

The Influence Of Metal Ions On Cellulolytic Activities Of Fungal Isolates From Palm Biomass

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Abstract

Numerous metal ions and chemical compounds can influence the activity of some fungi. Some have inhibitory effects whereas others have enhancing effects. In this study, the effect of metallic chlorides; lead II chloride (PbCl₂), potassium chloride (KCl), copper II chloride (CuCl₂), calcium II chloride (CaCl₂), manganese II chloride (MnCl₂), iron III chloride (FeCl₃) and mercury II chloride (HgCl₂) were evaluated for their influence on the cellulolytic activity of seven (7) fungal isolates at a concentration of 0.005M at 30°C for four (4) days using 3, 5-dinitrosalicylic acid (DNS) method. CaCl₂ had the highest influence on the cellulolytic activity for all the fungal isolates as compared to the control. In the presence of CaCl₂, *Trichoderma* species (C) showed a high cellulolytic activity of 2.28x10⁻⁵ mmol/min/ml, *Rhizopus*, *Trichoderma* and *Aspergillus* species (A, B and E) had activities of 1.72 x10⁻⁵, 1.65 x10⁻⁵ and 1.68 x10⁻⁵ mmol/min/ml respectively. HgCl₂ and CuCl₂ had the least influenced on the cellulolytic activity of all the fungal isolates. This, therefore, suggests that calcium ion activates the cellulase activity of the fungal species while HgCl₂, PbCl₂, KCl, CuCl₂, MnCl₂ and FeCl₃ had a retarding effect on the cellulolytic activity.

Keywords: Metallic ions, Cellulolytic activity, Fungal isolates

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Introduction:

Heavy metals have been shown to be one of the greatest hazardous pollutants in the environment brought about by human economic activities, both agricultural and non-agricultural (Goyer, 2001; On et al., 2005; Micó, et al., 2006). In the soil, they react with chemicals, accumulating in numerous forms with different bioavailability to plants (Singh and Kalamdhad, 2011). In natural concentrations, metals are generally crucial in the activities of living organisms, but frequently have noxious properties when in excess (Badura and Piotrowska-Seget, 2000; Singh and Kalamdhad,

2011). Furthermore, their existence in soil has a key influence on cell structure, growth and development, and the biological actions of soil microorganisms (Badura and Piotrowska-Seget, 2000; Tscherko et al., 2007; Hassn et al., 2014). Although heavy metals naturally transpire in the soil environment, currently there are many factors significantly fueling their increase, such as industrial activities, and imaginably most importantly, the increase of agricultural production using chemical-dependent farming methods (Lenart and Wolny-Koładka, 2013). Metals in soil are detected in different forms such

as soluble metal complexes (sequestered to ligands), unrestricted metal ions, transferrable metal ions, precipitated or insoluble compounds such as oxides, carbonates and hydroxides. Heavy metal toxicity depends on their transfer from the affected soil to the organisms (Juste, 1988). The effect of metals not only depends on their total concentration but also on biological (e.g. solubilisation, biosorption and bioaccumulation) and physico-chemical (e.g pH, organic matter, clay content) factors (Berthelin, et al., 1995).

Lignocellulolytic enzymes include cellulases, hemicellulases, and ligninases, which individually break down cellulose, hemicellulose, and lignin, which majorly make up the plant cell wall, altogether called lignocellulose. Cellulases are engaged in countless areas in the industry, like in the textile (Simic, et al., 2015), detergents, animal feed, and vinification (Podrepsek, et al., 2012; Karmakar and Ray, 2011; Liete, et al., 2008; Graminha, et al., 2008). In the past, the prospects of these enzymes in breaking down cellulose from lignocellulosic remains has been studied broadly targeting glucose for use in cellulosic ethanol making (de Cassia Pereira, 2016).

The impact of metal ions on activities of enzymes can be from the water in the soil or as a result of reagents used during et al., industrial processes or also come from equipment corrosion (Oviedo and Rodríguez, 2003). Metal ions and chemical compounds may enhance cellulolytic activity. There are reports that metal ions such as Hg^{2+} , Cu^{2+} , Zn^{2+} , Mg^{2+} , Fe^{3+} , Mn^{2+} , Ag^+ , Mn^{2+} , K^+ partially or entirely inhibit cellulolytic activities, although metal ions like Ca^{2+} , Na^+ and Co^{2+} enhance or moderately enhance cellulolytic activity (Bagga, et al., 1990; Yan and Lin, 1997; Dutta, et al., 2008; Nazir, et al., 2009). Riou et al. (1998) considered the influence of several metal ions and detergents on cellulase generated by *A. oryzae*, and discovered that Ag^+ , Hg^{2+} , Fe^{3+} , SDS, diethylpyrocarbonate, castanospermine, dithiothreitol slightly or entirely repressed the cellulolytic activity; while Mn^{2+} improved the activity. Furthermore, the level of cellulase produced by *A. terreus* M11 was enhanced on the addition of Mn^{2+} ; but Hg^{2+} , Cu^{2+} , Pb^{2+} and detergents to some extent reduced or repressed the cellulolytic activity (Gao, et al., 2008). Yang et al. (2008) established that the cellulase

produced by *Paecilomyces thermophila* was meaningfully reduced by Hg^{2+} ; while the cellulolytic activity was extremely improved on addition of Zn^{2+} (Yang, et al., 2008).

The purpose of this study is to investigate the effect of selected metallic ions in the production of cellulase from fungi associated with palm biomass.

Materials and Methods:

Sample Collection:

Samples of palm pressed fibre (PPF), decaying empty fruit bunch (DEFB) and decaying roots of the oil palm tree were collected from the Nigerian Institute for Oil Palm Research (NIFOR) main station, Benin City, Nigeria.

Isolation of Fungi and Preparation of Culture:

The samples were cut and placed in McCartney bottles which contained 9 ml of sterile distilled water. They were shaken vigorously on a vortex mixer for 10 minutes. Each McCartney bottle containing samples were serially diluted from which 1ml of 10^1 to 10^{-5} dilutions were plated separately on Petri dish plates of potato dextrose agar (PDA), pH 6, containing (0.02g per 200mL of medium) chloramphenicol. The plates were incubated aerobically at room temperature of 30°C. The developing fungi colonies were aseptically subcultured into gelled prepared potato dextrose agar (PDA, Difco Laboratories, Detroit, USA) plates until pure cultures were gotten). Pure fungi isolated were stored in slants.

Reagents and chemicals:

Cellulose powder, PDA, and metallic salts; lead II chloride ($PbCl_2$), potassium chloride (KCl), copper II chloride ($CuCl_2$), calcium II chloride ($CaCl_2$), manganese II chloride ($MnCl_2$), iron III chloride ($FeCl_3$) and mercury II chloride ($HgCl_2$) all of the analytical grades were purchased from Pyrex-IG Scientific Company, Benin City, Edo State, Nigeria.

Measurement of Cellulolytic Enzymes Activity:

The fungi isolates were grown on Czapek-Dox broth (Lekh, et al., 2014). Czapek-Dox broth was prepared and amended with 1% cellulose, 0.005M each of the metal salts and distributed into 100 ml conical flasks and the pH adjusted to 7.0 using NaOH and HCl. After sterilization, the

fungal spore were inoculated into it and incubated at 30°C for four (4) days on a rotary shaker at 120rpm. The mycelia were harvested by filtering, and the filtrate centrifuged and assayed by DNS method to get the activity of the enzymes (Sivaramanan, 2014), each measured using UV spectrophotometer at 540 nm.

Dinitrosalicylic acid (DNS) method:

The assessment of the reducing sugars in the samples was done using the DNS method. The DNS reagent is an alkaline solution of 3, 5-dinitrosalicylic acid and tartarate salt. On heating, the reducing sugars can reduce 3, 5-dinitrosalicylic acid to 3 amino-5-nitrosalicylic acid, which appears orange-red in colour. The concentration of the colour is relative to the quantity of reducing sugars existing in the sample. The orange colour developed is compared to standards in a spectrophotometer at 540nm (Miller, 1959).

Preparation of Dinitrosalicylic acid:

DNS reagent was made by adding 1 g 3, 5-dinitrosalicylic acid (DNS) in 50 mL of distilled water. About 200 mg crystalline phenol (elective) and 30 g of sodium potassium tartrate were poured into the solution turning the solution into yellow colour. Also, 20 mL of 2N NaOH was added producing a clear orange-yellow colour. The addition of distilled water was used to make up the stock solution to 100 mL. The stock was stored in a refrigerator, to prevent deterioration (Miller, 1959).

Results and Discussion

Seven (7) fungal species were isolated from the palm biomass. The fungi isolates were identified, and labelled A-G. They are one *Rhizopus*, two *Trichoderma*, three *Aspergillus* and one *Penicillium* species.

In Fig 1, it showed that isolate C produced a higher cellulolytic activity of 5.97×10^{-6} mmol/min/ml in the presences of $PbCl_2$, followed by isolate D (5.12×10^{-6} mmol/min/ml). Isolates F and G produced cellulolytic activities of 5.0×10^{-6} mmol/min/ml each. Isolate A produced the lowest activity of 4.02×10^{-6} mmol/min/ml, which is at variance with isolate E (4.75×10^{-6} mmol/min/ml).

In the presence of KCl, isolate B showed the highest cellulolytic activity of 9.88×10^{-6} mmol/min/ml; although isolate C (6.71×10^{-6} mmol/min/ml) cellulolytic activity was high but not significantly different when compared to A (6.34×10^{-6}), D(6.22×10^{-6}), E(5.61×10^{-6}) and F(4.63×10^{-6} mmol/min/ml) respectively. Isolate G produced the lowest cellulolytic activity of 4.02×10^{-6} mmol/min/ml when KCl was added, as compared to the control.

Isolate F caused the highest cellulolytic activity of 4.75×10^{-6} mmol/min/ml on addition of 0.005M of $CuCl_2$ when compared to isolate C with activity of 4.51×10^{-6} mmol/min/ml. Isolate A showed the lowest cellulolytic activity of 2.68×10^{-6} mmol/min/ml when compared to isolates B and D with activities of 3.05×10^{-6} and 3.29×10^{-6} mmol/min/ml respectively.

On addition of $CaCl_2$, the activities in all the fungal isolates were higher as compared to the other metal salts. Isolate C produced the highest cellulolytic activity of 2.28×10^{-5} mmol/min/ml, followed by isolate A (1.73×10^{-5} mmol/min/ml) when compared to isolates B (1.76×10^{-5}), E (1.68×10^{-5}), F (1.61×10^{-5}) and G (1.56×10^{-5} mmol/min/ml) respectively. Isolate D caused the lowest cellulolytic activity of 1.05×10^{-5} mmol/min/ml.

When $MnCl_2$ was added, isolate C produced the highest cellulolytic activity of 9.88×10^{-6} mmol/min/ml. Isolates A (6.71×10^{-6}), B (6.34×10^{-6}), F (6.22×10^{-6}) and D(5.6×10^{-6} mmol/min/ml) produced a high cellulolytic activity. Isolate E produced the lowest cellulolytic activity of 4.02×10^{-6} mmol/min/ml.

On addition of $FeCl_3$, isolate E triggered the highest cellulolytic activity of 7.32×10^{-6} mmol/min/ml when compared to isolate F (6.71×10^{-6} mmol/min/ml). Isolate B produced the lowest cellulolytic activity of 1.95×10^{-6} mmol/min/ml.

Isolate B produced the highest cellulolytic activity of 5.12×10^{-6} mmol/min/ml on addition of $HgCl_2$ when compared to isolates C (4.75×10^{-6}) and F (3.9×10^{-6} mmol/min/ml). Isolate A produced a lower cellulolytic activity of 2.8×10^{-6} mmol/min/ml when compared to isolate D (3.05×10^{-6} mmol/min/ml).

This study showed that all metal ions had different influence on the cellulolytic activity of the isolates compared to the control but the addition of Ca^{2+} increased the cellulolytic activities of the isolates. This suggests that Ca^{2+} has the capacity to stimulate the cellulolytic activity of the fungi, thus supporting the study of Bagga, et al., (1990), Sajith, et al., (2016) and Nazir, et al., (2009).

From this present study, Pb^{2+} , K^+ , Cu^{2+} , Mn^{2+} , Fe^{3+} and Hg^{2+} lowered the cellulolytic activity when compared to the control. This suggests that the metallic ions have the ability to cause an inhibitory effect which is in accordance with the study of Sajith, et al., (2016) and Riou, et al., (1998). This results obtained is contrary to the study of Gao, et al., (2008), who reported that Mn^{2+} increased the cellulase production of *A. terreus* M11. However, Hg^{2+} , Cu^{2+} and Pb^{2+} reduced the cellulase production as shown in Fig 1.

Key

- A= *Rhizopus* species
- B= *Trichoderma* species
- C= *Trichoderma* species
- D= *Aspergillus* species
- E= *Aspergillus* species
- F= *Aspergillus* species
- G= *Penicillium* species

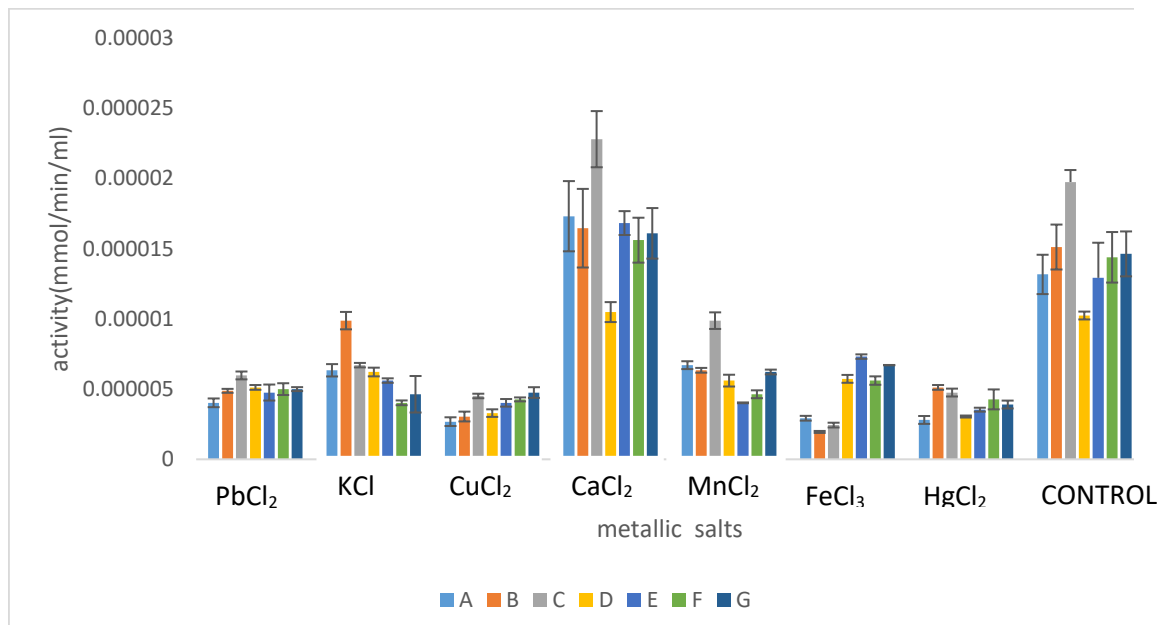


Fig.1: Cellulolytic activity of the fungi isolates

Conclusion

This work establishes the possibility of harnessing palm biomass which has been regarded as waste into a substrate for the growth of cellulolytic

fungi. There is the need to optimize the conditions necessary for the production of cellulolytic fungi in order to attain optimum yield. In this study, calcium ion plays a major role in

enhancing cellulolytic activity under the conditions studied. While the other metallic ions showed to be inhibitory under the same conditions.

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