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Isolation and Screening of Marine Bacteria for Industrially important Extracellular Enzymes

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Abstract : A total of 20 Marine Bacteria were isolated from sea water samples of three different Beaches viz Marina, Besant Nagar, Kovalam of Chennai and kanchipuram district, Tamil Nadu, India. Out of 20 Marine Bacteria Eight Bacteria were isolated from Marina Beach sample, 10 Bacteria were isolated from Besant Nagar Beach sample and only 2 Bacteria were isolated from Kovalam Beach sample. Morphology, Growth and colour of the isolated 20 Marine Bacteria were recorded. In Biochemical characters 12 Bacteria were Gram Negative and 8 Bacteria were Gram Positive, All the isolated Marine Bacteria were Motile, 10 Bacteria were Oxidase, Positive and seven Bacteria were Catalase positive. All the 20 Marine Bacteria were screened for five industrially important Extracellular enzymes. Comparative Analysis in three different Beaches (Marina, Besant Nagar, Kovalam) for the production of five industrially important extracellular enzymes like Amylase, Cellulase, Protease, Lipase and Chitinase shows that Besant Nagar isolates shows highest production of Amylase, protease and cellulose followed by Marina Beach isolates. Lipase enzyme was maximum produced in Marina isolates followed by Besant Nagar isolates. Kovalam Beach isolate were not able to utilize the CM-cellulose and Tween 20. Interestingly out of 20 isolates from three different Beaches (RMB1-RMB20) were not able to utilize the chitin substrate and produce chitinase enzyme.
Keywords : Marine Bacteria, Beaches, Extracellular enzymes.

Introduction

Marine microbes have enormous hope for bio discovery (5).They have distinctive 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. These unique characteristics have attracted many researchers to explore in depth since there is the potential of marine microorganisms used in industries (2). Research into natural products from the marine environment, including microorganisms, has rapidly increased over the past few years.

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It is estimated that global market for industrial enzyme grows by 10-15% annually with the current value of USD 4.1 billion (4, 11). With the unique ability of marine microorganisms, that capable catalyzing reactions at temperature near the freezing point of water, it have raise considerable interest for both industrial use and fundamental studies as alternative of common mesophilic enzyme since cold-adapted enzymes would contribute towards energy saving strategies (14). Besides, marine enzyme biotechnology can offer novel biocatalysts with properties like high salt tolerance, hyperthermostability, barophilicity, cold adaptively and ease in large scale cultivation (6). Enzymes are used as cost -effective and environmentally sensitive substitutes for chemical processing in several industries including pharmaceuticals, food, starch, laundry, detergents, for processing textiles, leather, wood pulp and paper, and for the production of fine and specialty chemicals, and industrial catalysis, organic synthesis and transformation of compounds and bioremediation.

Bacteria from marine sources have lot of application in biotechnology such as enzyme and metabolite production. A number of enzymes such as Amylase, Cellulase, Lipase, Protease and chitinase have been discovered from microbes isolated from extreme marine environments. In modern society, the proteases are widely used. Proteases are used in the detergent industry, leather industry, and also for pharmaceutical applications, such as digestive drugs and anti-inflammatory drugs [8,9,13, 18, and 24). Lipases have received increased attention recently, as evidenced by the increasing amount of information about lipases in the current literature. Also, many microbial lipases are available as commercial products, the majority of which are used in detergents, paper production, cosmetic production, food flavouring, organic synthesis and some other industrial applications [12, 21].

The intention of the present study was to investigate potential microorganisms present in Beaches of Chennai and Kanchipuram District, Tamil Nadu, which can be able to produce the industrially important Extracellular enzymes.

Materials and Methods

Collection of Marine Samples

Three marine water samples were collected from different locations in the Bay of Bengal from Marina, Besant Nagar, Kovalam Chennai and kanchipuram district, Tamil Nadu India.

Isolation of Marine Bacteria

Ten ml of collected marine water sample was suspended in 90 ml of sterile sea water and these suspensions were serially diluted. Nutrient medium (NA) prepared in natural aged sea water was used for isolation of marine bacteria and pour plate method was done. All the plates were incubated at 32˚c for 7 days(1).

Morphological and Growth Pattern of Marine Bacteria

All the isolated marine bacteria isolates were sub-cultured on NA medium the pigmentation for aerial and colony morphology produced was observed for all the isolates and the results were documented.

Biochemical Characterization

Cell shape and motility were examined on freshly prepared wet mounts by light microscopy of exponentially growing liquid culture. Gram nature of the isolated was determined by gram staining performed after fixing and simultaneously desalting the samples with 2% acetic acid for 5 min. Oxidase, Catalase test wasalso performed (16).

Production of Extracellular Enzymes

Twenty 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(22) as follows.

Screening of Marine Bacteria for Amylase Enzyme

The 20 bacterial isolates were screened for the production of amylase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % starch as a substrate and incubated at 32˚c for 3 days. The iodine indicator, with 2% of iodine and 1% potassium iodide was poured and the clear zone around the growth was observed, which indicated the hydrolysis of starch.

Screening of Marine Bacteria for Cellulase Enzyme

The 20 bacterial isolates were screened for the production of cellulase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % carboxy methyl cellulose as a substrate and incubated at 32˚c for 3 days. The After incubation, the plates were flooded with 0.1% Congo red solution, and development of clear zone around the colonies was observed.

Screening of Marine Bacteria for Protease Enzyme

The 20 bacterial isolates were screened for the production of protease enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % Gelatin as a substrate and incubated at 32˚c for 3 days. After incubation, the plates were flooded with saturated ammonium sulphate in 0.1 N HCl solutions and a clear zone around the colony was observed, which indicated the hydrolysis of Gelatin.

Screening of Marine Bacteria Forlipase Enzyme

The 20 bacterial isolates were screened for the production of lipase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % Tween 20 as a substrate and incubated at 32˚c for 3 days.After incubation, the plates were observed for opalescent zone formation around the colonies.

Screening of Marine Bacteria for Chitinase Enzyme

The 20 bacterial isolates were screened for the production of chitinase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % chitin as a substrate and incubated at 32˚c for 3 days.After incubation, the plates were flooded with 1.0% solution of Congo red. Then, the Congo red solution was poured off and the plates were again flooded with 1 N NaCl. The development of clear zone around the colonies was observed.

Comparative Analysis of Enzymes in Three Beaches

Comparative Analysis in three different Beaches (Marina, Besant Nagar, Kovalam) for the production of five industrially important extracellular enzymes like Amylase, Cellulose, Protease, lipase and Chitinase.

Result

Collection of Marine Water Samples

Three marine water samples were collected from different locations in the Bay of Bengal from Marina Beach, Besant Nagar, Kovalam Chennai and Kanchipuram District, Tamil Nadu India. The Marine water samples were collected aseptically in a clean and sterilized container from three Beaches. The collected samples were immediately transferred to the laboratory in an ice box for bacteriological examination**.**

Isolation of Marine Bacteria

Totally 20 Marine bacteria was isolated from Marina, Besant Nagar, Kovalam beaches sea water sample. The 20 Marine bacteria were streaked separately on Nutrient Agar medium.

Number of Marine Bacteria Isolated From Different Beaches

Eight Bacteria were isolated from Marina Beach sample, 10 Bacteria were isolated from Besant Nagar Beach sample and only 2 Bacteria were isolated from Kovalam Beach sample. (**Fig: 1)**

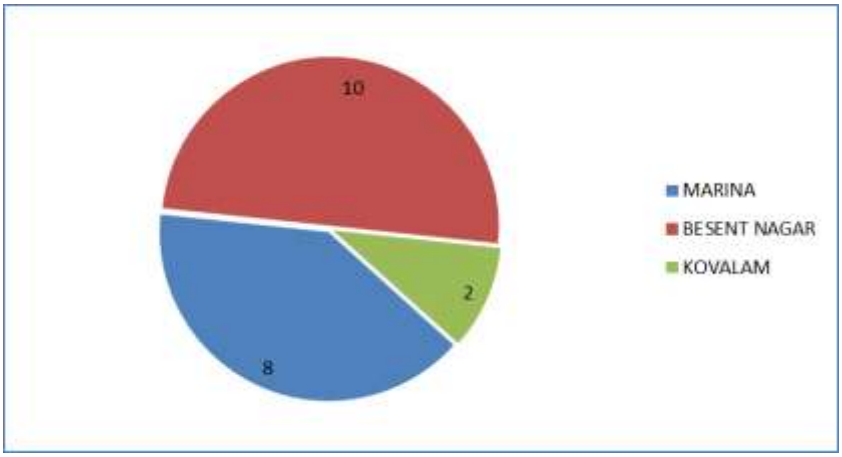

Fig: 1 Number of Marine Bacteria Isolated From Different Beaches

Morphological Characteristics of Marine Bacteria

All the isolated marine bacteria isolates were sub-cultured on NA medium the pigmentation for aerial and colony morphology produced was observed for all the isolates and the results were documented.The colonies were distinguished by morphological characters like shape, size, colour, 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 (**Table: 1).**

Table:1 Morphology of The Marine Bacteria

S.NO	SIZE	SHAPE	COLOUR
MB-1	Small	Round , Raised	white
MB-2	Big	Irregular , Flat	Transparent
MB-3	Moderate	Irregular , Raised	white
MB-4	Small	Raised	Yellow creamy
MB-5	Big	Flat	Creamy white
MB-6	Small	Flat , Irregular	white
MB-7	Moderate	Flat , Dried	yellow
MB-8	Big	Raised	Creamy yellow
MB-9	Small	Round	White creamy
MB-10	Big	Irregular , Raised	Dull White
MB-11	Moderate	Round , Raised	white
MB-12	Big	flat , Irregular	Dull white
MB-13	Small	Round , Raised	Creamy
MB-14	Small	Round	Yellow , bright
MB-15	Small	Round , Raised	Orange
MB-16	Moderate	Irregular , Raised	Creamy white
MB-17	Small	Round	White , Bright
MB-18	Moderate	Irregular , flat	Dull white

MB-19	**Big**	**Irregular , sticky**	**Creamy yellow**
MB-20	**Small**	**Round , Raised**	**white**

Growth Pattern of Marine Bacteria

All the isolated Marine Bacteria were studied for their growth on Nutrient Agar medium, among 20 isolates, most of them showed fast growth, few exhibited slow growth and rest of the isolates showed moderate growth.

Table: 2 biochemcial Characterization Of Isolated Marine Bacteria

S.No	Gram Staining	Motility	Oxidase	Catalase
MB-1	**Positive**	**Motile**	**Positive**	**Negative**
MB-2	**Negative**	**Motile**	**Negative**	**Positive**
MB-3	**Positive**	**Motile**	**Positive**	**Negative**
MB-4	**Negative**	**Motile**	**Negative**	**Negative**
MB-5	**Negative**	**Motile**	**Positive**	**Positive**
MB-6	**Positive**	**Motile**	**Positive**	**Negative**
MB-7	**Negative**	**Motile**	**Negative**	**Negative**
MB-8	**Negative**	**Motile**	**Positive**	**Negative**
MB-9	**Positive**	**Motile**	**Positive**	**Negative**
MB-10	**Negative**	**Motile**	**Negative**	**Positive**
MB-11	**Positive**	**Motile**	**Positive**	**Negative**
MB-12	**Positive**	**Motile**	**Negative**	**Negative**
MB-13	**Negative**	**Motile**	**Positive**	**Positive**
MB-14	**Negative**	**Motile**	**Negative**	**Negative**
MB-15	**Negative**	**Motile**	**Positive**	**Positive**
MB-16	**Negative**	**Motile**	**Positive**	**Negative**
MB-17	**Negative**	**Motile**	**Negative**	**Negative**
MB-18	**Negative**	**Motile**	**Positive**	**Positive**
MB-19	**Positive**	**Motile**	**Negative**	**Negative**
MB-20	**Positive**	**Motile**	**Negative**	**Positive**

Biochemical Characterization

Out of 20 Marine Bacteria 12 Bacteria were Gram Negative and 8 bacteria were Gram Positive, All the isolated Marine Bacteria were Motile, 10 Bacteria were Oxidase, positive and seven Bacteria were Catalase positive (**Table: 2).**

Extracellular Enzyme Screening on Solid Agar Media

Screening of Marine Bacteria for Amylase Enzyme

The 20 Marine bacterial isolates were screened for the production of amylase enzyme out of which MB1, MB3, MB7, MB8, MB11, MB12, MB15, MB16, MB17, MB18, MB20 were utilize the starch substrate and MB1 was found to be the highest amylase producing Marine bacteria (**Fig: 2& Table: 3)**

Screening of Marine Bacteria for Cellulase Enzyme

The 20 Marine bacterial isolates were screened for the production of Cellulase enzyme out of which MB2, MB3, MB11, MB13, MB15, MB16, MB17, MB18 were utilize the CM-Cellulose substrate and MB3 was found to be the highest cellulose producing Marine bacteria (**Fig: 2 & Table: 3)**

Screening of Marine Bacteria Forprotease Enzyme

The 20 Marine bacterial isolates were screened for the production of protease enzyme out of which MB1, MB3, MB4, MB5, MB11, MB12 MB15, MB16, MB17, MB18 were utilize the Gelatin substrate and MB12 and MB18 was found to be the highest protease producing Marine bacteria (**Fig: 2& Table: 3)**

Screening of Marine Bacteria for Lipase Enzyme

The 20 Marine bacterial isolates were screened for the production of lipase enzyme out of which MB4, MB6, MB8, MB10, MB12, MB13 were utilize the Tween 20 substrate and MB12 and MB13 was found to be the highest amylase producing Marine bacteria (**Fig: 2 & Table: 3)**.

Screening of Marine Bacteria for Chitinase

The 20 Marine bacterial isolates were screened for the production of chitinase enzyme. None of the Marine Bacteria were able to utilize the chitin substrate and produce chitinase enzyme (**Fig: 2 & Table: 3)**.

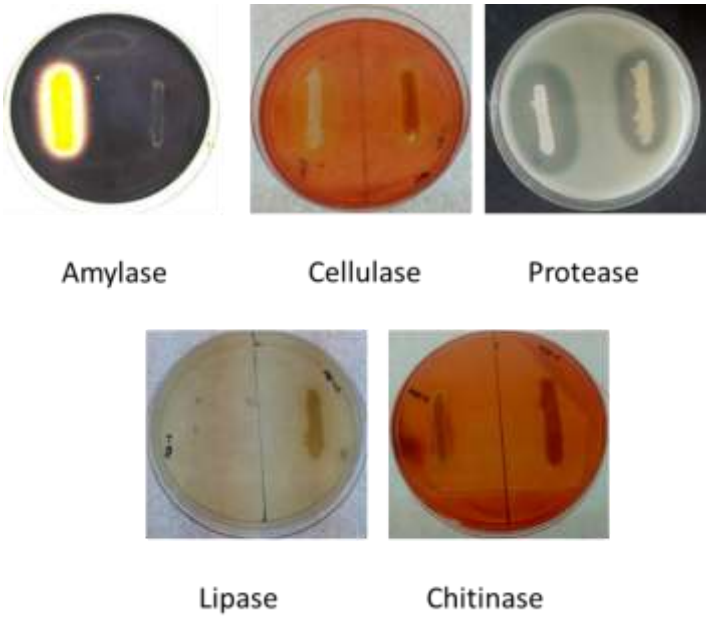

Fig: 2 Screening of Marine Bacteria For Various Extra Celluar Enzymes

Table: 3 Screening Of Marine Bacteria For Various Extra Celluar Enzymes

S.No	Amylase	Cellulase	Protease	Lipase	Chitinase
MB-1	+++	_	_	_	_
MB-2	_	+	_	_	_
MB-3	+	+ ++	+ +	_	_
MB-4	_	_	+ +	+	_
MB-5	_	_	+ +	_	_
MB-6	_	_	_	+	_
MB-7	+	_	_	_	_
MB-8	+	_	_	+	_
MB-9	_	_	_	_	_
MB-10	_	_	_	+	_
MB-11	+ +	+	+ +	_	_
MB-12	+ +	_	+ + +	+ +	_
MB-13	_	+	_	+ +	_
MB-14	_	_	_	_	_
MB-15	+	++	+	_	_
MB-16	+	+ +	+ +	_	_
MB-17	+ + +	+	+ +	_	_
MB-18	+ +	+ +	+ + +	_	_
MB-19	_	_	_	_	_
MB-20	+ +	_	+ +	_	_

Comparative Analysis of Enzymes In Three Beaches

Comparative Analysis in three different Beaches (Marina, Besant Nagar, Kovalam) for the production of five industrially important extracellular enzymes like Amylase, Cellulose, Protease, lipase and Chitinase shows that Besant Nagar isolates shows highest production of Amylase, protease and cellulose followed by Marina Beach isolates. Lipase enzyme was Maximum produced in Marina isolates followed by Besant Nagar isolates. Kovalam Beach isolate were not able to utilize the CM- cellulose and Tween 20 and produce Cellulase and Lipase enzyme. Interestingly out of 20 isolates from three different Beaches (MB1-MB20) were not able to utilize the chitin substrate and produce chitinase enzyme. (**Fig:3)**

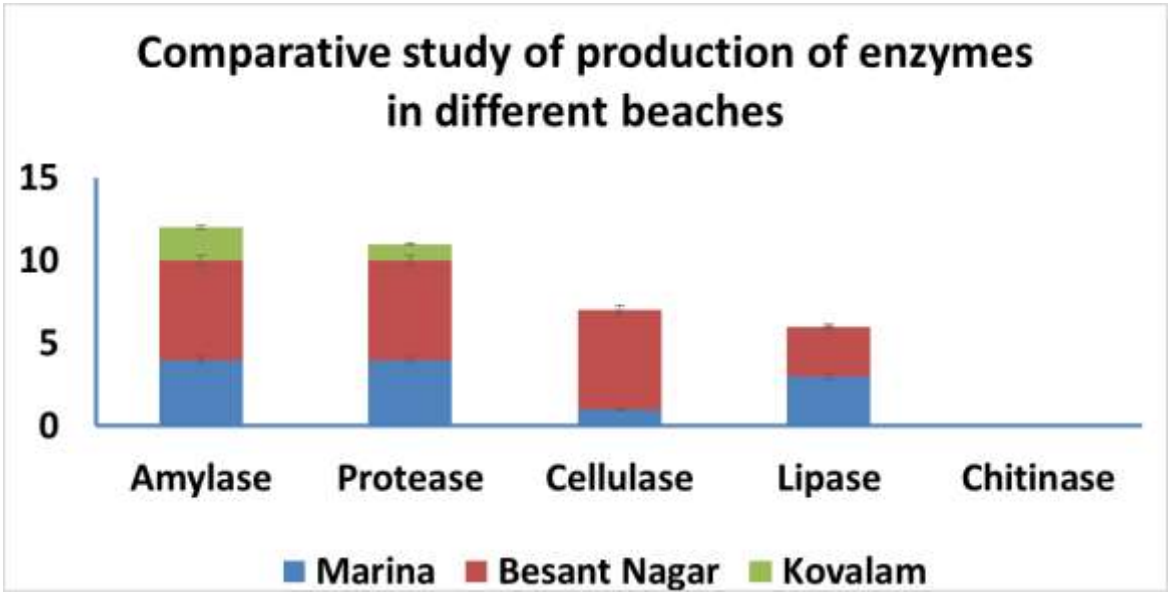

Fig: 3 Comparative Analysis Of Different Enzymes In Three Beaches

Discussion

Oceans include the greatest extremes of temperature, light and pressure encountered by life (15). It has been known for more than 30 years that many more bacteria are present in the surface ocean than can be cultured by the traditional microbiological approach of plating a sample onto selective media (10). The extracellular hydrolytic activity is performed by bacterial extracellular enzymes (3). Thus, in this study, the marine bacteria were screened for their ability to produce one or more extracellular hydrolytic enzymes using substrate amended plate assay.

In our study totally 20 Marine Bacteria were isolated from three different Beaches of Tamil Nadu and kanchipuramviz Marina, Besant Nagar, Kovalam. As like our research 13 marine bacterial isolates were isolated from marine water samples collected from Eastern, Western harbors' of Alexandria and Lake Mariout (12). The 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(7).

All the isolated marine bacterial 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. Similarly, schut reported that 37 Marine bacteria were isolated from North sea of Alaska and the morphologically different bacteria were studies for their growth in laboratory condition(20).

In Biochemical characterization out of 20 Marine Bacteria 12 Bacteria were Gram Negative and 8 bacteria were Gram Positive, All the isolated Marine Bacteria were Motile, 10 Bacteria were Oxidase, positive and seven Bacteria were Catalase positive. Among these the ratio of Gram positive and Gram negative were found to be varied as the dominance of Gram negative was comparatively more in all the three sites, this dominant Gram negative marine bacteria presence is supported by in the study (19), which is because of the ability to survive and grow in the water environment with low nutrient, high salinity and high pressure. Although these characteristics highlight the differences between marine and terrestrial microorganism, it remains difficult to separate bacterial genera on the basis of habitat due to the ubiquitous presence of similar species in both the environment.

Until now, it was found that bacteria can produce cellulase, including: *Cytophaga*, *Cellulomonas, Vibrio,* and *Clostridium, Nocardia, Streptomyces*, and for certain fungi it was found that *Trichoderma, Aspergillus, Fusarium, Chaetomium, Phoma, Sporotrichum, Penicillium*, etc. are also able to produce cellulase.

In our study also reveals that in 20 Marine Bacteria seven cultures were able to produce cellulose enzyme. In our study protease was produced by most of the Marine Bacteria, that 11 Bacteria can able to utilize the Gelatin substrate and produce protease enzyme. Qiu reported that selected 30 kinds of marine bacteria from the sea water, mud, fish and other samples; after UV mutagenesis they isolated the N1-35 strain, this strain produced protease that had significant advantages compared with the terrestrial ones(17).

Comparative Analysis in three different Beaches (Marina, Besant Nagar, Kovalam) for the production of five industrially important extracellular enzymes like Amylase, Cellulose, Protease, lipase and Chitinase shows that Besant Nagar isolates shows highest production of Amylase, protease and cellulose followed by Marina Beach isolates. Lipase enzyme was maximum produced in Marina isolates followed by Besant Nagar isolates. Kovalam Beach isolate were not able to utilize the CM- cellulose and Tween 20 to produce cellulase and lipase enzyme. Interestingly out of 20 isolates from three different Beaches (MB1-MB20) were not able to utilize the chitin substrate and produce chitinase enzyme. Compare to three Beaches Besant Nagar Beach isolates tend to produce the four industrially important enzymes in high number when compare to Marina and Kovalam Beach.

Conclusion

Marine microorganisms were proven already to have many beneficial bioactivities such as production of industrial enzymes and various metabolites.In the present study, the marine bacteria produced enzymes that can be further optimized and purified, to used in various industries for different Biotechnological Application.

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