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Molecular Characterization and Phylogenetic Analyses of Heavy Metal Removal Bacteria from the Persian Gulf

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Abstract: A total of 35 heavy metals resistance and removal bacterial strains were isolated from samples of marine environment and enclosed industrial areas. All isolates were characterized by molecular method. The diversity of isolated bacteria was examined by the phylogenetic analysis of 16S rRNA gene sequences. The phylogenetic analysis of the sequences revealed seven main taxonomic lineages. The phylogenetic tree illustrated discrimination between isolated bacteria from wastewater, industrials area and marine environment. Results showed new genetic differences and relationship between marine and industrial strains. Some *Pseudomonas* strains isolated from marine environment were well differentiated from those of industrial wastewater. Members of the genera *Delftia* and *Bacterium* formed a monophyletic group within the subdivision of the class. There was a clear differentiation between two groups of *Pseudomonas* and other groups of bacteria in the phylogenetic tree.

Key words: Phylogenetic, heavy metal resistance bacteria, 16S rRNA, *Pseudomonas*, Persian Gulf

INTRODUCTION

There have been numerous studies on heavy metal resistance of bacteria isolated from different habitats (Jansen *et al*., 1994; Shi *et al*., 2002). However, there was no information available on heavy metals bioaccumulation or heavy metals resistance bacteria from the Persian Gulf. It has been shown that molecular techniques are effective and fast technology for identification of microbial diversity in different environments (Pace, 1997; Hatamoto *et al*., 2008). Genetic diversity can identify individual organisms from some unique part of their DNA or RNA providing definitive information on its biodiversity (Elsayed and Elbestawy, 2008).

On the other hand, recent development in nucleic acid technology such as the Polymerase Chain Reaction (PCR), and analysis of 16S rRNA (Malik *et al*., 2008) has resulted in new methods that can be used for genotype analysis of bacteria. Comparative analysis of 16S rRNA has shown that highly conserved sequences are interspersed with region of variable sequences. Analysis of variable portion permits the determination of phylogenetic and evolutionary relationships (Malik *et al*., 2008). Conserved or variable region have been used as targets for primer directed DNA amplification by PCR for the identification of microorganisms (Boddinghaus *et al*., 1990; Jayarao *et al*., 1991). Nevertheless, it has also become evident in recent years that an analysis of the population structure is one prerequisite for understanding microbial processes in aquatic habitats, comparable to ecological studies of higher eukaryotes which were based mainly on population analysis. Prokaryotes are too small for a morphological classification and have to be put in axenic culture before characterization. Depending on the ecosystem only 0.001-15% of the total number of visible cells can be retrieved by isolation (Amann *et al*., 1995).

Hence, traditional microbiological methods are not suitable for the study of the full microbial diversity. The use of molecular techniques in the characterization and identification of bacteria has many advantages. It is not only sensitivity and reproducibility, but also are rapidity to obtain results (Min and Baeumner, 2002; Rompre *et al*., 2002). In the other hand, molecular techniques now allow researcher to investigate microbial community function and structure at increasingly finer resolution without cultivation (Mills *et al*., 2007).

In the present study, our objective was to identify heavy metals removal bacteria by using molecular

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methods and establish the phylogenetic relationship among heavy metals removal bacteria isolated from the Persian gulf and enclosed industrial areas.

MATERIALS AND METHODS

Uptake of copper, zinc, cadmium and lead by bacteria: Heavy metal removal bacteria obtained from Khuramshahr Marine Science and Technology University in October 2008, which were isolated by Zolgharnein *et al.* (2007). Bacteria cells were harvested by centrifugation at 9000xg for 20 min at 4°C minimums washed twice with de-ionized water. The cells were suspended in de-ionized water to a final concentration of 2.5 mg dry weight mL^{-1}. Forty milliliters of the suspension were added to 160 mL of selected concentrations of $CuSO_4$, $ZnSO_4$, $Cd(NO)_3$ and $Pb(NO)_3$ separately. The suspensions were incubated at room temperature on a shaker with a speed of 100 rpm for 1 h then centrifuged at 9000xg for 20 min. The bacterial cells were heated to 105°C overnight in an oven. After heating, the dry weight was measured.

Measuring of heavy metals in bacteria cell: Two milliliters of nitric acid were added into 25 mL plastic bottles that were previously rinsed with nitric acid and washed with de-ionized water. Sixteen milligrams of dried bacterial cells were added into the concentrated nitric acid separately and incubated in a water bath at 100°C for 1 h. The mixture was later cooled to 25°C. The volume of the mixture was raised to 20 mL with distilled water and the concentration of heavy metals was measured by flow injection Atomic Absorption Spectrophotometry (AAS). Determination of copper, zinc, cadmium and lead was done by using a special lamp for each metal at a specific wavelength.

Bacterial DNA preparation: Genomic DNA was extracted from isolated bacteria using a commercial kit (Bactozol®) according to manufacturer's instructions. Bacteria cells were grown overnight at 28°C in TB broth with shaking. One milliliter of bacterial fresh culture was transferred to 1.5 mL microcentrifuge tube and centrifuged at 6,000 xg for 4 min at 4°C. The supernatant was discarded and the bacterial pellet suspended in 100 μL of 1x bactozyme. The mixture was then vortexed to achieve a homogenous suspension and then incubated at 50°C for 30 min. Four hundred microliters of DNAZOL solution was added to the lysated bacterial suspension and mixed manually for 30 sec and then incubated at room temperature for 5 min. DNA was precipitated by adding 0.3 mL of 100% ethanol and mixed by inversion for 15 sec and then stored at room temperature for 5 min. White thread of DNA was spool a pipet tip and transferred to clean 1.5 mL microcentrifuge tube and washed with 75% ethanol three times with using briefly vortex and ethanol carefully removed from the tube, then DNA containing tube stored at inverted condition for 2 min. Finally DNA pellets rehydrated with 200 μL TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 7-8).

PCR amplification: Two universal oligonucleotide primers forward and reverse were synthesized based on standard 16S rRNA gene sequence (GenBank). The primers used to amplify the 16S rRNA samples were forward primer: 5'-CCGAATTCGTCG ACAACAGAGTTTGATCCTGGCTCAG-3'; and reverse primer: 5'-CCCGGGATCCAAGCTTACGGCTACCTTGT TACGACTT-3'

PCR amplification was performed in a total volume of 100 μL mixture, containing 10 μL chromosomal DNA (= 100 ng), 5 μL of 16S Forward Primer (= 20 pmol), 5 μL of 16S Reverse Primer (= 20 pmol), 4 μL of 5 mM 4 dNTP mix, 4 μL of 50 mM $MgCl_2$, 10 μL of l0x *Taq* Buffer (supplied with the Taq DNA polymerase), 61.5 μL sterile distilled water, 0.5 μL (2.5U) *Taq* DNA polymerase, mixed and microcentrifuged briefly. The tubes were subjected to 30 cycle in a thermal cycler with the following program: 2 min at 95°C (this denatures the DNA and primers), 30 cycles of 30 sec at 45°C, 2 min at 72°C and 30 sec at 95°C, 1 cycle of 1 min at 45°C, 2 min at 72°C. After it was finished all samples were removed from the thermal cycler and stored at -20°C until the next lab period. For some bacterial that was not detected on the gel after PCR the following method was performed for amplification of that types bacterial. A total volume 50 mL mixture, containing 5 μL of 10 x PCR Buffer, 0.5 μL of 10 x dNTP, 1 μL of forward and reverse primer each other, 1.3 μL of template DNA (10 ng), 1 μL of Taq DNA polymerase and sterile distilled water to adjust 50 μL. The tubes were subjected to 35 cycle in a thermal cycler with the following program: initial denaturation at 95°C for 3 min and was followed with 35 cycle consisting of denaturation at 94°C for 30 sec annealing at 60°C for 30 sec, elongation at 72°C for 2 min, the reaction was finished with an extension step at 72°C for 5 min.

Gel electrophoresis: The presence of PCR products was determined by electrophoresis of 10 μL of the PCR product in 1% of agarose gel in electrophoresis buffer TAE and a 1 kb DNA ladder as molecular marker. The remaining amount of PCR product was further used for 16S rRNA purification of gel.

Purification of PCR amplified 16S rRNA gene: The PCR product of 16S rRNA gene was run on 1% low melting gel for 1 h, gel was observed and precisely cut under UV with

in the shorted time of less than 15 sec, then 200 mg of cutting gel was transferred to a clean 1.5 microcentrifuge tube using commercial Genomic DNA purification kit (Geni, pin) according to manufacturer's instructions. Eight hundred microliter of binding buffer was added into the tube containing the gel slice and incubated at 60°C for 10 min, then centrifuged at 10,000xg for 1 min, 800 μL of incubated solution was added into column and centrifuged at 10,000x g for 1 min, 750 μL of washing buffer was added into column and centrifuged at 10,000xg for 1 min and repeated two times for obtaining best result. The empty column was centrifuged at 10,000xg for 1 min for drying the sample, column was put into clean 1.5 microcentrifuge tube and 30 μL of TE buffer was added into column and centrifuged for 1 min.

Sequence analysis: The 16S rRNA gene products (forward and reverse strands) of 35 bacteria isolates were sequenced. The clean PCR product was subjected cycle sequencing in both direction using universal primers. The sequencing was done with ABI PRISM Dye Terminator Cycle sequencing method (Perkin Elmer) that used four different colored fluorescence-dyes to label four ddNTPs terminators. Sequencing reactions were setup according to instructions manual. Briefly, a total of 20 μL reaction mixture consisting of 8 μL terminator ready reaction mix, 50 ng of PCR product and 3.2 pmole of primer. The cycle sequencing was performed on the GeneAmp PCR system 9600 (perkin Elmer) with the following parameters: 25 cycles at 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min. The nucleotide sequences were edited using the software chromas and compared to published sequences in the NCBI GenBank using the nucleotide BLAST. For phylogenetical analysis the bacterial 16S rRNA gene sequences from this work and other sequences retrieved from the database were aligned using the software CLUSTAL W 1.8 (Salomon *et al*., 2003). The phylogenetical analysis was made using the neighbour joining method. The analysis was performed with the software Phylo Win (Galtier *et al*., 1996). The output trees were prepared using the software Treeview 1.5 (Page, 1996).

RESULTS

A total of 35 bacterial strains resistant to heavy metals were isolated from samples of marine environment and enclosed industrial areas. All of the strains were resistant to all four metals tested in this study (Cu, Pb, Cd and Zn). The most of isolated strains belonged to *Pseudomonas* strains. However, *Pseudomonas* strains were capable of growing in the presence of heavy metals better than others species and could accumulate heavy metals very well. In a *Delftia tsuruhatensis* and *Pseudomonas* strain AU3411 were observed to have the highest accumulation of Pb.

Two universal oligonucleotides were used to determine and identify the 16S rRNA gene for all isolates. The primer amplified the 16S rRNA gene successfully from all heavy metal removal bacteria, although no conspicuous variations in the size of rRNA gene products between the 35 isolated bacteria were discernable. However, the size of the PCR amplified 16S rRNA gene product of all isolated bacteria investigated in this study was approximately 1.4 kb to the relative DNA size marker.

The amplified 16S rRNA gene products were purified using a commercial gel purification kit. The resultant 16S rRNA sequence was edited and aligned using Chromas and Bioedit program. The additional sequence data were obtained from the Gen Bank.

Comparison of the partial 16S rRNA gene sequence from the 35 bacterial isolates with sequences from the database showed that they belong to seven taxonomic lineages. Eighteen species belong to the gamma subdivisions of *Proteobacter*, eight species were related to beta subdivisions of *Proteobacter*, five strains were belonged to alpha subdivisions of *Proteobacter*. Two of the bacterial isolates were related to *Bacterium* Ph10, subdivision of these bacteria was not identified. The bacteria strains was first reported by Donachie *et al.* (2003) who determined 848 bases of the 16S rRNA of *Bacterium* Ph10, while two strain were related to the class *Actinobacteria* (*Actinomycete*) within the division *Firmicutes* (Gram-positive bacteria). Finally one isolate belonged to *Bacterium* RRP-E3 could not be assigned to any known phylum. All of them are members of the domain *Eubacteria*. The 16S rRNA analysis revealed that all isolates belonged to the genera *Pseudomonas, Delftia, Brevundimonas Methylobacterium*, *Bacillus*, *Arthrobacter*, *Staphylococcus* and species *Bacterium* Ph10. The most frequently observed species were of the *gamma Proteobacter* genus *Pseudomonas*. Sequences from 23 isolates had a similarity equal or higher than 95% with other 16S rRNA sequences from the database, while two had less than 90% similarity and the remaining were between 90 and 95%. Only *Pseudomonas* sp. Fa27 showed 99% similarity.

The phylogenetic analysis based on the partial 16S rRNA sequences was able to discriminate the seven main taxonomic lineages using DNA neighbor phylogenetic tree program. Within the main lineages, the sequences obtained from the bacterial strains associated with *Pseudomonas* were often formed in branches separated from the sequences of other bacteria isolated

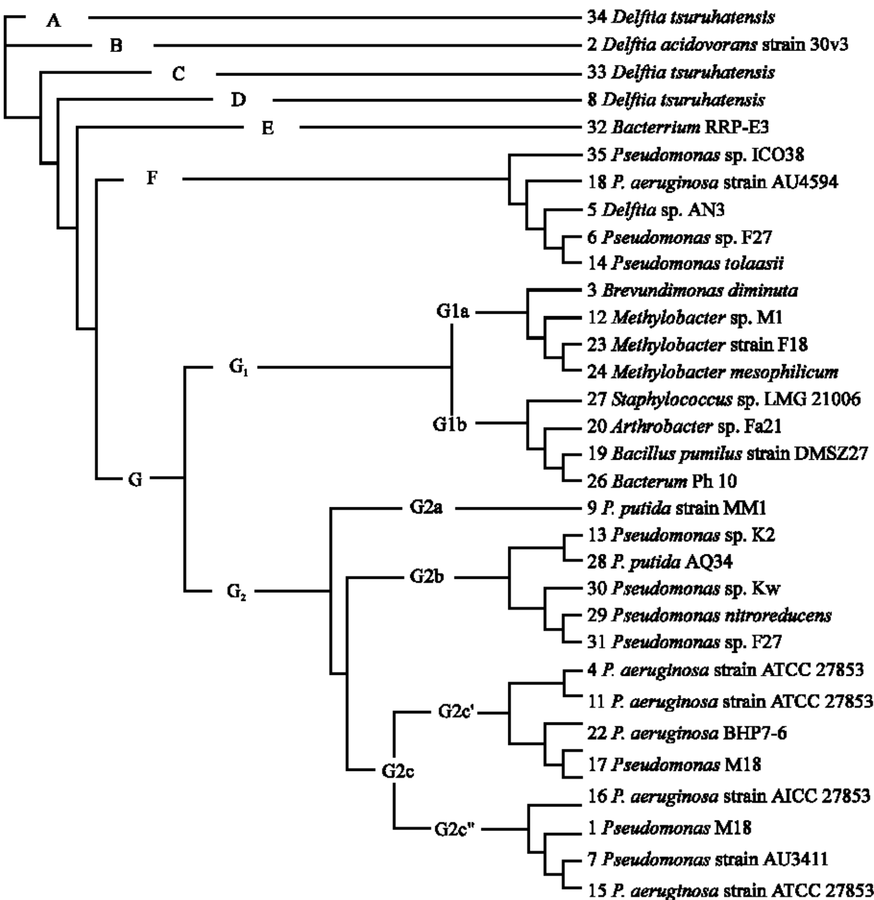

Fig. 1: Phylogenetic tree based on partial 16S rRNA gene sequences of heavy metal removal bacteria by using DNADIST Neighbor phylogenetic tree program

from the Persian gulf. This feature was clear within the branch enclosing the sequences belonging to the gamma, beta and alpha proteobacter subdivision. Others belonged to bacillus and unknown family. There are three phylogeny branches belonged to *Pseudomonas* strains, which are *p. putida* strain MM1, *Pseudomonas* sp. K2 two strains, *P. putida* AQ34, *Pseudomonas nitroreducens*, *Pseudomonas* sp. Fa27, *P. aeruginosa* strain ATCC 27853 consist four strains, *P. aeruginosa* BHP7-6 consist two strains, *Pseudomonas* sp. M18 consist two strains and *P. aeruginosa* strain AU3411 (Fig. 1).

Neighbor-joining analysis revealed the presence of seven well-resolved lineages according to 16S rRNA sequence analysis, designated clusters A to G. Clusters G was further subdivided into G_1 and G_2 subclusters, subclusters were again divided into more subcluster as G_1 consists G_{1a} and G_{1b}, respectively and G_2 includes $G_{2a,}$ G_{2b} and G_{2c}, in addition G_{2b} and G_{2c} divided more small subclusters. Clusters A, B, C and D contained four Delftia strains, where clusters A, C, D contained *Delftia tusurhatensis* that derived from β-subclass *Proteobacteria*, as these tree lineages have the same strains and formed different tree subclasses. Cluster B contained *Delftia acidovoranc* strain 30V3 which derived from α-subclass *Proteobacteria*. Cluster E contained sequences derived from special bacteria *(Bacterium* RRP-E3), cluster F comprised *Pseudomonas* sp. IC038, *Pseudomonas* sp. F27, *Pseudomonas* strain AU4594, *Pseudomonas tolaasii* and *Delftia* sp. AN3. Clusters G were closely related to each other and include; two subclusters G_1 and G_2, that were subdivided into other small subcluster as G_1 divided to two small subcluster $G1_{a,}$ G_{1b} and G_2 divided to three small subcluster G_{2a}, G_{2b}, G_{2c}, the *Pseudomas* strains majority were placed in subcluster G_2.

Gene sequences of heavy metals removal bacteria were grouped within *γ-proteobacteria* (19 sequences), *β-proteobacteria* (6 sequences), *α-proteobacteria* (5 sequences), *Firmicutes* (2 sequences) and *Actinobacteria* (1 sequence). *Bacterium* Ph10 and

Bacterium RRP-E3 could not be assigned to any known phylum. Cluster F mostly belong to *γ- proteobacteria* that consist *Pseudomonas* sp. IC038, *Pseudomonas* sp. F27, *Pseudomonas* strain AU4594 and *Pseudomonas tolaasii*, with the exception of *Delftia* sp. AN3 which belong to *β-proteobacteria*. In cluster G, small subcluster G_{1a} was grouped as *α-proteobacteria* that include; *B. dininuta*, *Methylobacter* sp. Mil, *Methylobacter* strain F18 and *Methylobacter mesophilicum*. Small cluster G_{1b} comprised *Staphylococcus* sp. *LMG 21006*, *Bacillus pumiilus* strain DSMZ27, *Artrobacter* sp. Fa21 and *Bacterium* Ph10. *Staphylococcus* sp. LMG 21006 and *Bacillus pumiilus* strain DSMZ27 belong to *firmicutes*, *Artrobacter* sp. Fa21 belong to *Actinobacteria* and *Bacterium* Ph10 unknown phylum. Subcluster G_2 completely belonged to *γ- proteobacteria* and comprises; *p. putida* strain MM1, *Pseudomonas* sp. K2 two strains, *P. putida* AQ34, *Pseudomonas nitroreducens*, *Pseudomonas* Fa27, *P. aeruginosa* strain ATCC 27853 consist of three strains, *P. aeruginosa* BHP7-6 consist of two strains, *Pseudomonas* sp. M18 consist of two strains and *P. aeruginosa* strain AU3411.

DISCUSSION

The present study exhibited that most of the bacteria isolated from the Persian Gulf belonged to the gram negative, which disagreement with the finding of Barzanti *et al.* (2006), who is reported that most of the heavy metal resistance isolates belonged to gram-positive genera. In the current study most bacteria isolated belonged to *Pseudomonas* strains, these results are in agreement with Wong *et al.* (1993) and Hussein *et al.* (2004). Most of *Pseudomonas* strains isolated from waste water of industrial plants, while *Delfetia* and *Methylobacter* were entirely isolated from the marine environment. This study reported for the first time the role of these bacteria in the removal of heavy metals. Lead accumulation by *Delftia tsuruhatensis* showed maximum concentration at 120 mg g^{-1} dry weight of cells when it was exposed at 1 mM of lead concentration. On the other hand, *Methylobacter mesophilicum* and *Methylobacter* sp. Mil absorbed lead at 92.5 and 34.16 mg g^{-1}, respectively. In this study, *Delftia tsuruhatensis* showed higher capabilities to remove heavy metals (Pb) compared to the findings of other reports. Studies showed that metal removal capability could be modified in a variety of metal concentration and bacteria species. Maximum Pb removal by *Delftia tsuruhatensis* and *Pseudomonas* strain AU3411 was 120 mg g^{-1} dry weight of bacteria cells, the removal capability was found to be up to 90%. The maximum amount of absorption of Cu by bacteria was 54.2 mg g^{-1} dry weight of cells. In the present study one of the marine bacteria *Delftia tsuruhatensis* accumulated lead even more than *Pseudomonas* strains. This bacterial was isolated from the water of Persian Gulf.

It consists of regions with different levels of conservation which allows determining phylogenetic relationships from distantly related species as well as from highly related ones. These features make rRNA a very powerful phylogenetic marker (Woese, 1987; Ludwig *et al*., 1998).

The present study mainly focuses on heavy metals resistance and removal bacteria in the Persian Gulf. All high resistance bacteria were isolated by using 1 mM of multi-mix heavy metal containing media. Therefore the bacterial isolates were not representative of all Persian Gulf bacteria. However, partial 16S rRNA sequence analysis in the present study showed that most isolates belonged to *Pseudomonas*, which is in agreement with most other reports about heavy metal removal bacteria. 16S rRNA sequences of isolated bacteria showed conformed to remove heavy metals with all isolates, particularly with *Methylobacter* and *Delftia* species which revealed high ability uptake when grown in the presence of heavy metals. Interestingly the nucleotide sequence comparison of 16S rRNA of isolates presented seven (A-G) phylogenetically distinct lineages.

Microbial communities in Calyptogena sediment and microbial mats of Sagami Bay, Japan, were characterized using 16S rRNA gene sequencing and lipid biomarker analysis. Characterization of 16S rRNA gene isolated from these samples suggested a predominance of bacterial phylotypes related to Gammaproteobacteria (57-64%) and Deltaproteobacteria (27-29%) (Fang *et al*., 2006).

The phylogenetic analysis demonstrated proteobacteria as the most abundant group in all the samples examined. This experiment is also in agreement with the finding of Williams *et al.* (2004) and Spring *et al.* (2000) that showed that most isolated bacteria from drinking water and fresh water lakes belong to proteobacter. As other bacteria and Gram positive bacteria were found in the present study, these results indicate that most heavy metal removal bacteria that has been isolated from marine environment and enclosed industrial area belong to the proteobacter subclass.

In bacterial taxonomy, it is generally accepted that two bacteria do not belong to the same species when the similarity of the 16S rRNA gene is below 97% (Stackebrandt and Goebel, 1994). Interestingly, 16S rRNA analysis showed several bacterial isolates described in the present study were *Pseudomonas* strains ACTT 27854

that belong to the same strain but were not grouped at same lineage. These results clearly revealed inter-species differences so one of *Pseudomonas* strain ACTT 27854 was located in separate branches and showed close genetic relationship with other *Pseudomonas* strains. It may thus be concluded that the characterization of most strains were close.

There was clear separation of *Pseudomonas* and other group of bacteria in the phylogenetic tree. Phylogenetic analysis of the sequences in this study found two separate *Pseudomonas* groups that indicated a clear discriminate to 16S rRNA genes. The phylogenetic tree in Fig. 1. shows that all small subcluster bacteria G_{2c} were *Pseudomonas* that include; *P. aeroginosa* strain ATCC 27853, *P. aeroginosa* BHP7-8, *Pseudomonas* M18, *P. aeroginosa* BHP7-6 p, *P. aeroginosa strain* and isolated from the industrials areas except one, while all small subcluster G_{2b} were also *Pseudomonas* that comprised *Pseudomonas* sp. K2, *P. putida* AQ34, *Pseudomonas* Fa27 isolated from the marine environment except one. The phylogenetic tree also showed the cluster belonging to the strains and marked with 6, 14, 18, 35 to all belong to *Pseudomonas* but are separated from other *Pseudomonas* groups and located on different lineage. These kinds of bacteria were isolated from waste water of industrials area and form a special separate lineage. In the phylogenetic tree those strains with marked 34, 2, 33, 8, 32 (cluster A-E) were isolated from marine environment and form a separate cluster. Those strains that marked with 6, 14, 18, 35 formed one lineage (F) with *Dolftia* AN3 which is isolated from marine environment. These results clearly revealed separation between some marine strains and industrial strains genetically. Therefore, strong discrimination was observed between some *Pseudomonas* strains isolated from marine environment and from industrial waste water, as most marine bacteria showed tendency toward other marine bacteria.

The 16S rRNA analysis for characterization of bacteria is excellent. In this method, the discriminatory power of this technique depends on genetic diversity of class of isolates being examined (Blanc *et al*., 1993; Maslow *et al*., 1993). Although no marker fulfils all of the requirements for epidemiological-ecological comparison of bacteria strain, 16S rRNA offers a clear advantage over other molecular methods because it is based on the rRNA sequences which are highly conserved among eubacteria. In addition, identification of diversity and the ecological spread of environmental bacteria are difficult using conventional methods.

Based on near full-length 16S rDNA sequence analysis, all isolated *Pseudomonas* strains form four distinct clusters using the DNADIST neighbor phylogenetic tree. Comparison of 16S rRNA gene sequences revealed a greater genetic diversity in *Pseudomonas* strains. As expected, cluster I absolutely belong to industrial strains but cluster H divided in to two sub cluster belong to *Pseudomonas* strains that were mostly isolated from marine environment and other marine isolated bacteria. *Pseudomonas* sp. ICO38 form a separate cluster (G), while the other three *Pseudomonas*, *Pseudomonas tolaasii*, *Pseudomonas* sp. F27 and *Pseudomonas strain* AU4594 showed close relationship with *Delfetia* sp. *AN3* and form separate cluster(F). These results suggest that the *Pseudomonas* genus grouped at four sub clusters is very heterogeneous.

Feris *et al*. (2003) suggested that physiological stress caused by the toxic effects of metals leads to selection of less diverse communities comprising metal-resistant populations and a general suppression of metabolic activity. There was no apparent correlation between sediment metal content and either diversity or total productivity (Feris *et al*., 2003). Rather, the structure microbial communities' changes (i.e., population and group-level composition and relative abundance). Another prior study suggested that compensatory changes in response to metal toxicity may alter genotypic and/or phenotypic characteristics of a community at metal concentrations below thresholds that impact metabolic activity (Baath *et al*., 1998). Species diversity and the structure of microbial communities was affected by the heavy metals pollution. When, microorganisms exposed to long-term heavy metals contaminated areas, their community tolerance to the heavy metals increased, for instance, forest soils exposed for centuries to naturally lead concentrations were observed to support an abundance of lead-tolerant species (Baath *et al*., 2005). The present study clearly showed differences between location of sampling and diversity, as all isolated bacteria from industrial area belonged to *Pseudomonas* strains, while bacteria from marine environment were more diverse, as it may be increasing the population of heavy metal resistance bacteria in the industrial waste water and heavy metal contaminated areas.

In conclusion the present study clearly showed differences in the types of bacteria between location of sampling and diversity, as all isolated bacteria from industrial area belonged to *Pseudomonas* strains. Meanwhile bacteria strains isolated from marine environment were more diverse. As it may be consequent of increasing the population of heavy metal resistance bacteria in the heavy metal contaminated areas.

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