PRODUCTION AND PURIFICATION OF CELLULASE FROM *Trichoderma viride* BY OPTIMIZED MEDIUM USING VARIOUS AGROWASTE FOR POULTRY FEED SUPPLEMENT

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Abstract— **In India, Tamilnadu ranks second in poultry meat and egg production. Poultry feed manufacture has undergone an enormous expansion and development during the past half century throughout the country. The cellulase was produced from Trichoderma viride which can degrade cellulose .Then it was given as feed supplementary for the poultry animals. Trichoderma viride was isolated from soil and is allowed to grow in Potato dextrose Agar (PDA). For the effective production of T.viride, various agro wastes such as wheat bran, rice brawn, molasses, saw dust, and beet root were used. The produced Trichoderma viride and cellulase were given as feed supplementary to the chicks in order to increasing their digestibility and growth. The maximum biomass production of Trichoderma viride was observed in beetroot media. With these facts in the background, the present study has been designed to explore the technology to synthesize cocktail fibro lytic enzymes at farm gate level and make the farmers as successful entrepreneurs.**

***Index Terms*— *Trichoderma viride,* Cellulase, Gel filtration chromatography, Ammonium precipitation method.**

I. INTRODUCTION

Feeding enzymes to poultry is one of the major nutritional advances in the last fifty years. Plants contain some compounds that either the animal cannot digest or which hinder its digestive system, often because the animal cannot produce the necessary enzyme to degrade them. So they need exogenous enzymes in feed to aid digestion. Cellulose is the most abundant biomass on the earth. Cellulose, a crystalline polymer of D-glucose residues connected by β-1, 4 glycosidic linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature. A number of fungi and bacteria are capable of producing multiple groups of enzymes, which are collectively known as cellulases that act in a synergistic manure to hydrolyze the β-1, 4-D-glycosidic bonds within the cellulose molecules. Fungi are the main cellulase-producing microorganisms, though a few bacteria and actinomycetes have also been recently reported to yield cellulase activity. Microorganisms of the genera *Trichoderma* and *Aspergillus* are thought to be major cellulase producers, and crude enzymes produced by these microorganisms are commercially available for agricultural use. The fungus *Trichoderma viride* is a single-celled fungus that can reproduce by splitting them and describe the active cellulose contained in the soil. Some newly developed agro industrial wastes used for cellulase production are banana wastes, rice straw, corn cob residue, rice husk, wheat straw, banana fruit stalk, and coconut coir. One of the main reasons for supplementing wheat- and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility**.** In addition, this review demonstrates that enzymes are a very useful tool in the study of physiological and metabolic mechanisms of poultry animals. Our aim is to convert each and every farmer into an entrepreneur who can be benefitted even from the agro waste to produce cellulase using *T.viride* at lower economic cost.

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II. METHODS

2.1 Isolation and identification of *Tricoderma viride*

The fungal microorganism *Tricvhoderma viride* was isolated from the agricultural land soil and serial diluted with sterile saline water. Examination of the shape, size, arrangement and development of conidiophores or phialides provided a tentative identification of *Trichoderma sp*. Characteristics were observed for identification and the plates were stored at 4˚C.

2.2 Prodution of *Tricoderma* viride using various substrates

In this study *Trichoderma sps.*was produced by liquid state fermentation by using five different substrates such as rice brawn, wheat bran, saw dust, molasses, carrot and beetroot. The selection of this substrate is specified because those rich in the source of carbohydrate and it helps in the production of *Trichoderma viride*. These five substrates are stored in sanitary condition after the sterilization in sanitary condition for further use.

2.3 Biomass production

Biomass production was measured as dry weight (mg). After filtering, the substrate and the retained cell mass was dried at 70 ^{0}C in an oven until constant weight and then measured.

2.4 Production and extraction of cellulase

After stipulated fermentation time period, cellulase was extracted from the fermented biomass by adding distilled water as extraction solvent in 1:10 (w/v) ratio and the flasks were shaken at 120 rpm for 30 minutes. The contents were filtered through muslin cloth and washed thrice with distilled water. The filtrates were centrifuged at 10,000 × g (4°C) for 10 minutes and carefully collected supernatants were used for enzyme activity determinations and also used for purification purposes.

2.5 Screening of cellulase activity using Congo red clearing zone assay

Grow isolated microbes, which is needed to be screened for cellulase production in CMC agar, (carboxy methyl cellulose it is soluble form of cellulose) $NaNO_3$, K_2HPO_4, KCl, $MgSO_4$, yeast extract, glucose. The medium should be solidified using 1.7 % w/v agar. Grow culture 2-3 days at 25-30^oC and the screening can be done using congo red clearing zone assay.

Congo red clearing zone assay is suitable for qualitative display of cellulase activity. After incubation plates which contain the circular batches of isolated microorganisms are flooded with 0.1% congo red solution and left for 15 min with intermittent shaking. Then destained with 1M NaCl solution and washed with HCl solution. The clearing zone of enzymatic activity will be visible around the batch of growth.

2.6 Protein estimation by Lowry's method

Different adequate concentration of standard protein solution is taken. The volume in all test tubes is made up to 3ml by using distilled water. Then Folin Ciocalteau reagent is added to each test tube and a standard graph was plotted.

2.7 Enzyme assay

Cellulase activity was measured by DNS method through determination of reducing sugar liberated. A set of five test tube taken, CMC solution, crude enzyme and Citrate was added to the respective tubes to the blank and all the tubes were incubated at 50C for 30 minutes. After incubation, DNS solution was added to all the tubes. The treated samples were boiled for 15 minutes prior to cool down in cold water for color stabilization. The optical density was read at 540 nm against reagent blank UV-Vis spectrophotometer.

2.8 Purification

The control and UV samples were centrifuged for 10 minutes at 10,000 rpm consecutively at 4ºC. The supernatant was collected and enzyme assay was performed as above. Finally, samples were taken for purification by using ammonium sulphate precipitation, dialysis and Gel filtration chromatography.

2.9 Salt precipitation/ salting out

For cellulase, ammonium precipitation was carried out, 33.15 g of ammonium sulphate for 75 ml of control and 30.498 g of ammonium sulphate for 69 ml of test sample was added pinch, under ice cold conditions with continuous stirring on a magnetic stirrer for 45 minutes .After incubation, the precipitated enzymes were centrifuged at 10,000 rpm for 10 minutes. Pellet was collected and dissolved in 10 ml of 10mM Tris –HCL buffer which was later subjected to dialysis. Before the dialysis, the bag was weighed and the value was noted.

2.10 Determination of molecular weight

The protein samples and protein marker (Broad range Marker) were loaded into the wells. The tank was then connected to power supply and the gel was run at 100V until the dye reaches the bottom of the gel. The gels were detached from the glass plates and then subjected for staining. The gels are placed in staining solution for 2 hrs. This was allowed by placing in the de-staining solution for overnight. The protein bands were observed and recorded.

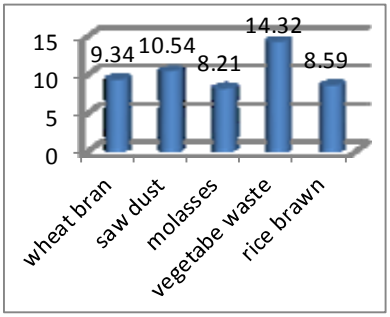
III. RESULTS AND DISCUSSION

3.1 Isolation of *T.viride* on PDA agar medium:

Figure I - *Trichodermaviride*

The fungi was isolated from a sample of soil taken from agro-field and grown in a Potato Dextrose Agar plate and incubated at 37ºC and green colonies were observed.

3.2 Biomass production of *Trichodermaviride* observed in various media:



Graph I - Biomass production

Among the five medium 14.32 gram per litre of *Trichodermaviride* was produced from beetroot.

3.3 Conformation of cellulose activity by Congo red method:

Figure II – Congo red method

Enzyme activity for crude sample:

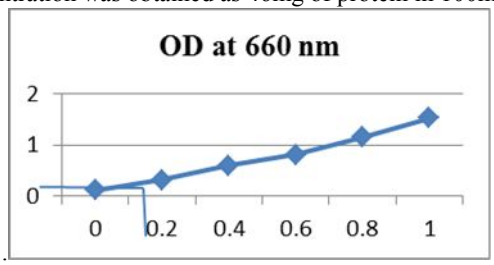
Table I – Enzyme activity

S.NO	OD at 540nm CONTROL	OD at 540nm SAMPLE
1.	0.454	0.670

Thus the congo red method was performed and the enzyme activity of crude sample was obtained as 0.251micromole/100ml or 1U/100ml

3.4 Lowry method for protein estimation:

From the lowry's method for protein estimation the concentration was obtained as 46mg of protein in 100ml



Graph II - Protein estimation

Cellulases are the group of hydrolytic enzymes and they are capable of degrading all types of lignocellulosic materials. Cellulases have wide range of applications. Present work focuses on the factors for improvement of enzymatic hydrolysis of various agricultural wastes by using different fungal species. It was the aim to study the Cellulase enzyme production ability of fungal strains against the lignocellulosic bio-waste like Rice husks, Millet husks, Banana peels, Wheat bran, and Coir waste and saw dust.

CONCLUSION

Agricultural waste in the form of cellulose which is the most abundant renewable biomass in the biosphere has been shown to be used in the production of valuable products by microorganism. Some of these agricultural wastes used in this work as fermentation substrate which produced a large amount of cellulase enzymes by *Trichoderma viride*

From the study it was clear that beetroot is the substrate that was yield high amount of of *Trichoderma viride* on liquid medium about 14.32gm/250 ml when compared to other substrates and it shows high enzyme activity. The purified cellulase has molecular weight of 58 kDa with an optimum activity at pH 8 and 55°C.

Our aim of this study involves the cost effective production of cellulase from *Trichoderma viride* by using various agro waste in order to make each and every farmer as an successful entrepreneurs.Then the produced cellulase can given as feed supplement for poultry animals.

REFERENCE

[1] Adams, E.A. and R. Pough.(1993). Non-starch polysaccharides and their digestion in poultry. Feed Compounder 13: 19-21.

[2] Ahmed, I., Zia, M.A. and Iqbal, H.M.N. (2010) Bioprocessing of proximally analyzed wheat straw for enhanced production of cellulase through parameters optimization with *Trichoderma viride* under SSF. *International Jour-nal of Biological and Life Sciences*, 6, 164-170.

[3] Ahsanur rahman1, most. ferdousi begum1, Matiur rahman1, M. A. bari1,Akiba,S.Kimura,.Yamamoto, and Kumagai, H. 1995. Purification and characterization of protease resistant cellulose from Aspergillusniger. J. Ferment. Bioeng. 79:125-130.

[4] Ali, Nagham1, Yazaji, Sabah hajali, A.2and azmeh, M.F.1 National Commission for Biotechnology, Damascus, Syria (corresponding author: N.Ali Department of Food Science, faculty of Agriculture, Damascus University

[5] A.S. Ponnambalam1, R.S. Deepthi2, A.R. Ghosh Qualitative Display and Measurement of Enzyme Activity of Isolated Cellulolytic Bacteria Research Article, Biotechnol. Bioinf. Bioeng. 2011, Society for Applied Biotechnology.

[6] Beauchemin, K., A., L. M. Rode, and V.J.H. Sewalt. 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. 75:641-644.

[7] Bedford, M.R. and H. L. Classen. (1993). An in-vitro assay for prediction of broiler intestinal viscosity and growth when fed rye based diets in the presence of exogenous enzymes. Poultry science 72: 137-143.

[8] Bhatt, R.S., S. Manoj and B. S. Katoch. (1991). Effect of supplementation of diet with fibre degrading enzyme on performance and nutrient utilization in broilers. Indian Journal of animal Nutrition 8(2): 135-138.

[9] Choct, M. and G. Annison. (1990). Anti-nutritive activity of wheat pentosans in broiler diets. British Poultry Science 30: 811–821.

[10] Creswell, D.C. (1994). Upgrading the nutritional value of grains with the use of enzymes. Technical bulletin, American Soybean Association, 341 Orchard Road No.11-03 Liat Towers, Singapore.

[11] Dunn, N. (1996). Combating the pentosans in cereals. World Poultry 12(1): 24-25.

[12] F. M. Khattak, T. N. Pasha, Z. Hayatand A. Mahmud, Department of Poultry Production. Department of Animal Nutrition ,University of Veterinary and Animal Sciences, Lahore, Pakistan .J. Anim. Pl. Sci. 16(1-2): 2006

[13] Gamal,M.Abdel-Fattah,Yasser M.Shabana,Adel E.*T* .harizianu*m:* mycophathalogy,2007,164-81-89.

[14] Gielkens, M. M. C., Dekkers, E., Visser, J. and Graaff, L. H.1999. Twocellubiohydrolase-ncodinggenes from Aspergillusniger require D-xylose and the xylanolytic transcriptional activator XlnR for their expression.

[15] Mantyla, A., Paloheimo, M. and Suominen,P. 1998. Industrialmutants and recombinant strains of Trichodermareesei. In:Harman, G. F. and Kubicek, C. P. (eds) Trichoderma&Gliocladium-Enzymes, biological control and commercial applications (2nd ed), Taylor & Francis, Ltd. London.