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Identification of Lipase Producing Yeast Strains from Fresh Mango Fruit Juice

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ABSTRACT

The utilization of enzymes including lipase in the industry has intensified search for lipase-producing microorganisms from natural sources. This study aimed at identifying lipase producing yeast strains from mango fruit juice. The isolation of yeast strains from mango fruit juice was carried out using streaking method on potato dextrose agar (PDA) and was incubated at 30^0C for 48h. The yeast isolates were screened for lipolytic activity by point inoculation on tributyrin agar. The yeast strains which had lipolytic activity were identified and characterized phenotypically by morphological characterization, Gram staining, biochemical tests, sugar fermentation and molecular approaches including DNA extraction, electrophoresis, gene amplification and sequencing. The obtained results revealed that all isolated yeast strains hydrolyzed the tributyrin in the agar by recording mean zone of hydrolysis of ±30mm. The phenotypic and molecular characterizations revealed the lipolytic yeast strains as *Pichia kudriavzevii* FJIB1- FJIB3. *Pichia kudriavzevii* FJIB1-FJIB3 had 99.35% pairwise similarity with Pichia kudriavzevii strain KBP: YE-1321 which has NCBI accession number MW856016.1. These findings highlight the potential of indigenous *Pichia kudriavzevii* FJIB1- FJIB3 from mango juice as cost-effective sources of lipases which can be utilized for lipase production in relevant industries including food.

KEYWORDS

Pichia kudriavzevii, Mango Juice, Isolation, Identification, Lipase

INTRODUCTION

Enzymes are biological macromolecules that catalyze a wide range of biochemical reactions, facilitating processes essential to life. They are derived from various natural sources including plants, animals, and microorganisms. Plant-derived enzymes such as papain (from papaya), bromelain (from pineapple), and ficin (from figs) have been used traditionally in food and pharmaceutical industries. Trypsin and chymotrypsin (from the pancreas), and pepsin (from the

stomach) which are from animal play vital roles during digestion and industrial bioprocesses [1]. However, enzymes sourced from plants and animals often suffer limitations such as seasonality, ethical issues, and lower stability during industrial processing.

Microorganisms, on the other hand, offer a more sustainable and controllable source of enzymes. Microbial enzymes, especially those produced by bacteria, fungi, and yeasts, are preferred in industrial biotechnology due to their high productivity, genetic manipulability, and adaptability to a wide range of environmental conditions [2]. These microbial enzymes include lipases, proteases, amylases, cellulases, and pectinases which have applications in food processing, pharmaceuticals, leather tanning, paper production, textiles, and biofuel generation [3].

The advantages of using microorganisms for enzyme production are numerous. Firstly, microbial growth is rapid and not influenced by seasonal fluctuations, unlike plant or animal sources. Secondly, microbes can be cultivated in inexpensive media, often using agricultural waste as substrates, making production cost-effective [4]. Thirdly, microbial strains can be genetically improved through mutagenesis and recombinant DNA technologies to enhance enzyme yield and performance. Furthermore, enzymes from microbes often display greater stability under extreme pH, temperature, and salinity conditions, increasing their suitability for industrial applications [5].

Among microbial producers, yeasts have emerged as an important group for enzyme biotechnology. Yeasts such as *Saccharomyces cerevisiae, Candida rugosa, Yarrowia lipolytica*, and *Pichia pastoris* are widely known for producing high-value enzymes like lipases, amylases, and proteases. Their eukaryotic nature makes them suitable for expressing complex proteins, while their unicellular form allows for easy cultivation [6]. Yeasts also tolerate higher alcohol concentrations, osmotic pressures, and acidic environments better than most bacteria, making them ideal candidates for industrial fermentation processes.

Among these, non-Saccharomyces yeasts like *Pichia kudriavzevii* are gaining prominence due to their unique physiological properties. *P. kudriavzevii* is a facultative anaerobic and osmotolerant yeast capable of thriving in acidic and high-temperature conditions, often where traditional yeasts fail to survive [7]. It has been isolated from fermented foods, beverages, dairy products, and tropical fruits, and is known to produce diverse enzymes such as lipases, amylases, and proteases [8]. Its robustness under stress conditions and its ability to utilize a wide range of carbon sources make it a versatile candidate for enzyme production in both submerged and solid-state fermentation systems.

Lipases (EC 3.1.1.3), in particular, have attracted significant industrial interest due to their role in hydrolyzing fats and oils into glycerol and fatty acids. These enzymes are vital in industries such as biodiesel production, detergent formulation, food processing, and bioremediation [2]. The use of lipase-producing yeasts offers several benefits, including high enzyme yield, extracellular secretion for easy recovery, and reduced production costs compared to bacterial systems. Moreover, lipases from yeasts such as *P. kudriavzevii* often show desirable properties like thermostability, solvent tolerance, and broad substrate specificity [9].

Tropical fruit juices like mango (*Mangifera indica*) provide an excellent ecological niche for isolating such beneficial microorganisms. Mangoes are rich in sugars, organic acids, vitamins, and minerals, creating a nutrient-rich medium for microbial growth. They are widely available in Nigeria and other West African countries, and have been previously reported as sources of diverse yeast communities [10,11]. Utilizing mango juice as a natural substrate not only reduces production costs but also supports eco-friendly bioprospecting strategies aligned with circular bioeconomy principles.

MATERIALS AND METHODS

Source of sample and preparation

Fresh mango fruits (*Mangifera indica*) were purchased from Eke Agbani Enugu State. The mango fruits were washed thoroughly under running tap water to remove surface debris and contaminants. The peels were aseptically removed using a sterile knife and the pulp was excised and blended with a sterile electric blender to obtain the juice. The resulting juice was filtered through sterile muslin cloth into sterile conical flasks and used immediately for yeast isolation.

Isolation of the yeast from fresh mango juice

The freshly prepared mango juice was aseptically inoculated onto Potato Dextrose Agar (PDA) plates supplemented with chloramphenicol (50 µg/mL) to inhibit bacterial growth and incubated at 30°C for 48 hours under aerobic condition.

Screening for Lipolytic Activity of the Isolated Yeast Strains

The isolated yeast strains were screened for lipase production using tributyrin agar as described by Mbah and Nwachukwu [12]. The yeast isolates were point-inoculated onto tributyrin agar which was composed of peptone (0.5%); yeast extract (0.3%); tributyrin (0.1%) and agar (2%) and incubated at 30°C for 48 h. Thereafter the culture plates were observed for the zones of hydrolysis around colonies and then measured using a meter rule in millimeters (mm)

Identification of the lipolytic Yeast Strains

The yeast strains that produced lipase were identified by phenotypic and molecular approaches. The colony morphological characterization was done by observing shape of the yeast colonies on the PDA medium. Gram staining, biochemical and sugar fermentation, yeast were also carried out on the isolates. The molecular characterizations including DNA extraction, electrophoresis, gene amplification and sequencing were done as described by George-Okafor [13].

RESULTS AND DISCUSSION

Identification of the lipolytic Yeast Strains Isolates

All the four isolates (FjiB1–FjiB3) showed similar morphological and physiological patterns. All fermented glucose, sucrose, and maltose, but did not ferment lactose. The isolates were Gram-positive and showed creamy-coloured colonies on potato dextrose agar (PDA). Amplification of the ITS rRNA gene yielded distinct bands of approximately 650bp for all isolates. The Gel electrophoresis revealed a single sharp band of approximately 650 base pairs, consistent with the expected size for yeast ITS amplicons. This confirmed the presence and quality of fungal DNA, and served as a basis for downstream sequencing and phylogenetic analysis.

The sequence obtained from the ITS amplification was subjected to BLAST analysis using the NCBI database. The isolate showed 99.35% similarity with Pichia kudriavzevii strain KBP: YE-1321 (Accession No. MW856016.1).

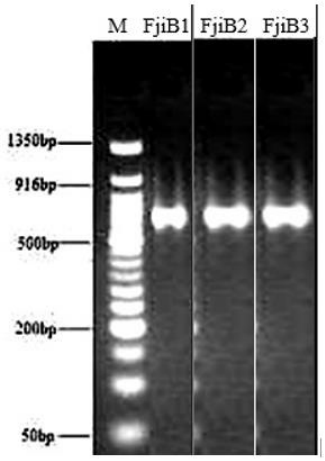
A phylogenetic tree was constructed using the ITS sequence and aligned against closely related yeast strains obtained from GenBank. The neighbor-joining method revealed that Pichia kudriavzevii FjiB clustered tightly with other *P. kudriavzevii* strains, confirming its taxonomic identity (Table 1 & 2 Figure 1).

Organism Code	Gram reaction/ Morphological appearance on PDA	Glucose	Fructose	Galactose	Sucrose	Maltose	Lactose
FjiB1	Gram + Circular, smooth cream colored	+	+	+	+	+	_
FjiB2	Gram + Oval shaped smooth white colonies	+	+	+	+	+	_
FjiB3	Gram +Circular creamy colonies	+	+	+	+	+	_

Table 1: Phenotypic characterization of the isolated yeast strains.

Isolate Code	Isolated Yeast	Closest specie	Identity	E Value
FjiB1	Pichia kudriavzevii	Pichia kudriavzevii Strain KBP:YE-1321	99.35%	0
FjiB2	Pichia kudriavzevii	Pichia kudriavzevii Strain KBP:YE-1321	99.35%	0
FjiB3	Pichia kudriavzevii	Pichia kudriavzevii Strain KBP:YE-1321	99.35%	0

Table 2: Molecular characterization of the isolated yeast strains.



Key: Amplification at 650bp.

Lane M is a 50bp DNA ladder

Figure 1: The amplification of the internal transcribed spacer (ITS) of Pichia kudriavzevii FjiB1- FjiB3.

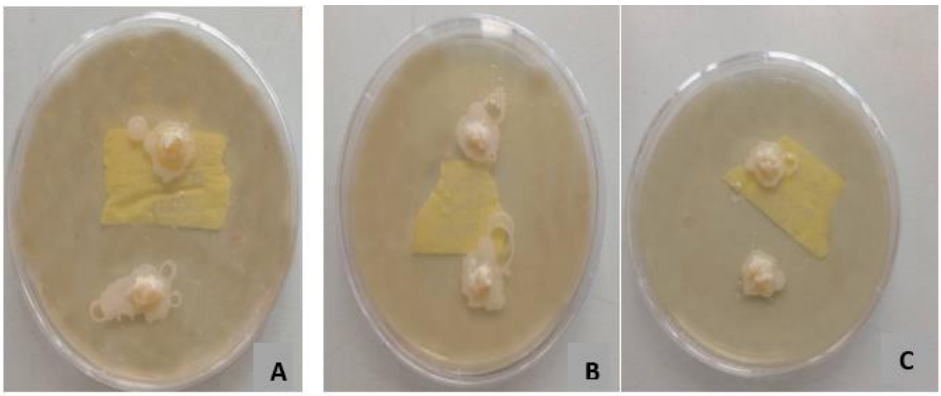
Screening for Lipase producing yeast strain

The yeast isolates were screened for extracellular lipase activity using tributyrin agar and the zones of hydrolysis were observed. All the yeast isolates demonstrated the high lipase activity, with zones of hydrolysis measuring ±30 mm. The presence of clear zones indicates effective lipase secretion capable of hydrolyzing the tributyrin substrate.

The high lipase activity observed can be attributed to its osmotolerant nature and ability to metabolize a wide variety of lipid substrates. This is in line with the findings of Sandhya et al. (2005), who reported that *P. kudriavzevii* strains isolated from fruit juice exhibited notable extracellular lipase activity. Also Patel et al. (2014) reported lipase production with zones of hydrolysis ranging from 22 mm to 30 mm in Pichia isolates recovered from agro-waste, supporting the industrial enzyme potential of this yeast. Similarly, Sharma et al. (2001) recorded high lipolytic activity in Pichia species, emphasizing their application in dairy processing and biodiesel production.

In contrast, Hasan et al. (2006) reported lower lipase activity of same yeast strain. This demonstrated lipolytic activity suggests *P. kudriavzevii* FjiB as a valuable biocatalyst for industrial applications such as biodiesel production, dairy processing, waste management, and food technology. Its ability to grow under ambient conditions and utilize diverse sugars in mango juice adds to its industrial appeal, particularly in settings where cost-effective, indigenous enzyme sources are desirable.

These findings contribute to the expanding search for microbial enzymes from underexplored natural environments, particularly tropical fruits. The study underscores the potential of mango juice as a low-cost substrate for the isolation of enzyme-producing yeasts and supports sustainable bioresource utilization in enzyme technology (Figure 2).



KEY: A- Pichia kudriavzevii FjiB1= ±30mm
B-Pichia kudriavzevii FjiB2= ±29mm
C-Pichia kudriavzevii FjiB3= ±20mm
Figure 2: Lipase production of Pichia kudriavzevii FjiB1-FjiB3.

CONCLUSION

This study revealed the presence of lipase producing *pichia kudriavzevii* in mango juice and hence recommended for lipase production in the industry.

CONFLICT OF INTEREST

There is no conflict of interest to disclose by the authors.

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