



**Primary Screening Of Cellulase Producing Microorganisms From Soil Of
Banana Cultivated Cropland For Saccharification Of The Agrowaste**

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Abstract:

A total of forty microbial cultures having ability to utilize agro-waste of banana were isolated and screened for cellulase activity. The banana agro-waste is rich in cellulose, hemicellulose, protein, and minerals. These nutrients can be used for production of value-added products only after its bioconversion. To bring about this bioconversion, in present work, an attempt has been made to isolate cellulase producing microorganisms from the soil of banana plantation area of Nanded district (Maharashtra). Out of 40 isolates; three bacterial, five actinomycetes and two fungal isolates were found efficient for cellulase activity. Similarly, all these microbial isolates were screened for hemicellulase activity. The isolates efficient in cellulase production were also found efficient in hemicellulase production.

Key words: Cellulase, hemicellulase, banana agro-waste, screening, bioconversion

Introduction:

Cellulose constitutes the most abundant unit of plant biomass on earth; it is degraded by cellulases, specific enzymes produced by microorganisms. Nevertheless, cellulases of bacterial origin attract more interest because of their natural diversity and ability to occupy a variety of niches, allowing the selection of cellulolytic strains (Amel Balla et al, 2022). Banana is grown in more than 130 countries across the world in an area of 8.251 million hectare with total production of 97.378 million tones. India ranks second with respect to productivity (34.0 MT/hectare). It is estimated that approximately 30 MT/ha/year banana lignocellulosic agro-waste is generated after harvest. The lignocellulosic waste(biomass) generated from agricultural residues provides a wide range of affordable renewable value-added products (Pandey et al 2001, Van Wyk 2001,). The bioconversion of agricultural crop residues rich in cellulose, hemicellulose, protein, minerals into fermentable substrate is best for microbial production of various value-added products like fine chemicals, enzymes, amino acids, vitamins and microbial biomass (Howard et al, 2002). A number of biomass conversion

methods are employed ranging from direct chemical methods viz. acid hydrolysis and pyrolysis to biological methods i.e. application of enzymes (Walsh G, 2002). It is investigated that, the efficient enzyme system for agro-waste saccharification is available with different microorganisms which play an important role in recycling of nutrients in soil. These microorganisms include majorly bacteria, fungi and actinomycetes. The microorganism's activity in decomposition of agricultural crop residues is induced by constituents of crop plant residues. Therefore, microbial enzymes efficient in degradation of agro-waste are best for carrying out saccharification of the same agro-waste. The present study was focused on the isolation and screening of microorganisms producing the major enzyme i.e. cellulase acting on cellulose the core constituents of banana agro-waste.

Material and Methods:

Materials:

1. Agro-waste Sample: Banana agro-waste samples were collected from the Ardhapur region of Nanded district. The samples comprised of leaves, pseudo stem and rhizome of the popularly grown

- cultivar of banana plant, viz. Ardhapuri-1
2. Soil sample from above mentioned banana plantation area.
 3. Chemicals: All the chemicals of analytical grade were procured from Qualigens, S.D. Fine Chemicals and Spectrochem whereas all the culture media were procured from Hi-Media Laboratories Pvt. Ltd.

Methods:

Sample preparation:

The banana plants were collected from the Ardhapur region of Nanded district where this crop is grown for successive 10 years. Plants of the cultivar i.e. Ardhapuri-1 collected from the field. The whole plant, which is to be discarded, was brought in the laboratory. Leaves, pseudo stem and rhizome were separated. Each part was thoroughly washed with tap water to remove extraneous dust. Each part was separately chopped into pieces of 2 cm. Chopped pieces were air dried for 72 hours and then oven dried at 45°C to constant weight. The samples were ground into fine powder by using electric grinder and stored in polythene container.

Determination of cellulose, hemicellulose and lignin content in the agrowaste :

Cellulose, Hemicellulose and Lignin content of the powdered sample was determined according to the methods described in AOAC . by determining Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL).

Isolation of Banana agro-waste utilizing Microorganisms:

Isolation of agro-waste utilizing microorganisms was carried out by selective enrichment and isolation method as:

Enrichment for banana agro-waste utilizing microorganisms:

One gram of the soil sample was inoculated into minimal broth containing banana agro-waste and incubated at room temperature for one week. Re-enrichment was done twice by inoculating 5 % inoculum of pre enriched sample into fresh media. The flasks were incubated at room temperature for one week. Enriched broth was stained to confirm the growth of microbes.

Isolation of cellulose utilizing Microbes:

Bacteria: A loopful of enriched broth was streaked on cellulose mineral agar plate.

Plates were incubated at 30°C for 96 hours for the development of colonies. Morphological and cultural characteristics of well isolated colonies were recorded and the pure cultures were maintained on nutrient agar slants for further study.

Actinomycetes: A loopful of the enriched broth was streaked on cellulose casein nitrate agar (Kuster and Williams) plate. This media was fortified with the antibacterial agents i.e. penicillin, streptomycin and the antifungal agent griseofulvin before pouring the media into sterile plates. The plates were incubated at 30°C for 5 to 7 days for the development of colonies. Well isolated colonies were maintained on the slants of respective media for further use.

Fungi: A loopful of enriched broth was streaked on cellulose agar containing Rose Bengal and Chloramphenicol as selective agent. The plates were incubated at 30°C for 5 to 7 days for the development of fungal growth. Pure culture of the fungal isolates was maintained on potato dextrose agar slant till further use.

Identification of Bacterial isolates:

Bacterial isolates were identified by recording morphological and cultural and biochemical characters by comparing the characteristics with *Bergey's Manual of Systematic Bacteriology* (Williams et al, 1989)).

Identification of Actinomycete isolates:

Actinomycete isolates were grown on the cover-slips by agar block method and identified by microscopic observations of the morphological structure, mycelial structure and spore arrangement and then comparing the characteristics with *Bergey's Manual of Systematic Bacteriology*.

Identification of Fungal isolates:

Fungal isolates were grown on coverslips as mentioned above and then the coverslip was observed under microscope and identified by the morphology and spore arrangement of organism. The identification of fungal isolates was done on the basis of cultural and morphological characteristics.

Screening of isolates for cellulase activity:

Isolates were screened for cellulase activity by their ability to utilize carboxy methyl cellulose (CMC) in the CMC agar plate (Apun et al, 2000). Each isolate was

spot inoculated on CMC agar plate. Plates were incubated at 30°C for 4-6 days. After incubation the plates were flooded with 1 % aqueous Congo red solution for 15 min. The stain was discarded and plates were washed with 1 M NaCl solution for 15 minutes. Clear zone around the colony was recorded. The ratio of CMC hydrolysis zone to colony diameter, hydrolysis capacity (HC) was calculated to check the efficiency of cellulose degradation by the isolates.

Screening of isolates for hemicellulase activity:

Isolates were screened for hemicellulase activity by their ability to utilize oat spent xylan in the xylan mineral agar plate. Each isolate was spot inoculated on xylan mineral agar plate. Plates were incubated at 30°C for 4-6 days. After

incubation the plates were flooded with 1 % aqueous Congo red solution for 15 min. The stain was discarded and plates were washed with 1 M NaCl solution for 15 minutes. Clear zone around the colony was recorded. The ratio of diameter of xylan hydrolysis zone to colony diameter, (hydrolysis capacity, HC) was calculated to check the efficiency of xylan degradation by the isolates.

Result and Discussion:

A total 40 microbial isolates belonging to bacteria, actinomycetes and fungi were isolated by providing banana agrowaste as a sole source of carbon and energy through enrichment. Gram's staining of 16 bacterial isolates revealed that indicate that eight bacterial isolates were Gram positive and eight were Gram negative. (Table.1)

Table 1: Morphological characteristics of cellulose utilizing Bacterial isolates.

Sr. No.	Isolate No.	Size mm.	Shape,colour,margin,elevation,opacity	Consistency	Gram's nature	Motility
1	BI-1	3	Circular, white, dentate, low convex, translucent colony.	Butyrous	Gram +ve long rod	Motile
2	BI-2	2	Circular, off white, entire, low convex, transparent colony.	Butyrous	Gram -ve short rod	Motile
3	BI-3	3	Circular, off white, entire, low convex, opaque colony.	Butyrous	Gram +ve short rod	Motile
4	BI-4	2	Circular, lemon yellow, entire, low convex, translucent, colony.	Butyrous	Gram +ve very short thin rod	Motile
5	BI-5	3	Irregular, off white lobate, high convex, opaque colony.	Butyrous	Gram +ve long slender sporulating rod	Motile
6	BI-6	2	Circular, off white, entire, low convex, translucent colony.	Butyrous	Gram -Ve very short thin rod	Motile
7	BI-7	2	Circular, light yellow, entire, low convex, translucent colony.	Butyrous	Gram -ve Very short rod	Motile
8	BI-8	3	Circular, circular, off white, entire, high convex, translucent colony.	Butyrous	Gram -ve short thin rod	Motile
9	BI-9	2	Circular, bright yellow, entire, low convex, translucent colony.	Butyrous	Gram -ve coccobacillary rod	Motile
10	BI-10	3	Irregular, off white lobate, planar, opaque colony.	Papery	Gram +ve sporulating thick rod	Motile
11	BI-11	2	Circular, bright yellow, entire, low convex, opaque colony	Butyrous	Gram +ve cocci in clustre	Non-Motile
12	BI-12	2	Circular, off white, entire, planar, transparent colony.	Butyrous	Gram -ve coccobacillary rod	Motile
13	BI-13	2	Circular, golden yellow, entire, high convex, translucent colony.	Butyrous	Gram -ve cyst forming rod	Motile

14	BI-14	4	Circular, off white, entire, high convex, opaque colony.	Butyrous	Gram -ve short thin slender rod	Motile
15	BI-15	2	Circular, orange, entire, low convex, opaque colony.	Butyrous	Gram +ve thick nonsporulating rod	Motile
16	BI-16	3	Irregular, cream white, lobate, umbonate, opaque colony.	Papery	Gram +ve non sporulating rod	Motile

Legend BI = Bacterial Isolate

Morphological studies of 13 actinomycete isolates showed that these comprise the species of *Spirillospira*(2), *Streptomyces*(3), *Actinomadura*(5) and

Nocardia (2) This was based upon *Bergey's Manual of Systematic Bacteriology*, (Table.2)

Table 2. Morphological characteristics of cellulose utilizing Actinomycete isolates.

Sr. No.	Isolate No.	Colony Characteristics	Microscopic Features	Morphologically Identified as
1	AI-1	Light Orange, Powdery colony.	Spherical sporangia on aerial mycelium.	<i>Spirillospira sp.</i>
2	AI-2	Mucilaginous, transparent, gum forming colony.	Long chains of spores in spiral or hook form on aerial mycelium.	<i>Streptomyces sp.</i>
3	AI-3	Dark green, firm colony.	Chains of spores on aerial mycelium in loops.	<i>Actinomadura sp.</i>
4	AI-4	Dark green, firm colony.	Chains of spores on aerial mycelium in loops.	<i>Actinomadura sp.</i>
5	AI-5	Greyish white, Powdery colony.	Substrate mycelium fragmenting into rod, coccoid elements.	<i>Nocardia sp.</i>
6	AI-6	White, cottony colony.	Chains of spores on aerial mycelium in loops.	<i>Actinomadura sp.</i>
7	AI-7	Greenish colony.	Long chains of spores in spiral or hook form on aerial mycelium	<i>Streptomyces sp.</i>
8	AI-8	White, cottony colony.	Spherical, sporangia on aerial mycelium	<i>Spirillospira sp.</i>
9	AI-9	Creamish white powdery colony.	Chains of spores on aerial mycelium in loops.	<i>Actinomadura sp.</i>
10	AI-10	Light Orange, Powdery colony	Chains of spores on aerial mycelium in loops.	<i>Actinomadura Sp.</i>
11	AI-11	Green, powdery colony.	Spherical, sporangia on aerial mycelium	<i>Spirillospira sp.</i>
12	AI-12	Light Orange, Powdery colony	Substrate mycelium fragmenting into rod, coccoid elements.	<i>Nocardia sp.</i>
13	AI-13	White, powdery colony.	Long chains of spores in spiral or hook form on aerial mycelium	<i>Streptomyces sp.</i>

Legend

AI - Actinomycete Isolate.

Similarly 11 fungal isolates were also identified morphologically by the features mentioned in illustrated kingdom of fungi [9] as species of *Aspergillus* (4), *Mucor* (1),

Neurospora (1), *Cladosporium* (1), *Penicillium* (2) and *Fusarium* (2) (Table.3)

Table 3. Morphological characteristics of cellulose utilising Fungal isolates.

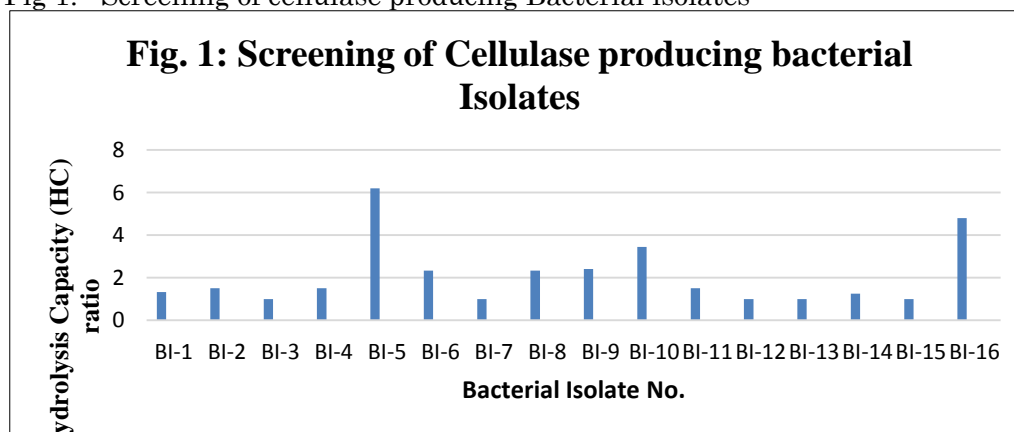
Sr. No.	Isolate No.	Colony Characteristics	Microscopic Features	Morphologically Identified as
1	FI-1	Yellowish green colony.	Erect hypha, globose vesicle at the tip, chains of spherical conidia on the vesicle.	<i>Aspergillus sp.</i>
2	FI-2	Deep green colony.	Erect hypha, globose vesicle at the tip, chains of spherical conidia on the vesicle.	<i>Aspergillus sp.</i>
3	FI-3	Greyish black, cottony colony	Rhizoids absent, sporangiophore arise singly from mycelium, sporangia spherical, many spored.	<i>Mucor sp.</i>
4	FI-4	Whitish orange, Cottony colony.	Mycellium highly branched, pigmented, macro and micro conidia present in branched chains.	<i>Neurospora sp.</i>
5	FI-5	Greyish white, firm colony.	Conidiophores tall, dark, upright, branched near apex, conidia variable in shape and size.	<i>Cladosporium sp.</i>
6	FI-6	Greyish white, cotton colony.	Hyphae hyaline branched, septate. Conidiophores hyaline, short, simple branched with terminal phialides which are subulate (broad at base, narrow at apex).	<i>Fusarium sp.</i>
7	FI-7	Deep green spongy colony.	Septate, branched hyphae, conidiophore unbranched, conidial chain bearing phialids forming brush like structure, foot cell absent.	<i>Penicillium sp.</i>
8	FI-8	Bluish green colony.	Septate, branched hyphae, conidiophore unbranched, conidial chain bearing phialids forming brush like structure, foot cell absent.	<i>Penicillium sp.</i>
9	FI-9	Yellowish green, cottony colony.	Erect hypha, globose vesicle at the tip, chains of spherical conidia on the vesicle.	<i>Aspergillus sp.</i>
10	FI-10	White, cottony colony.	Hyphae hyaline branched, septate. Conidiophores hyaline, short, simple branched with terminal phialides which are subulate (broad at base, narrow at apex).	<i>Fusarium sp.</i>
11	FI-11	Black, cottony colony with loose spores.	Erect hypha, globose vesicle at the tip, chains of spherical conidia on the vesicle.	<i>Aspergillus niger</i>

Legend

FI - Fungal Isolate

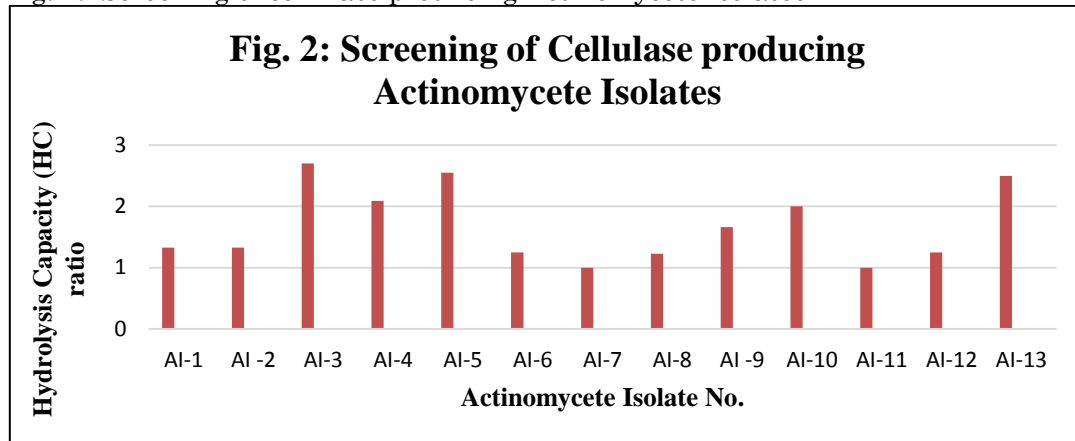
Analysis of our results on the production of cellulase by microbial isolates showed that out of 40 isolates tested, only 10 showed areas of hydrolysis around the revealed colonies. These microbial isolates therefore possess cellulases capable of degrading CMC (Sharma et al, 2016). Three bacterial isolates out of 16, The isolates efficient in cellulase production were also efficient in hemicellulase production viz. BI-5, BI-10 and BI-16 were efficient for cellulase activity having the hydrolysis capacity ratio of 6.2, 3.4 and 4.8 respectively (Fig. 1).

Fig 1. Screening of cellulase producing Bacterial isolates



Five actinomycete isolates viz. AI-3, AI-4, AI-5, AI-10 and AI-13 showed highest hydrolysis capacity ratio of 2.7, 2.09, 2.5, 2.0 and 2.5 respectively (Fig. 2)

Fig. 2. Screening of cellulase producing Actinomycete isolates



Two fungal isolates FI-4 and FI-11 were efficient for cellulase activity having the hydrolysis capacity ratio of 1.66 and 1.68 respectively (Fig.3).

Fig. 3. Screening of cellulase producing Fungal isolates

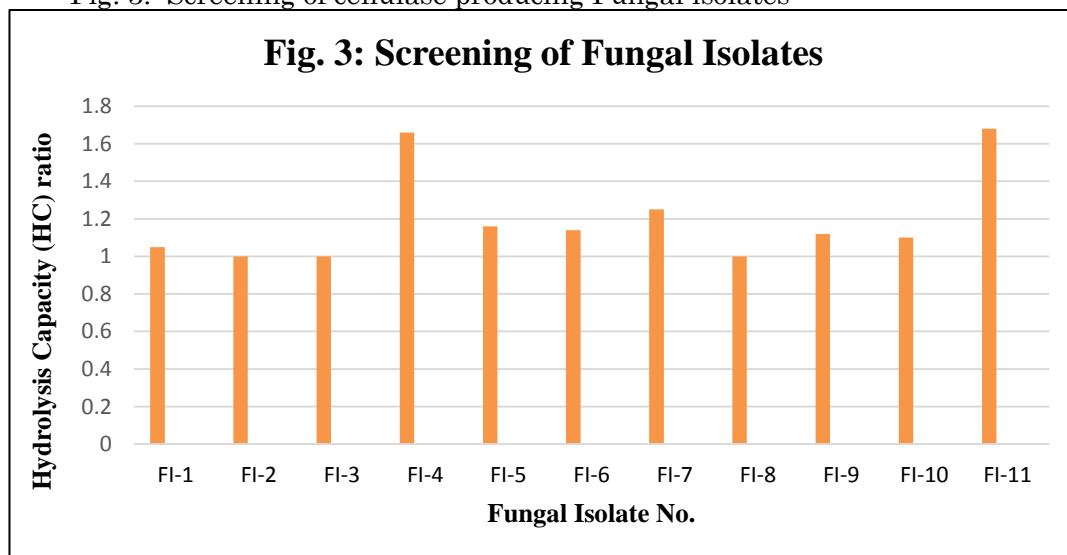


Fig. 4. Illustration of CMC hydrolysis zones by the tested isolates revealed by Congo red



Aspergillus niger KK₂ also produced cellulase and hemicellulase on agro-waste of rice straw and wheat bran (Kang et al, 2004). The isolates efficient in cellulase production were also efficient in hemicellulase production *Cellulomonas flavigena* also demonstrated both cellulase and hemicellulase activity on *Leptochloa fusca* L. Kunth (Kallar grass) and concluded that kallar grass components were best inducer for enzyme production (Rajoka & Malik 1984).

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