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FERULOYL ESTERASE PRODUCTION DURING DEGRADATION OF DIFFERENT FIBRES BY *Aspergillus oryzae*

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Abstract: **Feruloyl esterase production by *Aspergillus oryzae* was investigated using plant fibre materials as substrates. The fibre samples (Sugarcane bagasse and brans of wheat, rice, maize, and barley) were purchased from retailers at Lafenwa market in Abeokuta, Ogun state. They were allowed to decay spontaneously for the isolation of *Aspergillus oryzae*. The fungus (*Aspergillus oryzae*) was isolated and characterised using phenotypic and molecular techniques and screened for production of feruloyl esterase using all the fibre materials as substrate. Optimum conditions for feruloyl esterase production by *Aspergillus oryzae* was determined with standard methods using all the bran as substrate. The inoculum size for feruloyl esterase production was 1.40 x10^4 spores/ml and the enzyme produced ranged from 0.48 U/mL with rice-bran and maize-bran to 1.26 U/mL with wheat-bran. The optimum temperature, pH, time of incubation and inoculum concentration for the production of feruloyl esterase by the fungus using wheat-bran as substrate were found to be 30°C, 7.0, 120hrs and 1.0 respectively.**

***Keywords: Aspergillus oryzae*, feruloyl esterase, fibre materials, optimum conditions.**

1. INTRODUCTION

Plant cell walls are composed of complex polysaccharides such as cellulose, hemicellulose, lignin, and pectins, which confer structural integrity and resistance to enzymatic hydrolysis. Ferulic acid-mediated cross-linking between lignin, cellulose, and hemicellulose contributes significantly to this recalcitrance (Faulds and Williamson, 1999; Rumbold et al., 2003). These phenolic linkages must be disrupted to enhance the valorization of agro-residues into fermentable sugars and value-added products (Grabber et al., 2000).

Feruloyl esterase, also known as ferulic acid esterase (FAE) is an enzyme capable of cleaving ester linkages between hydroxycinnamic acids and polysaccharides, thereby facilitating delignification and hemicellulose depolymerization. FAEs have been identified in diverse microorganisms, including bacteria and fungi such as *Streptomyces*, *Penicillium*, *Trichoderma*, and several *Aspergillus* species (Maheshwari and Bhat, 1987; Castanares et al., 1992; Smith et al., 1991; Kroon et al., 2000). Beyond biomass degradation, FAEs also catalyze the synthesis of sugar–phenolic esters and the functionalization of biopolymers, broadening their industrial applications (Topakas et al., 2003).

Members of the genus *Aspergillus* are prolific producers of polysaccharide-degrading enzymes and are widely used in food fermentation and enzyme biotechnology (Nwodo-Chinedu et al., 2005; Sooriyamoorthy et al., 2004). Their ecological diversity and established industrial relevance make them attractive candidates for FAE production. However, reports on FAE production and characterization from locally sourced *Aspergillus* isolates remain limited, particularly with respect to optimizing conditions for industrial applications. This study therefore investigates the potential of *Aspergillus* species for efficient FAE production and their suitability for biotechnological exploitation.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Plant fibers including rice bran, maize bran, wheat bran, barley bran, and sugarcane bagasse were obtained from Lafenwa market, Abeokuta, Ogun State, Nigeria. Samples were moistened with water and left at room temperature to undergo natural decay prior to fungal isolation.

2.2 Isolation of Fungi

Fungal isolates were obtained from decayed fibers using the dilution plate technique on Potato Dextrose Agar (PDA) supplemented with 200 mg/L chloramphenicol to suppress bacterial growth (Harrigan and McCance, 1966; Greben et al., 2007). Plates were incubated at 27 °C for 3 days, and emerging colonies were sub-cultured onto PDA slants to obtain pure cultures (Abe et al., 1988; Kausa et al., 2010). Pure isolates were maintained at 4 °C and sub-cultured biweekly (Swetha et al., 2007).

2.3 Identification of *Aspergillus oryzae*

Morphological identification was based on cultural characteristics and microscopic examination of lactophenol cotton blue-stained samples, as described by Deacon (1980) and Domsch et al. (1980). Molecular identification was performed by sequencing the internal transcribed spacer (ITS) region of rDNA. Genomic DNA was extracted from mycelial biomass using a phenol–chloroform method, and the ITS region was amplified by PCR with universal primers ITS1 and ITS4. PCR products were purified, sequenced, and compared against the NCBI database using BLAST for species confirmation.

2.4 Inoculum Preparation

Spore suspensions were prepared from 7-day-old PDA slants by adding 10 mL sterile distilled water and gently dislodging spores. The suspension was filtered through sterile muslin, and spore concentration was determined with a haemocytometer.

2.5 Feruloyl Esterase Production

Feruloyl esterase (FE) was produced in a chemically defined medium (Shin and Chen, 2006) containing bran substrates (30 g) and basal salts (KH2PO4, CaCl2, MgSO4·7H2O, Na2HPO4·2H2O, NaH2PO4, and ammonium tartrate). Media were sterilized separately and inoculated with 1 mL spore suspension. Cultures were incubated at 30 °C for 5 days (Ganapati, 2009).

2.6 Enzyme Extraction and Assay

After incubation, fermented substrates were extracted with 0.05 M citrate buffer (pH 5.0) under shaking at 150 rpm for 20 min. Filtrates were clarified and used as crude enzyme. Feruloyl esterase activity was assayed by measuring ferulic acid released from methyl ferulate as substrate (Trzcinska et al., 2005). Reaction mixtures were incubated at 40 °C for 20 min and terminated with methanol. One unit of activity was defined as the amount of enzyme releasing 1 µmolferulic acid per min. Ferulic acid was quantified by HPLC on a C18 column with detection at 320 nm (Lu et al., 2005).

2.7 Optimization of Feruloyl Esterase Production

Since wheat bran yielded the highest FE activity, it was used for optimization studies. The effects of incubation temperature (28–36 °C), initial medium pH (6.0–8.0), inoculum concentration (0.6–1.4 mL spore suspension), and fermentation time (72–168 h) on FE production were evaluated.

3. RESULTS

Table 1. Feruloyl esterase activity (U/mL) of *Aspergillus oryzae* under different cultivation conditions

Parameter	Condition	FAE Activity (U/mL)
Substrate	Rice bran	0.48
	Maize bran	0.48
	Wheat bran	1.26
	Barley bran	0.72

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Parameter	Condition	FAE Activity (U/mL)
	Sugarcane bagasse	0.18
Temperature (°C)	28	0.00
	30	1.26
	32	0.30
	34	0.00
	36	0.00
Ph	6.0	0.00
	6.5	0.48
	7.0	1.26
	7.5	0.00
	8.0	0.00
Incubation time (h)	72	0.00
	96	0.00
	120	1.26
	144	1.00
	168	0.82
Inoculum concentration (mL)	0.6	0.36
	0.8	0.48
	1.0	1.26
	1.2	0.42
	1.4	0.24

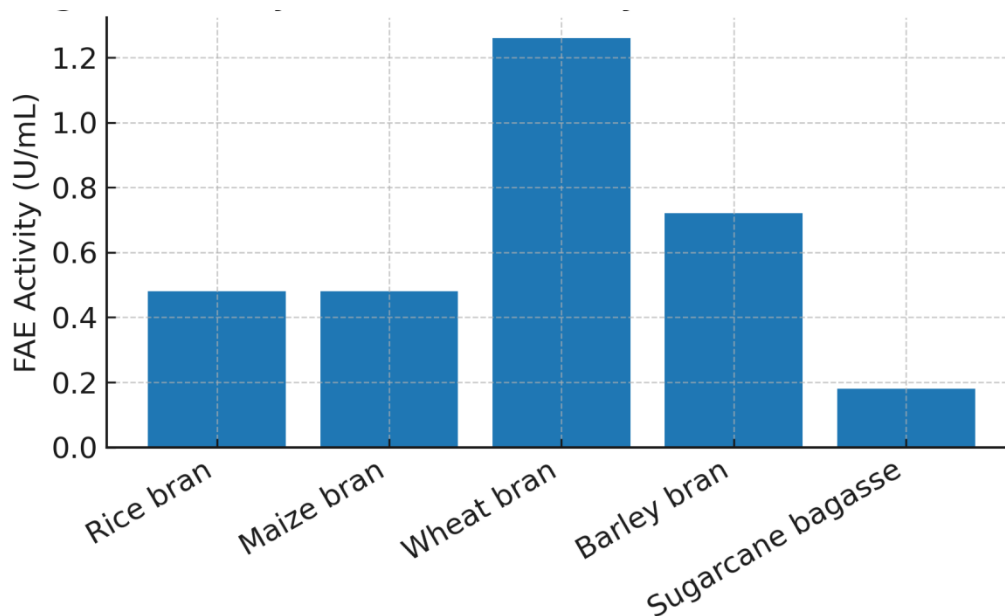

FIGURE 1: FERULOYL ESTERASE (FAE) PRODUCTION BY ASPERGILLUS ORYZAE

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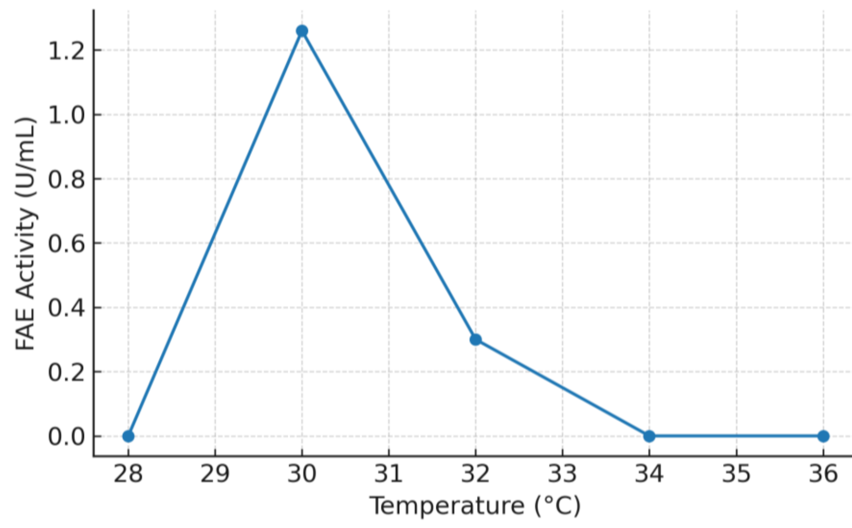

FIGURE 2: EFFECT OF TEMPERATURE ON FEROLOYL ESTERASE PRODUCTION.

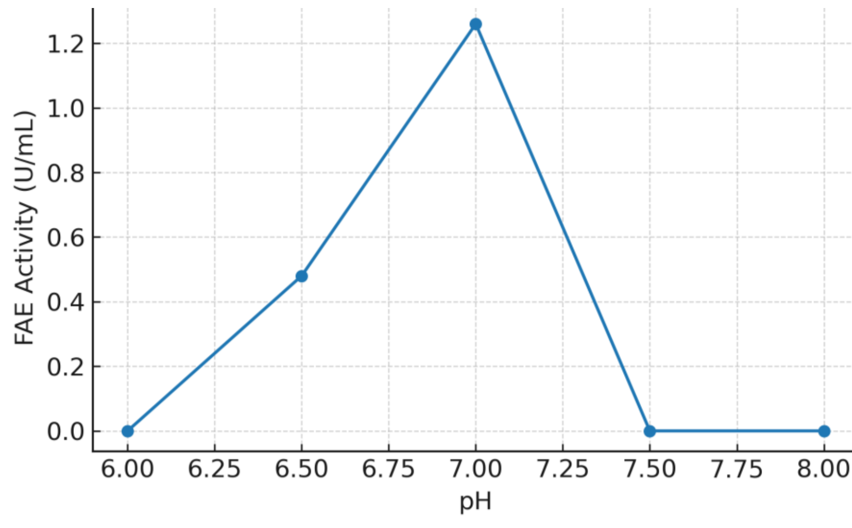

FIGURE 3: EFFECT OF PH ON FERULOYL ESTERASE PRODUCTION

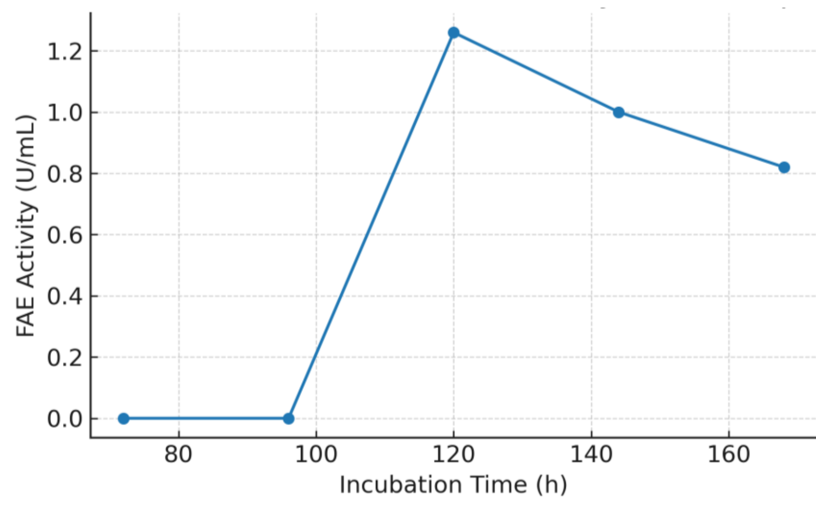

Figure 4: EFFECT OF INCUBATION TIME ON FERULOYL ESTERASE PRODUCTION.

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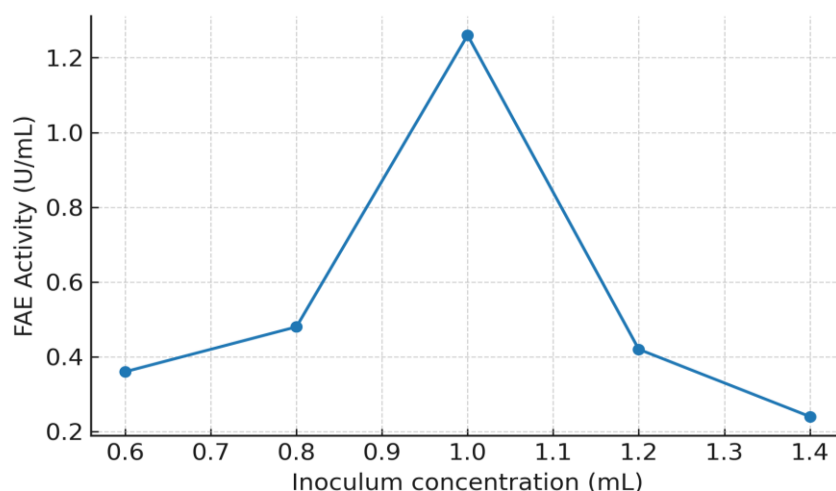

FIGURE 5: EFFECT OF INOCULUM CONCENTRATION ON FERULOYL ESTERASE PRODUCTION

4. DISCUSSION

Morphological and Molecular Identification

The isolate showed rapid mycelial growth with brown conidia borne on spherical, rough-walled, and colorless conidiophores, consistent with the description of *Aspergillus oryzae*. Molecular identification by ITS rDNA sequencing further confirmed the species identity. The spore suspension contained 1.4×10^4 spores/mL.

Substrate Screening

Among the tested substrates, wheat bran supported the highest feruloyl esterase (FE) activity (1.26 U/mL), followed by barley bran (0.72 U/mL) and maize bran (0.48 U/mL), whereas sugarcane bagasse yielded the lowest activity (0.18 U/mL) (Fig. 1; Table 1). The superior performance of wheat bran is likely due to its rich nutrient composition and availability of amino acids that enhance fungal growth and enzyme biosynthesis (Nochure et al., 1993).

Effect of Temperature

FE production was strongly influenced by incubation temperature. Maximum activity was obtained at 30 °C, while no enzyme activity was detected at 26, 28, 34, and 36 °C (Fig. 2; Table 1). Reduced activity at higher temperatures has been attributed to negative effects on microbial metabolism and enzyme stability (Venkateswarlu et al., 2000; Pandey et al., 2001).

Effect of pH

The optimum pH for FE production was 7.0 (1.26 U/mL). Slightly acidic conditions (pH 6.5) supported moderate activity, whereas no activity was detected at pH 6.0, and enzyme production declined at alkaline pH values (7.5–8.0) (Fig. 3; Table 1). These findings align with previous reports that fungal growth and enzyme secretion are favored by near-neutral to slightly acidic conditions (Haltrich et al., 1996).

Effect of Incubation Time

Maximum enzyme production occurred at 120 h (1.26 U/mL), after which activity declined with prolonged incubation (144–168 h) (Fig. 4; Table 1). The decrease in activity beyond 120 h may be linked to nutrient depletion and metabolic stress, which can impair the secretion machinery of fungi (Nochure et al., 1993).

Effect of Inoculum Concentration

An inoculum size of 1.0 mL spore suspension produced the highest FE activity (1.26 U/mL). Lower (0.6–0.8 mL) or higher inoculum concentrations (1.2–1.4 mL) resulted in reduced enzyme yields (Fig. 5; Table 1). Insufficient inoculum likely delays fermentation, while excessive inoculum can lead to nutrient competition and metabolic burden, ultimately lowering enzyme productivity (Dada et al., 2012; Lee et al., 2008).

Summary of Findings

This study demonstrated that *Aspergillus oryzae* isolated from decayed plant fibers is an efficient producer of feruloyl esterase, with wheat bran serving as the most effective substrate. Optimal production was achieved at 30 °C, pH 7.0, 120 h incubation, and an inoculum size of 1.0 mL. These findings confirm the suitability of *A. oryzae* as a promising source of FE for biotechnological applications, particularly in biomass degradation.

5. CONCLUSION

This study established *Aspergillus oryzae* as an effective producer of feruloyl esterase (FAE) under optimized fermentation conditions. Wheat bran was identified as the most suitable substrate, while maximum enzyme yield was achieved at 30 °C, pH 7.0, 120 h of incubation, and with an inoculum size of 1.0 mL spore suspension. The findings highlight the ability of *A. oryzae* to utilize agro-residues for enzyme production, thereby offering a cost-effective approach to FAE synthesis. The enzyme's neutral pH optimum and mesophilic temperature preference also suggest its adaptability for diverse industrial applications.

6. RECOMMENDATIONS

1. **Scale-up studies** should be conducted in bioreactors to validate the optimum conditions identified in this work under industrial settings.
2. **Purification and characterization** of the enzyme are necessary to determine its kinetic properties, stability, and potential for application in lignocellulosic biomass conversion.
3. **Genetic and molecular studies** on *A. oryzae* may be explored to enhance FAE yield through strain improvement or recombinant expression.
4. **Application trials** using agricultural residues such as wheat bran, rice bran, and sugarcane bagasse should be expanded to assess the enzyme's performance in biofuel production, food processing, and synthesis of value-added biochemicals.
5. Given its cost-effective production and functional versatility, FAE from *A. oryzae* holds promise for sustainable industrial biotechnology, especially in biomass valorization and green chemistry.

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