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ISOLATION AND EVALUATION OF EFFECT OF CARBON AND NITROGEN ON CELLULOSE DEGRADING MICROBES

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ABSTRACT
The production and utilization of cellulolytic enzymes is a topic of great interest in the world searching for renewable resources. A study was done to determine the effect of Carbon and nitrogen sources on the growth of different microbes isolated from different soil samples. Among the 40 isolates, 7 isolates showed higher activity as compare to others .To carry out the study, nine different carbon sources and seven different nitrogen sources were used. Among the carbon sources used, maximum growth was observed in glucose followed by other carbon sources, whereas urea, showed potent result in comparison to other nitrogen sources for better survival of cellulose degrading bacteria.

INTRODUCTION

Cellulose is the most abundant biopolymer in the world. Discarded cellulosic biomass derived from forestry, agriculture and municipal sources are potential feed stock for the synthesis of biofuels that could displace fossil fuel consumption and reduced greenhouse gas emission (Levin *et al.,* 2004, 2006). Industrial bioconversion of lignocelluloses to ethanol occurs in multiple steps, where hydrolyzing enzymes are added after pretreatment of the lignocelluloses and than in an additional step, microorganisms are capable for fermentation to produced bioethanol from sugar hydrolystate (Maki *et al.,* 2009). Cellulose produced by plants is composed of both highly amorphous regions containing large voids and other irregularities as well as tightly packed crystalline regions. Cellulose, because it is resistant to most forms of degradation, accumulates within the environment, (Carol Carere *et al., 2008*).Cellulolytic enzymes also plays an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by Cellulolytic fungi, bacteria, actinomycetes and protozoa (Barman *et al.,* 2011).The lack of an economic processes for saccriharification of waste. Degradation of cellulosic material using microbial enzymes is one of the major problems yet to be solved by fermentation technology. Fermentative processes stand out, where microbial metabolism is used for the transformation of simple raw materials in products with aggregates value. (Cazetta *et al.,* 2007) Reduction of cost will involved the proper selection of strains yielding high levels of the enzymes (Tong *et al.,* 1992). When organic matter, such as compost or wood residuals, is added to topsoil, the natural organic matter content is raised considerably and it increases food sources (carbon) availability for beneficial soil microorganisms (Munten, 2005).Microorganisms brings about most of the cellulose degradation occurring in nature.

MATERIALS AND METHODS

Sample Collection: Different soil samples were collected from forest and garden area. From March 2010 to June 2011. Soil samples were dried and sieved to remove stones and other impurities and stored until use.

Enrichment procedure: Enrichment of media was done by adding 1g of soil in about 250mL of MSM broth with 1% carboxymethly cellulose. This media was inoculated with soil samples, incubated at 150 rpm for 5 days at 37ºC on orbital shaker incubator. The solution was used as stock solution .From, this 10mL sample was serially diluted upto 10^8 and intermediate dilutions (*i.e.* 10^3,10^4, 10^5, 10^6) were used for plating on MSM agar plates (2% agar). From enrichment media loopful of sample was taken and streaked over agar plates and incubated at 37ºC for 24 to 48h. This procedure was repeated till pure culture of cellulolytic bacterium was obtained. The isolated pure cultures were preserved for further study on solid MSM media at 4ºC.

Screening of cellulose degrading microbes: For screening of cellulose degrading microbes, freshly grown cultures of isolates were streaked on sterilized MSM agar plates containing 1% CMC. The plates were incubated at 37ºC for 24 to 48h. After completion of incubation period the plates were flooded

with Grams iodine solution and the zone of clearance was observed around the line of growth (Balamurugan *et al.,* 2011). Zone of diameter was measured using zone meter scale.

Effect of different carbon and nitrogen sources on cellulose degrading microbes: To study the utilization of different carbon sources Mineral Salt Broth (MSB) with 1% of different carbon sources such as glucose, lactose, galactose, fructose, mannitol, sucrose, maltose, xylose, starch were added in Mineral salt broth and their pH was adjusted to neutral *i.e.* at 7. The medium was distributed in test tubes .The tubes were autoclaved and inoculated with test organisms. The tubes were incubated at 37ºC for 24 to 48h. After completion of incubation at period, turbidity (*i.e.* growth of organism) was measured by colorimeter at 560nm, (Fig. 1).

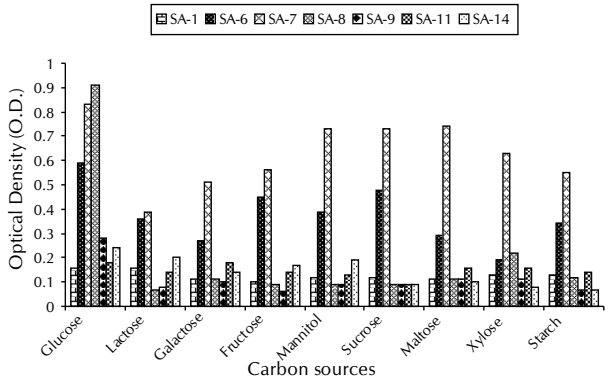


Figure 1: Effect of different carbon sources on microbial growth

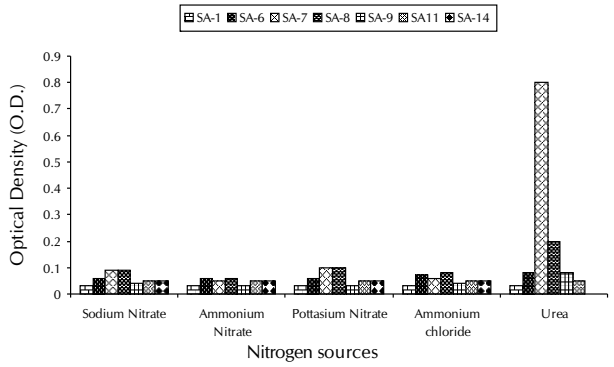


Figure 2: Effect of different Nitrogen sources on microbial growth

To study the utilization of different nitrogen sources Mineral Salt Broth with 1%of different nitrogen sources such as sodium nitrate, ammonium nitrate, potassium nitrate, ammonium chloride and urea, were added in mineral salt broth. The pH of the medium was adjusted to 7. The medium was distributed in test tubes. The tubes were autoclaved and Inoculated with test organisms. The tubes were incubated at 37ºC for 24 to 48h. After completion of incubation period, turbidity (*i.e.* growth of organism) was measured by colorimeter at 560nm (Fig. 2).

RESULTS AND DISCUSSION

Cellulose is a major component of wood. Cellulose fibers in wood are bound in lignin, a complex polymer. Cellulosic waste-materials including agricultural, forestry, and municipal wastes are among the Earth's most abundant and available as renewable resources. The decomposition of cellulose is brought about by complex communities of interacting microorganisms. Because the substrate cellulose, is insoluble, bacterial and fungal degradation occurs exogenously or endogenously. Products of cellulose hydrolysis are available as carbon and energy sources for other microbes that inhabit environments in which cellulose is biodegraded, and this availability forms the basis of many microbial interactions that occur in these environment.

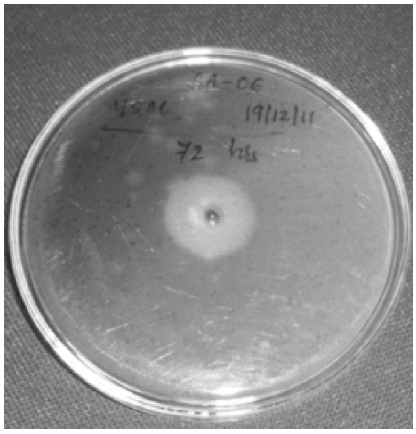
In the present investigation Forty, cellulose degrading bacterial isolates were isolated and screened out of which only 7 strains showed great enzymatic activity. Our preliminary studies indicates that these screened species have ability to degrade quickly wide range of cellulosic material.However, the zone production in cm or mm formed by cellulose degrading microbes in MSM medium. The enzyme production was carried out by plate assay method using MSM medium containing 1% CMC in order to study cellulose degrading microbes. The enzyme activity was determined by zone production in mm as shown in Table 1.The results indicated that the selected 7 strains, *i.e. Pseudomonas* Sp., *Cellulomonas* Sp., *Celluvibrio* Sp., *Sporophytophaga* Sp., *Zymomonas* Sp., *Micrococcus* Sp., *Bacillus* Sp., produced higher level of enzyme activity when compare to other strains. In present study, the effect of carbon and nitrogen sources had been observed on the growth and cellulose production.

To estimate effect of carbon utilization, 9 types of carbohydrates as a sole carbon source have been used and incorporated in MSM broth and incubated at optimum conditions.Amongs 9 carbon sources maximum growth was observed in glucose, lactose, galactose, etc. as shown in Fig. 1 where O. D. is taken at 560nm. Similar results were reported by Balamurugan *et al.,* 2011, where glucose was the most potent carbon source. The highest activity of xylanase was reported in cellulomonas species, when rice straw was used as carbon source followed by waste and xylan Sangkharak *et al.,* 2011.Our results are also in agreement with the findings of Tong and Rajendra, 1992, where effect of different carbon sources on the growth of cellulose degrading microbes was studied.

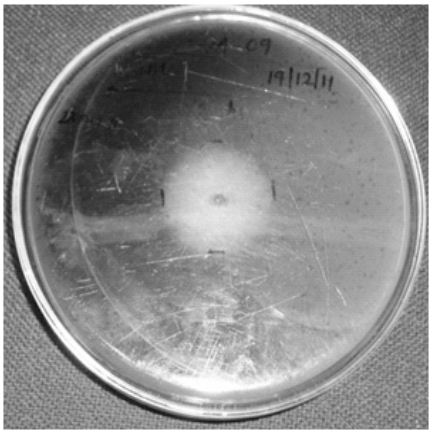
Table 1: Zone of clearance of isolated Strains

Strain No.	Incubation after 24h	After 48h	After 72h	After 96h	After 120 h
Pseudomonas spp.	11mm	18mm	33mm	35mm	40mm
Cellulomonas spp.	14mm	26mm	35mm	38mm	34mm
Celluvibrio spp.	15mm	24mm	34mm	31mm	38mm
Sporophytophaga spp.	17mm	24mm	26mm	30mm	28mm
Zymomonas spp	15mm	21mm	26mm	30mm	35mm
Micrococcus spp.	11mm	23mm	26mm	28mm	35mm
Bacillus spp.	14mm	26mm	27mm	27mm	28mm

***Pseudomonas* spp.**

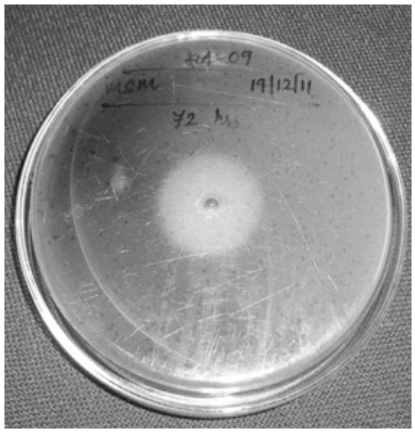


Cellulomonas spp.

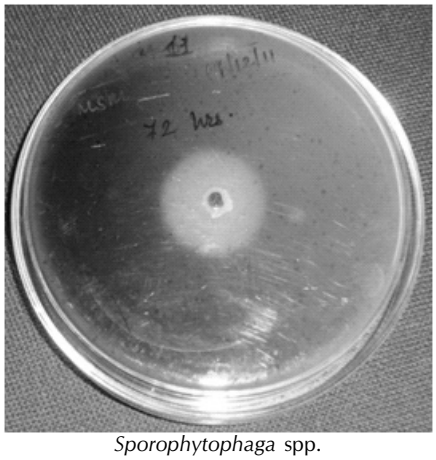


Celluvibrio spp.

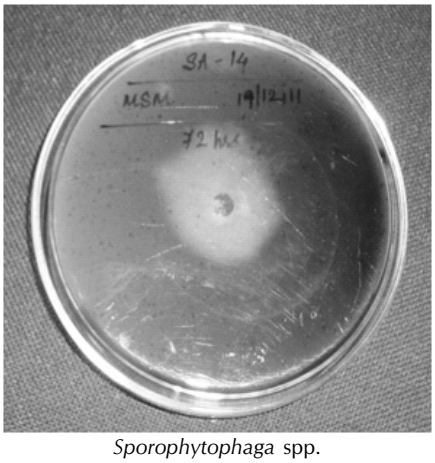
Sporophytophaga spp.



Zymomonas spp.



Sporophytophaga spp.



Sporophytophaga spp.

Figure 3: Cellulose degrading bacteria derived from soil and grown on MSM Agar. The plates were flooded with Gram's Iodine solution and zone of clearance was observed around the line of incubation

In the present investigation seven cellulose degrading bacteria viz: *Pseudomonas* Sp., *Cellulomonas* Sp., *Celluvibrio* Sp., *Sporophytophaga* Sp., *Zymomonas* Sp., *Micrococcus* Sp., *Bacillus* Sp. were characterized and confirmed by Bergey's Manual of determinative bacteriology (9th Edition).The result showed that all the well characterized bacteria were grown in different nitrogen sources. Among 5 nitrogen sources, urea has been best utilized by organisms for their better survival and among 7 strains, strain no. Bacillus sp.shows potent results as depicted in Fig. 2. The effect of 5 different nitrogen sources on the growth of cellulose degrading microbes on production of cellulase enzyme in MSM is shown in Table 2. Tong and Rajendra, 1992, worked on effect of different nitrogen source on production of cellulase activity in their attempt. Ammonium nitrate supported best mycellial growth but did not promote high level of cellulase production in comparison to other nitrogen sources which was supported by Wang, 1982; Balamurugan *et al.*, 2011, also showed the good effect of nitrogen sources for better survival of cellulose degrading microbes isolated during study.

Many fungi were able to break down polysaccharides such as celluloses and were able to convert these polymeric

compounds into sugars due to their capability to produce extracellular enzyme and cellulose Abdelnasser and Ahmed, 2007. According to Lee and Hung, 2000, *Zymomonas* sp.is able to obtain an ethanol production close to the theoretical one from glucose through Entner-Doudroff pathway under aerobic conditions. In present study, 7 potential cellulose degrading microbial strains have been isolated from of 40 strains. These 7 strains showed highest potential of enzymatic activity. Also the effect of different carbon and nitrogen sources on growth and cellulase production was carried out which showed promising results.

ACKNOWLEDGEMENTS

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