Journal of Science Research

Isolation and identification of thermophilic cellulolytic yeast from cassava waste dump

Onilude, A. A.,[1] Adekoya, A.O.,[1] Wakil, S. M.,[1] Fasiku, S. A.[2] and **Ja'afaru, I. M.[3]**
[1]Department of Microbiology, University of Ibadan, Ibadan. Oyo State
[2]Department of Biological Sciences, Ajayi Crowther University, Oyo
[3]Department of Microbiology, Madibbo Adama University of Technology, Yola, Adamawa State
Correponding author: shemowak@yahoo.com

Abstract

Cellulase is an enzyme complex which breaks down cellulose to glucose. The need for economical, complete and fast industrial processes necessitates the use of very active starters able to operate at high temperature of production to degrade cheap nutrients most of which are cellulolytic; hence the search for thermophilic cellulolytic yeasts from the environment. Isolation and identification of thermophilic yeasts were made from the soil samples using standard procedures and obtained isolates were screened for cellulolytic enzymes production. The enzymes were characterised using different parameters such as temperature, pH, substrate concentrations, enzyme concentrations and metal ion concentrations. A total of seven thermophilic yeasts were isolated from cassava waste dump sites and identified as *Torulopis sphaerica, Kloeckera apiculata, Pichia canadensis, Pichia* species, *Candida krusei*, *Candida utilis* and *Rodotorula rubra.* They all had optimum growth at temperature and pH of 55ºC and 6.0 respectively. The optimum temperature and pH for cellulolytic activities ranged from 45-55ºC and 5-6 respectively. Cellulolytic activities increased with increase in the concentration of substrate, enzymes and metal ions.

Keywords: Cellulolytic activities; thermophilic yeasts; substrate concentration; ion concentration.

Introduction

Cellulases refer to a group of enzymes which act together to hydrolyze cellulose into soluble sugars. They are distributed throughout the biosphere and are found in plants, animals and microorganisms. However, microorganisms are considered to be the main source of cellulases with novel and highly specific activities [1]. Microbial conversion of cellulose/lignocellulosic biomass into useful by-products is a complex process involving combined action of three enzymes namely endogluconase, exogluconase and β-glucosidase [2]. They are produced by different groups of organisms: psychrophiles, thermophiles and mostly by mesophiles. Developments of heat during industrial processes which destabilizes and denatures enzymes from mesophiles thus prompt the need for thermophilic enzymes.

Microorganisms, like all living things, adapt to the condition in which they live and survive. Thermophiles are reported to contain proteins which are thermostable and resist heat-denaturation and proteolysis [3]. Temperature along with other important environmental factors affects microbial activity. Yeasts are ascomycetous or basidiomycetous fungi that reproduce vegetatively by budding or fission, and that form sexual states which are not enclosed in a fruiting body [4]. They are single cell microorganisms and can be differentiated from bacteria morphologically by their larger cell size and their oval, elongate, or spherical cell shapes [5]. Yeast colonies show colours from creamy to red [6]. There have been reports of isolation of cellulose degrading yeast from environmental samples and thermophilic yeasts are also found in the environment [7-9].

Cellulases are widely used in the textile industry for the manufacture and finishing of cellulose-containing materials [10]. They have been successfully used for

the bio-stoning of jeans and bio-polishing of cotton and other cellulosic fabrics [10]. Industrially, it is a potential detergent additive to remove rough protuberances for a smoother, glossier and brighter coloured fabric [11]. Cellulase enzymes are used in the food industry to decrease viscosity of pulp mush, increase filtration rate and reduce processing time, increase juice yield and improve extraction of valuable components. Also, pre-treatment of agricultural silage and grain feed by cellulases can improve its nutritional value [10].

Thermophilic fungi are potential source of enzymes of scientific and commercial interest [12]. They have a powerful ability to degrade polysaccharide constituents of biomass. Their extracellular enzymes are generally more heat-stable than those of mesophilic microbes [13]. There is a need for isolating cellulolytic thermophilic yeasts. This work aims at isolating and identifying thermophylic cellulolytic yeasts from cassava waste dump and characterising the produced cellulolytic enzymes.

Materials and methods

Sample collection

Samples of cassava peels and root fibres were collected in sterile bottles from three cassava processing waste dump sites located at Iyana Agbala, Mokola and Agodi Gate, all within Ibadan Metropolis, Oyo State, Nigeria. Samples were collected at 2 cm and 4 cm depths from each site on three consecutive occasions and transported to the laboratory in an ice pack.

Isolation procedure

Thermophilic yeasts were isolated on yeast extract dextrose agar containing 30 μgml^{-1} of streptomycin using pour plate method. The plates were incubated at 55ºC for 96 hours and pure cultures obtained by streaking were maintained on yeast extract agar slant (pH 6) containing 5% (w/v) glucose at 4ºC. The themophilic yeast isolates were identified using morphological, physiological and biochemical characteristics [14].

Cellulase production

Erlenmeyer flasks (250 mL) containing modified Mandels and Weber basal medium [15] were autoclaved at 121ºC for 15 minnutes. The carbon source used was 5% (w/v) glucose which was filter-sterilized. Equal amounts of sterile carbon solution and basal medium were mixed together and inoculated with the test yeast isolates. After inoculation, the conical flasks (250 mL) containing the mixture were incubated at 55ºC in an incubator with shaking at 150 rpm for 96 hours. The growth medium was filtered using Whatman No. 4 filter paper, and the filtrate taken as crude enzyme was used for further enzyme studies.

Enzyme assays

Assay for the production and characterization of the different cellulases was by the optimized method of Haki and Rakshit [16]. Carboxymethyl cellulose (CMC-ase) was assayed by the procedure of Wood and McCrae [17] using carboxylmethyl-cellulose (CMC) as substrate. 9 mL of the filtrate (crude enzyme) added to 1mL of the solution containing 1.2% (w/v) carboxylmethyl-cellulose (CMC) in 0.2 M acetate buffer at pH 6.0 were incubated at 50ºC for 60 minutes. The reaction was stopped by the addition of 3mL Dinitrosalicylic acid (DNSA) solution.

β-glucosidase activity was determined using the modified method of Wood and McCrae (1977) with salicin as substrate[18]. To 9 mL of culture filtrate, 1 mL of a solution containing 0.5% (w/v) salicin in 0.2 M acetate buffer at pH 6.0 was added and incubated at 50ºC for 60 minutes.

Exo-β-1, 4 glucanase activity was assayed by the method described by Mandels and Weber [19] using filter paper as substrate. 1mL of culture filtrate was added to 1 mL of a solution containing 0.05% (w/v) Whatman No. 1 filter paper in 0.2 M acetate buffer at pH 6.0 and incubated at 50ºC for one hour.

The amount of reducing sugars produced was determined by the dinitrosalicylic acid procedure [20] with glucose as standard. The released reducing sugar was measured spectrophotometrically at 540 nm using UNISPEC 23D spectrophotometer. One unit (U) of enzyme activity was defined as 1 micromole of reducing sugar released per minute under the described assay conditions.

Total extracellular protein was measured by the method of Lowry *et al* [21] using casein as standard. All experiments were performed in duplicates and readings were done in triplicates.

Effect of temperature on cellulase enzyme production

The effect of temperature on the production of the three enzymes described above was studied by incubating the conical flasks at 40, 45, 50, 55 and 60ºC for one hour. The amount of reducing sugar produced was estimated according to the procedure described by Miller [20].

Effect of pH on cellulase enzyme production

One millilitre (1 mL) of each culture filtrate added to prescribed quantities of each substrate (Carboxylcellulose, salicin and Whatman No. 1 filter paper) in acetate buffer of varying pH (4.5, 5.0, 5.5, 6.0 and 6.5) was incubated at 50ºC for 1 hour. The reaction was stopped by the addition of 3 mL of DNSA, followed by heating at 100ºC for 15 minutes. The

amount of reducing sugar produced was determined spectrophotometrically [20].

Effect of substrate concentration on cellulase activity

Different concentration of each substrate (CMC, Salicin, Whatman No 1 filter paper) were dissolved in 0.2 M acetate buffer (pH 6.0) and incubated at 50ºC for 1 hour. The amount of reducing sugar produced was determined by the dinitrosalicylic acid procedure [20].

Effect of enzyme concentration on cellulase activity

Effect of enzyme concentration on cellulose activity was determined by varying the concentrations of culture filtrate added to the different substrate at pH 6.0 and incubated at 50ºC for 1 hour. Amount of reducing sugar produced was determined spectrophotometrically by the dinitrosalicylic acid procedure [20].

Effect of ion concentration on cellulase activity

The effect of cations on cellulase activity was determined using Manganese sulphate and Zinc sulphate as component of the enzyme substrate mixture separately. The different enzyme was assayed for as described earlier.

Statistical analysis

The data obtained in the present study were subjected to Analysis of Variance (ANOVA) using SPSS 19.0 version software. The significance of differences was tested at the level $p = 0.05$.

Results

Seven thermophilic yeast isolates that were cellulolytic as a result of their ability to degrade carboxymethyl cellulose were characterized and identified as *Torulopis sphaerica*, *K. apiculata*, *P. canadensis*, *Pichia* species, *C. krusei*, *Candida utilis* and *R. rubra*. The highest growth rate was observed at pH 6.0 for all the isolates except *P. canadensis* which showed the highest growth rate at pH 5.5. Generally, all the isolates grew favourably well at temperature of 55ºC except *Pichia* species which was favoured with temperature of 50ºC (Table 1).

Cellulolytic activities and cellulose production on the various substrates by the seven thermophilic cellulolytic yeasts isolated from the cassava waste dump are shown in Figure 1. From the three cellulolytic enzymes produced (carboxy-methylcellulase, exo-β-1, 4 glucanase and β-glucosidase), β-glucosidase had the highest yield of 6.50 mg/ml of reducing sugar concentration followed by carboxymethylcellulase producing a total of 6.41 mg/ml while exo-β-1, 4 glucanase gave the least reducing sugar yield in total of 6.02 mg/ml. The highest reducing sugar yield was recorded for β-glucosidase (1.2mg/ml) produced by *Pichia* species while the least reducing sugar yield was observed for exo-β-1, 4 glucanase (0.83 mg/ml) produced by *Rodotorula rubra*. Also from the figure, the highest yield of reducing sugar (0.97 mg/ml) from carboxymethylcellulase was by *P. canadensis* while the least value (0.85 mg/ml) was recorded for *K. apiculata*.

In terms of the protein content of the culture filtrates, the cellulolytic yeast isolate with highest specific activity of 0.3 U/mg proteins was observed to be *C. krusei* and the least value of 0.015U/mg was recorded for *Rodotorula rubra* (Figure 2).

Effect of temperature on the cellulolytic activities of thermophilic yeast isolates is as shown in Table 2. Highest carboxylmethyl cellulase activity was recorded at 55°C by *T. sphaerica* and *Rodotorula rubra* while *C. krusei* and *C. utilis*, *K. apiculata* and *Pichia* species and *P. canadensis* had their highest activities

Table 1. Effect of varying pH and temperature on the growth of the yeast isolates.

Isolates	Temperature of growth (ºC)			pH (growth culture incubated at 55ºC) (nm)		
	50	55	60	5.0	5.5	6.0
Torulopsis sphaerica	++	+++	+	*0.365±0.007^{bc}	0.400±0.004^{ab}	0.420±0.007^{b}
Candida krusei	+++	+++	+	0.365±0.004^{bc}	0.400±0.007^{ab}	0.400±0.003^{a}
Candida utilis	++	+++	++	0.370±0.003^{bc}	0.405±0.006^{b}	0.405±0.001^{a}
Kloeckera apiculata	+++	+++	+	0.350±0.004^{a}	0.420±0.002^{b}	0.440±0.004^{c}
Pichia spp.	+++	+	++	0.375±0.006^{c}	0.375±0.007^{a}	0.440±0.006^{c}
Pichia canadensis	+	+++	++	0.390±0.006^{d}	0.425±0.004^{b}	0.400±0.003^{a}
Rodotorula rubra	++	+++	++	0.360±0.007^{ab}	0.415±0.004^{b}	0.435±0.004^{c}

*Each value is a mean of duplicate measurements. Mean values with similar superscript along the column are not significantly different ($p \leq 0.05$).

Key: + = less than 10 colonies. ++ = less than 20 colonies. +++ = more than 20 colonies.

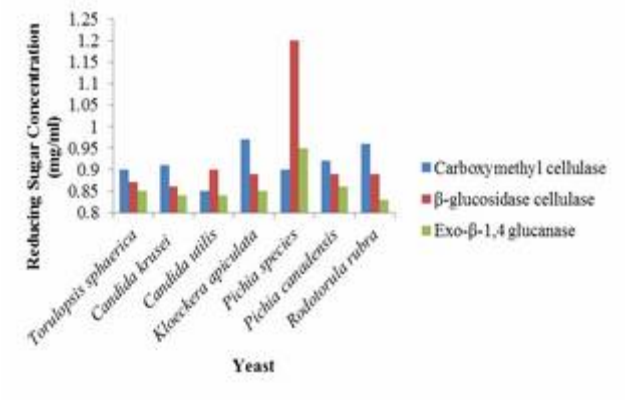

Figure 1. Reducing sugar concentration from Carboxymethyl cellulase, β-glucosidase and exo-β-1, 4 glucanase of seven cellulolytic yeast isolates from cassava waste dump.

at 50, 45 and 40°C respectively. The least carboxymethyl cellulase activities by all the isolates was observed at 60°C except for *K. apiculata* (40°C). For â-glycosidase enzyme, *C. utilis*, *K. apiculata* and *Pichia canadensis* had their highest activity at 55°C, *Torulopsis sphaerica* and *Pichia* species at 50°C while *Candida krusei* and *R. rubra* had theirs at 40°C. The β-glycosidase activities of all the yeast isolates at 40, 45 and 60°C were not significantly different ($p \leq 0.05$) from each other within each isolate except for *C. krusei* and *R. rubra*. *Pichia* species had the highest Exo-β-1-4-glucanase activity at the highest temperature of 60°C followed by *Kloeckera apiculata* at 55°C while *C. krusei* and *R. rubra* had theirs at 50°C which were not significantly different with other temperatures.

Generally, cellulolytic activities of all the yeast at varying temperature ranged from 0.0122-0.0500 unit/ml. Carboxylmethylcellulase, β-glucosidase and exo-β-1, 4 glucanase activities of all the isolates ranged from 0.0172-0.0360, 0.0122-0.0500 and 0.0168-0.0390 unit/mL respectively. The optimum temperature for Carboxylmethylcellulase, β-glucosidase and exo-β-1, 4 glucanase production ranged from 50-55ºC for all isolates.

Table 3 shows the effect of pH on cellulolytic activities of the thermophilic yeasts. *T. sphaerica* showed cellulolytic acitvities that ranged from 0.0168-0.0460 units/mL with the least for carboxyl-methylcellulase and the highest for β-glucosidase. Highest enzyme activity for carboxylmethylcellulase (0.0198Units/mL) and â-glucosidase (0.0460 units/mL) were recorded at pH 5.5 while that of exo-β-1, 4 glucanase (0.0450 units/mL) was at 6.0. Highest carboxylmethyl cellulase activities by most yeast was observed at pH 5.5 while others had theirs at pH 6.5 and differences in the enzyme activities were not significantly different ($p \leq 0.05$) within each strain at different pH. Optimum β-glucosidase activities by the yeasts were recorded at pH 5.5 except for *C. utilis*, *C. krusei* and *K. apiculata* which had theirs at pH 5 and pH 6 respectively. pH had significant effect on the β-glucosidase activities of the yeast isolates and their activities within each isolate were significantly different from each other except for *R. rubra*. All the yeast isolates had their highest exo-β-1, 4 glucanase activities at pH 5 which were significantly different from other pHs within each isolates except for *Pichia* species and *T. sphaerica* had theirs at pH 4.5 and pH 6.0 respectively.

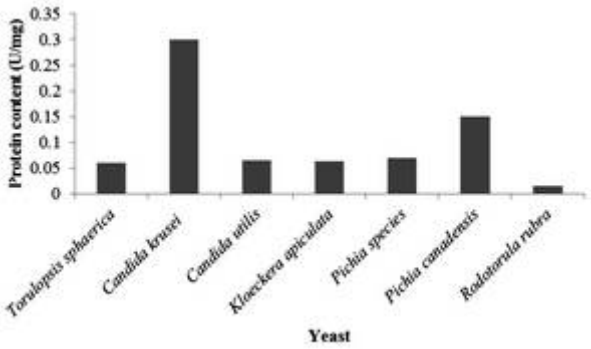

Figure 2. Protein estimation in filtrates of each cellulolytic yeast isolates from cassava waste dump.

Effect of substrate concentration on cellulolytic activities of thermophilic yeasts is shown in Table 4. There was increase in cellulolytic activities with increase in substrate concentration by all the isolates. Cellulolytic activities of *T. sphaerica*, *C. krusei*, *C. utilis*, *K. apiculata*, *P.* species, *P. canadensis* and *R. rubra* ranged from 0.0160-0.0856, 0.0230-0.0970, 0.0188-0.0714, 0.0176-0.0624, 0.0284-0.0910, 0.0252-0.0850 and 0.0250-0.0710 units/ml respectively. Carboxylmethyl cellulase, β-glucosidase and exo-β-1,4 glucanase activities of all the yeasts ranged from 0.0160-0.0536, 0.0176-0.0970 and 0.0176-0.0850 unit/ml respectively. The highest cellulolytic activities of all the yeast isolates were observed at the highest substrate concentration except for *C. utilis* which had its highest carboxylmethyl cellulase and â-glucosidase enzyme activities at substrate concentration of 1.4% and 0.6% respectively. Also *K. apiculata* had its highest β-glucosidase and exo-β-1,4 glucanase enzyme activities at 0.6% and 0.060% respectively. Statistical analysis revealed significant different ($p>0.05$) in enzyme activities of each yeast isolate at different substrates concentration except for carboxylmethyl cellulase activities of *T. sphaerica*.

Table 5 shows the effect of enzyme concentration on cellulolytic activities of thermophilic yeasts. The cellulase activity of *T. sphaerica*, *C. krusei*, *C. utilis*, *K. apiculata*, *Pichia* species, *P. canadensis* and

Table 2. Effect of temperature on cellulolytic activities (unit/mL) of thermophilic yeast,

Isolates	Temperature (ºC)	Cellulolytic activities		
		Carboxylmethylcellulase	β-glucosidase	Exo-β-1,4 glucanase
Torulopsis sphaerica	40	*0.0190±0.00071[a]	0.0260±0.00071[b]	0.0220±0.00071[c]
	45	0.0172±0.00071[a]	0.0250±0.00071[b]	0.0174±0.00057[a]
	50	0.0330±0.00071[b]	0.0400±0.00000[c]	0.0192±0.00071[b]
	55	0.0360±0.00057[b]	0.0220±0.00071[a]	0.0174±0.00057[a]
	60	0.0170±0.00071[a]	0.0250±0.00071[b]	0.0176±0.00071[ab]
Candida krusei	40	0.0340±0.00071[c]	0.0500±0.00000[e]	0.0172±0.00071[a]
	45	0.0174±0.00014[a]	0.0196±0.00071[a]	0.0172±0.00071[a]
	50	0.0254±0.00057[b]	0.0242±0.00071[c]	0.0184±0.00057[a]
	55	0.0176±0.00057[a]	0.0260±0.00071[d]	0.0172±0.00028[a]
	60	0.0174±0.00057[a]	0.0220±0.00071[b]	0.0184±0.00057[a]
Candida utilis	40	0.0176±0.00042[a]	0.0176±0.00057[a]	0.0190±0.00071[bc]
	45	0.0174±0.00071[a]	0.0174±0.00057[a]	0.0196±0.00057[c]
	50	0.0254±0.00071[b]	0.0180±0.00000[a]	0.0176±0.00071[ab]
	55	0.0176±0.00042[a]	0.0258±0.00057[b]	0.0170±0.00000[a]
	60	0.0174±0.00057[a]	0.0174±0.00028[a]	0.0184±0.00057[abc]
Kloeckera apiculata	40	0.0220±0.00000[a]	0.0170±0.00057[a]	0.0188±0.00042[a]
	45	0.0320±0.00071[c]	0.0170±0.00057[a]	0.0196±0.00042[ab]
	50	0.0310±0.00000[c]	0.0224±0.00057[b]	0.0204±0.00057[b]
	55	0.0310±0.00000[c]	0.0242±0.00028[c]	0.0220±0.00071[c]
	60	0.0260±0.00071[b]	0.0176±0.00057[a]	0.0188±0.00057[a]
Pichia species	40	0.0184±0.00057[a]	0.0170±0.00071[a]	0.0196±0.00042[b]
	45	0.0332±0.00071[c]	0.0170±0.00000[a]	0.0260±0.00057[c]
	50	0.0240±0.00071[b]	0.0190±0.00057[b]	0.0176±0.00000[a]
	55	0.0174±0.00000[a]	0.0184±0.00057[ab]	0.0176±0.00000[a]
	60	0.0174±0.00000[a]	0.0170±0.00057[a]	0.0390±0.00071[d]
Pichia canadensis	40	0.0280±0.00071[d]	0.0170±0.00071[a]	0.0188±0.00028[b]
	45	0.0270±0.00000[cd]	0.0170±0.00000[a]	0.0270±0.00071[c]
	50	0.0260±0.00071[bc]	0.0172±0.00042[a]	0.0174±0.00000[a]
	55	0.0250±0.00042[b]	0.0220±0.00071[b]	0.0174±0.00000[a]
	60	0.0180±0.00000[a]	0.0170±0.00057[a]	0.0172±0.00028[a]
Rodotorula rubra	40	0.0184±0.00057[ab]	0.0160±0.00057[c]	0.0170±0.00071[a]
	45	0.0190±0.00071[b]	0.0142±0.00028[b]	0.0170±0.00000[a]
	50	0.0192±0.00042[b]	0.0128±0.00000[a]	0.0172±0.00071[a]
	55	0.0240±0.00057[c]	0.0122±0.00000[a]	0.0170±0.00000[a]
	60	0.0174±0.00057[a]	0.0122±0.00000[a]	0.0168±0.00000[a]

*Each value is a mean of duplicate measurements. Mean values with similar superscript along the column of the same isolate are not significantly different ($p \leq 0.05$).

Table 3. Effect of pH on cellulolytic activities (unit/mL) of thermophilic yeast.

Isolates	pH	Cellulolytic activities		
		Carboxylmethylcellulase	β-glucosidase	Exo-β-1,4 glucanase
Torulopsis sphaerica	4.5	*0.0168±0.00071[a]	0.0178±0.00000[a]	0.0184±0.00071[a]
	5.0	0.0170±0.00057[a]	0.0176±0.00042[a]	0.0174±0.00071[a]
	5.5	0.0198±0.00028[b]	0.0460±0.00000[c]	0.0300±0.00000[b]
	6.0	0.0174±0.00057[a]	0.0310±0.00000[b]	0.0450±0.00000[c]
	6.5	0.0176±0.00057[a]	0.0172±0.00057[a]	0.0184±0.00071[a]
Candida krusei	4.5	0.0168±0.00071[a]	0.0176±0.00057[a]	0.0190±0.00042[b]
	5.0	0.0168±0.00057[a]	0.0200±0.00000[c]	0.0580±0.00000[c]
	5.5	0.0196±0.00057[b]	0.0250±0.00000[d]	0.0178±0.00071[a]
	6.0	0.0172±0.00071[a]	0.0300±0.00000[e]	0.0168±0.00042[a]
	6.5	0.0170±0.00042[a]	0.0186±0.00056[b]	0.0190±0.00000[b]
Candida utilis	4.5	0.0172±0.00071[a]	0.0176±0.00042[a]	0.0190±0.00000[b]
	5.0	0.0168±0.00028[a]	0.0380±0.00000[c]	0.0280±0.00000[c]
	5.5	0.0192±0.00057[b]	0.0174±0.00057[a]	0.0186±0.00057[b]
	6.0	0.0172±0.00028[a]	0.0200±0.00000[b]	0.0166±0.00057[a]
	6.5	0.0170±0.00000[a]	0.0174±0.00071[a]	0.0190±0.00000[b]
Kloeckera apiculata	4.5	0.0166±0.00071[a]	0.0176±0.00042[a]	0.0174±0.00057[a]
	5.0	0.0170±0.00000[a]	0.0187±0.00057[ab]	0.0350±0.00000[d]
	5.5	0.0196±0.00057[c]	0.0197±0.00042[b]	0.0298±0.00014[c]
	6.0	0.0186±0.00042[bc]	0.0220±0.00000[c]	0.0264±0.00057[b]
	6.5	0.0178±0.00071[ab]	0.0182±0.00057[a]	0.0174±0.00057[a]
Pichia species	4.5	0.0168±0.00071[a]	0.0172±0.00042[a]	0.0178±0.00071[a]
	5.0	0.0172±0.00028[ab]	0.0176±0.00056[a]	0.0176±0.00057[a]
	5.5	0.0184±0.00057[b]	0.0420±0.00000[c]	0.0176±0.00057[a]
	6.0	0.0280±0.00000[c]	0.0230±0.00000[b]	0.0168±0.00042[a]
	6.5	0.0168±0.00071[a]	0.0172±0.00042[a]	0.0178±0.00028[a]
Pichia canadensis	4.5	0.0168±0.00071[a]	0.0174±0.00057[a]	0.0180±0.00028[a]
	5.0	0.0168±0.00042[a]	0.0176±0.00057[a]	0.0396±0.00042[d]
	5.5	0.0170±0.00000[a]	0.0240±0.00000[b]	0.0334±0.00028[c]
	6.0	0.0172±0.00057[a]	0.0186±0.00057[a]	0.0270±0.00000[b]
	6.5	0.0172±0.00057[a]	0.0186±0.00057[a]	0.0180±0.00000[a]
Rodotorula rubra	4.5	0.0170±0.00057[a]	0.0174±0.00057[a]	0.0174±0.00057[a]
	5.0	0.0176±0.00071[ab]	0.0176±0.00057[a]	0.0184±0.00071[a]
	5.5	0.0180±0.00000[ab]	0.0184±0.00057[a]	0.0180±0.00000[a]
	6.0	0.0183±0.00071[ab]	0.0180±0.00000[a]	0.0178±0.00057[a]
	6.5	0.0186±0.00042[b]	0.0176±0.00057[a]	0.0174±0.00057[a]

*Each value is a mean of duplicate measurements. Mean values with similar superscript along the column of the same isolate are not significantly different ($p \leq 0.05$).

Table 4. Effect of substrate concentration on cellulolytic activities (unit/mL) of thermophilic yeast.

Isolate	Cellulolytic activities					
	Carboxylmethylcellulase		β-glucosidase		Exo-β-1,4 glucanase	
	Sub. Conc. (%)	Enzyme activities	Sub. Conc. (%)	Enzyme activities	Sub. Conc. (%)	Enzyme Activities
Torulopsis sphaerica	1.0	*0.0160±0.00042[a]	0.2	0.0448±0.00057[a]	0.050	0.0270±0.00071[a]
	1.2	0.0162±0.00071[a]	0.4	0.0502±0.00028[b]	0.055	0.0378±0.00028[b]
	1.4	0.0168±0.00057[a]	0.6	0.0712±0.00071[c]	0.060	0.0640±0.00042[c]
	1.6	0.0170±0.00014[a]	0.8	0.0856±0.00057[d]	0.065	0.0734±0.00071[d]
Candida krusei	1.0	0.0230±0.00071[a]	0.2	0.0440±0.00028[a]	0.050	0.0394±0.00057[a]
	1.2	0.0306±0.00042[b]	0.4	0.0624±0.00057[b]	0.055	0.0618±0.00028[b]
	1.4	0.0380±0.00071[c]	0.6	0.0730±0.00071[c]	0.060	0.0642±0.00042[c]
	1.6	0.0390±0.00042[c]	0.8	0.0970±0.00057[c]	0.065	0.0714±0.00057[d]
Candida utilis	1	0.0188±0.00000[a]	0.2	0.0382±0.00042[a]	0.050	0.0240±0.00028[a]
	1.2	0.0310±0.00028[b]	0.4	0.0500±0.00028[b]	0.055	0.0264±0.00000[b]
	1.4	0.0470±0.00071[d]	0.6	0.0660±0.00071[d]	0.060	0.0642±0.00042[c]
	1.6	0.0390±0.00071[c]	0.8	0.0518±0.00042[c]	0.065	0.0714±0.00028[d]
Kloeckera apiculata	1	0.0188±0.00057[a]	0.2	0.0176±0.00057[a]	0.050	0.0176±0.00057[a]
	1.2	0.0284±0.00057[b]	0.4	0.0392±0.00071[b]	0.055	0.0220±0.00071[b]
	1.4	0.0370±0.00071[b]	0.6	0.0624±0.00042[d]	0.060	0.0296±0.00042[d]
	1.6	0.0536±0.00057[b]	0.8	0.0512±0.00057[c]	0.065	0.0260±0.00071[c]
Pichia species	1	0.0284±0.00028[a]	0.2	0.0396±0.00057[a]	0.050	0.0306±0.00071[a]
	1.2	0.0382±0.00014[b]	0.4	0.0710±0.00071[b]	0.055	0.0330±0.00071[b]
	1.4	0.0520±0.00071[c]	0.6	0.0842±0.00057[c]	0.060	0.0440±0.00028[c]
	1.6	0.0520±0.00042[c]	0.8	0.0910±0.00071[d]	0.065	0.0714±0.00071[d]
Pichia canadensis	1	0.0252±0.00028[a]	0.2	0.0380±0.00071[a]	0.050	0.0300±0.00014[a]
	1.2	0.0306±0.00042[b]	0.4	0.0550±0.00071[b]	0.055	0.0440±0.00042[b]
	1.4	0.0500±0.00028[c]	0.6	0.0674±0.00042[c]	0.060	0.0712±0.00042[c]
	1.6	0.0500±0.00042[c]	0.8	0.0674±0.00057[c]	0.065	0.0850±0.00042[d]
Rodotorula rubra	1	0.0250±0.00057[a]	0.2	0.0328±0.00028[a]	0.050	0.0252±0.00042[a]
	1.2	0.0284±0.00057[b]	0.4	0.0440±0.00071[b]	0.055	0.0320±0.00042[b]
	1.4	0.0398±0.00028[c]	0.6	0.0540±0.00071[c]	0.060	0.0420±0.00071[c]
	1.6	0.0454±0.00071[d]	0.8	0.0710±0.00000[d]	0.065	0.0660±0.00071[d]

*Each value is a mean of duplicate measurements. Mean values with similar superscript along the column of the same isolate are not significantly different ($p \leq 0.05$).

Key: Sub. Conc. = Substrate Concentration.

Table 5. Effect of enzyme concentration on cellulolytic activities (unit/mL) of thermophilic yeasts.

Isolates	Enzymes concentration (%)	Cellulolytic activities		
		Carboxylmethylcellulase	β-glucosidase	Exo-β-1,4 glucanase
Torulopsis sphaerica	0.5	*0.0136±0.00071^{bc}	0.0162±0.00057^{a}	0.0172±0.00057^{bc}
	1.5	0.0146±0.00042^{c}	0.0184±0.00057^{b}	0.0160±0.00000^{ab}
	2.0	0.0130±0.00071^{ab}	0.0168±0.00071^{a}	0.0158±0.00042^{a}
	2.5	0.0120±0.00000^{a}	0.0160±0.00000^{a}	0.0174±0.00057^{c}
Candida krusei	0.5	0.0150±0.00071^{b}	0.0160±0.00071^{b}	0.0176±0.00042^{b}
	1.5	0.0128±0.00000^{a}	0.0172±0.00000^{c}	0.0178±0.00057^{b}
	2.0	0.0128±0.00000^{a}	0.0164±0.00057^{bc}	0.0158±0.00028^{a}
	2.5	0.0130±0.00071^{a}	0.0140±0.00042^{a}	0.0186±0.00042^{b}
Candida utilis	0.5	0.0140±0.00071^{a}	0.0160±0.00071^{ab}	0.0172±0.00028^{a}
	1.5	0.0140±0.00071^{a}	0.0170±0.00042^{b}	0.0166±0.00042^{a}
	2.0	0.0140±0.00071^{a}	0.0150±0.00057^{a}	0.0172±0.00042^{a}
	2.5	0.0140±0.00000^{a}	0.0166±0.00057^{ab}	0.0192±0.00071^{b}
Kloeckera apiculata	0.5	0.0160±0.00071^{ab}	0.0162±0.00028^{a}	0.0128±0.00071^{a}
	1.5	0.0170±0.00042^{b}	0.0172±0.00057^{a}	0.0166±0.00057^{b}
	2.0	0.0148±0.00057^{a}	0.0164±0.00057^{a}	0.0172±0.00028^{b}
	2.5	0.0162±0.00000^{ab}	0.0168±0.00000^{a}	0.0177±0.00000^{b}
Pichia species	0.5	0.0160±0.00057^{ab}	0.0168±0.00071^{a}	0.0158±0.00057^{a}
	1.5	0.0172±0.00042^{b}	0.0160±0.00042^{a}	0.0166±0.00057^{a}
	2.0	0.0166±0.00057^{ab}	0.0162±0.00028^{a}	0.0172±0.00028^{a}
	2.5	0.0150±0.00071^{a}	0.0162±0.00000^{a}	0.0166±0.00057^{a}
Pichia canadensis	0.5	0.0150±0.00071^{a}	0.0164±0.00000^{a}	0.0162±0.00042^{a}
	1.5	0.0178±0.00028^{b}	0.0168±0.00057^{a}	0.0164±0.00057^{a}
	2.0	0.0160±0.00042^{a}	0.0160±0.00000^{a}	0.0178±0.00000^{b}
	2.5	0.0162±0.00028^{a}	0.0172±0.00071^{a}	0.0196±0.00042^{c}
Rodotorula rubra	0.5	0.0142±0.00000^{b}	0.0166±0.00057^{a}	0.0250±0.00000^{c}
	1.5	0.0146±0.00042^{b}	0.0160±0.00000^{a}	0.0185±0.00057^{b}
	2.0	0.0126±0.00042^{a}	0.0162±0.00042^{a}	0.0172±0.00042^{a}
	2.5	0.0128±0.00071^{a}	0.0160±0.00057^{a}	0.0162±0.00042^{a}

*Each value is a mean of duplicate measurements. Mean values with similar superscript along the column of the same isolate are not significantly different ($p \leq 0.05$).

Table 6. Effect of $ZnSO_4$ concentration on cellulolytic activities (unit/mL) of thermophilic yeasts.

Isolates	$ZnSO_4$ concentration (mM)	Cellulolytic activities		
		Carboxylmethylcellulase	β-glucosidase	Exo-β-1,4 glucanase
Torulopsis sphaerica	0.1	*0.0172±0.00071[a]	0.0174±0.00057[a]	0.0166±0.00057[a]
	0.2	0.0180±0.00000[a]	0.0190±0.00000[b]	0.0170±0.00000[a]
	0.3	0.0194±0.00042[b]	0.0194±0.00071[b]	0.0170±0.00000[a]
	0.4	0.0196±0.00057[b]	0.0220±0.00000[c]	0.0172±0.00071[a]
	0.5	0.0270±0.00000[c]	0.0290±0.00000[d]	0.0184±0.00042[b]
Candida krusei	0.1	0.0170±0.00000[a]	0.0174±0.00071[a]	0.0168±0.00028[a]
	0.2	0.0170±0.00000[a]	0.0190±0.00000[b]	0.0170±0.00000[a]
	0.3	0.0176±0.00057[a]	0.0194±0.00071[b]	0.0172±0.00042[a]
	0.4	0.0192±0.00057[b]	0.0224±0.00071[c]	0.0176±0.00057[ab]
	0.5	0.0220±0.00000[c]	0.0300±0.00000[d]	0.0184±0.00057[b]
Candida utilis	0.1	0.0168±0.00071[a]	0.0170±0.00000[a]	0.0168±0.00028[a]
	0.2	0.0172±0.00071[a]	0.0180±0.00000[b]	0.0170±0.00000[a]
	0.3	0.0180±0.00000[ab]	0.0194±0.00071[c]	0.0172±0.00028[a]
	0.4	0.0190±0.00000[b]	0.0240±0.00000[d]	0.0174±0.00014[a]
	0.5	0.0196±0.00057[c]	0.0302±0.00028[a]	0.0184±0.00071[b]
Kloeckera apiculata	0.1	0.0170±0.00000[a]	0.0180±0.00000[a]	0.0168±0.00014[a]
	0.2	0.0170±0.00000[a]	0.0192±0.00071[b]	0.0168±0.00028[a]
	0.3	0.0172±0.00071[a]	0.0240±0.00000[c]	0.0170±0.00000[a]
	0.4	0.0178±0.00028[a]	0.0312±0.00071[d]	0.0172±0.00071[a]
	0.5	0.0188±0.00028[b]	0.0390±0.00000[e]	0.0176±0.00057[a]
Pichia species	0.1	0.0192±0.00071[a]	0.0330±0.00000[a]	0.0168±0.00071[a]
	0.2	0.0196±0.00057[a]	0.0350±0.00000[b]	0.0172±0.00071[a]
	0.3	0.0198±0.00028[a]	0.0360±0.00000[c]	0.0174±0.00071[a]
	0.4	0.0220±0.00000[b]	0.0424±0.00071[d]	0.0180±0.00000[ab]
	0.5	0.0306±0.00071[b]	0.0510±0.00000[e]	0.0192±0.00028[b]
Pichia canadensis	0.1	0.0172±0.00071[a]	0.0170±0.00000[a]	0.0168±0.00057[a]
	0.2	0.0174±0.00071[a]	0.0176±0.00042[a]	0.0168±0.00057[a]
	0.3	0.0176±0.00057[a]	0.0220±0.00000[b]	0.0170±0.00000[a]
	0.4	0.0194±0.00071[b]	0.0296±0.00057[c]	0.0172±0.00057[a]
	0.5	0.0196±0.00057[b]	0.0308±0.00071[d]	0.0174±0.00057[a]
Rodotorula rubra	0.1	0.0170±0.00000[a]	0.0170±0.00000[a]	0.0166±0.00028[a]
	0.2	0.0176±0.00057[a]	0.0180±0.00000[a]	0.0168±0.00028[a]
	0.3	0.0180±0.00000[ab]	0.0192±0.00071[b]	0.0168±0.00028[a]
	0.4	0.0188±0.00028[bc]	0.0198±0.00028[b]	0.0170±0.00000[a]
	0.5	0.0192±0.00071[c]	0.0296±0.00057[c]	0.0172±0.00071[a]

*Each value is a mean of duplicate measurements. Mean values with similar superscript along the column of the same isolate are not significantly different ($p \leq 0.05$).

Table 7. Effect of $MnSO_4$ concentration on cellulolytic activities (unit/mL) of thermophilic yeasts.

Isolates	$MnSO_4$ Concentration (mM)	Cellulolytic activities		
		Carboxylmethylcellulase	β-glucosidase	Exo-β-1,4 glucanase
Torulopsis sphaerica	0.1	*0.0172±0.00071[a]	0.0250±0.00000[a]	0.0220±0.00028[a]
	0.2	0.0230±0.00000[b]	0.0270±0.00000[b]	0.0308±0.00071[b]
	0.3	0.0252±0.00071[c]	0.0310±0.00000[c]	0.0370±0.00057[c]
	0.4	0.0298±0.00028[d]	0.0440±0.00000[d]	0.0390±0.00057[d]
	0.5	0.0398±0.00028[e]	0.0510±0.00028[e]	0.0452±0.00071[e]
Candida krusei	0.1	0.0240±0.00028[a]	0.0240±0.00071[a]	0.0186±0.00042[a]
	0.2	0.0250±0.00071[a]	0.0260±0.00071[b]	0.0252±0.00071[b]
	0.3	0.0270±0.00071[b]	0.0280±0.00071[c]	0.0272±0.00071[c]
	0.4	0.0308±0.00071[c]	0.0392±0.00071[d]	0.0312±0.00057[d]
	0.5	0.0394±0.00071[d]	0.0500±0.00000[e]	0.0420±0.00071[e]
Candida utilis	0.1	0.0184±0.00071[a]	0.0186±0.00057[a]	0.0184±0.00071[a]
	0.2	0.0240±0.00071[b]	0.0220±0.00071[b]	0.0192±0.00043[a]
	0.3	0.0250±0.00071[b]	0.0280±0.00071[c]	0.0300±0.00000[b]
	0.4	0.0368±0.00071[c]	0.0384±0.00071[d]	0.0380±0.00071[c]
	0.5	0.0500±0.00000[d]	0.0500±0.00000[e]	0.0512±0.00071[d]
Kloeckera apiculata	0.1	0.0184±0.00071[a]	0.0196±0.00057[a]	0.0240±0.00071[a]
	0.2	0.0196±0.00057[a]	0.0198±0.00028[a]	0.0500±0.00000[d]
	0.3	0.0198±0.00028[a]	0.0306±0.00028[b]	0.0298±0.00028[b]
	0.4	0.0300±0.00000[b]	0.0480±0.00071[c]	0.0310±0.00071[b]
	0.5	0.0420±0.00071[c]	0.0570±0.00071[d]	0.0450±0.00071[c]
Pichia species	0.1	0.0264±0.00071[a]	0.0280±0.00071[a]	0.0270±0.00071[a]
	0.2	0.0280±0.00071[ab]	0.0306±0.00071[b]	0.0280±0.00071[a]
	0.3	0.0296±0.00057[b]	0.0450±0.00071[c]	0.0390±0.00071[b]
	0.4	0.0460±0.00071[c]	0.0540±0.00000[d]	0.0472±0.00000[c]
	0.5	0.0660±0.00071[d]	0.0630±0.00071[e]	0.0800±0.00000[d]
Pichia canadensis	0.1	0.0186±0.00071[a]	0.0220±0.00071[a]	0.0220±0.00000[a]
	0.2	0.0198±0.00028[a]	0.0262±0.00000[b]	0.0250±0.00071[b]
	0.3	0.0220±0.00000[b]	0.0296±0.00057[c]	0.0270±0.00071[c]
	0.4	0.0324±0.00057[c]	0.0308±0.00071[c]	0.0274±0.00071[c]
	0.5	0.0440±0.00071[d]	0.0512±0.00071[d]	0.0470±0.00071[d]
Rodotorula rubra	0.1	0.0172±0.00071[a]	0.0224±0.00042[a]	0.0184±0.00071[a]
	0.2	0.0186±0.00057[ab]	0.0268±0.00042[b]	0.0332±0.00071[b]
	0.3	0.0196±0.00057[b]	0.0280±0.00071[b]	0.0340±0.00071[b]
	0.4	0.0308±0.00071[c]	0.0306±0.00057[c]	0.0380±0.00071[c]
	0.5	0.0440±0.00071[d]	0.0500±0.00000[d]	0.0460±0.00071[d]

*Each value is a mean of duplicate measurements. Mean values with similar superscript along the column of the same isolate are not significantly different ($p \leq 0.05$).

R. rubra ranged from 0.0120-0.0184, 0.0128-0.0186, 0.0140-0.0192, 0.0128-0.0177, 0.0150-0.0172, 0.0150-0.0196 and 0.0126-0.0250 unit/ml respectively. Carboxylmethylcellulase, β-glucosidase and exo-β-1, 4 glucanase of all the yeast isolates ranged from 0.0120-0.0178, 0.0140-0.0184 and 0.0128-0.0250 unit/ml respectively.

Cellulase activity of all the yeasts ranged from 0.0120-0.0250 unit/ml. With the exception of *C. krusei*, all other isolates recorded highest value of carboxylmethylcellulase at 1.5 enzyme concentration and highest exo-β-1, 4 glucanase at 2.5 enzyme concentration except for *Pichia* species and *R. rubra*. The least values of cellulolytic activities of all isolates were recorded in carboxylmethylcellulase for all the isolates with the exception of *K. apiculata* that recorded the least cellulolytic activities (0.0128 unit/ml) in exo-β-1, 4 glucanase. Statistical analysis showed there is little or no significant difference ($p \leq 0.05$) in the cellulolytic activities of all the isolates at different concentration of enzymes.

Cellulululolytic activity increased with increase in concentration of $ZnSO_4$ with the highest cellulase activities recorded at 0.5 mM of $ZnSO_4$ (Table 6). The enzyme activity of each isolate increased in the order, exo-β-1, 4 glucanase activity < carboxylmethycellulase activity < β-glucosidase activity. *Pichia* species recorded the highest cellulase activity for all concentration of $ZnSO_4$. Statistical analysis revealed that there was little or no significant difference in carboxylmethylcellulase and exo-β-1,4 glucanase but there is significant difference (p>0.05) in â-glucosidase with different concentration of $ZnSO_4$ in each of the isolates.

The enzyme activity of all the isolates increased with increase in concentration of $MnSO_4$ and the highest was recorded at 0.5 mM for each isolate (Table 7). Carboxylmethylcellulase, β-glucosidase and exo-β-1, 4 glucanase of all the isolates ranged from 0.0172-0.0660, 0.0186-0.0630 and 0.0184-0.0800 unit/mL respectively. At different concentration of $MnSO_4$, cellulase activity of all the isolates ranged from 0.0172-0.0800 unit/mL. *Pichia* species recorded highest cellulase activity at all concentration of $MnSO_4$. The relative decreasing order of cellulolytic activities of the three cellulolytic enzymes is exo-β-1, 4 glucanase > β-glucosidase > carboxylmethylcellulase. Change in concentration of $MnSO_4$ had significant effect (p>0.05) on the enzyme activities of all the isolates.

Discussion

Seven cellulolytic thermophilic yeast species were isolated from two cassava dump sites. Yeasts have been known to utilize agro-industrial peel and pulp wastes to produce enzymes and proteins [22]. The cellulolytic thermophilic yeasts were identified as *T. sphaerica*, *C. krusei*, *C. utilis*, *K. apiculata*, *Pichia* species, *P. canadensis* and *R. rubra* and their enzymes were stable at high temperature. Maheshwari [13] reported that thermophilic fungi extracellular enzymes are generally more heat stable than that of mesophilic microbes.

Temperature had significant effect on cellulolytic enzyme activities with optimum temperature ranging from 45-55ºC. This is in accordance with the work of Azzeddine *et al* [23] and Sharma *et al* [24], who recorded 50ºC as the optimum temperature for cellulolytic activities but contrary to the work of Jaradat *et al* [25] and Trinh *et al* [26] who reported 28 and 60ºC respectively as the optimum temperature for cellulolytic activities.

It was observed that pH had significant effect on cellulolytic activities of all the isolates. The optimum pH in this work ranged from pH 5-6 which was supported by Jaradat *et al* [25], Azzeddine *et al* [23] and Sharma *et al* [24] but contrary to the reports of Padmavathi *et al* [2] (2012) and Trinh *et al* [26] who reported pH 3 and 7 respectively as the optimum pH for cellulolytic enzyme activity.

Substrate concentration is very important and it was observed in this research that it played a significant role in cellulolytic activity in all the isolated thermophilic yeasts. Cellulolytic activity increased with increase in substrates concentration in all the three forms of cellulolytic enzymes (carboxylmethylcellulase, β-glucosidase and exo-β-1, 4 glucanase). This is in line with the work of Ahmed *et al* [27], Padmavathi *et al* [2] (2012) and Sharma *et al* [24] who reported that substrate concentration had significant effect on cellulase activities. They all reported that increase in substrates concentration resulted in increase in cellulolytic activities however, Sharma *et al* [24] reported that further increase beyond optimum concentration of substrate resulted in decrease in cellulolytic activities of the microorganisms.

Significant effect of metal ions on cellulolytic activities was observed as there was increase in cellulolytic activities with increase in metal ion ($MnSO_4$ and $ZnSO_4$) concentration. This is in line with the report of some workers [28, 29]. Ramanathan *et al* [30], Andrade *et al* [31] and Trinh *et al* [26] reported contrarily that metal ions repressed cellulolytic activities. A higher cellulolytic activity was observed with $MnSO_4$ than $ZnSO_4$ which was contrary to the report of Shankar and Isaiaras [28] where $ZnSO_4$ performed better than $MnSO_4$.

In conclusion, cellulolytic thermophilic yeasts with optimum growth's temperature and pH of 55ºC and

6.0 respectively were isolated and identified. The optimum temperature and pH of their cellulolytic activities ranged from 45-55ºC and 5-6 respectively; and optimum Metal ion concentration is 0.5 mM of $ZnSO_4$. Cellulolytic activities increased with increase in the concentration of substrate, enzymes and metal ions.

References

[1] Korish M. 2003. Production, purification, properties and application of the cellulases from a wild type strain of a yeast isolate. Dissertation for attaining the degree of doctor of Natural Sciences, Faculty of Biology, Johannes Gutenberg-University, Mainz.

[2] Padmavathi, T, Vaswati, N. and Puneet, A. 2012. Optimization of the medium for the production of cellulases by *Aspergillus terreus* and *Mucor plumbeus*. *European J. Exp. Biol. 2 (4):* 1161-1170.

[3] Kumar, H. D. and Swati, S. 2001. *Modern Concepts of Microbiology, Second Revised Ed.* Vikas Publishing House Prt. Ltd., New Delhi.

[4] Boekhout, T. and Kurtzman, C.P. 1996. Principles and methods used in yeast classification, and an overview of currently accepted yeast genera. In: Wolf, K. *Nonconventional Yeasts in Biotechnology: A Handbook.* Springer-Verlag: Heidelberg, pp. 1-99.

[5] Chomsri, N. 2008. Impact of protease activity of yeast on wine fermentation and formation of volatile and non-volatile metabolites. Thesis of degree of Doctor Institute of Nutritional Science, Justus Liebig-University Giessen, Germany, p. 4.

[6] Kurtzman, C. P. and Fell, J. W. 2006. Yeast systematics and Phylogeny: Implications of molecular identification methods for studies in ecology. In C. Rosa and G. Péter (Eds.), *Biodiversity and ecophysiology of yeasts: The yeast handbook*. New York: Springer, pp. 11-30.

[7] Mangunwardoyo, W., Aprilismulan, Oetari, A. and Sjamsuridzal, W. 2011. Screening Cellulose Activity of Yeast Isolated from Soil, Sediment and Water River from Taman Nasional Gunung Halimun, West Java, Indonesia. *Malaysian J. Microbiol. 7(4):* 210-216.

[8] Phuong, D. N. T., Thanonkeo, P. and Phong, H. X. 2012. Screening useful isolated yeasts for ethanol fermentation at high temperature. *Inter. J. Appl. Sci. Tech. 2(4):* 65-71.

[9] Sulman S. and Rehman, A. 2013. Isolation and Characterization of Cellulose Degrading *Candida tropicalis* W2 from Environmental Samples. *Pak. J. Zool., 45(3):* 809-816.

[10] Weldesemayat, G. 2011. Production and Optimization of cellulase enzyme under Submerged and Solid State Fermentation from *Trichoderma* Isolates. A Thesis of Degree of Master of Science in Microbiology, Addis Ababa University.

[11] Bhat, M. K. 2000. Cellulases and related enzymes in biotechnology. *Biotechnol. Adv. 18:* 355-383.

[12] Nazir, N., Mirza, J. H., Akhtar, N., Bajwa, R. Nasim, Ghazala. 2007. Some studies on thermophilic and thermotolerant fungi from Lahore, Pakistan. *Mycopath 5(2):* 95-100.

[13] Maheshwari, R. 1997. The ecology of thermophilic fungi. In: Janardhan. K. K., Rajendran, C., Natarajan, K, Hawksworth, D. L. Editors. *Tropical mycology.* New Delhi, India: Oxford and IBH Publishing Co. Pvt. Ltd. 277-289.

[14] Van der Walt, J. P. and Yarrow, D. 1984. Methods for isolation, maintenance and classification of yeast. In: *The Yeast, a Taxonomy study, 3rd edn.*, Edited by N. J. W. Kreger-Van Rij. Amsterdam: Elsevier, pp. 45-104.

[15] Onilude, A. A. 1996. Effect of cassava cultivar, age and pretreatment processes of cellulase and xylase production from cassava waste by *Trichoderma harzianum*. *J. Basic Microbiol. 36 (6):* 421-431.

[16] Haki, G. D. and Rakshit, S. K. 2003. Developments in industrially important thermostable enzymes: a review. *Biores. Rev. 89:* 17-34.

[17] Wood, T. M. and McCrae, S. I. 1977. Cellulase from *Fusarium solani*. Purification and properties of C1 component. *Carbohydrate Res. 57:* 117-133.

[18] Kader, A. J. and Omar, O. 1998. Isolation of cellulolytic fungi from Sayap-Kinasalu Park, Sabah. *Asean Review of Biodiversity and Environmental Conservation.*

[19] Mandel, M. and Weber, J. 1969. The production of cellulases. In cellulases and their applications, *Adv. Chem. Ser 95:* 391-413, Ed. E. J. Hajnj and E. T. Reese. Washington, USA.

[20] Miller, G. L. 1959. Use of dinitrosalisylic acid reagent for determination of reducing sugar. *Anal. Chem. 31:* 426-28.

[21] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem. 193:* 265-75.

[22] Attyia, S. H. and Ashour, S. M. 2002. Biodegradation of agro-industrial orange waste under solid state fermentation and natural environmental conditions. *Egyptian J. Biol. 4:* 23-30.

[23] Azzeddine, B., Abdelaziz, M., Estelle, C., Mouloud, K., Nawel, B., Nabila, B., Francis, D. and Said, B. 2013. Optimization and partial characterization of endoglucanase produced by *Streptomyces* sp. B-PNG23 *Arch. Biol. Sci., Belgrade, 65 (2):* 549-558.

[24] Sharma, V., Vij, H., Singh, P. K. and Bhatt S. 2013. Potential Cellulase Production, Optimization and Sachharification Study by Novel Thermophilic Microbes. *ABS J. Sustainable Biotech. 1 (1):* 19-26.

[25] Jaradat, Z., Dawagreh, A., Ababneh, Q. and Saadoun, I. 2008. Influence of Culture Conditions on Cellulase Production by *Streptomyces* Sp. (Strain J2) *Jor. J. Biol. Sci. 1 (4):* 141-146.

[26] Trinh, D. K., Quyen, D. T., Do, T. T., Nguyen, T. T. H , Nghiem, N. M. 2013. Optimization of Culture Conditions and Medium Components for Carboxymethyl Cellulase (CMCase) Production by a Novel Basidiomycete Strain *Peniophora* sp. NDVN01. *Iranian J. Biotech.* 11(4): 251-259.

[27] Ahmed, I. and Zia, M. A. and Iqbal, H. M. N. 2010. Bioprocessing of proximally analyzed wheat straw for enhanced cellulase production through process optimization with *Trichoderma viride* under SSF *Inter. J. Biol. Life Sci. 6(3):* 164-170.

[28] Shankar, T. and Isaiarasu, L. 2011. Cellulase production by *Bacillus pumilus* EWBCM1 under varying cultural conditions. *Middle-East J. Scient. Res. 8 (1):* 40-45.

[29] Sreeja, S. J., Jeba, M. P. W., Sharmila, J. F. R., Steffi, T., Immanuel, G. and Palavesam, A. 2013 Optimization of cellulase production by *Bacillus altitudinis* APSMSU and *Bacillus licheniformis* APS2 MSU, gut isolates of fish *Etroplus suratensis*. *Inter. J. Adv. Res. Tech.*, *2(4):* 401-406.

[30] Ramanathan, G. Banupriya, S and Abirami, D. 2010. Production and potimization of cellulase from *Fusarium oxysporum* by submerged fermentation. *J. Scient. Indust. Res. 69:* 454-459.

[31] Andrade, J. P., Bispo, A. S. R., Marbach, P. A. S. and Nascimento, R. P. 2011 Production and partial characterization of cellulases from *Trichoderma* sp. IS-05 isolated from sandy coastal plains of northeast Brazil *Enzy. Res.* vol. 2011, 7 pages. doi:10.4061/2011/167248.

Journal of Science Research
Volume 14, 2015, ISSN 1119 7333
Citation: Onilude, A. A., Adekoya, A.O., Wakil, S. M., Fasiku, S. A., and Ja'afaru, I. M.
Isolation and identification of thermophilic cellulolytic yeast from cassava waste dump.

Textflow Limited
Ibadan, Nigeria