Isolation and Identification of Predominant Microorganisms from Palm Oil

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A B S T R A C T

Palm oil is a critical economic crop in tropical regions and is widely used in cooking, food production, and industrial applications. However, the quality of palm oil is often compromised by microbial contamination, posing significant health risks to consumers. Therefore the aim of the study was to isolate and identify predominant microorganisms from palm oil. The palm oil was inoculated into nutrient agar, mannitol salt agar, MacConkey agar, Salmonella shigella agar (SSA) and potato dextrose agar (PDA). All were incubated for 24h at 37℃ except for PDA, which was incubated at 25℃ for 7days. The isolated organisms were subjected to phenotypic and molecular characterization. The phenotypic characterization revealed four isolates; Escherichia coli, Staphylococcus aureus and Aspergillus species. The molecular characterization revealed the predominant Aspergillus spp. as Aspergillus niger which has 89.98% pairwise similarity with Aspergillus niger strain TMPSI with NCBI accession number DQ316605.1. The result obtained gave an insight that palm oil sold in the market may harbour several microorganisms including pathogenic ones and hence proper hygiene practices should be maintained by the processors and handlers to avoid contamination to ensure consumer's safety.

Keywords: Isolation, characterization, microorganisms, palm oil

1. INTRODUCTION

Palm oil (*Elaeis guineensis)* is a resourceful economic oil-bearing plant which has been extensively cultivated all over the world especially in the humid tropical and subtropical regions of the world, including Indonesia, Malaysia, Thailand, Columbia and Nigeria((Izah & Ohamain,2013) . It has red colour and this could be as a result of its large content of beta-carotene and it is derived from the mesocarp (reddish pulp) of the fruit of oil palms. It is a rich source of vitamin A and E (Nwachukwu et al., 2019; Adebayo-Tayo et al., 2012).

Palm oil can be contaminated by various microorganisms and some of the sources of contamination include environment, raw materials and equipment utilized during processing, storage and distribution. Microbial contamination of palm oil can lead to its spoilage which may pose health risks to consumer (Okogbenin et al., 2014).

During palm oil processing, traditional methods and rudimentary equipment are usually used. Some of these manufacturers have little or no knowledge about microbial implication of poor sanitation, handling and storage methods (Abdullahi et al., 2020 ; Izah & Ohamain, 2016).

Microbial contamination can be unintentional introduction of microorganism including bacteria and fungi. Several microorganisms have been implicated in the spoilage and deterioration of palm oil. These microorganisms include *Proteus* sp.*, Staphylococcus* sp.*, Enterobacter* sp. , `*Aspergillus* spp. and many other organisms (Ngangjoh et al.,2020).

Palm oil sold in the market has been reported to harbour many microorganisms due to poor handling and production practices (Okogbenin et al., 2014). It is usually used in cooking and the cooking temperature is adequate to destroy most of the pathogenic organisms that are likely to be present in it . However, this palm oil can often be eaten raw in many food including boiled white yam and "abacha".

Some fungi including *Aspergillus* spp have been reported to survive in palm oil due to their ability to produce the enzyme lipase and spores. The production of spores by these microorganisms have enabled them survived the anaerobic nature of palm oil and makes them resistant to heat (Agu et al., 2013).

The consumption of palm oil is common in many states in Nigeria including Enugu state, however assessing the microbiological quality of palm oil sold in their markets is essential. The assessment will provide insights on the safety of the oil consumed by the public and regulatory measures to take in order to improve food safety standards.

2. MATERIALS AND METHODS

2.1. Collection of Sample

Palm oil sample used in this study was purchased from Eke market in Agbani Enugu State, Nigeria.

2.2. Isolation of microorganism

The palm oil was inoculated into nutrient agar, mannitol salt agar, MacConkey agar, salmonella shigella agar and potato dextrose agar (PDA) respectively and was incubated at 37ºC for 24h except of PDA at 25ºC for 7days.

2.3. Identification of the isolates

The isolates were identified as follows;

2.3.1. Phenotypic characterization

Bacterial isolates were identified by observing their colonial appearance on the agar plates, Gram's reaction and biochemical tests including catalase test, oxidase test and voges proskauer (VP) test .

The fungal isolate was identified according to its micro-morphology, as well as the colonial appearance in the PDA medium.

2.3.2. Molecular characterization

The molecular characterization of the predominant organism (*Aspergillus* sp.) was carried out as follows;

2.3.2. 1. DNA Extraction

The DNA extraction using ZR fungal/bacterial DNA miniprep was carried out as described by Nwachukwu et al. (2023). The internal Transcribed Spacer (ITS) gene amplification and sequencing of the fungal isolate was carried out as described by Edeh et al. (2024)

3. RESULTS AND DISCUSSION

3.1. Phenotypic characterization

Table **1** indicates the phenotypic characterization of the palm oil bacterial isolates. A total of two bacterial isolates which included *Escherichia coli* and *Staphylococcus aureus* were recovered from the palm oil sample. *Escherichia coli* is a well-known indicator of faecal contamination, and its presence in food products, including palm oil, could result from poor handling and processing practices. This finding underscores the need for stringent sanitary measures in the production and handling of palm oil to prevent contamination and ensure consumer safety. The detection of *E. coli* in palm oil aligns with studies by Adebayo et al. (2012) and MacArthur et al. (2021) who reported contamination of palm oil with this pathogen.

The second isolate, *Staphylococcus aureus* is a bacterium commonly associated with food-borne illness (Mirzababaei et al., 2021). *Staphylococcus aureus* is a significant pathogen in the context of food safety due to its ability to produce enterotoxins that can lead to severe gastroenteritis (Le Loir et al., 2003; Al-Bahry et al., 2014). Identifying this bacterium in palm oil suggests potential lapses in food hygiene, as it is often introduced through contact with contaminated hands, equipment, or surfaces. Tong et al. (2015) highlighted that *S. aureus* can survive in various environmental conditions, making it a persistent threat in food processing environments. The presence of *S. aureus* in palm oil thus necessitates comprehensive hygiene protocols to mitigate the risk of food poisoning.

Table 1: The Phenotypic Characterization of the Bacterial Isolates

| Isolates | Colonial Appearance | | | | | Biochemical Test | | | | | | | |
	NA	MAC	SSA	MSA	Gram reaction	Catalase	citrate	indole	coagulase	oxidase	urease	VP	Suspected organism
RS$_1$	Smooth circular white colonies	Pink colonies	-	-	Gram -ve rod-shaped	+	-	+	-	-	-	-	*Escherichia coli*
RS$_2$	Large white colonies	-	-	Yellow colonies	Gram +ve cocci shape in clusters	+	+	-	+	-	+	+	*Staphylococcus aureus*

KEY: **NA=** Nutrient Agar, MSA= Mannitol Salt Agar, MAC= MacConkey Agar, SSA= Salmonella Shigella Agar

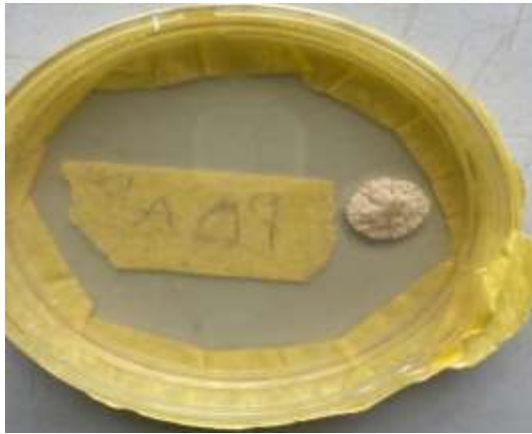**Figure 1: Colonial Appearance of the Bacterial Isolates on Various Media**

The phenotypic characterization of the palm oil fungal isolates (Table 2) revealed two fungal isolates (*Aspergillus* spp.). Similar result was reported by Okechalu et al. (2011) and Mounjouenpou et al. (2018) who identified this organism from processed palm oil. The identification of *Aspergillus* spp. in palm oil is particularly of great concern due to its potential for mycotoxin production, notably aflatoxins, which are potent carcinogens. Klich (2007) emphasized the dangers posed by aflatoxin-producing *Aspergillus* species, which can contaminate various food products, including oils, under favourable conditions such as high humidity and temperature. The presence of *Aspergillus* spp. in palm oil highlights the importance of monitoring for fungal contamination, especially in storage and processing environments where moisture control is crucial to prevent fungal growth.

Table 2: The Phenotypic Characterization of the Fungal Isolates

Isolates	Colonial Appearance on PDA	Microscopic Appearance	Suspected Organism
RS$_1$	white colonies but changed to black after a few days	Hyphae are septate, and hyaline conidial head are black radiate with a tendency to split into loose columns at maturity	*Aspergillus* sp.
RS$_2$	white colonies which later became black with conidial production	Smooth coloured conidiophores and conidia. The conidia heads appear radial and they split into columns	*Aspergillus* sp.

KEY: PDA= Potato Dextrose Agar

Colonial Appearance of Staphylococcus aureus on Mannitol salt agar   **Colonial Appearance of Escherichia coli on MacConkey agar**
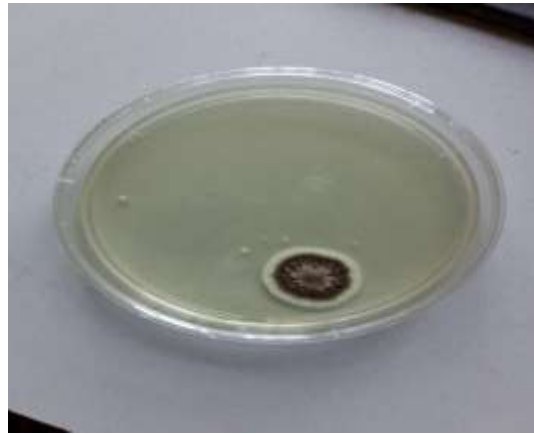

Figure 2: Colonial Appearance of Aspergillus spp. on potato dextrose agar

Molecular characterization

The molecular characterization of *Aspergillus* sp. was revealed in Table 4. The isolate was confirmed to be *Aspergillus niger* which has 89.98% pairwise similarity with *Aspergillus niger* strain TMPSI with NCBI accession number DQ316605.1.

The gel Image showing the amplification of the Internal Transcribed Spacer (ITS) region of the fungal isolate (*Aspergillus niger* RS) as stipulated in figure 3 revealed the amplification at 650bp. Lane M is a 50bp DNA ladder used for estimation of the amplification size.

The phylogenic tree of *Aspergillus nige*r RS (figure 4) revealed related families as *Aspergillus niger* strain TMPSI, *Aspergillus niger* strain SAR-6, *Aspergillus niger* strain 1805 and *Aspergillus niger* strain 56.

Table 4: Molecular Characterization of *Aspergillus* sp. (Dominant Isolate)

Isolate code	Accession number	Closely related organism	% identity	Kingdom	Family	Genus	Specie
RS3	DQ316605.1	*Aspergillus niger* strain TMPSI	89.98%	Fungi	*Trichocomaceea*	*Aspergillus*	*A. niger*

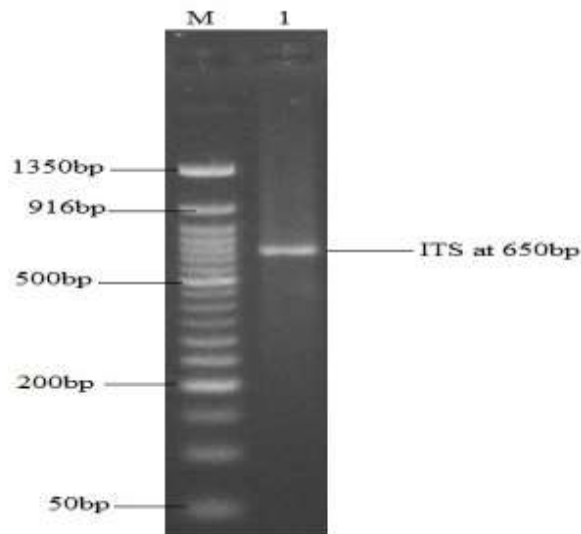**Figure 3:** The gel Image showing the amplification of the Internal Transcribed Spacer (ITS) region of the fungal isolate (*Aspergillus niger* RS)
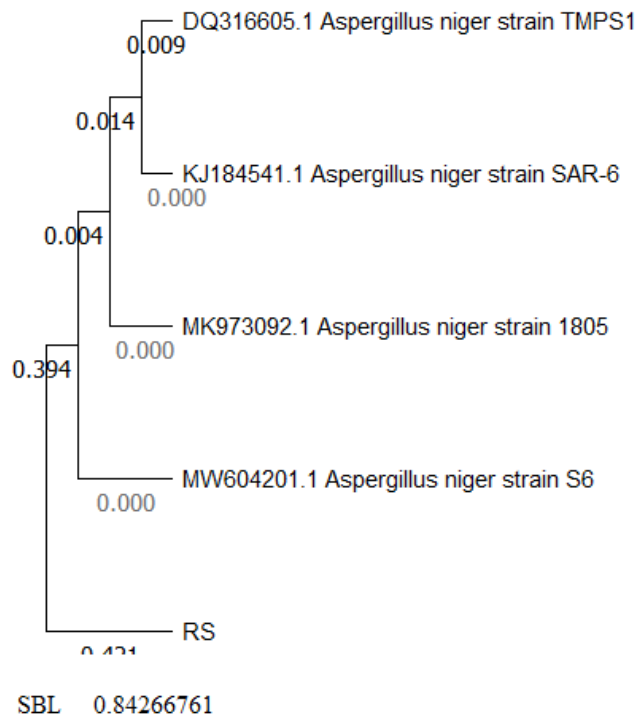
```
        ┌── DQ316605.1 Aspergillus niger strain TMPS1
   0.009│
  0.014 │
        │  └── KJ184541.1 Aspergillus niger strain SAR-6
        │       0.000
   0.004│
        │  ┌── MK973092.1 Aspergillus niger strain 1805
        │  │    0.000
   0.394│  │
        │  └── MW604201.1 Aspergillus niger strain S6
        │       0.000
        │
        └── RS
         0.421

   SBL    0.84266761
```

Figure 4: The phylogenic tree of *Aspergillus niger* RS

CONCLUSION

The obtained results gave an insight that the palm oil sold in the market may harbor many microorganisms including pathogenic one. Hence, it is necessary to maintain hygienic practices during their processing and handling to prevent these pathogens for the consumer's health safety.

Disclosure of conflict of interest

There is no conflict of interest among the authors.

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