, Tel. +90 312 210 58 58; Fax: +90 312 210 26 46; email: bicgen@metu.edu.tr (B. Icgen)*

Received 11 October 2013; Accepted 5 February 2014

ABSTRACT

The first major challenge for the bioremediation field is to select the most promising types of biomass. Hence, the objective of this study was to isolate and identify a novel species which has the potential to remediate tin in freshwaters. For this reason, a bacterium designated as Sn11, with 59 μg ml^{-1} maximum tolerable concentration of tin was isolated selectively from the freshwater samples collected along the river Kırıkkale-Kızılırmak, Turkey. Identification of the isolate was done using biochemical tests, fatty acid methyl ester analysis, and 16S rRNA sequencing. Fatty acids produced by the isolate investigated were assumed as typical for the genus *Pantoea*. *Pantoea* were highly homogeneous by dominant $C_{16:0}$ and $C_{16:1}$ cis 9 fatty acids. 16S rRNA sequence analysis also confirmed that the isolate Sn11 had 98% homology with *Pantoea agglomerans*. The complete sorption of 59 μg ml^{-1} tin by *P. agglomerans* was recorded at about 20 h incubation. Due to higher affinity toward tin sorption, *P. agglomerans* can be an important isolate for the natural attenuation of tin toxicity in contaminated freshwaters.

Keywords: *Pantoea agglomerans*; Tin resistance; Bioremediation; FAME analysis; 16S rRNA sequencing

1. Introduction

Tin (Sn) is one of the toxic metals found in most wastewaters. Many industries produce large quantities of waste streams containing Sn such as Sn electroplating, aluminum anodizing, printed circuit board manufacturing, and metal pickling [1]. Sn compounds are also found in various environmental media in both inorganic and organic forms. Gases, dusts, and fumes containing Sn may be released from smelting and refining processes, industrial uses of Sn, waste incineration, and burning of fossil fuels [2,3]. In general, organotin compounds are released into the environment from anthropogenic sources; however, methyltin compounds can be produced in the environment by biomethylation of inorganic Sn [4]. Although the toxicological pattern of organotin compounds is very complex, Sn in its inorganic form is accepted as being non-toxic. It has been established that the toxicity of Sn is highest for triorganotins like tributyltin and triphenyltin [5]. Use of antifouling paints represents the major source of tributyltin into the coastal environment. On the other hand, triphenyltin enters

*Corresponding author.

Presented at the International Conference WIN4Life, 19–21 September 2013, Tinos Island, Greece

the environment directly from its use as fungicides, algicides, and molluscicides [6,7].

Wastewaters and industrial effluents containing Sn are major concern of environmental pollution. It has been shown that the remediation of organotin compounds could be successful using a number of different processes ranging from biological treatment to ultrafiltration, reverse osmosis, adsorption, and solvent extraction [8]. Inorganic Sn cannot be degraded in the environment, but may undergo oxidation–reduction, ligand exchange, and precipitation reactions [9]. It has been established that inorganic Sn can be transformed into organometallic forms by microbial methylation [10]. Inorganic Sn may also be converted to stannane (H_4Sn) in extremely anaerobic conditions by macroalgae [11]. Degradation of organotin compounds involves the breaking of the Sn-carbon bond, which may occur by UV irradiation or by biological or chemical cleavage [12]. In water, tributyltin can be degraded by photochemical and biological processes relatively rapidly; however, adsorption onto suspended particulate material in water followed by sedimentation is a key removal process [13]. Biodegradation is the major process in seawaters rich in suspended solids, but photolysis exceeds biodegradation in clean seawater. Calculated half-lives range from 6 d in summertime waters rich in suspended particles to 127 d in clean winter waters [14].

It has been established that one of the most important low-cost mechanism of Sn elimination is biodegradation due to activity of bacteria, algae, and fungi [5]. Particularly, micro-organisms able to survive well in high concentrations of Sn are of great interest as bioremediation agents for Sn-contaminated residues. Therefore, it is necessary to isolate and identify the microbes which are able to eliminate Sn from contaminated waters. Hence, the aim of this study was to isolate and identify a novel species which has the potential to bioremediate Sn.

2. Materials and methods

2.1. Sampling, isolation, and biochemical identification

Water samples were collected along the river Kızılırmak extending from 39˚22´16.39´´N, 33˚26´49.26´´E, 890 m to 39˚57´22.98´´N, 33˚25´04, 35´´E, 679 m of the city Kırıkkale, Turkey. The samples were put into sterile screw-capped bottles aseptically, kept in an icebox containing ice packs, and taken immediately to the laboratory. Inductively coupled plasma mass spectrometry ICP-MS (Perkin Elmer, USA) was used for Sn measurements. A quantity of 1 ml of water from each of the collected samples was dissolved in 9 ml sterile distilled water and serial dilutions were made. Each dilution was plated on Luria Bertani (LB) agar plates supplemented with increasing concentrations of $SnCl_2 2H_2O$ by the standard pour plate method. Plates were incubated at 30˚C for 3 d and colonies differing in morphological characteristics were selected. After the growth of different micro-organisms on the plate, each bacterial colony on the basis of its morphological characteristics was picked up and further purified by repeated streaking on nutrient agar plates and identified with gram staining. Each bacterial culture was then inoculated in nutrient broth, incubated and glycerol stocks were made, and frozen at –70˚C. For isolation and purification, strains were routinely grown in LB medium containing Sn at 30˚C. The Analytical Profile Index (API 20E) strep micromethod for the primary identification of isolates, using eight conventional biochemical tests and 12 carbohydrate assimilation tests, was performed as described by the manufacturer (Bio Merieux, France) and the results were analyzed using Apiweb, version 1.2.1.

2.2. 16S rRNA sequence analysis

Confirmation of the taxonomical status of the selected strain was done by molecular methods. Genomic DNA was isolated and analyzed by the method of Chen and Kuo [15]. Bacterial 16S rDNA was amplified using the universal bacterial 16S rRNA primers, 27F (5´-AGAGTTTGATCMTGGCTCAG-3´), and 1492R (5´-ACCTTGTTACGACTT-3´). PCR was performed with a 50 µl reaction mixture containing 1 µl (10 ng) of DNA extract as a template, each primer at a concentration of 5 mM, 25 mM $MgCl_2$, and dNTPs at a concentration of 2 mM, as well as 1.5 U of *Taq* polymerase and buffer used as recommended by the manufacturer (Fermentas, Germany). After the initial denaturation for 5 min at 94˚C, there were 35 cycles consisting of denaturation at 94˚C for 1 min, annealing at 55˚C for 1 min, extension at 72˚C for 1 min, and final extension at 72˚C for 5 min. PCR was carried out in a gene Thermal Cycler (Techne TC-Plus, USA). The obtained PCR products were purified, using the GeneJET™ PCR Purification Kit (Fermentas, Germany), according to restrictions of the manufacturer, and sequenced. The PCR product was sequenced by 3730x1 DNA synthesizer (Applied Biosystems, USA). The 16S rRNA sequences were aligned and compared with other 16S rRNA genes in the GenBank using the ClustalW program [16]. A distance matrix was generated using the Jukes-Cantor corrected distance model. The 16S rRNA gene sequences have been deposited to GenBank using BankIt submission tool and has been assigned with NCBI accession numbers.

2.3. Analysis of fatty acid methyl esters

Pure cultures were streaked on trypticase soy broth agar (Difco) and incubated at 30°C for 24 h. fatty acid methyl esters (FAME) were produced in five or more replicates using the standard MIDI procedures [17]. A loopful (20–40 mg) of cells, from the third streaked quadrant after the 24 h culture, was harvested and placed into a clean screw-capped glass tube. Cellular fatty acids were saponified by boiling at 100°C with 1 ml of 3.75 N certified ACS-grade NaOH (Fisher) in 1:1 (v/v) HPLC-grade methanol: H_2O for 30 min [18]. Methylation occurred after 2 ml of 1.18:1 (v/v), 6 N HCl (VWR): HPLC-grade methanol was added and the mixture heated at 80°C for 10 min. The FAME were extracted into the organic phase by adding 1.25 ml of 1:1 (v/v), HPLC-grade hexane: HPLC-grade methyl tert-butyl ether (J.T. Baker, Phillipsburg, NJ) followed by a base wash (0.3 N NaOH in H_2O) to remove any free fatty acids and residual reagents. The purified FAME were analyzed immediately using a gas chromatograph (GC model 6850, Agilent Technologies, Wilmington, DE) coupled to a flame ionization detector using the protocol recommended by MIDI [17]. The GC Chemstation enhanced integrator program (rev. A. 10.02, 1757, Agilent Technologies) was used to integrate the chromatogram peaks and to electronically transfer results to the MIS for library comparison with the TSBA method 5.0 (TSBA50) aerobe database. The output contained a list of fatty acids corresponding to percent composition and identifications with similarity indexes ranging from 0 to 1 in which the higher number being the closest match to the library of fatty acids for aerobic bacteria (RTSB50). The GC was calibrated twice before the start of each analysis sequence and after every 11th sample injection [17], using rapid calibration standards (No. 1300-AA, MIDI). During all the calibration analyses, the peak percent named for the standard was >99% with the root mean square error <0.003.

2.4. Growth studies

Growth curve of bacterial isolates was studied in 250 ml flasks containing 50 ml LB medium supplemented with $SnCl_22H_2O$ for the total Sn concentration 59 μg ml^{-1}. Control flask was not supplemented with $SnCl_22H_2O$. Flasks were inoculated with 0.5 ml of overnight culture and agitated on a rotary shaker (150 rev min^{-1}) at 30°C. Growth was monitored as a function of biomass by measuring the absorbance at 600 nm using the spectrum SP-2000UV spectrophotometer (Hangzhou Chincan Trading Co., China).

2.5. Experiments of biosorption

Experiments of heavy metals biosorption were done in Erlenmeyer flasks containing 250 ml of each samples and 15.0 ± 1.0 mg of cells. To ensure equilibrium, cells and waste were maintained in contact for 48 h, under constant agitation, at 30°C. In all experiments, cells were obtained from only one cultivation and collected from the same flask at the same growth stage. After 40 h, cells were separated from the medium and residual metal concentrations were monitored by ICP-MS (Perkin Elmer, USA). Experiments were done in triplicate. The optimum pH and temperature maintained for the growth of micro-organisms in the batch culture [18].

2.6. Scanning electron microscopy measurements

The surface morphologies of isolate were investigated by scanning electron microscopy (SEM) instrument (JEOL JSM-6060 SEM) combined with energy dispersive X-ray spectroscopy. The samples mounted on the plate were kept under vacuum, and then coated with gold particles before the SEM measurements. SEM micrographs of biosorbent were analyzed before and after biosorption of Sn. The results were the mean values of three replicate measurements.

2.7. Analysis of total and outer membrane proteins

The preparation of total and outer membranes with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Djkshoorn et al. [19]. Briefly, cell envelope fractions were prepared by ultrasonic disruption of cells and subsequent fractional centrifugation. A discontinuous system of a 3% stacking and an 11% acrylamide separating gel was used for electrophoresis, and after staining, the profiles were examined visually. The molecular weights of the total and outer membrane proteins were estimated from calibration curves prepared using PageRulerTM Plus Prestained Protein Ladder protein weight marker (Fermentas, Germany). The gels were stained with coomassie blue. The coomassie blue-stained bands on the SDS-polyacrylamide gel were scanned to estimate the relative amounts of proteins using an LKB 2202 Ultrascan laser densitometer, and the absorbance of the peaks was recorded on a LKB 2220 recorder with integrator. The values for each band scanned at three different places in each lane were averaged. The area was computed by the LKB 2220 by dropping a perpendicular from the trough between the two peaks to the horizontal

baseline. The reliability of these ratios was confirmed independently using a graphics tablet with computer programmed analysis [20].

2.8. Statistical analysis

The data were statistically analyzed using one-way analysis of variance at $p = 0.05$. Before statistical analyses were performed, data were tested for normality, if needed. The obtained data had a normal distribution and were not distorted before statistical analysis [21]. All statistical analyses were performed with Statistical Analysis System programs [22].

3. Results and discussion

3.1. Isolation and identification of tin-resistant bacteria

Kırıkkale is a recent province of Turkey in Central Anatolian region nearby the river Kızılırmak. Kırıkkale is metallurgically very active since decades. Main industries in Kırıkkale include state-owned military arms and ammunition factories, petroleum refineries, seamless steel pipe plant, mobile power plant, and chemical plants. The river has been affected by industrial and agricultural heavy metal pollution from the surrounding facilities and domestic effluents from the city. Considering natural adaptation theory, industrial drainage samples were collected from Kızılırmak around the city Kırıkkale to screen and develop microbial communities having a potency for Sn tolerance and bioremediation activities. Presumably, bacteria thriving in wastewater, where heavy metals may be discharged, face different challenges than those surviving in a drinking water supply system, where the organic content and chemical contamination is absent or at trace levels. Such environmental pressures may also be responsible for the prevalence of specific groups of organisms able to deal with the environmental conditions imposed. Although water analysis results with ICP-MS showed very low concentrations of Sn (<0.005 mg/l), attemps were still made to isolate Sn-tolerant bacteria by considering the environmental pressures. For the selective isolation of Sn-tolerant bacteria, increased concentrations of $SnCl_2 2H_2O$ incorporated media were used. After the incubation period, the plates were observed for any kind of growth on the media. The isolated and distinct colonies on these selective media were subcultured repeatedly on the same media for purification. The pure culture was tentatively identified on the basis of their morphological and biochemical characters. Out of 17 bacterial isolates, only one bacterial isolate showed the maximum tolerable concentration of 59 μg ml^{-1} of Sn. This might indicate that spontaneous tin-resistant mutant occurred at a lower frequency. This single isolate was designated as Sn11. Gram staining of colonies of Sn11 showed Gram-negative rods, which were motile, catalase positive and oxidase negative with fermenting sugars and phenotypically identified as *Pantoea agglomerans*.

For the further confirmation, cellular FAME profiles of the isolate were determined. The most abundant fatty acids in strain Sn11 were $C_{16:0}$ (26.05%), $C_{16:1}$ cis 9 (21.16%), $C_{14:0}$ (4.21%), $C_{17:0}$ cyclo (2.85%), and $C_{12:0}$ (2.49%). However, large amount of the summed fatty acids could not be separated by the GC of MIDI system. Interpretation of FAME profiles isolated from

Table 1
Comparison of the fatty acid compositions (%) of the isolate Sn11 and other *Enterobacteriaceae* family members. Taxa: 1, the isolate Sn11; 2, *P. agglomerans* LMG 1286, *E. amylovora* LMG 2024, and *P. dispersa* LMG 2603, The data for *P. agglomerans* LMG 1286, *E. amylovora* LMG 2024, and *P. dispersa* LMG 2603 were obtained from Mergaert et al. [24]

Fatty acids	Sn11	*P. agglomerans* LMG 1286	*E. amylovora* LMG 2024	*P. dispersa* LMG 2603
$C_{12:0}$	2.49	3.8	5.1	4.2
$C_{14:0}$	4.21	6.0	4.9	3.0
$C_{15:0}$	<1.0	1.1	<1.0	<1.0
$C_{16:0}$	26.05	27.1	34	30.8
$C_{16:1}$ cis 9	21.6	24.2	24.5	10.6
$C_{17:0}$	<1.0	<1.0	1.5	<1.0
$C_{17:0}$ cyclo	2.85	13.2	9.6	11.3
Summed feature 3[a]	26.87	11.6	9.8	24.4
Summed feature 3[b]	14.06	11.2	9.1	9.0

*Summed feature 3 comprises 18:1 trans, 18:1 trans 9, and 18:1 cis.
[a]16:1 iso and 14:0 3OH.
[b]The summed fatty acids cannot be separated by the GLC of MIDI system.

environmental samples is complicated by the large number of fatty acids commonly identified and by the fact that environmental conditions impact the nature of lipid components that constitute biological communities [23]. The fatty acid pattern of the strain Sn11 is shown in Table 1 in comparison with other representative *Enterobacteriaceae* species. The fatty acid profile of strain Sn11 was in agreement with data obtained for other members of the family [24]. Thereby, confirming the identity of the isolate Sn11 as a member of the genus *Pantoea*. *P. agglomerans* is member of *Enterobacteriaceae* that inhabits plants, soil, water, and such species includes bacteria reported as both commensal and pathogen of animals and humans [25]. Previously known under the different names of *Enterobacter agglomerans* and *Erwinia herbicola*, this bacterium is known to be an opportunistic pathogen in the immunocompromised persons, causing wound, and blood and urinary tract infections. It is commonly isolated from plant surfaces, seeds, fruit (e.g. mandarin oranges), and animal or human feces [26]. It is difficult to differentiate *Pantoea* species from other members of this family, such as *Enterobacter*, *Klebsiella*, and *Serratia* species. However, *Pantoea* does not utilize the amino acids lysine, arginine, and orinthine, a characteristic that sets it apart from the other genera. *Pantoea* species are clearly opportunistic pathogens and rarely cause disease in the otherwise healthy individuals [27]. As *P. agglomerans* is frequently found in water and soil, our study highlight the fact that surface waters contaminated with hospital wastes could be a source of infection.

Distance matrix-based phylogenetic analysis resulted in the highest correlation of Sn11 with *P. agglomerans* DSM 3493 (Table 2). Multiple sequence alignment analysis of the 16S rRNA sequences with already available database also confirmed that the strain Sn11 had 98% homology with *P. agglomerans* DSM 3493. Phylogenetically, the closest relatives of the strain was *P. ananatis* (97%) as shown in Fig. 1. 16S rRNA gene sequences allow differentiation between organisms at the genus level across all major phyla of bacteria, in addition to classifying strains at multiple levels, including species and subspecies level.

3.2. Growth characteristics and biosorption ability

The growth curve of the isolate was also constructed to determine the difference in their growth characteristics. The growth of *P. agglomerans* isolate was monitored for 44 h and no significant variations in their growth curve patterns were observed in the presence 59 μg ml^{-1} of Sn. The highest cell number 20×10^8 was obtained around 38 h in the presence of

Table 2
Distance matrix analyses for the identification of Sn11

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Sn11															
2. *Pantoea agglomerans* DSM 3493	1.107														
3. *Pantoea ananatis* 1846	1.127	0.012													
4. *Pantoea stewartii* ATCC 8199	1.121	0.021	0.018												
5. *Erwinia tasmaniensis* Et1/99	1.107	0.026	0.037	0.030											
6. *Citrobacter gillenii* CDC 4693-86	1.121	0.031	0.033	0.034	0.027										
7. *Enterobacter kobei* CIP 105566	1.121	0.029	0.033	0.027	0.029	0.014									
8. *Buttiauxxella noackiae* NSW 11	1.124	0.030	0.036	0.034	0.020	0.015	0.017								
9. *Erwinia amylovora* DSM 30165	1.114	0.033	0.037	0.029	0.011	0.030	0.035	0.029							
10. *Pantoea dispersa* LMG2603	1.121	0.030	0.024	0.020	0.023	0.032	0.032	0.034	0.023						
11. *Klebsiella oxytoca* ATCC 13182	1.135	0.034	0.028	0.027	0.034	0.016	0.017	0.025	0.036	0.029					
12. *Buttiauxxella warmboldiae* NSW326	1.121	0.032	0.037	0.036	0.022	0.017	0.018	0.002	0.030	0.036	0.027				
13. *Kluyvera intermedia* 256	1.117	0.036	0.029	0.029	0.030	0.014	0.016	0.018	0.035	0.022	0.017	0.020			
14. *Escherichia vulneris* ATCC 33821	1.127	0.029	0.026	0.029	0.030	0.023	0.017	0.024	0.037	0.025	0.019	0.025	0.020		
15. *Citrobacter youngae* GTC 1314	1.135	0.036	0.037	0.035	0.029	0.022	0.020	0.021	0.027	0.029	0.025	0.023	0.027	0.028	

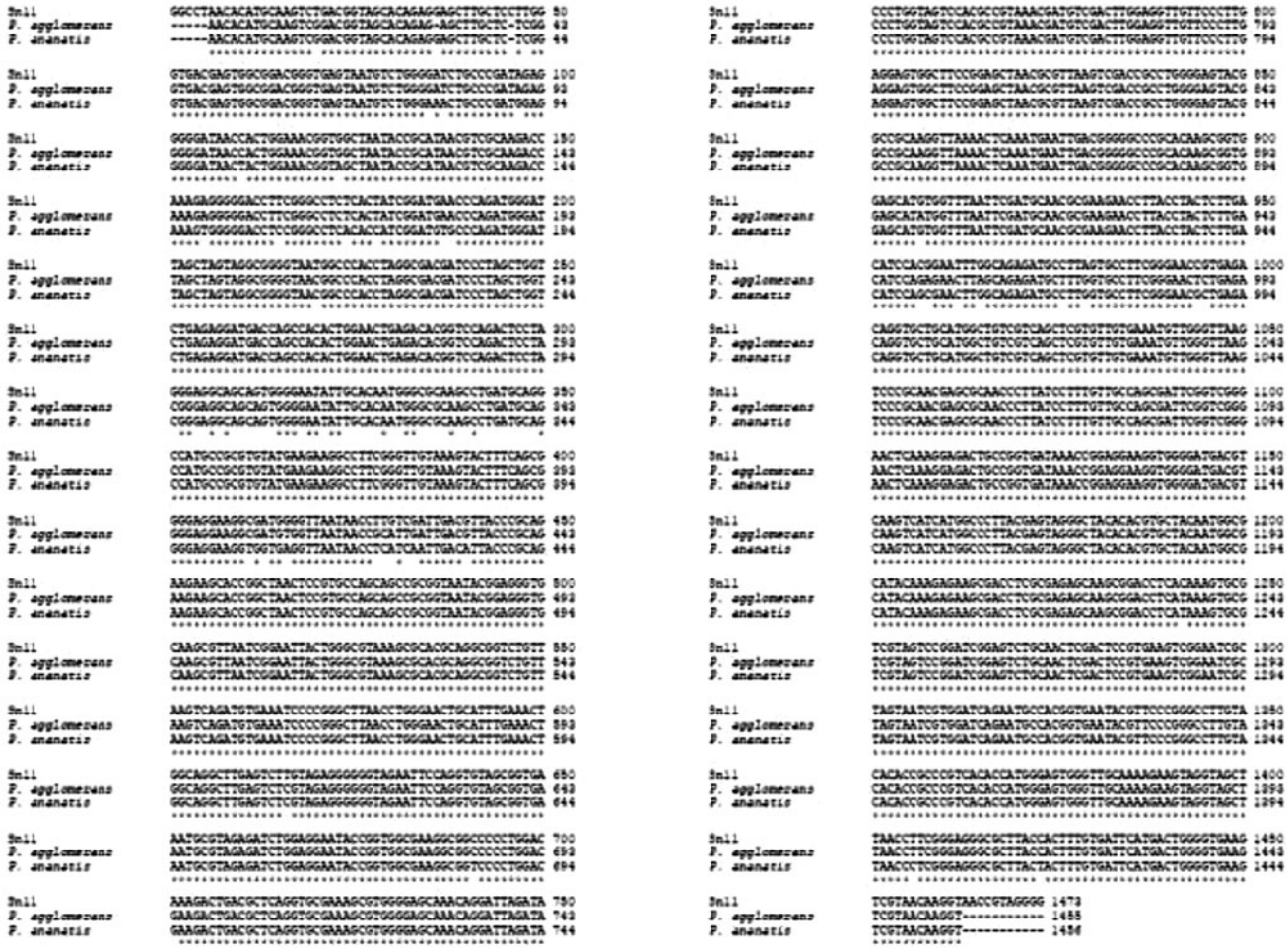

Fig. 1. Multiple nucleotide sequence alignment analysis. Asterisks denote identical residues.

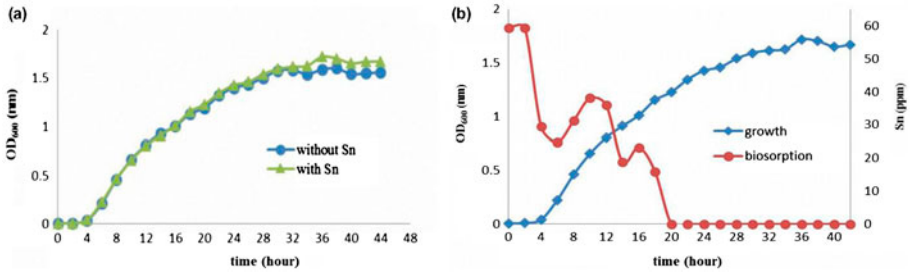

Fig. 2. Growth (a) and Sn biosorption (b) curves of *P. agglomerans*.

Sn, while it was at around 21×10^8 at the same hour without Sn (Fig. 2(a)). Sn sorbed by *P. agglomerans* was also investigated. The complete sorption of 59 μg ml^{-1} Sn was recorded at about 20 h which corresponded to mid-log phase of the growth at 30°C (Fig. 2(b)).

The biosorption of heavy metal depends on the higher heavy metal solution taken for the study. Microbial biomass offers an economical option for removing heavy metals by the phenomenon of biosorption [28], therefore, bacterial species are tested for heavy metal removal and it is found that *Pseudomonas* species are able to remove copper and nickel better than others and lead is removed better by *Staphylococcus* species. Wong and So [29] also

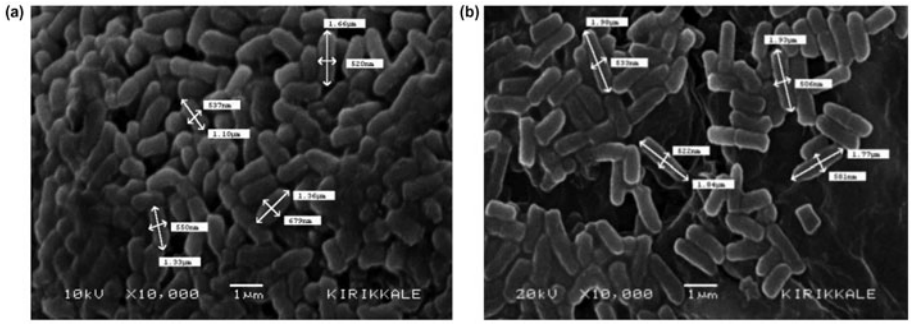

Fig. 3. SEM images of *P. agglomerans* incubated in a medium without (a) and with Sn (b), (10.000 × magnification).

reported the similar result with *Pseudomonas* species for removal of copper. Lopez et al. [30] reported that *Pseudomonas fluorescence* 4F39 accumulated heavy metals very rapidly in the order Cu > Cd > Co > Cr > Pb [30]. Although it has demonstrated that microalgae, fungi, and bacteria are able to biosorb and debutylate tributyltin into less toxic dibutyltin, monobutyltin, and inorganic tin [31–35], information is still severely limited [33]. The tributyltin removal efficiency and degradation ability seemed vary from species to species [31,34–36].

3.3. Total and outer membrane protein profiles

Micro-organisms possess an abundance of functional groups that can passively adsorb metal ions. The surface envelopes of bacterial cells can adsorb various heavy metals by virtue of ionic bonds to their intrinsic chemical groups. The sites for metal binding are different according to bacterium species and metals. SEM evaluation of Sn-resistant *P. agglomerans* also indicated an association of bound Sn with the cell envelope. Although the average cell size was around 1.38 × 605 nm in the absence of Sn, it was found out to be 1.45 × 521 nm in the presence of Sn (Fig. 3(a) and (b)).

SEM micrographs of *P. agglomerans* indicated accumulation of Sn on the cell surface. For this reason, the cell envelope proteins of *P. agglomerans* grown on LB broth in the absence and presence of Sn were also extracted and analyzed in our study. Higher expressions were observed in almost all of the total and outer membrane protein bands observed depending on the presence of Sn (Fig. 4(a) and (b)).

The walls of bacteria are efficient metal chelators though a wide spectrum of uptake capacities may be exhibited. Metal binding may be at least a two-stage process; first, involving interaction between metal ions and reactive groups followed by inorganic deposition

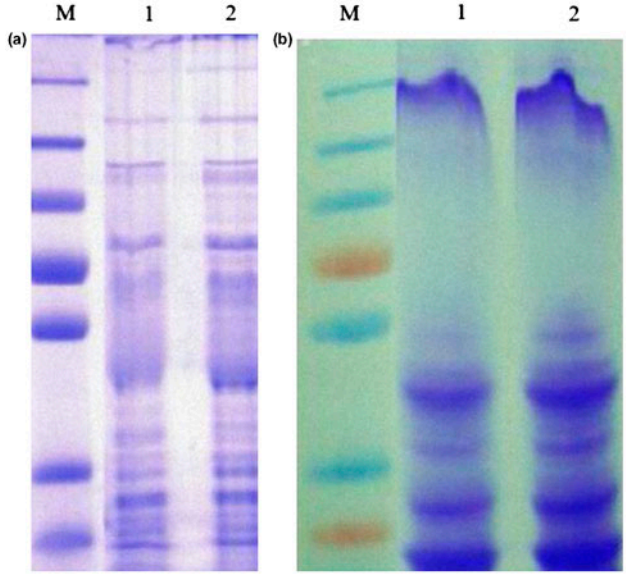

Fig. 4. Total (a) and outer (b) membrane protein profiles of *P. agglomerans* without (lane 1) and with Sn (lane 2). (M) protein weight marker; PageRulerTM Plus Prestained Protein Ladder (from top to down 250, 130, 100, 72, and 55 kDa, respectively).

of increased amounts of metal. In some bacteria, metabolism-independent biosorption may be the most significant proportion of total uptake. Although biosorption is independent of metabolism, it is possible that a metabolism-dependent microenvironment may enhance metal deposition. Growing cells have unlimited capacities to cleave organo-metallic complexes, degrade organic compounds, as well as take up other inorganic ions such as ammonium, nitrate, and phosphate. Further, dissolved and fine-dispersed metallic elements can also be removed via immobilization. The isolation and selection of metal-resistant strains shall be a crucial aspect to overcome the prime constraint of employing living cell systems. Incidentally, resistant

cells are expected to bind substantially more metals, which in turn is a prerequisite for enhanced bioprecipitation, intracellular accumulation, and development of an efficient process [37].

4. Conclusions

Due to industrial expansion, large quantities of industrial wastes are accumulating in many countries and cannot be disposed without prior special treatments. In particular, waste products from the mining and metal refining industries, sewage sludges, and residues from power station and waste incineration plants can contain heavy metals at high concentrations. Usually, these heavy metals can be leachated from the soil to the surface water system at higher concentrations than they are allowed. For this reason, they cannot be disposed into wastewaters plant and must be submitted to special treatment in order to reduce metal contents. Therefore, the management of waste sludge produced from the industrial activity becomes the most important issue of environmental protection. Micro-organisms play a significant role in adsorption of heavy metal from contaminated wastewater. Among heavy metals presents in sludge, Sn is one of the most common. The present investigations elucidated that the remediation of Sn-contaminated freshwaters could be carried out using *P. agglomerans*. The species may provide an interesting option to remediate Sn and act as an effluent bioremediator.

References

[1] K.L. Wasewar, S. Kumar, B. Prasad, Adsorption of tin using granular activated carbon, J. Environ. Prot. Sci. 3 (2009) 41–52.

[2] WHO, Tin and Organotin Compounds: A Preliminary Review, Environmental Health criteria 15, World Health Organization, Geneva, 1980. http://www.inchem.org/documents/ehc/ehc.ehc015.htm 6 June 2013.

[3] G.S. Senesi, G. Baldassarre, N. Senesi, B. Radina, Trace element inputs into soils by anthropogenic activities and implications for human health, Chemosphere 2 (1999) 343–377.

[4] K. Fent, Ecotoxicology of organotin compounds, Crit. Rev. Toxicol. 26 (1996) 1–117.

[5] O.S. Ayanda, O.S. Fatoki, F.A. Adekola, B.J. Ximba, Fate and remediation of organotin compounds in seawaters and soils, Chem. Sci. Trans. 1 (2012) 470–481.

[6] C. Alzieu, Tributyltin: Case study of a chronic contaminant in the coastal environment, Ocean Coast. Manage. 40 (1998) 23–36.

[7] WHO, Concise International Chemical Assessment Document 13: Triphenyltin compounds, Geneva, 1999. http://www.inchem.org/documents/cicads/cicads/cicad13.htm 30 July 2013.

[8] A. Abbott, P.D. Abel, U.D.W. Arnold, A. Milne, Cost benefit analysis of the use of tributyltin: The case for a treatment approach, Sci. Tot. Environ. 258 (2000) 5–19.

[9] HSDB, Tin. Environmental Standards and Regulations, Hazardous Substances Data Bank, Bethesda, MD, 2004. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.htm 6 January 2004.

[10] L.E. Hallas, J.C. Means, J.J. Cooney, Methylation of tin by estuarine microorganisms, Science 215 (1982) 1505–1507.

[11] O.F.X. Donard, J.H. Weber, Volatilization of tin as stannate in anoxic environments, Nature 332 (1982) 339–341.

[12] S.J. Blunden, L.A. Hobbs, P.J. Smith, The environmental chemistry of organotin compounds, Environ. Chem. 3 (1984) 49–77.

[13] S.J. De Mora, E. Pelletier, Environmental tributyltin research: Past, present, future, Environ. Technol. 18 (1997) 1169–1177.

[14] N. Watanabe, S. Sakai, H. Takatsuki, Examination for degradation paths of butyltin compounds in natural waters, Water Sci. Technol. 25 (1992) 117–124.

[15] W.P. Chen, T.T. Kuo, A simple and rapid method for the preparation of gram negative bacterial genomic DNA, Nucleic Acids Res. 21 (1993) 2260.

[16] D.A. Benson, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, B.A. Rapp, D.L. Wheeler, GenBank, Nucleic Acids Res. 1 (2000) 15–18.

[17] R. Paisley, Training Manual MIS Whole Cell Fatty Acid Analysis by Gas Chromatography, MIDI, Newark, DE, 2004.

[18] K.A. Hietala, T.M. Roane, Microbial remediation of metals in soils in advances in applied bioremediation, Soil Biol. 17 (2009) 201–220.

[19] L. Djkshoorn, I. Tjernberg, B. Pot, Numerical analysis of cell envelope protein profiles of *Acinetobacter* strains classified by DNA-DNA hybridization, Syst. Appl. Microbiol. 13 (1990) 338–344.

[20] A.M. Mukhallad, P.S. Malcolm, The distribution of heavy–chain isoforms of myosin in airways smooth muscle from adult and neonate humans, Biochem. J. 260 (1989) 421–426.

[21] K. Ashok, B.S. Bisht, K. Manish, K. Lalit, Effects of Ni and Zn on growth of *Vigna mungo, Vigna radiata* and Glycine max, Int. J. Pharma. Biosci. 1 (2010) 1–7.

[22] Statistical Analysis Systems, SAS User's Guide: Statistics, Version 5 Edition, SAS Institute, Cary, NC, 2001.

[23] S.K. Haack, H. Garchow, D.A. Odelson, L.J. Forney, M.J. Klug, Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities, Appl. Environ. Microbiol. 60 (1994) 2483–2493.

[24] J. Mergaert, L.V. UGent, K. Kersters, Transfer of *Erwinia-ananas* (synonym, Erwinia-uredovora) and *Erwinia-stewartii* to the genus Pantoea emend as *Pantoea-ananas* (serrano 1928) comb-nov and *Pantoea-stewartii* (smith 1898) comb-nov, respectively, and description of *Pantoea-stewartii* subsp *indologenes* subsp nov., Int. J. Syst. Bacteriol. 43 (1993) 162–173.

[25] F. Gavini, J. Mergaert, A. Beji, C. Mielcarek, D. Izard, K. Kersters, J. De Ley, Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov, Int. J. Syst. Bacteriol. 39 (1989) 337–345.

[26] Y. Aktan, S. Tan, B. Icgen, Characterization of lead-resistant river isolate *Enterococcus faecalis* and assessment of its multiple metal and antibiotic resistance, Environ. Monit. Assess. 185 (2013) 5285–5293.
[27] W.E. Sander, C.C. Sanders, *Enterobacter* spp.: Pathogens poised to flourish at the turn of the century, Clin. Microbiol. Rev. 10 (1997) 220–241.
[28] R. Gupta, H. Mahapatra, Microbial biomass: An economical alternative for removal of heavy metals from waste water, Ind. J. Exp. Biol. 41 (2003) 945–966.
[29] P.K. Wong, C.M. So, Copper accumulation by a strain of *Pseudomonas putida*, Microbiol. 73 (1993) 113–121.
[30] A. Lopez, N. Lazaro, J.M. Priego, A.M. Marques, Effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39, J. Ind. Microbiol. Biotechnol. 24 (2000) 146–151.
[31] S.V. Avery, G.A. Codd, G.M. Gadd, Biosorption of tributyltin and other organotin compounds by cyanobacteria and microalgae, Appl. Microbiol. Biotechnol. 39 (1993) 812–817.
[32] S. Kawai, Y. Kurokawa, H. Harino, M. Fukushima, Degradation of tributyltin by a bacterial strain isolated from polluted river water, Environ. Pollut. 102 (1998) 259–263.
[33] L. Zhang, G.L. Huang, Y.T. Yu, Immobilization of microalgae for biosorption and degradation of butyltin chlorides, Artif. Cells, Blood Substitues, Immobilization Biotechnol. 26 (1998) 399–410.
[34] C.K. Tsang, P.S. Lau, Biodegradation capacity of tributyltin by two *Chlorella* species, Environ. Pollut. 105 (1999) 289–297.
[35] N.F.Y. Tam, A.M.Y. Chong, Y.S. Wong, Removal of tributyltin by live and dead microalgal cells, Mar. Pollut. Bull. 45 (2002) 362–371.
[36] R.F. Lee, A.O. Valkirs, P.F. Seligman, Importance of microalgae in the biodegradation of tributyltin in estuarine waters, Environ. Sci. Technol. 23 (1989) 1515–1518.
[37] A. Malik, Metal bioremediation through growing cells, Environ. Int. 30 (2004) 261–278.