ORIGINAL ARTICLE

CrossMark

Al^{3+} and Fe^{2+} toxicity reduction potential by acid-resistant strains of *Rhodopseudomonas palustris* isolated from acid sulfate soils under acidic conditions

Khuong Quoc Nguyen[1,2] · Duangporn Kantachote[1,3] · Jumpen Onthong[4] · Ampaitip Sukhoom[1]

Abstract

This research aimed to evaluate the capacity of acid-resistant purple nonsulfur bacteria, *Rhodopseudomonas palustris* strains VNW02, TLS06, VNW64, and VNS89, to resist Al^{3+} and Fe^{2+} and to investigate their potential to remove both metals from aqueous solutions using exopolymeric substances (EPS) and biomasses. Based on median inhibition concentration (IC$_{50}$), strain VNW64 was the most resistant to both metals under conditions of aerobic dark and microaerobic light; however, strain TLS06 was more resistant to Al^{3+} under aerobic dark conditions. High metal concentrations resulted in an altered cellular morphology, particularly for strain TLS06. Metal accumulation in all tested PNSB under both incubating conditions as individual Al^{3+} or Fe^{2+} was in the order of cell wall > cytoplasm > cell membrane. This was also found in a mixed metal set only under conditions of aerobic dark as microaerobic light was in the degree of cytoplasm > cell wall > cell membrane. Of all strains tested, EPS from strain VNW64 had the lowest carbohydrate and the highest protein contents. Metal biosorption under both incubating conditions, EPS produced by strains VNW64 and TLS06, achieved greater removal (80 mg Al^{3+} L^{-1} and/or 300 mg Fe^{2+} L^{-1}) than their biomasses. Additionally, strain VNW64 had a higher removal efficiency compared to strain TLS06. Based on the alteration in cellular morphology, including biosorption and bioaccumulation mechanisms, *R. palustris* strains VNW64 and TLS06 demonstrated their resistance to metal toxicity. Hence, they may have great potential for ameliorating the toxicity of Al^{3+} and Fe^{2+} in acid sulfate soils for rice cultivation.

Keywords Bioaccumulation · Biopolymer · Bioremediation · Biosorption · Paddy field · Purple nonsulfur bacteria

✉ Duangporn Kantachote
duangporn.k@psu.ac.th

Khuong Quoc Nguyen
nqkhuong@ctu.edu.vn

Jumpen Onthong
jumpen.o@psu.ac.th

Ampaitip Sukhoom
ampaithip.s@psu.ac.th

1 Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand

2 Department of Soil Science, College of Agriculture and Applied Biology, Can Tho University, Can Tho, Viet Nam

3 Center of Excellence on Hazardous Substance Management, Bangkok 10330, Thailand

4 Department of Earth Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai 90112, Thailand

Introduction

It is well-recognized that acid sulfate soils (ASS) cause adversely impact on crop productivity, particularly on rice (Choudhury et al. 2017), and the most common metal toxicities are Al^{3+}, Fe^{2+}, and Mn^{2+} (Jones et al. 2016). In addition, these toxicities have a high potential to produce an adverse effect on human's health via food chains (Tóth et al. 2016) and also damage ecosystems through contamination in water environment (Auger et al. 2013). Specifically, diseases of neurological-related disorders (Alzheimer) were resulted from accumulating of highly concentrated aluminum in the human brain by alum-treated drinking water (McLachlan 1995). In fish, aluminum is considered as a stimulant of oxidative stress and as a disruption of antioxidant enzymes and neurotransmitter synthesis (Fernández-Dávila et al. 2012). As values above the safe threshold of heavy metals are unacceptable, remediation should be considered to remove the toxicity (Gautam

et al. 2014). It has long been known that there are many approaches for reducing toxicities of aluminum and ferrous iron (Panhwar et al. 2016), but the most recent researches have focused on the concept of bioproducts for sustainable agricultural intensification. The utilization of soil microorganisms would be attractive to minimize the aluminum and ferrous toxicities and to enhance the environmental-friendly production. Among beneficial microbes, purple nonsulfur bacteria (PNSB) should be considered as they have high possibility to reduce methane emission in wetland rice fields for preventing global warming as well (Kantachote et al. 2016). Hence, it would be worth to use PNSB for decreasing toxicities of Al^{3+} and Fe^{2+} in Al- and Fe-rich paddy fields like ASS. Recently, we obtained the acid-, aluminum-, and ferrous iron-resistant PNSB strains isolated from acid paddy fields, and this led to investigate their potential to remove both metallic ions for applying on acidic farmed rice.

Biosorption is a process of heavy metal removal by inactive or dead biomass under aqueous solution (Ilamathi et al. 2014); the retaining of heavy metals within living cells, however, is known as bioaccumulation (Liang et al. 2014). Biosorption is highly effective in binding metals of microbes, and they can use exopolymeric substances (EPS) to bind with heavy metals for increasing their resistance to them (Panwichian et al. 2011; Nookongbut et al. 2016). EPS matrix is normally excreted by microbes under physiological stress progress, which are presented under natural environmental state and laboratory conditions (Yuan and Wang 2013) to play the key roles in adsorptive and hydrophilous capacity for microbial aggregation (More et al. 2014). EPS can be categorized into cell-bound EPS and free EPS based on the proximity to the cell surface. Cell-bound EPS are tightly linked via a covalent or non-covalent bond as capsular EPS; however, free EPS as slime are not directly enclosed to the surface of the cell (Wingender et al. 1999). EPS as biopolymers, with the components including carbohydrates, proteins, and nucleic acids, are able to form a complex with heavy metals including sodium ion (Xuan et al. 2010; Nunkaew et al. 2015). Several researchers have studied on EPS produced under the various stress conditions by several PNSB strains, such as *Rhodopseudomonas acidophila* in the presence of heavy metals (Cu^{2+}, Cr^{6+}, Cd^{2+}) and 2,4-dichlorophenol (2,4-DCP) (Sheng et al. 2005) and *Rhodopseudomonas palustris* under the salinity stress (Nunkaew et al. 2015). A lot of work has been done on EPS produced by different bacterial genera under acidity stress (Aguilera et al. 2008) for investigating the removal of aluminum (Mikutta et al. 2011) and ferrous iron (Tapia et al. 2013). However, no work has been done on the production of EPS by *R. palustris* strains under an acidic condition to remove both metallic ions. Therefore, the aims of this study were as follows: (a) to determine resistant ability to Al^{3+} and Fe^{2+} of acid-resistant *R. palustris* strains isolated from ASS; (b) to investigate cell morphology under metal stress and the

accumulation of Al^{3+} and Fe^{2+} contents in cellular components; and (c) to compare the biosorption capacity between EPS and biomass of the promising PNSB for gaining better understanding of resistant mechanisms, prior to use for bioremediation in wetland rice fields on ASS.

Materials and methods

Preparations of PNSB inoculums and metal solutions

R. palustris strains, VNW02, TLS06, VNW64, and VNS89, as the potential acid-, aluminum-, and ferrous iron-resistant PNSB isolated from paddy fields on ASS (Khuong et al. 2017), including the reference strain *Rhodoblastus acidophilus* ATCC 25092 were used in this study. Basal isolation medium (BIM), pH 4.50, was used to grow isolated PNSB and ATCC® medium 650, pH 5.00, for the reference strain (Khuong et al. 2017). They were grown in culture broths and incubated under microaerobic light conditions for 48 h. After twice washing with 0.1% peptone water, cell pellets were used to make inoculums by adjustment with 0.1% peptone water to reach an optical density of 0.50 at OD_{660}. All experiments in this study used BIM or ATCC® medium 650 with no added phosphate composition, except for inoculum preparation.

The Al^{3+} stock preparation was diluted from aluminum chloride hydrated, $AlCl_3.6H_2O$, at 2500 mg Al^{3+} L^{-1}, and iron stock solution was attenuated by ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) at 5000 mg Fe^{2+} L^{-1} concentration. They were diluted with deionized water before filtration using a filter with 0.45 μm pore size. The ferrous solution was kept under the dark to avoid photo-oxidation of ferrous ion forming to ferric iron state. A 1 M HCl was filtered using a 0.45 μm sterile filter and used for pH adjustment of aqueous mixtures in all experiments of this study. For evaluating the possibility to use tested PNSB in paddy fields with high variation of both metal ions, concentrations used in this study were designed to be higher than critical contents for rice growth (68 mg Al^{3+} L^{-1} and 250 mg Fe^{2+} L^{-1}) (Attanandana and Vacharotayan 1986; Samaranayake et al. 2012). Hence, the initial concentrations in all experiments were set at 80 mg L^{-1} for Al^{3+} and 300 mg L^{-1} for Fe^{2+} including a mixture of both metallic ions at 80 and 300 mg L^{-1} for Al^{3+} and Fe^{2+}, unless otherwise stated.

Quantification of threshold value causing inhibitory effects of Al^{3+}, Fe^{2+} on acid-resistant PNSB

Minimum inhibitory concentration (MIC) of each PNSB was investigated in culture broth without phosphate composition as previously mentioned. The toxic metal stock solutions for determination of MIC were prepared by chemical salts as

previously described. The concentration of each stock was added into BIM or ATCC® medium 650, ranging from 0 to 1000 mg L^{-1} for Al^{3+} and 0 to 2000 mg L^{-1} for Fe^{2+}. A 10% of each PNSB was inoculated into 18.0 mL toxic BIM broth, containing designed levels of Al^{3+} or Fe^{2+}, pH 4.50 in test tubes (20 mL) for microaerobic light (3000 lx) and serum bottles (100 mL) for aerobic dark (150 rpm, 30 °C) to follow optimal growth incubating conditions (Khuong et al. 2017). For a reference strain, ATCC® Medium 650 broth at pH 5.00 was used instead of BIM broth. After 72 h incubation, for MIC assay, bacterial growth was measured based on cell turbidity using a spectrophotometer. MIC is defined as the lowest concentration of Al^{3+} or Fe^{2+} that inhibits the growth of the test microorganism as the clear culture broth, while minimum bactericidal concentration (MBC) is the lowest concentration that kills as no growth after streaking the clear culture broths from the MIC assay onto BIM or ATCC® medium 650 agar plates by separating incubation under both conditions for one week. As we aim to apply PNSB in the paddy fields on ASS, median inhibition concentration (IC_{50}) of Al^{3+} and Fe^{2+} was also determined. IC_{50} is defined as the concentration of the Al^{3+} or Fe^{2+} that inhibits 50% PNSB growth; this value was used to evaluate the capacity of Al^{3+} or Fe^{2+} resistance of each PNSB strain under acidic condition. For accuracy in this assay, bacterial growth was measured by counting viable cells instead of using a spectrophotometer to measure growth as turbidity. To determine IC_{50}, growth inhibition percentages based on viable cells in log CFU mL^{-1} of all tested PNSB were computed by comparison with their control sets as without Al^{3+} or Fe^{2+} using probit analysis in SPSS software (SPSS, version 16.0).

Cellular morphology of tested PNSB under influence of toxicity (Al^{3+} or Fe^{2+})

Only two PNSB strains (TLS06 and VNW64) that showed very high resistance to both metals from the previous experiment were used to investigate their cell morphologies under stress of metal ions. Scanning electron microscope-energy dispersive X-ray spectrometer (SEM-EDS) was used to observe bacterial cell morphology under stress of each metal and confirmed Al^{3+} or Fe^{2+} concentration in the biomass. A 10% of each culture was inoculated into 45 mL BIM broth, pH 4.25 containing either individual of Al^{3+} or Fe^{2+} at initial concentrations as previously stated, and this pH was adjusted due to metals producing more toxicity to bacterial cells compared to pH 5.00. They were grown under microaerobic light conditions for 48 h; culture broths were centrifuged at 8000 rpm for 15 min. After twice washing cell pellets with 0.1% peptone water, each biomass was used to investigate toxicity of Al^{3+} or Fe^{2+} on bacterial cells and metal absorption by SEM technique (JSM-5800LV, JEOL), with X-ray microanalysis—EDS (Oxford ISIS

300). Bacterial cells were fixed with 2% $C_5H_8O_2$ (glutaraldehyde) in 0.1 M sodium phosphate buffer (pH 7.00) for 1 h, followed by washing for three times with the above mentioned buffer. After that, cells were mixed with 1% OsO_4 (osmium tetraoxide) for 90 min and subsequently washed with 0.1 M sodium phosphate buffer. Then, samples were dehydrated with ethanol concentrations of 70–100% for 15 min in each level. Finally, the samples were coated with gold for SEM and without gold coating for SEM-EDS detection.

Uptake and allocation of Al^{3+} and/or Fe^{2+} toxicity in different cellular components

Each PNSB at 10% inoculum size was inoculated into 450 or 950 mL BIM broth (pH 4.25) in 1000 mL flasks that contained individual of Al^{3+} or Fe^{2+} and a mixture of both metallic ions at initial concentrations as previously mentioned. Culture flasks with 450 mL were shaken to provide aeration at 150 rpm, 30 °C under dark for aerobic conditions, while culture flasks with 950 mL as a little head space to provide microaerobic conditions under light intensity at 3000 lx. Both incubating conditions as above were set for the reference strain with the change of medium used as previously stated. After 48 h, all culture broths were centrifuged at 8000 rpm for 15 min to separate cell pellets and supernatants for determining Al^{3+} and/or Fe^{2+} in each fraction using inductively coupled plasma-optical emission spectroscopy (ICP-OES) as described by Soltanpour et al. (1996).

To investigate the uptake of Al^{3+} and/or Fe^{2+} in bacterial cell wall, each cell pellet was thrice bathed with 0.1 M sodium citrate (preparation using deionized water) for 10 min at each time by a three-fold volume of the solution (10 mL) to release ionic aluminum and/or iron from the cell wall and followed by centrifugation (8000 rpm, 15 min). Afterwards, the concentrations of Al^{3+} and/or Fe^{2+} in supernatants were determined. After washing cell pellets as in the previous step, bacterial cell walls were hydrolyzed for determination of Al^{3+} and/or Fe^{2+} in the cellular membrane by mixing with 4.0 mL of lysozyme (1.0 mg mL^{-1}) and 6.0 mL of 0.01 M sodium phosphate buffer. This mixture was incubated under the hypertonic conditions containing 10.3% of sucrose for 60 min at 30 °C to prevent the fracture of protoplast. Following this step, the suspensions were centrifuged at 8000 rpm for 15 min to measure the concentration of Al^{3+} and/or Fe^{2+} in the solutions. After centrifugation, protoplast pellets were separated by 10 mL of nitric acid (35%) to measure the accumulation of Al^{3+} and/or Fe^{2+} in the cytoplasm. For calculation, the loss of Al^{3+} and/or Fe^{2+} from culture supernatants was assumed as the accumulated amounts in biomasses (cell wall, cell membrane, and cytoplasm) and undetected amount.

Removal of Al^{3+} and/or Fe^{2+} by biomass and EPS from acid-resistant PNSB

To prepare biomass and EPS for metal biosorption, PNSB used were grown in 1000 mL of BIM, pH 4.50 (ATCC® Medium 650, pH 5.00 for reference strain) with the same medium composition for preparing inoculums under both aerobic dark and microaerobic light conditions for 72 h to reach stationary phase. All PNSB culture broths were harvested by centrifuging at 8000 rpm for 15 min to separate the cell pellets as biomasses and supernatants, in which, each cell pellet was further used for evaluating the removal efficiency of aluminum and ferrous iron, while each supernatant was used to extract EPS for further investigating its removal efficiency.

For soluble EPS extraction, the protocol was modified from the method described by Eboigbodin and Biggs (2008) as follows. Briefly, the volumetric ratio of 1:2.2 of culture supernatant and cold ethanol (4 °C) was incubated for 24 h at − 20 °C to precipitate soluble EPS. Then, the suspension was subsequently centrifuged at 8000 rpm for 15 min at 4 °C to obtain EPS, and some portions were used to determine dry weight (DW) (Ferreira et al. 2016). EPS extract was kept at − 20 °C until use for composition analysis as the following provided.

Total carbohydrate was analyzed by a colorimetric method with phenol-sulfuric acid reaction (DuBois et al. 1956) using glucose as a standard at 485 nm. Total protein content was measured by bicinchoninic acid at the wavelength of 562 nm (Smith et al. 1985), and bovine serum albumin was used for preparing standard protein solution. The nucleic acid concentration was assessed according to Johnson (1981) using optical density at 260 nm. The EPS concentration was defined as the total of the following components: total carbohydrate, protein, nucleic acid, and unidentified component.

According to the results of previous experiments on metal resistance and EPS component, only strains TLS06 and VNW64 were selected to study metal biosorption; the initial concentrations for Al^{3+} and Fe^{2+} including a mixture of both metallic ions were set as previously described. The experiment was performed by adding wet EPS, obtained from cells grown under aerobic dark or microaerobic light, at an amount equivalent to 50 mg DW to the aliquots volume (20 mL) containing individual Al^{3+}, Fe^{2+}, and both metal ions at pH 4.25 for 30 min under conditions of aerobic dark (150 rpm, 30 °C) and microaerobic light (at 3000 lx, room temperature) to follow the growth conditions. The pH 4.25 was designed because the metals under a lower pH give a higher toxicity to organisms including rice; also, this pH is normally found in ASS (Khuong et al. 2017). Both incubating conditions were investigated for metal biosorption by EPS as well, and this is because the redox conditions and light-mediated reactive oxygen species generation affect on iron forms and also iron binding (Swanner et al. 2017; Wang et al. 2017) that iron would

compete with other metals like Al. This is also due to the major component of EPS being exopolysaccharides; these compounds are important in transparent exopolymeric particles (TEP) (Engel et al. 2014). EPS or TEP is normally secreted by aquatic microbes and the compounds, particularly TEP are degraded by solar radiation, specifically in the UVB range (Ortega-Retuerta et al. 2009; Wurl et al. 2011). For biomass, the cell pellets were used instead of EPS by twice washing with 0.1% peptone, and a portion of biomass was used to determine DW, and designed amount of fresh cells that equaled to 2.50 mg DW was mixed in each metal, and a mixture of Al^{3+} and Fe^{2+} solution for biosorption study under the same condition as EPS biosorption. After 30 min exposure, the EPS or cell suspension was centrifuged at 8000 rpm for 15 min to remove EPS or biomass. Finally, the supernatant was used to determine Al^{3+} and/or Fe^{2+} contents for quantification of Al^{3+} and/or Fe^{2+} biosorption by EPS or biomass. The metal removal was calculated from the different amounts between the initial and the remaining concentrations in the solution. The biosorption capacity was defined as milligram toxicity removed per gram dry weight EPS or biomass.

Statistical analysis

All data shown in this study, otherwise stated, are mean values of three replications with their standard deviations. The data were subjected to one-way analysis of variance using SPSS software, version 16.0. Means were separated by analysis of variance (ANOVA), and the significant differences were assessed by Duncan's multiple range tests at $P < 0.05$. Independent sample t test analysis was used to compare means between Al and Fe contents in each pair. The function of Command Syntax in SPSS 16.0 was used to analyze data for comparing the removal of metals by EPS between microaerobic light and aerobic dark conditions.

Results

Inhibitory effects of Al^{3+} and Fe^{2+} on acid-resistant PNSB and cell morphology

Table 1 shows the sensitivity of tested PNSB grown in BIM broth (without phosphate composition) containing various metal toxicities at pH 4.50 and 5.00 for the reference strain under both incubating conditions. Based on values of MIC, MBC, and IC_{50}, it was found that *R. palustris* strains TLS06 and VNW64 had the greatest resistance to both Al^{3+} and Fe^{2+}. In contrast, the reference strain, *R. acidophilus* ATCC 25092 was the most sensitive to both metallic ions. Under Al^{3+} stress with aerobic dark conditions based on MIC values in a range of 100–850 mg L^{-1}, the resistant capacity was ranked as TLS06 ~ VNW64 > VNS89 > VNW02 > ATCC 25092;

Table 1 Sensitivity of acid-resistant PNSB to Al^{3+} and Fe^{2+} grown in acidic BIM or ATCC 650 (for reference strain) broth with no added phosphate composition containing various concentrations of individual metal ion under aerobic dark and microaerobic light conditions for 72 h

Metal	Strain	Growth condition					
		Aerobic dark			Microaerobic light		
		MIC (mg L^{-1})	MBC	IC_{50}	MIC	MBC	IC_{50}
Al^{3+}	VNW02	750[c]	750[d]	85.2[c]	500[c]	550[d]	79.2[d]
	TLS06	850[a]	900[b]	175.1[a]	600[b]	650[b]	142.3[b]
	VNW64	850[a]	950[a]	128.6[b]	650[a]	750[a]	180.4[a]
	VNS89	800[b]	800[c]	116.7[c]	600[b]	600[c]	96.5[c]
	ATCC 25092	100[d]	100[e]	40.2[d]	100[d]	100[e]	30.4[e]
	Sig	*	*	*	*	*	*
Fe^{2+}	VNW02	1500[b]	1400[c]	579.4[c]	1500[b]	1600[b]	753.5[b]
	TLS06	1700[b]	1800[b]	769.3[b]	1600[b]	1750[ab]	886.4[a]
	VNW64	2100[a]	2200[a]	908.1[a]	2000[a]	2100[a]	880.2[a]
	VNS89	1100[c]	1200[c]	521.9[c]	1000[c]	1150[c]	427.4[c]
	ATCC 25092	350[d]	400[d]	216.6[d]	400[d]	450[d]	154.3[d]
	Sig	*	*	*	*	*	*

Significant difference at $P < 0.05$ (*) is used to analyze mean values ($n = 3$) in each column as indicating by different lowercase letters

under microaerobic light conditions, the resistant capacity (100–650 mg L^{-1}) was in the order of VNW64 > TLS06 ~ VNS89 > VNW02 > ATCC 25092. In Fe^{2+} stress under both incubating conditions, strain VNW64 was the most resistant to Fe^{2+}, followed by descending order of TLS06 ~ VNW02, VNS89, and ATCC 25092. On the basis of MBC values, a similar result was observed for the sensitivity of tested PNSB to both metal ions under both incubating conditions as strain VNW64 was the most resistant to individual Al^{3+} and Fe^{2+}; while strain ATCC 25092 was the most sensitive. The IC_{50} values for Al^{3+} of our PNSB strains were in a range of 85.2–175.1 mg L^{-1} and 79.2–180.4 mg L^{-1} under conditions of aerobic dark and microaerobic light, respectively; and they were 40.2 and 30.4 mg L^{-1} for the reference strain. For the Fe^{2+} set, IC_{50} values under conditions of aerobic dark and microaerobic light were between 521.9–908.1 and 427.4–886.4 mg L^{-1} for our PNSB strains and 216.6 and 154.3 mg L^{-1} for the reference strain. Overall result showed that strain VNW64 was the most resistant to both metal ions under both incubating conditions although based on IC_{50} strain TLS06 was better to resist Al^{3+} under aerobic dark conditions.

Bacterial cell morphology using SEM after growing PNSB cells in BIM medium at pH 4.25 with or without Al^{3+} or Fe^{2+} under microaerobic light conditions for 48 h is shown in Fig. 1. For control sets, both PNSB strains (TLS06 and VNW64) showed rod-shaped cells as normal cells (Fig. 1a, d). In contrast, strain TLS06 under stress condition with either Al^{3+} or Fe^{2+} cells was altered as cell deformations with a wrinkle at the exterior surface (Fig. 1b, c); abnormal cells of strain VNW64 differed from strain TLS06 (Fig. 1e, f). Table 2 shows the accumulated amount of aluminum or iron in percent of the total element components found in PNSB cells with significantly higher accumulations found in only strain TLS06 with the mean of 1.10% for aluminum and only 0.16% for iron.

Uptake and allocation of Al^{3+} and/or Fe^{2+} toxicity in different cellular components

The uptakes of Al^{3+} and/or Fe^{2+} in different cellular components of tested PNSB and a reference strain are illustrated in Table 3. Accumulation of Al^{3+} or Fe^{2+} as individual in all tested PNSB under both incubating conditions was in the order of cell wall > cytoplasm > cell membrane. This accumulated metal order was also observed in a mixed metal set only under conditions of aerobic dark as microaerobic light was in the degree of cytoplasm > cell wall > cell membrane. In details, under aerobic dark conditions, Al^{3+} accumulation in cell wall, cytoplasm, and cell membrane for sets of individual Al^{3+} and a mixture of both metal ions were in the range of 4.70–15.48, 0.31–16.93, and 0.10–12.51%, respectively. A similar trend was found for accumulated Al^{3+} (%) in an individual Al^{3+} set under microaerobic light conditions; 14.78–41.60 in cell wall, 0.36–26.49 in cytoplasm, and 0.38–18.43 in plasma membrane. On the other hand, percent Al^{3+} in a mixture of Al^{3+} and Fe^{2+} under microaerobic light conditions was in the degree of cytoplasm > cell wall > cell membrane; 0.48–37.75, 12.11–20.84, and 1.09–16.20, respectively.

Individual Fe^{2+} uptake in cell wall and cell membrane was similar under both incubating conditions as the maximum level found under microaerobic light conditions,

Fig. 1 SEM photomicrographs showing morphological changes of PNSB grown in BIM broth with no added phosphate composition, pH 4.25 containing 80 mg Al^{3+} L^{-1} or 300 mg Fe^{2+} L^{-1} (no metal addition served as control) for 48 h under microaerobic light conditions: (**a**, **b**, **c**) for strain TLS06 and (**d**, **e**, **f**) for strain VNW64
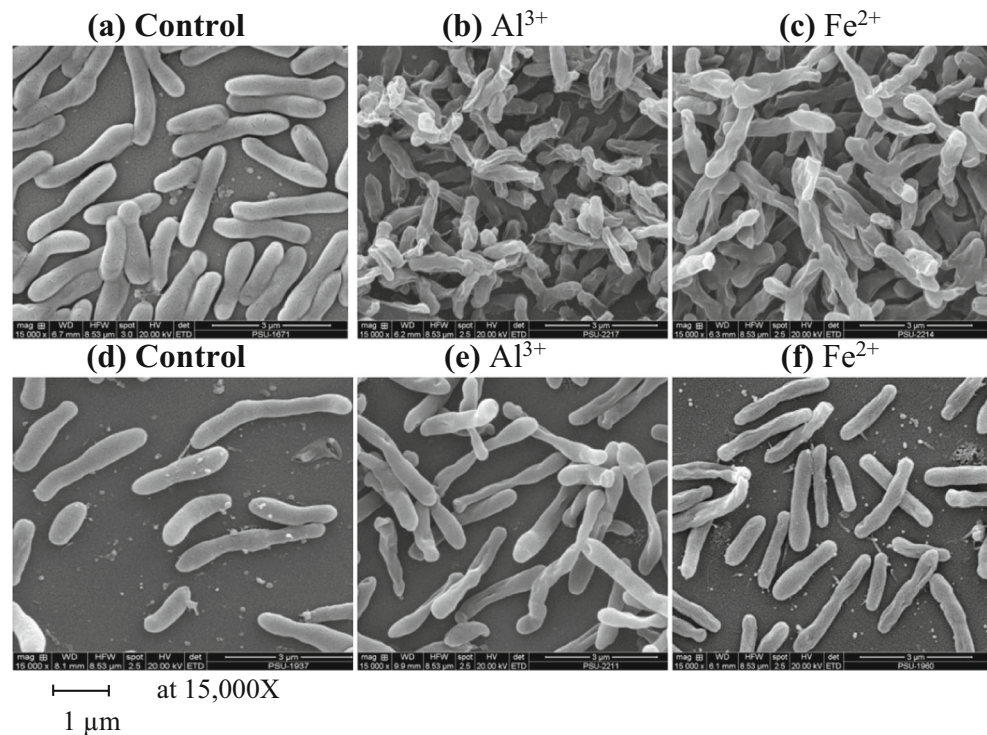
but no different uptake in cytoplasm for both incubating conditions (see details in Table 3). On the other hand, uptake of Fe^{2+} or Al^{3+} in a mixture of both metallic ions was similar as the metal uptake in cell wall > cytoplasm > cell membrane under aerobic dark conditions, while under microaerobic light conditions found metal accumulated in cytoplasm > cell wall > cell membrane. However, the metal uptakes based on the loss of metal ions from the culture broths included undetected metals as well. High percentages of undetected metals (Al^{3+} and Fe^{2+}) are illustrated in Table 3. Under aerobic dark conditions, the undetected Al^{3+} in an individual Al^{3+} set (5.56–46.19%) was lower than in a mixed metal set (47.70–69.11%); while under microaerobic light conditions, it was 14.68–38.33% and 13.00–28.93%, respectively. However, undetected Fe^{2+} in conditions of aerobic dark and microaerobic light in sets of individual Fe^{2+} (7.31–18.27 and 1.80–8.36%) and a mixture of both metals (8.67–14.58 and 3.32–10.68%) was much lower than that of undetected Al^{3+}.

Removal of Al^{3+} and/or Fe^{2+} by biomass and EPS from acid-resistant PNSB

EPS production under the optimum growth conditions of all tested PNSB grown in acidic BIM or ATCC® Medium 650 broth provided biomass that ranged from 0.52 to 0.98 g DW L^{-1} under aerobic dark conditions and 0.42 to 0.75 g DW L^{-1} under microaerobic light conditions. These PNSB released EPS in a range of 0.26–0.84 g DW L^{-1} under microaerobic light and 0.15–1.00 g DW L^{-1} under aerobic dark. The reference strain showed the minimum for both biomass and EPS under both incubating conditions. All data were used to calculate for setting biosorption experiment to obtain a final suspension of 2.50 mg dry weight EPS mL^{-1} in deionized water.

EPS composition of each PNSB under both incubating conditions as shown in Table 4 found that a major composition was carbohydrate (42.5–55.8%), followed by nucleic acid (25.4–31.2%), protein (8.5–17.5%), and unidentified compound (8.1–10.5%). However, under both incubating

Table 2 Analysis of Al^{3+} or Fe^{2+} using SEM-EDS for its accumulation in PNSB cells grown in acidic BIM broth with no added phosphate composition containing 80 mg Al^{3+} L^{-1} or 300 mg Fe^{2+} L^{-1} under microaerobic light conditions for 48 h

Metal	Al			Fe		
Strain	Minimum (%)	Mean	Maximum	Minimum	Mean	Maximum
TLS06	0.96	$1.10^a \pm 0.12$	1.31	0.10	$0.16^b \pm 0.05$	0.27
VNW64	1.37	$1.54^a \pm 0.11$	1.69	1.17	$1.45^a \pm 0.28$	2.08

Each value is a mean of ten randomly detected values ± standard deviation (S.D.). Different lowercase letters in each row indicate significant difference at $P < 0.01$

Table 3 Uptake of Al^{3+} and/or Fe^{2+} in PNSB cellular parts when grown in acidic BIM or ATCC 650 broth with no added phosphate composition containing 80 mg Al^{3+} L^{-1}, 300 mg Fe^{2+} L^{-1}, and both metals (80 mg Al^{3+} L^{-1} + 300 Fe^{2+} mg L^{-1}) under aerobic dark and microaerobic light conditions for 48 h

Metal	Strain	Percent of Al^{3+} and Fe^{2+} in culture supernatant and PNSB cellular parts									
		Aerobic dark					Microaerobic light				
		Culture supernatant	Cell wall	Cell membrane	Cytoplasm	Undetected	Culture supernatant	Cell wall	Cell membrane	Cytoplasm	Undetected
Al^{3+}	VNW02	17.08[c]	15.01[ab]	11.31[b]	14.11[c]	42.49[ab]	14.43[b]	32.84[c]	19.39[a]	18.66[b]	14.68[c]
	TLS06	17.79[c]	15.48[a]	10.95[c]	15.05[b]	40.73[bc]	13.55[c]	41.60[a]	9.86[b]	11.50[c]	23.49[b]
	VNW64	9.56[d]	14.81[b]	12.51[a]	16.93[a]	46.19[a]	10.42[d]	39.50[b]	7.75[b]	4.00[d]	38.33[a]
	VNS89	65.52[a]	7.98[c]	9.71[d]	11.23[d]	5.56[d]	9.43[e]	26.81[d]	18.43[a]	26.49[a]	18.84[bc]
	ATCC 25092	56.62[ab]	4.73[d]	0.30[e]	0.31[e]	38.04[c]	60.10[a]	14.78[e]	0.38[c]	0.36[e]	24.38[b]
	Sign	**	**	**	**	**	**	**	**	**	**
Fe^{2+}	VNW02	81.69[bc]	0.42[d]	0.13[c]	0.37[b]	17.39[ab]	89.60[c]	1.15[b]	0.34[a]	0.55[a]	8.36[a]
	TLS06	82.36[b]	0.65[a]	0.18[a]	0.51[a]	16.30[b]	95.71[b]	0.93[c]	0.18[b]	0.19[b]	2.99[b]
	VNW64	80.58[c]	0.48[c]	0.16[b]	0.51[a]	18.27[a]	89.83[c]	1.44[a]	0.33[a]	0.55[a]	7.85[a]
	VNS89	82.87[b]	0.54[b]	0.18[a]	0.32[c]	16.09[b]	95.91[b]	0.92[c]	0.16[c]	0.19[b]	2.83[b]
	ATCC 25092	92.12[a]	0.27[e]	0.15[b]	0.15[d]	7.31[c]	97.66[a]	0.28[d]	0.12[d]	0.14[c]	1.80[c]
	Sign	**	**	**	**	**	**	**	**	**	**
Al^{3+} + Fe^{2+}	Al^{3+}										
	VNW02	16.02[d]	8.53[a]	1.86[d]	4.48[b]	69.11[a]	21.89[c]	17.34[b]	14.96[b]	31.23[b]	14.53[c]
	TLS06	22.17[b]	7.90[a]	3.81[a]	5.08[a]	61.04[c]	23.34[b]	15.65[c]	16.20[a]	27.49[c]	13.00[d]
	VNW64	16.18[d]	8.90[a]	3.51[b]	4.64[b]	66.76[b]	16.44[e]	18.75[b]	14.06[c]	37.75[a]	20.33[b]
	VNS89	18.72[c]	7.85[a]	2.73[c]	3.16[c]	67.54[ab]	18.22[d]	20.84[a]	13.61[d]	26.95[c]	20.39[b]
	ATCC 25092	44.72[a]	4.70[b]	0.10[e]	2.78[d]	47.70[d]	57.39[a]	12.11[d]	1.09[e]	0.48[d]	28.93[a]
	Sign	**	**	**	**	**	**	**	**	**	**
	Fe^{2+}										
	VNW02	86.78[b]	2.19[b]	0.62[a]	1.74[a]	8.67[c]	94.05[ab]	0.89[b]	0.44[a]	1.31[b]	3.32[d]
	TLS06	85.94[b]	2.22[a]	0.61[a]	1.72[a]	9.51[bc]	93.20[b]	0.81[c]	0.40[b]	1.10[c]	4.51[c]
	VNW64	81.33[c]	2.65[a]	0.37[c]	1.07[b]	14.58[a]	89.89[c]	1.61[a]	0.40[b]	1.40[a]	10.68[a]
	VNS89	84.72[b]	2.18[b]	0.51[b]	0.65[c]	11.94[b]	85.90[d]	0.85[bc]	0.42[ab]	1.39[a]	7.46[b]
	ATCC 25092	90.26[a]	0.67[c]	0.06[d]	0.15[d]	8.86[c]	94.79[a]	0.71[d]	0.20[c]	0.84[d]	3.45[d]
	Sign	**	**	**	**	**	**	**	**	**	**

Each value is an average of three determinations. Different lowercase letters in each column indicate significant difference at $P < 0.01$ (**)

conditions, strain VNW64 had the minimum carbohydrate content but the maximum contents for nucleic acid and protein; while the opposite result for all component contents was observed in a reference strain. Among the other PNSB strains compared with strain VNW64 and the reference strain, it was found that under both incubating conditions, strain TLS06 showed significant differences for almost EPS composition contents with strain VNW64 but similar with the reference strain, except for protein content as a significantly higher content. However, only strains VNW64 and TLS06 showed the highest resistant to both metal ions as previously described.

Therefore, only strains VNW64 and TLS06 were chosen for testing biosorption to remove Al^{3+} and/or Fe^{2+}.

The use of biomass and EPS of the great potential PNSB strains for biosorption (Al^{3+} and Fe^{2+}) under both incubating conditions is presented in Fig. 2. Regarding an initial concentration of 80 mg Al^{3+} L^{-1}, the removal efficacy of Al^{3+} by EPS of both strains under both incubating conditions at pH 4.25 was significantly higher than biomass, and a significantly higher removal efficiency was found in VNW64 compared with strain TLS06 (Fig. 2a). For example, Al^{3+} removal efficiency by EPS from strains TLS06 and VNW64 under

Table 4 The component of exopolymeric substances (EPS) produced by PNSB strains of *R. palustris* and *R. acidophila* ATCC 25092 grown in BIM, pH 4.50 and ATCC 650 broth, pH 5.00 under aerobic dark and microaerobic light conditions

Strain	Growth condition							
	Aerobic dark				Microaerobic light			
	Carbohydrate (%)	Nucleic acid	Protein	Unidentified	Carbohydrate	Nucleic acid	Protein	Unidentified
VNW02	$48.5^a \pm 1.11$	$29.7^{ab} \pm 1.35$	$13.1^b \pm 1.30$	8.7 ± 0.26	$49.9^{bc} \pm 1.2$	$28.6^{ab} \pm 1.9$	$12.3^b \pm 0.3$	9.2 ± 2.0
TLS06	$49.6^a \pm 2.79$	$27.5^{ab} \pm 1.82$	$13.5^b \pm 1.23$	9.4 ± 1.75	$52.2^{ab} \pm 2.4$	$27.0^{bc} \pm 1.6$	$11.0^c \pm 0.7$	9.8 ± 1.5
VNW64	$42.5^b \pm 3.70$	$31.2^a \pm 2.00$	$17.5^a \pm 1.31$	8.8 ± 1.08	$46.6^c \pm 3.5$	$30.1^a \pm 2.0$	$15.2^a \pm 0.6$	8.1 ± 1.7
VNS89	$54.2^a \pm 2.69$	$27.0^b \pm 1.18$	$9.2^c \pm 1.06$	9.6 ± 1.65	$54.9^a \pm 2.1$	$26.8^{bc} \pm 0.9$	$8.9^d \pm 0.4$	9.4 ± 1.7
ATCC 25092	$53.1^a \pm 3.66$	$26.8^b \pm 3.29$	$9.6^c \pm 0.20$	10.5 ± 2.05	$55.8^a \pm 1.5$	$25.4^c \pm 0.6$	$8.5^d \pm 0.7$	10.3 ± 0.6
Sig	**	*	**	ns	**	**	**	ns

Values are means and their S.D. ($n = 3$). Different lowercase letters in each column indicate significant difference at $P < 0.05$ (*) and $P < 0.01$ (**); ns, non-significant ($P > 0.05$)

microaerobic light conditions was 36.12 and 60.10% for individual set and 27.33 and 52.47% for a mixed metal set (Fig. 2a). On the other hand, under the same condition, the removal efficiency by both biomasses was 8.55 and 12.98% for individual and 8.47 and 13.46% for a mixture of Al^{3+} and Fe^{2+}. Similarly, for aerobic dark conditions, removal percentages by both EPS (TLS06 and VNW64) for individual Al^{3+} and a mixture of both metal ions were 29.71 and 59.63% and 36.56 and 57.06%, respectively; on the other hand, removal efficiencies by both biomasses were 12.70 and 26.11% for sets of individual and 9.92 and 20.20% for a mixture of Al^{3+} and Fe^{2+}. Overall result showed that strain VNW64 was the most effective for removing Al^{3+} by EPS and its biomass under individual Al^{3+} or a mixture ($Al^{3+} + Fe^{2+}$) under both incubating conditions.

The removal efficiency of Fe^{2+} (initial 300 mg L^{-1}) by EPS of both PNSB strains under both incubating conditions had the similar trend for individual Fe^{2+}, and a mixture of Al^{3+} and Fe^{2+} with the removal efficiency of Al^{3+} and a mixture of Al^{3+} and Fe^{2+} (Fig. 2a, b). However, in the comparison between both PNSB, strain VNW64 achieved a higher efficiency to remove individual Fe^{2+} and also mixed metals than strain TLS06 by only EPS under both incubating conditions as removal efficiency by their biomasses the significant differences was found only in a mixed metal under aerobic dark conditions (Fig. 2b). For instance, the removal efficiencies of Fe^{2+} under microaerobic light conditions by EPS from strains TLS06 and VNW64 were 18.99 and 50.96% in individual and 22.71 and 47.54% in mixed metal ions; while by their biomasses were 9.58 and 9.75% for the individual and 8.04
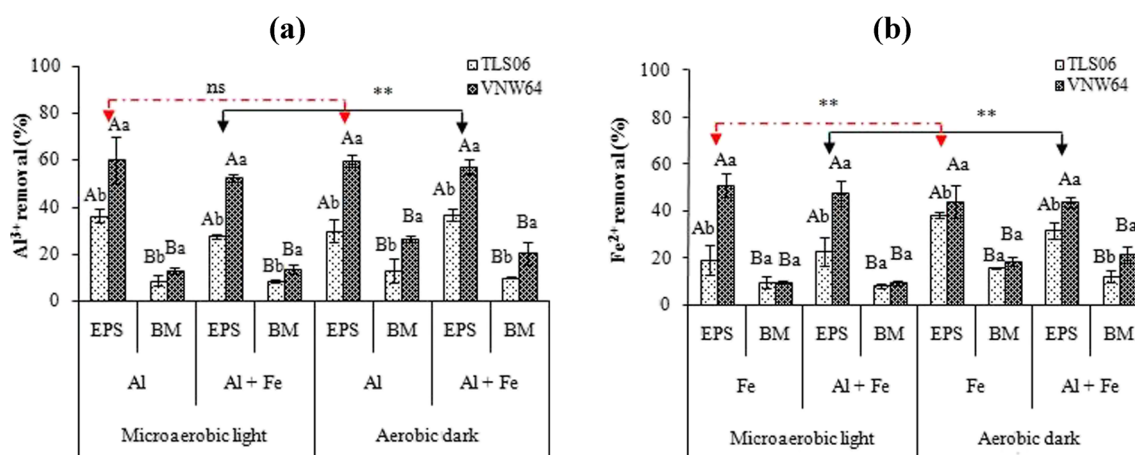

(a)

(b)

Fig. 2 Removal of metals (**a**) for Al^{3+} and (**b**) for Fe^{2+} using biosorption by exopolymeric substances (EPS) or biomass (BM) of PNSB in acidic condition (pH 4.25) containing 80 mg Al^{3+} L^{-1} and/or 300 mg Fe^{2+} L^{-1} under conditions of aerobic dark and microaerobic light. Different uppercase and lowercase letters indicate significant differences for removal efficiencies by a pair of EPS–biomass and a pair of strains TLS06–VNW64, respectively. No significant difference (ns) and ** ($P < 0.01$) are used to indicate effect of incubating conditions on removal of metal ions by EPS from both strains

and 9.37% for the mixed metal ions. Under aerobic dark conditions, removal percentages of Fe^{2+} by EPS (TLS06 and VNW64) were 38.21 and 44.23% as compared to biomasses (15.79 and 18.49%) for individual Fe^{2+}. On the other hand, removal percentage of Fe^{2+} was lower in a mixed metallic ion as 31.55 and 43.64% by EPS (TLS06 and VNW64) and 12.19 and 21.44% by both biomasses.

Removal of individual Al^{3+} by EPS from both PNSB (TLS06 and VNW64) showed no significant difference under both incubating conditions (microaerobic light and aerobic dark); however, its removal in a mixed metal set under conditions of aerobic dark was significantly higher than microaerobic light (Fig. 2a). Removal of Fe^{2+} by EPS from both PNSB in sets of individual and a mixed metal resulted to significant increase under conditions of aerobic dark compared to microaerobic light that was observed only in EPS from strain TLS06 (Fig. 2b).

Discussion

Capacity of PNSB resistance to toxicities of Al^{3+} and Fe^{2+} under acidic condition

Results in Table 1 found that all tested PNSB grew up under both metal toxicities at the contents which were much higher than the highest values found in paddy fields on ASS (Khuong et al. 2017) and also the critical levels for rice growth as previously stated. For example, under conditions of microaerobic light and aerobic dark, IC_{50} values from Fe^{2+} set showed up to 886 and 908 mg L^{-1}, but their values from the Al^{3+} set were maximum at 180 and 175 mg L^{-1}, respectively. This indicates that our acid-resistant PNSB might be used for bioremediation of rice grown on ASS due to their resistance to high concentrations of both toxic metals under acidic condition at pH 4.50. It is not surprising that all PNSB strains tested were more sensitive to Al^{3+} than Fe^{2+} under both incubating conditions; this is because of their biological functions. Aluminum is unnecessary for the metabolism of organisms; while iron is used as a micronutrient for the growth and metabolism of microbes (Bose and Newman 2011). This leads to aluminum being more toxic than ferrous iron for both bacteria and rice.

All organisms require an amount of trace elements including some heavy metals for their growth, but excessive levels of metabolic metals can be detrimental to organisms. For PNSB, the high concentrations of both aluminum and ferrous showed an adverse effect on shaped cells (Fig. 1) although they were able to resist on Al^{3+} and Fe^{2+} under acidic conditions. As a result, the present study revealed that the morphological characterizations of bacterial cells were changed into wrinkle at the exterior surface and bleb-like structures on the surface in BIM broth at pH 4.25 containing 80 mg Al^{3+} L^{-1} or

300 mg Fe^{2+} L^{-1} concentrations under microaerobic light conditions for 48 h. The results are in accordance with the previous study as heavy metals, such as Cu and Zn, altered the cellular morphology of *Rhodobium marinum* NW16 and *Rhodobacter sphaeroides* KMS24 from rods to filamentous or dumbbell shapes (Panwichian et al. 2011). The change of cell surface to cell elongation resulted from the reduction of surface area–volume ratio, and this reduction ratio significantly decreased the uptake sites on the cell surface for the heavy metals (Neumann et al. 2005). Hence, cell elongation might be an adaptive response of bacteria under stress conditions. This is supported by the evidence that more accumulation of aluminum in strain TLS06 caused more abnormal shaped cells compared to iron with less accumulation (Fig. 1b, c and Table 2).

Uptake and allocation of Al^{3+} and/or Fe^{2+} toxicity in different cellular components

In general, the biosorption process of heavy metals pertained to two stages. Firstly, it is defined as passive biosorption (biosorption) due to metabolism independent of metal ions that are absorbed to the cell surface by functional groups (carboxyl, amine, hydroxyl, phosphate, sulfur, sulfide, etc.) on the cell wall component (Boeris et al. 2016). This step takes immediately less than 30 min by principles of metal binding, which included coordination, complexation, ion exchange, physical adsorption (e.g., electrostatic) or inorganic microprecipitation (Goyal et al. 2003). Subsequently, it is called active biosorption (bioaccumulation) as metals were transported to the cell membrane and enter into the cytoplasm. This step might take more than one month (Wang and Chen 2006). Both steps of Al^{3+} and/or Fe^{2+} biosorption were studied in this work, and Table 3 shows that both metal ions were the most to uptake using passive biosorption as the most abundant found in the cell wall. However, high metal levels in this study allowed cells to uptake metal ions using active biosorption for increasing cell resistance to metals as some of the metals changing to be complex as an evidence of undetected metal ions. The results are in accordance with *R. marinum* NW16 and *R. sphaeroides* KMS24 as amounts of Cu^{2+} and Zn^{2+} accumulated in cell wall > cytoplasm > cell membrane (Panwichian et al. 2011).

As the loss of metal ions was the difference between initial concentration and the remaining amount in the culture supernatant. This means that undetected metal ions might be in a complex form or acting for biological roles, particularly for Fe^{2+} (Bose and Newman 2011). However, the amount of undetected Al^{3+} was much higher than undetected Fe^{2+} (Table 3); this could be explained by the lower removal efficiency for Fe^{2+} compared to Al^{3+}. For example, under microaerobic light and aerobic dark conditions, strain VNW64 gained the highest uptake of Al^{3+} roughly 90% but

only 10.17 and 19.42% of Fe^{2+}, respectively. The lower removal efficiency was due to the higher initial concentration of Fe^{2+} compared to Al^{3+}, including competition between cations with bivalency compared to trivalent cations. This led to more accumulation of aluminum than iron in bacterial cells (Table 2) although aluminum is an unnecessary element for biological functions. As the ability of metal accumulation under aerobic dark was higher than under microaerobic light conditions were found only for Fe^{2+}, this suggests that Fe^{2+} might be used for biological functions in a higher level under aerobic conditions. It should be noted that for Al^{3+} resistance of PNSB-like strain TLS06 in addition of both metal biosorptions, other mechanisms should be involved. This is supported by altered cell shape under stress of Al^{3+}, which was much more frequent than under stress of Fe^{2+} compared to the control (Fig. 1a, b, c).

Removal of Al^{3+} and/or Fe^{2+} by biomass and EPS from acid-resistant PNSB

The bacterial EPS has negative functional groups, such as carboxyl (–COOH), hydroxyl (–OH), and amide (N–H) for binding metal ions (Hou et al. 2013). As the EPS proportion in *R. palustris* strains VNW64 and TLS06 was similar with *R. palustris* PP803 (Table 4 and Nunkaew et al. 2015, carbohydrate > nucleic acid > protein > unidentified compound), strain PP803 had galacturonic acid as the most important part in EPS for binding Na^+ in aqueous solution (Nunkaew et al. 2015). This suggests that *R. palustris* strains also used their EPS for binding other cations, such as Al^{3+} and Fe^{2+}. Bacterial EPS has the ability for Al^{3+} adsorption which was also explained via a binding mechanism as Al^{3+} that was embedded by functional groups; –OH, N–H, C=O, and C–N are found in loosely bound EPS and tightly bound EPS (Ruan et al. 2013). For aluminum biosorption by biomass or EPS, the efficiency of adsorption of Al^{3+} was significantly different between strains VNW64 and TLS06 (Fig. 2). This reflected to the EPS component of both strains as significantly higher of protein content in the former strain resulting to higher removal efficiency (Table 4 and Fig. 2). In addition, strain VNW64 might have the tightly bound EPS being attached with biomass that provided higher efficiency to its biomass. This indicates why strain VNW64 was the most resistant to both metal ions among the tested PNSB (Table 1). It is not surprising that the removal efficiency of EPS was much better than biomass (Fig. 2a); this is due to a limitation of time exposure as only 30 min; so too little for bioaccumulation by live biomass. The maximum adsorption capacity of *Chryseomonas luteola* TEM05 was 55.2 mg Al^{3+} g^{-1} for 1 h incubation with initial concentration of 5 mg L^{-1} under optimal condition (Ozdemir and Baysal 2004). In this study, biosorption capacity of VNW64 biomass was roughly 13 mg Al^{3+} g^{-1} for only 30 min, but the initial concentration was much higher. This is

because the removal efficiency in this study was not high as the maximum was only 60%; and this suggests that optimal conditions of biomass or EPS dose, exposure time, pH value, and initial concentration of toxicity should be investigated to achieve the greatest efficiency.

Biosorption of Fe^{2+} by EPS as individual was higher than that of a mixture with Al^{3+} (Fig. 2b); this is due to that in the presence of Al^{3+}, EPS might have impact by Al^{3+} separation from Al–EPS mixture to form $[Al_6(OH)_{12}(H_2O)_{12}]^{6+}$ and $[Al_{13}(OH)_{32}]^{7+}$ which prevented other cations in exposure to the binding sites (Lee et al. 2004). The offered mechanism can be explained that a component of EPS as carboxyl group (R-COOH) cause to be the formation of ferrous oxalates (FeC_2O_4) including both ferrous formate dihydrate ($FeC_2O_4.2H_2O$) and ferrous formate hydrate ($FeC_2O_4.H_2O$) (Tapia et al. 2016), and other organic compounds (Tapia et al. 2013). Due to Fe^{2+} has a biofunction for organisms, this led to no significant difference observed for the removal efficiency by biomasses from both PNSB (Fig. 2b). However, strain VNW64 showed significantly higher efficiency to bind with Fe^{2+} in mixed metal ions than strain TLS06 under aerobic dark conditions. This implies that the former strain might have tightly bound EPS as previously described; and this led to its more metal resistance.

It is clearly shown that oxygen levels affect the removal of metals by EPS from both PNSB in the presence of iron as removal of Al^{3+} in a mixed metal with Fe^{2+}, which was significantly higher in aerobic dark compared to microaerobic light conditions (Fig. 2a). This is because redox values affect iron forms to less solubility under higher oxygen levels (Swanner et al. 2017); thus, this led to increase in binding sites for Al^{3+} to bind with EPS. The presence of iron significantly changed removal of Fe^{2+} and Al^{3+} by both EPS under aerobic dark compared to microaerobic light conditions (Fig. 2) indicating that oxygen levels produced more influence on metal removal than that found for light intensity. This is an evidence of no significantly different for individual Al^{3+} by both EPS under both incubating conditions (Fig. 2a). As EPS, particularly TEP are degraded by light (Wurl et al. 2011), this could be a short exposure time to light of EPS as only 30 min biosorption so no damage of both EPS by light. EPS from strain TLS06 showed significant increases to remove Fe^{2+} in both sets of individual and a mixed metal under aerobic dark conditions, suggesting that its EPS property differed from EPS from strain VNW64 (Table 4). This might be that EPS from strain TLS06 was sensitive to light as higher metal (Al^{3+} and Fe^{2+}) removal efficiency was found under aerobic dark (Fig. 2a, b).

Conclusions

The resistance capacity of potential acid-resistant PNSB to the toxicity of Al^{3+} and/or Fe^{2+} was found at a higher level than

critical concentration for rice growth; this is because they used both biosorption and bioaccumulation mechanisms including adaptation of cell shape. The highest protein content in EPS component of strain VNW64 corresponded to the highest resistant to both metal ions and also metal biosorption efficiency. The overall result proved that the tested acid-resistant PNSB, especially strains VNW64 and TLS06, have the high possibility to be applied for bioremediation of paddy fields on ASS to improve rice yield.

References

Aguilera A, Souza-Egipsy V, San Martín-Úriz P, Amils R (2008) Extraction of extracellular polymeric substances from extreme acidic microbial biofilms. Appl Microbiol Biot 78(6):1079–1088

Attanandana T, Vacharotayan S (1986) Acid sulfate soils: their characteristics, genesis, amelioration and utilization. Southeast Asian Stud 24:155–180

Auger C, Han S, Appanna VP, Thomas SC, Ulibarri G, Appanna VD (2013) Metabolic reengineering invoked by microbial systems to decontaminate aluminum: implications for bioremediation technologies. Biotechnol Adv 31:266–273

Boeris PS, Agustín MR, Acevedo DF, Lucchesi GI (2016) Biosorption of aluminum through the use of non-viable biomass of *Pseudomonas putida*. J Biotechnol 236:57–63

Bose A, Newman DK (2011) Regulation of the phototrophic iron oxidation (*pio*) genes in *Rhodopseudomonas palustris* TIE-1 is mediated by the global regulator, Fix K. Mol Microbiol 79(1):63–75

Choudhury BU, Malang A, Webster R, Mohapatra KP, Verma BC, Kumar M, Das A, Islam N, Hazarika S (2017) Acid drainage from coal mining: effect on paddy soil and productivity of rice. Sci Total Environ 583:344–351

DuBois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3):350–356

Eboigbodin KE, Biggs CA (2008) Characterization of the extracellular polymeric substances produced by *Escherichia coli* using infrared spectroscopic, proteomic, and aggregation studies. Biomacromolecules 9(2):686–695

Engel A, Piontek J, Grossart HP, Riebesell ULF, Schulz KG, Sperling M (2014) Impact of CO$_2$ enrichment on organic matter dynamics during nutrient induced coastal phytoplankton blooms. J Plankton Res 36(3):641–657

Fernández-Dávila ML, Razo-Estrada AC, García-Medina S, Gómez-Oliván LM, Piñón-López MJ, Ibarra RG, Galar-Martínez M (2012) Aluminum-induced oxidative stress and neurotoxicity in grass carp (Cyprinidae—*Ctenopharingodon idella*). Ecotoxicol Environ Saf 76:87–92

Ferreira ML, Casabuono AC, Stacchiotti ST, Couto AS, Ramirez SA, Vullo DL (2016) Chemical characterization of *Pseudomonas veronii* 2E soluble exopolymer as Cd (II) ligand for the biotreatment of electroplating wastes. Int Biodeter Biodegr 119:605–613

Gautam RK, Mudhoo A, Lofrano G, Chattopadhyaya MC (2014) Biomass-derived biosorbents for metal ions sequestration: adsorbent modification and activation methods and adsorbent regeneration. J Environ Chem Eng 2:239–259

Goyal N, Jain SC, Banerjee UC (2003) Comparative studies on the microbial adsorption of heavy metals. Adv Environ Res 7:311–319

Hou W, Ma Z, Sun L, Han M, Lu J, Li Z, Mohamad OA, Wei G (2013) Extracellular polymeric substances from copper-tolerance *Sinorhizobium meliloti* immobilize Cu^{2+}. J Hazard Mater 261:614–620

Ilamathi R, Nirmala GS, Muruganandam L (2014) Heavy metals biosorption in liquid solid fluidized bed by immobilized consortia in alginate beads. Int J Chem Tech Res 6:652–662

Johnson JL (1981) In: Gerhardt P, RGE M, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GB (eds) Manual of methods for general bacteriology. American Society for Microbiology, Washington, DC 20006, p 456

Jones AM, Xue Y, Kinsela AS, Wilcken KM, Collins RN (2016) Donnan membrane speciation of Al, Fe, trace metals and REEs in coastal lowland acid sulfate soil-impacted drainage waters. Sci Total Environ 547:104–113

Kantachote D, Nunkaew T, Kantha T, Chaiprapat S (2016) Biofertilizers from *Rhodopseudomonas palustris* strains to enhance rice yields and reduce methane emissions. Appl Soil Ecol 100:154–161

Khuong NQ, Kantachote D, Onthong J, Sukhoom A (2017) The potential of acid-resistant purple nonsulfur bacteria isolated from acid sulfate soils for reducing toxicity of Al^{3+} and Fe^{2+} using biosorption for agricultural application. Biocatal Agric Biotechnol 12:329–340

Lee HS, Suh JH, Kim IB, Yoon T (2004) Effect of aluminum in two-metal biosorption by an algal biosorbent. Miner Eng 17(4):487–493

Liang X, He CQ, Ni G, Tang GE, Chen XP, Lei YR (2014) Growth and Cd accumulation of *Orychophragmus violaceus* as affected by inoculation of Cd-tolerant bacterial strains. Pedosphere 24:322–329

McLachlan DRC (1995) Aluminium and the risk for Alzheimer's disease. Environmentrics 6:233–275

Mikutta R, Zang U, Chorover J, Haumaier L, Kalbitz K (2011) Stabilization of extracellular polymeric substances (*Bacillus subtilis*) by adsorption to and coprecipitation with Al forms. Geochim Cosmochim Ac 75(11):3135–3154

More TT, Yadav JSS, Yan S, Tyagi RD, Surampalli RY (2014) Extracellular polymeric substances of bacteria and their potential environmental applications. J Environ Manag 144:1–25

Neumann G, Veeranagouda Y, Karegoudar TB, Sahin Ö, Mäusezahl I, Kabelitz N, Kappelmeyer U, Heipieper H (2005) Cells of *Pseudomonas putida* and *Enterobacter* sp. adapt to toxic organic compounds by increasing their size. Extremophiles 9(2):163–168

Nookongbut P, Kantachote D, Megharaj M (2016) Arsenic contamination in areas surrounding mines and selection of potential As-resistant purple nonsulfur bacteria for use in bioremediation based on their detoxification mechanisms. Ann Microbiol 66(4):1419–1429

Nunkaew T, Kantachote D, Nitoda T, Kanzaki H, Ritchie RJ (2015) Characterization of exopolymeric substances from selected *Rhodopseudomonas palustris* strains and their ability to adsorb sodium ions. Carbohyd Polym 115:334–341

Ortega-Retuerta E, Passow U, Duarte CM, Reche I (2009) Effects of ultraviolet B radiation on (not so) transparent exopolymer particles. Biogeosciences 6(12):3071–3080

Ozdemir G, Baysal SH (2004) Chromium and aluminum biosorption on *Chryseomonas luteola* TEM05. Appl Microbiol Biot 64(4):599–603

Panhwar QA, Naher UA, Shamshuddin J, Radziah O, Hakeem KR (2016) Management of acid sulfate soils for sustainable rice cultivation in Malaysia. In: Hakeem K, Akhtar J, Sabir M (eds) Soil science: agricultural and environmental prospectives. Springer International Publishing, Cham, pp 91–104

Panwichian S, Kantachote D, Wittayaweerasak B, Mallavarapu M (2011) Removal of heavy metals by exopolymeric substances produced by resistant purple nonsulfur bacteria isolated from contaminated shrimp ponds. Electron J Biotechnol 14(4):2–2

Ruan X, Li L, Liu J (2013) Flocculating characteristic of activated sludge flocs: interaction between Al^{3+} and extracellular polymeric substances. J Environ Sci 25(5):916–924

Samaranayake P, Peiris BD, Dssanayake S (2012) Effect of excessive ferrous (Fe^{2+}) on growth and iron content in rice (*Oryza sativa*). Int J Agric Biol 14:296–298

Sheng GP, Yu HQ, Yue ZB (2005) Production of extracellular polymeric substances from *Rhodopseudomonas acidophila* in the presence of toxic substances. Appl Microbiol Biot 69(2):216–222

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150(1):76–85

Soltanpour PN, Johnson GW, Workman SM, Jones JB, Miller RO (1996) Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry. In: Sparks DL, Page AL, Helmke PA, Loeppert RH (ed.) Methods of soil analysis. Part 3—chemical methods. SSSA Book Ser. 5.3. SSSA, ASA, Madison, WI. doi:https://doi.org/10.2136/sssabookser5.3. Pp 91-139

Swanner ED, Bayer T, Wu W, Hao L, Obst M, Sundman A, Byrne JM, Michel FM, Kleinhanns IC, Kappler A, Schoenberg R (2017) Iron isotope fractionation during Fe (II) oxidation mediated by the oxygen-producing marine cyanobacterium *Synechococcus* PCC 7002. Environ Sci Technol 51(9):4897–4906

Tapia JM, Muñoz J, González F, Blázquez ML, Ballester A (2013) Sorption of ferrous and ferric iron by extracellular polymeric substances (EPS) from acidophilic bacteria. Prep Biochem Biotech 43(8):815–827

Tapia JM, Muñoz J, González F, Blázquez ML, Ballester A (2016) Sorption of ferrous iron by EPS from the acidophilic bacterium *Acidiphilium* sp.: a mechanism proposal. Rev Metal Madrid 52(3): e076. https://doi.org/10.3989/revmetalm.076

Tóth G, Hermann T, Da Silva MR, Montanarella L (2016) Heavy metals in agricultural soils of the European Union with implications for food safety. Environ Int 88:299–309

Wang J, Chen C (2006) Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. Biotechnol Adv 24(5):427–451

Wang K, Garg S, Waite TD (2017) Light-mediated reactive oxygen species generation and iron redox transformations in the presence of exudate from the cyanobacterium *Microcystis aeruginosa*. Environ Sci Technol 51(15):8384–8395

Wingender J, Neu TR, Flemming HC (1999) Microbial extracellular polymeric substances. Springer, Berlin Heidelberg

Wurl O, Miller L, Vagle S (2011) Production and fate of transparent exopolymer particles in the ocean. J Geophys Res 116(C7):C00H13

Xuan W, Bin Z, Zhiqiang S, Zhigang Q, Zhaoli C, Min J, Junwen L, Jingfeng W (2010) The EPS characteristics of sludge in an aerobic granule membrane bioreactor. Bioresour Technol 101(21):8046–8050

Yuan DQ, Wang YY (2013) Effects of solution conditions on the physicochemical properties of stratification components of extracellular polymeric substances in anaerobic digested sludge. J Environ Sci 25(1):155–162