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FULL LENGTH ARTICLE

Characterization and bioremediation potential of marine *Psychrobacter* species

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Abstract Three marine *Psychrobacter* strains were isolated from seawater and sediments in Mediterranean Sea, Egypt, using culture-dependent techniques. Genotypic characterization for the three strains was performed using 16S rDNA sequence analysis. The *Psychrobacter* strains were screened for some physiological, biochemical characters, resistance to some antibiotics and heavy metals. All tested *Psychrobacter* 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. Lead ions were easier to be bioaccumulated than the other two metals. *Psychrobacter* sp. H41A was the most potent strain in accumulation of the different metals. *Psychrobacter* sp. H41A accumulated 91.47 mg Pb^{2+}/g fresh cells at optimum conditions of 60 min contact time, at 600 ppm and 30 °C. Plackett–Burman experimental design was applied to optimize the nutritional factors. The growth of *Psychrobacter* sp. H41A strain in the optimized culture medium increased the lead bioaccumulation 1.12-fold. The *Psychrobacter* strains were monitored for their ability to decolorize three different azo-dyes (fast orange, methanil yellow and acid fast red). *Psychrobacter* sp. H62 and H65 recorded the highest decolorization percentages (85% and 65%) with fast orange and methanil yellow, respectively.

Introduction

Mesophiles contribute essentially to nutrient turnover processes, litter decomposition and biomass production in cold habitats. There is proof of a high range of metabolic activities in cold ecosystems. (Trotsenko and Khmelenina, 2005). At low temperatures, the metabolic fluxes are similar to those displayed by cold-adapted bacteria at moderate temperatures (Margesin et al., 2003).

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Only sporadic studies reported the presence of mesophilic bacteria outside extremely cold habitats (Martiny et al., 2006). For example, the study of the transcriptome of the cold-adapted genus *Psychrobacter* exhibited adaptations to significant variations in temperature (Bergholz et al., 2009). The presence of *Psychrobacter* was significantly accompanied with temperature and variable environmental factors such as high salinity, pH near neutrality and high concentrations of magnesium and potassium (Rodrigues et al., 2009).

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).

The occurrence of *Psychrobacter* may also be influenced by anthropogenic-related factors such as, the enriched presence of *Psychrobacter* in contaminated aquatic ecosystems (Prabagaran et al., 2007; Lo Giudice et al., 2010).

Due to the high potential of the marine bacteria in bioremediation, great attention has been drawn to these marine microorganisms for ecosystem restoration of hazardous compounds thus leading to reducing their toxic levels (Dash and Das, 2012). The biotransformation or bioremediation methods have been employed to tap the naturally occurring metabolic ability of marine bacteria to accumulate, transformer degrade hazardous compounds including heterocyclic compounds, hydrocarbons, toxic metals and pharmaceutical substances (Karigar and Rao, 2011).

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 (Bej et al., 2000; Philip et al., 2005; Paniker et al., 2006).

The release of heavy metals into the marine ecosystems causes many environmental pollution problems because of their unique characteristics (Banat et al., 2005). Heavy metals may reach water sources by direct discharge of industrial, agricultural and municipal wastewater or naturally through a different of geochemical processes (Semerjian, 2010; Srinivasa-Rao et al., 2010), which causes the discharge of a variety of toxic metals such as Cu^{2+}, Ni^{2+}, $Zn^{2+}Co^{2+}$, Cd^{2+} and Pb^{2+} into the environment (Malik, 2004). Many chemical and physical methods have been proposed to remove such toxic metals from the contaminated ecosystems, but they are effectiveness because of cost, limitations, and generation of harmful substances (Wuana and Okieimen, 2011). Marine bacteria solve these problems as they are highly efficient even at low concentrations of metal and they do not produce any byproducts (De et al., 2008). Several mechanisms are developed by the bacteria to tolerate few high heavy metal concentrations. One of these mechanisms is heavy metals bioaccumulation which is dependent upon catabolic and anabolic energy of bacteria (Banerjee et al., 2015).

Unlike conventional optimization (one-variable-at-a-time approach), optimization by statistical methods present a more balanced alternative method, since it takes into account the interaction of variables in generating the process response. (Hao et al., 2006). Statistical experimental designs can be adopted on several steps of an optimization strategy, such as searching for the optimal conditions of a targeted response or for screening experiments (Yue et al., 2012). The application of statistical designs to bioprocessing include the Plackett–Burman design, which is considered one of the most popular choices (Kiran et al., 2010).

Little was known about *Psychrobacter* species in Mediterranean Sea, Egypt. The present study aimed to investigate the characterization, heavy metal accumulation and azo dye decolorization by *Psychrobacter* species isolated from Mediterranean Sea, Egypt.

Materials and methods

Bacterial isolation

One hundred marine bacterial isolates capable of growing at room temperature were selected and purified. They were screened for growth at different temperatures. Twenty-three isolates that showed good growth at 5 °C were chosen for studying. The isolated bacteria were subjected to phylogenetic analysis.

Bacterial strains

The psychrotolerant bacterial strains used in the present study were isolated from Mediterranean Sea, Egypt, using culture-dependent techniques. *Psychrobacter* sp. H41 was isolated from sediments of Alexandria Eastern Harbor, *Psychrobacter* sp. H65 from seawater of Abu Qir and *Psychrobacter* sp. H62 from seawater of Rashid.

Medium

Nutrient agar (Oxoid LTD, England) and tryptone yeast extract were used for isolation and growth of psychrotolerant bacteria. Media were prepared with aged seawater and distilled water (1:1, v/v). Tryptone yeast extract contained in 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).

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 polymerase chain reaction (PCR), using Maxima Hot Start PCR Master Mix (Fermentas), was carried out in a thermal cycler (Multigene Optimax, Labnet International, Inc.). The PCR thermocycler was programed as follows: 95 °C for 5 min for initial denaturation, 30 cycles at 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 10 min. The PCR mixture contained 25 pmol of each primer (Sigma Scientific Services Company, Egypt, 2013), 10 ng chromosomal DNA, 200 mmol l^{-1} dNTPs and 2.5 U of Taq polymerase in 50 ml of Taq polymerase buffer (10X standard Taq reaction buffer).