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# Characterization and Bioremediation potential of marine psychrotolerant *Pseudomonas* spp. isolated from the Mediterranean Sea, Egypt.

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**ABSTRACT**

Fourteen psychrotolerant *Pseudomonas* strains were isolated from seawater and sediments in the Mediterranean Sea, Egypt, using culture-dependent techniques. Genotypic characterization for the fourteen strains was performed using 16S rDNA sequence analysis. The *Pseudomonas* strains were screened for some physiological, and biochemical characters, also resistance to some antibiotics and heavy metals were tested. Moreover, heavy metals bioaccumulation and azo-dyes removal were estimated. All tested *Pseudomonas* strains were able to resist and accumulate several metals ($Pb^{2}$+, $Cu^{2+}$ and $Cd^{2+}$) with variable degrees, depending on bacterial strains and metal ion species. The highest tolerance (MICs) was observed with lead ions as all strains grew in presence of 750-800 ppm of lead, also, lead ions were easier to be bioaccumulate than the other metals, while cadmium bioaccumulation was relatively low with respect to the other two metals. *Pseudomonas* sp. H69A was the most potent strain in accumulation of the different metals. It supports the highest accumulated values of lead and cupper (2.95 and 1.837 mg /g fresh cells, respectively).The *Pseudomonas* strains were monitored for their ability to decolorize three different azo-dyes (fast orange, methanil yellow and acid fast red). All *Pseudomonas* strains achieved powerfull decolorization activity with the tested dyes. The maximum decolorization activities were recorded in fast orange. *Pseudmonas* sp. H26S recorded the highest decolorization percentages (91%) with fast orange and their biosorption capacity was 4.8 mg/g.

## INTRODUCTION

Marine ecosystem has a higher diversity of living organisms compared to the land that provides numerous resources to human societies (Hill and Fenical, 2010), and the marine microorganisms are highly abundant in nature. It is realistic to assume that we know less than 0.1%, probably only 0.01% (Simon and Daniel, 2009) of all microbes in the oceans. Marine microorganisms inhabit all kinds of available niches. They can be isolated from the marine water, sediments, and mangroves associated with the marine habitats, normal flora of the marine organisms, and deep sea hydrothermal vents (Yang *et al*., 2013; Zhang *et al*., 2013). Cold adapted organisms, psychrophiles and psychrotrophs, inhabit both terrestrial and aquatic environments in

polar and alpine regions, in the bulk of the ocean, in shallow subterranean regions, in the upper atmosphere, in refrigerated environments, and on plants and animals living in cold regions (Margesin *et al*., 2007). Psychrotolerant organisms grow well at temperatures close to the freezing point of water, but have the fastest growth rates above 20 ºC (Margesin *et al*., 2007;Orellana-Saez *et al*., 2019), whereas psychrophilic organisms grow faster at a temperature of 15 ºC or lower, but are unable to grow above 20ºC (Margesin *et al*., 2007).

Both physical and chemical factors affect the distribution and activity of different microbial types (Shimshon and Rita, 2005). Cold-adapted organisms have successfully evolved features, genotypic and/or phenotypic, to surmount the negative effects of low temperatures and to enable growth in these extreme environments (Salvino *et. al*., 2006). They contribute essentially to the processes of nutrient turnover, biomass production, and litter decomposition in cold ecosystems. There is evidence of a wide range of metabolic activities in cold habitats, e.g., nitrogen fixation, photosynthesis, methanogenesis, and degradation of natural or xenobiotic organic compounds such as proteins, carbohydrates, lignin, and hydrocarbons (Trotsenko and Khmelenina, 2005).

Until now, about 100 species of the genus *Pseudomona*s have been reported from various habitats including Antarctica (Vásquez-Ponce, *et al*., 2018; Yarzabal *et al*., 2019). Kriss *et al*., (1976) were the first to report the existence of *Pseudomonas* species in Antarctica.

The biotechnological applications of psychrotolerant and psychrophilic bacteria have been studied by Huston (2007). Psychrotolerant bacteria are great value for bioremediation of contaminated ecosystems in Antarctica. Psychrotolerant bacteria have the ability to maintain activity under the extreme conditions of the polar ecosystems (Paniker *et al*., 2006).

The extent of cold habitats is decreasing due to global warming thus affecting the evolution of mesophilic bacteria. The extreme biotechnological importance of cold-adapted bacteria along with their essential role in biogeochemical cycles (Feller and Gerday, 2003) emphasizes the importance of understanding to what extent these bacteria can adapt to ecosystems warming. The research of cold-adapted bacteria in temperate ecosystems will add to the knowledge about this topic (Azevedo *et al.*, 2013).

In a previous work (Abd-Elnaby *et al.*, 2016), reported the isolation and identification of *Psychrobcter* species from work Mediterranean Sea, Egypt. The present work aimed to throw some light on the characterization of *Pseudomon*as species and their potentiality in metal accumulation and azo dye decolorization

## MATERIALS AND METHODS

### Bacterial strains

Fourteen *Pseudomonas* strains used in the present study were isolated from Mediterranean Sea, Egypt, using culture dependent techniques. They were able to grow over a temperature range (5 to 30°C) showing good growth at 5°C.

### Media

Nutrient agar (Oxoid LTD, England) and tryptone yeast extract were used for isolation and growth of the isolated bacteria. Media were prepared with aged seawater and distilled water (1:1, v/v). Tryptone yeast extract contained (g/l): Tryptone, 5.0; yeast extract, 2.5; glucose, 1.0; dipotassium hydrogen orthophosphate, 0.2 and

magnesium sulfate, 0.05. For solid medium 15 g/l agar was added (Lyudmila *et al.*, 2002).

**Isolation of bacteria**

Samples of water and sediment collected from different sites along the Mediterranean coast were plated on nutrient agar plates and incubated at different temperatures to select those showing psychrophilicity.

**Bacterial identification**

The bacterial isolates were cultured in tryptone yeast extract medium overnight and the genomic DNAs were extracted with the genomic DNA extraction protocol of GeneJET genomic DNA Purification Kit (Fermentas). The PCR clean-up of the PCR product was performed using GeneJETTM PCR Purification Kit (Fermentas) at Sigma Scientific Services Company, Egypt. The sequencing of the PCR product was made by the GATC Company using ABI 3730xl DNA sequencer with universal primers (16S 27F and 16S 1492R), (5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-GGT TACCTTGTTACGACTT-3'). Genotypic characterization was performed using 16S sequence analysis. Multiple alignments with sequences of the most closely related members and calculations of levels of sequence similarity were carried out using BioEdit (software version 7) (Hall, 1999). Sequences of rRNA genes, for comparison, were obtained from the National Center for Biotechnology Information database.

**Phenotypic characterization**

The characters of the *Pseudomonas* strains were studied following the standard microbiological methods as reported in Bergey's Manual of Systematic Microbiology (Holt *et al.,* 2005). Gram reaction, colony morphology, vegetative cell and spore characteristics were tested. Also, production of degradative enzymes and antibiotic sensitivity were tested (Finegold and Martin, 1982).

**Growth measurement**

Growth patterns of *Pseudomonas* strains were tested by allowing cells to grow on tryptone medium at different temperatures (5, 12, 20 and 30°C)**.** Growth of the *Pseudomonas* strains were monitored by measuring the optical density (OD) of cultures using Spectrophotometer, Model (SP-300) Optima, at wavelength 550 nm using the medium as blank.

**Bioaccumulation of heavy metal**

**Preparation of heavy metal solutions**

Stock solutions of chloride salts of zinc, nickel, cadmium and lead in addition to copper sulfate were individually added in equivalent weights to di-ionized water to final concentration of 50–400 ppm for $Zn^{2+}$, $Ni^{2+}$, $Cd^{2+}$, and $Cu^{2+}$ and 10–2000 ppm for $Pb^{2+}$. Solutions were sterilized by filtration through 0.22 µm Millipore bacterial membrane filters (Forbes *et al.,* 1998).

**Determination of the minimal inhibitory concentrations (MICs)**

Agar plates supplemented with different concentrations of each metal cations were prepared and dried at 37 °C for 30 min. Plates were then spot-inoculated with the tested organisms and incubated for 2 days (Mergeay *et al.,*1985). Plates containing media without metals were used as controls. The MICs are measured as the lowest concentration of the metal ion preventing bacterial growth (Sabry *et al.,* 1997).

**Metal bioaccumulation assay**

For metal bioaccumulation studies, aliquots (0.05 g) of fresh bacterial biomass were placed in 100 ml screw-caped tubes containing 50 ml of metals solutions with a known initial concentration. Bacterial cells were then kept in contact with the metal solution in a shaker incubator at 120 rpm for 1 h. For the estimation of the residual

metal ions, the metal biomass suspensions were centrifuged at 7000 rpm for 20 min and the supernatants were (in most cases) diluted by deionized water to a final concentration of 1/10, then submitted to instrumental analysis using an Atomic Absorption Spectrophotometer (Shimadzu -AA-6800). Bioaccumulation of metals ions (q) is calculated according to the following equation:

Metal bioaccumulation (q) = V(IC -FC)/w

where V, volume of reaction (l); IC, initial metal concentration(mg/l); FC, final metal concentration (mg/l); and W, total biomass (g), (Abd-Elnaby *et al*., 2011).

**Decolorization of dyes**

Stock solutions of dye were prepared in water and sterilized at 121 °C for 15 min, 1 ml of dye solution contains 1.5 mg dye/l. The strains were monitored for their capacity to decolorize 3 different azo-dyes (fast orange, methanil yellow and acid fast red). The experiment was conducted according to El-Ahwany (2008). Each bacterium was grown in 250 ml Erlenmeyer flask containing 50 ml of tryptone yeast extract medium. The flask was inoculated from a pre-culture of the same organism and incubated at 30 °C shacked till late stationary phase ($OD_{550}$ 1.0). Cell biomass (0.3 mg) was transferred to a test tube containing 1 ml of dye solution (15 $x10^{-4}$ g dye/l). Tubes containing dye solutions and biomass were shacked for 24 h, followed by centrifugation to remove cells. The residual dye concentration was measured in the clear supernatants. The spectral profiles as well as absorbance values of individual dyes at their $k_{max}$ were monitored using spectrophotometer. Control experiments were carried under similar conditions without biomass addition. All experiments were carried out in duplicates and the results are the mean value. Decolorization percentage was calculated as follows:

Decolorization% = $(C_0 - C_e)/ C_0$ x100

where Co= Initial absorbance reading before decolorization; and Ce= final absorbance reading after decolorization.

Biosorption capacity by cells (mg/g) = concentration of dye biosorbed/mg biomass

The concentrations of the residual dyes in the supernatants were determined using a standard curve. All results are the mean of replicates.

## RESULTS AND DISCUSSION

Fourteen bacterial isolates showing good growth at 5°C were subjected to identification by molecular tools. DNA sequences were deposited in Genbank. Accession number, sequence homology and similarity percent to closest strains are as given in Table 1. Strains showed similarity percent to different *Pseudomonas* 99% and were this assigned as members of Genus Pseudomonas, Gamma proteobacteria. Five strains (H26S, H69A, H20S, H20 and H45A) were isolated from sea water of Alexandria Eastern Harbor, three (H73, H50 and H49) from sediments of Alexandria Eastern Harbor, three (H60, H63A and H44) from Abu-Qir sediments, two (H67 and H67S) from Rashid sea water one *strain*.(H64) from sediments of Rashid.

Numerous psychrophilic and psychrotolerant *Pseudomonas* strains have been isolated from Antarctica, (Reddy *et al*., 2004; Ma *et al*., 2006; Bozal *et al*., 2007, Orellana-Saez *et al*., 2019, Yarzabal *et al*., 2019, Divya *et al*., 2019).

Table 1: *Pseudomonas* strains, accession numbers and similarity percentage to nearest neighbors.

| Identification & source of isolation | Homology strain | Homology % | Accession number | Phyllum |
|---|---|---|---|---|
| ***Pseudomonas* sp. H20 E(W)** | *Pseudomonas brenneri* partial rrs gene, strain CCUG 60601 | 99% | SeqH20 KF207753 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H26S E(W)** | *Pseudomonas* sp. H-12 16S ribosomal RNA gene, partial sequence | 99% | SeqH26S KF207763 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H44 A(S)** | *Pseudomonas* sp. SOK33 16S ribosomal RNA gene, partial sequence | 99% | SeqH44 KF207764 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H45A E(W)** | *Pseudomonas* sp. S5-28 16S ribosomal RNA gene, partial sequence | 98% | SeqH45 KF207765 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H49 E(S)** | *Pseudomonas gessardii* strain CIP 105469 16S ribosomal RNA, partial sequence | 99% | SeqH49 KF207766 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H50 E(S)** | *Pseudomonas brenneri* strain G10 16S ribosomal RNA gene, partial sequence | 99% | SeqH50 KF207767 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H60 A(S)** | *Pseudomonas brenneri* strain G11 16S ribosomal RNA gene, partial sequence | 99% | SeqH60 KF207768 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H63A A(s)** | *Pseudomonas brenneri* strain G96 16S ribosomal RNA gene, partial sequence | 99% | SeqH63A KF207769 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H64 R(S)** | *Pseudomonas panacis* strain CG20106 16S ribosomal RNA, partial sequence | 99% | SeqH64 KF207770 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H73 E(S)** | *Pseudomonas* sp. H-12 16S ribosomal RNA gene, partial sequence | 99% | SeqH73 KM194720 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H67S R(W)** | *Pseudomonas* sp. Lyh1B 16S ribosomal RNA gene, partial sequence | 99% | SeqH67S KM052578 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H67 R(W)** | *Pseudomonas* sp. LV-5 16S ribosomal RNA gene, partial sequence | 99% | SeqH67 KM052581 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H20S E(W)** | *Pseudomonas* sp. SRI2 partial 16S rRNA gene, strain SRI2 | 76% | SeqH20S KM052580 | Gamma Proteobacteria |
| ***Pseudomonas* sp. H69A E(W)*** | *Pseudomonas plecoglossicida* strain FPC951 16S ribosomal RNA, partial sequence | 89% | SeqH69A KF207761 | Gamma Proteobacteria |

* E(W) = sea water of Alexandria Eastern Harbor, E(S) = sediments of Alexandria Eastern Harbor, A(S) = sediments of Abu-Qir, R(S) = sediments of Rashid, R(W) = sea water of Rashid

## Phenotypic characterization

The *Pseudomonas* strains were screened for tolerance to a range of temperatures, salinities and pH, in addition to some physiological and biochemical characterization (Table 2).

To understand these microorganisms' role in this extreme environment, the characterization and description of new species is vital. Vásquez-Ponce *et al.* (2018) investigated the Phylogenetic and phenotypic analysis identification of three probable novels *Pseudomonas* species isolated on King George Island, South Shetland, Antarctica.

Table 2: Phenotypic characteristics of *Pseudomonas* strains

| **Characterstics features** | ***Pseudomonas* sp. H45** | ***Pseudomonas* sp. H73** | ***Pseudomonas* sp. H69** | ***Pseudomonas* sp. H70** | ***Pseudomonas* sp. H67** | ***Pseudomonas* sp. H60** | ***Pseudomonas* sp. H63A** | ***Pseudomonas* sp. H44** | ***Pseudomonas* sp. H50** | ***Pseudomonas* sp. H67C** | ***Pseudomonas* sp.H20** | ***Pseudomonas* sp. H64** | ***Pseudomonas* sp. H49** | ***Pseudomonas* sp. H45A** | **% of positive tests** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Morphological characters | | | | | | | | | | | | | | | |
| Colony Color | | | | | | | | | | | | | | | |
| Cream | + | + | + | + | + | + | + | + | + | - | + | - | - | + | 78 |
| White | - | - | - | - | - | - | - | - | - | + | - | - | + | - | 14 |
| Orange | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 7 |
| Cell shape | | | | | | | | | | | | | | | |
| Rods | + | + | + | + | + | + | + | + | + | + | + | - | + | + | 93 |
| Cocci | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| Coccobacilli | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 7 |
| Physiological tests | | | | | | | | | | | | | | | |
| Catalase production | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| Oxidase production | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| Nitrate reduction | - | + | + | + | - | + | + | + | + | - | - | + | + | - | 64 |
| Urease production | - | + | + | + | - | - | - | - | + | - | - | + | - | - | 35 |
| $H_2S$ production | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| Growth at different temp.(°C) | | | | | | | | | | | | | | | |
| 5 – 30 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 40 | + | + | + | + | + | + | - | - | + | + | + | + | - | + | 78 |
| 45 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 |
| Growth at pH | | | | | | | | | | | | | | | |
| 5-9 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| Growth in presence of NaCl (%) | | | | | | | | | | | | | | | |
| 4 – 7 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 9 -13 | - | - | + | - | - | + | - | + | + | - | - | + | + | + | 50 |

**Production of degradative enzymes**

The ability of *Pseudomonas* strains to secrete six different extra-cellular hydrolytic enzymes was tested. They produced one or more hydrolytic enzymes, chitinase being the one secreted by most strains (100%). Lipase (64%), protease (64%) and amylase (50%) were moderate in prevalence (Table 3).

Until now, researchers have found a wide range of marine microorganisms including *Psychrobacter* and *Pseudomonas* that can produce degradative enzymes (Zhang and Kim, 2010).

**Sensitivity to antibiotics**

The variability in antibiotic resistance profiles could indicate important strain-level. The antibiotic's different patterns susceptibility would suggest strain-level differences in accessory or antibiotic-resistance genes either encoded on plasmids, within the genome, or transposons in the bacterial strains (Babaeekhou *et al*., 2018; Pang *et al.*, 2019).

In the present study, sensitivity of *Pseudomonas* strains to various antibiotics was tested. Maximum resistance was observed with Ampicillin, 20µg (100%),

followed by ampicillin, 10µg and Ceftriaxone, 30µg (93%). Occurrence of antibiotic resistance were extremely low with cefoperazone, 5µg (29%), (Table 4). The data in the present study was in partial agreement with those reported by Reddy *et al*. (2004).

Table 3: Production of degradative enzymes by *Pseudomonas* strains.

| **Degradation of** | ***Pseudomonas* sp. H26 S** | ***Pseudomonas* sp. H73** | ***Pseudomonas* sp. H69 A** | ***Pseudomonas* sp. H20 S** | ***Pseudomonas* sp. H67** | ***Pseudomonas* sp. H60** | ***Pseudomonas* sp. H63A** | ***Pseudomonas*sp. H44** | ***Pseudomonas* sp. H50** | ***Pseudomonas* sp. H67S** | ***Pseudomonas*sp.H20** | ***Pseudomonas*sp. H64** | ***Pseudomonas*sp. H49** | ***Pseudomonas*sp. H45A** | **% of positive tests** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Starch | | | | | | | | | | | | | | | |
| 5°C | + | + | + | - | + | - | + | - | - | + | - | + | - | - | 50 |
| 10°C | + | + | + | - | + | - | + | - | - | + | - | + | - | - | 50 |
| 20°C | - | + | - | - | - | - | - | - | - | + | - | - | - | - | 14 |
| Gelatin | | | | | | | | | | | | | | | |
| 5 -20 °C | - | + | - | + | + | - | + | - | + | + | - | + | + | + | 64 |
| Chitin | | | | | | | | | | | | | | | |
| 5 -10 °C | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 20°C | + | + | + | + | - | + | + | + | + | + | + | + | - | + | 85 |
| Tween 20 | | | | | | | | | | | | | | | |
| 5 - 10 °C | - | - | - | - | - | - | - | + | - | - | - | - | - | + | 14 |
| 20 °C | - | + | - | - | - | - | - | - | - | - | - | - | - | + | 14 |
| Tween 80 | | | | | | | | | | | | | | | |
| 5 - 10 °C | + | + | + | + | - | + | - | + | + | - | + | + | - | - | 64 |
| 20 °C | + | + | + | + | - | - | + | - | + | - | + | + | + | - | 64 |
| Skim milk | | | | | | | | | | | | | | | |
| 5°C | - | - | + | - | - | - | - | - | - | - | - | - | - | - | 7 |
| 10°C | - | - | + | + | - | - | - | + | + | - | - | + | - | - | 35 |
| 20°C | + | - | + | + | - | - | + | + | + | - | + | + | + | - | 64 |

Table 4: Sensitivity of *Pseudomonas* strains to some antibiotics

| **Antibiotics(µg)** | ***Pseudomonas* sp. H26 S** | ***Pseudomonas* sp. H73** | ***Pseudomonas* sp. H69 A** | ***Pseudomonas* sp. H20 S** | ***Pseudomonas* sp. H67** | ***Pseudomonas* sp. H60** | ***Pseudomonas* sp. H63A** | ***Pseudomonas*sp. H44** | ***Pseudomonas* sp. H50** | ***Pseudomonas* sp. H67S** | ***Pseudomonas*sp.H20** | ***Pseudomonas*sp. H64** | ***Pseudomonas*sp. H49** | ***Pseudomonas*sp. H45A** | **% of positive tests** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amikacin, 30 | - | + | + | - | + | - | + | + | - | + | + | - | + | - | 57 |
| Ceftazidime, 30 | - | + | + | + | + | + | + | + | + | + | + | - | + | + | 86 |
| Cefoperazone, 5 | - | + | - | + | + | - | + | - | - | - | - | - | - | - | 29 |
| Ampicillin, 10 | + | + | + | + | - | + | + | + | + | + | + | + | + | + | 93 |
| Ampicillin, 20 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| Amipenem, 10 | - | + | - | - | - | + | + | - | + | - | - | - | + | - | 35 |
| Rifampicin, 30 | - | + | - | - | + | + | + | + | + | + | + | + | + | + | 78 |
| Doxycycline, 30 | + | + | + | + | - | + | + | + | + | + | + | - | + | + | 86 |
| Ceftriaxone, 30 | - | + | + | + | + | + | + | + | + | + | + | + | + | + | 93 |
| Subphamethoxazole, 25 | - | + | + | + | + | + | + | + | - | + | + | + | + | + | 86 |

**Growth of *Pseudomonas* strains at different temperatures**

Almost a similar pattern of growth was observed for all *Pseudomonas* strains as shown in Fig. 1. All *Pseudomonas* strains grew at the temperature range tested. It is noticeable that no lag phase was observed except for 4 strains (H49, H50, H67 and H20S). It is worth mentioning that growth in cultures incubated at 5 and 10°C for 10 days reached the similar values of those incubated at 20 and 30 °C for 10 days. At these temperatures growth rate was higher compared to that at 5 and 10 °C.

Psychrotolerant bacteria grow well at temperatures near the freezing point of water, but the rate of growth increases at temperature above 20 °C (Margesin *et al.*, 2007; Orellana-Saez *et al*., 2019). Bakermans *et al*. (2006) reported that facultative psychrotolerant or cold adapted bacteria such as Psychrobacter cryohalolentis can grow in temperature ranges from 10 to 30 °C. Also Bozal *et al.*, (2007) isolated two psychrotolerant stains namely *Pseudomonas peli and Pseudomonas anguilliseptica*, these two psychrotolerant strains grew between -4 and 30 °C.

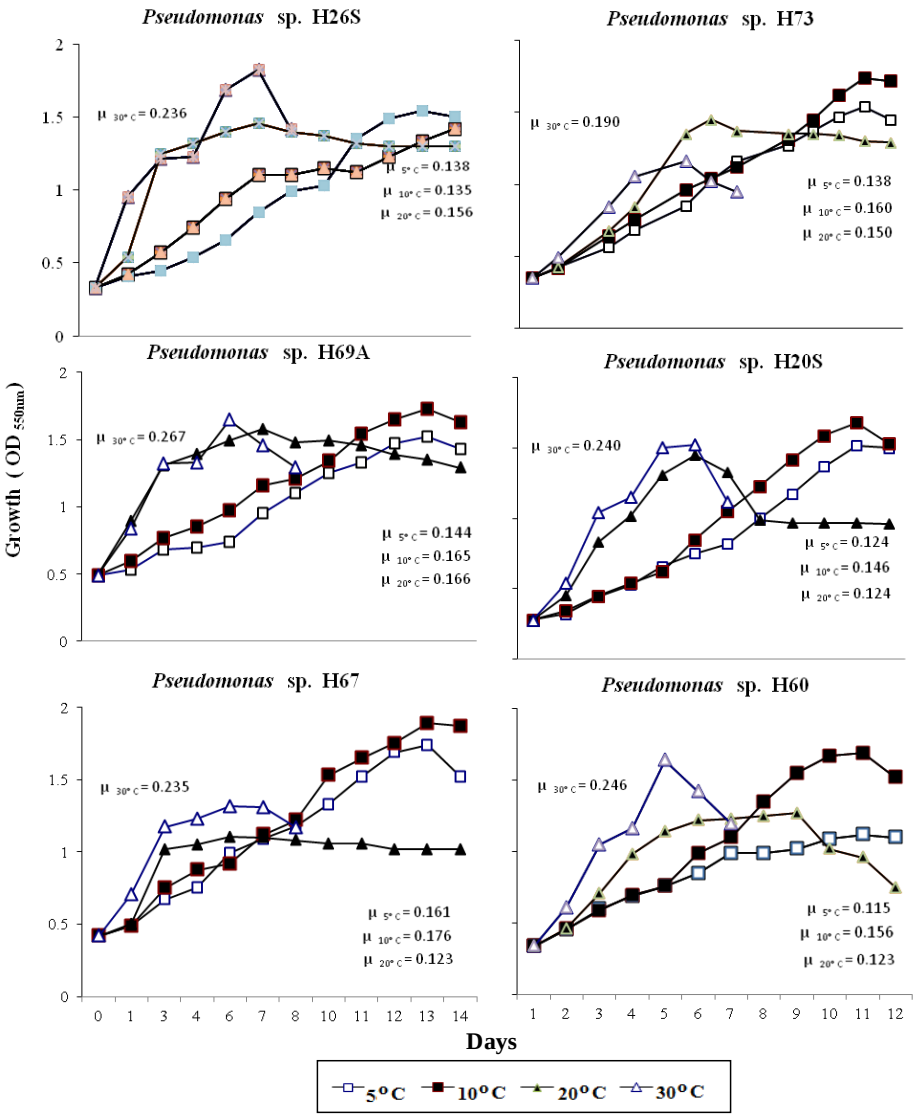


Fig. 1. Growth of *Pseudomonas* strains on tryptone yeast extract medium at different temperatures

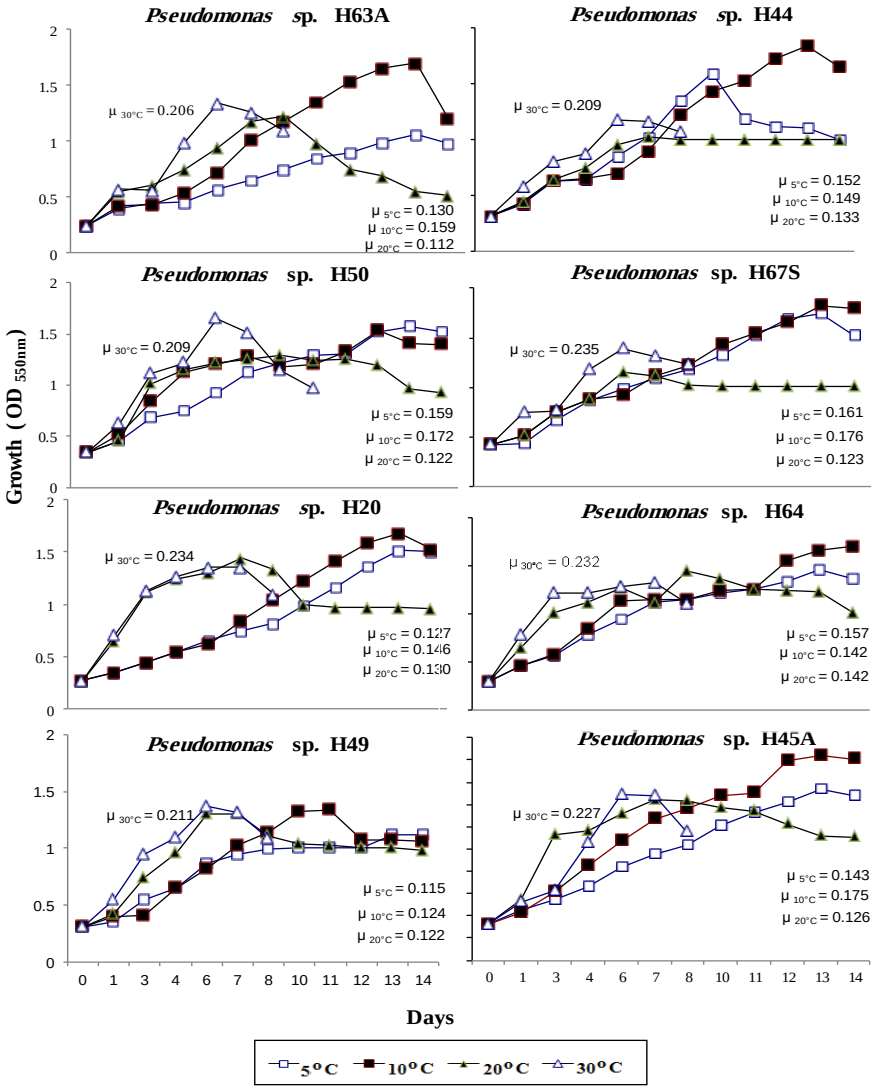


Fig. 1: Growth of *Pseudomonas* strains on tryptone yeast extract medium at different temperatures (cont.)

## Heavy metal accumulation by *Pseudomonas* strains

### Minimum inhibitory concentration (MIC)

The MIC values of each metal against *Pseudomonas* strains showed that, the resistance to metal ions was species dependent i.e. varied according to strain tested. The highest tolerance was observed with lead ions as all strains grew in presence of 750-800 ppm, for zinc the MICs ranged from 50 to 400 ppm, while for nickel they varied from 100 to 400 ppm. Also, *Pseudomonas* strains were resistance to cupper and cadmium (150 to 400 ppm). The tested *Pseudomonas* stains were suitable for the multiple metals resistance. The two *Pseudomonas* strains H67 and H60 were the most resisted strains isolated in this work (Table 5).

Other studies are in good agreement with our data (Ashraf *et al.*, 2007; Rajbanshi, 2008; Selvi *et al.*, 2012). Moreover, Anyanwu and Nwachukwun (2011) reported that some isolates had MIC over 1000 µg/ ml against lead.

Table 5: Minimum inhibitory concentration of heavy metals for *Pseudomons* strains

| Strains | Heavy metal concentrations (ppm) | | | | |
|---|---|---|---|---|---|
| | $Zn^{2+}$ | $Ni^{2+}$ | $Cu^{2+}$ | $Cd^{2+}$ | $Pb^{2+}$ |
| *Pseudomonas* sp. H26S | 400 | 250 | 300 | 250 | 800 |
| *Pseudomonas*sp.H73 | 350 | 350 | 250 | 400 | 800 |
| *Pseudomonas*sp.H69A | 300 | 400 | 250 | 350 | 800 |
| *Pseudomonas*spH20S | 250 | 150 | 200 | 200 | 750 |
| *Pseudomonas*sp. H67 | 400 | 400 | 400 | 250 | 800 |
| *Pseudomonas*sp. H60 | 400 | 400 | 300 | 150 | 800 |
| *Pseudomonas*sp.H63A | 350 | 400 | 350 | 300 | 750 |
| *Pseudomonas*sp. H44 | 300 | 400 | 300 | 200 | 800 |
| *Pseudomonas*sp. H50 | 50 | 250 | 250 | 300 | 800 |
| *Pseudomonas*spH67S | 150 | 200 | 200 | 250 | 800 |
| *Pseudomonas*sp. H20 | 300 | 400 | 200 | 350 | 750 |
| *Pseudomonas*sp. H64 | 50 | 400 | 150 | 300 | 800 |
| *Pseudomonas*sp. H49 | 400 | 250 | 400 | 300 | 800 |
| *Pseudomonas*sp.H45A | 300 | 100 | 250 | 400 | 800 |

## Metal bioaccumulation

Few publications dealt with the removal of heavy metals by psychrotolerant bacteria Data in Fig. 2 depict that all psychrotolerant *Pseudomonas* strains were able to accumulate tested metal ions with a variable degree, depending on bacterial strains and metal ion species. In general, lead was the most readily accumulated metal compared to other metal ions. It is worth to mention that *Pseudomonas* sp. H69A cells exhibited the highest accumulated values to lead and cupper ions (2.95 and 1.837 mg /g wet cells, respectively).Cadmium bioaccumulation was relatively low with respect to the other two metals. Bioaccumulation values ranged from 0.217 to 1.690 mg /g fresh cells.

Marine bacteria transform, adsorb and accumulate heavy metals in many food chains (De Souza *et al.,* 2006). Shakibaie *et al*., (2008) and Odokuma and Akponah (2010) reported that *Pseudomonas* strains were predominant bacteria which could tolerate high concentrations of copper and zinc. Krishna *et al*., (2012) mentioned that *Pseudomonas* sp. has high efficiency to accumulate cadmium ions and a high adsorption yield for the treatment of wastewater containing cadmium ions.

## Dyes decolorization by *Pseudomonas* strains

Data in Fig. 3 depict that all *Pseudomonas* strains achieved powerfull decolorization activity with tested dyes (fast orange, methanil yellow and acid fast red).The decolorization percentages of the tested dyes by *Pseudomonas* strains ranged from 33% to 91% for fast orange, 5% to 73% for methanil yellow and 24% to 82% for acid fast red. The maximum decolorization activity was recorded in fast orange. *Pseudmonas* sp. H26S recorded the highest decolorization percentages (91%) with fast orange and their biosorption capacity were 4.8 mg/g. whereas, *Pseudomonas* sp. H63A was more effective in decolorization of methanil yellow with decolorization percentage 65% and biosorption capacity 3.23 mg/g. Most of the tested isolates recorded low biosorption percentages with acid fast red (24% - 57%), except *Pseudomonas* sp. H20S recorded high biosorption percentage (82%) with biosorption capacity reached to 3.7 mg/g.

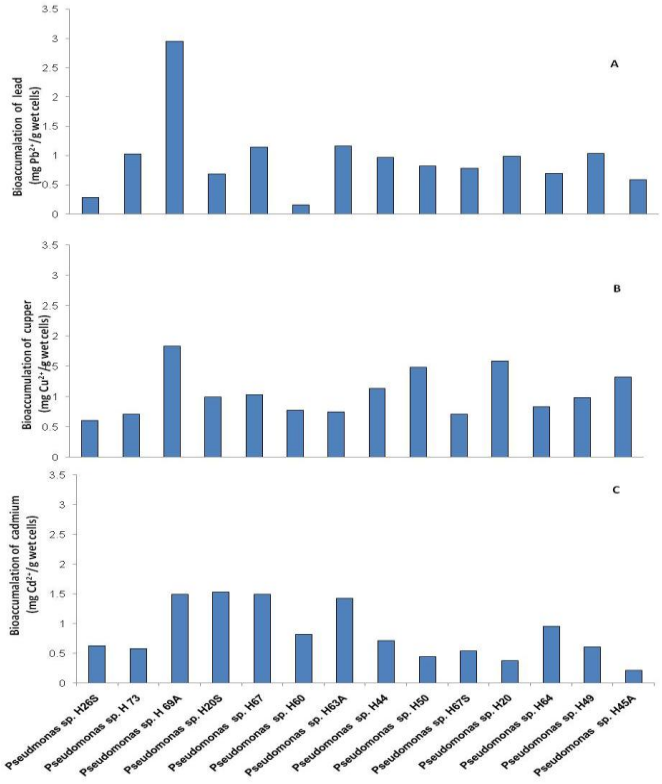


Fig. 2: Bioaccumulation of $Pb^{2+}$, $Cu^{2+}$ and $Cd^{2+}$ by *Pseudomonas* strains

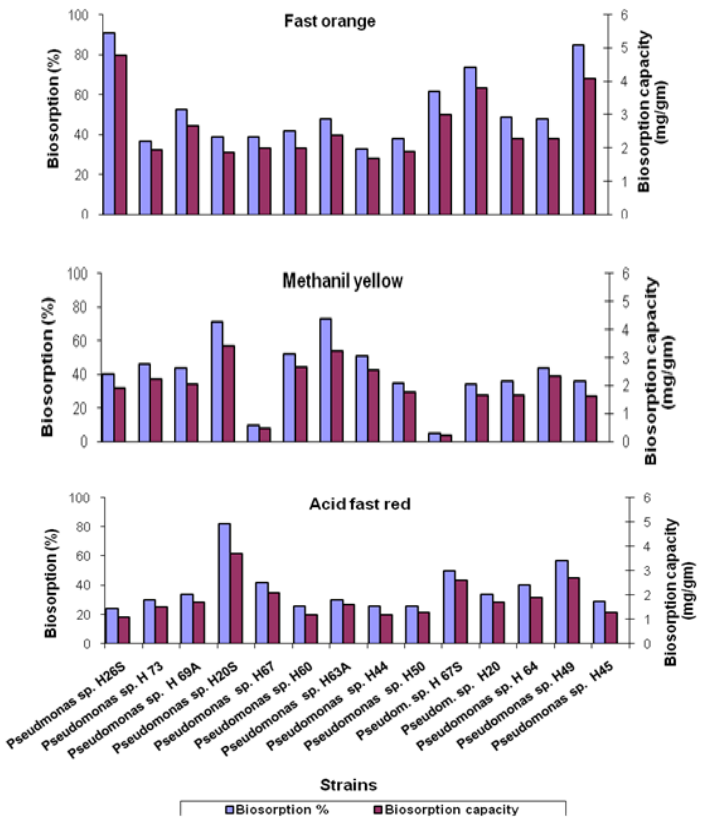


Fig. 3. Decolorization percentage and biosorption efficiency of dye removal by *Pseudomonas* strains.

Tom-Sinoy *et al*., (2011) examined the potential of aerobic mixed culture of *Pseudomonas* sp. for decolourization of Brilliant Green Malachite Green, Carbol Fuchsin and Crystal Violet dyes. Ponraj *et al*., (2011) showed that, maximum decolorization efficiency (89 %) was achieved at pH 6.0 for *Pseudomonas* sp. Rajamohan and Rajasimman (2013) reported that, the biodegradation studies of dye effluent were performed utilizing *Pseudomonas stutzeri* in a controlled laboratory environment under anoxic conditions.

## CONCLUSION

Few studies reported the presence of psychrotolerant (cod-adapted bacteria) bacteria outside extremely cold habitats. This paper presents *Pseudomonas* strains isolated from moderate temperatures marine ecosystem using culture-dependent techniques. It provides important information about the physiological capabilities and phylogenetic analysis of these strains. Moreover, it provides the importance of *Pseudomonas* strains for bioremediation of different contaminants as heavy metals and azo-dyes.

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