Research Paper Zoology

Extraction of Cellulase Enzyme From Isolated Actinomycetes

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ABSTRACT

Enzyme Cellulase has various applications in industries such as pulp and paper, textile, laundry, biofuel production, food and feed industry, brewing, and agriculture. Microorganisms are considered to be the most common source of enzymes because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation. So an attempt was made in the present investigation to isolate the enzyme Cellulase from Actinomycetes and to study the enzyme producing potential of the isolates. The results were highly encouraging

KEYWORDS Actinomycetes, Cellulase, Industrial enzymes

INTRODUCTION

Enzymes are special types of proteins that catalyze specific chemical reactions. Many industrial processes are simplified due to the use of enzymes. Enzymes are having applications in several industries such as biofuel, paper, animal feed, biomedicine, agriculture and food.

Cellulase enzyme plays an important role in converting cellulosic biomass in high value products and is known to have its applications in a number of industries especially in the food industry (Sukumaran et al., 2005).

There are various sources for obtaining enzymes of industrial applications. These sources include bacteria, fungi, Actinomycetes, microalgae, animals and plants. Among all these sources, microorganisms represent the most common source of enzymes because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation (Niehaus et al. 1999).

Actinomycetes also known as Actinobacteria are prokaryotes with extremely various metabolic possibilities. They are best known for their ability to produce antibiotics and are gram positive bacteria which comprise a group of branching unicellular microorganisms. Actinomycetes, one of the well known cellulase producer group of bacteria, has attracted considerable research interest due to its potential application in the recovery of fermentable sugars from cellulose that can be of benefit to human consumption and to the ease of their growth (Jang and Chen, 2003; Arunachalam et al., 2010).

In the present investigation, Actinomycetes were isolated from sea sediment samples and then further explored for the extraction of cellulose enzyme.

MATERIALS AND METHODS

1) Isolation and culture of actinomycetes:

Mud samples from the sea shore and mangrove areas of Goa were collected in sterile zip lock bags and processed within 24 hrs. 1 g of the sample was serially diluted and then spread onto the surface of Actinomycete Isolation agar, GYM Streptomyces medium, Glycerol Yeast extract agar using the standard spread plate technique (Ghose, 1987). All the media were inoculated with two kinds of antibiotics. Rifampicin was used for the inhibition of bacterial growth while cycloheximide was used for the inhibition of fungal growth. All the plates were incubated at 40°C for a minimum period of 2-3 weeks to allow growth of slower growing microorganisms and also for the purpose of sporulation.

About 20 actinomycetes were collected by differentiating them depending on the morphological characteristics. The colonies were allowed to fully develop and sporulate. Once developed these were transferred to ISP2 slants in duplicate. The spores were stored as 15% glycerol stocks cultures maintained at -20°C.

The morphological characteristics of the colonies on plate were noted down. Individual colonies obtained on plate were picked up and a smear was prepared in saline. Smears were observed under 100X oil immersion microscope for their mycelial and spore structure.

Gram staining was performed as per the standard protocol. The colonies on the plate were directly observed under 40X lens of phase contrast microscope.

2) Screening for cellulase enzyme by plate assay:

The 20 isolated actinomycete cultures were spot inoculated on Carboxymethyl cellulose agar plates (Sukumaran et al., 2005). The media was then incubated at room temperature for 48 hours.

The plates were stained with 0.1% Congo red staining solution. 1M NaCl was used to wash off the excess unbound stain.

Glucose formed during the reaction stains yellow with Congo red while the unused Carboxymethyl cellulose stains red. Diameter of clear yellow zones on red background were measured. The values were recorded.

FINDINGS

1) Morphological studies of isolated cultures

Some typical characteristics for actinomycetes were noted down for the 20 isolates that were obtained as depicted in the Table 1. Actinomycetes isolated in this study were all of medium consistency. Actinomycetes colonies and the smear of selected isolates are shown in Fig. 1 -3.

Fig. 1 Fully grown actinomycete colonies

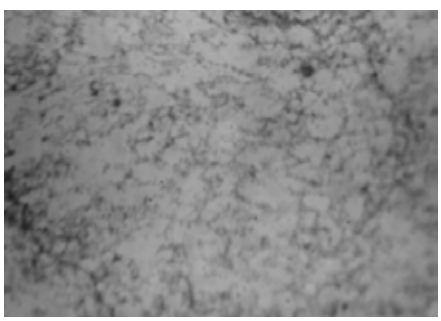

Fig. 2 Smear of Ac1 under 100X oil immersion

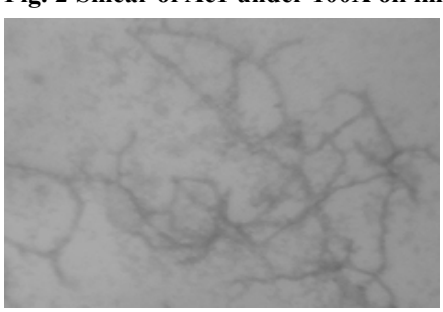

Fig. 3 Smear of Ac6 under 100X oil immersion

Gram staining indicated that all the cultures were found to be gram positive. Aerial and substrate mycelia from selected isolates are shown in the fig. 4 and 5. The colonies of both the selected cultures were off white in colour and raised. The colonies of Ac1 were found to be rough while that of Ac6 were smooth. No podery sporulation was observed as is seen in most of the actinomycete colonies. On preparing smear of these two cultures, the culture Ac1 showed fine branched mycelia while Ac6 showed long and thicker mycelia which were also branched.

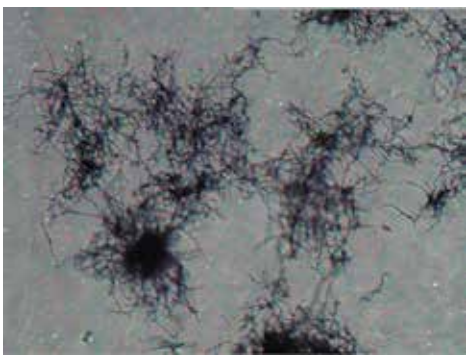

Fig. 4 Aerial and substrate mycelia for Ac 1 from ISP2 plate

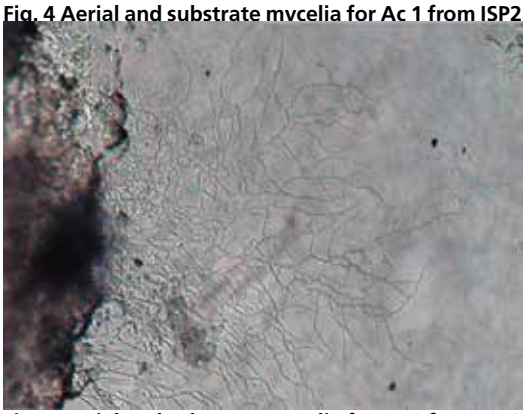

Fig. 5 Aerial and substrate mycelia for Ac 6 from ISP2 plate

Table 1 Characteristics of the isolated actinomycetes

Culture code	Source	Morphology		Medium of growth
		Shape	Colour	
	Sea sediment	Rough surface, round edge	white	GYM
	Sea sediment	Rough raised surface, round edge	Off white	GYM
	Sea sediment	Raised folded	Off white	GYEA
	Sea sediment	Rough raised tiny	Off white	GYM
	Sea sediment	Smooth round slightly raised	Off white	GYM
	Sea sediment	Smooth round	white	GYEA
	Sea sediment	Flat round	Orange peripheral white sporulation	GYM
	Sea sediment	Tiny round	Yellow with white sporulation	GYEA
	Sea sediment	Flat round peripheral sporulation	orange	GYEA
	Sea sediment	Rough round	Off white with white sporulation	GYM
	Sea sediment	Round surface breaks with growth	Off white with off white sporulation	GYM
	Sea sediment	Flat point sized	Orange with off white sporulation	GYM
	Sea sediment	Round differentiated centre	Off white with white central sporulation	GYM
	Sea sediment	Tiny translucent substrate mycelia	orange	GYEA
	Sea sediment	Umbrella, central depression	Off white	GYM
	Sea sediment	Pont sized Raised substrate mycelia	orange	GYM
	Sea sediment	Round raised	Off white with white peripheral sporulation	GYM
	Sea sediment	Peripheral sporulation flat	Brown	GYM
	Sea sediment	Round smooth flat	Pink colony with complete grey sporulation	GYEA
	Sea sediment	Round rough raised	Brown complete grey sporulation	GYM

2) Screening for cellulose producing activity

Out of 20 isolates screened for cellulase production 14 were found to be cellulase producers. As shown in the Table 2, isolates Ac1, Ac6, Ac13, Ac15 and Ac20 showed zone of clearance of above 30 mm and above. Ac3 showed the smallest zone of 10mm.

Table 2 Zone of Clearance for cellulase producing actinomycete

Sr. no.	Cultures	Zone of clearance in mm
	Ac1	32
	Ac2	---
	Ac3	10
	Ac4	---
	Ac5	25
	Ac6	34
	Ac7	---
	Ac8	---
	Ac9	25
	Ac10	24
	Ac11	25
	Ac12	---
	Ac13	30
	Ac14	28
	Ac15	31
	Ac16	27
	Ac17	---
	Ac18	25
	Ac19	25
	Ac20	30

CONCLUSIONS

So the present investigation reports successful isolation of Actinomycetes from samples taken from mangrove regions and sea shores. The activity for cellulose production for the screened isolates was also encouraging. Therefore, suggestion made in earlier reports that actinomycetes are a good source of cellulase enzyme is vindicated. Further studies are ongoing to study the structural and functional activity of the extracted enzyme and to phylogenetically characterize the isolated microbes using sophisticated molecular markers. It is hoped that this study will act as a first step towards isolating some unique Actinomyctes which meet the criteria for the commercial production of the cellulases.

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