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MARINE ENVIRONMENT A POTENTIAL SOURCE FOR CELLULOSE DEGRADING MICROORGANISM.

PRASAD M. P.[1]* AND REKHA SETHI[2]

[1] Department of Biotechnology, Sangenomics Research Lab, Domlur Layout, Bangalore, India.
[2]Department of Microbiology, Jain University, Bangalore, India

ABSTRACT

In the present study microorganisms have been isolated from coastal areas of southern parts of India using standard microbiological techniques. The isolates obtained were subjected to screening assay utilizing carboxy-methyl cellulose (CMC) as a substrate. CMC is a substrate for endoglucanase and so can be used as a test for endoglucanase and β-glucosidase activity. This assay is a good indicator of cellulolytic ability since endoglucanase is generally produced in larger titres by fungi than cellobiohydrolase. 92 bacterial isolates, 43 fungal and 11 actinomycetes were obtained out of which 42 bacteria, 16 fungi and 11 actinomycetes showed the ability to degrade cellulose. The screening assay clearly indicated that the isolates from the marine world could be of industrial importance for biodegradation of lignocellulosic biomass to release sugars which can be used as an alternative source of fuel such as ethanol. In addition the organisms could be used for the industrial production of celluloytic enzymes.

KEYWORDS: **Cellulose, Celullases, Marine sediments, Actinomycetes, Carboxy-methyl cellulose.**

PRASAD M. P.
Department of Biotechnology, Sangenomics Research Lab,
Domlur Layout, Bangalore, India.

INTRODUCTION

Most marine environments contain only dilute substances that can be used for metabolism and growth. In contrast, natural surfaces tend to collect and concentrate nutrients by charge-charge or hydrophobic interactions [1]. Assessing microbial biodiversity is a daunting task. Exploration of microbial diversity is clearly a topic of considerable importance and interest. Besides, analysis of microbial biodiversity also helps in isolating and identifying new and potential microorganisms having high specificity for recalcitrant compounds [2]. Cellulose is decomposed by cellulolytic bacteria, e.g. Cytophaga, Sporocytophaga. Chitin, which is synthesized by several marine organisms as extracellular material from algae, cell walls of some chlorophytes [3], exoskeletons, including molts from copepods and other marine invertebrates [4] is a structural polysaccharide. However, it is not degraded easily [5] as there is a report on chitin preservation in fossils [6]. Cellulosic materials are among the Earth's most abundant, renewable resources, and their degradation and utilization by microbes are considered very important [7]. Micro-organisms that degrade cellulosic materials, and the enzymes involved (e.g. cellulase, xylanase and peroxidase), have been well studied [8,9] and several microbial-related applications have been developed for textile, food and paper-pulp processing. However, utilizing these microorganisms and enzymes to process natural cellulosic materials without pre-treatment and/or sterilization is difficult. In nature, cellulosic materials are degraded by the cooperation of many micro-organisms. It has been reported that a mixed culture comprising a cellulolytic bacterium and a non-cellulolytic bacterium is ideal for degrading cellulose [10, 11]. Many microorganisms that produce various cellulolytic enzymes have been studied for several decades; the genus of Trichoderma has been especially famous for producing cellulolytic enzymes with relatively high enzymatic activity. However, it is also well-known that the Trichoderma enzymes do not effectively hydrolyze cellulose biomass alone because of their enzyme composition. The saccharification activity of enzymes is specifically important to produce reducing sugars, especially glucose, from cellulolytic biomass. This ability is influenced by the composition of the enzyme components, particularly crystalline cellulose-hydrolyzing enzyme D-glucanase and beta-glucosidase. Incontrast, *Acremonium cellulolyticus*,which was isolated in 1987 and developed to increase performance, is able to produce both enzymes, besides carbomethyl cellulose hydrolyzing enzyme (CMCase) and a small amount of xylanase, endo-1,4-beta-D-glucanase, and amylase (a).

MATERIALS AND METHODS

(i) Sampling and Isolation

Marine samples were collected from Pondicherry, Tamil Nadu and Karnataka sea coasts. These included sea water samples, wood scrap, rock scrap, sand, algae/ sea weeds and sediment samples. The samples were collected in sterile polythene bags and were preserved in the refrigerator for further studies. Standard microbiological methods were followed for the purpose of isolation [12]. One ml of the desired dilution was transferred aseptically into Potato Dextrose Agar (PDA)/Marine agar plates. Plates were incubated at room temperature/37^{0}C for 5 to 10 days. After incubation, the organisms were subcultured and characterized.

(ii) Cellulolytic Enzyme Assay

A single agar disc cut from the actively growing colony margin of a culture was used to inoculate each assay medium. Carboxy methyl cellulose (CMC) is a substrate for endoglucanase and so can be used as a test for endoglucanase and β-glucosidase activity. This assay is a good indicator of cellulolytic ability since endoglucanase is generally produced in larger titres by fungi than cellobiohydrolase. [13, 14, 15].
Cellulose Basal Medium (CBM) was prepared and supplemented with 2%w/v low viscosity

CMC and 1.6% w/v agar and aseptically transferred to the Petri dishes. The media was inoculated with test organisms and incubated at room temperature for 5-7 days for fungi and 37^0C for 48hrs for bacterial cultures. The Plates were flooded with 2%w/v aqueous Congo red (C.I.22120) and left for 15mins and then the Stain was removed, the agar surface was washed with distilled water which were later destained with 1M Nacl for 15 min. CMC degradation around the colonies appeared as yellow-opaque area against a red color for un degraded CMC which indicated cellulose degradation.

RESULTS AND DISCUSSION

The results recorded for isolation from different samples showed the bacteria in the range of 14 to 83 CFU/gm or ml for different samples. 92 bacterial isolates were obtained from marine samples in sea water supplemented agar medium like the isolations and findings of [16], the Actinomycetes isolates were from the sediment samples and marine backwater soil samples and no Actinomycetes were isolated from the sea water or the wood samples which clearly indicated that the presence of organic matter and availability of the same for the growth and proliferation of the Actinomycetes was present only in these samples. In the present study, 11 Actinomycetes were isolated. [17] isolated 289 actinomycetes from sea shore sediments of 15 different locations in an island. This number looks high, and this may be due to that particular islands richness in vegetation. A total of 43 fungal species were isolated which were numbered based on the place and type of sample. The screening activity which was assessed based on dye staining and zone of hydrolysis similar to the findings of [18 and 19]. The present study resulted in finding 42 bacterial and 11 fungal isolates out of the total 92 bacterial and 43 fungal isolates to be cellulase producers which is comparable to a similar screening study made by [20] showed large number of fungal isolates capable of cellulase activity.

Figure 1
Fungal colony on Potato Dextrose Agar in Marine water

Figure 2
Bacterial colony on Marine Isolation agar

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