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Isolation and Identification of Yeasts from Soil in Caleb University, Lagos, Nigeria

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Abstract

Yeast cells are unicellular microorganisms that can be found in various environments, including soil, plants, and animals. They are important in the food and fermentation industry, where they are used for production of various alcoholic beverages, bread, and cheese. Yeast cells have also been used for their role in biotechnology and as model organisms for genetic research. Yeast cells play a critical role in soil ecosystems, contributing to nutrient cycling, plant health, and overall soil ecology. This study focuses on the isolation and characterization of yeast cells from soil samples in the study location. Soil samples were collected from five locations in Caleb University, representing diverse habitats and soil types. Yeasts were isolated using selective media that favored yeast growth while inhibiting the growth of other microorganisms. Isolated yeasts were characterized based on the colonial, morphological and biochemical characteristics. Twenty yeasts isolates were isolated and identified which fell into the genera; Candida species, Geotrichum species and Saccharomyces species. Candida species was most abundant with isolation rate of 45%. This research contributes to our understanding of yeast within soil ecosystems. This study provides insights to soil as a cheap source of yeasts cells. These yeasts could be harnessed due to their potential applications in agriculture, biotechnology, and environmental remediation.

Keywords: yeasts, soil, Caleb University, Lagos

INTRODUCTION

Yeast cells are unicellular microorganisms that can be found in various environments, including soil, plants, and animals. They are important in the food and fermentation industry, where they are used for production of various alcoholic beverages, bread, and cheese (Legras *et al*., 2007). Yeast cells have also been used for their role in biotechnology and as model organisms for genetic research (Legras *et al*., 2007).

The isolation and identification of yeast cells in different environments have been the subject of several studies (Chao *et al.,* 2019). The identification of yeast cells in soil in

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particular, is of great interest due to the potential impact of yeasts on the agricultural industry. Yeast cells can have both beneficial and harmful effects on plants and their identification can help in the development of strategies to improve crop yield and reduce plant diseases (Chao *et al.*, 2019).

Soil is a complex environment that provides nutrients and support for plant growth. It is also home to a diverse range of microorganisms, including yeast cells (Jumpponen, 2003). The identification of yeast cells in soil can provide insights into their role in nutrient cycling and soil health. Yeast cells in soil have been found to play a role in the decomposition of organic matter and the release of nutrients for plant uptake (Jumpponen, 2003). Thus there is need to source for cheap sources for the isolation of yeasts cells. The soil because of its complex mix could provide a cheap source for the isolation of these yeast cells.

MATERIALS AND METHOD

Sample Collection

Soil samples were collected from 5 locations in Caleb University. Caleb University is a privately owned University located in Imota, Lagos State, Nigeria. Imota is located aroumdgps coordinates of 6°39'48.9888"N and 3°40'11.7444"E (Available at latlong.net/place/imota). A surface sterilized mini shovel was used to collect soil from each sampling location carefully following sample collection safety rules and regulations. The surface debris was removed from the sampling area to avoid contamination then 0-15 cm of topsoil was collected. Samples were collected in a zig-zag pattern at each sampling site to ensure even coverage and representative samples. Enough soil was collected to fill the petri dish adequately. Each petri dish was clearly labelled with name of sampling site. This was carefully transferred to the laboratory as soon as possible. Ensuring that they are securely sealed and packaged to prevent spillage or contamination during transit.

Culture Media Preparation

Yeast Extract Agar (YEA) was prepared according the manufacturer's instructions and all media used were sterilized using the autoclave for 15 minutes at 121 °C according to the method described by Onyeze *et al*. (2013).

Isolation of Yeast from Soil

The isolation of yeast from soil was done according to the method described by Kurtzman *et al*. (2011). Pour plate technique was used. The soil samples were serially diluted to reduce microbial load. In washed test tubes, 9 mL of distilled water was poured into the test tubes and sterilized for 15 minutes at 121 °C using the autoclave. The test tubes were then allowed to cool. One gram each of the five soil samples were aseptically dispensed into 9 mL of sterile distilled water in a test tube and then homogenized. 1ml of the stock solutions were serially diluted up to five-fold (10^{-5}). The 10^{-3} and 10^{-5} dilution factors were inoculated onto Yeast extract agar in Petri dishes using pour plate method and then incubated at 25 °C for 5 days. Colonies that developed on the plates after incubation were observed for their morphological characteristics. The colonies were counted and sub-cultured by streaking onto a fresh potato dextrose agar plate containing chloramphenicol (10µg) to obtain a pure culture of yeast, the sub-cultured isolates were incubated for 5 days at 25 °C.

Characterization of Yeast Based on Colonial Morphology

Preliminary macroscopic assessment of the incubated culture plates was performed to distinguish and characterize colonies. The colonial appearance; size, elevation, shape,

border, form, consistency, colour, odour, and opacity of the colony plates were observed according to the description given by Kurtzman *et al*. (2011).

Characterization of Yeast Based on Cellular Morphology
Microscopic examination was performed according to the method of Kurtzman *et al*. (2011). A drop of lactophenol blue was placed on a glass slide, an inoculating loop was used to pick up a colony of the yeast and smeared it onto a glass slide with lactophenol blue. A cover-slip was placed onto the smeared portion of the glass slide to reduce air bubbles. The glass slide was viewed under the microscope using the x40 objective lens, and the cellular morphology of the yeasts were observed and recorded.

Growth at Different Incubating Temperatures
The test to show the ability of the yeast isolates to grow at different temperatures was carried out according to the method as described by Nayak (2011). Potato dextrose agar (PDA) was prepared with the addition of 1.0 g of yeast extract. The yeast isolates were inoculated and incubated for 5 days at 15 ℃ and 45 ℃ in a refrigerator and oven respectively.

Sugar Fermentation Test
Sugar fermentation test was performed according to the method described by Onyeze *et al*. (2013). 10 mL of peptone water was dispensed into sterile test tubes each. One (1) g of each carbohydrate; glucose, fructose, sucrose, and galactose were homogenized into separate test tubes containing peptone water and labeled appropriately. They were stirred over a Bunsen flame until fully dissolved, 3 drops of phenol red was then added to each of the test tubes. Durham's tubes were placed inverted position in the tubes and corked with cotton wool coated with aluminum foil before sterilization in an autoclave at 115 ℃ for 15 minutes. After autoclaving and allowing to cool, the freshly prepared yeast isolates were inoculated into each test tubes and incubated for 24 h at 37 ℃. A change in the color of the medium from pink to yellow after incubation suggested a positive test caused by the ability of the yeast cells to ferment the sugar, whereas the retention of the pink color suggested a negative test (the yeasts cells were unable to utilize the sugars).

RESULTS

Colonial Characteristics
The yeast isolates appeared small, oval-shaped in their cell morphology, while their colony morphology ranged from cream to white with flat, smooth, irregular, raised, undulating, glossy, opaque, small, medium colonies on the Potato Dextrose Agar (Table 1).

Physiological and Biochemical Characteristics of Yeast Isolates
The physiological tests carried out showed that a variety of the yeast isolates grew at temperatures of 15 ℃ and 45 ℃ respectively (Table 2).
The biochemical tests performed showed that the yeast isolates had variation in the fermentation of glucose, fructose, sucrose, and galactose (Table 2).

Twenty yeast isolates were isolated from Soil. Identification was achieved based on microscopic, macroscopic, colonial, physiological and biochemical characteristics and compared with the Mycology manual. The species of yeasts suspected were; *Candida* species, *Saccharomyces* species, *Geotrichum* species. In this study, *Candida* specie had the

highest occurrence of 45% while *Geotrichum* species had the least occurrence of 15% (Figure 1).

Table 1: Morphological Characteristics of Yeasts Isolated from Soil in Caleb University

Isolate Code	Colony morphology	Cell morphology
Y01	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y02	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y03	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y04	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y05	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y06	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y07	White, flat, medium-sized, dry, round, opaque colony	Cylindrical, elongated cells
Y08	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y09	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y10	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y11	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y12	White, flat, medium-sized, dry, round, opaque colony	Cylindrical, elongated cells
Y13	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y14	White, flat, medium-sized, dry, round, opaque colony	Cylindrical, elongated cells
Y15	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y16	Creamy, flat, dull, small-sized, opaque colony	Oval-shaped, elongated cells
Y17	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y18	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y19	White, round, small-sized, smooth colony	Small, oval-shaped cells
Y20	White, round, small-sized, smooth colony	Small, oval-shaped cells

Key Y01-Y20 represents different yeast isolates

Table 2: Physiological and Biochemical Characteristics of Yeats Isolates

Isolate Code	15°C	45°C	Glucose	Fructose	Sucrose	Galactose	Suspected Organism
Y01	−	+	+	+	+	−	*Candida spp*
Y02	+	+	+	+	+	+	*Saccharomyces spp.*
Y03	−	+	+	+	+	−	*Candida spp.*
Y04	+	+	+	+	+	+	*Saccharomyces spp*
Y05	+	+	+	+	+	+	*Saccharomyces spp*
Y06	+	+	+	+	+	+	*Saccharomyces spp*
Y07	+	+	+	+	+	+	*Geotrichum spp.*
Y08	−	+	+	+	+	−	*Candida spp.*
Y09	+	+	+	+	+	+	*Saccharomyces spp.*
Y10	−	+	+	+	+	−	*Candida spp.*
Y11	+	+	+	+	+	+	*Saccharomyces spp.*
Y12	+	+	+	+	+	+	*Geotrichum spp.*
Y13	−	+	+	+	+	−	*Candida spp.*
Y14	+	+	+	+	+	+	*Geotrichum spp.*
Y15	+	+	+	+	+	+	*Saccharomyces spp*
Y16	+	+	+	+	+	+	*Saccharomyces spp*
Y17	−	+	+	+	+	−	*Candida spp.*
Y18	−	+	+	+	+	−	*Candida spp.*
Y19	−	+	+	+	+	−	*Candida spp.*
Y20	−	+	+	+	+	−	*Candida spp.*

Key Y01-Y20 represents different yeast isolates

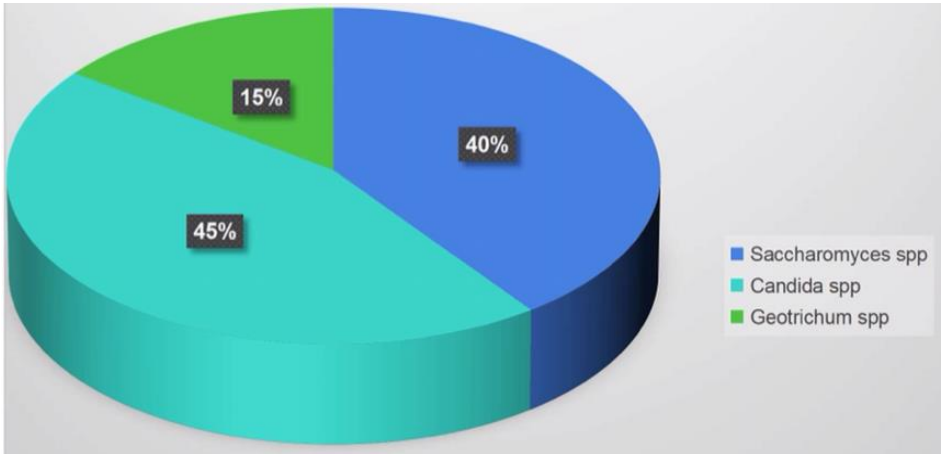

Figure 1: Percentage Occurrence of Yeasts Isolated from Soil in Caleb University

DISCUSSION

The study of yeasts in soil has gained significant importance due to their crucial ecological roles and diverse applications in various fields such as industry, agriculture, biotechnology, and medicine. Isolating and identifying yeasts in soil helps us understand their distribution, diversity, and potential functions in different ecosystems (Mohd *et al*., 2011).

In addition to other yeasts, several workers such as Silva *et al.* (2018), Pagnocca *et al*. (2017), and Davidson *et al.* (2015) have isolated *Saccharomyces* spp, *Candida* spp. and *Geotrichum* spp. from soil. Yeasts such as *Saccharomyces cerevisiae* has been extensively used in industrial fermentation for the production of bioethanol (Mohd *et al*., 2011). Species of yeast like *Candida* have not been extensively reported as fermentative yeast for industrial utilization such as the production of bioethanol nor in the production of other useful organic compounds except as causal agents of human diseases. Ellis *et al*. (2007) reported *Candida tropicalis* as the causal agent of candidiasis in man; they are opportunistic fungi which live in most human organs. However, reports by Kathiresan and Saravanakumar (2011) and Senthilraja *et al*. (2011) have shown that species of *Candida* are not just pathogens but can be useful tools for bioethanol production, as they were able to use *Candida tropicalis* and *Candida albicans* isolated from marine environment to produce bioethanol. *Geotrichum candidum* has been reported as pathogenic to tomato (Bourret *et al*., 2013). The yeast *Geotrichum candidum* has also been used as starter culture in the dairy industry. It has also been administered as a probiotic nutritional supplement in fish indicating improvements in developmental and immunological parameters. Strain of this species also produce a plethora of biotechnologically important enzymes including cellulases, β-glucanases, xylanases, lipases, proteases and α-amylaes. Moreover, strains that produce antimicrobial compounds capable of bioremediation have been identified (Kamilari *et al*., 2023).

CONCLUSION

Twenty yeasts isolates were isolated from the soil in Caleb University, Lagos. These yeasts were identified and fell into the genera; *Candida* species, *Geotrichum* species and *Saccharomyces* species. This study provides insight to soil as a cheap source of these yeasts cells which could be harnessed due to their potential applications in agriculture, biotechnology, and environmental remediation

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