

Isolation and biochemical characterization of industrially applicable bacteria from the eastern coast of Chilika lake



Biotechnology

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ABSTRACT

Microbes isolation and characterization helps us to study microbial diversity as well as for bio prospectus utilization from different environmental source so our current aim to isolation and characterization the bacteria from the eastern coast of Chilika lake which is a most famous hotspot for both prokaryotic as well as eukaryotic organism. For this purpose we took lake sediment and isolate bacteria and performed pure culture and finally biochemically characterized by growing in different substrate contain medium.

INTRODUCTION

Isolation and biochemical characterization of microorganism is a most important work for identification as well as biotechnological and industrial application of microorganism. Microorganism are widely using in different industrial sector for the human beneficial use like therapeutic purpose and production of food beverage or agricultural application. The micro organism for industrial importance is generally, bacteria, fungi, algae and actinomycetes. These organism are Omni present eg -in air, in water and soil, on the surface of animal and plant , some bacteria are present inside the different organism and their tissues which may be pathogenic or non pathogenic .But most common sources of industrially applicable micro organism are soil, lake and river mud . Before industrial application it is most important to know the characteristic of that micro organism .Often the desired characteristic of the product also depends on the type of microorganism as well as the ecological habitat from which the microorganism has isolated. So our current study is isolation and biochemical characterization of bacteria from the lake mud of Chilika lake, which is a brackish water lagoon, spread over the Puri, Khurda and Ganjam districts of Odisha state on the east coast of India, at the mouth of the Daya River, flowing into the Bay of Bengal, covering an area of over 1,100 km² It is the largest coastal lagoon in India and the second largest lagoon in the World. It is the largest wintering ground for migratory birds on the Indian sub-continent. The lake is home to a number of threatened species of plants and animals.

The lake is an ecosystem with large fishery resources. It sustains more than 150,000 fisher-folk living in 132 villages on the shore and islands. The lagoon hosts over 160 species of birds in the peak migratory season. Birds from as far as the Caspian Sea, Lake Baikal, Aral Sea and other remote parts of Russia, Kirghiz steppes of Mongolia, Central and southeast Asia, Ladakh and Himalayas come here. These birds travel great distances; migratory birds probably follow much longer routes than the straight lines, possibly up to 12,000 km, to reach Chilika Lake.

Material and methods

Sample collection: for characterization of bacteria by biochemical method soil sample were collected from the different site of eastern side of Chilika lake mud. The sample were brought to laboratory safely and kept aseptically.

Sample preparation and serial dilution:

For reduce microbial load of sample we performed serial dilution. For serial dilution we took 10 sterilized tube with distilled water and marked each tube. 1st tube contains 1gm of soil in 10 ml of distilled water and second tube contains 9 ml of distilled water and so on. From first tube 1ml of diluted sample were transferred to second and continue till last tube and all these tube were marked from 10¹ and 2nd 10⁻² and so on.

Culture technique:

For culture of bacteria we performed pour plate method and spread plate method and from these pour plate and spread plate we performed streak plate method for the purpose of pure culture .for pure culture NA media was prepared and solidified on petridishes after solidification an isolated colony from pour plate was picked out by sterilized inoculating loop and make a point on agar plate and from the point different street line were stretched in quadrant shape. Then the plate covered and kept on inverted position and allowed to incubate it for 24hours at 30 C .

Result:

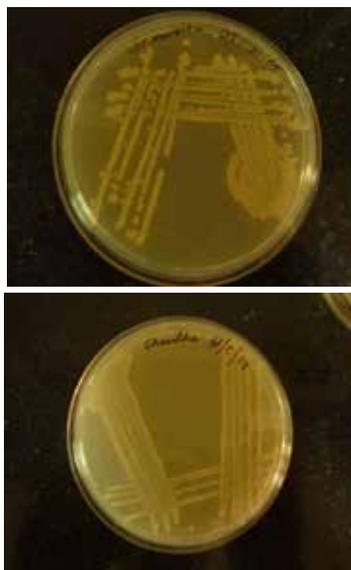
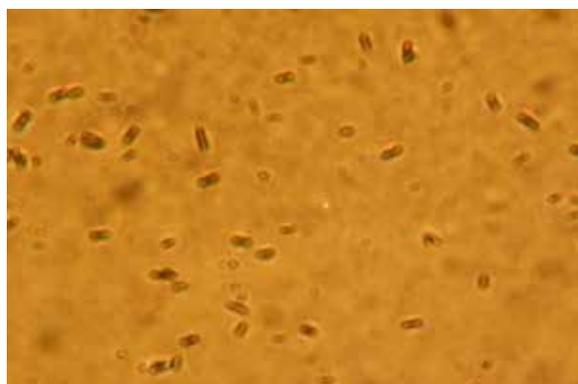


Fig- pure culture of bacteria by streak plate Characterization by Gram staining technique:

Slides were prepared by smearing of single isolated colony from streak plate and heat fixed and allowed to air dry. Few drops of crystal violet was added on smeared slide which act as primary stain for staining and kept for 2 minute and then washed with distilled water. Then safranin was added which act as counter stain and washed by destaining agent such as methanol. Then the slides were allowed air dried for few minutes and viewed on microscope.

Result: Fig- Gram staining showing gram negative bacillus bacteria



Biochemical characterization:

For biochemical characterization medium were prepared by using different carbon sources like chitin, cellulose, starch, gelatine, Cassin as a substrate. Among these each carbon source were added to different plate at concentration of 5 ml of broth medium with agar and allowed to solidify. After solidification the plate was divided into four parts and marked it as A, B, C, D. Then an isolated colony from pure culture was picked by micro-pipette and placed on the newly prepared medium each part of the medium contain one type of colony and then incubated the plate at 30 °C for 24 hours.

Result:



Fig-formation of zone of clearance on the medium due to catalitic action of the bacterial enzyme

Starch hydrolysis test

Capacity of the organism to hydrolyze starch into simple substrate like dextrin, glutamine, maltose etc .by amylase enzyme were detected by spot inoculating the bacterial culture on the NA plates containing 1% soluble starch. After incubation for 24 hours at 30 °C . The plates were flooded with iodine solution for 5 minute, excess solution were decanted off and starch hydrolysis were noted from the clear zone from around the colony. Reddish brown area around the colonies indicated partial hydrolysis of starch. The diameter of the clear zone & the diameter of bacterial growth gave the activity level.

Gelatine hydrolysis test

Gelatine liquefaction was tasted by spot inoculation the bacteria on NA plates containing 1% gelatine followed by incubation at 30 °C for 24 hours. The plates were flooded with acetic HgCl₂ solution (15%) and allowed to stay for 5 minutes clear zone formation were obtained due to hydrolysis of gelatine by gelatinease enzyme. Diameter of the clear zone depicted activity levels of the organism.

Casein hydrolysis test

Casein hydrolyzing activity of the bacteria was recorded from liqification of casein by bacteria on nutrient agar plate containing 1% casein, spot inoculated and incubated at 30 °C , the plate were flooded with HgCl₂, clear zone formation was obtained, ratio of the clear zone and bacterial growth gave enzymatic activity of the organism.

Chitin hydrolysis test

Chitin hydrolysis of the microorganism was assayed by the test. Chitin at 1% level is added to NA medium organism at 30 C for 72 hour incubated. A clear zone formation was obtained after incubation the ratio of clear zone to the bacterial growth gave activity of levels of the organism.

Result:-



A.Caseinmedium

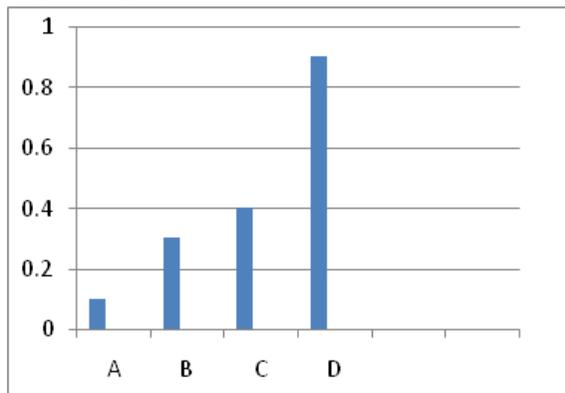


(D) Total diameter-1.3cm
Colony diameter-0.4 cm
Zone of clearance:- T.D-C.D=0.9cm

TABLE

Total diameter	Colony diameter	Zone of clearance (Total diameter-zone of clearance)
0.2	0.2	0.1
0.3	0.2	0.3
0.4	0.2	0.4
1.3	0.4	0.9

Graphical representation.



X. Substrate and medium, Y. zone of clearance

Discussion:

Through Biochemical characterization of the bacteria by the above mentioned method results that we got gram negative bacillus with multi substrate utilizing activity like casein, chitin, gelatine and starch. From these the bacteria have high efficiency of degradation of starch which is due to production of starch degradable enzyme i.e amylase. Which can be use for further study by downstream processing of the product of bacteria? It is also possible to molecular characterization of the bacteria by 16S rRNA sequencing.

Conclusion:

From the above study we concluded that the isolated and biochemical characterized bacteria is a gram negative bacillus and amylase producing bacteria as well as is a malty enzyme producing bacteria.

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We complete this work by the kind co operation and valuable suggestion of Dr. Bhaskar Behera, faculties of P.G Dept. of Bio-sciences and Biotechnology, Fakir Mohan University and proper guidance with provide lab by Dr. Snehashis Mishra faculty of Dept. of Biotechnology KIIT University. So we convey our deep sense of gratitude and healthy thanks to both of them.

B.Chitin medium



C.Gelatinmedium



D.Starch medium

- (A) Total diameter- 0.3cm
Colony diameter-0.2 cm
Zone of clearance: - T.D- C.D=0.1
- (B) Total diameter- 0.3cm
Colony diameter- 0.6 cm
Zone of clearance:- T.D-C.D=0.3cm
- (C) Total diameter-0.8 cm
Colony diameter-0.4cm
Zone of clearance:- T.D-C.D=0.4 cm

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