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STATISTICAL FILTERING OF INTERMEDIATE COMPONENTS WITH AGRO-INDUSTRIAL WASTES TO PRODUCE CHITINASE

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ABSTRACT

The medium components for the production of chitinase were optimized through the use of statistically based experimental designs. Optimization experiments were conducted on strains of *T.viride* that generate the enzyme chitinase utilizing sugarcane bagasse as a substrate, taking into account the industrial value of enzymes. Eight variables were found to be significant for the synthesis of chitinase based on preliminary investigations conducted on the parameters. Fractional factorial was then used to determine which factors were most significant for each strain. Generally speaking, KH_2PO_4, yeast extract, $MgSO_4.7H_2O$, and colloidal chitin impacted the synthesis of chitinase. Using CCD to create the model, it was discovered that, while yeast extract by itself had a quadratic effect on the strain's chitinase production, chitin and yeast extract together had an interaction effect on the synthesis of chitinase in *T.viride.* Likewise, yeast extract and colloidal chitin exhibited a quadratic impact.

Keywords: *Chitinase, Trichoderma viride, Optimization, Sugarcane bagasse*.

1.INTRODUCTION

Agro-industrial wastes fall into three main categories: hazardous agricultural and agro-industrial wastes, non-recyclable and non-compostable agricultural and agro-industrial wastes, and recyclable and compostable or naturally occurring agricultural and agro-industrial wastes. Wastes that can be recycled at recycling facilities or utilized again on farms are known as compostable wastes. Pit, shell, peels, husk, cake, slurry, and slaughterhouse wastes are classified as secondary wastes because they come from agro-allied industrial processing, whereas pruning, straw, leaves, stover, stalk, bagasse, cob, and animal dung or manure are classified as primary residues because they are directly from crop and animal production activities. Primary and secondary residues are typically regarded as the least troublesome to control.

The 1→4 β-glycoside bond of N-acetyl d-glucosamine is hydrolyzed by chinases, which belong to the glycosyl hydrolase family. Unexpectedly, chitinase appears to be more common in nature; numerous chitinases that have been isolated from various organisms have been documented. Many bacterial strains that produced chitinases have been identified, isolated, and purified over the last 20 years; the majority belongs to the genera Erwinia, Pseudomonas, Bacillus, Paenibacillus, Serratia, and Streptomyces. Conversely, there are remarkably few chitinases that are sold commercially. Consequently, a rise in chitinase production is directly associated with their future. Here, we discuss issues that arise during the synthesis of chitinases and their uses. The most important variables affecting biosynthesis are examined.

2.MATERIALS AND METHODS

2.1.MICROORGANISM & SUBSTRATE PREPARATION

The Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology in Chandigarh, India, is the source of *Trichoderma viride* (MTCC167). Before they were used to produce the inoculum for the synthesis of chitinase, they were cultured on Potato Dextrose Agar.

The EID Parry Cooperative sugar mill in Puliyur, Tamilnadu, India is the source of sugarcane bagasse. The laboratory grinder is used to powder the sugarcane bagasse at 3000 rpm. In order to stop any potential deterioration or spoiling, all of the substrates are sieved through a 100 mesh screen, and the fine powder is stored at 4°C in a sealed plastic bag.

2.2.PLACKETT-BURMAN DESIGN

Plackett-Burman, a two factorial design identifies critical chemical and physical parameters required for maximum enzyme production by screening N variables using N + 1 experiment.

2.3.SOLID STATE FERMENTATION

In 250 ml Erlenmeyer flasks, solid state fermentation (SSF) is performed with 1 g of powdered sugarcane bagasse and the nutrients specified by the experimental design. The flasks are incubated for 144 hours at 30°C in a rotary shaker with 200 rpm. Samples are taken out every 24 hours, and the concentration of the enzyme chitinase is measured on a regular basis.

2.4.ENZYME EXTRACTION

The fermented materials are combined with 25 milliliters of distilled water and shaken in a reciprocating shaker for 20 minutes to extract the crude chitinase. Following filtering, it undergoes a 20-minute centrifugation. The crude enzyme in the supernatant is used for enzymatic assays using the DNS method (Miller, 1959).

2.5.RESULT AND DISCUSSION

Using sugarcane bagasse as the substrate listed in Table 1, 12 rounds of Plackett-Burman design experiments are carried out to screen the media components for the synthesis of chitinase by *T.viride.* The Plackett-Burman design outcomes can be easily viewed using the Pareto chart.

Table 1: Plackett-Burman design matrix for screening of variables for Chitinase Production by *T.viride* using sugarcane bagasse

Run	A	B	C	D	E	F	G	H	Chitinase Activity, U/gds
1	1	-1	-1	-1	1	1	1	-1	11.5
2	-1	1	1	-1	1	-1	-1	-1	19.5
3	-1	1	-1	-1	-1	1	1	1	22.5
4	1	1	-1	1	1	-1	1	-1	15.5
5	1	1	-1	1	-1	-1	-1	1	24.5
6	1	-1	1	1	-1	1	-1	-1	12.5
7	-1	-1	1	1	1	-1	1	1	15.0
8	-1	-1	-1	-1	-1	-1	-1	-1	16.5
9	1	1	1	-1	1	1	-1	1	22.5
10	-1	1	1	1	-1	1	1	-1	12.0
11	-1	-1	-1	1	1	1	-1	1	20.5
12	1	-1	1	-1	-1	-1	1	1	15.5

Where A-$(NH_4)_2SO_4$, B-KH_2PO_4, C-$MgSO_47H_2O$, D-urea, E-$CaCl_2$, F-NaH_2PO_4, G-yeast extract, H-colloidal chitin

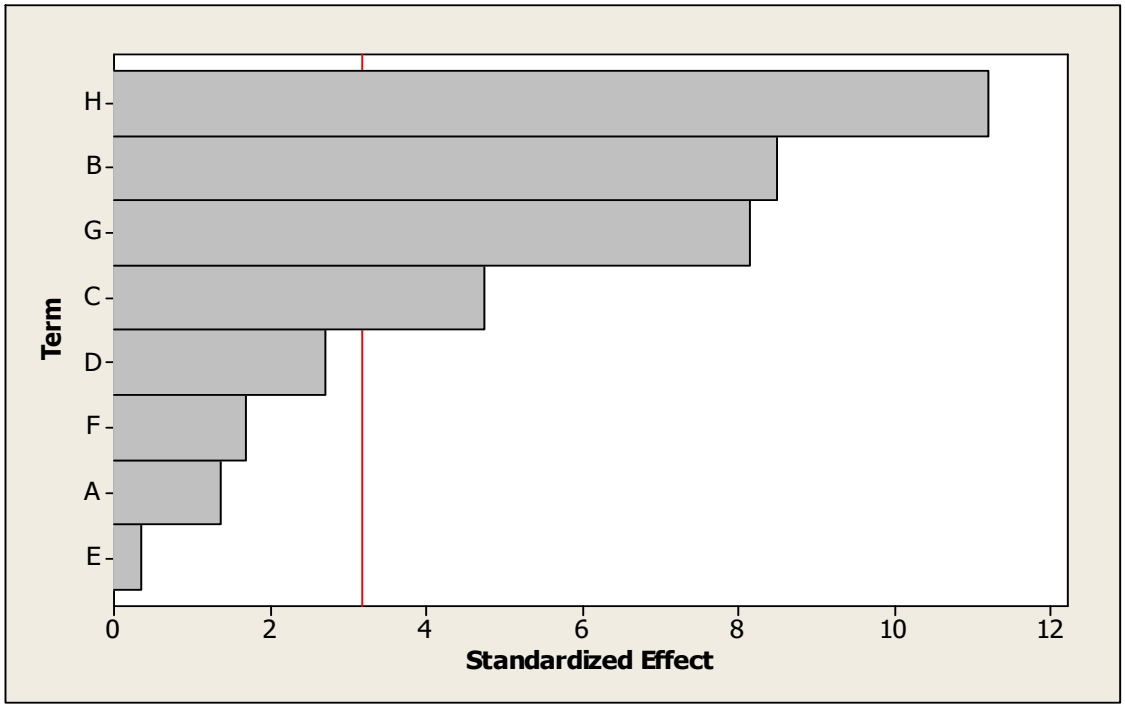

Fig.1 Pareto chart for screening of nutrients for the production of chitinase by *T.viride* using sugarcane bagasse

Pareto chart figure 1 shows the following in order of significance: urea, NaH_2PO_4, $(NH_4)2SO_4$, yeast extract, $MgSO_47H_2O$, colloidal chitin, KH_2PO_4, and $CaCl_2$. The Pareto chart suggests that the nutrients $MgSO_4.7H_2O$, KH_2PO_4, yeast extract and colloidal chitin have a major impact on the synthesis of chitinase.

The best circumstances for *T.viride* to produce chitinase from sugarcane bagasse are found using CCD. Centric composite design is used to further enhance the screened nutrients from the Plackett-Burman design for medium optimization (colloidal chitin, KH_2PO_4, yeast extract, and $MgSO_47H_2O$). Thirty-one tests are run in various configurations. The design matrix and the anticipated and observed responses are identified.

3.CONCLUSION

In the current study, it is established that the nutrients, sugarcane bagasseas substrate, and solid state fermentation (SSF) can be utilized to increase the production of chitinase. The following are the ideal amounts for maximum chitinase production: 4g of colloidal chitin, 1.0g of yeast extract, 0.5g of KH_2PO_4, and 0.12g of $MgSO_4.7H_2O$. For chitinase synthesis, the actual reaction is 34.30U/gds, compared to the projected response of 33.02U/gds.

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