Production, Purification and Characterization of Mannanase Obtained from Yeasts Isolated from Different Citrus Wastes

Afolabi, F.T. & Jimoh, Y.Z.
Department of Microbiology
University of Ibadan
Ibadan, Oyo State, Nigeria.
E-mail: folakeojo1@yahoo.com
Phone: +2348034218552

ABSTRACT

Microorganisms are currently the primary sources of industrial enzymes, with fungi and yeasts contributing 50%, bacteria contributing 35%, while the remaining 15% are either of phytopathogenic fungi and some plant parasitic nematodes or animal origin. The potential of using these microorganisms as sources of biotechnological and industrial relevant enzymes stimulated interest in the study of extracellular enzymes from several microorganisms including various fungi, bacteria, actinomycetes and yeasts. One hundred and two (102) yeasts were isolated from different citrus wastes obtained from five different markets and screened for their mannanase producing ability. Gum Arabic was used as a substrate for the production of mannanase enzyme by yeasts isolates using submerged fermentation. *Candida* sp. LES2, *Candida* sp. OS12, *Pichia* sp. and *Pichia kudriavzevii strain AUMC 10190* were the yeast isolates with the best potential of mannanase production. Incubation period, pH, temperature, carbon and nitrogen source were optimized under submerged fermentation for the production of mannanase. The mannanase produced by *Pichia kudriavzevii strain AUMC 10190* was optimally active at pH 7.0 (202.10 U/ml) and its temperature is stable above 60^0C. *Pichia kudriavzevii strain AUMC 10190* was optimally active at 35^0C (147.91U/ml). Mannanase enzyme produced by *Pichia kudriavzevii strain AUMC 10190* had a purification fold of 2.14 with a specific activity of 134.53U/ml proving that yeasts obtained from citrus wastes have the ability to produce mannanase enzyme under optimized conditions. Therefore, it can be concluded that *Pichia kudriavzevii strain AUMC 10190* isolated from citrus waste is a potential yeast for the production of mannanase under suitable condition for higher yield.

Keywords: Mannanase, Gum Arabic, *Pichia kudriavzevii strain AUMC 101190,* Yeasts

iSTEAMS Proceedings Reference Format
Afolabi, F.T. & Jimoh, Y.Z. (2019): Production, Purification and Characterization of Mannanase Obtained from Yeasts Isolated from Different Citrus Wastes. Proceedings of the 18th iSTEAMS Multidisciplinary Cross-Border Conference, University of Ghana, Legon, Accra, Ghana. 28th – 30th July, 2019 Pp 197-202. www.isteams.net - DOI Affix - https://doi.org/ 10.22624/AIMS/iSTEAMS-2019/V18N1P23

1. BACKGROUND OF STUDY

Enzymes are the known catalytic agents of metabolism that have become important tools in biotechnology industry. Enzymes can be produced from various sources like plants, animals and microorganisms. Microbial enzymes are preferred for industrial application because of their easy and economical production and novel properties such as activity in wide range of temperature and pH. After proteases, cellulases and hemicellulases are the major industrially important enzymes (Polizeli *et al.,* 2005; Dhawan and Kaur, 2007). Mannans are the major constituents of the hemicelluloses fraction in softwoods and show widespread distribution in plant tissues. The major mannan-degrading enzymes are β- mannanases, β-mannosidases and β-glucosidases. In addition to these, other enzymes such as α-galactosidases and acetyl mannan esterases are required to remove the side chain substituents.

Yeasts are unicellular eukaryotic microorganisms, belonging to the fungus group. Yeasts have been isolated from varying habitats such as plants, soil, water, glaciers, geothermal regions and intestinal tracts of animal and insects (Stefanini *et al.,* 2012). It is estimated that about 1% of all yeast species has been identified today, corresponding to about 1500 species (Blackwell, 2011). Yeast cells are typically in the micrometer (µm) size range, although, under certain circumstances, some yeasts are able to form multicellular-like forms, so called pseudo hyphae. Yeasts and yeast like fungi are widely distributed in nature. They are present in orchards and vineyards, in air and soil, and in the intestinal tract of animals. Like bacteria and moulds, yeasts can have beneficial and non-beneficial effects in food fermentations. Some of the yeasts like *Pichia* are viewed as spoilage of food products while those like *Candida* are utilized for the single cell protein production (Aziz *et al.,* 2008).

Citrus are the most important crops in the world in terms of production according to the Food and Agricultural Organization (FAO), with 240,780 million metric tons produced in 2013 (FAO Statistics, 2016). Citrus plants are grown in many countries all over the world and among the major African citrus-producing countries is Tunisia. Thus, Citrus would be considered as one of the most economically important crops in Tunisia. The genus Citrus belongs to the Rutaceae family that comprises of about 140 genera and 1300 species and, for instance, Citrus limon (Lemon) is among important species of genus Citrus (Kamal *et al.,*2011).

2. STATEMENT OF PROBLEM

The production of mannanase by chemical means is expensive therefore a means of producing mannanase using biological method which is a better means and eco-friendly is being sought for. However, reports on the microbial production of mannanase have centered majorly on bacteria and molds, hence there is a dearth of information on the production of mannanase by yeasts

3. OBJECTIVE

The research aimed at isolating and screening for Mannanase-producing yeasts from citrus wastes and to produce, optimize, purify and characterize the enzyme.

4. METHODOLOGY

4.1 Microorganisms

Yeasts: *Candida* sp. LES2, *Candida* sp. OS12, *Pichia* sp. and *Pichia kudriavzevii strain AUMC 10190* were isolated from different spoilt citrus (orange, grape, lemon and lime) obtained from Ojoo, Oje, Bodija, Dugbe Mall and Abeokuta road in Ibadan, Oyo State, Nigeria.

4.2 Screening for mannanase producing yeasts

The isolates were screened for mannanase producing ability by inoculating them in a sterile medium containing 1% Gum Arabic, 0.1% yeast extract, 0.1% peptone, 0.1% NH_4NO_3, 0.14% KH_2PO_4, 0.02% $MgCl_2$, 1% Congo Red and 3% Agar (Rattanasuk and Ketudat-Cairns, 2009). The plates were incubated at 30ºC for 24 hours, 48 hours and 72 hours. The mannanase activity of each isolate was measured based on the ratio of the clearing zone (dark blue-black colouration) formed. The colonies with highest clear zone were collected and maintained as frozen stocks in the presence of 20% glycerol (Adebayo-Tayo *et al.,* 2013).

4.3 Mannanase production in submerged fermentation

The Mannanase Production Medium (MPM) containing in g/l: Bacteriological peptone 0.1, yeast extract 0.1, MgCl2 0.02, KH2PO4 0.14, NH4NO3 0.1, and locust bean gum (LBG) 1.0, Distilled water 1 liter and pH 5.5 was used. 100 mL of the sterile mannanase production medium was inoculated with 0.5 mL of the isolate and incubated for 24 hrs. After incubation, the fermentation medium was harvested by centrifugation at 4000rpm for 30 minutes at 4°C.

The supernatant was used to assay for mannanase activity (Adebayo-Tayo *et al.,* 2013). The production of mannanase enzyme by the yeast isolates *Pichia sp*., *Pichia kudriavzevii strain AUMC 10190*, *Candida sp.*OS12, *Candida sp*. LeS2 were affected by several factors which include the following parameters; incubation time, temperature, carbon sources, nitrogen sources and pH under submerged fermentation. One of the parameters used was effect of temperature.

5. RESULTS

One hundred and two yeasts were isolated from different citrus wastes. All the isolates were screened for mannanase activity. Thirty-one isolates showed mannanase degrading ability. Secondary screening was carried out for isolates with the highest zones of clearance. Ten yeast isolates showed the highest zones of clearance, therefore, four most promising isolates were selected for the production of mannanase. These four isolates with the code OS8, LeA5, OS12 and LeS2 showed mannanase activity of 121.10U/mL, 155.50U/mL, 138.50U/mL and 107.60U/mL respectively and production using submerged fermentation. The four best isolates were selected based on secondary screening for further studies as shown in Table1.

Probable identity of yeast isolates

A total of four isolates were obtained after secondary screening for mannanase activity and were identified tentatively using their morphological and biochemical characteristics. The morphological features of isolates OS8, LeA5, LeS2 and OS12 were irregular, creamy, dull, circular, glistening, and smooth and butryous. They were elongated, ovoid and spherical in shape when viewed under microscope. Isolates OS12 and LeS2 were able to tolerate acetic acid, positive for glucose, sucrose, fructose, mannitol and citrate utilization, while they are negative for inositol. OS8 and LeA5 are negative for sucrose, inositol and mannitol while positive for glucose, fructose and galactose. All the four isolates are negative for urease, inositol, starch and lactose while they are weakly positive for maltose and all are positive for mannose. Only LeA5 showed negative result to citrate utilization as shown in Table 2.

Effect of temperature on enzyme production

Mannanase production by *Pichia sp*., *Pichia kudriavzevii strain AUMC 10190*, *Candida sp.* OS12, *Candida sp*. LeS2 was found to be optimal at an incubation temperature of 30ºC and 35ºC. The values at the optimal temperature which is 35ºC are 136.02U/mL for *Pichia sp.*, 147.91U/mL for *Pichia kudriavzevii strain AUMC 10190*, 110.03U/mL for *Candida sp.* LeS2 and 141.01U/mL for *Candida sp.* OS12. Therefore, *Pichia kudriavzevii strain AUMC 10190* gave the highest mannanase activity of 147.91U/mL at 35ºC which is the optimal temperature as shown in Fig.1

Table 1: Yeast isolates positive for primary and secondary screening of mannanase

Isolate code	Citrus waste	Diameter of clearance (mm)
0S8	Orange	22.0
LEA5	Lemon	20.0
OS12	Orange	16.0
LES2	Lemon	16.0
OS2	Orange	15.0
LEA6	Lemon	14.0
LA2	Lime	14.0
LES12	Lemon	14.0
LA1	Lime	14.0
LES10	Lemon	14.0

Table 2: Morphological and Biochemical Identification of yeasts obtained from different citrus wastes.

Yeast code	Morphological characteristics	50% Glucose	Glucose	Sucrose	Mannnitol	Fructose	Maltose	Inosositol	Galactose	Mannose	Lactose	KNO_3	Starch	Citrate Utilization	Urease	Probable Organism
OS8	Circular, creamy, dull and flat.	+ve	+ve	-ve	-ve	+ve	W	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	*Pichia sp.*
LEA5	Circular, creamy, dull and flat.	+ve	+ve	-ve	-ve	+ve	W	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	*Pichia sp.*
LES2	Irregular, creamy, glistering, smooth and butyrous.	+ve	+ve	+ve	+ve	+ve	W	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	*Candida sp.*
OS12	Irregular, creamy, glistering, smooth and butyrous.	+ve	+ve	+ve	+ve	+ve	W	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	*Candida sp.*

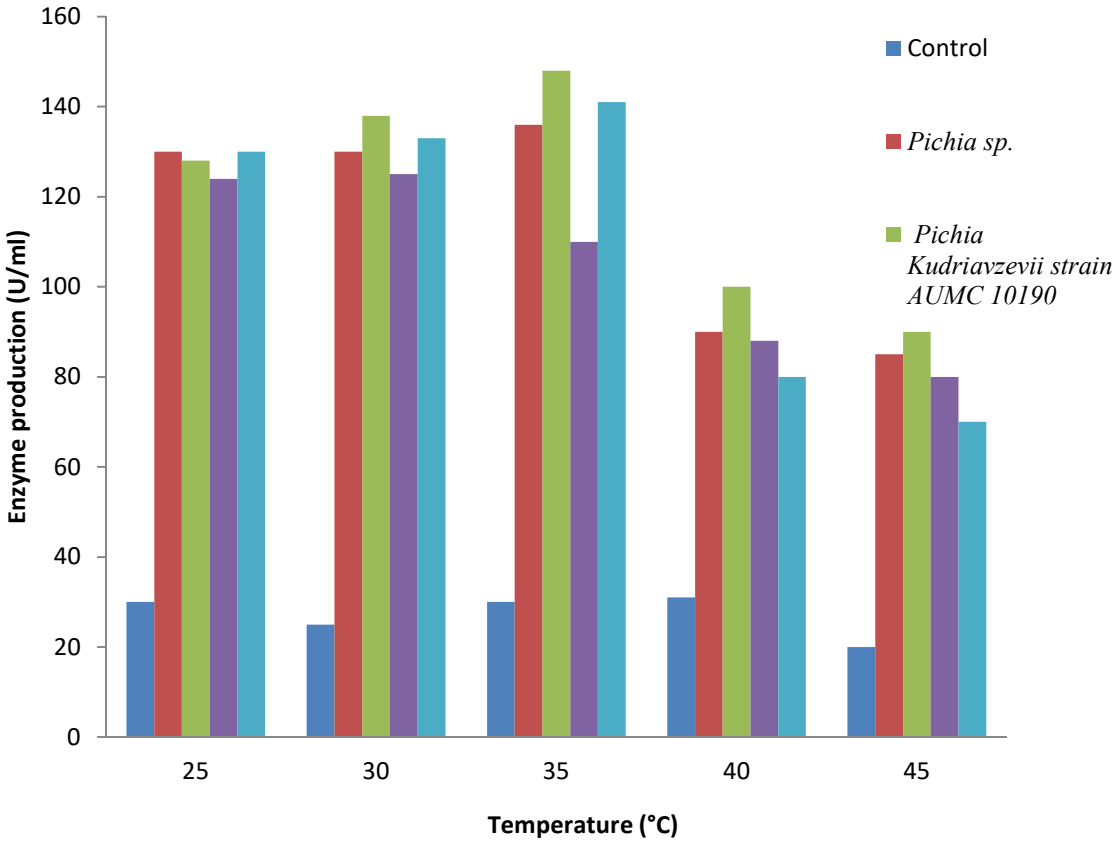

Fig 1: Effect of Temperature on the production of mannanase by the Yeasts obtained from citrus wastes

6. DISCUSSION OF FINDINGS

In this study, one hundred and two yeast isolates were obtained from different citrus wastes (lemon, lime, orange and grape). After preliminary screening of the one hundred and two (102) isolates for their enzyme producing ability, thirty-one (31) isolates, which represented 30% of the total, were positive for mannanase producing ability. This was almost similar to the report of Ernesto *et al*. (2006) and Obinna-Echem *et al*. (2014) that a number of fungal isolates such as *Saccharomyces* sp., *Kluyveromyces* sp., *Candida* sp. *Pichia sp.* etc. isolated from fermented foods and fruits possess inherent ability to produce mannanase.The biochemical and molecular identification study of the selected yeast strains showed that *Pichia spp* remain a dominant group of yeasts that colonize citrus wastes and *Pichia kudriavzevii strain AUMC 10190* has been identified to be one of the most frequently occurring yeast in citrus wastes. According to Kurtzman, (2011) and Chan, (2012), this yeast strain was mainly associated with food and fruits spoilage to cause surface biofilms in low pH products. An optimum temperature of 35ºC was observed for maximum mannanase activity but there was a decrease above this optimum temperature. According to Rosso *et al.,* (1995), when the temperature of the environment is higher or lower to the temperature required, the microbial activity is reduced, causing a decrease in enzyme activity. This is in line with the work of Oskay and Yalcin (2014) and Adebayo-Tayo *et al.* (2013) who also reported temperature of 35ºC.

7. CONCLUDING REMARKS

The yeast isolates obtained from citrus wastes (Lemon, Orange, Lime, Grape) particularly *Pichia kudriavzevii strain AUMC 10190* yielded high activity of mannanase enzyme when supplied with adequate nutritional and optimized conditions.

8. CONTRIBUTION TO KNOWLEDGE

This research established the fact that *Pichia kudriavzevii strain AUMC 10190* isolated from citrus waste is a potential yeast for the production of mannanase under suitable condition for higher yield. Yeast mannanase are promising for use in various fields of biotechnology, in particular in the processing of mannan-containing raw materials.

REFERENCES

1. Adebayo-Tayo, B., Elelu, T., Akinola, G. and Oyinloye, I. 2013. Screening and production of Mannanase by *Bacillus* strains isolated from fermented food condiments. *Innovative Romanian Food Biotechnology.* 13: 53-62.
2. Aziz, S.A, Ong, L.G.A, Hassan, M.A and Karim, M.I.A. 2008. Production parameters
3. optimisation of mannanase production from *Aspergillus niger FTCC* 5003 using palm kernel cake as carbon source. *Asian Journal of Biochemistry.* 3(5):297–307
4. Blackwell, M. 2014. Research coordination networks: A phylogeny for kingdom fungi. *Mycologia.* 98: 829-837.
5. Chan G.F, Gan H.M, Ling H.L and Rashid N.A. 2012. Genome sequence of *Pichia kudriavzevii* MI2, a potential producer of bioethanol and phytase. *Eukaryot Cell*, 11: (10)
6. Dhawan, S., Kaur, J. 2007. Microbial mannanases: an overview of production and applications. *Critical Review of Biotechnology.* 27(4):197–216
7. Ernesto, J., Favela-Torres, C., Tania Volke, B. and Viniegra- González, D. 2006. *Food Technology and Biotechnology.* 44 (2): 221–227.
8. FAO. Statistics. 2016; http://faostat.fao.org/. Accessed 5 Jan 2016
9. Kamal, G.M., Anwar, F, Hussain, A.I, Sarri N. and Ashraf, M.Y. 2011. Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. *International Food Research Journal.18:1275*–82.
10. Kurtzman, C.P., Fell, J.W. and Boekhout, T. 2011. The yeasts, a taxonomic study. Volume 1, fifth edition, Elsevier Science, Amsterdam. The Netherlands
11. Obinna-Echem, P.C., Kun, V. and Beal, J. 2014. Evaluation of the microbial community, acidity and proximate composition of *Akamu*; a fermented maize food. *Journal of the Science of Food and Agriculture*. 94: 331-340.
12. Oskay, M. and Yalcin, H.T. 2014. Screening of yeast strains for pectinolytic activity: Effects
13. of different carbon and nitrogen sources in submerged fermentations. *Online Journal of Biological Sciences*. 15(3): 89-96.
14. Polizeli, M.L.T.M, Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A. and Amorim, D.S. 2005. Xylanase from fungi: properties and industrial applications. *Applied Microbiology*
15. *Biotechnology.* 67:577–591pp. 64-118.
16. Rattanasuk, S. and Cairns, M.K. 2009. *Chryseobacterium indologenes* novel mannanase-
17. producing bacteria. *Journal of Science and Technology.* 31 (4):395–399
18. Rosso, L., Lobry, J.R., Bajards, S. and Flandrois, J.P. 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. *Applied and Environmental Microbiology* 61: 610-616.
19. Stefanini, I., Dapporto, L., Celabretta, A., Di paola, M., Turillazi, S. and Cavalieri, D. 2012. Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution. *Proceeds of the National Academy of Science.* 109: 13398-13403.