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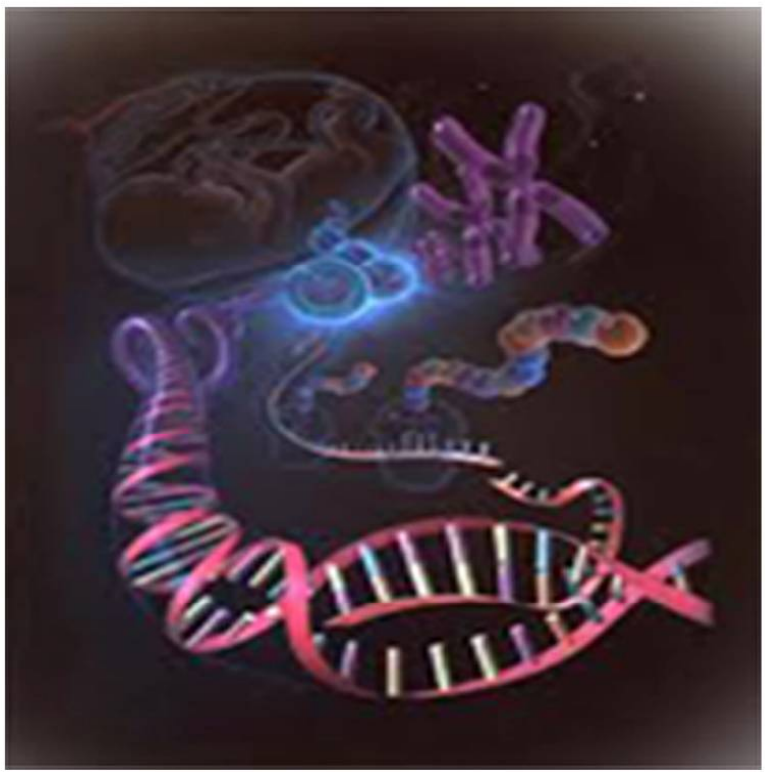

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Research Paper

EFFECT OF SUPPLEMENTARY CARBON AND NITROGEN SOURCES ON AMYLASE PRODUCTION BY *Trichoderma Viride* IN SOLID STATE FERMENTATION

Lalitha P[1*], Shravya K[1], Arunalakshmi K[2]

Corresponding Author:* ***Lalitha P,* ✉ *pappulalitha@gmail.com*

The combinatorial effect of supplementary carbon and nitrogen source (carbon source – glucose, fructose, maltose and sucrose, nitrogen source – ammonium nitrate, sodium nitrate, yeast extract and peptone) in the production of amylases by *Trichoderma viride* was studied in solid state fermentation using corn cob residue as the substrate. We have observed that potential of solid state systems is immense when the combination of fructose and sodium nitrate as supplementary carbon and nitrogen sources respectively.

Keywords: Amylase, *Trichoderma viride*, Supplementary carbon and nirogen sources, solid state fermentation.

INTRODUCTION

The variety of micro organisms that have been reported to produce amylolytic enzymes includes bacteria, yeasts and filamentous fungi (Guzman – Madonado, 1995).

Amylases play a role of paramount importance in industrial processes requiring efficient saccharification of raw starch (Bowler, 1996). Not only they are important industrially but also are of biotechnological significance and are now used in food as well (Joshi et al., 1999). Amylases are now used in production of glucose syrup, high fructose corn syrup and alcohol (James and Lee, 1977).

Production of amylases using several fungal species by submerged fermentation is well documented (Berka et al., 1992). The cost of enzyme production by submerged fermentation is higher compared to solid state fermentation (Kiro M, 2010). Use of low cost fermentation medium for the production of amylase by using agricultural byproducts has been reported to be

[1] Department of Microbiology, GITAM Institute of science, GITAM University, Rushikonda, Vishakapatnam – 530045.
[2] Department of biotechnology, GITAM Institute of science, GITAM University, Rushikonda, Vishakapatnam – 530045

advantageous (Ikram-ula-haq etal 2003). Substrates such as wheat bran, paddy husk, rice processing wastes or other starchy substances have gained importance as supports for fungal growth during amylase production (Arasaratnam etal, 2001). But these substances can be used as animal feed and fodder. On the other hand, due to the presence of high percentage of lignin, the corn cob residue is not digestible and hence cannot be used as animal feed. Despite containing a high percentage of carbon, the sugar in corn cob residue is not put to use. Optimization of amylase production has been reported terms of pH, temperature, inoculum size, incubation duration, moisture content, supplementary carbon and nitrogen sources etc, but the data regarding combinatorial effect of supplementary carbon and nitrogen sources is scanty. The current paper deals with the combinatorial effect of supplementary carbon and nitrogen sources on amylase producing activity of *Trichoderma viridae* using an agricultural waste corn cob residue by solid state fermentation.

MATERIALS AND METHODS

Culture and its Maintenance

Trichoderma viride was propagated on potato dextrose agar medium, slants were grown at 25° C,90% relative humidity and 16:8 light: dark regime for 5 days and stored at 4° C. All chemicals used were of analytical grade purchased from Himedia.

Inoculum Preparation

An aqueous conidial suspension was made from a 6 days old slant in 50ml of distilled water (sterile) containing 0.1% of tween 80. Spore count was determined using heamocytometer.

Substrate Preparation

Corn cob residue was cut into 1 inch pieces, dried to complete dryness and ground to fine powder.

Solid State Fermentation

Corn cob residue (5 gm) was taken into each of 16 Erlen mayer flasks, moisture content was adjusted to 50% with sterile distilled water, pH adjusted to 5. A combination of four supplementary carbon and four nitrogen sources resulting in total of 16 combinations was made into each of the 16 flasks. The supplementary carbon sources used were glucose, fructose, maltose and sucrose. The supplementary nitrogen sources used were yeast extract, peptone, ammonium nitrate and sodium nitrate. The fermentation process was started by adding 10^7 spores into each flask. The contents were mixed thoroughly and incubated at 30°c for 7 days in a stationary condition.

Enzyme Extraction

To the fermented dough acetate buffer was added and kept in a rotatory shaker for 1 hr. The suspension was filtered through Whattman filter paper no.1 and filtrate was used for assaying amylase activity.

Enzyme Assay

The enzyme activity was determined by incubating at 50 °C for 20 minutes, a reaction mixture containing 0.9 ml of 50mm citrate buffer (pH 5), 1.0 ml starch solution (1%w/v) and 0.1 ml of crude enzyme. The reaction mixture was incubated at 50°c for 20 minutes and then released. Reducing sugars were estimated with 3,5 Dinitrisalicylic acid reagent (Miller et al., 1959) using maltose as a standard. Amylase activity unit was expressed as the amount of enzyme releasing 1 mM of maltose equivalent per minute under assay conditions and enzyme activity was

expressed in terms of units per gram of protein in 1 gram of dry fermented substrate. Estimation of protein was done using Lowry's method.

RESULTS

The analysis showed that a combination of fructose and sodium nitrate produced highest amount of amylase whereas that of maltose and peptone produced the least. On an average sodium nitrate combination with any carbon source proved to give a high yield. Such kind of constancy was not observed with carbon sources. Different combinations yielded different results. Figures 1 and 2 schows the enzyme activity and soluble protein respectively in different combination of substrates.

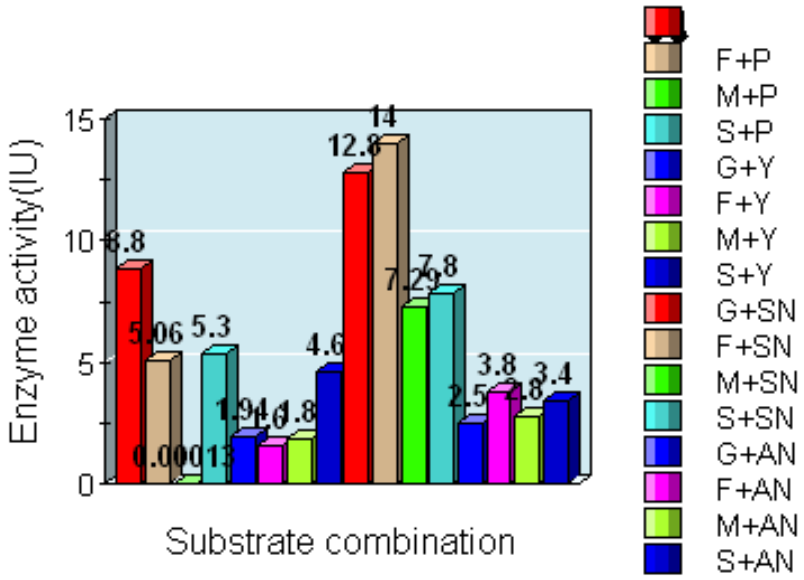
PROTEIN PRODUCTION

The protein production also was highest in the combination of fructose with sodium nitrate.

DISCUSSION

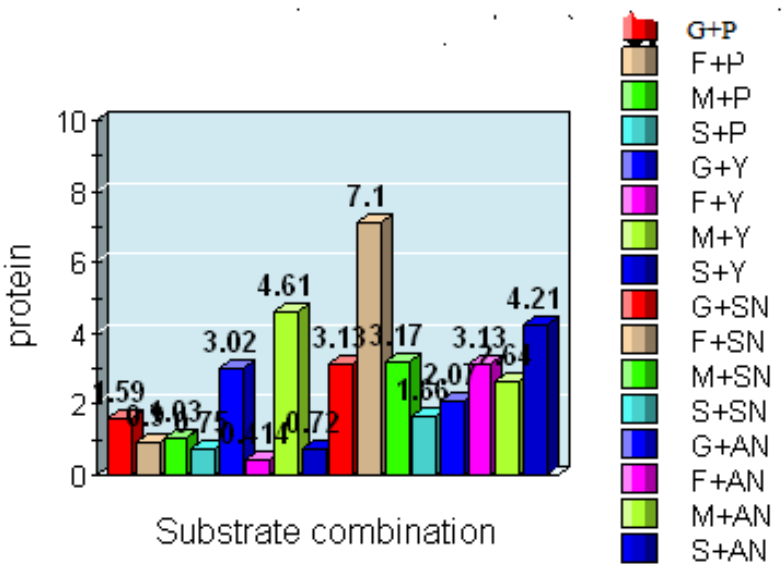
The choice of fructose over any other sugar suggests that the use of dissacharides being energy expensive. The organism as obvious chooses the least expensive pathway. However there exists a option of using glucose which also the organism didn't opt as glucose has ultimately get converted to fructose 6 phosphate which would again be energy saving as entry of fructose into glycolysis would minimize the energy. On the same lines the incorporation of nitrogen from sodium nitrate is less energy expensive than that of yeast extract or peptone. Ammonium nitrate can also be incorporated and hence it is the second choice. The use of sodium nitrate over ammonium nitrate suggests the role of Na^+ ions in transport mechanisms.

Figure 1: Graph Showing the Enzyme Activity in Different Combinations of Substrates



Note: G-Glucose, F- Fructose, M- maltose, S- Sucrose, P- Peptone, YE- Yeast extract, SN- Sodium nitrate and AN-Ammonium nitrate.

Figure 2: Graph Showing the Total Soluble Protein in Different Substrate Combinations



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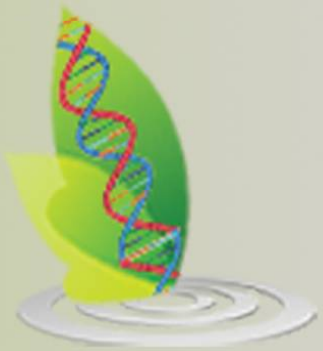

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Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
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