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Isolation, screening and characterization of Xylose-fermenting yeasts isolated from sawdust

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ABSTRACT

Yeasts have been less frequently reported as xylanase producers compared to bacteria and filamentous fungi. Different cellulosic materials including sawdust are produced on a large scale and these can be used for the production of useful enzymes such as xylanases. Xylanases are a group of enzymes that hydrolyze plant fibers made of xylan hemicellulose. Xylose-fermenting yeasts isolated from soil at a wood processing factory were isolated and qualitatively and quantitatively screened for xylanase production using xylose supplemented medium and congo red as indicator. Xylanase enzyme was produced using different xylose concentrations (0.5%, 1.0%, 1.5% and 2.0%). *Pichia chambardii* isolate, which was later identified as *Wickerhamomyces chambardii* by molecular techniques, showed the highest xylanase activity of 199.31U mL^{-1}. Maximum xylanase activity (275.83U mL^{-1}) was achieved at 1.5 %w/v xylose. This study showed that yeasts have a high potential for the production of xylanase enzymes.

Keywords: Congo red, Lignocellulosic, Sawdust, *Wikerhamomyces chambardii*, Xylanase

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INTRODUCTION

Xylanases are enzymes that degrade the linear polysaccharide β-1,4-xylan into simpler compounds, which mainly consist of xylose monomeric units, and as a result, hemicellulose is broken down (Bhardwaj et al 2019).The use of xylanase enzymes in various sectors has increased in the past decade due to their application in a wide range of industrial processes such as the pulp and paper industry, animal feed, pharmaceutical, biofuel, drinks, bakery, and fermentation (Kalim et al 2015). Industrial wastes such as wheat bran, corn cobs, and wheat straw are commonly used as carbon sources in the nutrient media for industrial production of xylanase enzymes which leads to low production costs (Kalim et al 2015, Burlacu et al 2016).

Xylanases can be sourced from animals, plants, and microorganisms. These enzymes are present in the digestive tract of ruminant animals and also in the intestines of wood-eating insects. In plants, xylanases are involved in cell wall extension, cell division, germination of seeds, as well as other physiological and morphological events (Sharma et al 2019). Xylanases are derived from bacteria, marine algae, and fungi, which include aerobes, anaerobes, mesophiles, thermophiles, and extremophiles (Mandel 2015, Sharma & Sharma 2017), but the major producers of xylanase enzymes are fungi and bacteria (Lee et al 2014).

Microorganisms are considered good sources for the production of new enzymes in fermentation bioprocesses because their growth rate is fast and they can be genetically manipulated to produce new enzymes with desirable abilities (Shankar et al 2021). Different yeasts such as *Saccharomyces diasticus, Wikerhamomyces chambardii*, *Trichosporon beemeri, Debaryomyces hansenii, Candida species* have been reported to produce extracellular enzymes such as amylase and cellulase (Papagianni & Papamichael 2017). Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoa, snails, crustaceans, insect, seeds, etc (Maryam et al 2018). Bacterial genera, such as Bacillus, *Micrococcus Cellulomonas, Staphylococcus, Paenibacillus, Arthrobacter, Microbacterium, Pseudoxanthomonas,* and *Rhodothermus* have been reported to produce xylanases (Burlacu et al 2016). Filamentous fungi are useful producers of xylanase because they are capable of producing high levels of extracellular enzymes and can be cultivated very easily. On an industrial scale, xylanases are mainly produced using *Aspergillus* spp and *Trichoderma spp* (Burlacu et al 2016).

Lignocellulose is the most abundant biopolymer accessible on earth as a renewable biomass (Burlacu et al 2015). This is present in most common organic materials, such as corn straw, corn cob, wheat straw, sugarcane bagasse, and sawdust among others. Lignocellulosic biomass is made up of three biopolymers: cellulose (~30-50% by weight), a linear polymer of glucose molecules linked together in a highly crystalline structure; hemicellulose (~19-45% by weight), a highly branched polymer consisting of several sugars, eg, arabinose, glucose, galactose, mannose and xylose; and lignin (~15-35% by weight), a complex phenyl-propane polymer (Lee et al 2014). Lignocellulosic biomass is the feedstock for the pulp and paper industry, textile industry, agricultural industry and is the precursor for liquid fuels (Burlacu et al 2015) Large sources of lignocellulosic biomass, such as sawdust from wood processing factories, are industrial residues that are sustainable, readily available and less expensive. These can used in the manufacture of a variety of commercial or industrial products (Maryam et al 2018).

MATERIALS AND METHODS

Isolation and Characterization of Yeasts

Soil samples from a wood processing factory in Abeokuta, Ogun State, Nigeria, were collected with the aid of a sterilized metal hand trowel into sterile sample bottles, at a depth of about 10cm after removal of the superficial layer (approximately 0.5mm). Serial dilution technique was used for isolation. Dilutions to 10^{-2} and 10^{-4} were plated on Yeast Extract Peptone Dextrose Agar (YEPDA) and incubated at 30°C for 72h. All yeast isolates were subjected to morphological characteristics such as colour, size, elevation and margin for identification. Subculturing of yeasts with different colony morphologies was carried out using YEPDA and incubated for 48h at 30°C. Pure cultures were stored on YEPDA. The isolated yeasts were identified using colony morphologies and different biochemical characterization (Table 1) (Barnett & Yarrow 2000).

Table 1. Biochemical characteristics of yeast isolated from sawdust

Isolate code	Sucrose	Lactose	Glucose	Xylose	Urease	10% NaCl/5% glucose	50% glucose	Ethanol Growth	Probable organism
. .	+	-	+G	-	+	+	-	+	*S. cerevisiae*
Y2	+	-	+	+	+	+	-	+	*P. stipilis*
Y3	+	-	-	+	+	+	-	s	*T. lutetiae*
Y4	+	-	+G	+G	+	+	-	+	*C. sheatae*
Y5	+	-	-	+	+	+	-	+	*C. parapsilosis*
Y6	-	-	+	+	+	+	+	+	*P. stipilis*
Y7	+	-	-	+	+	s	-	s	*T. lutetiae*
Y8	-	+	+G	+G	+	+	-	+	*T. asahii*
Y9	+	-	+	+	+	+	-	+	*C. tropicalis*
Y10	-	+	-	+	+	+	-	+	*S. cerevisiae*
Y11	+	-	-	+	+	+	-	+	*T. beigelil*
Y12	+	-	+	-	w	+	s	+	*C .sheatae*
Y13	+	-	-	+	+	+	-	+	*P. chambardii*
Y14	-	+G	-	+G	+	+	+	+	*P. stipilis*

Key: +G =gas production, + =Positive, - =Negative, w =weak, s= slow.

Screening for Xylanolytic Yeast

Qualitative screening

The isolated yeasts were screened qualitatively for xylanase production using Congo red solution. Pure cultures of yeasts obtained were transferred to YEPDA containing 1% D-xylose and incubated for 72h at 30°C and flooded with 1% Congo red solution (Adelabu et al 2019). Culture plates with clear zones around the line of growth showed xylanase hydrolysis.

Quantitative screening

Yeasts were screened quantitatively for xylanase production by growing them in YPD broth containing 1% D-xylose; 0.8g L^{-1} yeast extract; 2.0g L^{-1} KH_2PO_4; 0.75g L^{-1} peptone; 1.4mg L^{-1} $ZnSO_4.7H_2O$; 0.3g L^{-1} $MgSO_4.7H_2O$; 1.4g L^{-1} $(NH_4)_2SO_4$; 5.0g L^{-1}

glucose; 0.3g L^{-1} urea; 0.4g L^{-1} KCl and 5.0mg L^{-1} $FeSO_4.7H_2O$. The cultures were incubated in an orbital shaker operating at 150rpm, 30°C for 72h.

Extraction of enzyme

The flasks containing the culture were withdrawn and the contents were filtered through Whatman No.1 filter paper. The filtrate was centrifuged at 4000rpm for 15mins and the supernatant was collected and used as the enzyme source for the assay of xylanase activity (Adelabu et al 2019).

Xylanase assay

Xylanase assay was performed according to the modified method of Kareem et al (2009) by measuring the reducing sugar liberated from the xylan. The reaction mixture consisting of 0.5mL of 4%w/v D-xylose in 0.2M Acetate buffer at pH5.0 was placed in test tubes and the reaction commenced with the addition of 0.5mL of crude enzyme extract. The reaction mixture was incubated in a water bath at 60°C for 60mins and the reaction was terminated by adding 1.0mL of 3,5-dinitrosalicylic acid (DNSA) reagent. The contents were thereafter heated in test tubes for 5min at 80°C in a water bath and then distilled water (10mL) was added. The absorbance of the reaction mixture was read at 540nm using spectrophotometer (Biobase 752N) to determine the concentration of sugar released by the enzyme. One unit (U) of xylanase was defined as the amount of enzyme that released 1.0µmol reducing sugar as xylose equivalent per minute in the reaction mixture under the specified assay conditions.

Selection of starter cultures

Two yeasts with the best xylanolytic activity were selected for further study and identified using molecular tools.

Genotypic Characterization

DNA isolation

For DNA extraction, yeasts were grown on YEPDA plates at 28°C for 24–48h. Each colony was then grown overnight on YPD broth (1% yeast extract, 2% peptone, 2% dextrose) at 28°C with shaking at 200rpm. Extraction of genomic DNA was carried out with Zymo DNA extraction kit following instructions from the manufacturer. Evaluation of the DNA concentration and purity was accomplished with the use of Thermo Scientific NANODROP (ND 1000) Spectrophotometer (Gupta 2019).

PCR Amplification of the ITS gene

To identify the yeasts genotypically, the internal transcribed spacers (ITS) and 18S rDNA gene regions were amplified using specific primers ITS-1 (5'-TCCGTAGGT GAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (Zymo Research). Polymerase chain reaction was accomplished with the use of a master mix (Solis Biodyne) in a total reaction mixture of 25µL. The PCR reaction mixture was made up

of 2.5µL 10x buffer, 0.5µL dNTP, 0.25µL Taq DNA Polymerase, 0.75µL $MgCl_2$, 0.5µL forward and reverse primer and 1µL DNA Template.

Polymerase chain reaction was carried in a Nexus Series thermal cycler. Initial denaturation was carried out at 94°C for 5min, denaturation at 95°C for 30s, annealing at 5°C for 45s and extension at 72°C for 5min. Agarose gel (1.5%) was used to separate amplified DNA products. Gel electrophoresis was carried out for 1h 30mins at 80V. Ethidium bromide was used to visualize the DNA bands, using 100bp DNA ladder as the molecular weight standard.

DNA sequencing

BLAST programs were used to compare sequence data to the gene sequences in libraries (GenBank). CLUSTALW program was used to carry out multiple sequence alignment of the gene. Maximum parsimony with bootstrap method used to used to estimate Genetic distances (Apiradee 2006).

Effect of different D-xylose concentrations on xylanase activity

The effect of different D-xylose concentrations on xylanase activity was carried out using 0.8g L^{-1} yeast extract; 2.0g L^{-1} KH_2PO_4; 0.75g L^{-1} peptone; 1.4mg L^{-1} $ZnSO_4.7H_2O$; 0.3g L^{-1} $MgSO_4.7H_2O$; 1.4g L^{-1} $(NH_4)_2SO_4$; 5.0g L^{-1} glucose; 0.3g L^{-1} urea; 0.4g L^{-1} KCl and 5.0mg L^{-1} $FeSO_4.7H_2O$ (Adelabu et al 2019), into which varying concentrations of D-xylose sugar (0.5%, 1.0%, 1.5% and 2.0%) had been separately added. Each 250mL flask containing 100mL of the medium was sterilized and inoculated with 5% inoculum of the two selected yeasts.

Analysis of data

Data generated during the course of this study were analyzed with statistical package for social sciences (16.0) for Windows (SPSS, Chicago IL, USA).The means of the data obtained were analyzed by analysis of variance (ANOVA), and the means were separated using least significant difference (LSD) at $p \geq 0.05$) (Taiwo et al 2021).

RESULTS

Isolation and Characterization of Yeast Isolates

A total of 14 yeast isolates were obtained. All the yeast isolates were positive for urease test and 5% ethanol (Table 1). Physiological and biochemical tests identified the yeasts as *S. cerevisiae, Pichia chambardii*, *P. stipilis, Trichosporon lutetiae, T. asahii, Trichosporon beigelil, Candida tropicalis, C. sheatae, C. parapsilosis.*

Qualitative Screening of Yeast Isolates for Xylanase Production

Fourteen (14) isolates tested positive for xylanase enzyme by showing clear zones on xylose agar flooded with Congo red solution. The xylanase-positive isolates showed clear zones from 2.00mm to 36.09mm in diameter (Table 2). *P. chambardii* had the highest halo zones 46.09mm followed by *C. tropicalis* 40.50mm, *T. asahii* 13.54mm, *T. beigelil* 12.50mm, and *S. cerevisiae* (2.03mm).

Table 2. Qualitative screening for xylanase production in yeast isolates

Yeast isolates	Zones of Clearance x 10^2mm
S. cerevisiae	0.0203±0.010^{m}
P. stipilis	0.2604±0.10^{e}
T. lutetiae	0.1590±0.12^{ijk}
C. sheatae	0.3043±0.00^{cd}
C. parapsilosis	0.2248±0.01^{f}
P. stipilis	0.1973±0.00^{h}
T. lutetiae	0.1100±0.11^{jk}
T. asahii	0.1354±0.01^{ij}
C. tropicalis	0.4050±0.00^{b}
S. cerevisiae	0.071±0.10^{l}
T. beigelil	0.1250±0.00^{ijk}
C. sheatae	0.3275±0.01^{c}
P. chambardii	0.4609±0.12^{a}
P. stipilis	0.2142±0.00^{fg}

Key: +G =gas production, + =Positive, - =Negative, w =weak, s=slow.

Quantitative screening of yeast isolates for xylanase production

Quantitative screening of fourteen (14) yeast isolates showed that all the isolated yeasts produced xylanase and had different levels of enzyme activity. Table 3 shows that highest xylanase activity was from *P. chambardii* (199.31U mL^{-1}), followed by *C. tropicalis* (198.22U mL^{-1}), T. *lutetiae* (192.00U mL^{-1}) and *S. cerevisiae* (122.03U mL^{-1}) (Table 3).

Table 3. Quantitative screening for xylanase production in yeast isolates

Yeast isolates	Xylanase activity x10^3 U mL^{-1}
S. cerevisiae	0.122±0.01^{i}
P. stipilis	0.184±0.10^{c}
T. lutetiae	0.184±0.12^{c}
C. sheatae	0.172±0.00^{d}
C. parapsilosis	0.152±0.01^{g}
P. stipilis	0.149±0.00^{gh}
T. lutetiae	0.192±0.11^{b}
T. asahii	0.163±0.01^{ef}
C. tropicalis	0.198±0.09^{a}
S. cerevisiae	0.167±0.10^{e}
T. beigelil	0.152±0.00^{g}
C. sheatae	0.172±0.01^{d}
P. chambardii	0.199±0.11^{a}
P. stipilis	0.2142±0.00^{fg}

Results are values of mean ± standard error of mean for three replicates.
Values in a column that are followed by various letters indicate significant differences in xylanase activity of organisms according to LSD test at $\alpha \leq 0.05$

Molecular Characterization of Yeast Isolates

Gel electrophoresis of the DNA shows that a fragment of amplified DNA with 800bp was generated for *W. chambardii* and 500bp for *C. tropicalis* (Figure 1). Organisms were identified by searching databases using the BLAST sequence analysis tool (www.ncbi.nhi.gov/BLAST/) (NCBI). The two isolates with NCBI Accession number EF190223.1 and EF550482.1 were identified as *W. chambardii* and *C. tropicalis* respectively (Figure 2). The identity of yeast strains by sequences of genomic DNA shows 99% sequence identity (Table 4).

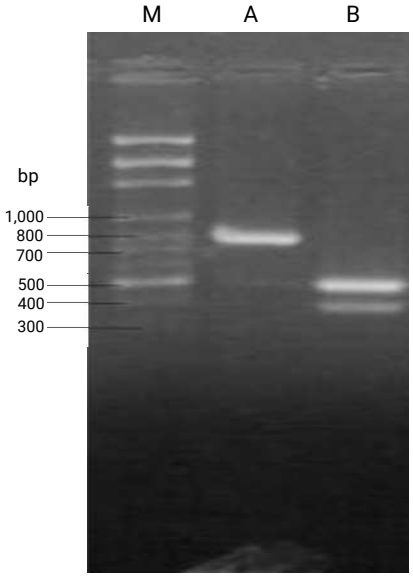

Figure 1. Electrophoretogram of PCR-amplified fragment of 18s rDNA of selected yeast strains. M=PCR marker, A and B=PCR products of DNA of selected yeast strains

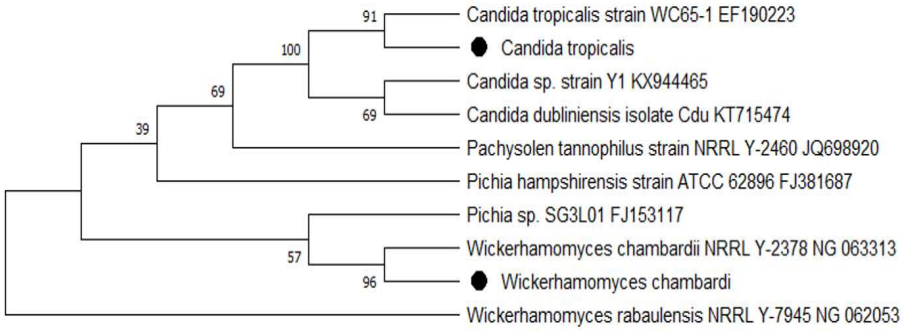

Figure 2. Phylogenetic tree of *W. chambardii* and *C. tropicalis* and related specie constructed using neighbor-joining method.

Table 4. Identity of yeast strains by sequences of genomic DNA

Isolate	Accession No	Name of Organisms	Identity
1	EF190223.1	*Wickerhamomyces chambardii*	99%
2	EF550482.1	*Candida tropicalis*	99%

Effect of D-xylose concentration on yeast isolates

W. chambardii and *C. tropicalis* showed different xylanase activity with varying concentrations of D-xylose. Xylanase activity increased with increase in concentration of D-xylose from 0.5% (w/v) up to 1.5% (w/v). Decrease in xylanase activity was observed at concentrations higher than 1.5% (Figure 1). At 1.5 % (w/v) D-xylose, *W. chambardii* had higher xylanase activity (275.83U mL^{-1}) compared to that of *C. tropicalis* (253.61U mL^{-1}) (Figure 3).

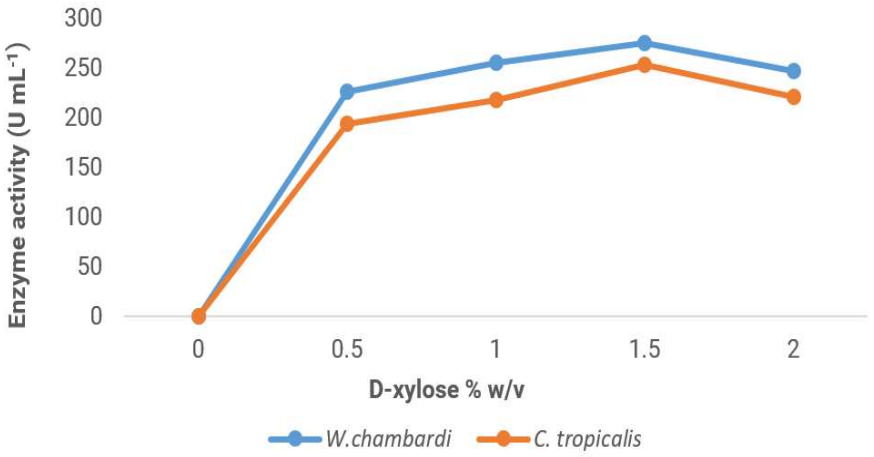

Figure 3. Effect of different concentrations of D-xylose on xylanase activity

DISCUSSION

Xylanase enzyme catalyzes the hydrolysis of 1,4-β-D-xylosidic linkages present in xylan, the major component of hemicellulose in plant cell walls (Adelabu & Kareem 2022). Yeasts are ubiquitous unicellular microorganisms used industrially for the production of enzymes. The results collated during the isolation of microorganisms in this study revealed that fourteen (14) isolates were obtained. Of all the isolates, 28.6% belonged to *Pichia* spp., 28.6% to *Trichosporon* spp., 28.6% to *Candida* spp. and 14.3% to *Saccharomyces* spp.

One of the factors responsible for isolation of only 14 yeasts may have been due to the availability of xylan, the carbon source of yeast, in the environment. The *Trichosporon* and *Pichia* spp growth on the samples may be due to their improvement in growth ability under stress conditions due to temperature (Agbogbo & Coward-Kelly 2008). The presence of Candida could be due to the depth of the different soil samples collected for isolation. Yeast, especially

Saccharomyces and *Candida* spp. are known to adapt best in high temperature and low pH (4-5) (Sadaf & Abdul 2013). The yeasts isolated, except *Saccharomyces* spp, are known to be abundant in various environments, such as sawdust and have no special nutritional requirements and hence, can be cultivated in most organic and inorganic environments. They utilize a wide range of nutrients due to their ability to secrete a wide range of digestive enzymes (Giese et al 2017). These yeasts also have the ability to live in acidic pH.

All of the fourteen (14) isolates were shown to produce xylanase enzyme in D-xylose supplemented Yeast extract Agar medium. The results suggest that some of the yeasts found in the soil of wood processing factory can directly digest xylose. The differences in their ability to hydrolyze xylose may possibly be due to the quantity of enzymes secreted into the growth medium and different growth rate of the yeasts (Giese et al 2017). The result of this study is consistent with that earlier reported by Adelabu et al (2019). Moubasher et al (2017) isolated yeasts that were xylanolytic from compost soil.

The result of ITS for identification of the yeasts shows a 99% identification rate. Results suggest that this approach provides an accurate alternative for specie description of xylanase producing yeasts. Biochemical characterization identified one of the yeast isolates as *P. chambardii* but the Polymerase Chain Reaction (PCR)- based method identified the isolate as *W. chambardii*. This finding agrees with the work of Kahve et al (2022) where PCR was also used to identify yeasts obtained from fermented juice extracted from black carrot.

Increase in xylanase activity was observed with increases in D-xylose concentration up to 1.5% D-xylose and decreased thereafter. The observed trend may be product inhibition where the accumulation of complex sugar in the fermentation medium inhibits the growth of yeast (Mardawati et al 2019). The complex sugar-rich fermentation medium could be toxic to the yeasts hence reduced the xylanase activity (Amadi et al 2020).

CONCLUSION

The result showed that yeasts found in sawdust have the potential to produce xylanase enzyme. The isolated yeasts produced xylanase enzyme from the fermentation of D-xylose. The potential of these yeasts to use D-xylose to synthesize xylanase will help various industries to reduce costs. This study has also shown that sawdust can be converted by yeasts into a useful enzyme and hence reduce pollution caused by composting or burning this waste product.

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AUTHOR CONTRIBUTIONS

BA designed the research, assisted in carrying out the research in the laboratory, she wrote the manuscript, she reviewed the manuscript till the final approval stage.

MT analyzed and interpreted the data. FS carried out the research in the laboratory.

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The research was carried out with personal funding.

AVAILABILITY OF DATA AND MATERIALS

Data generated during the study are included in this article and also available from the corresponding author upon request.

ETHICAL CONSIDERATION

This study does not involve vertebrates or invertebrates, thus there is no need for ethical approval.

COMPETING INTEREST

The authors declare no competing interests.

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