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Identification and Characterisation of Citric Acid-Producing Fungi from Soil and Melon (*Cucumeropsis mannii*) Rind Samples Collected from Melon Farm in Lafia, Nasarawa State, Nigeria

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Abstract

The essential and important organic acid used industrially worldwide for microbial fermentation, especially filamentous fungi, is citric acid. In this investigation, soil and melon rind (*Cucumeropsis mannii*) samples from melon farms in Lafia, Nasarawa State, Nigeria were obtained. These were used to isolate, screen, identify, and characterize citric acid-producing fungi. Standard microbiological culture protocols were used for serial dilution followed by plating on Potato Dextrose Agar (PDA). The samples were also cultivated on Czapek-Dox Agar (CZA) enriched with bromocresol green to specifically achieve acidogenicstrain isolates. This specialized medium allows for the identification of potent isolates with observable translucent halo zones around the colonies. Green colonies were the most common of the 212 fungi colonies that were obtained. Morphological and microscopic characterization revealed the presence of several fungi genera, including *Aspergillus niger, Aspergillus flavus, Aspergillus tamarii, Penicillium* spp., *Fusarium* spp. and *Rhizopus stolonifer*. Out of the characterized and identified fungus, *Aspergillus niger* showed highest potential for citric acid production. The results highlighted the possibility to use melon rind as an inexpensive substrate for industrial fermentation operations and revealed the abundance of native fungi in soil and agricultural leftovers capable of generating citric acid. This research shows how local microbial sources can be effectively used in industries and biotechnological projects.

Keywords: Citric acid, Fungi isolate, *Aspergillus niger*, Melon rind substrate, Fermentation process, Agricultural waste

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Introduction

The major organic acid produced worldwide is citric acid ($C_6H_8O_7$), which plays an essential role in many biochemical and industrial processes [1]. It is a weak organic acid which occurs naturally in different fruits and vegetables, particularly citrus fruits such as lemons, oranges, and limes, and is an essential intermediate of the tricarboxylic acid (TCA) cycle, which is fundamental to cellular energy metabolism [2]. It is known to have distinguished physicochemical properties such as very high solubility and low toxicity, strong metal chelating capacity. Also, it is biodegradable, and this made it an important industrial compound with diverse applications in different industries such as food, cosmetics, pharmaceutical and chemical industries [3, 4].

The demand for citric acid continues to increasing which exceed 2.8 million tons per year due to its many uses [5, 6]. The food and beverage industries are major users of citric acid globally, they rely on it to preserve food, improve taste and as an antioxidant [7, 8]. Citric acid serves as a buffer and stabilizer in the pharmaceutical industry. Its metal chelating ability makes it an important component in cosmetics and cleaning products [3, 4]. Microbial fermentation produces more than 90 % of the world's citric acid today. This shows how essential microbial biotechnology has become for meeting industrial needs. Many organisms, such as bacteria, yeast, and filamentous fungi, produce citric acid, but *Aspergillus niger* remains the most widely used industrially [2]. This is because of its high yields, stability during growth, and ability to survive on cheap materials like molasses, starch, and agricultural waste [9, 10]. Under low pH and specific conditions, such as high sugar concentration and few trace metals *A. niger* produces the highest amount of citric acid. These advantages make *Aspergillus niger* the most used organism for large scale citric acid production worldwide [9, 10].

Fungi are a varied group of microorganisms that play a vital role in recycling nutrients in the ecosystem as natural decomposers. They are found everywhere in the soil and plant waste, and they play a great role in the recycling and decomposition of organic material [10]. Fungi can break down tough materials like cellulose and lignin by releasing enzymes. This process helps to recycle nutrients back into the ecosystem. Because of these metabolic properties, fungi are great resources for making antibiotics, industrial enzymes, and organic acids like citric, gluconic, and malic acid [10]. High

performing fungi in nature are still an important way of improving industrial fermentation. Soil and agricultural leftovers are the most excellent sources for finding these fungal communities that produce valuable metabolites [11]. Farm waste is highly rich in nutrient with support growth of microorganisms used in the fermentation process. One great example is the rind of the egusi melon (*Cucumeropsis mannii*), which is a common food source across West Africa. While the seeds are used for cooking and local spices by many, the leftover rinds provide a home for active fungi to grow [10].

The oldest biotechnological process involved in food preservation biochemical changes of substrates is fermentation [12, 13]. Microbial activities help in the production of metabolites which had effect on the sensory and functional properties of food in many African traditions of fermented foods [9]. Beyond its industrial uses, citric acid is vital in medicine (biomedical and physiological processes). Citrate ions are part of several metabolic reactions and are commonly used as anticoagulants for blood storage. It is a key factor in the blood coagulation cascade, which prevent clotting [14]. Despite multiple reports on strains of *Aspergillus niger* as industrial citric acid producer, there are fewer reports on local fungi isolates from soil and farm waste of developing countries [9, 10]. Therefore, this study aimed to isolate citric acid producing fungi from soil and melon (*Cucumeropsis mannii)* rind samples within Lafia metropolis of Nasarawa State, and to characterized the isolates using biochemical and molecular approaches. Findings from this research are expected to reveal new fungi strains with the potential for industrial use.

Materials and Methods

Reagents and culture media

All chemicals used were of analytical grade.

Sample collection

Soil and fresh melon rind samples were collected from melon farms around Lafia metropolis, Nasarawa State, Nigeria. Soil samples were obtained from the topsoil layer at a depth of (0 to 15 cm) using sterile tools. While fresh melon rinds (*Cucumeropsis mannii*) were collected aseptically from the same locations.

Preparation of culture media

All media were prepared according to the manufacturer's instructions and were sterilized at 121 °C for 15 min. Media used include Potato Dextrose Agar (PDA), Czapek-Dox Agar (CZA) fortified with Chloramphenicol (60 mg/L) to suppress bacterial growth.

Isolation of fungi

The isolation of fungi was done using the dilution plate technique as described by Chilaka *et al*. (2025). The samples of the melon rind and soil were collected to isolate fungi following a serial dilution standard and direct plating methods. One gram (1 g) of each rind sample was added to 9mL of sterilized distilled water and was done under aseptic conditions. This was further diluted to make 10^{-9} dilution and 0.1 mL of an aliquot was collected and inoculated onto Potato Dextrose Agar (PDA) and Czapek-Dox agar (CZA) supplemented with Chloramphenicol (60 mg/L) To suppress bacterial growth, chloramphenicol (60 mg/L) was used as a supplement. The inoculum was spread evenly using sterilize glass rod spreader to ensure even distribution of the fungi inoculum. Plates were incubated at 25 °C for 5 days under aerobic conditions.

Purification of isolates

Pure cultures of fungal isolates were obtained by repeated sub-culturing of distinct colonies onto fresh PDA plates using aseptic techniques and these plates were incubated at 25 °C. Pure isolates were preserved on PDA slants at 4 °C for subsequent screening and identification.

Morphological and microscopic identification

Macroscopic and microscopic characteristics were used in the identification of the pure fungi isolates. For the macroscopy, morphological characteristics such as colour, size, margin, texture, and pigmentation of the fungi were characteristics used for the identification PDA medium. The fungi were microscopically identified by harvesting small portion of the fungi growth and introducing it into lactophenol cotton blue (LPCB) on cleaned greased free slide and was observed using a light microscope. Hypae structure, conidiophore, the conidia morphology were examined and compared with the report of Pitt *et al*. (2022).

Screening for citric acid production

In accordance with Ajiboye *et al.* (2024), fungi isolates were screened by plating on a Czapek-dox media using bromocresol green (BCG) as an indicator of citric acid-producing fungi. The resulting halo zoned formation and colour change in the medium at 28 ℃ after 5 days indicate citric acid producers. Those with a halo zone are selected for further characterization.

Results and Discussion

Fungal isolation

Six (6) different fungi colony counts were identified from both melon rind and soil samples. Table 1 shows the total colony count of fungi isolated in rind and soil samples across different concentration of aliquots which also indicate keys of macroscopic identification. A total of 212 colonies was counted with green with white edge (GWE) most predominant across all aliquot concentration.

Table 1: Distribution of fungal colonies in rind and soil samples based on morphological appearance isolated from soil and melon rind obtained from the farmland within Lafia metropolis

S/N	Sample (aliquot)	Morphological description with colony count						
		GWE	GY	YE	WT	BL	BWE	PK
1	Rind Sample (10^5)	22	2	2	2	6	0	**3**
2	Rind Sample (10^7)	28	7	1	5	4	0	**2**
3	Rind Sample (10^9)	18	1	0	10	0	0	**1**
4	Soil Sample (10^2)	2	0	1	4	4	1	**2**
5	Soil Sample (10^3)	21	0	1	8	8	3	**7**
6	Soil Sample (10^5)	9	4	0	7	5	1	**8**

In this study, isolates were categorized using the following phenotypic markers: BL for black pigmentation; BWE for black colonies with white borders; G.Y for greenish-yellow; Y.E for yellow; G.W.E for green with rounded marginal edges; and G.R for green

Table 2: Distribution and relative dominance of fungal isolate

S/N	Fungi Observed (Colony Description)	Total Colonies Isolated (%)	Relative Dominance
1	Green with round edge	100 (47.17)	High
2	White colour	36 (16.98)	Moderate
3	Black colour	32 (15.09)	Moderate
4	Brown with white edge	23 (10.85)	Low
5	Greenish Yellow	10 (4.72)	Low
6	Pink colour	6 (2.83)	Trace
7	Yellow colour	5 (2.36)	Trace
	Total	**212**	

Distribution of fungal isolate

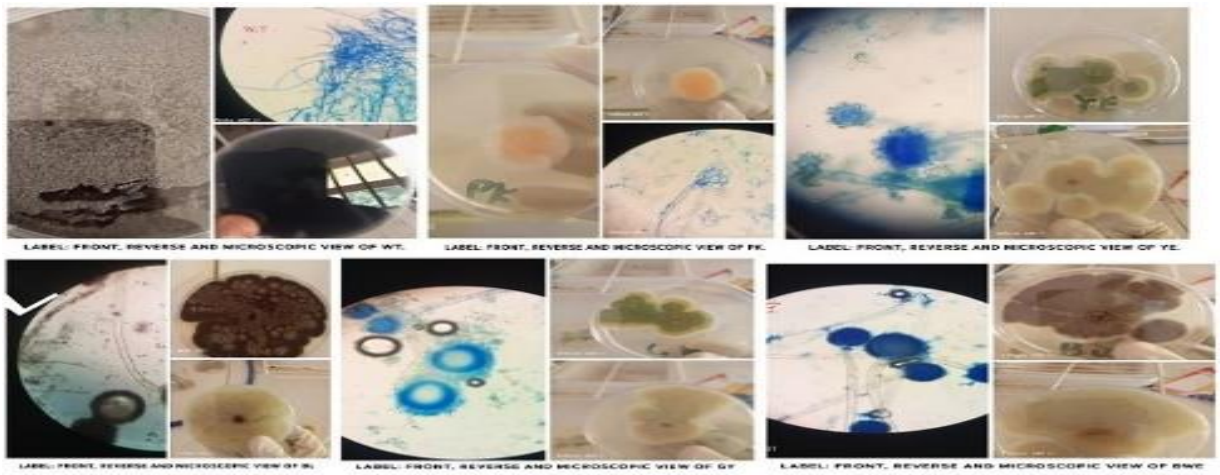
Table 2 displays percentage of dominance of which green with round edge (GWE) with highest dominance with 47.17 % of the entire population, which suggests it is the most ecologically or industrially adapted strain in the sampled environment. This favours fungi communities such as *Aspergillus* or *Penicillium* which maybe as a result of substrate composition or environmental filters [15, 16]. This is followed by White with 16.98 % and Black with 15.09 % morphological type sequentially. Black is most frequently identified as a high-performing citric acid producer which is mostly true for *Aspergillus niger* group [17]. On the other hand, isolates such as brown, greenish yellow, pink, and yellow are less common, with a collective dominancy of 20.76 % of the total isolates. This pattern proves even with many fungi types, the dominant species take over which common to use agricultural waste in a solid-state fermentation also certain species have a better metabolic efficiency than others [15, 17].

Morphological characteristics of fungal isolates

Combination of both microscopic and macroscopic examination in refrences to report of Pitt *et al*. (2022), the fungi were identified to be *Aspergillus sp., Fusarium sp.*, *Penicillium sp.* and *Rhizopus stolonifer* respectively as detailed in Table 3 and Plate 1. The basis of the identification was based on colony morphology such as hyphae structure and reproductive features such as conidia and sporangia which are widely used for the preliminary identification of filamentous fungi in environmental and food samples [18], and this approach remains consistent with recent studies that combine morphological traits with modern identification techniques [19].

Table 3: Morphological identification of fungal isolates

S/N	Sample Code	Macroscopic Characteristics	Microscopic Characteristics	Identified Organism
1	BWE/RB	Colonies appeared chocolate brown with a white edge; granular texture; reverse pale yellow	Non-septate hyphae; radiate conidial heads; smooth, round conidiophores	*A. amarii*
2	BL	Black granular colonies; reverse pale yellow	Non-septate hyphae; radiate conidial heads; rough conidiophores; black spores	*A. niger*
3	Pk	Pink, woolly colonies; reverse pinkish	Hyaline, septate hyphae; sickle-shaped macroconidia with distinct apical and foot cells	*Fusarium*
4	GWE/GR	Forest-green colonies with narrow white margin; wrinkled; reverse pale yellow	Septate hyphae; brush-like conidiophores (penicillus); branched into metulae; long chains of conidia	*Penicillium sp.*
5	WT	White to greyish, fluffy aerial mycelium; reverse dark/black	Broad, coenocytic (non-septate) hyphae; large globose sporangia with numerous sporangiospores	*R. stolonifer*
6	YE/GY	Yellow-green velvety colonies with white margin; wrinkled; reverse yellowish	Rough conidiophores; globose vesicles; uniseriatephialides; yellow-green conidia	*A. flavus*



(BL): Black; **(B.W.E):** Black with white edge; **(G.Y):** Greenish yellow; **(Y.E):** Yellow; **(G.W.E):** Green with round edge; **(G.R):** Green

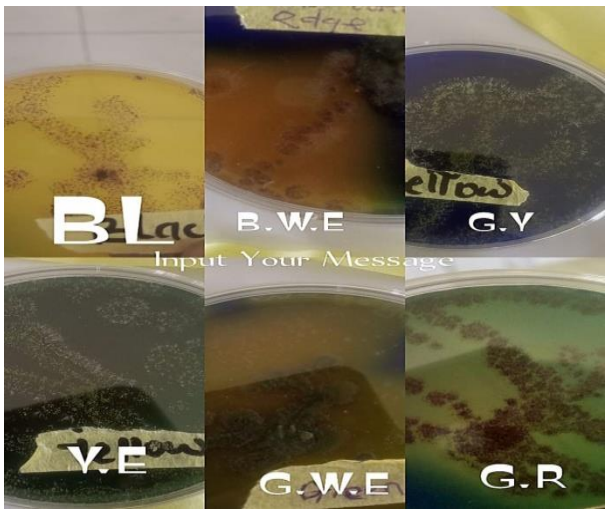
Plate 1: Morphological identification of fungi isolates form soil and melon rinds

Screening for citric acid production

The identified isolates were screened for acid production potential using Czapek-Dox Agar (CZA) supplemented with Bromocresol green (BCG). The desired biological activity was confirmed by the formation of clear halo zones or a color change in the medium, indicating citric acid secretion. Six distinct citric acid producing fungal isolates were identified in (Plate 2).

Citric acid-producing fungi included black colonies, brown with white edge, greenish yellow, yellow, and green colonies. The identified organisms include *Aspergillus niger, Aspergillus flavus, Aspergillus tamarii, Penicillium spp., Fusarium spp., and Rhizopus stolonifer.*

Aspergillus species were the most dominant, particularly *Aspergillus niger*.



(BL): *Aspergillusniger*; **(B.W.E):** *Aspergillus tamarii*; **(G.Y):** *Aspergillus flavus*; **(Y.E):** *Aspergillus flavus* (early stage); **(G.W.E):** *Penicillium sp.*; **(G.R):** *Penicillium sp.* (green variant)

Plate 2: Qualitative screening for citric acid production on BCG-supplemented agar

Table 4: Quantification of citric acid production by fungal isolates sourced from soil and *Cucumeropsis mannii* rind

S/N	Fungi Observed	Sample ID	Citric Acid Yield (mg/mL)	Performance Category
1	Green	G.R	0.319	High
2	Black	B.L	0.197	Moderate
3	Green with white round edge	G.W.E	0.188	Moderate
4	Greenish Yellow	G.Y	0.166	Low
5	Brown with round edge	B.W.E	0.120	Low
6	Yellow	Y.E	0.087	Minimal

Quantitative screening of fungal isolates

Table 4 shows varying strength in citric acid production and the amount yields ranged from 0.248 mg/mL to a high of 0.463 mg/mL. The yellow Y.E produced lowest with 0.087 mg/mL and green (G.R) isolate produced highest with 0.319 mg/mL. This makes it the best choice for future fermentation. Other included black (BL) and green with white edge (G.W.E) which are strong performing types. They produced 0.350 and 0.342 mg/mL. These results have proven that strong citric acid-producing fungi live in the local area. Some certain strains are much better at making organic acids than others [17].

The generally accepted method for measuring the concentration of organic acid is spectrophotometry which includes citric acid too [20, 21]. This was adapted in other to quantify the amount of citric acid produced. With a known concentration, a standard calibration curve was prepared and absorbance measured at 420 nm in Table 1. The optical density was compared to the concentration of the samples given rise to result in Table 4 and Fig. 2 which gives chart of comparison between both to determine the unknown concentration.

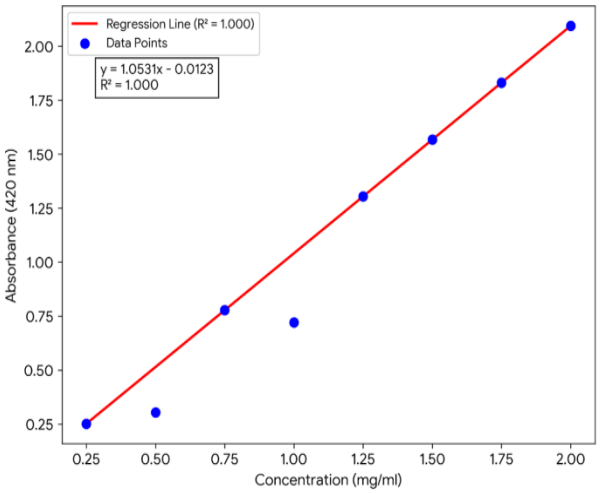


Figure 1: Standard calibration plot of citric acid (420 nm)

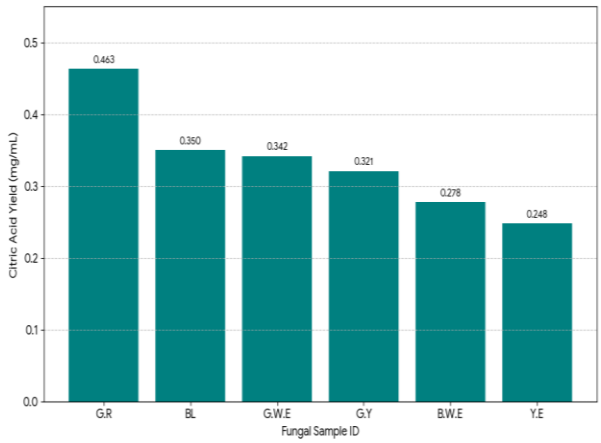


Figure 2: Showing quantification values of citric acid samples

A wide variety of fungi species were found in both the soil and melon rinds, as seen in the result, although the rinds had a much higher concentration of fungi load. Green colonies, especially *Aspergillus niger*, match earlier studies. This fungus is generally known for producing citric acid efficiently due to its tolerance and metabolic adaptation to different environments. This helps it handle industrial fermentation conditions easily [1, 17]. The presence of many *Penicillium*, *Fusarium*, and *Rhizopus* species shows that the soil and farm waste are perfect environments for acid producing fungi. This matches the results published by Dos Santos *et al*. [20], Adomi and Anozie [23]. The observed dominance of the green with a round edge account for 47.17 % of total colonies. This suggests a selective advantage likely due to faster growth rates and competitive substrate utilization or efficient metabolic pathways for organic acid production [17, 20]. Even the less common fungi isolate contribute to the overall diversity. This supports the theory that even rare species can be hidden sources of novel metabolites, and enzymes and chemicals for biotech applications [2].

Quantitative analysis has shown that the green isolate (*Penicillium* spp., G.R) has the most citric acid production at 0.319 mg/mL. Followed by black isolate (*A. niger*, B.L) with a moderate yield of 0.197 mg/mL. This study has confirmed that these organisms have high industrial potential for making organic acids, especially in an optimized condition [2, 1]. Other types, such as the green with white edge (G.W.E) and greenish yellow (G.Y), show a lower production yield of 0.188 mg/mL and 0.166 mg/mL respectively. Brown (B.W.E) and yellow (Y.E) isolates had the lowest yields, producing only about 0.120 mg/mL and 0.087 mg/mL. Species such as *A. flavus* and *A. tamarii* may improve production if fermentation parameters are optimized or adaptive evolution been used [8]. The use of czapek-dos mixed with bromocresol green is an indicator of acid producing fungi. The halo-coloured appearance around colonies shows that. This remains a dependable first step of screening method which matches with the methods used by Zakry *et al*. [22], Pitt and Hocking [19]. The link between the halo zone size and the actual citric acid yield is essential.

This shows why it is very important to use both visual and measured tests when choosing strains for industrial uses. This study also shows that melon rinds are a great and low-cost material for fermentation. This supports earlier work that focuses on using farm waste as a sustainable way to cut costs while finding a use for waste materials [10, 23]. The fact that local fungi grow so well on these materials shows a great opportunity for a cheap and local biotechnological process. Using physical and microscopic traits allowed us to identify the species accurately. This proves that traditional methods are still very useful in microbiology today. Especially when backed by modern molecular assay [20]. The variety of fungi we found, along with their ability to produce citric acid. This shows why we must keep exploring local microbes for industrial use industrial and pharmaceutical applications, especially in regions where access to commercial strains may be limited. These results prove that local fungal strains, especially *Aspergillus niger*, are excellent candidates for making citric acid. This is possible by combining physical traits, yield measurements, and environmental data. That will create a solid way to pick the best fungi from nature. This study supports the idea that using local fungi isolates is a cheap and sustainable way to produce citric acid and other biotech products.

Conclusion

The study shows how rich and abundant of native and highly potent acidogenic fungi present within Nasarawa State's soil and agricultural leftovers. Furthermore, it highlights that *Cucumeropsis mannii* rinds can serve as an exceptionally viable, low cost, and sustainable bioprocessing substrate for localized solid-state or submerged fermentation operations. Utilizing these indigenous strains offers an economic and eco-friendly blueprint for bio-based industries in regions where commercial strains are restricted or costly.

Conflict of interest: The authors affirm that there are no financial or personal associations that could be perceived as having influenced the findings or conclusions presented in this research.

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