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Identification and Characterization of Cellulose Degrading Bacteria and Estimation of Its Cellulolytic Capacity

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Abstract: Cellulosic biomass is one of the foreseeable sustainable sources of fuels and is also one of the dominating waste materials in nature resulting from human activities. Keeping in view the environmental problems like disposal of large volumes of cellulosic wastes and a shortage of fossil fuel in the world, the main aim of the present investigation was to characterize and study the cellulolytic activity of the selected isolate on natural cellulosic substrates viz. finely grated vegetable peels. The cellulose degrading capacity of the isolate was confirmed by Congo red test. By the selection of efficient cellulolytic microorganisms and cost- effective operational techniques, the production of useful end products from the biodegradation of the low-cost enormous stock of cellulose in nature can be very beneficial.

Keywords: Cellulolytic Potential, Cellulosic Biomass, Congo red Test, Sustainable Fuel, Waste Material.

I. INTRODUCTION

Cellulose is a linear polysaccharide of glucose residues with beta-1,4-glycosidic linkages. With the help of the cellulolytic system, cellulosic wastes which are often disposed of by biomass burning can be restricted. Cellulose can be converted into glucose which is simpler and a multi utility product in a much cheaper and biologically favourable process. Cellulase due to its massive applicability has been used in various industrial processes. The present work concentrates on identification and characterization of cellulolytic bacteria and estimation of degradation of various natural cellulosic products by the isolate [3].

II. MATERIALS AND METHODS

A. Media

Media used during the course of the present investigation were sterilized by autoclaving. Nutrient Agar (Peptone 5 g/L, beef extract 3 g/L, sodium chloride 5 g/L, agar 15 g/L) was used for isolation and preservation of cellulose degrader. Milk Agar, Methyl Red, Voges Proskauer broth, Nitrate broth, Phenol red dextrose broth, Phenol red lactose broth, Phenol red sucrose broth, Fructose broth, Maltose broth, Cellulose broth Simmons citrate Agar, Starch Agar, Tryptone broth, Mannitol motility agar, gelatin tubes, Sim agar, Casein agar, Nitrate reduction broth and TSI slants were used for specific biochemical tests (Cappuccino & Sherman, 2005). Stanier's basal medium [(NH4)2SO4 1 g/L, K2HPO4 1 g/L, MgSO4 0.2 g/L, CaCl2 0.1 g/L, FeCl3 0.02 g/L]; CMC agar (carboxymethylcellulose 0.5 g/L, NaNO3 0.1 g/L, K2HPO4 0.1 g/L, MgSO4 0.05 g/L, yeast extract 0.05 g/L, agar 15 g/L) and Modified Cellulose agar replacing carboxymethylcellulose in CMC agar with cellulose for cellulose degrading efficiency test. Stanier's Basal broth supplemented separately with different natural cellulosic wastes viz. finely grated vegetable peels for estimating the cellulolytic activity of the selected cellulose degrader.

B. Purification and maintenance of isolate

The isolate was streaked on Nutrient agar and kept in an incubator at 37°C for 48hrs. The isolate was maintained on Nutrient agar slants at 4 °C with periodic sub culturing.

C. Morphological characterisation of selected isolate

The selected cellulose degrader was critically examined for its morphology. The colony was orange in colour with a regular margin, slightly elevated, opaque. Gram Staining [2] and simple staining was performed. Morphology of the isolate was confirmed by negative staining.

D. Effects of various growth parameters on the isolate

The influence of temperature was studied by incubation of the media at 4°C, 25°C, 37°C and 42°C for 24 h. Similarly the influence of NaCl was studied using Nutrient agar with different NaCl concentrations (2 %, 5 %, 10 %, 15 %, 20 % and 25 %) prepared by dissolving 2 g, 5 g,10 g, 15g, 20g and 25g of NaCl in 100 mL of sterilized distilled water to obtain 2 % − 25 % concentration of NaCl [5]. All the experiments were done in triplicates.

E. Biochemical characterization of the selected isolate

The culture was characterized morphologically and physiologically by Gram's staining and different biochemical tests as per Bergey's Manual of Systematic Bacteriology which included Indole, methyl red, Voges- Proskauer, citrate utilization test, catalase, oxidase, starch, gelatin hydrolysis, sugar fermentation, caseinase, hydrogen and nitrate reduction test. The fresh culture was used for all the tests [9].

F. Influence of carbon sources on growth of the isolate

The isolate was inoculated separately in mineral salt medium supplemented with 0.5 % (w/v) of each of the different carbohydrates as substrates that included dextrose, fructose, sucrose, lactose, maltose, mannitol, starch, and cellulose. The isolate was incubated at 37 °C for 24h. The culture characteristics of the isolate on different carbohydrate substrates were observed and recorded.

G. Estimation of antibiotic sensitivity

The selected isolate was tested for antibiotic sensitivity using three different antibiotic discs on MHA media. The antibiotic discs used were Tetracyclin, Chloramphenicol, and Ampicillin. Clear zones were observed after incubation which indicates that the microbe is sensitive to these antibiotics.

H. Screening of cellulose degrading microorganisms from the isolate

Screening of cellulose degrading microorganism was performed by using Congo red test. The isolates were grown on CMC Agar (pH 7.0) and incubated at 37 °C for 5 days to allow for the secretion of cellulase. The agar medium was flooded with an aqueous solution of Congo red (1 % w/v) for 15 min to visualize the hydrolysis zone. The Congo red solution was then poured off and the plates were further treated by flooding with 1 N NaOH for 15 min. The cellulase activity was seen as clear zones around the colonies.

I. Estimation of degradation of finely grated vegetable peels by the isolate

10 mL of Stanier's Basal broth with 100 mg of different cellulosic substrates as sole carbon source viz. Vegetable peels were prepared separately [11]. Finely grated vegetable peels were weighed on the Electronic balance. The broths were autoclaved and inoculated with the selected strain. The tubes were incubated in the shaker incubator at 37 °C for 2days. After the incubation period, the culture broths were filtered through previously weighed filter papers. The filter papers with the residues were dried in the hot air oven for 15 min and re- weighed. The difference between the initial and the final weights gave a number of cellulosic substrates degraded by the isolate.

III. RESULTS

A. Morphological and biochemical characterization of the selected isolate

The selected cellulose degrader was critically examined for its morphology. The colony was orange in colour with a regular margin, slightly elevated, opaque. Gram Staining of the isolate resulted in gram positive cocci. Simple staining of isolated also resulted in gram positive cocci. Morphology of the isolate was confirmed by negative staining.

The biochemical tests were performed on the selected isolate. The isolate showed a positive result for citrate test, nitrate reduction test and negative for all the rest of the specified biochemical tests [10]. The morphological and cultural characteristic of the isolate were compared with Bergey's Manual of Systematic Bacteriology, Vol. 4 and was identified as *Micrococcus* genus [1].

B. Effect of various growth parameters on the isolate

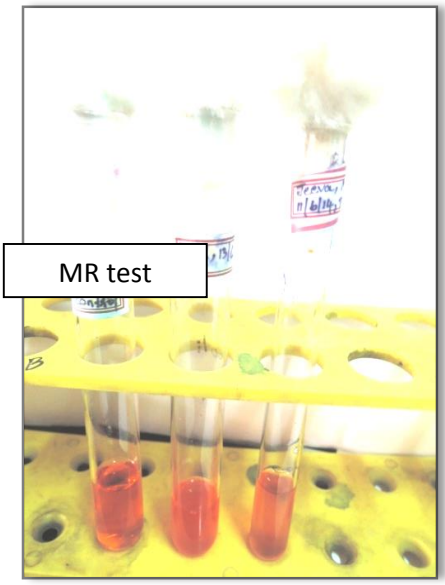
Growth parameters of the isolates were established with respect to temperature and saline tolerance [6]. Micrococcus sp. showed growth on a wide range of temperatures between 25 °C and 42 °C, though luxuriant growth was restricted between 30 °C and 42 °C. The strain showed growth at all NaCl concentrations (2 % − 10 %), except 15%, 20%, and 25%. [8]

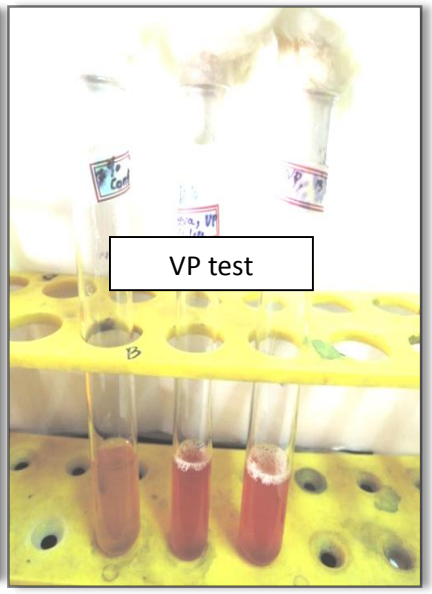
C. Influence of carbon sources on growth of the isolate

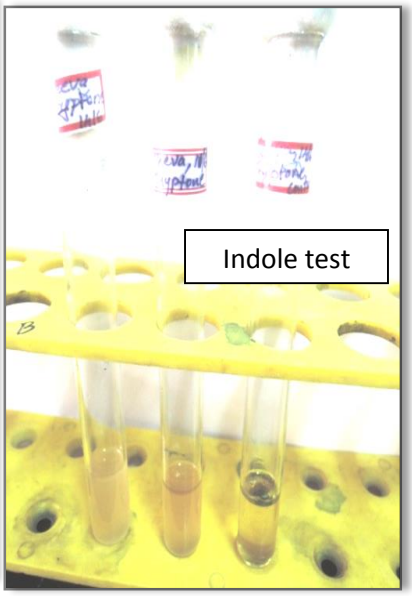
The culture characteristics of the strain indicated that it was capable of utilizing all tested carbon sources except lactose. No gas production was observed in any of the tested carbon sources except cellulose.

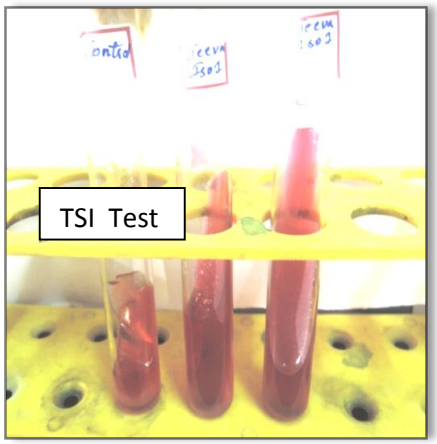
D. Screening of cellulose degrading microorganisms from the isolate

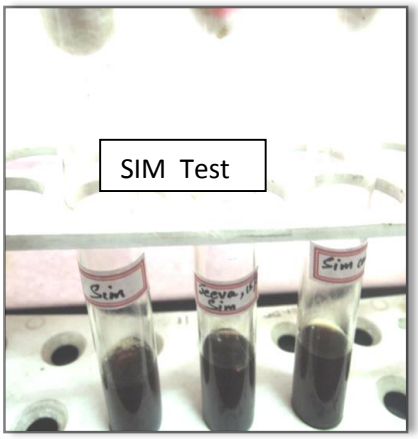
Screening of isolate was conducted by using the Congo red test as a preliminary study for identifying cellulose degraders [4]. Since the sole carbon source in CMC agar was carboxymethyl cellulose, the clear zone in the medium indicated cellulose degradation by the isolate.

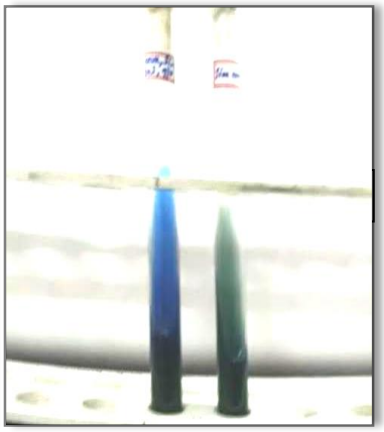


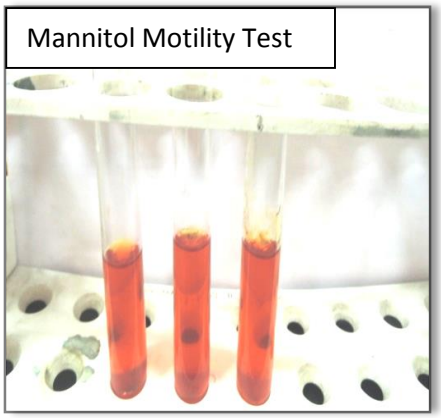


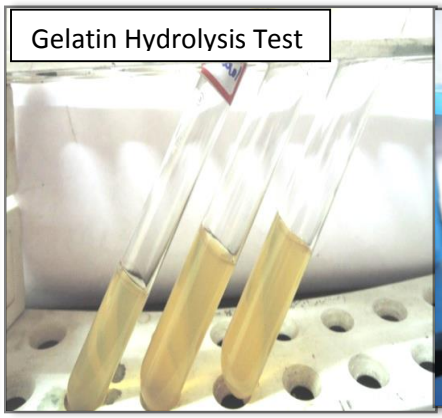


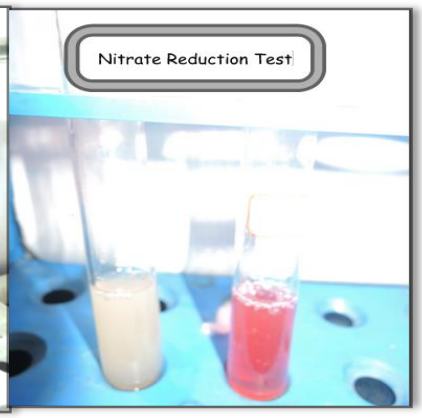












E. Estimation of degradation of finely grated vegetable peels by the isolate

10 mL of the Stanier's Basal broth was dispensed in tubes and 100 mg of finely grated vegetable peels was added as sole carbon source in each tube. The tubes were autoclaved and inoculated with the strain and kept in the shaker incubator for a period of 2 days at 37 °C. On the 2nd day, tubes were taken out from the shaker incubator and the culture broth was filtered through a previously weighed filter paper. The filter paper with the residue was dried in the oven for 15 min and re-weighed. The difference between the initial and the final weights gave the amount of vegetable peel degraded by the isolated in two days.

CONCLUSION

Cellulases are one of the most widely used enzymes in various industries. The present study focused on isolation, screening, and characterization of cellulolytic bacteria and determination of their cellulolytic potential. From different cellulosic samples used for the study, several isolates were obtained, out of which ten of them were found to be better cellulose degraders having higher enzyme activities. By the selection of efficient cellulolytic organisms and cost-effective operational techniques, production of useful end products from cellulose degradation can be very beneficial. Further improvement in cellulase performance can be imparted using various techniques for industrial applications.

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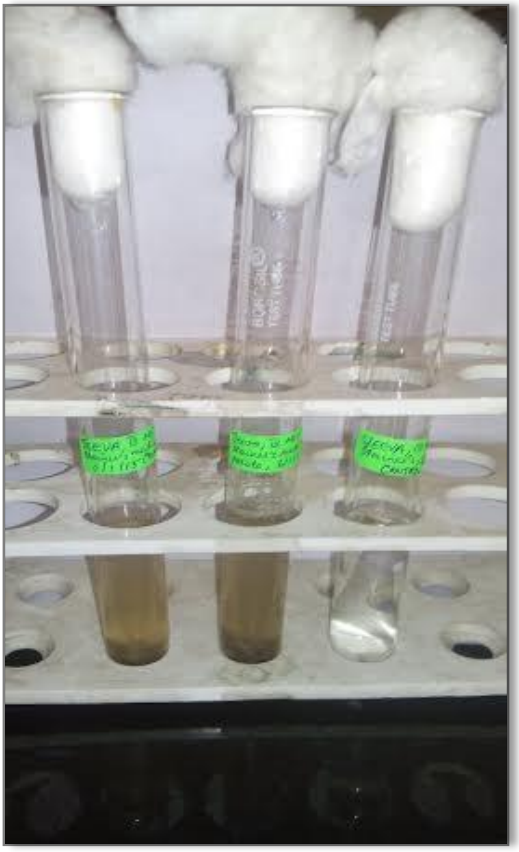
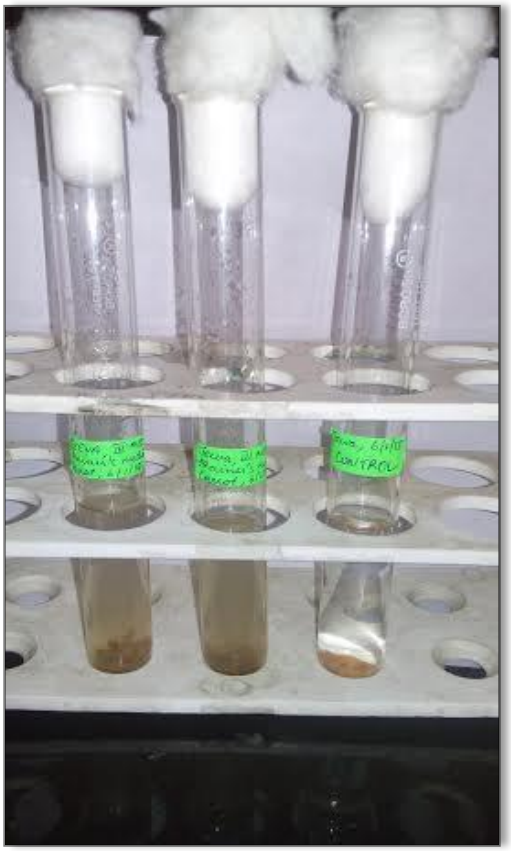
Table 1- BIOCHEMICAL CHARACTERIZATION OF THE SELECTED ISOLATE

SL NO	Tests	Results
1	MR	-
2	VP	-
3	Indole	-
4	Simmon's Citrate	+
5	TSI	Alkaline Slant and butt
6	Sim agar	-
7	Starch agar	-
8	Mannitol motility	-
9	Gelatin Test	-
10	Casein agar	-
	Carbohydrate Fermentation:	
11	Sucrose	+
12	Dextrose	+
13	Fructose	+
14	Lactose	-
15	Maltose	-
16	Cellulose	+
17	Nitrate Reduction	+
18	Oxidase	-
19	Catalase	-

Degradation of Vegetable Peels by the Isolate:



Potato peel degraded by iso1



Carrot peel degraded by iso1

