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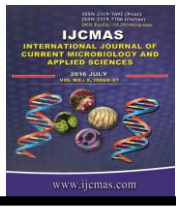

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Screening of Isolated Marine Bacteria for Multiple Biotechnological Applications

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A B S T R A C T

A total of thirteen bacterial isolates were isolated from Eastern, Western harbors' of Alexandria and Lake Mariout. The isolates were screened for various biotechnological applications such as the ability to produce antibacterial compounds and extracellular enzymes (including, protease, lipase, amylase and cellulase), as well as their ability to degrade crude oil and chemical dyes and to solubilize phosphate. Results revealed that 9 isolates were protease producers, 6 of them showed lipase activity and none of them produced cellulase or amylase. Four of the isolated bacteria exhibited antibacterial activity against Staphylococcus aureus ATCC 25923 and two bacterial isolates against Escherichia coli ATCC 8739 .None of them showed the ability to solubilize tri-calcium phosphate or to decolorize and degraded the tested dyes saffranin and crystal violet. Three isolates showed the potential ability to degrade crude oil. The study has overall concluded that marine bacteria could serve as potential bio resources for many biotechnological applications.

Introduction

Marine biotechnology is the science in which marine organisms are used in full or partially to make or modify products, to improve plants or animals or to develop microorganisms for specific uses. With the help of different molecular and biotechnological techniques, human has been able to elucidate many biological methods applicable to both aquatic and terrestrial organisms (Jha and Zi-rong, 2004). Marine microbes offer great opportunities for biodiscovery (Bull *et al.*, 2000), as they are not yet fully characterized or evaluated for their potential biotechnological application.

Despite of a huge microbial diversity of marine ecosystem, there is a lack of laboratory cultures of the microbes that are most abundant in the marine environment that severely limits development of biodiscovery research. Research into natural products from the marine environment, including microorganisms, has rapidly increased over the past few years. They are considered highly valuable as they produce various antibiotics and other therapeutically useful compounds with diverse biological activities (Ramesh and Mathivanan, 2009). Marine microorganisms were proven already

to have many beneficial bioactivities such as production of industrial enzymes (Chatellier *et al.,* 2011; Manasi, 2011). All species have a role in our biosphere. However trying to understand that role is not an easy task. With the help of experimentations we can try to understand the activities of microbes and implement them to our advantage in the industrial field or bioremediation. Marine microorganisms have unique properties since they have to adapt to extreme marine environment conditions such as high or low temperature, alkaline or acidic water, high pressure and limited substrate in the deep-sea water. These distinctive characteristics have attracted many researchers to explore in depth since there is the potential of marine microorganisms used in industry (Baharum *et al.,* 2010). In their review, the authors have focused on marine microorganisms that provided biotechnological applications in enzymes industry and pharmaceutical products and also provided an overview of the challenge faced by researchers in order to explore and exploit the marine reservoir. The purpose of the present study was to investigate potential microorganisms present in marine environment which can be utilized for various enzyme processes and biodegradation processes.

Materials and Methods

Collection of marine water sample

Collection of marine water samples was carried out from Western and Eastern Alexandria Harbors and Lake Mariout (Egypt). For bacterial isolation, samples were collected in 150 ml previously sterilized glass bottles.

Isolation of marine bacteria

Marine water bacteria were isolated using nutrient agar. The plates were incubated at (30±2°C) for two days. After incubation, well separated colonies were selected and sub-cultured on the nutrient agar.

Production of extracellular enzymes

Thirteen morphologically distinct bacterial were subjected for the screening of extracellular enzymatic activities namely protease, amylase, lipase and cellulase using simple quantitative plate assay described by Vijayan *et al*. (2012) as follows:

Protease activity

Nutrient gelatin medium containing (g/l) peptone: 5 g, Beef extract: 3g, gelatin: 120 g, and final pH was adjusted at 6.8. An 18 hrs old pure culture was streaked on nutrient gelatin medium and were incubated at 30±2°C for 48 hrs.

Lipase activity

Nutrient agar containing butter fat was used for screening bacterial isolates for lipase activity. The cultures were streaked on nutrient agar medium and the plates were incubated at 30±2°C for 24 hrs. following incubation, the plates were observed in a UV transilluminator for a clear zone around the colony.

Cellulose activity

The cultures were streaked on carboxymethyl cellulase agar and the plates were incubated 30±2°C for 24 hrs. The plates were then flooded with 10% NaCl for 10 min. Clear zone around the reddish background indicates the production of cellulose by the tested bacteria.

Amylase activity

The bacterial isolates were streaked on starch agar plates containing peptone (0.1%

wt/vol), NaCl (0.5% wt/vol), agar (2.0% wt/vol), and soluble starch (1% wt/vol) and final pH was adjusted at 7.0. Plates were incubated at 32°C for 24 hrs. A clear zone of hydrolysis after Lugol's Iodine solution addition gave an indication of amylolytic microorganisms.

Antibacterial Activity

Nutrient agar was prepared and autoclaved and poured in petridishes. Tow bacterial pathogen, Staphylococcus aureus ATCC 25923 and *Escherichia coli* ATCC 8739 obtained from Marine Microbiology at National Institute of oceanography and fisheries (NIOF). All the thirteen isolates were placed at 4 corners of the petridish as patch inoculums and kept for incubation at 30°C. After 24 hrs, the zone of inhibition around the bacterial colonies was observed if any (Vijayan *et al.*, 2012).

Dyes Degrading Ability

Nutrient broth (4 ml) was prepared and 1 ml of chosen dye (0.1% of crystal violet and saffranin) was added in test tubes and sterilized. Medium was then inoculated with the tested isolate. Control was prepared with 4 ml of nutrient broth added with 1 ml of chosen dye with no added bacteria. Tubes were incubated at 30°C for 48 hrs after which they were visually observed for decolorization if any. In addition, the growth of bacteria in the presence of dye was estimated by taking absorbance of the culture broth at 600 nm in Elico UV Vis Spectrophotometer (Ayed *et al.*, 2009).

Oil Degrading Ability

The isolates were checked for their oil degrading ability using Bushnell Haas agar medium as described by Singh *et al*. (2015). The molten medium was tempered at 47±1°C and inoculated with the tested isolate at 1% (v/v) and crude oil samples were poured in lid of plates. The plates were incubated at 30±2°C for 48 hrs.

Phosphate Solubilization Activity

For the screening procedure quarter strength of nutrient agar was prepared and 1 % of tri-calcium phosphate was added before autoclaving the medium. This resulted in a milky white medium. The medium was poured into petri plates and left to solidify under laminar flow. The bacterial isolates were patched on 4 corners of the plate and incubated at for 7 d at 30°C (Nautiyal and Mehta 2001). A clear zone around the bacterial patches indicates their ability to solublize phosphate in agar medium.

Results and Discussion

Isolation of Marine Bacteria

Thirteen marine bacteria ware isolated using nutrient agar. The colonies were distinguished by morphological characters like shape, size, colour, margin, elevation and capacity. The morphologically distinct bacteria were further subcultured on nutrient agar to screen them for various applications.

Thirteen marine bacterial (MB) isolates were isolated using nutrient agar. Eight of them were isolated from Eastern Alexandria harbor and given the following codes (MB1, MB2, MB3, MB4, MB5, MB6, MB7 and MB8). Four other isolates were isolated from Western Alexandria harbor and given codes (MB9, MB10, MB11 and MB12), while the last isolate was recovered from water sample taken from Lake Mariout (MB13). The colonies were distinguished by morphological characters like shape, size, color, margin, elevation and opacity. The morphologically distinct bacteria from nutrient agar were further subculture on nutrient agar to screen them for various potential biotechnological applications.

Production of Extracellular Enzymes

The thirteen bacteria were subjected for their ability to produce 4 different enzymatic activities (Table 1). The results revealed that six of them showed positive results for lipase activity and nine were positive for protease activity (Figure1) but none showed the ability to have either amylase or cellulase activity.

The thirteen bacterial isolates were screened for their antibacterial activity against both *Staphylococcus aureus* ATCC 25923 (Gram positive) and *Escherichia coli* ATCC 8739 (Gram negative). Among tested isolates, MB2 and MB11 showed potential antibacterial activity against *E. coli* ATCC 8739, while isolates MB2, MB3, MB4 and MB11 showed antibacterial activity against *S. aureus* ATCC 25923as shown in Figure 2.

Dyes Degrading Ability

None of the tested isolates was able to decolorize the tested dyes (crystal violet and saffranin) which may indicate the inability of the marine isolates to degrade hydrocarbons from the dyes which are necessary for growth of bacteria.

Oil Degrading Ability

The thirteen isolates were tested for their ability to degrade oil using Bushnell and Haas medium containing petrol. Three isolates (MB6, MB8 and MB13) were able to grow and degrade oil, indicating their ability to utilize the oils as a source of carbon.

Phosphate Solubilization Activity

None of the tested isolates was able to produce a clearance zone around their colony grown for 5 days in tri-calcium phosphate amended medium, indicating that none of them has the ability to solubilize phosphate by an extra-cellularly mechanism.

The marine environment is the largest habitat on Earth, representing more than 70% of the surface of our planet. Oceans have the greatest extremes of temperature, light and pressure encountered by life (Munn, 2004). These extremes increase biodiversity among marine bacterial ecosystems and make them vary widely in composition and activity. Consequently, marine bacterial ecosystem can be regarded as an excellent source of new bioactive compounds with potential industrial, environmental, pharmaceutical and medical applications (Debnath *et al*. 2007). Despite a huge microbial diversity, there is a lack of laboratory cultures of the microbes that are most abundant in the environment that severely limits development of biodiscovery research. Baharum *et al*. (2010) focused on marine microorganisms that provide biotechnological applications in enzymes industry and pharmaceutical products and also provided an overview of the challenge faced by researchers in order to explore and exploit the marine reservoir. In that study, 13 marine bacterial isolates were isolated from marine water samples collected from Eastern, Western harbors' of Alexandria and Lake Mariout. Dionisi *et al*. (2012) stated that microorganisms may be able to tolerate rapid and repeated fluctuations in environmental conditions including temperature, light and salinity, and are exposed to wave action, ultraviolet radiation, as well as periods of drought. Hence, microbes from such harsh environments may exhibit potential properties which can be exploited for biotechnological applications. There has been a great interest from researchers to explore marine microorganisms as new source of antibacterial compounds as

increasing resistance of pathogen to present antibiotics. One example of studies that has been carried out is purification and partial characterization of marinocine, a new broad-spectrum antibacterial protein produced by Marinomonas mediterranea (Lucas-Elio *et al.*, 2005). Marine microorganisms were proven already to have many beneficial bioactivities such as production of industrial enzymes (Chatellier *et al.*, 2011; Manasi, 2011).

Table.1 Production of extracellular enzymes

Strain code	Lipase	Protease	amylase	Cellulose
MB1	+	-	-	-
MB2	+	+	-	-
MB3	+	-	-	-
MB4	++	-	-	-
MB5	-	+	-	-
MB6	-	+	-	-
MB7	-	+	-	-
MB8	-	+	-	-
MB9	++	-	-	-
MB10	-	+	-	-
MB11	++	+	-	-
MB12	-	+	-	-
MB13	-	+	-	-

Fig.1 Ability of extracellular protease activity of marine isolates exhibited on nutrient gelatin medium. Numbers 1, 2, 3 and 4, refer to isolates MB1, MB2, MB3 and MB4, respectively.

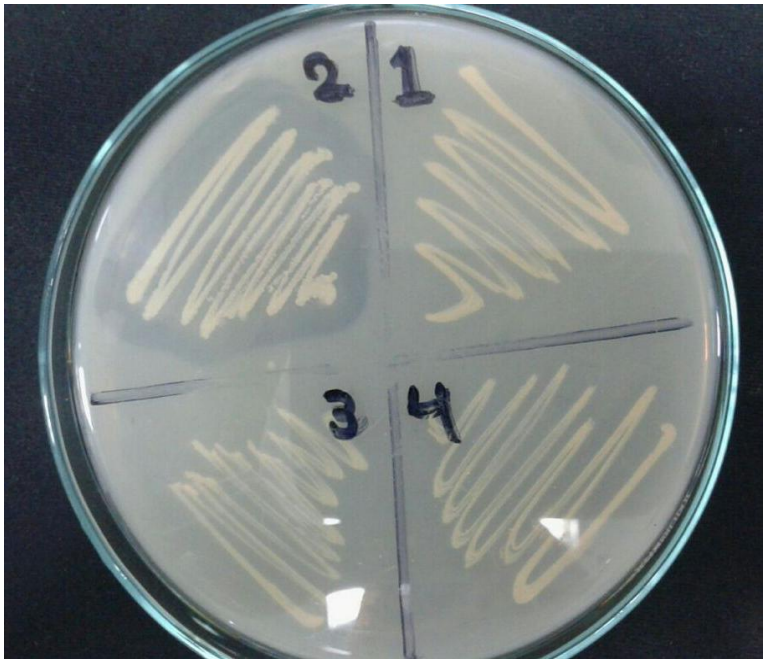
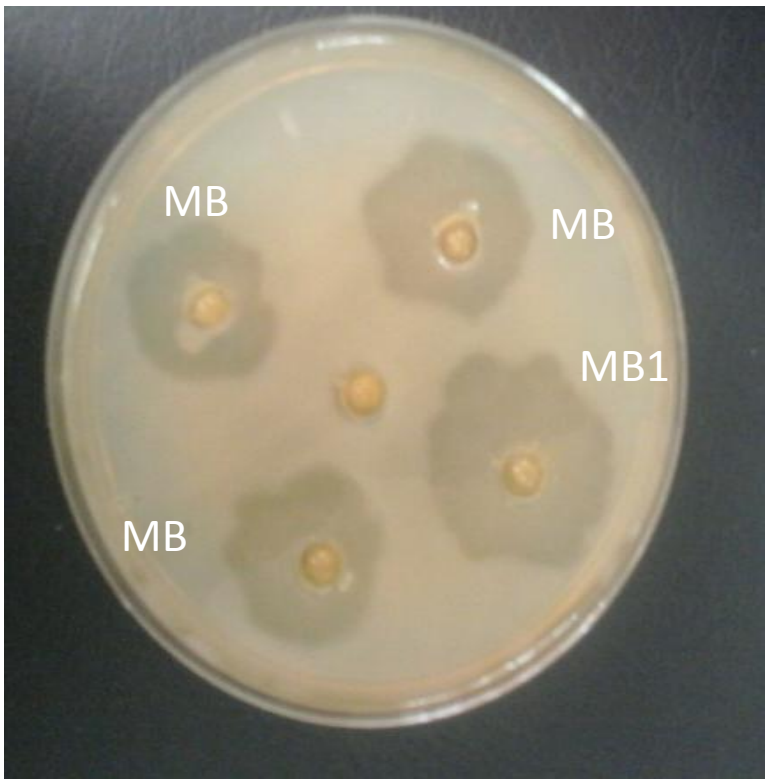


Fig.2 Antibacterial activity of bacterial isolates coded MB2, MB3, MB4 and MB11 against *Staphylococcus aureus* ATCC 25923.



In conclusion, results reported in the present study on marine bacterial isolates have given various insights into the ecology of microorganisms in aquaculture. Preliminary screenings of 13 bacterial isolates have given a brief idea of some of their biotechnological abilities. The potential capabilities of some isolates to produce extracellular protease, lipase and antibacterial activities and to degrade crude oil make them promising for aquaculture applications. Further study is needed to characterize such activity and to identify the promising isolates.

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