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Research Article

Isolation and Identification of Yeasts from Tomato (*Solanum lycopersicum*) Fruit and Cassava (*Manihot esculenta*) Tuber

***Onoja Oda[1] and Aisha Mohammed[2]**

[1]Department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota, Lagos
[2]Department of Pharmaceutical Microbiology and Biotechnology Faculty of Pharmaceutical Sciences Bayero University Kano, Nigeria
****Corresponding Author's email*:** lexono007@gmail.com

ABSTRACT

Different species of yeast inhabit various plant types and parts owing to variations in their nutrient needs. Thus, an effort to identify ideal yeast species for a given industrial process would require a certain level of research input directed toward their habitats. Therefore, this study aimed to identify and isolate yeast cells from the fruit and tuber of tomato and cassava respectively. Yeasts were isolated from the extract of cassava and tomato tuber and fruit respectively. Morphological, biochemical and microscopic assessments were determined using standard procedures. Results obtained from this study showed *Saccharomyces* constituted (66.7%) of the microbial populations, while *Candida* constituted (33%) of the sample. In conclusion, it can be deduced from this study that tomatoes can be relied upon as a dependable source of *Saccharomyces* for certain industrial processes such as the production of brewery products.

Keywords: Isolation, Yeasts, Tomato, Cassava. Biochemical

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INTRODUCTION

Yeasts generally considered fungi are evolutionally diverse eukaryotic, single-celled microscopic organisms varying in size to a large extent, a phenomenon which could be attributed to habitat and species, normally measuring up to 3-4 µm in diameter even though some measure as much as 40 µm (Dzialo *et al*., 2017). They are basically of dual phyla which include Ascomycota and Basidiomycota (Ashiwini and Mallesha, 2019) and not less than 1500 species have been identified (Den *et al*., 2015) several of which have proven to be critical tools in the industrial production of foods, beverages, wine and pharmaceuticals (Elkhateeb *et al*., 2022). Generally, it is a known fact that yeasts reside in plants as much as in other habitats. However, variation abounds in constituent species of yeasts found within a given environment determined primarily by the nutrient supply of the host environment (Elkhateeb *et al*., 2022).

Tomatoes botanically known as *Solanum lycopersicum* L. is a member of the nightshade family. It is reddish when ripe and contains certain nutrients such as lycopene, minerals and vitamins which are all of immense health significance (Osae *et al*., 2022). Cassava also known as *Manihot esculenta* is predominantly grown in tropical countries such as Nigeria. Its tuber is considered a staple for the ever-growing urban and rural populations (FAO, 2010) and is processed into different end products for human consumption (Olopade *et al*., 2014). Cassava root or tuber is rich in nutrients such as carbohydrates, and protein, and contains relatively low amounts of vitamins and minerals (Nweke *et al*., 2002).

Because yeasts grow on a variety of food materials which undoubtedly influence the nature of the inhabiting species owing to the varying nutrient compositions, it becomes extremely imperative to unravel the yeast populations of different environments to increase the number of industrially relevant yeast sources.

MATERIALS AND METHOD

Sample Collection

Fresh samples of tomato fruits and cassava tubers used in this study were bought from Mangoro Market Ikeja, Lagos State Nigeria. The samples were transported to the Microbiology Laboratory, Department of Biological Sciences and Biotechnology, Caleb University, Imota, Lagos, for microbiological analysis.

Media Preparation

Potato Dextrose Agar (PDA) used in this study was prepared according to manufacturers' instructions.

Isolation of Yeast

Fresh tomato fruits and cassava tubers were allowed to ferment for 3 days at room temperature after which extracts were serially diluted. Exactly 9 mL of distilled water was introduced into test tubes and subsequently autoclaved for 15 min at 121^{o}C and afterward allowed to cool. Precisely 1 g each the samples were aseptically dispensed into 9 mL of sterile distilled water in a test tube and then homogenized. 1 mL of the stock solution was serially diluted up to eight-fold (10^{-8}). The 10^{-3},10^{-4},$10^{-5}$and 10^{-8}-fold serial dilutions were inoculated onto yeast extract agar in Petri dishes using pour plate and spread plate methods and then incubated at 32°C for 4 days. After incubation, colonies were observed for colonial characteristics. The colonies were counted and sub-cultured by streaking onto freshly prepared PDA plates to obtain pure cultures of yeast. The sub-cultured plate was incubated for 5 days at 32°C (Hutzler *et al*., 2005).

Morphological identification of isolates

A preliminary macroscopic assessment of the incubated culture plates was performed to distinguish and characterize colonies. The colonial appearance; size, shape, form, consistency, color, odor and opacity of the colony plates were observed by the method described by Kurtzman *et al*. (2011).

Microscopic Identification of Yeasts

An inoculating wire loop was used to pick up a colony of yeast which was subsequently smeared on a glass slide for gram staining. The stained smear was viewed under the microscope with the 40× and 100× objective lenses, and the cellular morphology of the yeast isolates was observed and recorded Kurtzman *et al*. (2011).

Biochemical Test

Catalase, oxidase, and sugar fermentation tests were performed to confirm yeast isolates.

Catalase test

To perform the catalase test, a sterile wire loop was used to pick up a colony of freshly prepared yeast culture, which was then emulsified in a few drops of hydrogen peroxide on a clean microscope slide. The appearance of effervescence was an indication of catalase activity while the absence of effervescence was suggestive of catalase activity Jay (2005).

Oxidase test

A wet filter paper was used. A strip of filter paper was dipped in a freshly prepared 1% Kovacs Oxidase Reagent (tetra-methyl-p-phenylenediamine dihydrochloride, in water). A suspected yeast colony was picked from a potato dextrose agar plate and rubbed on the filter paper using a sterile inoculating wire loop. A positive result was indicated by the formation of a deep-purple colour that appeared in 5-10 seconds. A negative reaction was indicated by the lack of colouration (Cappuccino, 2008).

Sugar fermentation test

Exactly 9 mL of peptone water was dispensed into sterile test tubes followed the introduction of 1 g of each carbohydrate; glucose, fructose, sucrose, and galactose prior to homogenization. They were stirred over a Bunsen flame until fully dissolved, after which 3 drops of phenol red were added to each of the test tubes. Before sterilization in an autoclave at 115^{o}C for 15 mins, Durham's tubes were placed in inverted position in the tubes and corked with cotton wool coated with aluminum foil. After autoclaving, the freshly prepared yeast isolates were inoculated into each test tube and incubated for 48 h at 37^{o}C. After 48 h, a change in colour of the medium from red to orange was an indication of a positive response caused by the ability of the yeast to ferment the sugar, whereas the retention of the red color suggested a negative response (the yeast was not able to utilize the sugars) (Onyeze *et al*., 2013).

Urease test

Urease test was carried out according to the method described by Kurtzman *et al*. (2011). Bijou bottles were sterilized using the autoclave. Urea Agar base was prepared according to manufacturers' instructions. The Urea solution was prepared in distilled water and mixed with the urea agar base in aseptic condition. The mixture was stirred and dispensed into the sterile bijou bottles and kept in a slanting position. After solidification of the agar slant, a sterile wire loop was used to pick a colony and carefully inoculated onto the slant and incubated for 48 h. A change in the colour of medium from yellow to pink suggested a positive result which shows the ability of yeast to breakdown urea into ammonia and carbondioxide, whereas retention of colour suggests a negative result.

RESULTS

Physical characteristics of yeast isolates

Examination to determine the characteristics of the yeast colony revealed that colonies of S*accharomyces specie* revealed that the colour of the colonies varied from white to cream owing the specific stains as well as growth conditions. The surface of the colonies which are generally small and small and round had a smooth texture. *Candida* specie was visualized as smooth, creamy and white. While some appeared raised or dome shaped in appearance

Microscopic characteristics of yeast isolates

The outcome of the microscopic studies on the isolates unveiled the oval to elongated shape of *Candida* when viewed the microscope with characteristic Pseudohyphae which are chains of budding yeast cells that resemble hyphae. On the other hand, *Saccharomyces* was visualized as single, round-oval cells with a prominent nucleus and a clear cytoplasm but lacking the ability to produce pseudohyphae, instead they produce true hyphae only under certain conditions (Table 1).

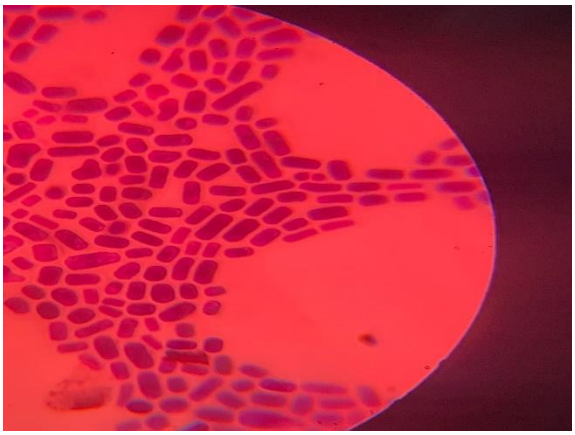

Fig 1: Microscopic view of *Saccharomyces* species

Biochemical analysis on yeast isolates

Biochemical analysis performed on the isolates revealed that the yeast isolates reacted positively to fermentation of fructose, sucrose, glucose, galactose indicating. All yeast isolates showed positive reaction to catalase test. However, while *Saccharomyces spp* tested negative to oxidase, *Candida spp* tested positive to it. All yeast isolates did not react to urea, clear indication yeast does not break down urea into ammonia and carbohydrate.

Fig 2: Plates of Yeasts on Yeast Extract Agar

Table 1: Morphological Characteristics of Yeasts Isolated from Tomato and Cassava Samples

Isolates Code	Colony Colour	Colony nature	Elevation	Budding	Cell Morphology
Y01	Creamy	Opaque, dull	Flat	Terminal	Oval-shaped, elongated cells
Y02	Creamy	Opaque, dull	Flat	Terminal	Oval-shaped, elongated cells
Y03	Creamy	Round, smooth	Raised	Lateral	Small, oval-shaped
Y04	Creamy	Opaque, dull	Flat	Terminal	Oval-shaped, elongated cells
Y05	Creamy	Opaque, dull	Flat	Terminal	Oval-shaped, elongated cells
Y06	Creamy	Wrinkled	Raised	Terminal	Small, oval-shaped

Y= Yeast

Table 2: Biochemical Characteristics of Yeasts Isolates

Isolate code	Catalase	Oxidase	Glucose	Fructose	Sucrose	Galactose	Urease	Probable Organism
Y01	+	–	+	+	+	+	–	*Saccharomyce sp*p.
Y01	+	–	+	+	+	+	–	*Saccharomyce spp.*
Y03	+	+	+	+	+	+	–	*Candida* spp.
Y04	+	–	+	+	+	+	–	*Saccharomyce sp*p.
Y05	+	–	+	+	+	+	–	*Saccharomyce sp*p.
Y06	+	–	+	+	+	+	–	*Candida* spp.

Y= Yeast

DISCUSSION

Tomato fruit and cassava tuber are both edible parts of the tomato and cassava plants respectively and are well valued for their nutritional and food values. They both wield the potential to habour diverse species of yeast owing to their varying nutritional profile and are therefore considered potential sources of industrially relevant yeasts. *Saccharomyces*, a genus of fungi encompasses a plethora of yeast species widely known for their relevance in food production where they are commonly referred to as brewer's yeast, baker's yeast and sourdough starter among others (Parapouli *et al*., 2019). The Candida species inhabit the normal microbiota of the human mucosal oral cavity, digestive tract as well as the vagina (Shao *et al*., 2007). They have been implicated in diverse arrays of clinical manifestations ranging from mucocutaneous overgrowth to infections of the blood stream (Eggimann *et al*., 2003). It operates a commensal relationship with a healthy human and may trigger a systemic infection in persons with a compromised immunity owing to their excellent ability to adapt different host niches. The genus is made up of a heterogenous group of organisms and not less than 17 different *Candida species* have been associated with human infections (Pfaller *et al*., 2007). This study revealed that *Saccharomyces species* are generally small and round. This is consistent with the finding of Aaron (2021) which reported the presence of small (3 µm× 2 µm) haploid round yeast cells which exist in two inverse mating types in a culture of *Saccharomyces*. It is possible to identify *Candida species* based on colour characteristics (Prakoeswa *et al*., 2018) this is substantiated by the findings made in this work which revealed the *Candida species* detected to be creamy and white or cream-coloured.

However, the *Candida species* are small, oval-shaped with characteristic pseudohypha which distinguishes it from the *Saccharomyces species*. This is in tandem with the observation made by Meyer *et al*. (1998) which reported the formation of pseudohyphae and septate by a *Candida* yeast. Yeast species may be characterized according to various criteria based on cell morphology (mode of cell division and spore shape etc), physiology (sugar fermentation test (Walker and White, 2005) etc. All isolates were oval in shape, an observation which agrees with the finding of Chavez *et al*. (2023) which revealed ovoid, spherical and ellipsoidal shapes to be the most common shapes of about 332 representative yeast species. The positive reaction of the isolates to the sugars suggested *Saccharomyces* species to be the probable organism. This is consistent with the findings of Umeh *et al*. (2022) who reported that *Saccharomyces* species were predominantly involved in the fermentation of carbohydrates.

CONCLUSION

From this study, it can be deduced that *Sacharomyces* sourced from tomato and cassava. However, it was unveiled from this study that more *Sacharomyces* can be obtained from tomato than cassava.

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