



STUDIES ON THE ANTIBIOTIC RESISTANT BACTERIA ISOLATED FROM MANGROVE SEDIMENTS

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ABSTRACT

The emergence of bacteria that are resistant to antibiotics is considered as a major public health threat and therefore isolation and studies of such bacteria hold significant importance. The present study was undertaken to identify some resistant bacteria from the samples collected from mangrove sediments from the coastal areas of Maharashtra. Out of the twelve bacterial isolates, MGS4 isolate

showed resistance against most of the antibiotics at 100µg/ml. When further identified by using 16S rRNA gene sequences, this antibiotic resistant bacterial isolate MGS4 showed sequence similarity with *Microbacterium* sp.

KEYWORDS: Antibiotics, Resistant bacteria, PCR, 16S rRNA, Mangroves.

INTRODUCTION

An antibiotic is a compound or substance that kills or slows down the growth of bacteria.^[1] The term antibiotic was coined by Selman Waksman in 1942 to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. Antibiotics can also be classified based on their target range; broad-spectrum antibiotics treat a wide range of infections whereas narrow-spectrum antibiotics are effective only against a few types or species of bacteria.

Antibiotics have transformed health care since they were introduced in the 1940s and have been widely used to fight bacterial infections. However, some infectious organisms have

developed resistance to the antibiotics used to treat patients with infections. When bacteria become resistant to an antibiotic, that medicine becomes less effective. Medical treatment of people infected with these drug-resistant organisms can become more complicated, leading to longer hospital stays, increased health care costs, and in extreme cases, to untreatable infections. Thus, antibiotic resistance of bacteria is nowadays becoming a very serious problem.^[2]

Scientists are trying their best to isolate such resistant bacteria from different sources.^[3-6] An attempt was made in the present study to isolate and study some antibiotic resistant bacteria from the mangrove sediment samples.

MATERIALS AND METHODS

Sample collection

Sediment samples were collected from mangrove areas of Ratnagiri (State of Maharashtra, West coast of India) at low tide using a plastic spatula and immediately poured into a plastic bag and carried to laboratory in an icebox. Sediment sample were stored at 4 °C till the further use.

Media used for bacterial isolation

The media used for isolation were nutrient agar and nutrient broth (Himedia Laboratories, India) prepared in seawater. The seawater was filtered through cotton and used in the media preparation.

Isolation of bacterial cultures

1g of soil sample dried at 40°C for 5 hours was suspended in 9 ml of sterile distilled water. Sediment samples were diluted and spread plated on nutrient agar, prepared in seawater. Antibiotics were added to above media in order to eliminate fungal growth. Plates were incubated at 30°C for 48 hours.

Antibiotic resistance assay by soft agar method

This method was conducted to measure the effects of different antibiotics on the growth of bacteria.

Inoculum was prepared by preparing the nutrient broth in seawater and dispensing the same into test tubes and autoclaved. These tubes were then inoculated with the sediment-associated bacteria, vortexed and kept on shaker for 24- 48 hrs at 30°C.

Nutrient agar agar (2%) plates were poured as basal layer overlaid with 1% Nutrient agar, containing 25µl inoculum of sediment associated bacteria. Six different antibiotics viz. Ampicillin, Amoxycilin, Azenam, Chloram, phenicol, Rifampicin and Streptomycin were selected to check their effect of sediment derived bacteria. Antibiotic discs of different concentration (100 µg/m and 50 µg/ml) were made and placed on the seed layer. The plates were then incubated at 30°C and observed after 24 hrs.

Morphological characteristics

Colony characteristics of each 48 hour grown colony were noted for the isolated cultures. The smears prepared were observed under oil immersion and cell type noted.

Phylogenetic analysis of antibiotic resistant bacterium

Antibiotic resistant bacterial isolate MGS4 was further identified by using 16S rRNA gene sequences amplified by PCR.

Amplification reactions were performed as given in the 5X qarta. Taq master mix protocol using 16s rRNA primers. Sequences for primers used are as follows: 519F (5'- CAG CAG CCG CGG TAA TAC -3') and 1385R (5'- CGG TGT GTA CAA GGC CC -3'). 50µl PCR reaction was prepared containing 10µl of 5X PCR master mix, 2µl each of 10pM 519F and 1385R primers, 1µl of DNA (80µg) and nuclease free water made up to 50µl. The conditions for thermal cycling were 30 cycles of denaturation at 94°C for 45 s, primer annealing at 52°C for 45s and primer extension at 72°C for 1 min 30s. All the PCR products were sequenced at MWG Biotech, Bangalore, India and chromatograms were obtained. The sequences of PCR products were analyzed by using Basic Local Alignment Search Tool (BLAST).

RESULTS

Twelve sediment associated bacteria were isolated. These bacteria were given codes from MGS1 to MGS 12 and their colony characteristics such as colour, form, elevation and margin were studied (Table 1).

Table 1: Colony characteristics of twelve bacteria isolated from mangrove sediments.

Bact. Code	Colour	Form	Elevation	Margin
MGS1	Cream	Filamentous	Flat	Entire
MGS2	Pale yellow	Circular	Umbonate	Entire
MGS3	Cream	Circular	Flat	Entire
MGS4	Cream	Circular	Flat	Entire
MGS5	Cream	Circular	Pulvinate	Entire

MGS6	Cream	Circular	Raised	Entire
MGS7	Cream	Circular	Flat	Entire
MGS8	Orange	Circular	Flat	Entire
MGS9	Cream	Irregular	Flat	Entire
MGS10	Yellow	Irregular	Flat	Lobate
MGS11	cream	Circular	Flat	Entire
MGS12	Cream	Irregular	Flat	Entire

Antibiotic resistance assay indicated that the isolate MGS4 showed strong resistance to all the six test antibiotics of the concentration of 100 µg/ml hence, it was selected for further identification with 16S rRNA gene marker. Other bacterial isolates failed to demonstrate significant antibiotic resistance except the isolate MGS1 which exhibited resistance against Azenam, MGS6 against Ampicillin, MGS7 against Chloramphenicol, MGS10 against Ampicillin and Azenam and MGS12 against Amoxycillin all having a concentration of 100 µg/ml. Bacterial isolates MGS2, MGS 3, MGS 5, MGS 8, MGS 9 and MGS 11 did not show resistance even against a single antibiotic under study (Table 2).

Table 2: Activity of different antibiotics against sediment derived bacteria.

Bact. Code	Conc ⁿ µg/m	Ampicillin	Amoxycillin	Azenam	Chloramphenicol	Rifampicin	Streptomycin
MGS1	50	-	-	-	-	-	-
	100	-	-	+	-	-	-
MGS2	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
MGS3	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
MGS4	50	+	+	+	-	+	-
	100	+	+	+	+	+	+
MGS5	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
MGS6	50	-	-	-	-	-	-
	100	+	-	-	-	-	-
MGS7	50	-	-	-	-	-	-
	100	-	-	-	+	-	-
MGS8	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
MGS9	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
MGS10	50	-	-	-	-	-	-
	100	+	-	+	-	-	-
MGS11	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
MGS12	50	-	-	-	-	-	-
	100	-	+	-	-	-	-

Phylogenetic analysis

In the phylogenetic analysis of antibiotic resistant strain MGS4 showed 98% sequence similarity with *Microbacterium testaceum* StLB037 strain when analyzed by the BLAST tool.

DISCUSSION

In spite of the efficacy of antibiotics in controlling the pathogenic bacteria and infectious diseases, infections are still the second-leading cause of death worldwide, causing over 13 million deaths each year. This fact is the result of the emergence of new diseases, the re-emergence of diseases once controlled and more specifically of the development of antimicrobial resistance.^[7]

Bacteria have a remarkable ability to adapt to adverse environmental conditions, which is an example of the ancient law of nature of 'survival of the fittest'. It appears that the emergence of antimicrobial resistant bacteria is inevitable to most every new drug and it is recognized as a major problem in the treatment of microbial infections in hospitals and in the community.^[8]

Mangrove ecosystem is considered to be an excellent source of unique microorganisms which is clearly vindicated in the present study.^[9-11] This study suggests that mangroves could be further explored to identify more such bacteria having unique properties which can have important therapeutic applications.

CONCLUSIONS

Total twelve bacterial strains were isolated from mangrove sediments collected from the coast of Ratnagiri. Out of the 12 isolates, MGS4 isolate showed resistance against most of the antibiotics at 100 µg/ml. The antibiotic resistant strain showed 98% similarity with *Microbacterium* sp. Therefore, encouraging the application of 16S rRNA gene markers in the reliable species identification is highlighted. So from the overall results, we can conclude that the mangrove sediments accumulate antibiotic resistance bacteria from the surrounding water.

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