EVALUATION OF β-1, 4 - ENDOGLUCANASE AND β-1, 4-EXOGLUCANASE PRODUCTION BY *FUSARIUM OXYSPORUM* F. SP. *ELAEIDIS*

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SUMMARY

***Fusarium oxysporum* f. sp. *elaeidis* has been established to be a pathogen responsible for vascular wilt disease of the oil palm. *Fusarium oxysporum* despite a pathogen of the oil palm, is also a good source of producing cellulases such as endoglucanase and exoglucanase. Endoglucanase and exoglucanase production by the fungus appears to be pH dependent. Three strains of *F. oxysporum* using pH parameter under the submerged culture conditions were comparatively examined for endoglucanase and exoglucanase production. The most suitable pH of the culture medium (pectin broth) for enzymes production was determined by adjusting the pH of the culture medium at different levels in the range of pH 4.5-7.5 using 1.5M NaOH and 1.0M HCl for pH adjustment. Crude enzymes extract obtained from the culture filtrates were used to evaluate for endoglucanase and exoglucanase using 3, 5-dinitrosalisylic acid (DNS). Optimum production of these enzymes occurred at pH 5.5. Result from this study show that pH influenced the production of these enzymes produced by the three strains of *F. oxysporum*.**

Keywords: Cellulases, endoglucanase, exoglucanase, *Fusarium oxysporum* f. sp. *elaeidis*

Fusarium oxysporum f. sp. *elaeidis* is a soil-borne fungus (Fraselle, 1951; Aderungboye, 1976) and has been established to be a pathogen responsible for vascular wilt disease of the oil palm. Vascular wilt disease is the most serious disease of the oil palm (*Elaeis guinensis* Jacq) in West Africa. At present, the control method of the disease is by planting tolerant oil palm seeds obtainable from the Nigerian Institute for Oil Palm Research (NIFOR). The Institute produces

vascular wilt tolerant oil palm seeds by screening oil palm progenies at seedling stage with live inoculum of the fungus and selecting disease tolerant ones using Prendergast (1963) technique. It is from the selected tolerant progenies that extension work seeds (EWS) and seedlings are produced and made available to farmers for planting in order to control the vascular wilt disease. However, studies conducted on vascular wilt disease in a number of plants have reported involvement of cell wall degrading enzymes in the disease (Hancock *et al.,* 1964; Bateman, 1968; Goel and Mehrotra, 1974; Cooper, 1983). Cell wall degrading enzymes produced by plant pathogens include endoglucanase, exoglucanase, β-glucosidase, pectinases, etc.

Cellulose is a major polysaccharide compound in the plant cell wall and is composed of repeating cellobiose units linked *via* -1,4-glycosidic bonds. It is one of the most abundant renewable bioresources in nature (Bhat and Bhat, 1997). Enzymes capable of degrading cellulose are the cellulases. The enzyme cellulases can be categorized into three major classes based on its catalytic action, namely; endo-1,4-glucanases (EC 3.2.1.4), cellobiohydrolases or β-1,4-exoglucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21). Endo-1, 4-glucanases randomly attack internal amorphous sites in cellulose polymers, generating new chain ends. Cellobiohydrolases remove cellobiose from reducing or non-reducing ends of cellulose polymers. B - Glucosidases hydrolyze cellodextrins and cellobioses to glucoses (Lynd *et al*., 2002). Cellulases have generated commercial interest in various sectors, including the food, energy, pulp, and textile industries (Bhat, 2000). Consequently, endo-1,4-glucanase and exo-1,4-glucanase have biotechnological potential in various industrial applications. This enzyme has been industrially applied in biomass waste management, pulp and paper deinking, and textile bio-polishing (Kuhad *et al*., 2011). In addition, this enzyme can be practically used to produce better animal feeds, improve beer brewing, decrease the viscosity of β-glucan solutions, and improve bio-finishing in the textile industry (Kuhad *et al*., 2011). The present investigation was based on screening and quantification of endo-1,4-glucanane and β-1,4-exoglucanase enzymes produced by three strains of *F. oxysporum* f. sp. *elaeidis*.

MATERIALS AND METHODS

Fungal isolates

Stock culture of three strains of *F. oxysporum* f. sp. *elaeidis* isolated from Cameroun and Nigeria with accession number: Abak 508.1746; Ndian CI0; Ndian 3AR6 (will be designated as strain N, C_A, C_1 respectively during the course of this study) were collected from Plant Pathology Division, N.I.F.O.R., and maintained on potato dextrose agar slant.

Media formulation

Czapeck Dox broth (sodium nitrate 2g, potassium nitrate 1g, potassium chloride 0.5g, magnesium sulphate 0.5g, ferrous sulphate 0.01g, sucrose 30g) was formulated such that its sucrose will be substituted with equivalent amount (30g/L of distilled water) of the appropriate carbon source (Okunowo *et al*., 2010).

Production of enzymes

The extracellular enzymes were produced through submerged fermentation. The inocula were inoculated at 1% (v/v) into the defined enzymes production media containing 30g/L for each carbon source in the modified Czapeck Dox broth. The initial pH was adjusted and sterilized under pressure (15 psi) at 121°C for 15 minutes. The medium inoculated in duplicates were optimized for enzymes production (Singh and Hayashi, 1995). The production of these enzymes was evaluated using pH at different range.

Effect of pH on enzymes production

The most suitable pH of the culture medium (pectin broth) for enzymes production was determined by adjusting the pH of the culture medium at different levels in the range of pH 4.5-7.5 using 1.5 M NaOH and 1 N HCl for pH adjustment (Hussain *et al*., 2012). Incubation was done at 30^0C for 4 days.

Enzymes extraction

Crude enzymes extract were obtained by filtering each enzyme medium through nylon cloth; then, the filtrates were centrifuged for 10 min at 12000rpm to remove fungi and substrate residues (Shamala and Sreekantiah, 1986). The supernatants were used to check for endoglucanase, exoglucanase, β-glucosidase and pectinase activity.

Enzyme assay

β-1, 4-Endoglucanase activity

The β-1, 4-endoglucanase activity was determined according to Zaldivar *et al.* (2001); using carboxymethylcellulose (CMC) as substrate and the formation of reducing sugars was measured by reaction with dinitrosalicylic acid (DNS). The reaction mixtures containing 10mg CMC in 1ml of 0.05M sodium acetate buffer (pH 5.0) and 1ml culture supernatant (enzyme extract) were incubated at 50°C for 30 minutes. The reducing sugar formed was measured with dinitrosalicyclic acid (DNS). One milliliter (1ml) of DNS reagent was added to 2ml of the test sample. The colour was developed by boiling the mixture in water bath for 5 minutes and diluted appropriately with distilled water. Absorbance was read at 540nm using spectrophotometer (SG8 072218, Spectronic GENESYS 8, England). Reducing sugar concentration was obtained from a standard glucose concentration curve (the net reducing sugar formation was determined by subtracting the value for the reaction with no enzyme addition). One unit of CMCase activity was defined as the amount of enzyme releasing 1µmol of reducing sugar per minute at 50^0C.

β -1, 4-Exoglucanase activity

The β-1, 4-exoglucanase activity was assayed as above using microcrystalline cellulose (Avicel) as substrate (Okunowo *et al*., 2010).

Note: Enzymes concentrations were determined from the mathematical equation below:

$$\text{E.A (IU/ml)} = \frac{\text{Absorbance of Enzyme soln. x Conc. of Standard (µM/mL) x Dilution Factor}}{\text{Absorbance of Standard}}$$

Statistical analysis

All the data obtained were analyzed by ANOVA (Analysis of variance), DMRCT (Duncan's multiple range comparison test) and LSD (Least significant difference) using GenStat Version 8.1.0 software.

RESULTS

Effect of pH on enzymes production

At pH 4.5; strain C_1 has the highest enzyme activity in both endoglucanse (9.824µg/ml) and exoglucanase (9.877µg/ml). At pH 5.5; strain C_1 has the highest enzyme activity of endoglucanse (10.103µg/ml) while strain N has the highest enzyme activity of exoglucanase (9.877µg/ml).

At pH 6.5; strain C_A has the highest enzyme activity of endoglucanse (6.849µg/ml) while strain N has the highest enzyme activity of exoglucanase (7.000µg/ml).

At pH 7.5; strain N has the highest enzyme activity in both endoglucanse (7.695µg/ml) and exoglucanase (7.053µg/ml).

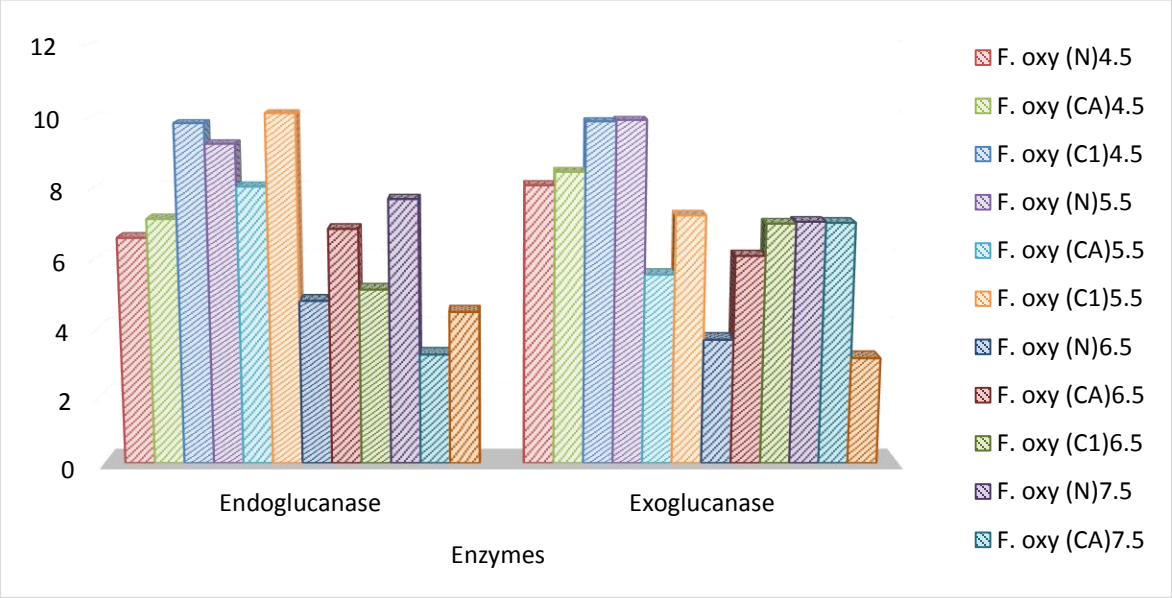

Figure 1: The effect of pH (4.5 - 7.5) on endoglucanase and exoglucanase production by each strains of *F. oxysporum*.

DISCUSSION

Cellulase refers to a class of hydrolases produced chiefly by fungi, bacteria, protozoans, and termites, which catalyzes the hydrolysis of cellulose (Lee *et al*., 2000; Watanabe *et al.*, 1998). The important of this enzyme cannot be underscored in that it can be produced by micro-organisms, termites as well as animals (Watanabe and Tokuda, 2001). Also, the use of cellulases to biodegrade cellulose containing biomass helps in mopping up agricultural biomass littered in every environment, thus, converting waste to wealth. Cellulase is a complex enzyme composed of three catalytic subunits which work in synergy to bring about the conversion of cellulose to a monomeric unit, glucose, which can subsequently be fermented for bio-ethanol production.

Fungi are well known agents of decomposition of organic matter in general and of cellulosic substrate in particular as reported by Lynd et al., (2002). The most common and most potent cellulose producers are *Aspergillus, Trichoderma, Penicillium, Paecilomyces* and *Fusarium* species Yalpani, (1987). Many research works had been focused on the optimization of enzyme production in fungi due to the continued demand for biotechnological and industrial application of enzymes. Therefore, further studies will also involve the development of mutant strains of these organisms with enhanced production of cellulase enzyme for lignocellulosic materials. The organisms used in this study where three strains of *F. oxysporum* f. sp. *elaeidis* using pH parameter under the submerged culture conditions were comparatively examined. Cellulase degrading enzymes appear to depend on pH value as reported in our previous study (Ekhorutomwen *et al*., 2018).

Duncan's multiple range comparison test (DMRCT) was used to evaluate the differences between each of the 3 strains of *F. oxysporum* with respect to endoglucanase and exoglucanase production along pH range of 4.5 to 7.5. At pH 4.5 (endoglucanase), there was no significant difference between strain N and C_A in endoglucanase production but, there was a significant difference between strain C_1 and the other 2 strains. At pH 5.5 (endoglucanase), there were a significant

differences among the 3 strains. At pH 6.5 (endoglucanase), there was no significant difference between strain N and C_1 but there is a significant difference between strain C_A and the other 2 strains. However, at pH 7.5 (endoglucanase) there were a significant differences among the strains.

There was no significant difference in between strains N and C_A but there was a significant difference between strains C_1 and the other 2 strains at pH 4.5. The production of exoglucanase at pH 5.5 was significantly different among the 3 strains, but not at pH 6.5 between strains C_A and C_1. However, at pH 7.5 exoglucanase production was significantly different among the three strains, but not between strains N and C_A. In this study, pH 5.5 influenced the highest production of both enzymes in the 3 strains of *F. oxysporum.* This agrees with the report of Juhasz *et al*. (2004) that the maximum cellulase production was obtained at pH ranging from 3.0 to 5.0.

CONCLUSION

Fusarium oxysporum f. sp. *elaeidis* despite a pathogen to the oil palm is good source of producing cellulases. This study has revealed the production of endoglucanase and exoglucanase by 3 strains *F. oxysporum.* In this study, the production of endoglucanase and exoglucanase by the 3 strains *F. oxysporum* was influenced most at pH 5. This pH value should be adopted for the production of these enzymes for further studies.

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