Zinc and Cadmium Resistance Mechanism of
Pseudomonas aeruginosa PDMZnCd2003

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Abstract: *Pseudomonas aeruginosa* PDMZnCd2003 is a Plant Growth Promoting Bacteria (PGPB) that was isolated from the rhizosphere of a Zn/Cd hyperaccumulative plant. This research aims to study the effect of Zn and/or Cd on the growth of *P. aeruginosa* PDMZnCd2003 and Zn and Cd tolerance mechanisms. The results showed that Zn and Cd treatments affected the growth of *P. aeruginosa* PDMZnCd2003 in Nutrient Broth (NB). Zn and Cd also induced the bacterium to secret yellow-green chemicals. Scanning Electron Microscopy (SEM) indicated that the bacterium produced large amounts of Extracellular Polymeric Substance (EPS) under the heavy metals treatments, especially Cd. The EPS were removed from native cells of *P. aeruginosa* PDMZnCd2003 by α-glucoamylase to become EPS-free cells. Statistical analyses of FT-IR spectra separated the native cells into control group and group with native cells treated with Zn and/or Cd by the shifts in α-helix and amide II. EPS-free cells were separated into three subgroups of control, EPS-free cells treated with Cd and EPS-free cells treated with Zn and Zn plus Cd. The α-helix and amide II peak shifts were found in the sub-groups of EPS-free cells treated with Zn and Zn plus Cd while the amount of α-helix was highest in EPS-free cells treated with Cd. In addition, there were shifts of sulfur groups and C-O bonds in both native cells and EPS-free cells after Zn and/or Cd treatments. Therefore, the biochemical structures involving in Zn and Cd accumulation were carbonyl, amine groups, protein containing thiol (-SH) groups and C-O bonds. Moreover, this study proposes *P. aeruginosa* PDMZnCd2003 has some specific mechanisms to tolerate Cd which are different from the mechanism responding to Zn and Zn plus Cd.

Key words: *Pseudomonas aeruginosa*, FT-IR, zinc, cadmium, bacterium, α-helix, Thailand

INTRODUCTION

Metal pollution problems occur when human activity either disrupts the normal biogeochemical cycles or concentrates metals; examples of such activities include mining and ore refinement (Kabata-Pendias and Pendias, 1992; Roane and Miller, 1994). Metal wastes can exist as individual metals or more often as metal mixtures. Baker *et al.* (1990) reported that Cadmium (Cd) never occurs in isolation in natural environments but rather, appears mostly as a guest metal in Zinc (Zn) mineralization. The soil in the fields of Phatat Phadaeng sub-district, Mae Sot, Tak province, Thailand is a source of Zn mineralization. The total Cd concentrations in the soil in Mae Sot are positively correlated with total Zn concentrations in the soil (Simmons *et al.*, 2005, 2009). The health impacts of Cd overexposure on the Mae Sot population have been reported since, 2007 (Swaddiwudhipong *et al.*, 2007, 2010; Teeyakasem *et al.*,

2007). Therefore, the problem of Cd and Zn contamination in the Mae Sot area needs to be remediated. Phytoremediation is a suitable method to remove heavy metals from contaminated soil. Phytoremediation takes advantage of a cost-effective, environmentally friendly, carbon neutral approach for the cleanup of toxic pollutants (Blaylock and Huang, 2000; Rascio and Navari-Izzo, 2011; Prasad *et al.*, 2010). In addition, the association of plant and microorganism in the rhizosphere can enhance removal of the contaminants. Therefore, microbial augmentations of Plant Growth-promoting Bacteria (PGPB) have been investigated to improve phytoremediation (Jing *et al.*, 2007; Zhuang *et al.*, 2007). Plant Growth-promoting Bacteria (PGPB) have the ability to promote a plant's growth (increase biomass) and increase tolerance to toxic heavy metals by nitrogen fixation, phosphate solubilisation, sulfate oxidation and synthesis of phytohormones such as Indole-3-Acetic Acids (IAA), cytokinins, gibberellins and

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Aminocyclopropane-1-Carboxylate (ACC) deaminase and Induce Systemic Resistance (ISR) mechanisms in the plant (Zhuang *et al.*, 2007; Mukerji *et al.*, 2006; Rajkumar *et al.*, 2009). In the previous research, Plant Growth-Promoting Bacteria (PGPB) were isolated from Zn/Cd contaminated soil near the rhizosphere of *Gynura pseudochina* (L.) DC., which is a Zn/Cd hyperaccumulator growing in a zinc mine (Panitlertumpai *et al.*, 2008; Phaenark *et al.*, 2009). API20E biochemical test and genetic characterization indicated that the isolated PDMZnCd2003 was *Pseudomonas aeruginosa*. The bacterium was able to tolerate high Zn and Cd concentrations and had plant growth promoting properties of IAA production, nitrogen fixation and phosphate solubilisation (Nakbanpote *et al.*, 2010). However, the Zn and Cd defense mechanism of *P. aeruginosa* PDMZnCd2003 should be researched before applying the bacterium for Zn/Cd phytoremediation.

Therefore, this research aims to study the effect of Zn and/or Cd on the growth of *P. aeruginosa* PDMZnCd2003. The Zn and Cd tolerance mechanisms of the bacterium were studied by Fourier Transform-Infrared (FT-IR) microspectroscopy, using a non-destructive method to indicate the change of functional groups in the treated cells. In addition, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy connected with Energy Dispersive X-ray spectroscopy (SEM/EDS) were carried out to study the bacterial cells.

MATERIALS AND METHODS

The bacterial cells of *P. aeruginosa* PDMZnCd2003 were separated into native cells and EPS-free cells. The native cells were untreated bacterial cells. The EPS-free cells were cells treated with α-glucoamylase (Fluka, USA) to remove the Extracellular Polymeric Substance (EPS) following the method of Ueshima *et al.* (2008). The native cells and EPS-free cells were cultivated in Nutrient Broth (NB) (Himedia, India) and NB supplemented with Zn (20 mg L^{-1}), Cd (20 mg L^{-1}) and Zn plus Cd (20 mg L^{-1} of Zn, Cd). The Zn and Cd were obtained from analytical grade ZnSO$_4$.7H$_2$O (Ajax Finechem, Australia) and CdSO$_4$.8H$_2$O (Ajax Finechem, Australia), respectively. The

sample code is shown in Table 1. The concentrations of Zn and Cd in the NB media were analyzed by Flame-Atomic Absorption Spectroscopy (FAAS) (Shimadzu AA-680, Japan) and the changing pH was detected with a pH meter (Denver Instrument, Model 215, USA). Bacterial growth curves were monitored by two methods: optical density at 660 nm by a Vis-spectrophotometer (ThermoFisher Scientific, Spectronic Genesys 20, USA) and total protein content by Bradford Protein Assay (Bradford, 1976). After cultivation, bacterial cells were collected by centrifugation and washed with 0.85% (w/v) NaCl and deionized water. The bacterial cells were centrifuged and washed with deionized water before being freeze dried using a Lyophilizer (Freeze-Dry system-77530 Labconco, USA). The dried bacterial sample was put on double sided carbon tape (Ted Pella, Inc., USA) on aluminum straps and analyzed with a Scanning Electron Microscope connected to an Energy Dispersive X-ray Spectroscopy (SEM/EDS) (Jeol JSM-6460 LV, Japan). The glucoamylase treated cells were studied for optimal EPS removal time by Transmission Electron Microscopy (TEM) (Joel JSM 1230, Japan). The cell samples were prepared by dropping a cell suspension on a formvar coated grid and straining with 5% (w/v) uranyl acetate. FT-IR microspectroscopy analysis was carried out by dropping a cell suspension on an IR reflected kevey slide and then vacuum drying for 24 h. The sample slides were analyzed by IR spectrometer (Tensor 27, Bruker Optic) connected to an IR microscope (Hyperion 2000, Bruker Optic) and Mercury Cadmium Tellride (MCT) detector (IR detection, 700-4000 cm^{-1}). Spectrums were obtained by reflected mode at a resolution of 4 cm^{-1} wavenumber, 128 scan and the spectrum was analyzed by OPUS 6.5 (Bruker Optic, German). The FT-IR spectra were pre-data processed by second derivative and vector normalized (S-Golay 3 polynomial at 9 point) and Extended Multiplicative Signal Correction (EMSC). The pre-data processed spectra were analyzed by Principle Component Analysis (PCA) using the Unscrambler 9.7 software package (CAMO, Norway) in order to distinguish different chemical components of the samples. The peaks were identified by IR Mentor Pro 6.5 (Bio-Rad, 1999) and an IR reference book (Mantsch and Chapman, 1996).

Table 1: Sample code of bacterial cells, non-treated and treated with Zn and/or Cd

Sample codes	Condition of treatment
Native cells	EPS-enclosed cells of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth
EPS-free cells	EPS-removed cells of *P. aeruginosa* PDMZnCd2003 by treatment with α-glucoamylase for 2 h, cultivated in nutrient broth
Native cells_Zn	EPS-enclosed cells of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth with Zn (20 mg L^{-1})
Native cells_Cd	EPS-enclosed cells of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth supplemented with Cd (20 mg L^{-1})
Native cells_ZnCd	EPS-enclosed cells of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth supplemented with Zn plus Cd (20 mg L^{-1} of Zn, Cd)
EPS-free-cells_Zn	EPS-removed cells of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth supplemented with Zn (20 mg L^{-1})
EPS-free-cells_Cd	EPS-removed cells of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth supplemented with Cd (20 mg L^{-1})
EPS-free-cells_ZnCd	EPS-removed cells of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth supplemented with Zn plus Cd (20 mg L^{-1} of Zn,Cd)

RESULTS AND DISCUSSION

Effects of Zn and Cd on growth curve: Figure 1 a-d shows the growth curves of *P. aeruginosa* PDMZnCd2003 cultivated in nutrient broth (control) and NB treated with Zn and/or Cd. Although, the growth curves obtained by turbidimetric method and protein assay were quite similar, the turbidimetric method was interfered by the EPS secreted by *P. aeruginosa* PDMZnCd2003. Therefore, the data from the protein assay represented the bacterial growth. The growth curve indicated that bacterial cells under Zn and Cd treatment required a longer time to accelerate to the exponential growth phase and the toxicity to bacterial growth was Zn<Zn plus Cd<Cd. Poirier *et al.* (2008) also reported that *Pseudomonas fluorescens* BA3d12 in NB containing Zn, Cu and Cd had a longer lag phase compared to the control and Zn stimulated the respiration of the bacteria whereas Zn plus Cd delayed the primary metabolism of the bacteria. In addition, the color of the NB (control) after 24 h bacterial cultivation was changed from pale yellow to blue green. Whereas, supernatant of bacterial cultures after treated with Zn, Cd and Zn plus Cd were orangey yellow, yellow-green and strongly yellowish green, respectively (Fig. 2a-d). *P. aeruginosa* PDMZnCd2003 might produce

and secrete different organic molecules under Zn and/or Cd stresses. The coloring chemicals could be siderophores and the different colors of the culture media might be cause by the different concentration ratios of pyomelanin (brown), pyorubrin (red) and pyocyanin (blue) (Turick *et al.*, 2010) or pyoverdine (yellow-green). *P. aeruginosa* PDMZnCd2003 was isolated from Zn/Cd contaminated soil by NB containing 20 mg L^{-1} of Zn and Cd (Nakbanpote *et al.*, 2010). Therefore, the concentration of Zn and Cd at 20 mg L^{-1} was used for bacterial treatment. In addition, the extractable amounts of Zn and Cd leached from the Zn/Cd contaminated soil of Padang mine by 1% (v/v) HNO_3 were 8.0 and 0.8 mg g^{-1}, respectively. Whereas available Zn and Cd leached from the soil with 0.005 M Diethylenetriaminepentaacetic Acid (DTPA) were 0.15 and 0.03 mg g^{-1}, respectively. Therefore, 20 mg L^{-1} of Zn and Cd used in this study was sufficient to study the effect of Zn and/or Cd on bacteria in the environment. The concentrations of Zn and/or Cd in Nutrient Broth (NB) at various pHs were investigated because the bacteria probably produced secondary metabolites that might increase the media pH and result in metal precipitation. Figure 3 shows that the pHs of the bacterial cultivation in NB and NB supplemented with Zn and/or Cd gradually increased from 7.3±0.5 (lag phase) to
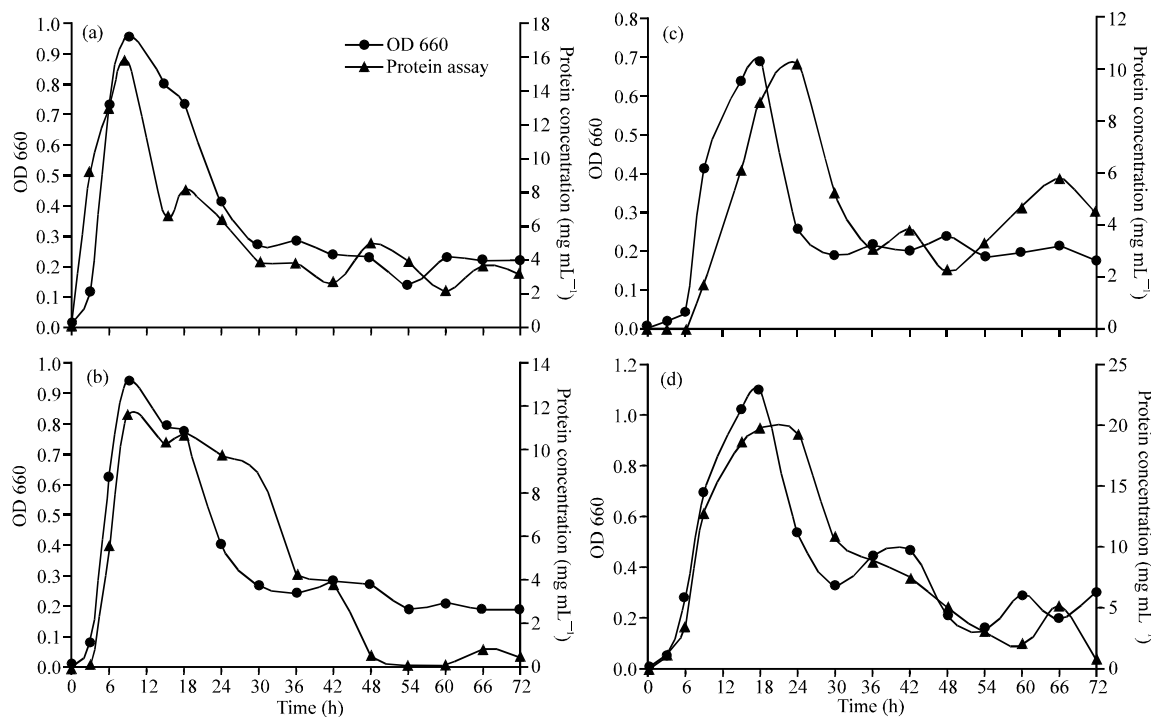

Fig. 1: Growth curves of *P. aeruginosa* PDMZnCd2003 in nutrient broth (control) and nutrient broth supplemented with Zn and/or Cd, monitored by turbidimetric method and protein assay; a) Nutrient Broth (NB); b) NB with Zn; c) NB with Cd and d) NB with Zn plus Cd

approximately 9.0 after 30 h (death phase). In the death phase, the stable pH values at ~9.0 might be caused by secondary metabolites secreted from *P. aeruginosa* PDMZnCd2003. The alkaline compounds in siderophores might be the metabolites that affected the alkaline pH. Dao *et al.* (1999) reported the production and secretion of pyoverdine (yellow-green) by *Pseudomonas aeruginosa* under Cd stress. The concentration of Zn and Cd in the media decreased within 18 h in which the bacteria grew from lag phase to stationary phase and the system pH >8.5 (Fig. 3 a, b). Then, the concentration of Zn and Cd were stable at 13 ± 2 and 14 ± 2 mg L^{-1}, respectively until the death phase. In the solution with a pH of 8-9, the form of Zn dissolved was probably Zn^{2+}, $ZnOH^+$, $Zn_2(OH)_3^+$ and the form of Cd dissolved could be Cd^{2+}, $CdOH^+$ (Stumm and Morgan, 1996). Siderophores secreted under heavy metal stress could be a chelating agent (Dao *et al.*, 1999). In addition, amino acids in nutrient broth might chelate with Zn and Cd to form soluble complexes at alkaline pHs. The experiments confirmed that there was no precipitation of Zn and Cd in the nutrient broth containing 20 mg L^{-1} of Zn and/or Cd with adjusted pH values of 5-9. Therefore, the decrease of Zn and Cd in NB might be
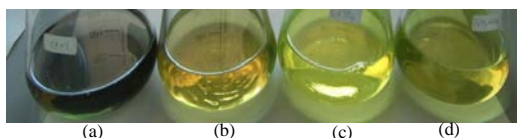

Fig. 2: Color of media after 24 h of bacterial cultivation in; a) Nutrient Broth (NB); b) NB with Zn; c) NB with Cd and d) NB with Zn plus Cd

caused by accumulation of Zn and Cd in dissolvable forms into bacterial cells and EPS (Roane and Miller, 1994; Ueshima *et al.*, 2008).

Bacterial cells studied by microscopy techniques: The SEM images (Fig. 4a-f) of *P. aeruginosa* PDMZnCd2003
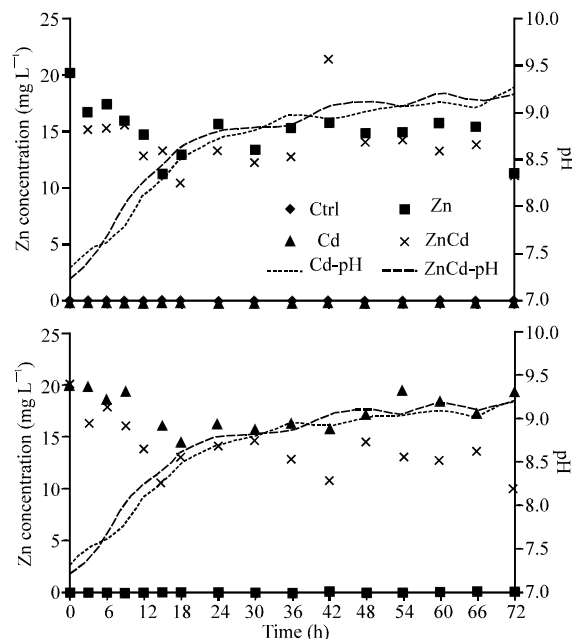

Fig. 3: Concentration of Zn and Cd during *P. aeruginosa* PDMZnCd 2003 cultivation in the Nutrient Broth (NB) (control), NB with Zn (20 mg L^{-1}), NB with Cd (20 mg L^{-1}) and NB with Zn plus Cd (20 mg L^{-1}) and changing pH; a) Zn concentration and b) Cd concentration
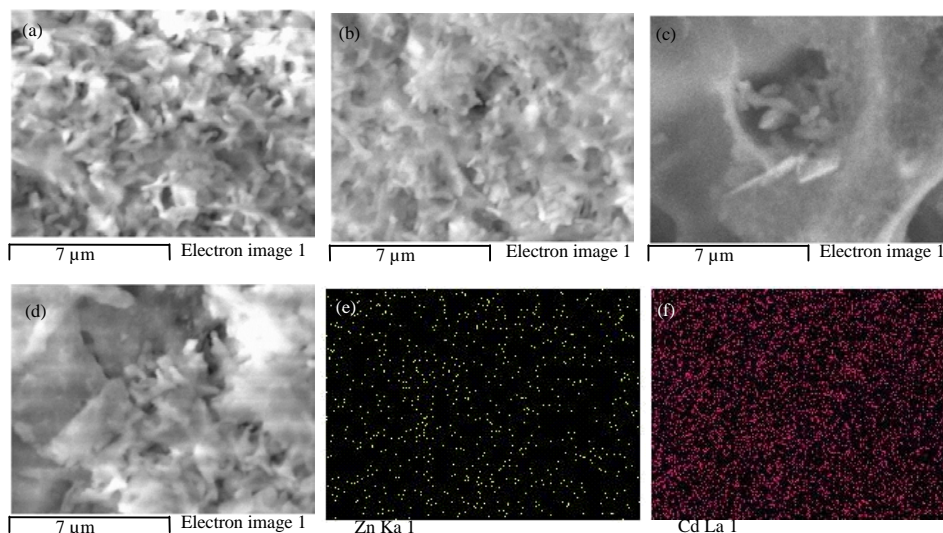

Fig. 4: SEM images of *P. aeruginosa* PDMZnCd2003 cultivated in; a) Nutrient Broth (NB); b) NB with Zn; c) NB with Cd; d) NB with Zn and Cd and the EDS mapping of; e) Zn and f) Cd of the native cells_ZnCd images
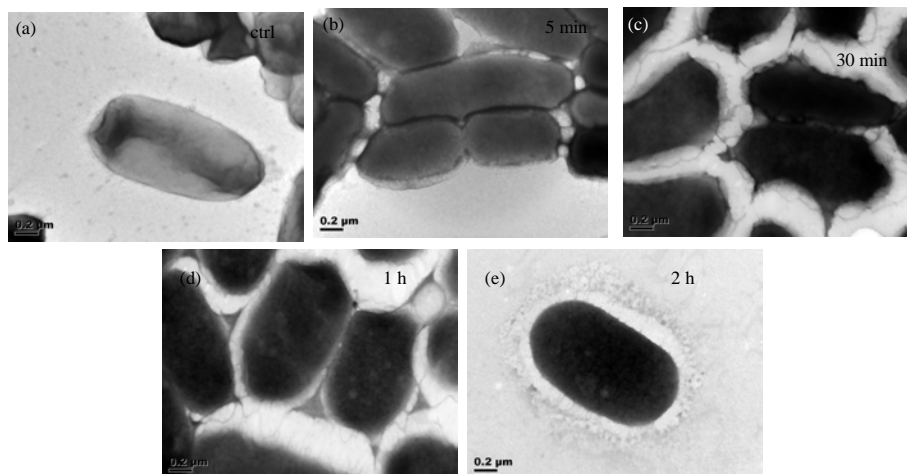
Fig. 5: TEM images of *P. aeruginosa* PDMZnCd2003 treated with α-glucoamylase enzymes at various incubation times of; a) 0 min; b) 5 min; c) 30 min; d) 1 h and e) 2 h (× 40,000)

showed a cloud of bacteria covered with numerous EPS. The appearance of a large amount of EPS under Cd treatment and Zn plus Cd treatment was found. The presence of large amounts of EPS indicated a mechanism to Zn and Cd toxicity. However, the evidence of Zn and Cd distribution in parts of the cells and/or EPS obtained by EDS mapping (Fig. 4e, f) was not clear because of lower electron energy and resolution. However, the important role of EPS to prevent heavy metal toxicity was published. Ueshima *et al.* (2008) reported *Pseudomonas putida* protected cells by absorbing the toxicity of Cd ions with EPS. Gonzalez *et al.* (2010) also showed the role of EPS in Cu adsorption of *Pseudomonas aureofaciens* as a protective barrier for cells. To study the role of EPS, *P. aeruginosa* PDMZnCd2003 was treated with α-glucoamylase enzymes to remove EPS. The EPS composition of *Pseudomonas* sp. was mainly alginate-like polysaccharides. Therefore, α-glucoamylase enzymes was applied to break α 1,4 glucosidic bonds within alginate. However, a suitable incubation time had to be optimized to remove EPS. TEM images showed that more EPS was removed when incubation times increased. EPS continued splitting out around the cells surface and 2 h was the appropriated time for EPS removal (Fig. 5a-e).

Effects of Zn and Cd on biochemical molecular changes investigated by FT-IR: Since, bacteria are tiny cells, the study of biochemical alterations was carried out by FT-IR connected to a microscope. The FT-IR spectra were pre-data processed by second .llderivative. The use of multivariate analysis, in particular Principle Component Analysis (PCA) has proven useful in the analysis of biospectroscopic data, providing two types of information: the visualization of the clustering of similar spectra within datasets in score plots and the

identification of variables (spectral bands representing various molecular groups within the samples) in loading plots to explain the clustering observed in the scores plots. Extended Multiplicative Signal Correction (EMSC) is a transformation method used to compensate for the multiplicative, additive scatter effect in the data and to account for the physical or chemical phenomena that affect the spectra.

The difference between native cells and EPS-free cells was determined by PC1 (78%) (Fig. 6a). The X-loading plot (Fig. 6b) indicated shifts in protein peaks, including anti-parallel, α-turn and α-helix in ~1693-1675 cm^{-1}, amide I at 1658 cm^{-1} (native cells) and 1646 (EPS-free cells), Amide I of α-pleated sheet structure at 1602 cm^{-1} (native cells) and 1623 cm^{-1} (EPS-free cells), Amide II 1560 cm^{-1} (native cells) and 1535 cm^{-1} (EPS-free cells), sulfur peak at 1074 cm^{-1} (native cells) and 1081 cm^{-1} (EPS-free cells).

The native cells control and native cells_Zn, native cells_Cd and native cells_ZnCd were analyzed in three ranges of wave numbers because the high intensity of amide peak hindered the lower intensity peak. In the lipid range (3000-2800 cm^{-1}), the separation between native cells and native cells treated with Zn and/or Cd by PC1 (71%) (Fig. 7a), the X-loading plot (Fig. 7b) show differences in shift of 2964 cm^{-1} (control) and 2952 cm^{-1} (native cells treated with Zn and/or Cd) and shoulder peaks at 2846 cm^{-1}. In the protein range (1800-1300 cm^{-1}), the separated native cells from the native cells treated with Zn and/or Cd by PC1 (92%) (Fig. 7c), the X-loading plot (Fig. 7d) show shifts in α elix peaks at 1664 cm^{-1} control) and 1,646 cm^{-1} (native cells treated with Zn and/or Cd) and shifts of the amide II peaks at 1556 cm^{-1} (control) and 1,537 cm^{-1} (native cells treated with Zn and/or Cd). In addition, the intensity of protein
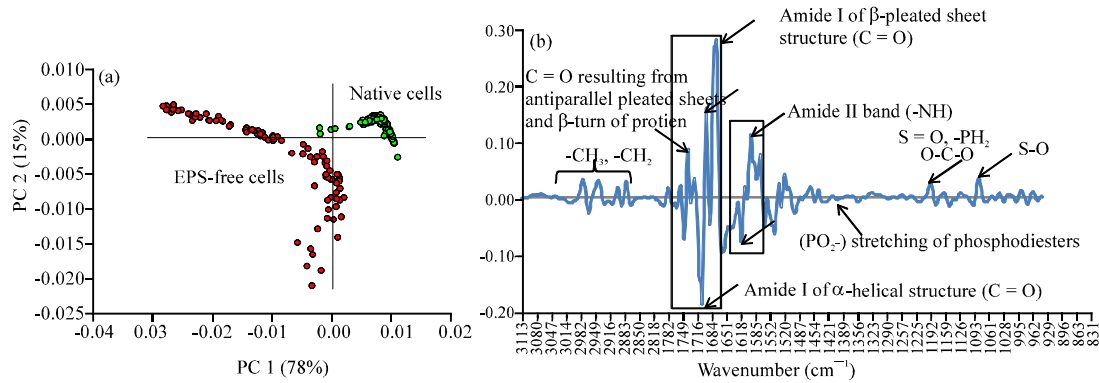
Fig. 6: Principal Component Analysis (PCA) applied to FT-IR spectra of native cells and EPS-free cells showing; a) Score plot and b) Loading plot in the range of 3200-800 cm⁻¹
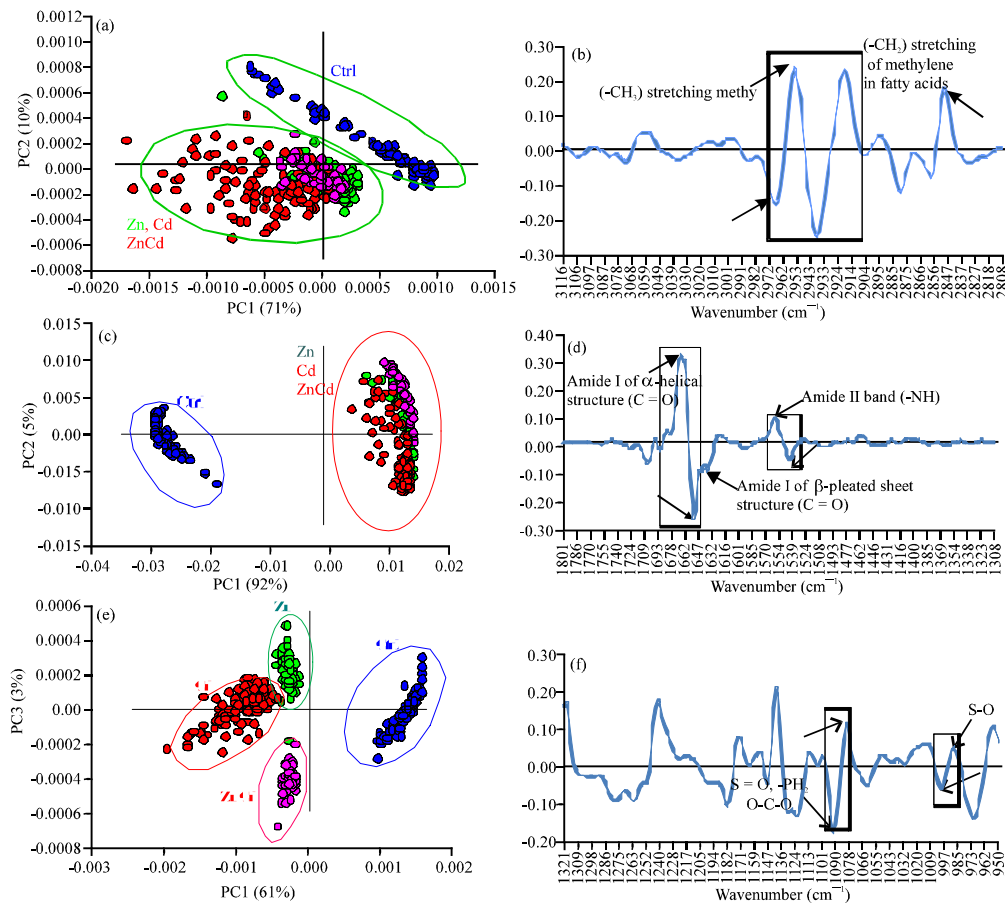

Fig. 7: Principal Component Analysis (PCA) applied to FT-IR spectra of native cells (control) and native cells_Zn, native cells_Cd and native cells_ZnCd showing the score plot and loading plot in; a, b) Lipid range (3000-2800 cm⁻¹); c, d) Protein range (1800-1300 cm⁻¹) and e, f) DNA band range (1300-800 cm⁻¹)

peaks in native cells treated with Zn and/or Cd was higher than the control (data not shown). In the DNA band range (1300-800 cm⁻¹), the separated samples divided into 4 groups by PC1 (61%) and PC3 (3%) (Fig. 7e) include

native cells, native cells_Zn, native_Cd and native cells_ZnCd. The X-loading plot (Fig. 7a) found shifts of the phosphorus peaks at 1085 cm⁻¹ (control), 1076 cm⁻¹ (native cells treated with Zn, Cd and Zd plus Cd) and
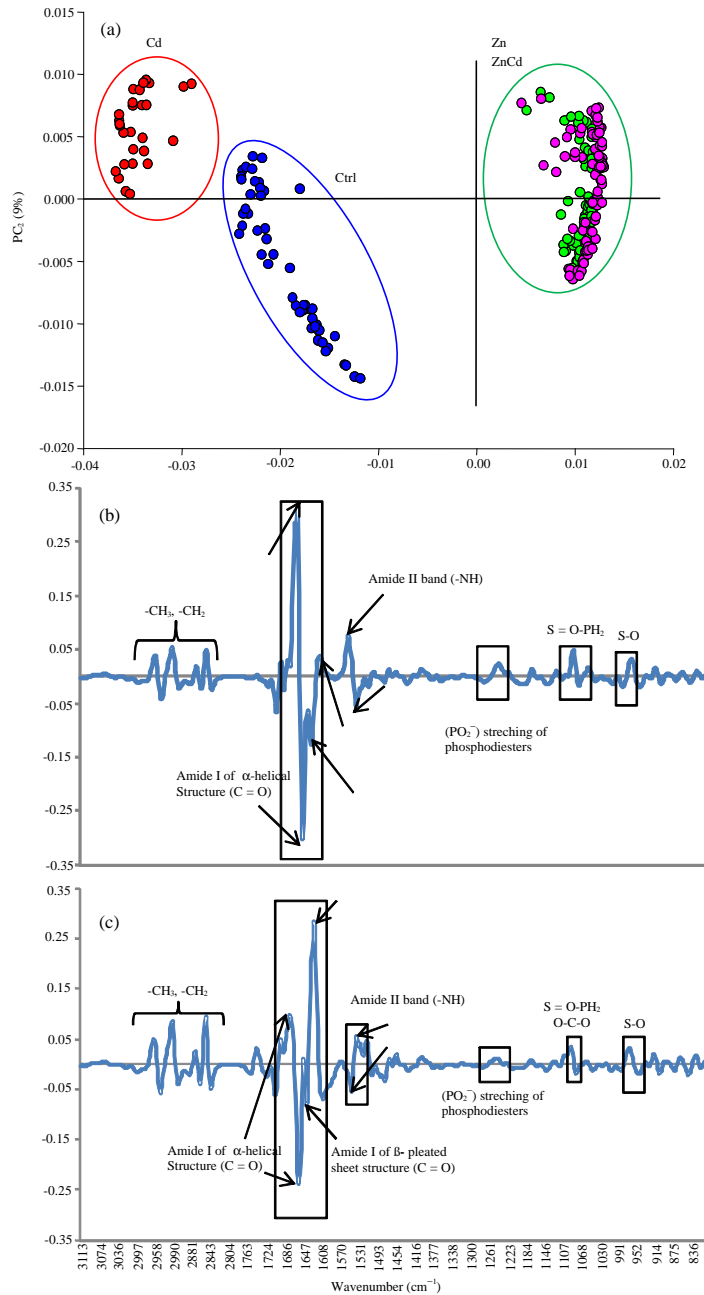
Fig. 8: Principal Component Analysis (PCA) applied to FT-IR spectra of EPS-free cells (control) and EPS-free cells_Zn, EPS-free cells_Cd and EPS-free cells_ZnCd showing the score plot; a) X-loading plots of PC1; b) PC2 and c) in the range of 3200-800 cm^{-1}

sulfur peaks at 1081, 993 cm^{-1} (control) and 1076, 981 cm^{-1} (native cells treated with Zn, Cd and Zd plus Cd). The EPS-free cells (control) and EPS-free cells_Zn, EPS-free cells_Cd and EPS-free cells_ZnCd were separated into three groups of; EPS-free cells (control), EPS-free cells_Cd and EPS-free cells_Zn and EPS-free cells_ZnCd by PC1 (88%) and PC2 (9%) (Fig. 8a). Control and EPS-free

cells_Cd were separated from EPS-free cells_Zn and EPS-free cells_ZnCd by the shifts of α-helix at 1660 cm^{-1} (EPS-free cells and EPS-free cells_Cd) and 1648 cm^{-1} (EPS-free cells_Zn and EPS-free cells_ZnCd) and shifts of amide II at 1552 cm^{-1} (EPS-free cells and EPS-free cells_Cd) and 1537 cm^{-1} (EPS-free cells_Zn and EPS-free cells_ZnCd) (Fig. 8b). EPS-free cells (control) were

separated from EPS-free cells treated with Cd by the PC2 axis. The X-loading (PC2) (Fig. 8c) and average spectra indicated the difference between EPS-free cells and EPS-free cells_Cd was at the α-helix (1656 cm^{-1}) and shifts of the α-plated sheet peak at 1621 cm^{-1} in EPS-free cells (control) and 1639 cm^{-1} in EPS-free cells_Cd. For FT-IR analysis, the shifts of the α-helix and amide II peaks might be caused by the functional groups of C = O and -NH binding to Zn and Cd ions. In addition, there were the shift of sulfur groups and C-O bonds in both native cells and EPS-free cells after Zn and/or Cd treatments. The lone pairs of electrons in the carboxyl groups and amine groups indicated coordinate covalent bonds with heavy metal ions. In addition, chelating agents and various metabolites secreted by the bacterium could bind to heavy metal ions. There have been many studies about the functional groups that bacteria use to bind with heavy metal ions. Choudhary and Sar (2009) showed the relationship between carboxyl groups and phosphoryl groups on heavy metal ions binding in *Pseudomonas* sp. Chen *et al.* (2007) showed that *Pseudomonas putida* CZ1 bound Cu and Zn ions by carboxyl groups mainly. Kazy *et al.* (2009) also reported *Pseudomonas* sp., binding with U and Th by phosphoryl, carboxyl and amide groups. These separations resulting from PCA (Fig. 8a) indicated that *P. aeruginosa* PDMZnCd2003 responds to Zn unlike Cd and the cells might have different mechanisms for Zn and Cd effects. The possible Cd tolerance mechanism of *P. aeruginosa* PDMZnCd2003 was the production and secretion of proteins and/or protein compounds due to the highest peak of protein in the EPS-free cells treated with Cd.

The secretions could prevent Cd ions binding to or entering the cells since Cd is more toxic than Zn which is an essential element for bacterial growth. The role of EPS in preventing cells from being damaged by heavy metal ions has been reported to occur by EPS enhancing heavy metal uptake and then retarding them to the cell wall (Fang *et al.*, 2011; Ha *et al.*, 2010; Ghannoum and O'Toole, 2004; Ueshima *et al.*, 2008; Gonzalez *et al.*, 2010). In addition, EPS-free cells had higher intensities of phosphoryl groups and nucleic acid than EPS-free cells treated with Cd because DNA might be damaged by toxic Cd (Zhou *et al.*, 2008).

Some enzymes and/or Metallothionines (MTs) might be responsible for binding with heavy metals. There have been many studies about proteins that bacteria expressed under heavy metal stress. Pereira *et al.* (2006) found that Cd could induce alterations in metabolite proteins in *Rhizobium* sp. Sample FT-IR spectra from the DNA band region showed the control samples had higher nucleic acid, phospholipids or phosphoryl groups than the Zn, Cd and ZnCd treatments. Heavy metals can damage nucleic acid and are toxic to cells. Zhou *et al.* (2008)

showed that an increase in As^{3+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} ion concentrations lead to damaged DNA of Effective Microorganisms (EMs) in wastewater.

CONCLUSION

Although, *Pseudomonas aeruginosa* PDMZnCd2003 was isolated from Zn/Cd contaminated soil near the rhizosphere of a Zn/Cd hyperaccumulative plant, Zn and Cd delayed the bacterial growth. However, the bacterium had Zn/Cd tolerance mechanisms. FT-IR indicated that the biochemical structures involved in Zn and Cd accumulation were carbonyl, amine groups, protein containing thiol (-SH) groups and C-O bonds. EPS and yellow-green chemicals secreted under Zn/Cd stress might help the bacterium decrease the metals' toxicity.

Moreover, *P. aeruginosa* PDMZnCd2003 showed some specific mechanisms to tolerate Cd which were different from the mechanism responding to Zn and Zn plus Cd. In addition, this research indicated that *P. aeruginosa* PDMZnCd2003 secreted specific siderophores as various shades of yellow-green chemicals to reduce the toxicity of Zn and/or Cd. Therefore, chemical analysis and properties of the siderophores in Zn/Cd tolerance mechanisms should be study further.

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