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The use of agricultural waste as substrate for cell growth

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Abstract : *Strains of Streptomyces albospinus & Streptomyces somaliensis are known to be cellulose degraders and were examined for cellulase production. Cellulase is a group of hydrolytic enzymes capable of degrading cellulose to the smaller glucose units and are produced by variety of bacteria and fungi as well as actinomycetes which convert cellulose into soluble sugars either by acid or enzymatic hydrolysis. Both the strains were found to grow in medium containing CMC as a sole carbon source. The highest cellulase activity obtained and observed on the 6th day of incubation from both the strains, Streptomyces albospinus and Streptomyces somaliensis were significantly not different. Comparing their growth in three different agricultural waste, maximum cell numbers were obtained from the medium containing pineapple peels followed by corn cob and vegetable residues. Pineapple peels stimulated higher production of cellulose than other agricultural waste. The value of these agricultural wastes can, therefore, be increased by its use not only in manufacture of cheap media but also in the production of valuable microbial biomass which is rich in protein and fatty acid.*

Keywords *: Carboxymethyl cellulose, CMCase, Streptomyces somaliensis, Streptomyces albospinus, agricultural waste.*

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Introduction :

A major problem experienced by agro – based industries, in developing countries is the management of wastes. The disposal of agricultural waste on land and into water bodies is common, and has been of serious ecological hazard (Smith *et al.*,1987). Today the emphasis is on the use of agricultural waste for the production of valuable products such as biofuel, single cell protein, pharmaceuticals.

Plant biomass contains cellulose as the major component (Haruta, S *et al.*, 2003). Cellulose, being an abundant and renewable resource, is a potential raw material for the microbial production of food, fuel and chemicals (Coughlan, 1985). It can be considered for biotechnological manipulation into eco-friendly component through the activity of the microorganisms.

The crystalline structure and insoluble nature of cellulose represent a formidable challenge for the enzymatic hydrolysis. Cellulose degrading microorganisms can convert cellulose into soluble sugars either by acid or enzymatic hydrolysis. Thus microbial utilization of cellulose is responsible for one of the largest material flows into the atmosphere. Various bacteria, actinomycetes and filamentous fungi produce extra cellular cellulases when grown on cellulosic substrates. However, many actinomycetes have been reported to have less cellulase activity than moulds (Ishaque and Kluepfel, 1980).

The complete degradation of cellulose is made by a cellulolytic enzyme system (Da Silva *et al*.,2005). Individual enzymes are not able to degrade cellulose completely while mixture of enzymes enhances efficiency of saccharification. A synergistic action of enzymes is required for the complete hydrolysis of cellulose.

This activity has been found in a variety of fungi and bacteria. Fungi are well known organic waste decomposers and are generally capable of hydrolysing complex organic compounds as a major source of energy. This potential has been utilized for biomass production, organic waste disposal and its conversion into biofertilizer (Moreira *et al*., 1981; Obuekwe and Okungbowa,1986) as well as production of valuable metabolites.

The objective of this study is to evaluate the potential of the cellulolytic strains of *Streptomyces* species for the production of cellulase enzyme and for upgrading agricultural wastes like corn cob, pineapple and vegetables residues as substrates and thus, to utilize agro waste as an alternative to costly media used in microbiological- laboratories and at the same time to have enhanced biomass production.

Materials and Methods :

Substrate collection : The agricultural wastes such as pineapple peel, corn cob and vegetables residues were used as substrate. Pineapple peels were collected from juice vendor, corn cobs from market and vegetable residues from home kitchen, respectively. All of these substrates were sun dried, blended into powder seperately and sieved with 0.5mm^2 wire mesh. The milled powders were stored in sterile airtight glass containers and used as substrate for source of carbon.

Strains : *Streptomyces albospinus* and *Streptomyces somaliensis* strains obtained from Patna Women's College microbiology laboratory as carboxy methyl cellulose degraders (CMC), were used for the study.

Plate assay : The carboxy methyl cellulose (CMC) agar plate were inoculated with *Streptomyces albospinus* and *Streptomyces somaliensis* strains and incubated at 37^{o}C. After the colonies were formed the plate assay were done for the production of carboxy methyl cellulase (CMCase) enzyme as cellulose degrader by the strains. For the confirmation, 1% Congo red was

prepared and then added to carboxy methyl cellulose (CMC) agar plates (Sudto *et al*., 2008) such as it completely covered the colony growth on plates. It was left for 10 minutes. The Congo red was drained out from plates and then 0.1N HCl was added to it and left for few minutes. A cleared zone was found below the colonies confirmed the cellulolytic activity of the strance. After the confirmation, the strains were inoculated on agar plates incorporated with pineapple, corn cob and vegetable residues and incubated at 37^{o}C. The colonies were counted with the help of Quebec colony counter manual (Electronic India).

Cellulase assay : The amount of cellulose degraded by the strains in different broths (CMC, pineapple, corn cob and vegetable residues) were determined by cellulase assay method described by Updegraff (1969). Moreover, the concentration of the reducing sugar in each broth were calculated to determine the strength of strains as cellulose degrader. For this 3 ml of acetic acid was added to 1 ml of sample (inoculated broth of CMC, Corn cob, Pineapple and Vegetable residue broth) in separate tubes and then the mixture was vortexed. The tubes were placed in water bath at 100^{o}c for 30 minutes. The content was cooled and centrifuged for15 to 20 minutes. Supernatant was discarded and residue was washed with distilled water. 67% of conc. H_2SO_4 was added to it and left for 1 hour. 1 ml of above solution was diluted to 100 ml with distilled water. To 1 ml of this solution 10 ml of anthrone reagent was added and mixed well. The tubes were heated in boiling water bath for 10 minutes and then allowed to cool. The O.D was taken at 430 nm using spectrophotometer (Thermo).

Results and Discussion :

Plate assay : Carboxy methyl cellulose (CMC) degradation was observed on carboxy methyl cellulose (CMC) agar plates inoculated with *Streptomyces albospinus* and *Streptomyces somaliensis*. The clearing occurred only below the colonies, which indicated that the enzyme remain attached to colonies. The clearing on carboxy methyl cellulose (CMC) agar plates after staining is shown in Figure 1.

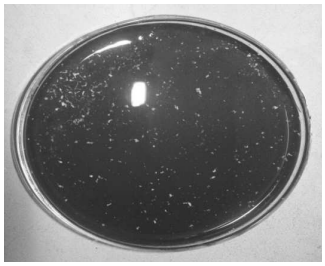


Fig 1:- Clear zones on CMC agar plates after staining indicates CMC degradation

Cellulase assay: Cellulase activity of *pinus* and *Streptomyces* rved at regular intervals of 2 ulase activity observed in *liensis* and S*treptomyces* 6µg/ml and 48.1µg/ml, e absorbance was measured as reducing sugars. The concentration of reducing sugars was determined by comparison with a glucose standard curve shown in Fig 2. The difference in celluloase activity between the two strains was negligible. The characteristic carboxy methyl cellulase (CMCase) production was in consistent with the results obtained from plate assay in which clearing occurred only below the colonies. It suggested that the enzyme remains attached to the actinomycetes. Cell bound carboxy methyl cellulase (CMCase) has been reported in many organisms such as, *Rhizobium leguminoserum* (Mateos *et al*., 1992).

Fig 2:- Standard graph for glucose estimation in different sample

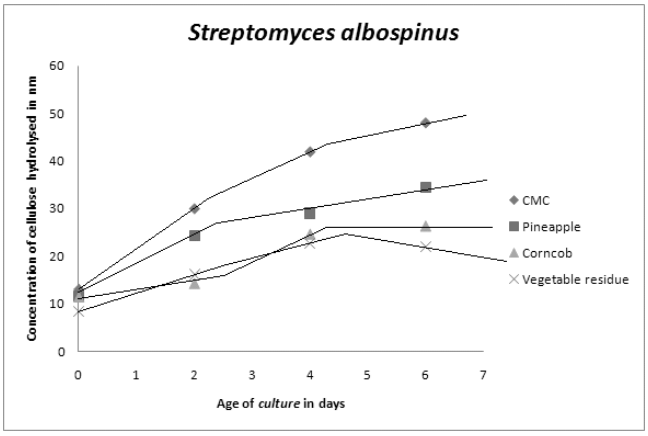


Fig 3 :- Growth curve and CMCase activities of Streptomyces albospinus

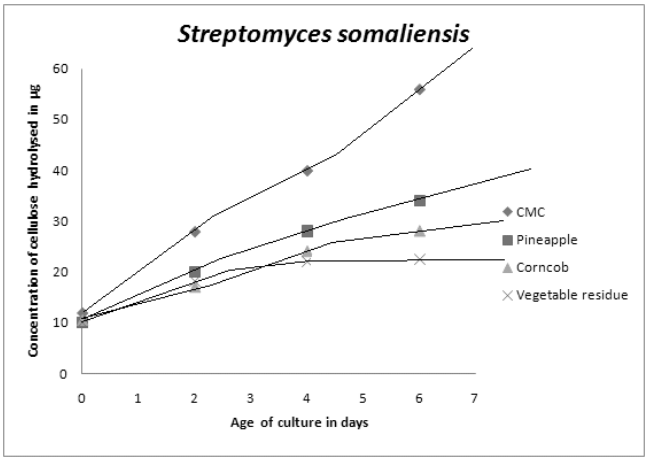
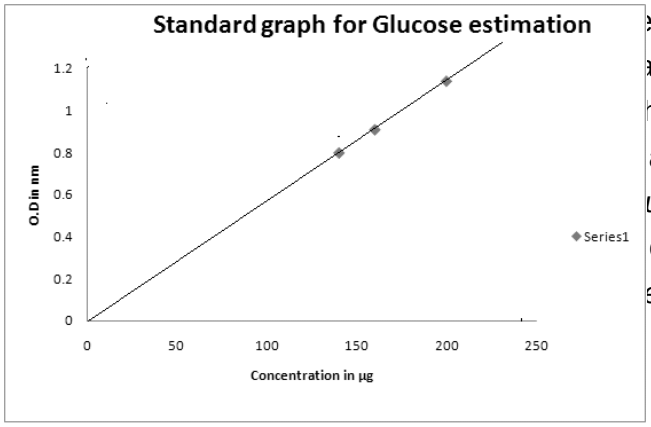


Fig 4*:- Growth curve and CMCase activities of Streptomyces somaliensis*

Maximum cellulase activity was shown from the medium containing pineapple peels and followed by corn cobs and the minimum cellulase activity was shown in the medium containing vegetable residues. This work was also illustrated by Sudto *et al.,* (2008) who worked on growth and cell bound carboxy methyl cellulase (CMCase) activity of *Bacillus subtilis*, *E coli* and *Rhizobium sp* and found maximum carboxy methyl cellulase (CMCase) activity on 6th day by *Bacillus subtilis* and *E coli* and on 2nd day by *Rhizobium sp* on pineapple peels followed by corn cob and vegetable residues. Initially reducing sugar of medium containing carboxy methyl cellulose (CMC), pineapple peels,corncobs and vegetable residues by *Streptomyces somaliensis* were 12 µg/ml, 10µg/ml,10.5µg/ml and 10.1µg/ml, respectively and by *Streptomyces albospinus* were 13µg/ml, 11.6µg/ml,11.4µg/ml and 8.4µg/ml, respectively, were calculated with the help of absorbance taken at 0 days. After incubation of 2 days the concentration



From the study conducted over a period of one month it is concluded that the agricultural waste in the form of cellulose an abundant and renewal biomass in the biosphere can be used for cell growth.

Cellulose degraders *Streptomyces albospinus* and *Streptomyces somaliensis* were found to be easily grown on agricultural waste with minor changes in its production protocol as compared to

their growth on carboxy methyl cellulose (CMC) media which was used as control in our study.

Further, as no significant morphological changes occurred in the actinomycetes strains used, agricultural waste has the potential to be used as substrate for the microbial biomass production as well as substrate for the production of metabolites like antibiotics and enzymes. Also the transformed organic waste can be used as manure.

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