CATRINA (2020), 21(1): 15-22

Isolation and identification new bacterial strains isolated from different sources of Al-Rafidiyah oil field in Iraq

Asaad Faraj Hamzah[1*], Wijdan Hussein Al-Tamimi[2], Saad Shakir Mahdi[2] and Najla'a.Z.Alameri[3]
[1] Technical institute of Basrah, Southern Technical University, Basrah, Iraq.
[2] Department of Biology, Science Collage, University of Basrah, Basrah, Iraq.
[3] Researches and Quality Control Department, Basrah Oil Company, Iraq.

ABSTRACT

In this study, twenty two pure bacterial strains isolates were isolated that can use crude oil as a carbon source. They occured at different frequency ratios for each of oil-contaminated soil samples, the produced water, and the crude oil of the Al-Rafidiyah oil field (Basrah south of Iraq). Bacterial isolates were characterized and identified based on phenotypic properties and molecular techniques. Fourteen strains, belonging to three genera of *Bacillus* sp., *Lysinibacillus* sp. and *Enterobacter* sp., were isolated from oil-contaminated soil and nine strains, belonging to *Bacillus* sp., *Lysinibacillus* sp., *Enterobacter* sp. and *Brevibacillus* sp., were isolated from produced water. Five strains, belonging to two genera, *Bacillus* sp. and *Pseudomonas* sp., were isolated from crude oil. New twelve strains were recorded as new strains and deposited in GenBank include *Bacillus cereus* strain ASWISA1, *Bacillus thuringiensis* strain ASWISA2, *Bacillus sonorensis* strain ASWISA3, Bacillus cereus strain ASWISA4, *Bacillus subtilis* strain ASWISA5, *Pseudomonas stutzeri* strain ASWISA6, *Bacillus subtilis* strain ASWISA7, *Bacillus cereu*s strain ASWISA8, Bacillus paramycoides strain ASWISA9, *Bacillus paramycoides* strain ASWISA10, *Brevibacillus brevis* strain ASWISA11, and *Enterobacter cloacae* strain ASWISA12.

Keywords: Oil filed bacteria, petroleum microbiology, Crude oil bacteria.

INTRODUCTION

Due to the high temperature, anaerobic conditions, high pressures, degree of salinity and different pH in the oil reservoirs, it has become as a new extreme environment for the growth of living organisms in these reservoirs (Elshafie *et al*., 2013; Cai *et al*., 2015). Although the extreme conditions support life, numerous studies over the past few years proved microbes are found in several oil reservoir environments. Divers groups of microbes detected in oil reservoirs. Microbial studies of such harsh environment have shown the presence of good different metabolic activities such as sulphate reducers, various hyperthermophilic fermentative microorganism, acetogens and methanogens from oil reservoirs worldwide (Magot *et al*., 2000; Orphan *et al*., 2003). By molecular techniques a wide diversity of bacteria depending in abiotic factors such as oxygen, temperature, pH, have been isolated from or have been detected in oil field samples (Telang *et al*., 1998; Al- Al-Tamimi 2015 Pannekens *et al*., 2019).

Oil reservoirs accommodate completely different phases wherever microorganisms will thrive, like formation water, and organic materials and crude oil (Kobayashi *et al*., 2012; Pannekens *et al*., 2019). Aerobic microorganisms have been found in oil reservoir with pH range (6.0 - 8.4) and temperatures ranging from (20 to 70°C). Some of these identified aerobic bacteria *Kocuria rosea*, *Rhodococcus ruber*, *Gordonia rubropertincta*, *Arthrobacter oxydans*, *Bacillus subtilis*, *B. cereus Cellulomonas cellulans*, *Pseudomonas fluorescens* and commonly bacteria of the genera *Clostridium*, *Bacteroides*, *Thermoanaerobacter*,*Thermotogales*, *Petrotoga*, *Thermotoga*, *Geosporobacter*, *Desulfotomaculum*, *Caminicella* represent anaerobic microflora were found in oil reservoirs (Pannekens *et al*.,2019).The current study aimed to isolate and identify some the bacterial strains from a variety of sources in the Al-Rafidia oil field.

MATERIALS AND METHOD

The bacterial strains were isolated from Al-Rafidiya oil field, Basra south of Iraq, 30.16°N 47.42°E. Twenty two samples of each oil contaminated soil, produced water and crude oil. The soil samples from the different sites of oil filed were collected in a sterile polythene bags at depth of 2-3 inches from the ground level using clean spatula, produced water samples were collected from separated tanks of water production. Meanwhile, the crude oil samples were collected from well head using 2.5 liter sterilized glass containers. All samples stored at 4°C in ice bag till transported to the laboratory. The daily reports of the oil field laboratories were used to for the physical and chemical properties of the samples, except the pH of soil which was measured in a 1:2 mixture of soil: water (0.01 M $CaCl_2$ solution) using pH electrode (Burghal, 2015).

Stimulation of indigenous microorganisms

To stimulate the indigenous microorganisms, soil samples were crushed and sieved through 2mm pore size (Fardoux *et al*., 2000 ; Dilmi *et al* ., 2017), then 5 ml soil suspension (5 g soil in 100 ml), 5 ml of produced water, and 5 ml of crude oil were add separately to 250 ml of Erlenmeyer flask contained 95 ml of mineral salt medium (MSM) composed of 2% of crude oil as a carbon source, 1g /l KH_2PO_4, 6 g/l $NaNO_3$, 1g/l K_2HPO_4, 0.02 g/l $FeSO_4$, 0.5 g/l $MgSO_4$, and 0.02 g/l Na_2MoO_4 at a pH of 7.0–7.2 (Zhao *et al*.,2017). The mixture was then shaken at 180 rpm at 35 °C for 48 hours using shaker incubator. Volume of 1 ml of the suspension was diluted serially and plated in triplicate on nutrient agar. The plates were incubated at

* *Corresponding author e-mail: asaadfaraj@yahoo.com*