IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Isolation And Identification Of Cellulose Producing Actinomycetes For Effective Degradation Of Solid Waste

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Abstract

In the present study samples were collected from various agricultural dumping sites of Shegaon Dist. Buldhana, Maharashtra, India and two strains of Actinomycetes were isolated from these samples. Actinomycetes produces several hydrolytic enzymes which is helpful for degradation of solid waste, further the isolates were identified as Actinomycetes on the basis of morphological and biochemical studies. These strains were selected on the basis of primary screening of strains. Cellulolytic activities were confirmed by formation of clear zone of hydrolysis around the colonies.

Keywords: Cellulase, Actinomycetes, cellulolytic activities.

Introduction:

Agriculture is the oldest occupation in both India and the world. In India, more than 620 million tons of agricultural waste are produced annually, it consists of crop waste (rice husk, wheat straws, sugarcane bagasse), animal waste (animal excreta, dead animals), processing waste (packaging material, fertilizer cans) and hazardous waste (pesticides, insecticides).

The current municipal solid waste (MSW)generation rate in India is 127,486 tons per day, consisting of organic matter (51%), recyclables (17.5%), and inert (31%). (Singh *et.,al.,* 2024) India currently employs six techniques to convert solid waste into renewable energy sources in an environmentally friendly manner. These techniques include incineration, gasification, composting, anaerobic digestion, and others. However, the rate of decomposition is slower than the rate of solid waste production, and there is a need for research to discover competitive hydrolytic enzymes. Microbes play a vital role in the biotransformation of the organic substrates during composting. Actinomycetes are a diverse group of gram-positive bacteria known for their ability to degrade complex organic compounds. (Bhatti *et.,al.,* 2017) Actinomycetes produces several hydrolytic enzymes which is helpful for degradation of solid waste, so plays an important role is sustainable developments toward green technology

Material and Methods

Sample collection from agricultural dumping site of Shegaon Dist. Buldhana, Maharashtra, India. The samples were collected in sterile zip lock bags using sterile spatula.

Enrichment and Isolation of soil actinomycetes:

One gram of sediment soil were transferred into Medium (Yeast extract: 4g/L, Malt extract powder: 10g/L, Glucose: 4g/L, Agar: 18g/L) in appropriate plate in duplicate. The plates were incubated for 7 days at 28^{o} C. Pure culture actinomycetes strains are stored in ISP-2 medium at 40 C and Glycerol stock was prepared for future use. (Mohanta 2014).

Screening of Cellulase producing actinomycetes:

Screening of Cellulase producing actinomycetes by using Cellulose agar plate containing Meat extract- 0.3g, Peptone-0.5g, Cellulose-1%, Agar-3%, and pH-7. Incubate plate 36 ˚C for 6 days (Mohanta 2014).After incubation add 1 % Congo red solution for 15 min (Brander *et.,al*., 1999).To visualize the zone formed by cellulose positive strains the plates were destained using 1M Nacl solution. In cellulose plate for observing cellulolyticzone near colony surface of actinomycetes. The colonies of actinomycetes having clear zone were selected for identification and cellulase production. Further bacterial strains were purified by repeated streaking. The purified colonies were preserved at 4^{0}C.

Identification of Bacteria
For the identification of strain of interest cultural characteristics, morphological characteristics and biochemical test were conducted and identified on the basis of characters as given in Bergey's manual of systematic Bacteriology (Bergey D.1957).

Biochemical characteristics of selected Actinomycetes colony:

After preliminary studies, the isolates which were found to be positive were selected for biochemical studies. The parameters investigated included Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Catalase test, Oxidase test, Carbohydrate Fermentation testf Motility test, Enzyme test, Nitrate reduction test, and test by standard methods. The various media was prepared in sterile distilled water and pH was adjusted accordingly. (Van *et.,al.,* 2001).
Further identification will be carried out on the basis of 16 srRNA analysis of selected strain for confirmation of Actinomycetes and enzyme assay of potential strain of actinomycetes.

Result:

Fig 1: Isolation of soil actinomycetes

Fig 2: Screening of cellulose producing actinomycetes.

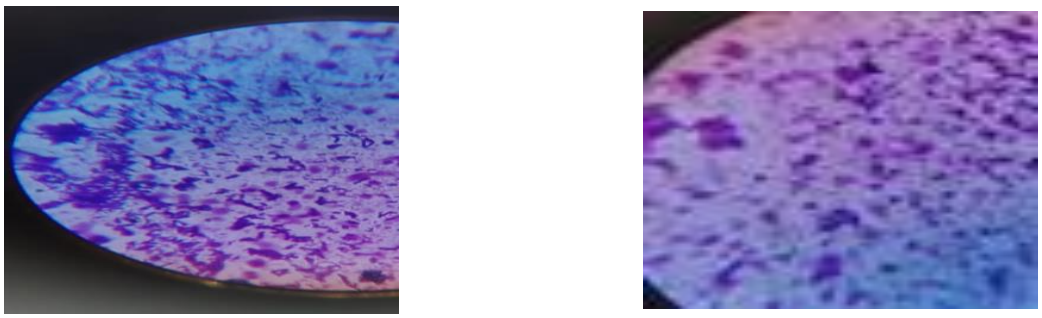

Fig. 3: Gram staining of selected islolated actinomycetes colony.

Table 1 Biochemical characteristic of selected Actinomycetes colony:

Biochemical characteristics			
Sr No.	Name of Test	Sample M1	M2
1	Indole	+	+
2	Methyl red test	+	+
3	Voges –Prauskar test	+	+
4	Citrate test	-	+
5	Urea hydrolysis	+	+
6	H2S	+	+
7	Nitrate reduction	+	+
8	Fermentation test		
	Dextrose	+	+
	Mannitol	+	+
	Glucose	+	+
	Sucrose	+	+
	Fructose	+	_
	Maltose	+	+
10	Enzyme test	+	+
	Amylase	+	+
	Cellulase	+	+
	Protease	+	+

Discussion

The genus Streptomyces is the largest generator of cellulases, which actively convert cellulose into simple fermentable sugars that are easily consumed by humans (Jang and Chang 2005).According to reports, a variety of actinomycetes produce cellulase enzymes. For example, Streptomyces longispororuber produces carboxymethylcellulase, while Streptomyces viridobrunnes SCPE-09 produces endoglucanase (Vinha *et.,al.*, 2011).Bedi and Prasad (2014). Another new Streptomyces species that has been reported to hydrolyze cellulose is *Streptomyces abietis,* which was isolated from pine forest soil (Fujji et al., 2013). Several investigations have shown that Streptomyces sp. cellulases are extremely thermostable and have an ideal alkaline pH (Jones *et al*., 2004).

Conclusion

For the purpose of solid waste management the current study focused on isolating cellulase-producing actinomycetes for possible cellulase activity of strains M1 and M2. Other microbial cellulase sources, such as bacterial and fungal cellulase, can be strengthened by these actinomycetes' cellulase enzymes. More research is necessary to fully utilize these organisms' potential for cellulase synthesis using contemporary protein engineering technologies.

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