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Comparative Study of Cellulase of *Trichoderma viride* and *Aspergillus niger*

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Aspergillus niger and *Trichoderma viride* were grown in wheat bran medium by both surface culture and submerged culture techniques. Some characteristics of cellulases produced under different conditions were compared. The results indicated that *Aspergillus niger* produces cellulase more than that which produces *Trchoderma viride* in surface culture On the other hand, *Trichoderma viride* produces cellulase more than that produced by *Aspergillus niger* in submerged culture. The comparison of profiles indicated that the production of cellulase by surface culture technique is better than that by the submerged culture technique. The temperature optima of cellulase of *Aspergillus niger* and *Trichoderma viride* were 50 and 30 ºC, respectively. The cellulase of *Aspergillus niger* was rather more thermostable.

Key Words: Cellulase, *Trchoderma viride*, *Aspergillus niger*.

INTRODUCTION

Cellulose is the most abundant biological material, which occurs in nature in the form of vegetable matter. It is also the major agricultural waste that can be used as a source of fuel. As the Third World needs security of energy supply in order to accelerate its future development, cellulose in the form of agricultural waste may be a potential source of energy for these countries in future. Its use as a source of energy is greatly simplified if cellulose is first hydrolyzed to its monomer that is glucose, which, being a fermentable sugar can be, subsequently, hydrolyzed to alcohol that is an important motor fuel. The best way of this conversion may be the enzymatic hydrolysis because the reaction takes place under moderate conditions. The enzyme involved for its breakdown into glucose is cellulase. The conversion of cellulosic biomass into fermentable sugar needs an economical process for the production of cellulase. This enzyme is reproduced microbiologically, by bacteria, fungi and some invertebrate animals[1].

Glucose is the major product of cellulysis and is also a major nutrient that supplies energy to the living organism. Thus, growing cellulytic microorganisms on cellulosic waste to convert cellulose into glucose increases their nutritive value. Moreover, glucose produced is a fermentable sugar that is converted into alcohol, a promising future motor fuel.

Keeping the utilization of cellulases for different purposes under focus, a large number of research studies have been carried out all over the world. The major projects of study have been the transformation of agriculture waste into sugar and characterization of cellulases of different origin[2-11].

Some work has also been carried in Pakistan to transform cellulosic wastes into sugar[12,13]. As the enzyme involved in this transformation is usually the cellulase of microbial origin, the work reported here was on the comparative production of this enzyme by *Aspergillus niger* and *Trichoderma viride*. The major objective of work was the increased production of cellulase.

EXPERIMENTAL

Culture and inoculum: *Trichoderma viride* was grown on solidified potato-dextrose-agar slants at 28 ºC. For the preparation of culture medium, 200 g potato slices were boiled for 1 h in *ca.* 500-700 mL distilled water. The resulting thick syrup was drained through a clean muslin cloth. To this was added glucose and agar. The final volume was made up to 1000 mL. The mixture was cooked for 1 h in a water bath. The pH of the medium was maintained at 4.5 *ca.* 10 mL of medium was poured in each test tube. All the test tubes were cotton plugged. The medium was sterilized in a pressure cooker at 15 psi for 15 min. The test tubes were allowed to set for 24 h to prepare the slants. The slants were inoculated with a sterilized needle loop and incubated at 30 ºC. The growth was allowed to occur for 4-6 days. The slants were preserved in a refrigerator. The culture medium had the following composition: Potatoes, 200 g/L, dextrose 20 g/L, Agar, 15 g/L, pH 4.5.

To prepare the inoculum, the slants were washed carefully with sterilized distilled water and a sporal suspension was obtained. The spores were centrifuged at 2500 rpm for 20 min in a sterilized centrifuge tube. The supernatant was discarded and the pallet was suspended in an adequate volume of sterilized distilled water. The optical density of the suspension was determined by a spectrophotometer. The suspension of the same optical density was transferred each time to keep the total population of spores constant; 10-20 mL of sporal suspension was transferred to each of the flasks containing 250 mL wheat bran medium and 30 mL glucose solution.

Fermentation medium: Wheat bran was chosen for the growth of *Trichoderma viride* as it was considered to be a suitable medium for the production of extra-cellular cellulase. The growth medium for *Trichoderma viride* was prepared by mixing the following quantities of ingredients per liter. Wheat bran 50 g, K_2HPO_4 2.0 g, KCI 0.54 g, $MgSO_4 \cdot 7H_2O$ 0.5 g.

The above quantities of ingredients were mixed in distilled water to make 1 L suspension. The pH of the suspension was adjusted to 4.5. The suspension was then sterilized for 0.5 h at 15 psi. 20 g glucose was dissolved in 250 mL of distilled water and was sterilized separately for 0.5 h. 250 mL of wheat bran medium was taken in different 500 mL conical flasks. To each flask then was added 30 mL of sterilized glucose solution to make final concentration of glucose 1 %. The flasks were cotton plugged and were ready for inoculation.

Fermentation: The organisms were initially grown by surface culture technique. The surface of the medium contained in flasks was inoculated using 10 mL of inoculum and subsequently incubated in an incubator. The growth temperature was 30 ºC. After 3 or 4 days when the growth of the organism had started, *ca.* 5 mL suspension was taken out with a sterilized pipette. The suspension was filtered and the enzyme activity of the extracellular cellulase present in the filtrate was assayed taking 0.2 mL filtrate.

For fermentation by submerged culture technique, the same procedure was followed except the flasks after transfer of inoculums were given continuous shaking in an orbital shaker at 150 rpm. The samples were similarly withdrawn and assayed.

Assay of cellulase activity: The cellulase activity was assayed by the method of Garge and Kantan[14] based upon the reaction of the enzyme on cellulose substrate and subsequent determination of reducing sugar colorimetrically.

To assay, 0.2 mL enzyme sample was incubated with 50 mg filter paper substrate and 0.8 mL 0.05 M citrate buffer in a 25 mL test tube for one hour at 40 ºC. 1 mL of 3,5-dinitrosalicylic acid reagent was then added to 1 mL aliquot of the mixture. The mixture was heated for 5 min in a boiling water bath and cooled under cold running tap water and filtered. A blank was prepared in another test tube as above but heat denatured enzyme was used in place of the enzyme sample. The optical density of the colour developed was read in a spectrophotometer at 575 nm.

A standard curve was constructed by plotting optical density against known concentrations of glucose. The optical density units were converted to the micromoles of glucose by comparison with the standard curve.

The unit of cellulase activity was defined as the amount of the enzyme that released 1 µm of glucose from the cellulosic substrate under the assay conditions defined.

Determination of enzyme characteristics

Effect of temperature: The effect of temperature on cellulase activity of the crude enzyme was studied within the range 30-70 ºC. 0.2 mL of the test sample was incubated with 50 mg filter paper in an incubator for 1 h at 30, 40, 50, 60 and 70 ºC. The enzyme activity was assayed as above. The experiment was repeated at 40, 45 and 50 ºC. The change in optical density was plotted as a function of temperature.

Thermostability: 0.2mL enzyme sample taken in different tubes was subjected to the temperature shock at 30, 40, 50, 60 and 70 ºC for 15 min. The residual activity of all samples was assayed at 40 ºC as above. The residual activity was plotted as percentage of original activity against rising temperature.

Effect of pH: The effect of pH on the cellulase activity was studied in the pH-range 3-8 using citrate-phosphate buffers of different pH. 0.2 mL of the enzyme sample was mixed with 0.8 mL of each buffer containing 50 mg filter paper and the mixture was incubated for one hour at 40 ºC. The change in optical density was plotted as a function of pH.

RESULTS AND DISCUSSION

The variation of cellulase activity with the incubation time during the growth of *Aspergillus niger* and *Trichoderma viride* in by surface culture technique is shown in Fig. 1.

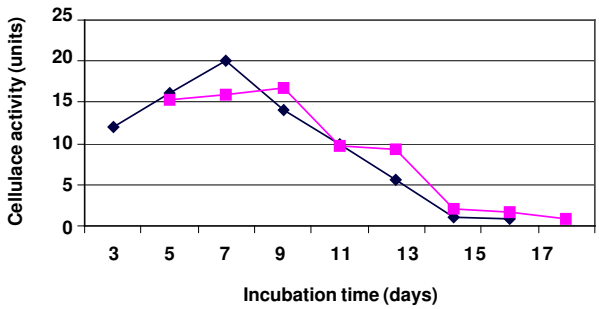

Fig. 1. Variation of cellulase activity with incubation time during the growth of *Aspergillus niger* (^) and *Trichoderma viride* by surface-culture method

The profiles indicate that both organisms produce significant quantities of cellulase when these are grown in 5 % wheat bran medium with glucose as major source of carbon and energy. *Aspergillus niger*, produces cellulase more than that produced by *Trichoderma viride*. The peak values for the organisms are 20.0 and 16.8, respectively.

The variation of cellulase activity with incubation time during the courses of fermentations of *Aspergillus niger* and *Trichoderma viride* by submerged culture technique is shown in Fig. 2.

The results indicate that both organisms in submerged culture produce significant quantity of cellulase. *Trichoderma viride*, of course, produces more cellulase activity than *Aspergillus niger*. The peak values, for the organisms are 7.2 and 5.6, respectively.

TABLE-1
CHARACTERISTICS CELLULASE OF *A. niger* AND *T. viride* ARE COMPUTED

	Temperature optima (ºC)	Thermostability	Total deactivation (ºC)	pH-optimum
Aspergillus niger	50	Stable up to 50 ºC	90	5.0
Trichoderma viride	40	Stable up to 40 ºC	85	5.0

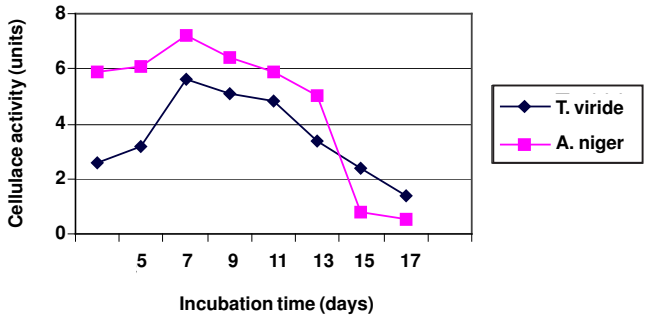

Fig. 2. Variation of cellulase activity with incubation time during the growth of *Aspergillus niger* and *Trichoderma viride* by submerged culture method

The comparison of the amounts of cellulase produced by *A. niger* and *T. viride* by both surface and submerged culture techniques is shown in Fig. 3.

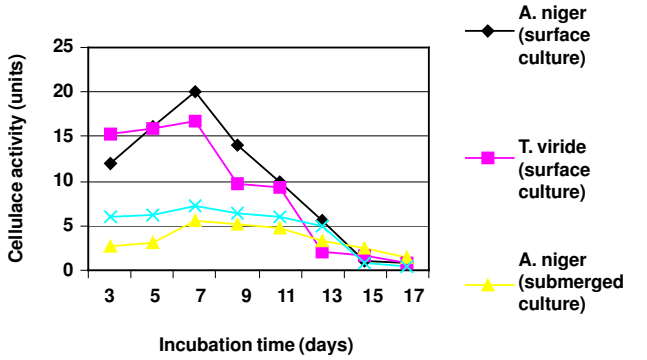

Fig. 3. Comparison of production of cellulase by *A. niger* and *T. viride* by both surface and submerged culture methods

The comparison of the profiles in Fig. 3 indicates that both organisms when grown in 5 % wheat bran medium with 1 % glucose by submerged culture technique produce significant quantities of cellulase. *Aspergillus niger*, of course, produces cellulase most favorably in the surface culture.

Our results are in good agreement with the results reported by Nuzhat and Khan[13], who reported that the production of cellulase by *Aspergillus niger* in surface cultures was significantly higher as compared to that produced by the organisms such as *Aspergillus terreus*[11], *Trichoderma viride*[5] and *Stachybutyra atra*[4].

The characteristics cellulase of *A. niger* and *T. viride* are given in Table-1.

The characteristic of the cellulase of *Aspergillus niger* reported by Nuzhat and Khan[12] are pH optimum 4.5, temperature optimum 45 ºC and thermostability up to 45 ºC and complete deactivation at 90 ºC, but here, pH-optimum was 5.0, temperature optimum was 50 ºC, while the enzyme completely deactivated at 85 ºC. The characteristics being reported by us are not much different from those reported by the said workers.

Ikram-ul-Haq[15] reported the pH-optimum for the cellulase of *Trichoderma viride* as 4.8 that is quite in agreement with that being reported by us that is 5. He reported the temperature optimum 28-32 ºC and that in our case is 40 ºC. Similarly, another worker Hameed has recently reported the pH-optimum of the cellulase of another *Trichoderma* species as 4.8[16].

REFERENCES

1. Symposium on Cellulases and Cellulosomes, Proctor Academy Andover, NH, July 29-Aug 3 (2001).
2. E.D. Cowling and W. Brown, Advances in Chemistry Series, American Chemical Society Publications Washington, DC, Vol. 93, p. 152 (1969).
3. S.H. Emert *et al*., Cellulases and Their Applications, Advances in Chemistry Series, American Chemical Society Publications Washington, DC, Vol. 126, 79 (1974).
4. M. Mandels, J.E. Medeiros, J. E. Andreotti and R.E. Bissett, *Biotechnol. Bioeng*., **23**, 2009 (1981).
5. Waliuzzaman, *Chem. Eng. Res. Bull. (Dacca)*, **1**, 17 (1980).
6. A. Jabbar and A. Ilahi, *Agric. Biol. Chem*., **45**, 2395 (1982).
7. C. David and P. Thiry, *J. Appl. Polym. Sci.*, **27**, 2395 (1982).
8. N. Tomoya, Proc. Int. Symp. Ethanol Biomass, 1982 Publication 1983, pp. 570-621 (1984).
9. S.M. Betrabet and K.M. Paralikar, *J. Sci. Ind. Res.*, **25**, 1311 (1983).
10. Y.A. El-Zawari and T.Y. Mustafa, *Isot. Radiat. Res*., **15**, 103 (1984).
11. A. Singh, *World J. Microbiol. Biotechnol*., **6**, 330 (1990).
12. J. Nuzhat and M.R. Khan, Proceedings of The First National Biochemistry Symposium (1991).
13. A.M. Majid and M.R. Khan, *Sci. Int*., **15**, 287 (2003).
14. S.K. Garge and N. Kantan, *Biotechnol. Bioeng*., **24**, 737 (1982).
15. I. Haq, PhD Thesis, University of Punjab, Lahore, Pakistan, pp. 57-59 (1992).
16. U. Hameed, M.Sc. Thesis, University of Punjab, Lahore, Pakistan, pp. 58-60 (2003).