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Article

Cellulase Production Using Biomass Feed Stocks by Indigenous Strains

S. Viswarupa' S. Akram, U. Umamaheswari, P. Suresh Yadav, B. V. Siva Prasad, M. Subhosh Chandra*

Department of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh, India

ABSTRACT

The feasible construction of chemicals and value-added products from inexhaustible, non-edible biomass has become a critical alternative to undertake pressing environmental problems created by our overdependence on fossil resources. The special strains of microorganisms called microbial cell factories are able to produce chemicals of interested through renewable carbon sources. Lignocellulosic biomass is considered a significant of such materials which can be used for the production of useful materials that are not only renewable but also do not have any edible values. This work reveals the ability of bacteria and fungi isolated from mango field soil samples to produce cellulolytic enzymes. In total, more than 100 bacterial colonies were obtained and 13 fungal strains were identified from the mango field soil sample. From these organisms, we choose one bacterium strain and one fungus strain after the secondary screening for hypercellulolytic microorganisms such as *Proteus mirabilis* and *Aspergillus.* Black gram husk, red cowpea husk, Bengal gram husk, and groundnut fodder were tested for cellulase production by bacteria under submerged condition fermentation and in solid-state fermentation with fungal strains. Red cowpea husk and identification of these cultures were done using biochemical and morphological features, and ground nut husk is the recommended solid substrate for maximum production of β-glucosidase, protein content and filter paperase (FPase) and carboxymethyl cellulase, respectively, by bacterial isolate.

Key words: Agricultural residues, Bacteria, Cellulase, Fungi, Solid-state fermentation, Submerged fermentation.

1. INTRODUCTION

The cellulase enzyme system comprises endoglucanases, exoglucanases, and β-glucosidase. It is an also intricate enzyme that can dissolve cellulose and hemicelluloses into monomers resembling simple sugar. In general, the current demand for cellulase enzymes is achieved by genetically modified *Trichoderma* strains under submerged fermentation (SmF). Nevertheless, the cost of producing these enzymes in SmF is very high. Hence, the production cost of cellulase is the limiting factor for the usage of cellulase in the production of ethanol from cellulosic biomass. The production cost of cellulase can be reduced by substituting solid-state fermentation (SSF) for SmF. According to cost estimates, SSF may result in lower unit costs than SmF. Depending on the analysis, enzyme cost estimates are not uniform [1]. Cellulases have broad range of applications in various fields like biofuels, food and animal feed, textiles, paper, waste management, medical and pharmaceutical industries, genetic engineering, and environmental pollution [2]. Furthermore, it has a market share of approximately 20% globally, making it an important industrial enzyme. In general, SmF serves as a means of satisfying the commercial necessity for cellulases. SmF has its own disadvantages such as the high cost of SmF production, which involves excessive water, strict sterility, and energy-intensive processing (such as purification to boost the activity of the enzyme). Alternatively, the production of cellulase by SSF, which uses solid waste as a substrate, has less resource utilization and is freely available [2]. Moreover, the cost of producing cellulases with SSF accounts for 0. 2 $/kg, which is almost 10 times less expensive than SmF. SSF can be a cost-effective means of producing biofuels at varying prices. Other than the above, the researcher's focus was on SSF potential to incorporate solid waste substrate usage and benefits such as improved productivity, reduced capital expenditure, low energy utilization, and a straightforward fermentation medium. Even though SSF has a high potential, it can only be explored in laboratories due to several limitations, such as the lack of inexpensive substrates for research purposes, inadequate heat and mass transfer equipment, and inadequate bioreactor designs [3].

Microorganisms that produce cellulase are commonly found in soil are classified as members of various bacteria and fungi genera [4]. Enhancement of new microbiota with high cellulase activity from uncultivated soils or forest floors is essential for investigating new species and functions related to fundamental questions. The presence of *micromonospora*, *Acidophilus*, *Paenibacillus,* and *Streptomyces*, as well as other unique or novel cellulolytic species, suggests that this may be a new ecosystem for studying polysaccharide or cellulose degradation [5,6]. One bacterial isolate or a group of microorganisms can both undergo bacterial degradation [7]. Microorganisms that produce cellulase are commonly found in soil. Although fungi are classified as microorganisms, they have become significant industrial

***Corresponding author:**
M. Subhosh Chandra,
E-mail: subhash_muni@yvu.edu.in

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raw materials for various purposes [8]. Earth's disintegration could be triggered by dynamic cellulolyzers. In addition, the resistance of this scaffold to environmental changes makes it more appropriate for fungal cellulosomes to produce cellulases [8]. Compared to *Aspergillus* and *Humicola*, *Trichoderma* is considered the most adaptable species for producing cellulase that can be used in industry [9]. A study was conducted on the production of cellulase by bacteria and fungi in both underwater and solid fermentation processes.

2. EXPERIMENTAL

2.1. Soil Sample Collection

Soil samples were gathered at a deepness of 10–15 cm from four places within the soil of a mango discipline positioned in Chennur, Kadapa, and Andhra Pradesh after doing away with the floor layer in sterile polyethylene bags. Of 6–10 samples were individually amassed shifted to the laboratory and preserved in a 4°C fridge until use. Composite soil samples were prepared by means of very well mixing equal quantities of soil for every place collected and those soil samples were used to isolate hypercellulose-degrading micro-organism.

2.2. Isolation of Hypercellulose-degrading Microorganisms

Every soil pattern was dried at ambient temperature in a shaded laboratory, sieved through a 2-mm sieve, after which mixed to create composite soil samples for isolation. 1 g of composite soil dissolved in 9 mL of sterile distilled water and was diluted in this solution decimally (10^{-1} to 10^{-6}). 0.1 mL of the precise bacterial dilution to the perfect Petri dish containing 20–25 mL of nutrient agar medium supplemented with 0.5% carboxymethyl cellulose (CMC) and spread uniformly with a sterile glass rod. After deployment, plates were placed at 37°C in an incubator for 24–48 h, and bacterial colonies determined on Petri dishes were subcultured onto fresh agar slants and saved at 4°C for screening experiments.

0.1 mL aliquots from the perfect dilutions were plated onto the Petri dish containing 20–25 mL of Czapek Dox agar medium added 0.5% CMC and spread uniformly with the help of a sterile glass rod. After spreading, the plates had been incubated in an incubator at 28–30°C for 7 days. Fungal colonies observed in Petri dishes were subcultured onto agar slopes and saved at 4°C for screening studies.

2.3. Screening of Hypercellulose-Generating Bacteria and Fungi

2.3.1. Bacterial screening

The selected bacterial strains were inoculated on a CMC agar plate and incubated at a specified temperature. After the culture was completed, the plate turned into flooded with 0.1% Congo red and left at room temperature for 15 min. Plates were counterstained with 1 M NaCl cautiously. A clean area appeared across the developing bacterial colonies, indicating cellulose hydrolysis. Bacterial colonies with the purest zones have been decided on to identify and bring cellulases.

2.3.2. Fungal screening

Plate screening was completed on Czapek Dox agar medium consisting of 1% CMC. A 6 mm well was made in the solidified medium and 0.1 mL of spore suspension was inoculated. Plates have been incubated at 30°C for 7 days and alternatively at 50°C for 18 h. The dish was then full of 10–15 mL of 0.1% Congo red solution [10], decolorized with 1 M NaCl, and the diameter of the obvious area fashioned around the fungal lifestyle turned into measured in centimeters. Fungal isolates with hypercellulase interest had been selected for identification experiments.

2.4. Identification of Isolates

All through the secondary screening process the use of plate analysis, isolated microorganisms were identified primarily based on morphological traits (microscopic and macroscopic) and biochemical reactions.

2.4.1. Microscopic strategies

a. Gram staining is used for the morphological identity of microorganisms
b. With the help of a sterile needle, a small amount of mycelium was placed on a clear glass slide, stained with lactophenol cotton blue, covered with a cover slip, and examined beneath a microscope for the phenotypic characteristics of mycelium, conidia, and spores.

2.4.2. Biochemical residences

Parameters investigated for biochemical properties encompass indole check, methyl red, Voges–Proskauer test, citrate usage test, and amylase test by the usage of standard protocols. The consequences have been compared with Bergey's determinative bacteriology guide of bacteria.

2.5. Pure Culture

a. Bacteria isolated from decomposing mango field soils and the isolated strains were grown on a nutrient agar medium. Inoculum was prepared from 24 to 48 h grown slants by pouring a sufficient quantity of sterile distilled water.
b. Isolated fungal strains were maintained on the Czapek Dox medium, and spore suspension was prepared from 7-day grown slants by supplementing the required quantity of sterile distilled water consisting Tween-20 (0.2%, v/v). Spore count in the prepared inoculums was determined by the Hemo cytometer method.

2.6. Substrate Collection for Fermentation Research

To select appropriate substrates for maximum production of cellulolytic enzymes, various substrates have been gathered from diverse resources. Lignocellulosic substrates which include black gram husk, red cowpea husk, pearl millet (Bajra) husk, Bengal gram husk, crimson gram husk, and groundnut feed have been selected as strong matrices because of their abundance and low fee in the local area. Black gram husk, red cowpea husk, pearl millet husk, Bengal gram husk, and red gram husk have been collected from local farmers in Chennur, Kadapa.

2.7. Substrate Screening

To pick an appropriate substrate, seven substrates together with black chickpea husk, red cowpea husk, pearl millet husk, Bengal gram husk, and crimson gram husk were tested for cellulolytic enzyme production. The substrates were dried at ambient temperature and screened with a 2-mm sieve to attain uniform particle size.

2.7.1. SmF

50 mL of medium (yeast extract 0.1 g, sucrose 0.2 g, substrate 1.0 g, K_2HPO_4 0.1 g and $FeSO_4$ 0.001 g, and dH_2O 100 mL) consists of 0.5 mL of basal salt solution ($NaNO_3$ 10.0 g, KCl 2.5 g, $MgSO_4$ 2.5 g, and distilled water 50 mL) in 250-mL Erlenmeyer flasks that were cultured with 0.5% (v/v) bacterial suspension after 3-day increase at the nutrient solution medium and incubated at 30°C and 120 rpm for 24 h in an orbital shaking incubator. The flasks were taken every 2 days over the duration of 8 days and centrifuged at 10,000 rpm for 10 min at 4°C. The clean supernatant attained was utilized for enzyme assay [11].

2.7.2. SmF for fungi

Fermentation is performed in 250 mL Erlenmeyer flasks consisting of 50 mL of Czapek Dox medium consisting of 0.5% cellulose. The flasks were sterilized at 121°C for 15 min and later cooled to room temperature, cultured with 2×10^6 spores/flask, and stored in a shaking incubator at 150 rpm at room temperature [12]. After that, fermented broth was filtered through Whatman No. 1 filter paper to separate mycelium. The filter paper with mycelium was dried and filtrate was employed for enzyme assay.

2.7.3. SSF

SSF was performed in 250 mL Erlenmeyer flasks and 10 g of different solid substrates were disbursed. Czapek Dox liquid medium consists of (g/L): NaNO3 – 2, K_2HPO_4 – 1, $MgSO_4$. $7H_2O$ – 0.5, KCl – 0.5, $FeSO_4$. $7H_2O$ – 0.01, sucrose – 30, and cellulose – 0.5 [13,14]. The substrates required different volumes of water, i.e., 10–15 mL to maintain 40% of the miniaturization of 10 g substrate. Flasks were covered with cotton plugs and autoclaved at 121°C for 15 min. A sterile solid medium inside the flasks was cultured with spores of *Aspergillus* spp. at the density of 2×10^6 spores/flask and incubated at room temperature. Spore inoculum was arranged from 7-day grown on Czapek Dox slants by adding 2 mL of sterile dH_2O containing 0.01% Tween-20 and the spores were suspended with a sterile loop. Everyday gap the flasks were withdrawn for further processing. Whole moldy bran inside the flask was mixed with distilled water or acetate buffer (0.2 M; pH 5.0); the slurry was filtered with nylon cloth and filtrate was centrifuged at 10,000 rpm for 20 min at 4°C. The clear filtrate attained was employed for enzyme assays up to 5 days of incubation. Extracellular protein content and activity of individual additives of the cellulase machine were estimated.

2.8. Analytical Strategies

At preferred durations, 250 mL Erlenmeyer flasks with a growing subculture of bacteria and *Aspergillus* spp. had been withdrawn at some point of the route of various experiments as cited above.

2.8.1. Extracellular protein content

An aliquot of traditional filtrate with suitable dilution is employed for the estimation of protein content in line with the controls [15]. Bovine serum albumin is used as protein control. Appropriate aliquots of filtrate/leachate had been combined with 5 mL of alkaline solution. After 10 min, 0.5 mL of accurately diluted Folin–Ciocalteu reagent was added. After 30 min, extinction becomes examined at 750 nm in a colorimeter.

2.8.2. Enzyme assays

Enzyme components of cellulase were secreted into the medium and were estimated according to strategies indexed [16]. The leachate recovered after centrifugation in SSF turned into used as a source of enzyme.

2.8.2.1. FPase assay

FPase activity was estimated by the standards method [17]. One unit of filter out paper unit (FPU) changed into described as the quantity of enzyme liberating 1 μm of lowering sugar from filter paper per min. The pastime of cellulase became expressed in FPUs.

2.8.2.2. Endoglucanase assay

The amount of endoglucanase within the leachate turned into quantified by way of the CMC approach [18]. The amount of reducing sugar was estimated by the dinitrosalicylic acid approach [19]. One unit of endoglucanase is described as the amount of enzyme releasing 1 μm of reducing sugar per minute.

2.8.2.3. β-D-glucosidase assay

The activity of β-glucosidase in the culture filtrate was determined according to the method [20]. One unit of β-glucosidase activity was defined as the amount of enzyme liberating 1 μm of p-nitrophenol per minute under standard assay conditions.

3. RESULTS AND DISCUSSION

3.1. Isolation of Bacteria and Fungi

One hundred thirty-one bacterial isolates and 13 fungal cultures were isolated from different places of mango discipline soil (MFS) placed in Chennur, Kadapa district, Andhra Pradesh, India [Tables 1 and 2; Figure 1].

3.2. Secondary Screening

a. Bacteria
The photographs of MFS-B1 and MFS-B4 cultures are shown in Figure 2a. The diameter of the colorless region was measured for all the colonies. The diameter of the clear zone on plates after de-staining was confirmed more than a few 1–4 cm. MFS 4 become exhibited a high zone of 0.5 cm. Therefore, MFS 4 turned into decided on for similar experiments.

b. Fungi
Fungal cultures (MFS-F1, MFS-F2 MFS-F3, MFS-F4, MFS-F5, and MFS-F6) were selected for the zone of clearance on plate assay approach according to Teather and Wooden (1982), the diameter of color less area shaped with the aid of each of the fungal cultures turned into measured. The diameter of the clean area on plates after de-staining was confirmed more than few 4.5 cm [Figure 2b]. MFS-F5 become exhibited a high activity zone of 4.5 cm. Therefore, the simplest MFS-F5 was selected for further experiments.

3.3. Morphological Identification of the Bacterial Isolates

All the isolates were observed to be Gram-advantageous in nature. The morphology of all the isolates was found to be rod-fashioned [Figure 3].

3.3.1. Morphological identity of the fungal isolates

The isolate MFS-F5 was purified by way of repeated sub-culturing on Czapek Dox agar medium and incubated at 30°C. The primary identification of fungal isolates is based on the microscopic and macroscopic characteristics. To observe fungal morphological characteristics such as colony morphology, i.e., characteristics of mycelia, conidia, and fruiting bodies by microscopy [21], Lactophenol cotton blue staining was used. The species produce colonies, composed of white or yellow flat; this is included with the aid of dark asexually produced fungal spores and it becomes tentatively identified as *Aspergillus* spp. [Figure 4a and b].

Table 1: Number of fungal colonies isolated from mango field soil sample.

S. no.	Name of the soil sample	Dilution	Number of fungal isolates
1	Mango field soil	10^{-2}	3
		10^{-2}	4
		10^{-3}	3
		10^{-4}	3

Table 2: Number of bacterial colonies isolated from mango field soil sample.

S. no.	Name of the soil sample	Dilution	Number of bacteria isolates
1	Mango filed soil	10^{-4}	4
		10^{-4}	5
			1
		10^{-5}	4
			4
		10^{-5}	3
			2

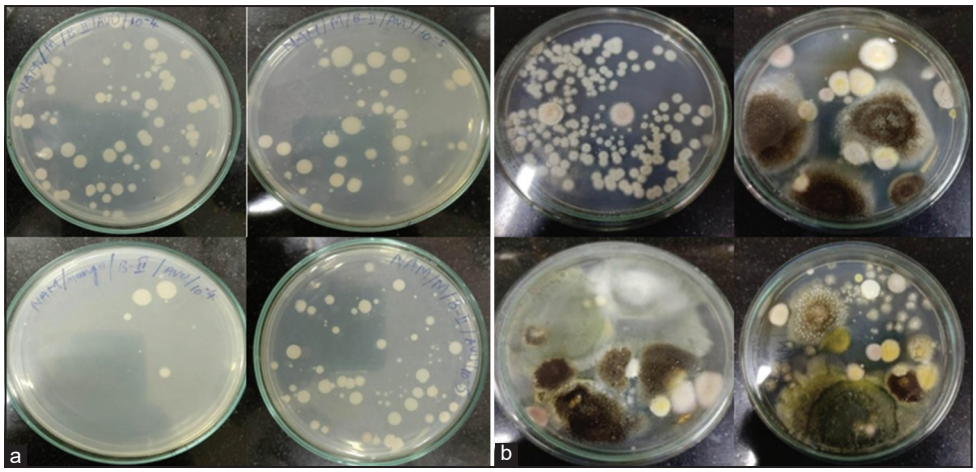

Figure 1: Isolated microorganisms from the mango field soil samples. (a) Isolated bacterial colonies on nutrient agar media (b) Isolate fungal strains on Sabouraud dextrose agar medium.

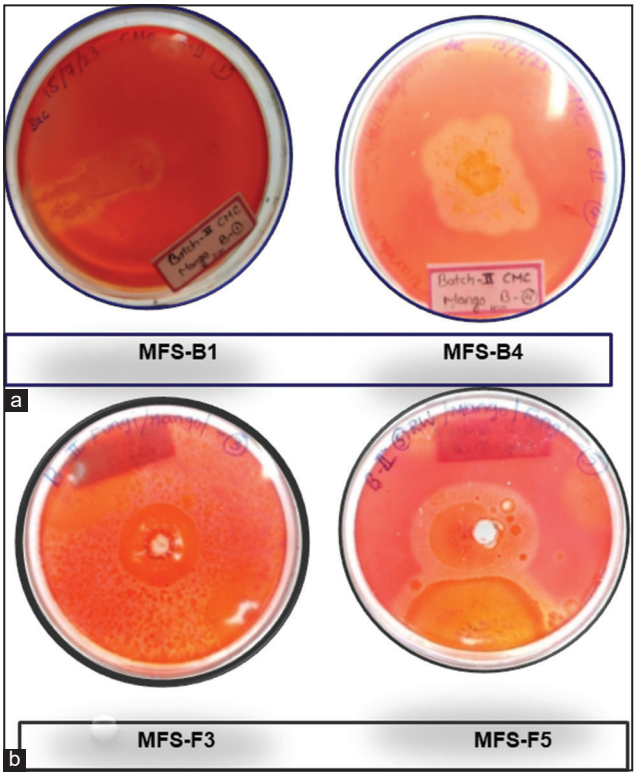

Figure 2: Zone of clearance by selected cultures by plate screening (a) Zone of clearance around the bacterial isolates, (b) Zone of clearance around the fungal isolates.

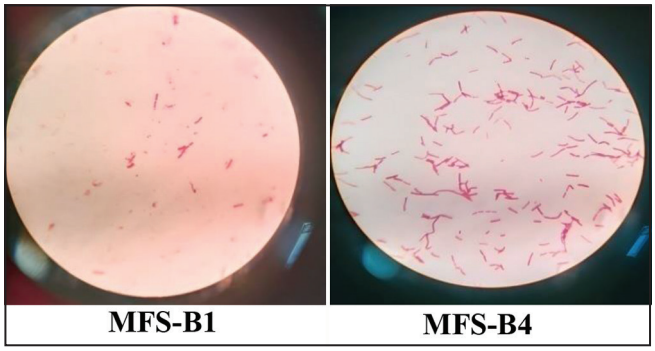

Figure 3: Microscopic observation of the cultures.

3.3.2. Biochemical characterization

The biochemical characterizations of bacterial isolates are stated in desk three and confirmed those isolates as rod-shaped organisms [Figure 5 and Table 3].

3.4. Evaluation of Enzyme Manufacturing

3.4.1. Production of cellulolytic enzymes through microorganisms in SmF

The substrates along with black gram husk, purple cowpea husk, Bengal gram husk, and groundnut fodder were screened for production of cellulolytic enzymes in SmF. Figure 6a shows the FPase production profile by way of microorganisms cultivated underneath SmF with the usage of one-of-a-kind strong substrates. Groundnut fodder was found to produce maximal FPase (0.54 FPU/mL), accompanied by black gram husk (0.32 FPU/mL), Bengal gram husk (0.08 FPU/mL), and purple cowpea husk (0.13 FPU/mL). Growth of bacteria on groundnut fodder gave the highest FPase and the lowest FPase activity was observed in crimson cowpea husks. Most titers of CMCase (0.55 U/mL) on groundnut fodder were registered on the 2nd day of incubation [Figure 6b]. In appreciation of different strong substrates, purple cowpea husk, black gram husk, and Bengal gram husk, the most CMCase activity changed into located on the 2nd and 8th day of incubation with 0.33, 0.43, and 0.17 U/mL of a solid substrate. The low CMCase interest changed into recorded Bengal gram husk. Production of β-glucosidase becomes to start with low or undetectable on the second 1 day of incubation. Maximum titers (0.043 U/mL) of β-glucosidase were recorded from cowpea husks on 2nd day of incubation, respectively [Figure 6c]. Production of β-glucosidase on red cowpea husk becomes highest while compared to different solid substrates at their respective time durations of top production. Groundnut fodder showed the lowest β-glucosidase activity on the 6th day of incubation (0.003 U/mL). Maximum secretion of extracellular protein (8 mg/mL) on cowpea husk at height time period on 6th day of incubation turned into recorded in contrast to protein content into low (1.78 mg/mL) on groundnut fodder at top time interval on 8th day of incubation [Figure 6d].

3.4.2. Production of cellulolytic enzymes through Aspergillus spp. in SmF

The stable substrates inclusive of black gram husk, crimson gram husk, Bengal gram husk, and pearl millet husk were screened for the

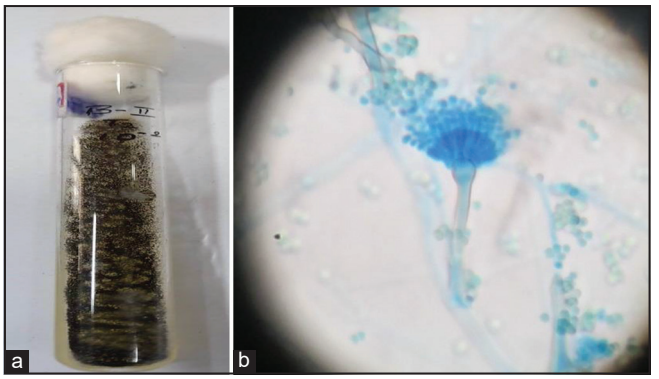

Figure 4: Morphological identification of *Aspergillus* spp. (a) Growth of *Aspergillus* spp. on Czapek Dox agar medium, (b) Microscopic observation of the culture.

production of cellulolytic enzymes by fungi in SmF. The increase of fungi on black gram husk received most titers of FPase activity (0.18 FPU/mL) on the 4th day of incubation [Figure 7a]. Similarly, higher FPase activity (0.15 FPU/mL black gram husk) was observed on 1st day of incubation on black gram husk and lowest production of FPase activity (0.012 FPU/mL) was noted on pearl millet husk on the 5th day of incubation. Among substrates examined, Bengal gram husk yielded maximum titers of 0.42 U/mL as toward 0.21 U/mL through red gram husk, 0.11 U/mL through black gram, and 0.06 U/mL by means of pearl millet husk. The low CMCase pastime changed into recorded with pearl millet with 0.014 U/mL on the 2nd day of incubation [Figure 7b]. Most titers (0.303 U/mL) of β-glucosidase were recorded from red gram husk on 1st day of incubation [Figure 7c]. Production of β-glucosidase with pearl millet husk was much less while compared to other solid substrates at their respective time intervals

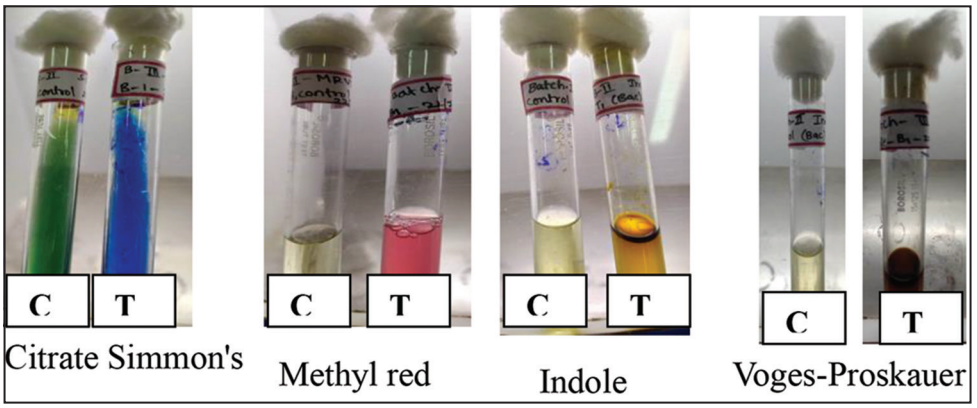

Figure 5: Biochemical characterization showed by selected bacterial cultures.

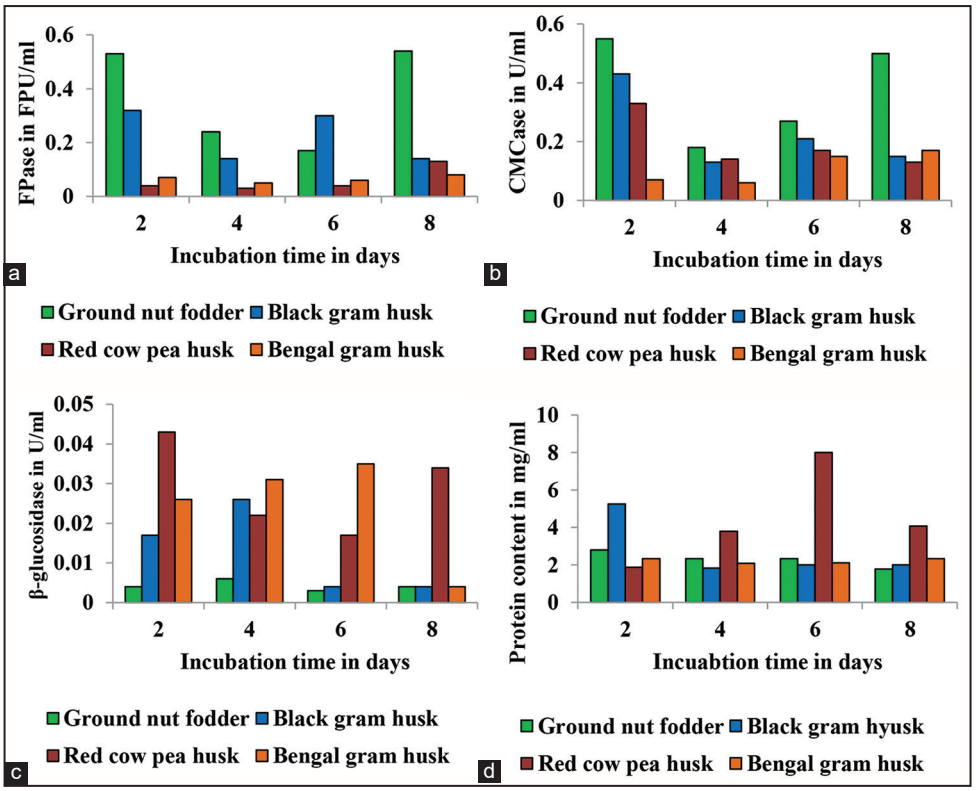

Figure 6: Production of different enzymes by *Proteus mirabilis* in submerged fermentation. (a) FPase, (b) Carboxymethyl cellulose, (c) β-glucosidase, and (d) Extracellular protein content.

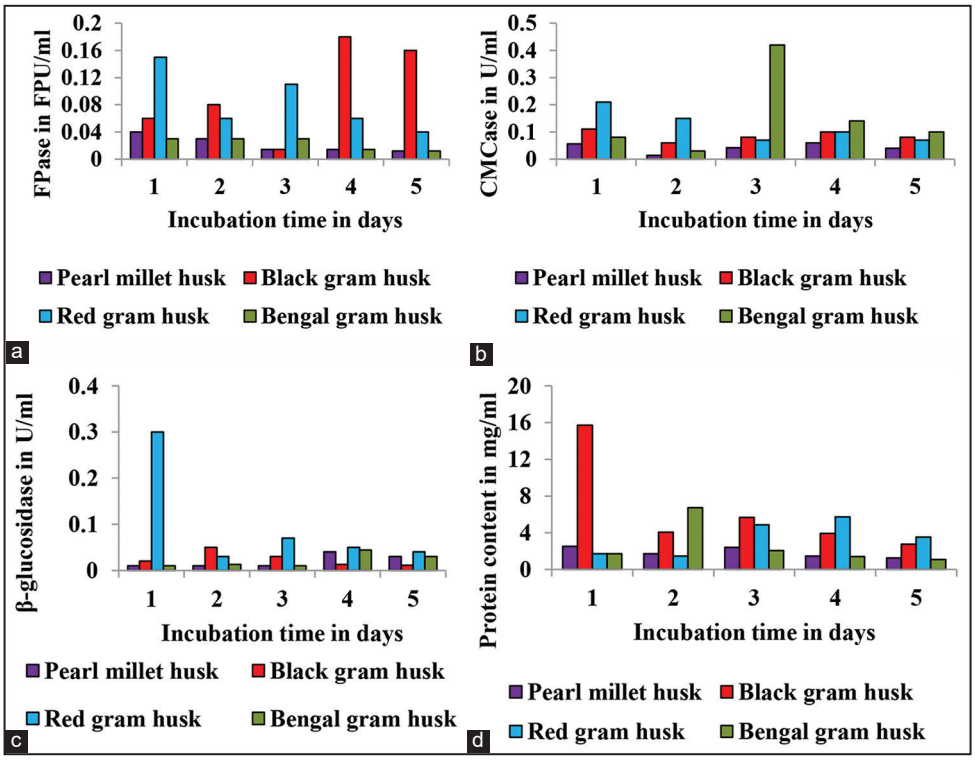

Figure 7: Production of different enzymes by *Aspergillus* spp. in submerged fermentation. (a) FPase, (b) Carboxymethyl cellulase, (c) β-glucosidase, and (d) Extracellular protein content.

Table 3: Biochemical results obtained for the selected bacterial culture.

S. no.	Name of the biochemical test	Result obtained
1.	Morphology	White and smooth texture
2.	Gram staining	Gram-negative
3.	Shape of bacteria	Rod-shaped
4.	Indole test	Negative
5.	Methyl red test	Positive
6.	Voges–Proskauer test	Negative
7.	Citrate Simmons test	Positive
8.	Amylase test	Positive

of top production. Among the screened solid substrates, black gram showed higher protein content (15.73 mg/mL) was recorded on 1st day of incubation whereas low protein content (1.09 mg/mL) was noted on bengal gram on 5th day of incubation [Figure 7d].

3.4.3. Production of cellulolytic enzymes using Aspergillus spp. in SSF

The solid substrates inclusive of black gram husk, pearl millet husk (Bajra), pink gram husk, and groundnut fodder were screened for the production of cellulolytic enzymes by *Aspergillus* spp. in SSF. Figure 8a proves the clear FPase production profile by *Aspergillus* spp. cultivated in SSF by the usage of distinct solid substrates. Groundnut fodder changed into discovered to bring about maximal FPase production (0.69 FPU/g of the substrate), an increase by way of black gram husk (0.64 FPU/g of the substrate), red gram husk (0.48 FPU/g of the substrate), and pearl millet husk (0.47 FPU/g of the substrate). An increase of *Aspergillus* spp. on groundnut fodder gave the best FPase and the bottom FPase titer was discovered in pearl millet husk. Most titers of CMCase (0.68 U/g of substrate) on black gram husk became registered at the 3rd day of incubation [Figure 8b]. In respect of other stable substrates, red gram husk, pearl millet husk, and groundnut fodder, the most CMCase pastime changed into observed on the 4th and 1st day of incubation with 0.67, 0.59, and 0.53 U/g of the stable substrate. The low CMCase pastime changed into recorded on pearl millet husk. Most titers (0.523 U/g of the solid substrate) of β-glucosidase were recorded from groundnut fodder husk on the 5th day of incubation, respectively [Figure 8c]. Production of β-glucosidase on groundnut fodder turned into maximum while as compared to other stable substrates at their respective time durations of peak production. The bottom β-glucosidase production changed into located on the red gram with 0.013 U/g of substrate on 1st day of incubation. The various strong substrates used inside the present look at most secretion of extracellular protein (6.66 mg/g of solid substrate) on black gram husk at top time on 3rd day of incubation turned into recorded while the protein content material was low (0.73 mg/g of solid substrate) on groundnut fodder at peak time interval on 1st day of incubation [Figure 8d]. Different substrates such as red gram husk, pearl millet husk, and groundnut fodder were shown maximum secretion of protein with 6.0, 5.2, and 1.87 mg/g of solid substrate at height time 3rd and 4th day of incubation. Substrates such as wheat straw, corn stover, and oat straw were tested among numerous agro-biomass samples. *Morchella spongiola* [22], *Aspergillus terreus* [23], and *Trichoderma reesei* [24] exhibited stronger cellulase pastime while exposed to those substrates. The variation in cellulase titer observed on this take a look at can be attributed to the variations in agrobiomass compositions. These findings align with the research carried out with the aid of Elisashvili *et al*. [25], who emphasized that the fantastic has an impact on the lignocellulosic biomass substrate on enzyme secretion. Considerably, the corn cob displayed decreased cellulase production as compared to alfalfa, oat straw, wheat straw, and corn stover. In a comparable vein, Reddy *et al*. [26], Ganash *et al*. [27], and Govumoni *et al*. [28] have pronounced the bottom cellulase interest in *Aspergillus*

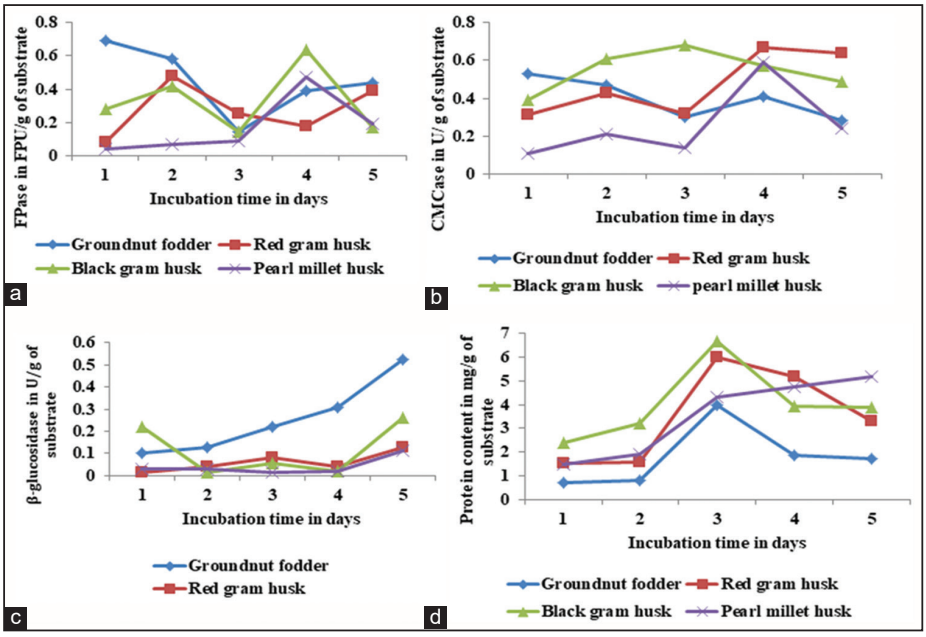

Figure 8: Secretion of different enzymes secreted by *Aspergillus* spp. into solid substrates in SSF. (a) FPase, (b) Carboxymethyl cellulase, (c) β-glucosidase, and (d) Extracellular protein content.

niger, *Pleurotus ostreatus*, and *Phanerochaete chrysosporeum* (MTCC 787) while grown on corn cobs, among numerous agro-biomasses. The reason at the back of corn cob's decreased cellulase production as compared to different agro-biomasses lies in its structural composition. Corn cob possesses a dense and compact micro-structural carbohydrate configuration. Therefore, the fungi mycelium faces trouble in penetrating and getting access to the hydrolyzable fibers important for cellulase enzyme biosynthesis, in evaluation to different agro-biomasses. Moreover, Reyes *et al*. [29] have advised that greater contact with cellulosic cloth can result in advanced enzyme production related to cellulose hydrolysis.

Cellulase production by *Aspergillus unguis* optimized the usage of groundnut fodder as a substrate for SSF. The consequences of different concentrations of NaOH, in addition to temperature, pH, and surfactants, were studied to increase cellulase manufacturing and yield of lowering sugars. The outcomes confirmed that beneath the optimized situations, groundnut fodder treated with 1% NaOH produced the very best FPase activity [5.45 FPU/g of substrate], CMCase interest [4.75 U/g of substrate], and β-glucosidase activity [19.0 U/g of substrate] with 1 ml of crude cellulase instruction [30,31]. In comparison, Sun *et al*. [32] pronounced most cellulase yield [FPase 5.56 FPU/g d.s; endoglucanase 10.31 U/g d.s; β-glucosidase 3.01 U/g d.s] the usage of banana peel because the substrate in SSF. However, *A. niger* did no longer show enormous cellulase production while prompted with a banana peel. The highest pronounced sports for cellulase (FPase), endoglucanase, and β-glucosidase were 1.96 FPU/g d.s, 2.0 U/g d.s, and 1.22 U/g d.s, respectively [33]. In addition, banana leaves were also located to be ineffective in inducing cellulase production. *Aspergillus* spp. B11 produced cellulase pastime of 0.94 FPU/g d.s and endoglucanase interest of 2.2 U/g d.s [34]. The low cellulose (11%), hemicellulose (9%), and lignin (3%) content material in banana peel and leaves can be the motive for their bad-inducing capability [33]. On the other hand, *Trichoderma harzianum* TF2, remoted from banana rhizosphere, confirmed high cellulase activity (38.5 U/g.ds) in the use of wheat bran because of the substrate [35].

4. CONCLUSION

In the present study, we successfully isolated bacterial and fungal strains from the mango fields soil samples and identified them based on the cultural characteristics and morphological features. We screened agricultural wastes such as black gram husk, red cowpea husk, Bengal gram husk, and groundnut fodder to choose higher renewable carbon sources for the production of cellulolytic enzymes by bacterial and fungal strains. Red cowpea husk and ground nut husk are the encouraged strong substrates for maximum production of β-glucosidase, protein content, and FPase and CMCase, respectively through bacterial isolate. The maximum FPase, CMCase, β-glucosidase and protein content was observed on black gram husk, Bengal gram husk, red gram husk and black gram husk at 4th, 3rd, 1st and 1st day of incubation respectively by Aspergillus spp. in SmF. Whereas in SSF, the higher FPase, CMCase, β-glucosidase and protein content was noticed on groundnut fodder, red gram husk, groundnut fodder and black gram husk at 1st, 4th , 5th and 3rd day of incubation respectively by Aspergillus spp.

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***Bibliographical Sketch**

Dr. M. Subhosh Chandra, Associate Professor and Raman Fellow of the Department of Microbiology, Yogi Vemana University, Kadapa. He has obtained a Ph.D from Sri Krishnadevaraya University, Anantapur. Immediately, he was joined as a Postdoctoral Fellow at the Indian Institute of Science (IISc), Bangalore. Later on, he moved to Dong-A University, South Korea, for his Postdoctoral studies. To his credit, he has published 49 research papers in reputed national and international journals and published 1 book and 27 book chapters. He has been to Forest Products Laboratory, United States Department of Agriculture, University of Wisconsin, USA, under UGC-Raman Fellowship during the year 2013–2014. He was awarded a Junior and Senior Research Fellowship funded by ICAR, New Delhi.