ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 01, pp. 296-298, January, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

CASE REPORT

Isolation and identification of microbes in the selected stations of Chennai coastal zone – A case study

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ARTICLE INFO

Article History:
Received 19th October, 2012
Received in revised form
24th November, 2012
Accepted 23rd December, 2012
Published online 14th January, 2013

Key words:
Staphylococcus aureus,
Pseudomonas aeruginosa,
Enterococcus sp.,
Escherichiacoli and *Candida sp.*

ABSTRACT

The present study is aimed to investigate identification of microbes in water samples in different sites. The places are; Kovalam, Kanathur, Injambakkam, Besant Nagar, Pattinapakkam – Foreshore estate, Santhome, Marina beach, Vailankanni. A total of 78 strains were isolated from nine different samples collected from the intertidal zone of the east coast road (ECR), India. The antibacterial effect for the strains AMET 6303, AMET 6308, AMET 6349, AMET 6361 and AMET 6374 were screened against the bacterial and yeast pathogen *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus sp., Escherichiacoli* and *Candida sp.*

INTRODUCTION

Ocean have borne most of the biological activities on our planet. A number of biologically active compounds with varying degrees of action, such as anti-tumour, anti-cancer, anti-microtubule, anti-proliferative, cytotoxic, photoprotective as well as antibiotic and antifouling properties, have been isolated to date from marine sources. The marine environment have represents a largely unexplored source for isolation of new microbes (bacteria, fungi, actinomycetes, micro algae – Cyanobacteria and dialoms) that are potent producers of bioactive potential of marine microbes (free living and symbiotic) that are potent producers of bioactive secondary metabolites. Extensive research has been done to unveil the bioactive potential of marine microbes (free living and symbiotic) and the results are amazingly diverse and productive. Some of these bioactive secondary metabolites of microbial origin with strong antibacterial and microbial origin with strong antibacterial and antifungal activities are being intensely used as antibiotics and may be effective against infectious diseases such as HIV, conditions of multiple bacterial infections diseases such as HIV, conditions of multiple bacterial infections (penicillin, cephalosporines, streptomycin, and vancomycin) or neuropsychiatric sequelae. Research is also being conducted on the general aspects of biophysical and biochemical properties, chemical structures and biotechnological applications of the bioactive substances derived from marine microorganisms and their potential use as cosmeceuticals and neutraceuticals. The review is an attempt to consolidate the latest studies and critical research in this field, and to showcase the immense competence of marine microbial flora as bioactive metabolite producers. In addition, the present review addresses some effective and novel approaches of procuring marine microbial compounds utilizing the latest screening strategies of drug discovery (Ira Bhatnagar and Se-Kwon Kim, 2010). Hence an attempt has been made to investigates the isolation, standardization and identification of microbes in marine water and also study the antibacterial activity using various station.

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MATERIALS AND METHOD

Collection of samples

The seawater samples were collected from the intertidal zone of east coast, Bay of Bengal in different sites. The places are; Kovalam, Kanathur, Injambakkam, Besant Nagar, Pattinapakkam – Foreshore estate, Santhome, Marina beach, Vailankanni.

Sampling and sample processing

The sea water samples were collected in clean sterile sample bottles. The sea waste samples were carried to the laboratory than the samples were serially diluted. The master blank was prepared by adding 10 ml of the sea water to 90 ml of distilled water. From the master blank 1 ml of the diluted mixture was transferred to the next tube (10^{-2}). The procedure was repeated up to the dilution 10^{-6}. The dilution 10^{-4}, 10^{-5} and 10^{-6} were selected for plating. The spread plate method was done using 0.1 ml of the dilutions 10^{-4}, 10^{-5} and 10^{-6} (Nutrient Agar).

Spread plate method

The nutrient agar was prepared and plated. 0.1 ml from the dilutions 10^{-4}, 10^{-5} and 10^{-6} was added the nutrient agar plates respectively and spread using a sterile L-rod.

Nutrient Agar composition/1000 ml

Peptone-5 gms
Beef extract-3 gms
Agar-20 gms

The pour plate method

1 ml of the dilution 10^{-4}, 10^{-5} and 10^{-6} was added on to the sterilized empty plates respectively, 20 ml of previously prepared and sterilized ago NA was poured onto the previously added diluted samples and rotated in the clockwise and anti-clock wise direction and the medium was allowed to solidify.

Pure culture and standardization of the growth medium

The colored colonies were taken and subcultured on 3 different growth media namely,

1) Sabouraud's dextrose Agar
2) Nutrient Agar
3) Yeast extract medium in order to standardize the growth medium.

Composition of sabouraud's dextrose Agar/1000 ml

Dextrose -20 gms
Peptone-10 gms
Agar-20 gms

Composition of yeast extract medium/1000 ml

Yeast extract-3 gms
Peptone-5 gms
Agar-20 gms

Standardization of the production medium/1000 ml

The production medium was standardized by inoculating the selected strains in the 3 different broth namely

1) Nutrient broth
2) Yeast extract broth
3) Sabauroud's dextrose broth

The strains AMET 3, AMET 8, AMET 30, AMET 44, AMET 61, AMET 65, AMET 72 and AMET 74 were selected. Among the 74 isolates and inoculated in the above said production medium.

Composition of sabouroud's dextrose broth/1000 ml

Dextrose -20 gms
Peptone-10 gms

Composition of yeast extract broth/1000 ml

Yeast extract-3 gms
Peptone-5 gms

Morphological identification

The morphological tests were done for the selected strains AMET 6303, AMET 6308, AMET 6349, AMET 6361 and AMET 6374.

Gram staining method

Smear was prepared from the strains AMET 6303, AMET 6308, AMET 6361 and AMET 6374. Gram stained smear were observed under microscope.

Motility test

24 hours broth culture of the strains AMET 6303, AMET 6308, AMET 6349, AMET 6361 and AMET 6374 were used for the testing of motility. A loop of culture was placed at the center of the cover slip and the paraffin wax was kept on the four corner of the cover slip. A cavity slide was inverted over the cover slip such that the drop hangs in the covertly of the slide. The drop was observed under the 4x objective of the microscope. The edge of the drop focused for the motile bacteria.

Antibacterial sensitivity assay

Live culture assay

The strains AMET 6303, 6308, 6349 and 6374 were selected for screening of antibacterial activity. The selected pathogens were *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus sp. Escherichiacoli* and *Candida sp.* Nutrient agar was prepared and the pathogens were swabbed. 8 mm diameter wells were cut using the cork borer and 100 µl culture of above said strains in the yeast extract broth was added in the wells. Ready methicillin discs were kept at the center as control. The plate were incubated at the room temperature. After 24 hours of incubation the plates were observed for the zone of inhibition and measured.

Cell free culture filtrate assay

The strains AMET 6303, 6308, 6349, 6361 and 6374 were selected for screening of antibacterial activity. The selected pathogens were *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus sp., Escherichiacoli* and *candida sp.* Nutrient agar was prepared and the pathogens were swabbed. Filter paper discs were impregnated with extracted pigment in the chloroform and were kept over the swabbed pathogen. Filter paper disc impregnated with chloroform was kept in the center as control. After 24 hours of incubation the plates were observed for the zone of inhibition and measured.

RESULTS

Isolation of bacteria

Colonies of bacteria appeared on the nutrient agar from the 2nd day onwards. A total of 78 strains were isolated from nine different samples collected from the intertidal zone of the east coast road (ECR), India. Morphologically 78 different strains were selected and designated as AMET 6301 to AMET 6378. The isolated bacteria were subcultured in 3 different media namely nutrient agar, sabouraud's dextrose agar and yeast extract medium.

Standardization of growth medium

On growing a 3 different media nutrient agar, sabouraud's dextrose agar and yeast extract medium, the pigmented colonies grew well both in sabouraud's dextrose agar and yeast extract medium. From among the 78 strains the bright pigment producing 9 strains (AMET 6303, AMET 6308, AMET 6330, AMET 6344, AMET 6349, AMET 6353, AMET 6361, AMET 6372, AMET 6574) were selected and quadrant streaked on yeast extract medium.

Standardization of production medium

The strains AMET 6303, AMET 6308, AMET 6330, AMET 6344, AMET 6349, AMET 6353, AMET 6366, AMET 6372, AMET 6374 were selected and inoculated in the yeast extracted broth, nutrient broth and sabouraud's dextrose broth in order to standardize media.

Morphological identification and motality test

Gram staining was done to study the morphology and hanging drop method was done for the strains AMET 6303, AMET 6308, AMET 6349, AMET 6361 and AMET 6374 and the results are shown in the

Screening for the antimicrobial effects

The antibacterial effect for the strains AMET 6303, AMET 6308, AMET 6349, AMET 6361 and AMET 6374 were screened against the bacterial and yeast pathogen *Staphylococcus aureus, Pseudomonas*

aeruginosa, Enterococcus sp., Escherichiacoli and *Candida sp.* The screening was done for both the extract and the live culture. In the live culture assay the strain AMET 6349 alone showed activity against *E. coli, Enterococcus* and *Staphylococcus aureus*. Regarding the extract assay none of the extract assay none of the extract showed activity against the pathogens.

DISCUSSION

Since the oceans are the largest sources of pigmented bacteria in the present study the sea water was used as the source. The literatures say that the bacteria produce pigments in the stress conditions such as sea high salinity and osmatic pressure the media used in the experiments was also prepared in the sea water. A characteristic of marine bacteria is that a large proportion of them are coloured (ZoBell, 1946); for example, ZoBell and Feltham (1934) examined several thousand colonies growing on agar inoculated with sea water or marine mud, and found that 69.4% were chromogenic. Later ZoBell and Upharn (1944) desoribed fully twenty coloured species. Many species of marine bacteria which spoil fish are also chromogenic; the considerable literature on this subject has been summarized by ZoBell (1946).

The different pigments producing bacteria was isolated and from that 2 pigment producing strains were selected for the screening for textile dyeing since it was brighter than the other pigments and also it was water soluble. On optimization using various growth factors and environmental parameters it grew well and produced increased amount of pigment in the presence of high salinity and increased source of nitrogen and in the average supply of ozone. On screening for the textile dyeing the dying process was effective when copper, iron and the salt sodium chloride was added as mordant, when the cotton cloth pieces were washed with water and detergent the pigment was not easily washed away.

The pink colour pigment of the strain AMET 6308 and the yellow colour of the strain 6349 is found to belong to the group of Astaxanthin whichis a carotenoid pigment. And the biochemical characteristics of both the bacteria resembles the genera *Erwinia sp.* Many carotenoid biosynthesis genes have been cloned from various organisms and their functions have been determined. Phytoene synthase genes which mediate the formation of the first colourless carotenoid phytoene from geranylgeranyl ppi (GGPP), have been isolated from the photosynthetic bacteria *Rhodobacter* species (5,31), the mono-photosynthetic bacteria *Erwinia sp* (4,45,60) and *Thermus thermophilus* (20), the cyanobacterium *Synechococcus sp.* strain PCC 7942 (11), the fungus *neurospora crassa* and higher plants (Norihiko Misawa, 1995).

The majority of the bacteria found in the sea water are chromogenic which produce industrially valuable pigments that can be used in textile dyeing. Totally 78 pigment producing strains were isolated from 9 different sites along Chennai coast, Pondicherry and Velankanni. From this 5 strains were selected which produced brighter pigments in the production medium. Various morphological and biochemical test were performed for the selected strains. The selected strains were screened for the production of extra cellular enzymes. All the strains produced cellulose enzymes. The strain AMET 6308, AMET 6349, AMET 6361 and AMET 6374 showed maximum pectinase activity. The strain AMET 6308 and AMET 6380 showed maximum lipase activity. By screening the culture filtrate the strain AMET 6303 and AMET 6308 showed maximum gelati activity.

The selected 5 strains and the culture filtrate were also screened for the antimicrobial activity. They were extracted in chloroform. The strains AMET 6303 showed average activity against all 5 pathogens *Staphylococcus aureuses, Pseudomonas aeruginosa, Escherichia coli, Enterococcus sp.* and *Candida sp.* In the culture filtrate assay the strains AMET 6308 were effective against *Stahylococcus aureus, Escherichia coli, Psuedomonas aeruginosa* and *Enterococcus.* The strain AMET 6303 was effective against the *Escherichia coli* and *Enterococcus sp.* None of the culture filtrate showed activity against *Candida sp.* From the 5 strains AMET 6308 and AMET 6349 were water-soluble and so they were selected for the screening of application in textile dyeing. They were optimized using various environmental parameters and growth factors. The strain AMET 6308 grew well in the presence of 1% of salinity pH 7 at room temperature, sucrose, ammonium chloride and nitrogen source, amino acid tryptophan, 3 min of ozone supply, sea food waste crab and fish. The strain AMET 6349 grew well in the presence of salinity 1, pH 11, room temperature, sucrose as carbon source, peptone and yeast extract as nitrogen source, amino acid leucine, 1 min of ozone supply and sea food waste prawn. It also grew well when it was grown it sea water enriched with prawn and crop waste.

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