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Screening of yeasts obtained from different fermented foods for their ability to produce pectinase

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ABSTRACT: In the present study, Citrus pectin was used for the production of pectinase enzyme by yeast isolates using submerged fermentation. Fifty yeasts were isolated from different fermented foods and screened for their producing ability. *Candida* sp. OG2 and *Candida tropicalis* strain AUMC 10275 were the yeast isolates with the best potential of pectinase production. Fermentation parameters such as incubation period, pH, temperature, carbon and nitrogen source were optimized under submerged fermentation. The optimal conditions for pectinase production were found to be incubation time 48 hours, pH 6.0 and temperature 40°C. Citrus pectin best induced the production of pectinase while yeast extract/peptone (1:1) was the best source of nitrogen. Pectinase produced by *Candida tropicalis* strain AUMC 10275 was purified at 4.00 folds with a specific activity of 63.99 U/ml. The yeasts obtained from fermented foods have the ability to produce pectinase enzyme under optimized conditions and can be used for industrial purposes.

Keywords: *Candida tropicalis*; Fermentation; Citrus pectin; Purification; Pectinase.

1. INTRODUCTION

Pectinase is one of the most important enzymes in food processing industries mainly for extraction and clarification of fruit juices and wines. Pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, and treatment of industrial wastewater [1]. Research has shown that pectinases are inducible and they can produce from different carbon sources. There have been various reports on the optimization of fermentation and microbiological parameters and different fermentation strategies for the production of pectinases.

Two types of fermentations can be carried out for the production of pectinase; they are solid state fermentation and submerged fermentation. The growth of organisms is very high with large quantities of enzyme being produced in solid- state fermentation [2]. However, in the production of extracellular pectinases, submerged fermentation is preferable as the extracellular pectinases are easier and cheaper to use in great quantities. Most important applications of these enzymes are in juice and wine making, and in the processing of vegetables. Although, submerged or solid state mediums are used for producing pectinolytic enzymes by fungi [3].

Pectinolytic enzymes are also classified and divided into three groups in general. Protopectinases are enzymes that catalyze the degradation of insoluble protopectin to highly polymerized soluble pectin.

Pectinesterases are the second major group of pectic enzymes that catalyze the de-esterification of pectin by removal of methoxyesters. Pectin methyl esterase (EC 3.1.1.11) is an example of this group of enzymes and hydrolyzes pectin into pectic acid and methanol.

The last group are depolymerases which catalyze the hydrolytic cleavage of the α-1,4-glycosidicbonds in the galacturonic acid [4]. Pectinases are commercialized to remove sizing agents from cotton with a combination of amylases, hemicellulases, and lipases. Waste water from vegetable processing plants contains pectin, and pectinases facilitate elimination of those by products. Pectinases are also used for production of animal feed, paper making and extraction of citrus oil [5].

Although, pectinase production used in industry has been reported from microorganisms including bacteria, actinomycetes [6], filamentous fungi in particular of the *Aspergillus niger*, *Penicillium pinophlilum*, *Penicillium viridicatum* and *Mucor circinelloides*. Some yeast has also been implicated [7].

Aspergillus niger is known worldwide for production of secondary metabolites and extracellular enzymes of commercial value, including industrial production of pectinases, yeasts have advantages compared to filamentous fungi with regard to the production of pectinases, because they are unicellular, the growth is relatively simple and the growth medium does not require an inducer. Yeasts have a great potential for the production of microbial enzymes for the food industry and they offer an alternative source of these enzymes. Co-production of tannase and pectinase by free and immobilized cells of the yeast *Rhodotorula glutinis* MP-10 isolated from tannin-rich persimmon (*Diospyros kaki* L.) fruits [8]. Although, some yeasts produced one or two types of pectinolytic enzymes, the production of pectinase is not so widespread in them. Very few yeasts show this ability namely those belonging to the genera *Saccharomyces*, *Kluyveromyces*, *Cryptococcus*, *Rhodotorula* and *Candida* [1]. Hence, the objective of this work was to isolate and screen yeasts for their ability to produce pectinase using submerged fermentation.

2. MATERIALS AND METHODS

2.1. Sample collection

Different fermented foods which include Ogi, Palm wine, Yoghurt, Kunnu, Fura de Nunu and Wara were purchased from Bodija market, Ibadan Oyo State, Nigeria. They were obtained in sterile polythene bags and brought to the Postgraduate laboratory of Department of Microbiology, University of Ibadan, Ibadan and were processed immediately.

2.2. Enumeration and isolation of yeasts

Each sample (1 mg/ml) was serially diluted in distilled water and pour plated into Yeast extract agar (YEA) media containing 0.5 mg/l streptomycin using the pour plate method and incubated at 30°C for 2-5 days. Afterwards, colonies with morphological differences such as color, shape, size and texture were randomly picked and sub cultured on fresh agar plates with the aim of obtaining pure cultures.

2.2.1. Maintenance of pure culture

The pure cultures of yeast was maintained by subculturing on YEA (Yeast Extract agar) slants, incubating for 48 hours at 30ºC and was thereafter stored in a refrigerator at 4ºC for subsequent use.

2.2.2. Characterization of yeast isolates

2.2.2.1. Macroscopic characterization

Morphological characteristics of the colonies of each isolate was examined on the surface of the media

to determine the size, color, elevation, opacity, texture, edge, pigmentation and shape on yeast extract agar plates according to Kurtzman and Fell [9].

2.2.2.2. Microscopic examination

This was carried out by wet mount. The yeasts isolates were stained with lactophenol in cotton blue dye stain and then viewed under x40 objective lens of the microscope [10].

2.2.2.3. Biochemical characterization

Sugar fermentation

This test was performed to determine the ability of yeast isolates to ferment different sugars. The basal medium consists of yeast extract broth and methyl red. 5 ml aliquot of basal medium was dispensed into test tubes containing inverted Durham's tubes. The medium was sterilized at 121°C for 15 minutes. After cooling, 1 ml of 1% concentrated, filter-sterilized solution of glucose, sucrose, fructose, galactose, lactose, maltose and mannitol and were aseptically added to each different tube preparation in duplicates and then inoculated with a loopful of yeast isolate followed by incubation at 30°C for 5-7 days. Twenty-four hours old cultures were used for inoculation. An uninoculated medium served as control for the experiment and the tube was observed daily for color change and gas production [9].

Urea hydrolysis

Test was done according to the method of der Walt and Yarrow [11]. Christensen's urea agar base was used. The slant was inoculated from the suspension of the active growing yeast culture using a sterile wire loop and incubated at 30°C for 2-5 days. Development of pink color in the agar indicated a positive result [12].

Nitrate assimilation test

Nitrate peptone water consisting of peptone water and 0.1% potassium nitrate was used. 5 ml portion of the medium was distributed into each screw-capped test tube containing an inverted Durham tube. The nitrate peptone water in test tubes was sterilized and allowed to cool before inoculating with the test isolates. Uninoculated tubes served as control. The tubes were incubated at 30°C for 4 days. The growth was measured based on turbidity of the solution.

2.3. Molecular characterization of the yeast isolate(s)

2.3.1. DNA extraction protocol

The extraction of the yeast genomic DNA for molecular analysis was carried out according to the method of Arnold et al. [13] with the following steps: 100 mg of fungal mycelia was taken into sterile mortal, and then 1 ml of DNA Extraction Buffer (DEB) containing proteinase K (0.05 mg/ml) was added and macerated with sterile pestle. The extract was transferred into 1.5 ml Eppendorf tube. 50 µl of 20% sodium dodecyl sulphate (SDS) was added and incubated in a water bath at 65°C for 30 minutes. The tubes were allowed to cool to room temperature. Afterwards, 100 µl of 7.5 M potassium acetate was added and mixed briefly. The mixture was centrifuged at 13000 rpm for 10 minutes.The supernatant were then transferred into fresh autoclaved tubes. To the supernatant 2/3 volumes of cold isopropanol/isopropyl alcohol was added, the tubes were inverted 3-5 times gently and incubated at -20°C for 1 hour. After incubation, the mixture was again centrifuged at 13000 rpm for 10 minutes and the supernatant discarded. Then, 500 µl of 70% ethanol was added and centrifuged for another 5 minutes at 13000 rpm.The supernatant was carefully discarded with the DNA pellet intact. Traces of ethanol were removed and the DNA pellets dried at 37°C for 10-15 minutes.

DNA pellets were then resuspended in 50 µl of Tris-EDTA (TE) buffer. Aliquot DNA was stored at -20°C for further laboratory analysis.

2.3.2. PCR analysis

To use the ITS gene for characterization of fungi, ITS universal primer set which flank the ITS1, 5.8S and ITS2 region can be used:
ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3'
ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3'

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each of the ITS 1 and ITS 4 primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8 µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 30 secs annealing of primer at 55°C and 72°C for 1 minute 30 second and a final termination at 72°C for 10 mins, and chill at 4ºC.

2.3.3. Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis.

2.4. Screening for pectinase producing yeast

2.4.1. Primary pectinase screening

This was done according to the method of Oskay and Yalcin [14]. Yeasts were spot inoculated into a pectinase screening agar medium (PSAM) containing citrus pectin (1%), di-ammonium orthophosphate ($(NH_4)_2HPO_4$), 0.3%); Potassium dihydrogenphosphate (KH_2PO_4, 0.2%); Di-potassium hydrogen phosphate (K_2HPO_4, 0.3%); $MgSO_4$ (0.01%) and agar-agar (2.5%). The initial pH of the medium was adjusted to 5.5. This medium was sterilized and distributed aseptically in Petri dishes. The Petri dishes containing PSAM were inoculated and incubated at 30ºC for 48 hrs. At the end of the incubation period, plates were stained with 50 mM iodine for the detection of clearance halos around the colonies. Strains presenting large clearing zones were used for enzyme production assays on liquid medium.

2.4.2. Secondary pectinase screening

The selected colonies from above were further screened in the Pectinase Screening Medium (PSM) which contains citrus pectin 5 g/L; peptone 5 g/L; yeast extract 5 g/L; $MgSO_4 \cdot 7H_2O$ 0.25 g/L; K_2HPO_4 1 g/L and $(NH_4)_2SO_4$ 0.25 g/L. 100 ml of the sterile pectinase screening medium was inoculated with 0.5 ml of the isolate and incubated for 48 hrs. After incubation, the medium was harvested by centrifugation at 8000 rpm for 15 minutes at 4ºC. The supernatant was used to evaluate pectinase activity [14].

2.4.3. Pectinase production and fermentation

For pectinase production, initially in a 100 ml Erlenmeyer flask containing 25 ml of propagation medium the strain was inoculated with a single loopful of a 48 h yeast culture from YEA plates for development of inoculums. The culture was then incubated at 30ºC in an orbital shaker at 150 rpm for 12 h,

after which the growing culture (1%, v/v) was transferred into a 250 ml Erlenmeyer flask containing 50 ml of propagation medium and incubated for an additional 10 h under the same conditions. This culture was used as the inoculum in subsequent experiments. Submerged fermentation was carried using 250 ml Erlenmeyer flasks with 150 ml of optimized production medium in a rotary shaker (150 rpm) at 30°C. After 48 h the biomass was separated by centrifugation at 8000 rpm for 15 min at 4°C and the supernatant was used to assay for pectinase activity [14].

2.5. Assay of pectinase activity

Assay of Pgase activity was determined by measuring the release of reducing groups using the modified dinitrosalicylic acid (DNS) method described by Miller [15]. Briefly, the reaction mixture containing 250 µL of 1% citric pectin in 250 µL citrate- phosphate, pH 6.0 buffer and 100 µL of cell free culture supernatant, was incubated at 30ºC for 5 min under static conditions. The reaction was stopped using the 3,5-dinitrosalicyclic acid reagent followed by keeping at 100°C for 5 min for development of color. After heating for 5 min in boiling water, the reaction mixture was centrifuged (8000 rpm for 5 min) to separate out the insoluble pectinolytic materials formed during reaction. A control mixture deprived of pectin and another mixture deprived of cell free supernatant were assayed in parallel tests. The absorbance read at 540 nm using UV-visible Spectrophotometer. One Unit (U) of Pgase activity was defined as the amount of enzyme required to liberate one µmol of galacturonic acid per minute under the assay conditions.

2.6. Optimization of the cultural parameters in submerged fermentation

The basal medium was optimized with various factors that influence pectinase production. The various physicochemical parameters of fermentation were optimized which included temperature, pH, incubation time, incubation temperature, carbon sources and nitrogen sources.

2.6.1. Effect of temperature

The effect of temperature on pectinase production using submerged fermentation was carried out at different temperatures. This was done between the range of 30-45°C [14].

2.6.2. Effect of pH

The effect of pH on enzyme production was also carried out. pH values ranging from 4.0-8.0 was used to investigate the effect of pH on the production of pectinase. Acetate, citrate and phosphate buffer were the buffers of choice used in this experiment [14]

2.6.3. Effect of incubation time

The production medium for pectinase were harvested at different incubation time 24-72 hrs and assayed to determine the best time that supports enzyme production [14].

2.6.4. Effect of incubation temperature

The effect of incubation temperature on pectinase production by the yeast isolates was also carried out. Temperature range of 35-50ºC was studied [14].

2.6.5. Effect of nitrogen sources

To investigate the effect of the different nitrogen sources on pectinase production, the only nitrogen sources in the basal medium which was peptone and yeast extract were replaced by different nitrogen sources

such as urea, casein, ammonium sulphate, ammonium chloride and yeast extract/peptone (1:1) at 1% (w/v) concentrations [14].

2.6.6. Effect of carbon sources

The effect of various carbon sources such as glucose, fructose, sucrose, maltose and mannitol at 1% (w/v) concentrations was also carried out by in addition to pectin which was the sole source of carbon in the basal medium [14].

2.7. Purification of enzyme

2.7.1. Ammonium sulphate precipitation

After determining the percentage saturation of ammonium sulphate salts that gave the highest activity, the equivalent amount of salt for 1 litre of crude enzyme is added. The salt is allowed to dissolve completely and the mixture is allowed to stand for 30 hrs. at 4ºC. It is then centrifuged at 4000 rpm for 30 mins. The pellets are collected and stored in a cool place for further studies [16].

2.7.2. Dialysis of enzymes

The dialysis tubes stored in 90% ethanol were used. However the tubes were rinsed thoroughly with distilled water and finally with 0.05 M phosphate buffer in order to remove traces of ethanol. An amount of the precipitated enzyme is poured into the dialysis tubes and placed in a beaker containing 0.05 M phosphate buffer. The beaker is placed on a magnetic stirrer which allows for a homogenous environment. The dialysis is carried out according to Dixon and Webb [17] for 12 hours and the buffer is changed after 6 hours which allows for the exchange of low molecular weight substances and left over ammonium sulphate salts that may interfere with the activity. After dialysis, Pectinase activity was measured in each fraction applying DNS method: 0.5 ml of dialyzed partially purified enzyme was added to 0.5 ml citrus pectin 1% (w/v) and 0.5 ml locust bean gum 1% (w/v) in 0.05 M phosphate buffer (pH 6.0) separately. Test tubes were covered and incubated for 5 mins at 65°C in a water bath. Then, 1 ml DNS reagent was added to each tube to stop the reaction and placed in boiling water bath for 5 mins. After cooling the samples in a cold water bath, the absorbance was read at 540 nm [16].

2.8. Studies on partially purified enzyme

2.8.1. Effect of pH change on pectinase activity

The effect of pH on enzyme activity was determined using 0.05 M sodium acetate buffer pH 3.5-5.5, phosphate buffer pH 6.0-7.5 and Tris-HCl buffer pH 8.0-10.0 at intervals of 1.0. 0.1% pectin solution and was prepared by dissolving 0.1 g pectin in 100 ml of 0.05 M of the respective buffers separately. Also 0.5 ml of the partially purified enzymes was added to 0.5 ml of each of the buffers. Then ultimately, 0.5 ml of each of the enzyme-buffer solution was mixed with 0.5 ml pectin solution at the corresponding pH for pectinase assay as described previously [14].

2.8.2. Effect of temperature change on pectinase activity

The optimum temperature was determined by incubating the enzymes with pectin solution between 35-50ºC at an interval of 5ºC for 1hour and at the pH with the highest activity. The activity was then determined as described previously [14].

2.8.3. Effect of substrate concentration on pectinase activity

The effect of substrate concentration on the activity of pectinase was determined by incubating the enzyme with 0.5 up to 2.0 mg/ml citrus pectin and at an interval of 0.5 using the buffer at the pH with highest activity and the temperature at which highest activity was observed [14].

2.8.4. Thermal stability of pectinase enzyme

For thermal stability, the partially purified enzyme was pre-incubated for 1 h at various temperatures (40-90˚C) before enzyme assay, and promptly cooled on ice and residual activity was determined under standard assay conditions.

3. RESULTS AND DISCUSSION

3.1. Yeasts isolated from fermented foods

Fifty yeasts were isolated from different fermented foods. The frequency of occurrence of the isolates is shown in Figure 1, with Palm-wine (30%) having the highest frequency of occurrence followed by Fura de Nunu (26%), while Yoghurt (14%), Ogi (12%), Kunnu (10%) and Wara (8%) showed relatively low numbers.

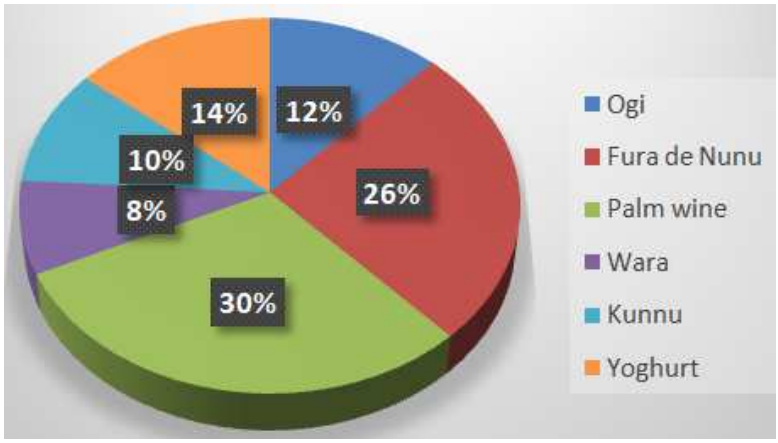

Figure 1. Frequency of occurrence of yeast isolates from different fermented foods.

3.2. Screening for pectinase producing isolates from fermented foods

All the isolates were screened for pectinase activity. Only ten isolates were positive for pectinase activity. Secondary screening was carried out for isolates with the highest zones of clearance (Table 1). Yeast isolates with the code OG2 and YG3 showed the highest zones of clearance (15 mm and 13.5 mm) respectively on the PSAM plate with enzyme activity of 23.92 U/ml and 24.40 U/ml respectively. The two isolates (OG2 and YG3) were selected based on this secondary screening for further studies.

3.3. Molecular characterization of the yeast isolates

Gene sequences from the characterized isolate showed 89% similarity to *Candida tropicalis* strain AUMC 10275 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene partial sequence.

3.4. Optimization of fermentation parameters

The production of pectinase enzyme by the yeast isolates *Candida tropicalis* strain AUMC 10275 and

Candida sp. (OG2) were affected by several factors. These factors include incubation time, temperature, carbon sources, nitrogen sources and substrate concentration under submerged fermentation.

Table 1. Yeast isolates positive for primary pectinase screening.

S/N	Isolate	Diameter of clearance
1	OG2	15
2	YG3	13.5
3	FN3	6
4	FN4	6.5
5	WR4	10
6	WR3	12
7	OG	7
8	WR1	8
9	WR2	8
10	PW1	7.5

3.4.1. Optimization of incubation period

Pectinase activity of all the yeast isolates increased as incubation period increased but was optimum at 48 hours. Pectinase activity by *Candida* sp. OG2 with the activity of 24.34 U/ml was found to be the highest after 48 hours of incubation (Fig. 2). This was in agreement with the study of Oskay and Yalcin [14] who reported 48 hours as the best incubation time for maximum pectinase production by yeasts. The period of fermentation depends on the nature of medium, fermenting organisms, concentration of nutrients and the process physiological conditions.

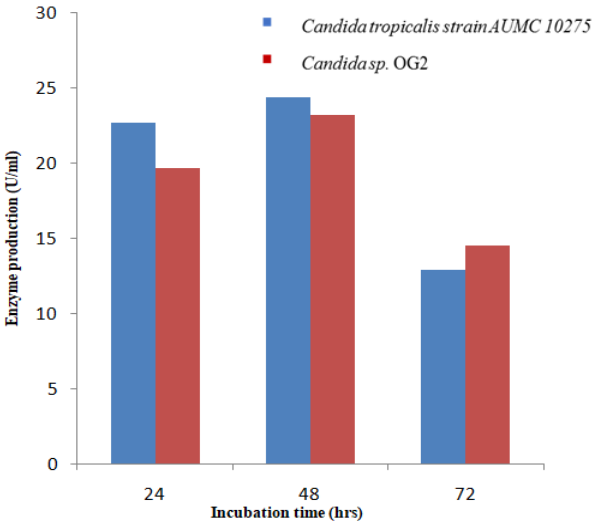

Figure 2. Effect of incubation time on pectinase production by the yeast isolates.

3.4.2. Optimization of temperature on enzyme production

Pectinase production by *Candida tropicalis* strain AUMC 10275 was found to be optimal at an

incubation temperature of 35ºC (Fig. 3). This was in agreement with the study of Oskay and Yalcin [14].

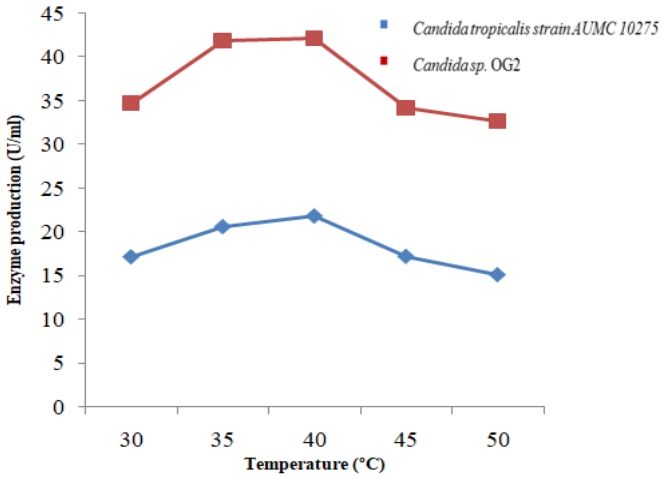

Figure 3. Effect of temperature on pectinase production by the yeast isolates.

3.4.3. Optimization of pH

The pH value of the fermentation medium for enzyme production is an important factor. For pectinase production, pH level of 6 was the optimum with a value of 22.55 U/ml by *Candida tropicalis* strain AUMC 10275 (Fig. 4).

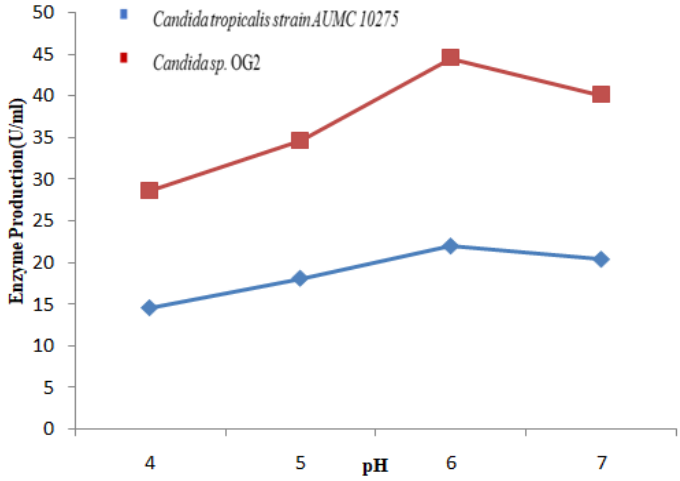

Figure 4. Effect of pH on pectinase production by the yeast isolates.

3.4.4. Optimization of carbon sources

The effect of different carbon sources on the production of pectinase enzyme was studied. It was observed that pectinase production by *Candida tropicalis* strain AUMC 10275 when supplemented with the control (pectin) was the highest with a value of 38.1 U/ml (Fig. 5). *Candida* sp. OG2 also had a value of 32.93 U/ml when supplemented with the same substrate (pectin). This was in accordance with the work of Oskay and Yalcin [14] who recorded the highest pectinase production in the medium containing citrus pectin as the sole carbon source.

The decrease in pectinase production maybe due to a repression effect caused by the additional sources of carbon which may have resulted to the utilization of carbon sources with fewer carbon chains as compared

to pectin which is an heteropolysaccharide. As such, it is metabolically economical for the yeast isolates to utilize these carbon sources albeit with low enzyme production.

This is also in line with the suggestion of Moyo et al. [18] that there is a repressive effect on pectinase activity when glucose, sucrose and other carbon sources were added to the medium. Although, this was in disagreement with the work of Hoa and Hung [19] who studied the production of pectinase using *Aspergillus oryzae*.

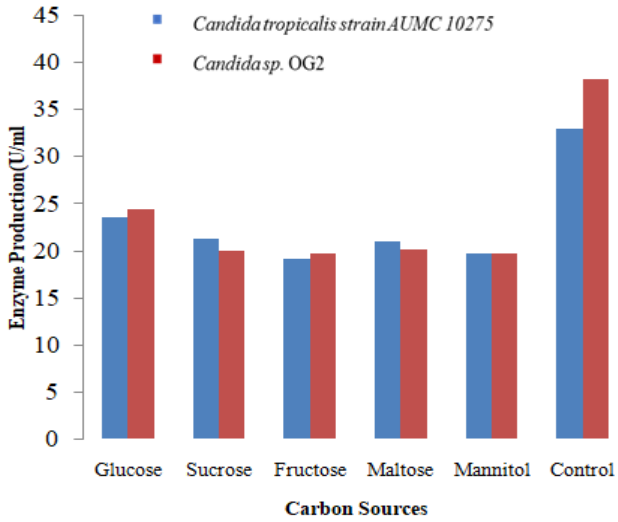

Figure 5. Effect of carbon sources on the production of pectinase by the yeast isolates.

3.4.5. Optimization of nitrogen sources

Nitrogen source is important in microbial growth as it plays a major role in the biosynthesis of cell metabolites and general maintenance of physiology of the cells. The effect of different nitrogen sources on the fermentation medium was studied. Optimal pectinase production was observed by *Candida tropicalis* strain AUMC 10275 when the medium was supplemented with yeast extract and peptone (1:1) showing a value of 23.5 U/ml. *Candida* sp. OG2 also showed a high value (22.23 U/ml) when the medium was supplemented with the same nitrogen source (yeast extract/peptone) (Fig. 6), which was also in line with the work of Oskay and Yalcin [14]. However this was in contrast with the work of Hoa and Hung [19] who reported urea as the best nitrogen source.

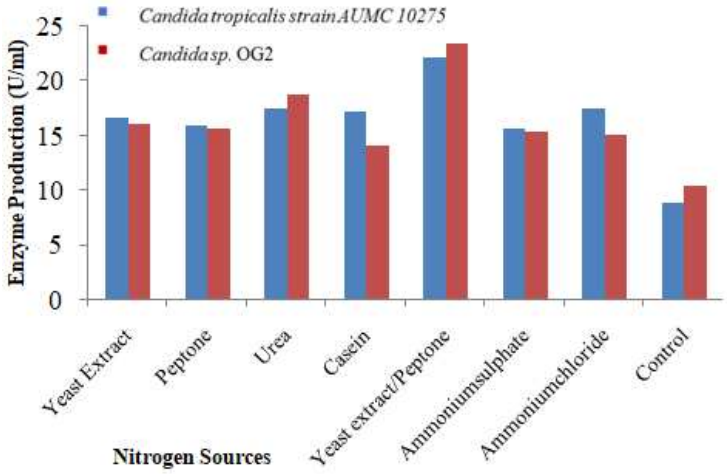

Figure 6. Effect of nitrogen sources on the production of pectinase by the yeast isolates.

3.4.6. Optimization of temperature on enzyme activity

The effect of temperature on the enzyme activity was also studied. The activity of pectinase enzyme was optimal at 50ºC. *Candida* sp. OG2 showed a value of 19.07 U/ml while *Candida tropicalis* strain AUMC 10275 recorded a value of 18.12 U/ml (Fig. 7).

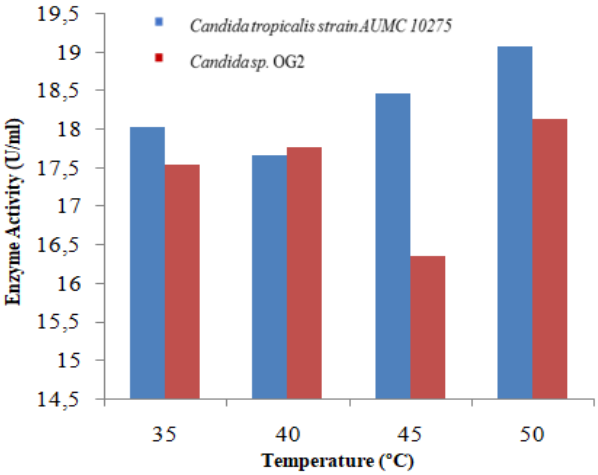

Figure 7. Effect of temperature on the activity of pectinase by the yeast isolates.

3.5. Characterization of crude pectinase

3.5.1. Optimum temperature for stability of crude pectinase

The effect of temperature on crude pectinase stability was determined by exposing the enzyme(s) to various temperatures ranging from 50ºC to 90ºC for 30 minutes. Pectinase stability was maintained above 60ºC by *Candida tropicalis* strain AUMC 10275 and *Candida* sp. OG2 (Fig. 8). This was however in contrast with the report of Chellegati et al. [20] who reported a drop in the activity of pectinase by *Aspergillus* sp. and *Pseudomonas* sp. at a temperature above 60ºC.

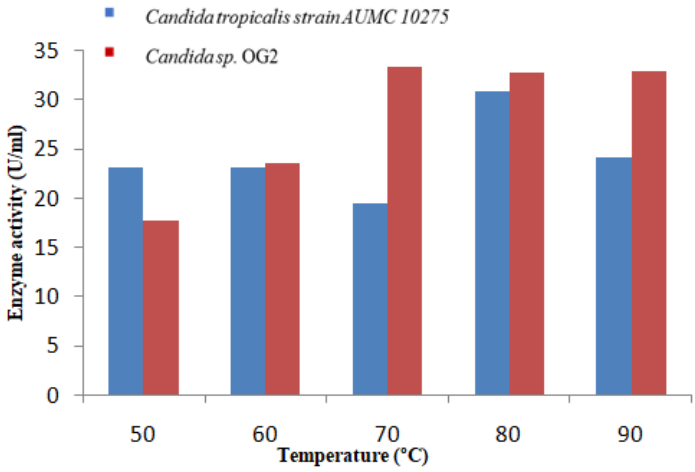

Figure 8. Stability of pectinase at different temperature.

3.5.2. Optimum pH

Results indicate that pectinase activity by *Candida tropicalis* strain AUMC 10275 and *Candida* sp. OG2 (30.51 U/ml and 34.52 U/ml) was optimal at pH 5.0 (Fig. 9). This was similar to the report of Freitas et

al. [21] who reported maximum pectinase activity around pH 5.5. This was also in agreement with the suggestion of Helms et al. [22] who suggested that extremely high or low pH concentrations usually results in the loss of enzyme activity.

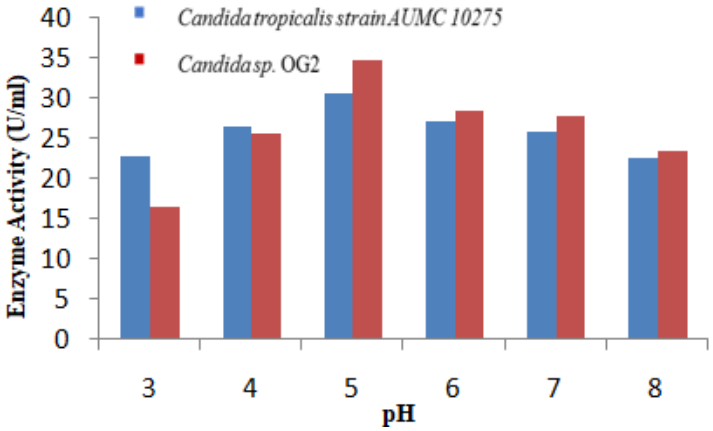

Figure 9. Stability of pectinase at different pH.

3.5.3. Optimum substrate concentration for pectinase activity

The effect of substrate concentration on the activity of enzyme was studied by determining the activity at different concentrations (0.5%-2.0% w/v). Results show that pectinase activity was optimal at a concentration of 2% (w/v) for *Candida tropicalis* strain AUMC 10275 (17.91 U/ml) while for *Candida* sp. OG2, optimal substrate concentration was 1% (w/v) with a value of 18.44 U/ml (Fig. 10).

This was in agreement with the work of Roosdiona et al. [23] who observed a progressive increase in pectinase activity as substrate concentration increases. This could be because the enzyme has a high maximum kinetic energy (Km) which consequently requires a high substrate concentration to become saturated. Hence, the maximum velocity is reached at high substrate concentration [24].

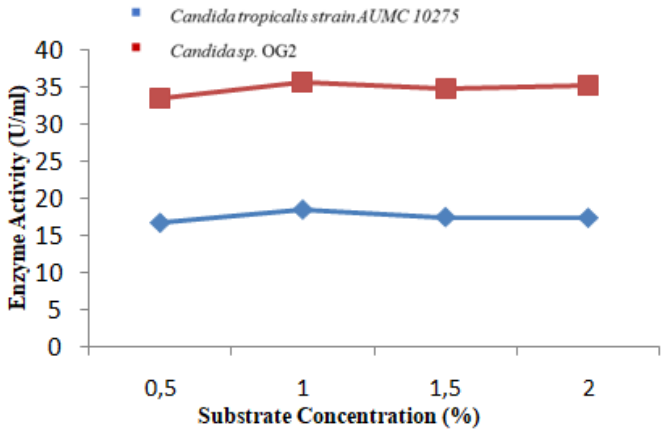

Figure 10. Effect of different substrate concentrations on pectinase activity.

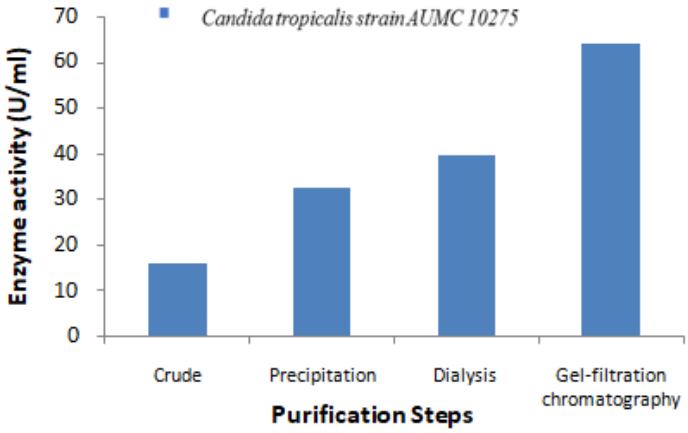

Figure 11. Effect of each purification steps on pectinase activity.

4. CONCLUSION

The result obtained from this study showed that yeast isolates obtained from fermented foods, particularly *Candida* sp. yielded high activities of pectinase enzyme when supplied with adequate nutritional and optimized conditions. Citrus pectin is a good substrate for pectinase enzyme production by the isolates under submerged fermentation. The optimum incubation period, temperature and pH for pectinase production for the *Candida tropicalis* isolates were 48 hours, 40ºC and 6.0, respectively. Citrus pectin was the best carbon source, while yeast extract/peptone (1:1) was the preferred source of nitrogen. Partially purified pectinases were stable at high temperatures signifying the possibility of their industrial application.

Authors' Contributions: FTA designed the study. FTA and YOS carried out the research work. YOS analysed the results. FTA corrected the draft. All authors read and approved the final manuscript.

Conflict of Interest: Authors declare that no conflict of interest exists.

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