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Research Article

Production of Cellulase from the Municipal Waste Residue by a Novel Cellulolytic Fungi

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Abstract

Cellulases catalyze the hydrolysis of 1,4-β-D-glycosidic linkages in cellulose and play a significant role in nature by recycling polysaccharide debris. This enzyme has enormous potential in industries such as textile wet processing, bio-stoning of denim fabric, biopolishing of textile fibres, softening of garments and removal of excess dye from the fabrics. Therefore, the research focused on obtaining new cellulose-producing microorganisms with higher specific activities and greater efficiency. By identifying a good strain, improving the production medium and using an alternative carbon source such as waste residue, this study aimed to lower the manufacturing cost of cellulase. This study was designed to assess the cellulase production by fungi isolated from water, soil, straw, dung, leaf and goat manure. In the present research, cellulase-producing fungal isolates obtained from waste samples were identified by morphological and microscopic features. On Congo red test, the largest zone of hydrolysis was found as 1.2 cm. From the morphological and microscopic test, the fungal strain was expected as *Aspergillus* sp. The assay of the enzyme cellulase was performed by measuring the release amount of reducing sugar. Optimization of process parameters was carried out for the isolate to maximize enzyme yield. On optimization, isolated fungal species showed maximum enzyme activity at a temperature of 30 °C and pH 6. Under optimized conditions of temperature and pH, agitation at 200 rpm with a 1 L/m air flow rate showed better cellulase activity. Cellulase production from *Aspergillus* sp. can be an advantage as the enzyme production rate is normally higher as compared to other fungi.

Keywords: *Aspergillus* sp., Cellulase, Fungi, Municipal wastes, Submerged state fermentation

Introduction

Exoglucanase, endoglucanase and β-glucosidase are necessary for the complete hydrolysis of cellulose into glucose. Exoglycanase also known as 1,4-D-glucan cellobiohydrolases are typically present on crystalline cellulose and split disaccharide units from either reducing or non-reducing ends. Endoglucanase (1,4-D-glucan-4-glucanohydrolase) may hydrolyze substituted celluloses such as carboxymethyl cellulose (CMC) and hydroxyethyl cellulose (HEC) and are particularly active against the amorphous portions of cellulose (Fermandes et al., 2022). Cellobiose and other soluble

oligosaccharides are converted to glucose by β-glucosidases (Bhat and Bhat, 1997).

Municipal waste management is one of the key challenges in environmental protection (Vergara and Tchobanoglous, 2012). The current methods for treating municipal trash are anaerobic digestion, composting, landfilling and incineration. Recently, the anaerobic conversion of municipal waste into biogas has attracted more attention because it reduces environmental impact of such wastes. However, some municipal waste contains over 50% lignocellulosic content (Li et al., 2012) which is difficult for digestion so that anaerobic digestion may not represent the most efficient process. A group of enzymes primarily cellulase, are needed for the enzymatic hydrolysis of lignocellulose into sugars. Various bacteria including *Bacillus subtilis* and *Bacillus circulans* (Ray et al., 2007), *Bacillus sphaericus*-JS1 (Singh et al., 2004), *Cellulomonas flavigena* (Rajoka, 2004), and fungi such as *Aspergillus* sp. (Guruchandran and Sasikumar, 2010) and *Trichoderma viride* (Zhao et al., 2013) can synthesize cellulase.

Here, we identified as cellulase-producing fungus from water, soil, straw, dung, leaf and goat manure, and optimized the conditions for better production of cellulase enzyme.

Materials and Methods

Sample collection

Municipal waste samples were collected from the Tribhuvan University premises, Kirtipur, Kathmandu. It was collected with a sterilized spatula and stored in sterile bottles.

Isolation of fungi

About 1 g of solid sample was transferred to 9 ml sterile distilled water and 1 ml of liquid sample was also transferred to 9 ml sterile distilled water in test tubes. It was then shaken vigorously at a constant speed for 15 min. The suspension was then subjected to serial dilutions in the plate in duplicate. The plates were incubated for 5 days at 30 °C in Accuma digital temperature controller incubator. Then, well-grown single colonies of isolated fungi were picked up and sub-cultured on potato dextrose agar slant.

Screening for cellulase enzyme

Municipal waste samples were tested using fungi having ability to produce the hydrolytic enzyme cellulase in a plate assay method using 1% carboxymethyl cellulose in a basal salt medium and incubated at 30 °C. After incubation, 0.1% Congo red solution was flooded on plate and counterstained with 1 M NaCl for 15–20 minutes. The zone of cellulose hydrolysis has appeared as a clear area around the colony (Gautam et al., 2011).

Production of cellulase enzymes

Enzyme production was done in liquid broth media. Fungal stains having largest hydrolysis zone in Congo red test were used for enzyme production on basal salt medium containing 1% cellulose as a sole carbon source. The medium was composed of KH_2PO_4 (2 g/L), $(NH_4)_2SO_4$ (0.14 g/L), $CaCl_2$ (0.30 g/L), $MgSO_4.7H_2O$ (0.30 g/L), $CoCl_2.6H_2O$ (2 g/L), $MnSO_4.H_2O$ (1.56 g/L), $FeSO_4.7H_2O$ (5 g/L), $ZnSO_4.7H_2O$ (1.4 g/L) and 1% CMC. This test was done for confirmation of cellulase enzyme production by isolated fungal strains in broth media which can be used in further experiments (Moura et al., 2020).

Optimization of culture conditions for enzyme production

For fermentation, a basal salt medium with CMC was used. Then optimization of physical parameters such as temperature, pH, agitation speed and aeration rate for better production of cellulase enzyme was done. The enzyme assay was carried out by the DNS method in triplicate (Karthika et al., 2020).

Effect of temperature on enzyme production

The optimal temperature for cellulase production by the isolated fungal strain was determined. A flask with 50 ml of basal salt broth was used for fermentation up to 6 days at intervals in the range of temperature 25, 30, 35, 40, and 50 °C. Then enzyme activity was determined by DNS method using UV-spectrophotometer.

Effect of pH on enzyme production

For the determination of optimal pH, the isolated fungus was cultivated in a 150 ml flask containing a

50 ml basal salt broth medium with different pH ranges from 4.0 to 8.0. The pH of the medium was adjusted by using 1 N HCl or 1 N NaOH. The flasks were kept in a stationary stage at an optimized temperature from above result. Then, enzyme activity was determined by DNS method using UV-spectrophotometer.

Effect of agitation on enzyme production

After finding of optimal temperature and pH for better production of enzyme, fermentation was carried out in bioreactor. About 3.5 L of sterilized basal salt media was taken in bioreactor at optimum temperature and pH. Then, medium in bioreactor was inoculated with a spore suspension of isolated fungal strain and mixed thoroughly. Agitation was carried out at 100 and 200 rpm respectively from which optimal agitation for enzyme production was determined. Enzyme activity was also determined by DNS method.

Effect of aeration rate on enzyme production

Submerged fermentation was carried out in the bioreactor for determination of effect of aeration rate in enzyme production. The temperature, pH and agitation were set according to the above results and aeration rate was varied. Three different aeration rates were used for determining the better condition of enzyme production; 0.5 L/min, 1 L/min and 2 L/min. Cell growth was monitored in every 24 h to evaluate the increase or decrease in the concentration of cellulase.

Statistical analysis

Data were presented as the mean of three replicates (±SE) obtained from their independent experiments using Graph pad prism 8.0.2.263.

Results and Discussion

Isolation and identification of organism

The fungal organisms grown on the potato dextrose agar plate was shown in Figure 1. The morphology of fungal isolates forms filamented hyphae. Microscopic observation of the organism was done using lactophenol cotton blue stain which shows smooth and colourless conidiophores and spores. On staining with cotton blue, the organisms were identified as *Aspergillus* sp. as shown in Figure 2.

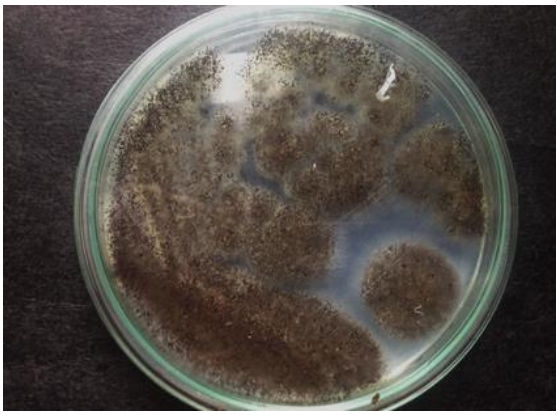

Figure 1: Pure culture of fungal strain isolated from the sample obtained after 5 days of incubation at 30 °C.

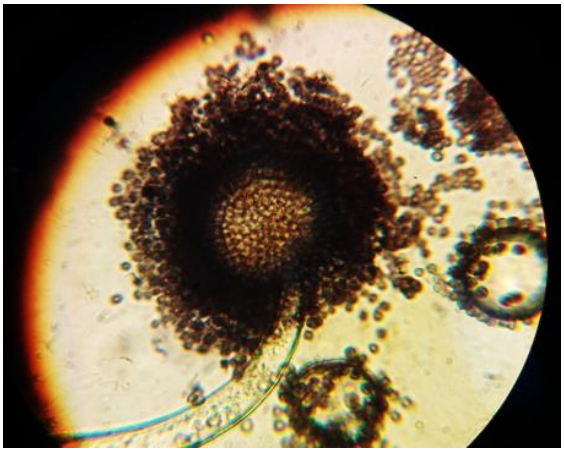

Figure 2: Microscopic view of isolated fungal strain.

Screening of enzyme

Screening of fungi for their cellulase activity was carried out by the hydrolysis of substrate incorporated into the basal salt medium. After the incubation period, enzyme activities were detected by the appearance of zones either by substrate clearances or colouration and discolouration around the fungal colonies as shown in Figures 3 and 4. The diameter of the hydrolysis of the cellulose zone was measured given by fungal isolates from different sources shown in Table 1.

Table 1: Cellulose hydrolysis zone formation by a fungal isolate of different sample.

S.N.	Sample	Diameter of zone
1	Cow dung	12 mm
2	Goat feces	10 mm
3	Straw	6 mm

Figure 3: Cellulose hydrolysis seen as clear area where fungal isolate was cultured.

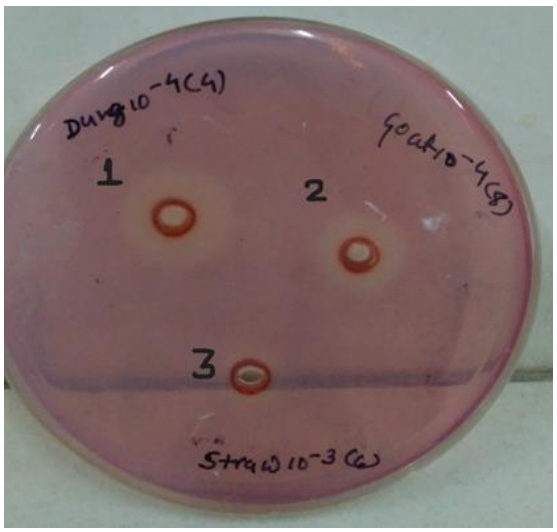

Figure 4: Zone of hydrolysis of isolated fungus strain from different samples.

Optimization of culture conditions for enzyme production

The effect of different temperatures on cellulase production was shown in Figure 5. The highest enzyme activity was 0.42±0.02 mg/ml shown by isolated fungal strain at 28-30 °C after 5 days of incubation at different temperatures. Due to high temperature, the results showed that the enzyme activity decreases when the temperature is increased above 30°C. Similarly, the result showed that the production medium having pH 6 yielded the highest concentration of glucose about 0.155±0.007 mg/ml after 5 days of incubation (Figure 6). According to this result, the isolated organism has optimum pH of 6 for better enzyme production capability. Then, the effect of agitation also varied in cellulase enzyme production (Figure 7). Production medium agitated with 200 rpm showed better enzyme activity i.e., 1.08±0.028 mg/ml glucose generation than with 100 rpm. From which, it was concluded that agitation at 200 rpm can be better than at 100 rpm for enzyme activity. Finally, the effects of aeration were also observed in the production of broth medium. For this, aeration was done with different flow rates (2, 1 and 0.5 l/min) showing the highest enzyme activity after 6 days of incubation with concentrations of 1.08±0.028 mg/ml, 1.28±0.1 mg/ml and 0.78±0.12 mg/ml respectively (Figure 8).

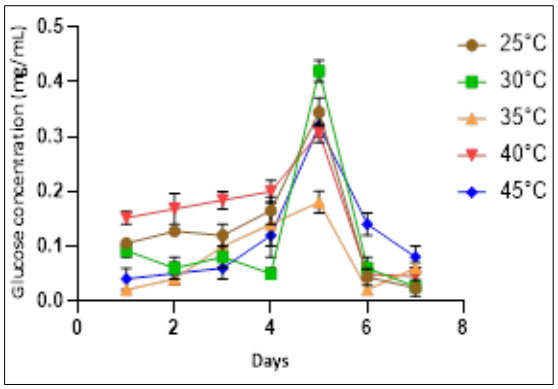

Figure 5: Effect of temperature on cellulase activity produced by isolated fungal strain.

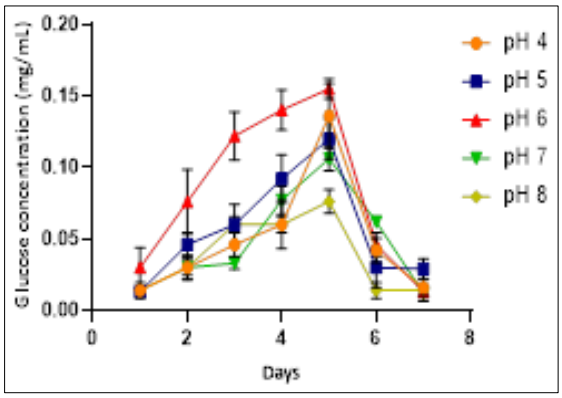

Figure 6: Effect of pH on cellulase activity produced by isolated fungal strain.

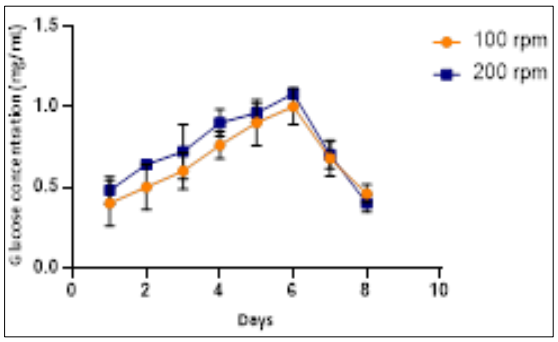

Figure 7: Effect of agitation on cellulase activity produced by isolated fungal strain.

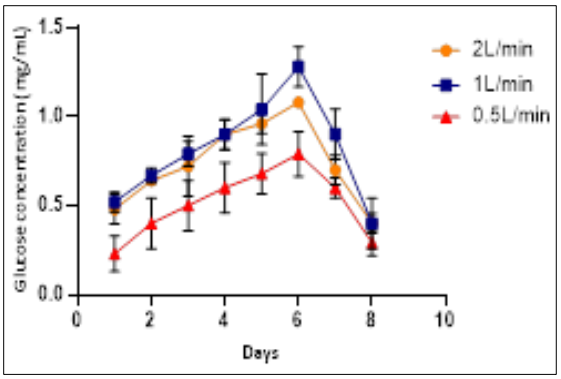

Figure 8: Effect of aeration on cellulase activity produced by isolated fungal strain.

From this study, the favourable conditions for better enzyme production were that the pH of the medium should be 6 at a temperature of 30 °C with the agitation of 200 rpm and a 1 l/min aeration rate. Cellulase production from *Aspergillus* spp. can be an advantage as the enzyme production rate is normally higher as compared to other fungi according to Gautam et al. (2011).

Conclusion

Among three isolated fungal strains, one strain was identified as *Aspergillus* spp. based on cultural, morphological and microscopic characteristics was used as inoculum. It effectively produced cellulase and showed the highest enzyme activity. Submerged fermentation was done for optimization of the culture condition of isolated fungal strain which has a higher activity of enzyme production. The more enzyme produced the higher amount of glucose in the medium because the cellulase enzyme breakdown the cellulose substrate into glucose monomers. It was determined from the results of the study that the best culture conditions for the production of cellulase enzyme were medium having pH of 6 incubated at 30 °C for 144 hours with agitation at 200 rpm and aeration at 1 L/min.

Acknowledgements

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