Microbial Profile from Isolates Present in Coconut Milk for Yoghurt Production

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Abstract

In recent years, there has been an increasing interest in plant-based milk alternatives.The nutritional properties of these products depend on the plant source, processing and fortification. Coconut milk can be consumed as a beverage when the mature coconut pulp (*Cocos nucifera* L.) is grated with water, resulting in a vegetable suspension. This study is to identify microbial isolates fond in coconut milk to be used for yoghurt production. Fresh mature coconuts were the primary raw material used in this study. Standard laboratory media and reagents such as MRS agar, MacConkey agar, Sabouraud Dextrose Agar (SDA), and Mueller-Hinton agar were used for microbial cultivation and testing. Antibiotic discs were used for antimicrobial susceptibility testing. A total of 22 isolates were recovered and characterized using Gram staining, cell morphology, and a battery of biochemical tests. These isolates included both Gram-positive and Gram-negative bacteria, with a predominance of rod-shaped, catalase-positive, Voges-Proskauer (VP)-positive organisms. The antimicrobial sensitivity screening was also conducted for isolates initially revealed a worrying trend of resistance among many of the bacterial isolates recovered from the raw coconut milk. Zones of inhibition were generally low often between 1.00 mm to 5.00 mm**m** and in several cases, no zone (NZ) was observed, indicating multi-drug resistance (MDR)**.** Based on the findings of this study the recommendations include the need to begin the use of coconut milk yogurt as an alternative to cow milk yoghurt. There is also need for improved sanitary conditions in the handling of milk and dairy product

Keywords: Coconut milk, Microbial profile, Isolates, Antibiotics Sensitivity

Introduction

In recent years, there have been an increasing interest in plant-based milk alternatives. The nutritional properties of these products depend on the plant source, processing and fortification. Plant-based milks are not substitutes to dairy products because they differ in protein and other nutrients content; however, they can offer alternatives or complements to the consumers, as they contain functionally active components with health-promoting properties (Kopf-Bolanz, et al., 2023)

Coconut milk can be consumed as a beverage when the mature coconut pulp (*Cocos nucifera* L.) is grated with water, resulting in a vegetable suspension. This beverage is associated with the presence of lauric acid, a medium-chain fatty acid that can have beneficial properties to the human body (Reyes-Jurado, et al., 2023). Studies suggest that although coconut milk consumption may increase low-density lipoprotein (LDL) levels, it has a proportionally greater effect on high-density lipoprotein (HDL) levels so that the total cholesterol/HDL cholesterol level decreases. The fatty acids present in coconut oil also showed evidence of neuroprotective antioxidant properties, influencing Alzheimer disease-related risk factors. raditionally, yoghurt is obtained from fermentation of cow milk and other dairy products (Daryani, et al., 2024). The use of appropriate starter culture preferably isolates from spontaneously fermented coconut milk or mixed culture, under optimum conditions could yield coconut milk yoghurt of good quality, which could compete favourably with yoghurt from dairy milk (Soumya et al., 2024). Development of yoghurt product from coconut milk would help reduce the problem of hunger and malnutrition in the country as well as serve as alternative diet for lactose intolerant people (Suryamiharja et al., 2024).

Coconut milk**,** extracted from the grated flesh of mature coconuts, presents a promising plant based substitute for dairy milk in yoghurt production. It is naturally lactose-free, cholesterol-free, and rich in medium-chain triglycerides (MCTs), which have been associated with various health benefits (Erem and Kilic□Akyilmaz, 2024). Despite its nutritional and functional potential, coconut milk remains underutilized in the production of functional food products**,** particularly in tropical regions where coconut is abundant (Anumba, et al., 2023). The limited exploitation of coconut milk for fermented food applications is a missed opportunity, especially given the rising global demand for plant-based, sustainable, and health-oriented food products. Therefore, the aim of this study is to identify microbial isolates fond in coconut milk to be used for yoghurt production.

Materials and Methods

Collection of sample

Coconut fruits were procured from Oje, Bodija, and Ayeye markets, Ibadan, Oyo state Nigeria.

The coconut fruits were transported aseptically into the laboratory for further analysis.

Preparation of the Sample

Coconut was broken using knife and the coconut water stored in a sterile plastic bottle for further use. The brown layer was carefully removed with knife and the remaining coconut meat (3.5 Kg) washed and grated using manual grater. Grated coconut meat (particle size < 1617 μm) was

mixed in a ratio of 1:1 with a solution containing 75 and 25 % of distilled water and coconut water respectively and allowed to stand in a water bath at 40^0C for 15 min (Edem, 2016).Thereafter, it was sieved using cheese cloth (folded four times) to obtain the milk. The milk was pasteurized at 90^0C for 30 min and allowed to assume room temperature (37^0C).

Production of Coconut Yoghurt

This study employed an experimental research design to explore the possibility of producing yoghurt from coconut milk using naturally occurring microorganisms. Fresh mature coconuts were the primary raw material used in this study. These were sourced from local markets and selected based on maturity and freshness. Deionized water was employed throughout the experimental procedures to ensure purity and eliminate interference from external ions. Standard laboratory media and reagents such as MRS agar, MacConkey agar, Sabouraud Dextrose Agar (SDA), and Mueller-Hinton agar were used for microbial cultivation and testing. Antibiotic discs including ampicillin, erythromycin, tetracycline, and ciprofloxacin were obtained for antimicrobial susceptibility testing. Cow milk was used as a control substrate to compare fermentation outcomes with those of coconut milk.

The coconuts were manually dehusked, cracked, and the flesh extracted. The coconut flesh was washed thoroughly, grated, and blended with warm deionized water in a 1:2 weight-to-volume (w/v) ratio. The mixture was filtered through muslin cloth to yield fresh coconut milk. This milk was then pasteurized at 85°C for 30 minutes to eliminate undesirable microorganisms and deactivate native enzymes. The pasteurized coconut milk was rapidly cooled to 42°C to prepare it for microbial inoculation. A portion of the coconut milk was used for microbial isolation. Indigenous lactic acid bacteria (LAB) were isolated by plating samples on MRS agar, followed by anaerobic incubation at 37°C for 48 hours. Pure colonies were subcultured, morphologically examined, and subjected to preliminary biochemical tests. These isolates were subsequently used as starter cultures to ferment fresh samples of coconut milk.

Total viable counts of LAB were performed using MRS agar incubated anaerobically at 37°C for 48 hours. To further evaluate the safety of the LAB strains used in fermentation, antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method**,** following Clinical and Laboratory Standards Institute (CLSI) guidelines. The LAB isolates were cultured and standardized to a 0.5 McFarland turbidity standard. A sterile swab was used to evenly spread the cultures onto Mueller-Hinton agar plates. Antibiotic discs containing ampicillin, erythromycin, tetracycline, and ciprofloxacin were placed on the agar surface using a disc dispenser. The plates were incubated at 37°C for 24 hours.

Molecular Characterization

Genomic DNA was also extracted from the cultures received using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) with the primers presented in Table 1. The PCR products were run on a gel and cleaned up enzymatically using the EXOSAP method. The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™TerminatorCycleSequencingKitV3.1, BRD3-100/1000) and purified (ZymoResearch, ZR-96DNA Sequencing Clean-up Kit™, Catalogue No. D4050) 3. The purified fragments were analysed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific).BioEdit SequenceAlignment Editor version7.2.5 was used to analyse

Parameters	Methyl red	Vogesprosekauer	Catalase	Sulphide	Indole	Motility	Glucose	Lactose	Cell shape	Gram reaction	Probable Identity

the. ab1 files generated by the ABI3500XL Genetic Analyzer and results were obtained by a BLAST search (NCBI).

Results

Table 1 Biochemical test for microbial identification and probable identity of isolates

C1	-	+	-	-	-	-	AG	NC	Cocci	+	*Leuconostoc spp.* or *Lactococcus lactis*
C2	-	+	-	-	-	-	A	A	Rod	+	*Lactobacillus spp.* (homofermentative)
C3	-	+	+	+	_	+	A	A	Rod	+	*Bacillus subtilis* or *B. licheniformis*
C4	-	-	+	_	_	+	AG	A	Rod	-	*Enterobacter spp.* or *Pantoea spp.*
C5	-	+			-	+	A	a	Rod	+	*Bacillus spp.*
C 6	-	_	+		-	+	NC	NC	Rod	-	*Pseudomonas spp.* (glucose NC)
C7	-	+	+		-	-	NC	A	Rod	-	*Acinetobacter spp.*
C8	-	+	+		-	--	A	NC	Rod	-	*Citrobacter spp.*
C9	-	-	+		-	-	A	NC	Cocci	+	*Lactococcus spp.*
C10	-	-	+		-	_	A	NC	Rod	+	*Bacillus spp.*

C11	-	+	+	_	_	_	A	a	Rod	+	*Bacillus cereus* group
C12	-	+	+	-	-	_	NC	NC	Rod	+	*Bacillus spp.*
C13	-	+	+	+	-	+	NC	NC	Rod	+	*Bacillus cereus* or *Clostridium spp.*
C14	-	+	+	-	-	+	NC	NC	Rod	+	*Bacillus subtilis* group
C15	-	+	+	-	-	+	NC	NC	Rod	+	*Bacillus spp.*
C16	-	+	+	-	-	+	NC	NC	Rod	+	
C17	-	+	+	-	-	-	NC	NC	Rod	+	*Bacillus spp.*
C18	-	+	+	-	-	-	NC	NC	Rod	+	*Bacillus spp.*
C19	-	+	+	-	-	-	NC	NC	Rod	+	*Bacillus spp.*
C20	-	+	+	-	-	-	NC	NC	Rod	+	*Bacillus spp.*
C21	-	+	+	-	-	-	NC	NC	Rod	+	*Bacillus spp.*
C22	-	+	+	-	-	-	NC	NC	Rod	+	*Bacillus spp.*

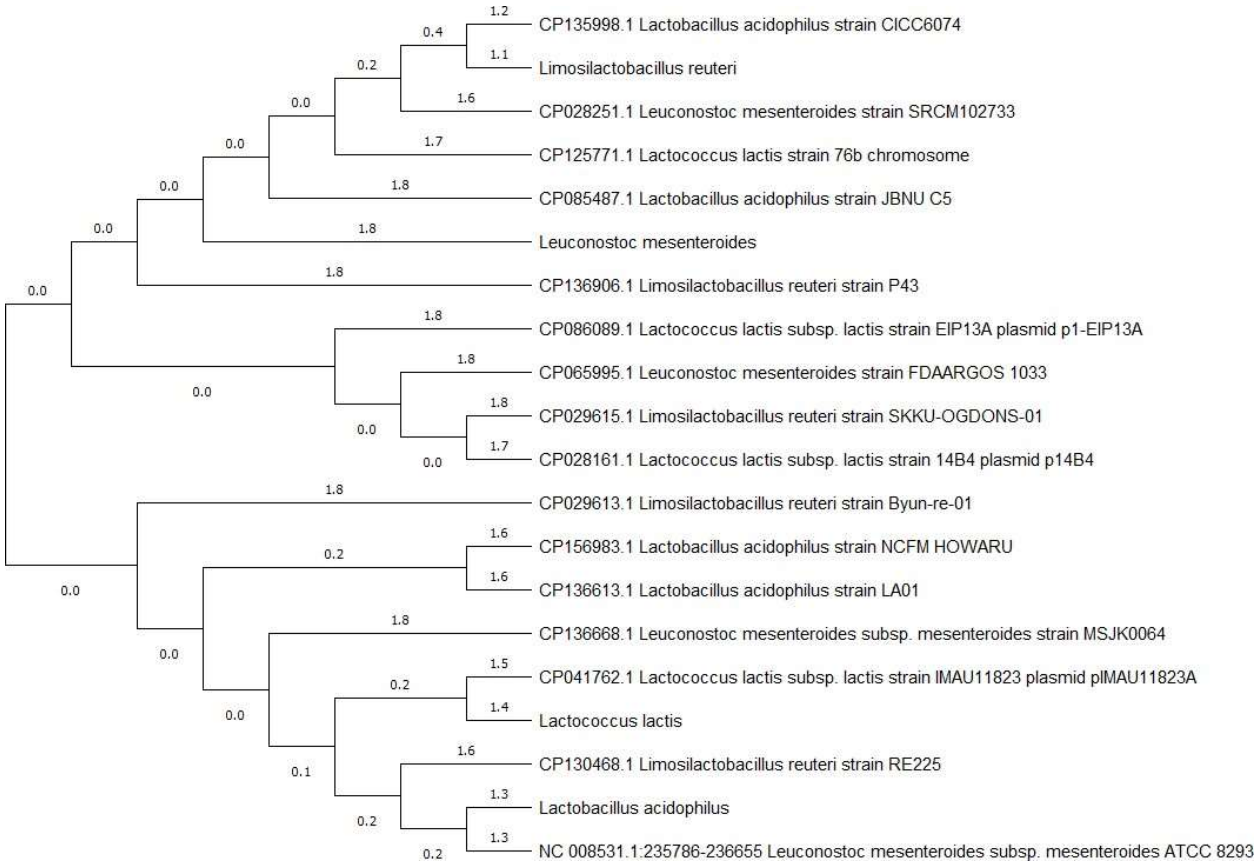
Table 2 BLAST predictions on the NCBI database

Sample ID	Length	Identified species	Details	Evalue	Alignment Score	Highest query coverage (%)
Yogurt 1	374 bp	*Lactobacillus acidophilus*	99.20% similarity using BLAST 2.10.0N+	0.0	>200	100
Yogurt 2	377 bp	*Limoilactobacillus reuteri*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
Yogurt 3	380 bp	*Leuconostoc mesenteroides*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
Yogurt 4	375 bp	*Lactococcus lactis*	99.47% similarity using BLAST 2.10.0N+	0.0	>200	100

Table 3 Anti-Microbial Sensitivity Screening results for isolates for this study

Anti-Microbial Sensitivity Screening - Antibiotics (mm)						
	%R	**%I**	**%S**	**%R**	**%I**	**%S**
Pefloxacin,	100`0		0	100	0	0
CN- Gentamycin	96	0	3	96	0	2
AM- Ampiclox	95	0	2	96	0	2
Z- Zinnacef	100	0	0	87	0	2
AM- Amoxacilin	98	0	0	82	0	0
Roceplin	100	0	2	98	0	2
Ciprofloxacin	100	0	0	87	0	2
Streptomycin	98	0	0	96	0	2
SXT- Septrin	100	0	0	98	0	2
E- Erythromycin	100	0	0	88	0	2
CH- Chloraphenicol	96	0	0	98	0	2
SP- Sparfloxacin	76	0	2	70	0	6
AU- Augmentin	64	6	6	64	0	6
Tarivid	62	4	8	62	2	8

Pefloxacin, CN- Gentamycin, AM- Ampiclox, Z- Zinnacef, AM- Amoxacilin, Roceplin, Ciprofloxacin, Streptomycin, SXT- Septrin, E- Erythromycin, CH- Chloraphenicol, SP- Sparfloxacin, AU- Augmentin, Tarivid



Phylogenetic Tree of Lactic Acid Bacteria Isolates Based on 16S rRNA Gene Sequences

Discussion

The search for healthy, sustainable, and inclusive food options has become increasingly important in the world's nutrition landscape as people adopt plant-based diets whether for health reasons, persnal values, environmental concerns, or medical conditions like lactose intolerance the demand for dairy alternatives continues to grow (Kopf-Bolanz et al., 2023). Yogurt, a staple dairy product consumed worldwide for its probiotic and nutritional benefits, has not been left out of this shift. In response, researchers and food technologists have explored a variety of plantbased ingredients as substitutes for cow's milk in yogurt production (Reyes-Jurado, et al., 2023).

Coconut milk also aligns with the growing interest in functional foods products that offer health benefits beyond basic nutrition. When fermented with suitable lactic acid bacteria, coconut milk not only acquires the characteristic tangy flavor of traditional yogurt, but it also becomes a carrier of probiotics, known for supporting gut health and immunity. This dual role as both a dairy substitute and a functional food positions coconut milk yogurt as a strong candidate for inclusion in modern diets (Anumba, et al., 2023).

The microbiological analysis of the raw coconut milk samples in this study revealed the microbial profile of the milk as having bacterial isolates. A total of 22 isolates were recovered and characterized using Gram staining, cell morphology, and a battery of biochemical tests. These

isolates included both Gram-positive and Gram-negative bacteria, with a predominance of rod-shaped, catalase-positive, Voges-Proskauer (VP)-positive organisms. Many of the isolates belong to genera that are well-known for their roles in fermentation, spoilage, or environmental persistence. (Suryamiharja, et al., 2024).

The isolates were identified as *Leuconostoc sp., Lactobacillus sp., Lactococcus sp., Bacillus sp.,* and a few Gram-negative genera like *Pseudomonas sp., Enterobacter sp., Citrobacter sp.,* and *Acinetobacter sp.* These findings are consistent with previous studies on the natural microbiota of plant-based milk, especially coconut milk. Studies have both reported the presence of lactic acid bacteria (LAB) such as *Lactobacillus spp.* and *Leuconostoc spp.* in freshly expressed and fermented coconut milk, confirming their natural occurrence and potential for fermentation.

Isolates such as C1, identified as *Leuconostoc spp.* or *Lactococcus lactis*, and C2, likely a homofermentative *Lactobacillus* are of interest. These genera are staples in traditional dairy fermentation and have been successfully applied in plant-based substrates to produce desirable acidification and flavor profiles. Their presence in raw coconut milk suggests that the substrate is naturally predisposed to fermentation, especially when handled under hygienic conditions and guided with starter cultures.

However, the detection of Gram-negative rods such as *Pseudomonas spp.* (C6), *Enterobacter spp.* (C4), and *Citrobacter spp.* (C8) indicates possible environmental contamination or poor post-harvest hygiene, which aligns with concerns raised researchers who worked on promoting coconut milk for yogurt production. (Antunes, et al., 2025).

These genera, although not necessarily pathogenic in small numbers, are not desirable in fermented food due to their spoilage potential and competition with beneficial fermentative microbes. Their presence reinforces the need for starter culture inoculation and pasteurization during coconut milk yogurt preparation.

The second group of isolates (C12–C22) was more homogenous, consisting entirely of Grampositive, catalase-positive, VP-positive, rod-shaped, non-sugar fermenting bacteria a classic biochemical signature of Bacillus sp. These organisms are spore-forming and can survive harsh environmental conditions, making them common contaminants in raw plant materials. While *Bacillus cereus* (C13) is a known opportunistic pathogen, others like *B. subtilis* and *B. megaterium* have potential biotechnological and probiotic applications, as highlighted in the work of various researchers .

The antimicrobial sensitivity screening was also conducted for isolates initially revealed a worrying trend of resistance among many of the bacterial isolates recovered from the raw coconut milk. Zones of inhibition were generally low often between 1.00 mm to 5.00 mm**m** and in several cases, no zone (NZ) was observed, indicating multi-drug resistance (MDR)**.**Gentamicin (CN) showed the most consistent inhibitory activity across the isolates, echoing results by many researchers who identified gentamicin as one of the few antibiotics retaining efficacy against environmental and foodborne Gram-positive organisms. Ciprofloxacin (CPX) also demonstrated moderate effectiveness, although resistance was noted in a number of isolates, further emphasizing the emergence of resistance even to broad-spectrum agents ·

In contrast, β-lactam antibiotics such as ampicillin (AM), amoxicillin (AMX), and augmentin (AU) exhibited limited or no inhibitory effects on many isolates. This mirrors findings by some researchers**,** who documented widespread resistance to these drugs among *Bacillus sp., Enterobacter sp.,* and *Pseudomonas spp.* isolated from plant-based and environmental samples in southwestern Nigeria. The presence of resistant strains such as *Citrobacter sp., Enterobacter sp.,* and *Pseudomonas sp.***,** inferred from earlier biochemical characterizations, is particularly concerning. These genera are known to harbor intrinsic resistance genes**,** and their presence in raw food substrates like coconut milk suggests a potential route for the dissemination of AMR (antimicrobial resistance) genes through the food chain. This raises important public health questions, particularly in low-resource settings where food safety monitoring is limited.

The susceptibility pattern observed also provides practical insight for starter culture selection**.** Strains used for food fermentation should ideally be sensitive to most clinical antibiotics, or at the very least, be devoid of transmissible resistance genes. In this regard, isolates such as *Lactococcus lactis* and some *Bacillus spp.***,** which showed limited resistance, may represent safer probiotic candidates for large-scale production

The patterns of antimicrobial resistance observed in this study are consistent with global reports of increasing MDR in foodborne isolates**,** especially from raw, unprocessed plant substrates. Studies report similar resistance levels in Bacillus and Pseudomonas strains isolated from soy milk and raw rice beverages. A study done with coconut water isolates in Lagos showed them to harbor resistance against tetracyclines, β-lactams, and macrolides, which supports the findings in this current study. (Trajkovska, et al., 2024)

Conclusion

A total of 22 bacterial isolates were initially recovered from raw coconut milk samples. Biochemical and microscopic characterization revealed a diverse microbial community, including both beneficial lactic acid bacteria and environmental or opportunistic strains such as *Citrobacter sp.*, *Enterobacter sp.*, *Pseudomonas sp.*, and various *Bacillus sp.*. Several isolates demonstrated probiotic-friendly traits, such as catalase negativity and Gram-positive rod morphology, while others exhibited traits typical of spoilage or potentially pathogenic organisms. The antimicrobial sensitivity testing also revealed high resistance levels across multiple antibiotic classes, particularly beta-lactams (e.g., ampicillin and augmentin), erythromycin, and sulfonamides. Only gentamicin and ciprofloxacin retained moderate effectiveness across most isolates. These findings are consistent with growing global concerns about antimicrobial resistance (AMR) in foodborne bacteria and highlight the importance of rigorous starter culture selection and substrate sanitization in plant-based fermentation systems.

Recommendations

Based on the findings of this study the recommendations include the need to begin the use of coconut milk yogurt as an alternative to cow milk yoghurt. There is also need for improved sanitary conditions in the handling of milk and dairy product.

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