Biotec.Res.J.2015; Vol 1(3):171-174
eISSN 2395-6763

ORIGINAL RESEARCH

Marine-bacteria *Actinomycetes* : A case study for their potential in bioremediation

Pratibha KULKARNI[1], Hameeda BEE[1*]
[1]Department of Microbiology, University College of Science, Osmania University, Hyderabad - 601 301, INDIA
*Corresponding author email: drhami2009@gmail.com

• Received: 20 September 2015 • Revised: 23 October 2015 • Accepted: 15 November 2015 • Published: 24 December 2015 •

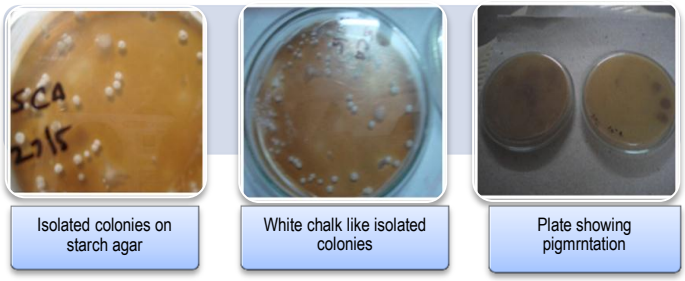
ABSTRACT
The present study highlights isolation, screening and characterization of marine microbial flora. Upon screening some six potential surface active agents producing isolates of *Actinomyces* with significant difference in morphology were isolated from marine soil of Chennai & Bombay . Results from optimization studies revealed, sucrose & yeast extract are suitable carbon and nitrogen sources, pH optimized 7.8+2, temp of 37° Celsius and agitation at 300 rpm. This study emphasizes the utilization of marine actino bacteria , having potential to produce various novel enzymes & antibiotics. Currently emerging and reemerging infectious diseases of humans, which are resistant to available drugs - is a case for future investigation of marine bacteria as source for bioactive molecules. Dead marine organisms or biomass of sea or ocean is converted into abiotic factor by marine bacteria hence serve as decomposers by their chitinolytic property. Chitin is the cell wall component of fungi, insects & worms which cause heavy loss of crops. These marine bacteria due to chitinolytic property serve as bio pesticides.

KEY WORDS: *Marine actinobacteria, Adaptation, Stress response, Bioremediation*

Introduction

Over the past 3.5 billion years, microorganisms have shaped and defined Earth's biosphere and have created conditions that allowed the evolution of macroorganisms and complex biological communities, including human societies. The first bacteria are *archae* bacteria also called extremophiles Adapted for life in sea, ocean and hot springs are marine bacteria with sea of opportunities & excitement. Marine environments are one of the most adverse environments owing to their varying nature of temperature, pH, salinity, sea surface temperature, currents, precipitation regimes and wind patterns. Due to the constant variation of environmental conditions, the microorganisms present in that environment are more suitably adapted to the adverse conditions, hence, possessing complex characteristic features of adaptation. Microorganisms play an important role in the maintenance and sustainability of any ecosystem as they are more capable of rapid adjustment towards environmental changes and deterioration. They are present everywhere, be it in the volcanic eruptions or Antarctic glacier or mars conditions; hence, marine environments are not far apart. 97 % of the Earth's water is saline in nature and it accounts for approximately 71 % of the earth's surface which generates 32 % of world's net primary production. Oceans are the source of wealth, opportunity, and abundance as they provide us food, energy, & water and are helpful in sustaining the livelihoods of hundreds of millions of people. Besides, oceans are also the main high-way of the international trade and are the main stabilizer of the world's climate. Sea with its diverse physical & chemical parameters is either feast or famine for microbes. Inter-tidal estuarine systems are a vital interface of ocean atmosphere and terrestrial environment. It is suggested that fluctuating environmental conditions or stress conditions may induce the marine bacteria to produce secondary metabolites and antimicrobial substances. Such strains may help in fighting antibiotic resistance. *Actinobacteria* are the most economically valuable prokaryotes which are well known to produce chemically diverse metabolites with wide range of activities such as pigments, antibiotics, and fungicides due to chitin degrading property, larvicidal property, enzymes - amylases, protease, cellulases & lipase. *Actinobacteria* from Bay of Bengal showed good oil degrading property. Oils like olive, sunflower, gingley were degraded, so also diesel & petrol. This shows that they have role in oil refineries. These are also good bio pesticide as they degraded chitin of worms,

cockroaches and fungi. *Actinobacteria* isolated from marine environments are better utilized in bioremediation of heavy metals, hydrocarbon oil spills, recalcitrant compounds, xenobiotic through biofilm formation and production of extracellular polymeric substances. The advantage of using marine bacteria for bioremediation in situ is the direct use of organisms in any adverse conditions without any genetic manipulation.



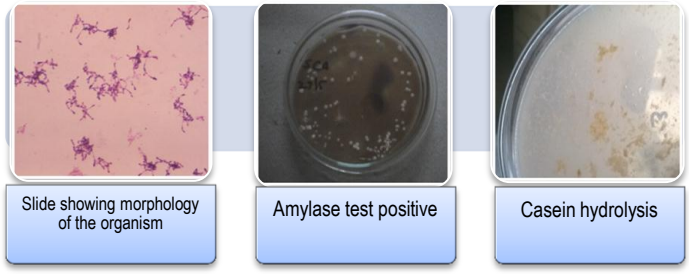
Materials and Methods

Soil sample collection

Soil sample were collected from kalpakkum near Chennai & Dadar beach in Mumbai. The samples were air dried under room temperature for about 10 days before isolation.

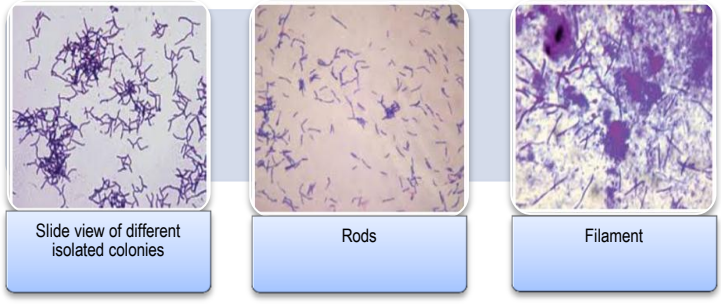
Isolation of *Actinomyces*

0.5 g of soil sample (which was air dried then boiled) was suspended in 9.5 ml sterile diluted water and was 1000 fold diluted. 1 ml of dilution was spread on various culture media as Bennet agar, L-glycine glycerol agar, L-Arginine glycerol agar, Starch-caesin agar, Humas agar with pH 7.8 and antifungal agent fluconazole & streptomycin. The plates were incubated at 28^{o}C for 2 weeks.



Morphology of the colonies

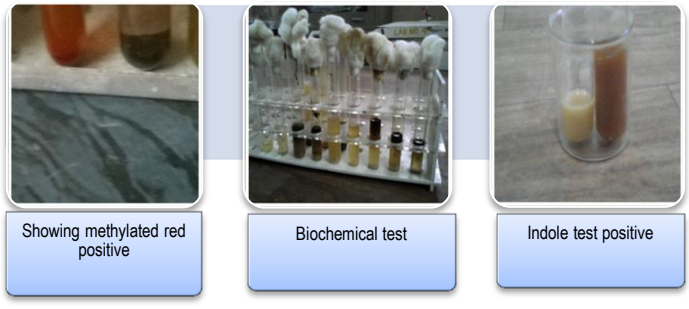
Colonies were opaque and variously pigmented. The colony surface was waxy white, chalky orange, red, purple grey. Pink pigment is seen with smooth and granular margins. Colonies were velvety depending on abundance of growth. Wrinkles which formed on incubation for 2 weeks reached diameter of several centimeters. Biochemical activities include catalase and acidification of carbohydrates. Several other biochemical properties for the isolates from two different sources.



Observations

The isolates of the two different places, one from Arabian sea & other from Bay of Bengal showed following biochemical properties . The amylase production was observed by iodine test & DNS method. Casein degradation in starch casein agar, cellulose release in cellulose agar is observed in the plate.

The isolate from Bay of Bengal sample was grown in mannitol salt broth with various oils as carbon source showed their lipolytic property (30 % in 8 days).



The isolate was also grown in chitin broth. Growth is recorded by optical method. O. D. is observed increasing. Glucose left at the end of the process is estimated by DNS method. Its growth is maximum in 2mg/ml salt concentration & pH 7.5 to 8.

Second isolate from Mumbai was grown in TCN-broth. Its positive growth showed its melanin producing property. Its Pigmentation is shown by its colonies on cellulose agar its keratin degradation & its larvicidal property is under study.

By this study we can say actinomycetes can be exploited without much genetic manipulation in bioremediation.

Table 1: Test result tabulation

Test	Result	
	Source 1	Source 2
Indole Production	_	+
Methyl red test	+	_
Nitrate reduction	+	+
Aerobic growth	+	+
Production of pigments	+	+
Lactose fermentation	+	_
Cellulase	+	+
Sucrose	_	+
Starch	+	+
Xanthine	+	+
Hypoxanthine	+	+
Antibiotic production	+	+
Fungicidal	+	+
Chitin degradation	+	+

The two soils from different marine sources showed growth in various selective media. Good growth was seen on growing for 7 to 8 days as shown by plate pictures. They showed characteristic pigment or zone of inhibition indicating either antibiotic or enzyme production. Well defined zone of inhibition was seen for starch agar, caesin agar& cellulase agar. Well isolated colonies were seen with wavy margin on simple staining cocci, filamentous bacilli and cocco bacilli. These were grown in different biochemical media to check their growth. They showed results as shown in the table 1. The plates which showed rods were cultured in manitol salt broth, chitin broth for its enrichment. The plates which showed filamentous form were sub cultured in tyrosine casein nitrate broth, xanthine broth. It showed growth in 7 to 8 days.

Pros and cons of using marine bacteria in bioremediation

Marine bacteria are found in a wide range of environmental conditions from sea floor to fish stomachs and develop unique mechanism of resistance in adverse and diverse conditions. Thus, it gives ample opportunity to employ as potential bioremediation agents. When a bacterium utilizes the contaminant as its food source, its number increases rapidly in the contaminated environments and on subsequent decontamination, the number decreases to produce harmless biomass. The process is cost-effective in comparison to the chemical processes, and they can be carried out onsite. Utilization of marine bacteria in bioremediation is highly specific; hence, the chance of forming harmful by products is less, which is the major advantage of utilizing these isolates However, there are some disadvantages in the process of using marine bacteria. In case of mixed contaminants, finding a suitable consortium becomes difficult. As far as, the potential of the microorganisms is concerned in bioremediation, marine bacteria have been proved to be the valuable and efficient candidates.

Results

The above study shows the organisms in sea are potent enough to produce bioactive molecules without much manipulation. Unlike the terrestrial resources, that have been already explored by academic researchers and industrial researchers , the rationale of searching for drugs from marine environment system from the fact that, the flora and fauna have adopted to all sorts of marine environment and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction. The study of marine chemical compounds produced by different organisms showed the strategies for their use for human benefit. Many sessile invertebrates such as sponges, corals and tunicates feed by filtering sea water. Since, sea water contains high concentrations of bacteria, these organisms produce antibiotics to defend themselves from potentially harmful microorganisms. Thus the production of antibacterial compounds by filter feeders such as sponges provides possibilities of novel antibiotics for human use.

Inference

Marine bacteria having immense genetic and biochemical diversity are likely to become rich source of novel effective drugs.

References

Ballav S., Dastager, S. G., and Kerkar S.,(2012), Biotechnological significance of Actinobacterial research in India. Recent Research in Science and Technology 2012, 4(4): 31-39: ISSN: 2076-5061.

Karthikeyan P., Senthilkumar G. and.Panneerselvam A.,(2014) Actinobacterial diversity of mangrove environment of the Palaverkadu mangroves, east coast of Tamil Nadu, India (2014), 3(1) pp. 145-154 ISSN*:* 2319-7706.

Quadri S. R. and Agsar D.,, Antimicrobial attributes of rare actino bacteria detected from lime stone quarries, *Int J Pharm Bio Sci* 2012 July; 3(3): (P) 137 – 147: ISSN 0975-6299.

MohanaPriya, Radhakrishnan M. and Balagurunathan R, 2011, Production and optimization of L-asparaginase from *Streptomyces sp* (TA22) isolated from Western Ghats, India. *J. Chem. Pharm. Res*., 2011, 3(4): 618-624: ISSN No: 0975-7384**.**