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Potential biodegradation of crude petroleum oil by newly isolated halotolerant microbial strains from polluted Red Sea area☆

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ABSTRACT

Two microbial isolates from oil polluted Red Sea water in Egypt, designated as RS-Y1 and RS-F3, were found capable of degrading Belayim mix (BX) crude oil. Strains RS-Y1 and RS-F3 were assigned to the genera *Lipomyces tetrasporus* and *Paecilomyces variotii* based on their morphological and physiological characteristics. Both isolates were compared for the biodegradation of crude petroleum-oil hydrocarbons in basal salt medium supplemented with 5% (*w*/*v*) of BX-crude oil. Gas chromatography profile showed that the biodegradation of total petroleum hydrocarbons (TPHs) inoculated with *L. tetrasporus* (68.3%) and *P. variotii* (58.15%) along with their consortium (66%) significantly reduced TPHs levels as compared to the control after 30 days. *L. tetrasporus* (44.5%) was more effective than *P. variotii* strain (32.89%) in reducing the unresolved complex mixtures (UCM) content from the medium. Both isolates exhibited a strong growth over a wide range of salinity (5–45 g/L NaCl).

1. Introduction

Petroleum is still the main global energy resource and one of the most important raw materials in the field of chemical industry (ElMekawy et al., 2013b). Nevertheless, oil spills during exploitation, conveyance and refining are triggering severe environmental complications, particularly when accidental spills occur on a large scale. These problems can seriously affect fishery, aquatic habitats and human health, as well as destroying the environmental balance which can take decades to recover (Zhang et al., 2011). Petroleum represents a blend of aromatic and aliphatic hydrocarbons (Wu et al., 2013), in which the polycyclic aromatic hydrocarbons (PAHs) are in the focus of interest as a result of their environmental persistence and possible harmful influences on human health. Several PAHs are carcinogenic and/or mutagenic rendering remediation of petroleum-polluted spots a primacy (Radwan et al., 2005).

Many studies have been carried out to diminish the ecological influences of petroleum and its threats to human health (Dellagnezze et al., 2014). Bioremediation is known as an effective, inexpensive and multipurpose substitute to physicochemical remedy (ElMekawy et al., 2013a). Throughout the last decade, much attention has been attracted towards the bioremediation of oil-contaminated sites, and research has been performed with pure or mixed cultures isolated from oil-polluted locations (Al-Mailem et al., 2010; Camilli et al., 2010; Valentine et al., 2010). However, there are a limited number of microbial strains that are capable to biodegrade all the constituents of crude oil. This can be attributable to the confined ability of each strain to only degrade one compound type or more of crude oil, and several factors can influence the bioremediation process of oil-polluted sites (Gandolfi et al., 2010; Zahed et al., 2010). The short chain compounds, e.g. cyclic, branched, straight alkanes and aromatic hydrocarbons, are among the typical constituents of petroleum oil, which are readily degraded by several microbial strains (Das and Mukherjee, 2007). However, PAHs and alkanes, with high molecular weight, were marginally biodegradable owing to their greater hydrophobic nature (Zhang et al., 2011). Accordingly, exploring new microbial strains, with high efficiency to degrade petroleum, has turned out to be a priority to petroleum microbiology.

Biodegradation of crude oil in polluted sites by microorganisms is often a lengthy process. As a result, the key solution for such a problem is the isolation and identification of oil-degrading microorganisms (Wu et al., 2013). There are around 70 microbial genera of recognized oil-degrading ability, including fungi, yeast and bacteria (Joo et al., 2008).

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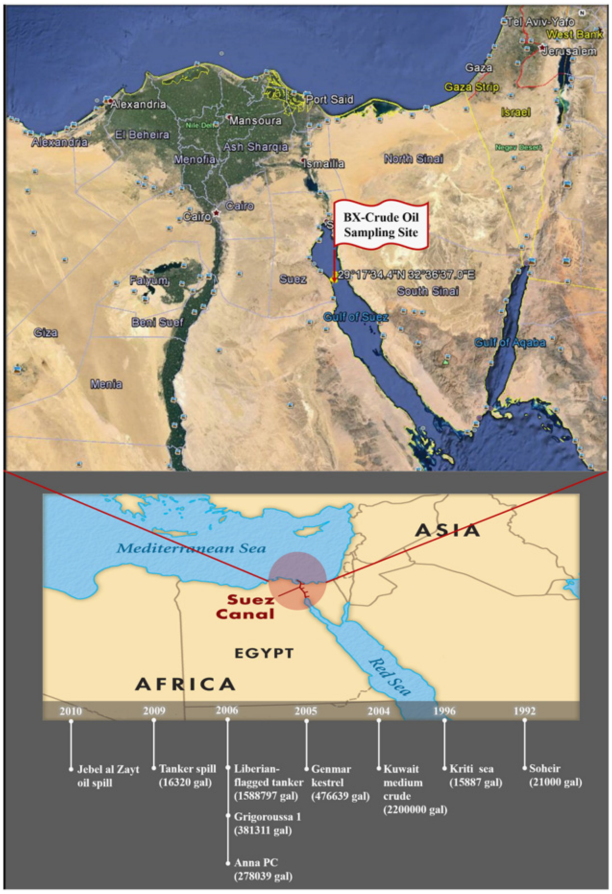

Fig. 1. Map showing the location of the Suez Canal within the habitable region of Suez governorate with the most oil spill incidents in the last decade. The red flag shows the location of the sampling site. Map was obtained from Google Earth (Fingas, 2011; REMIP, 2008).

The isolated bacterial strains, e.g. *Achromobacter*, *Acinetobacter*, *Actinomyces*, *Alcanivorax*, *Bacillus*, *Burkholderia*, *Cellulomonas*, *Dietzia*, *Exiguobacterium*, *Gordonia*, *Klebsiella*, *Marinobacter*, *Microbacterium*, *Microbulbifer*, *Micrococcus*, *Nocardia*, *Pseudomonas*, *Sphingomonas*, *Spirillum*, *Streptomyces* and *Vibrio* (Chaerun et al., 2004; Das and Mukherjee, 2007; Fahd et al., 2006; Hazen et al., 2010; Menezes Bento et al., 2005; Mohanty and Mukherji, 2008; Supaphol et al., 2006), have the capability to aerobically or anaerobically degrade alkanes and aromatic hydrocarbons. Moreover, fungal and yeast strains belonging to *Allescheria*, *Amorphoteca*, *Aspergillus*, *Candida*, *Debayomyces*, *Fusarium*, *Graphium*, *Mucor*, *Neosartorya*, *Paecilomyces*, *Penicillium*, *Pichia*, *Saccharomyces*, *Talaromyces*, *Trichoderma* and *Yarrowia* have also been involved in the hydrocarbon biodegradation process (Chaillan et al., 2004; Potin et al., 2004; Silva et al., 2009; Teng et al., 2010). This process involves their growth as they metabolize petroleum hydrocarbon as a source of carbon, although no single microbial strain is capable of metabolizing more than two to three types of compounds normally found in crude petroleum. Therefore, the efficient degradation of crude oil requires a consortium composed of various microbial strains (Wu et al., 2013).

Several locations are globally polluted with crude oil in many countries, and also in Egypt, especially in Suez Canal which is one of the most important waterways around the world for transporting the commercial oil tankers. It was reported that approximately 20,000 vessels/year are transported through the Suez Canal, including 2500 tankers, which represent about 14% of the international trade (Zaki et al., 2014). Sea oil spills became a major ecological problem in Suez Canal, with several incidents that occurred from the 1990s till now (Fig. 1), mainly due to increased petroleum exploitation and production worldwide and development of supertankers with transporting capacity of more than 153,930,000 gal of oil. Oil spills can have disastrous consequences for both national and international economies. In view of the fact that environmental conditions can differ due to the diverse climatic conditions in different locations, the application of indigenous microorganisms for the bioremediation of oil-polluted areas is required. Therefore, the aim of this study was to isolate new microbial species and test their efficiency for the bioremediation of petroleum-contaminated seawater. Accordingly, several novel microbial strains were isolated from oil-contaminated Red Sea water, and degradation of crude oil by these strains was investigated under wide range of salinity.

2. Materials and methods

2.1. Collection of oil-polluted water samples

Red sea water samples polluted with Belayim Mix (BX) crude oil were collected from the Advanced Petroleum Interceptor (API) of SUMED Cooperation (Suez governorate 29°17′34.4″N 32°36′37.0″E, Egypt). Two samples were collected, in screw-capped sterile glass

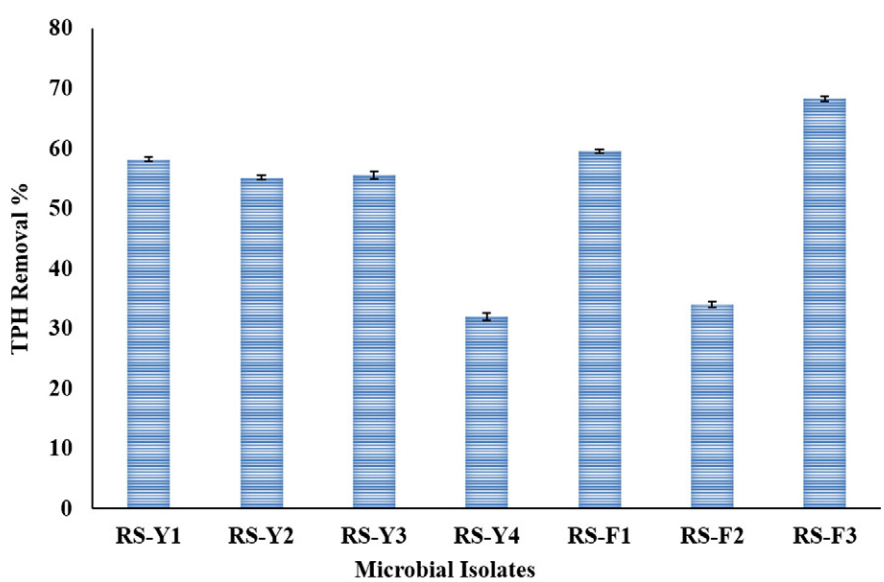

Fig. 2. The percentage of total petroleum hydrocarbons (TPH) removal of BX-crude oil using the isolated marine yeast and fungal isolates.