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Study of Isolated Marine Bacteria For Various Applications

KEYWORDS: Marine environment, antibacterial activity, PHB production, biotechnological potential.

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ABSTRACT *Bacterial isolates were isolated from marine environment of Mumbai beaches and were screened for various biotechnological applications viz. antibacterial activity, production of extracellularenzymes, production of PHB, tolerance of heavy metals and degradation of biologicaldyes. A total of twenty six bacteriawere isolated in pure culture form and their biotechnological potential was studied.These isolates were checked forvarious enzyme production namely, Protease, Lipase, Amylase, Cellulase, β-Galactosidase and Pectinase. 6 isolates showed activity against S.aureus whereas 2 isolates showed activity against E.coli. 8 bacterial isolates were positive for PHB production. The marine bacteria showed the potential to tolerate heavy metals viz. lead and chromium and were able to decolourize and degraded biological dyes. The study has overall concluded that marine bacteria could serve as potential bioresources for many biotechnological applications.*

Introduction

Research into natural products from the marine environment, including microorganisms, has rapidly increased over the past few years. Marine microorganisms are increasingly becoming an important source for industrially important molecules. They are considered highly valuable as they produce various antibiotics and other therapeutically useful compounds with diverse biological activities (Ramesh and Mathivanan, 2009). Despite facing various difficulties in isolating and harvesting marine bacteria, microbial metabolites are increasingly attractive to science because of their broad-ranging pharmacological activities (Azamjon *et al.,* 2010). The heterotrophic bacteria commonly present in the marine environment have received little attention, though specialized groups such as the agar digesters have been extensively investigated. Existing knowledge of marine bacteria is comprehensively summarized by Zobell and Upham (1944) and Zobell (1946), a significant addition since then, being the report of extensive investigations on bacteria from marine sources in Australia by Wood (1953).

Marine bacteria including actinomycetes have been isolated from intertidal sediments and studied for various biotechnological applications (Bhagat *et al.,* 2010b; Gopalakrishnan *et al.,* 2010; Jayaprakahsvel *et al.* 2011). Qualitatively, the bacterial flora of marine environments in different parts of the world recorded by different researchers shows some differences. Different commercial enzymes viz. L-glutaminase, α-galactosidase, amylase, cellulase, protease, lipase, L-asparaginase, have also been obtained from the marine actinobacteria (Lakshmanaperumalsamy, 1978; Jayaprakashvel et al., 2012).

All species have a role in our biosphere. However trying to understand that role is not an easy job. 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. 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.

Material and method

Collection of marine water sample

Marine water sample were collected from Mumbai coastal areas Juhu, Girgaon, Gateway of India and Bandstand, (Mumbai).

Isolation of marine bacteria

Marine water bacteria were isolated using Zobell marine agar and CAT medium. The plates were incubated (35 ± 2°C) for two days. After incubation, well separated colonies were selected and were subculture on the Zobell marine agar.

Production of extracellular enzymes

Ten morphologically distinct bacteria were isolated from the marine water samples which were subjected for the screening of extracellular enzyme namely protease, amylase, lipase, cellulase, pectinases and beta- Galactosidase using simple quantitative plate assay (Vijayan et al., 2012).

For protease producing bacteria, nutrient gelatin medium containing (g/l) peptin digests of animal tissue: 5 g, Beef Beef extract: 3g, gelatin: 120, and final pH (at 250 c): 6.8 was taken. An 18 hours old pure culture was stab-inoculated in Nutrient Gelatin medium with an inoculating needle directly down the centre of the medium to a depth of approximately one half an inches from the bottom of the tube. All tubes including un-inoculated control were incubated 35±2°C for 24-48 hours (Vijayan et al., 2012).

For lipase producing bacteria, nutrient agar containing ethidium bromide (10mg/ml) and oil was used. The cultures were spot and the plates were incubated for 24 hours at room temperature. After 24 hours incubation the plates were observed in a UV transilluminator for a clear zone around the colony (Vijayan et al., 2012).

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For cellulose activities, Carboxymethyl Cellulase (CMC) agar was used. The cultures were spot inoculated and the plates were incubated for 24 hours at room temperature. After 24 hours incubation, the plates were flooded with 10% Nacl for 10 min. Clear zone around the reddish background indicates the production of cellulose by the test bacteria (Vijayan et al., 2012).

For amylase production, the bacterial isolates were spot inoculated 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) pH-7.0. The plates were incubated at 37°C for 24 hrs. A clear zone of hydrolysis after Lugol's Iodine solution addition gave an indication of amylolytic microorganisms.

The isolates obtained were subjected for beta-Galactocidase production using Luria bertani agar, containing (g/l) casein hydrosylate: 10, yeast extract: 5, sodium chloride 10, agar15, final pH 7.5. X-gal and IPTG were added to the prepared LB agar. The isolates were spot inoculated in LB plate and incubated at 37°C for 24 hrs. After incubation blue colour colonies indicates the production of beta-Galactocidase (Vijayan et al., 2012).

For production of pectinase, yeast extract pectate agar medium (1% pectin, 1% yeast extract, 0.5%Nacl 1.5% agar and pH 7.0) was used. The isolates were spot inoculated and incubated at 37°C for 24 hrs. After the incubation, the plates were flooded with 1% solution of hexadecyltrimethyl ammonium bromide. Clear zone around colonies indicated pectinolytic actitivy (Baharum et al., 2010).

Antibacterial activity

The isolates obtained were screened for antibacterial activity using Wilkin's agar overlay method. The bacterial isolates were spot inoculated and incubation at room temperature for 24 hours. After the incubation, the plates was over laid with wilkin's agar containing test organisms *E. coli* and *S. aureus* respectively in different plates. The plates were reincubated at 37°C for 24 hrs (Vijayan et al., 2012).

Dyes degrading ability

The ten morphologically distinct bacterial isolates were tested for their ability to decolorize biological dyes using Bushnell Hass broth containing (g/L) 1g KH_2PO_4, 1g K_2HPO_4, 1g NH_4NO_3, 0.2g $MgSO_4.7H_2O$, 0.05g $FeCl_3$, 0.02g $CaCl_2.2H_2O$, 15g/L of agar. Biological dyes such as crystal violet, saffranin, malachite green and trypan blue were used. The tubes were inoculated with the bacterial culture. Control was prepared with 4ml of sterilized Bushnell Haas broth added with 1 ml of sterilized distilled water. Tubes were incubated for 48 hours after which they were centrifuged at 5000 rpm for ten min and the absorbance of the supernatant was determined. The decolourization of dye was measured using colorimeter [CL 157 (ELICO)]. The % decolourization of dye by each isolate was calculated (Gurulakshmi et al., 2008; Bhattiwal et al., 2013).

Decolourization (%) = Initial absorbance - Final absorbance × 100 / Initial absorbance

Oil degrading ability

The isolates were checked for their oil degrading ability using Bushnell Haas media. The plates were spot inoculated with the bacterial culture and oil samples (petrol, motor oil and diesel) were poured in lid of plates. The plates were incubated at room temperature for 48 hours at room temperature (Singh et al., 2015).

Bioaccumulation studies:

The isolates were checked for their bioaccumulation capacities using two heavy metal salts *viz.* Lead Acetate and Potassium chromate. Heavy metal salt solutions (500 ppm) of Lead Acetate and Potassium chromate were made in sterile nutrient broth. The solutions were inoculated with 24 hr old culture suspensions of the individual isolates. The inoculated cultures were kept at 37°C for 24 hrs at shaker conditions. After 24 hours, the broth was centrifuged at 5000 rpm for 15 min and the supernatant was sent for ICP-AES analysis at SAIF, IIT-Bombay (MS) (Durve et al., 2013)

Production of Polyhydroxybutyrate (PHB)

The bacterial isolates were spot inoculated on nutrient agar plates and incubated at 37°C for 24 hrs. After the incubation Sudan black B solution (0.2 % Sudan black B +96% ethanol) was poured over the colonies and incubate for 30 minutes at room temperature. Dark blue colonies indicated positive result for polyhydroxybutyrate production (Panigrahi and Badveli, 2013).

Results and Discussion

Isolation of marine bacteria

Twenty six marine bacteria ware isolated using Zobell marine agar and CAT marine agar. The colonies were distinguished by morphological characters like shape, size, colour, margin, elevation and opacity. The morphologically distinct bacteria from Zobell and CAT agar were further subculture on Zobell marine agar to screen them for various applications.

Production of extracellular enzymes

All the twenty six bacteria were subjected for their ability to produced 6 different enzyme activity (Table 1). Most of the isolates showed positive results for Amylase and Beta-galactosidase production. Protease activity was seen in 4 isolates, Lipase activity was seen in 3 isolates whereas cellulase was seen in 5 bacterial isolates.

Table 1: Production of extracellular enzymes

Strain code	Protease	Lipase	Amylase	Cellulase	Beta-Galactosidase	Pectinase
MB1	-	-	+	-	-	-
MB2	-	-	+		-	-
MB3	+		+	-	+	-
MB4	-	-	-	-	+	-
MB5		-	-	-	+	-
MB6	-	-	+	-	-	-
MB7	-	-	+	-	-	-
MB8	-	-	+	-	-	-
MB9	-	-	+	+	+	-
MB10		-	-	+	-	-
MB11	-	-	-	-	-	-
MB12		-	+	-	+	-
MBA	-	-	+	+	-	-
MAB	-	-	+	+	+	-
MBC1		-	+	-	+	-
MBD	-	-	-	-	-	-
MBE	-	-	+		-	-
MBF	-	-	+	-	-	-

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Strain code	Protease	Lipase	Amylase	Cellulase	Beta-Galactosidase	Pectinase
MBG	-	-	+	-	-	-
MBH	-	-	+	-	+	-
MBI	-	-	+	-	+	-
MBJ	+	+	+	-	+	-
MBK	+	+	+	-	+	-
MBL		-	+	-	-	-
MBM	-	-	+	-	+	-
MBC2	+	+	+	+	+	-

Keys:- + indicates the production of enzyme
– indicates no production

None of marine bacteria exhibited pectinolytic activity with substrate pectin. However, they cannot be considered as non-pectinase producer unless they are studied with different pectinase substrate.

Antibacterial activity

All the isolates were screened for test their ability to show antibacterial activity against the two test organisms *S.aureus* (Gram positive) and *E. coli* (Gram negative). Some marine bacterial strains have exhibited antibacterial activity.

Antibacterial activity was shown by bacterial isolate MBG and MB10 against *E.coli* while isolates MB10, MBC2, MBM, MBK, MB2 and MB3 showed antibacterial activity against *S. aureus*.

Dyes degrading ability

All the 10 bacteria decolorized the tested dyes considerably. Growth analysis indicated that the marine bacterial strains have decomposed and may have used hydrocarbons from the dyes which are necessary for growth of bacteria. The decolorized samples were colourimeterically analyzed (Figure 1).

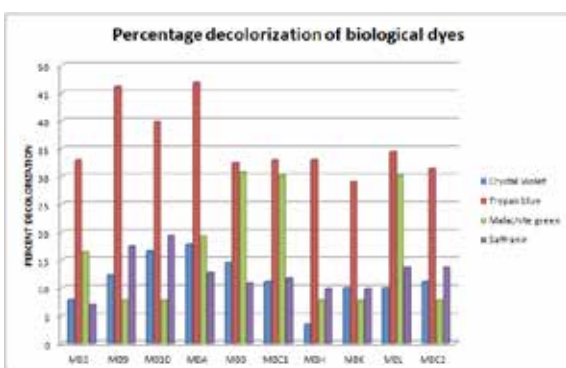

Figure 1: Percentage decolourization of biological dyes

Oil degrading ability

All 26 isolates were tested for their ability to degrade oil using Bushnell and Haas media. Some bacterial strains have grown on Bushnell Haas media in the presence of diesel (MB3, MB9, MBC2 and MBL), motor oil (MBA, MBB, MBC1, MBD, MB3, MBG, MBH, MB9, MB10, MBC2, MBL) and petro (MN3, MBA, MBB, MB9, MBC1. MBC2), indicating that they can utilize these oils as a source of carbon.

Bioaccumulation studies

Bioaccumulation studies were carried out using four bacterial isolates. The 24 hr old culture suspension of the bacterial isolates was subjected to accumulation of 500ppm heavy metal salt solutions. ICP-AES analysis was carried out (Figure 2). Maximum bioaccumulation of lead was seen in isolate MB9 whereas maximum chromium accumulation was seen in isolate MB3

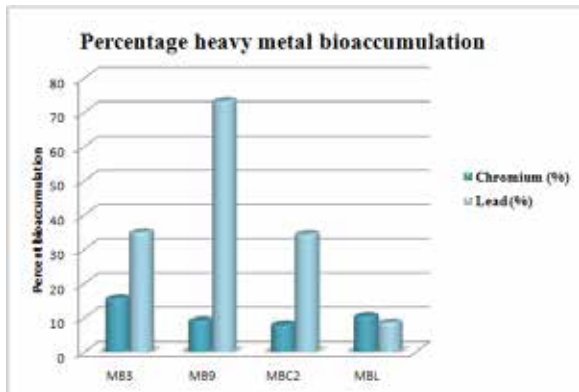

Figure 2: Percentage Heavy metal bioaccumulation

Production of Polyhydroxybutyrate (PHB)

Among 26 colonies, 8 colonies (MB8, MB4, MB5, MB12, MB9, MB11, MBC2, MBA) showed positive result for Sudan black staining indicating that these colonies produce polyhydroxybutyrate.

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). Marine bacteria can be a potential source of new bioactive compounds for industrial, agricultural, environmental, pharmaceutical and medical uses (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. In a review by Baharum et al. (2010), they 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.

In this study, 26 marine bacteria were isolated from marine water samples collected from of Mumbai coastal area. Dionisi et. al. (2012) stated that microorganisms from intertidal zones 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), plant growth promotion potentials such as production of phytohormones and phosphate solu-

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bilisation (Jayaprakashvel et al., 2011), antifungal activity (Jayaprakashvel et al., 2010), biocontrol activity for plant disease control (Gobalakrishnan et al., 2010; Bhagat et al., 2010a), antibacterial and probiotic activity (Kaarthikeyan et al., 2010).

Out of the 26 marine bacteria isolates, isolate MBC2 produced most of the extracellular hydrolytic enzymes using substrate amended plate assay. Studies have proved that marine bacteria including marine actinomycetes are exhibiting diverse pattern in secreting extracellular enzymes (Ramesh et al., 2008; Jayaprakashvel et al., 2008) Similarly, potential capability of heterotrophic bacteria for extracellular enzyme synthesis and their activity were determined in a transect from dunes to a water depth of 1 m in a sandy beach near spot on the southern Baltic coast. Among studied enzymes, alkaline phosphatase, esteraselipase, and leucine arylaminase were synthesized in a higher degree, whereas α-fucosidase, β–glucouronidase and α-galactosidase hadonly low level (Mudryk and Podgórska, 2006). Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants (Cooney, 1984). Ayed et al., (2009) studied the comparative abilities of marine bacteria for decolorization of crystal violet and saffranin.

Conclusion

Our research work about the isolated marine bacteria has given various insights into the ecology of microbes in aquatic sediments. Preliminary screenings of these 26 isolates have given a brief idea of their abilities. Further analysis can give information on the applications of these microbes. Since the marine bacteria obtained are not from deep sea levels, it proves that these microbes are easy to access and can be subjected to bioprospecting.

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