

Characterisation of Resistance Potential to Biofilms Microbial Contaminating *Thaumatococcus daniellii* and *Musa paradisiaca* Leaves Used as Food Packaging

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How to cite this paper: Benie, C.K.D., Kouame, N.D., Atobla, K., Attien, Y.P., Tuo, W.-T.A., Traore, A., Diarrassouba, N.J., Dadie, A. and Dosso, M. (2025) Characterisation of Resistance Potential to Biofilms Microbial Contaminating *Thaumatococcus daniellii* and *Musa paradisiaca* Leaves Used as Food Packaging. *American Journal of Plant Sciences*, 16, 180-194.

<https://doi.org/10.4236/ajps.2025.161016>

Received: September 21, 2024

Accepted: January 27, 2025

Published: January 30, 2025

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Abstract

Despite their biodegradability and economic advantage, plant leaves used as packaging can constitute a public health problem. The aim of this study was to characterize the microbial diversity contaminating plant leaves used as food packaging. In total, two hundred and forty (240) samples composed of *Thaumatococcus daniellii* and *Musa paradisiaca* leaves were collected and analyzed. Microbial diversity was assessed using specific medium and biochemical tests. The resistance profile was determined by the Müller-Hinton agar diffusion method. The resistance (*blaSHV*, *blaIMP*, *blaTEM*) and biofilm formation (*pslA*, *pelA*) genes were searched by PCR method. Plant leaves were contaminated by bacterial (68.7%) and fungal (100%) strains. Extreme bacterial (7.1 log₁₀ cfu/cm²) and fungal (3.5 log₁₀ cfu/cm²) loads were obtained on *Thaumatococcus daniellii* leaves. Bacterial prevalence was 45.1% (*S. aureus*), 38.8% (*E. coli*) and 16.1% (*P. aeruginosa*). In order of decreasing importance, the prevalence of fungal species was 41.1% (*A. flavus*), 33.1% (*A. fumigattus*), 13.7% (*A. niger*) and 12.1% *Candida sp.* Resistance of *E. coli* to penicillins ranges from 31.6% to 87.3% and to cephalosporins from 13.3% to 28%. The *P.*

aeruginosa strains were mainly resistant to aztreonam (87.6%). Those of *S. aureus* showed resistance to tetracycline (67.6), vancomycin (53), erythromycin (44.6) and levofloxacin (32.7). The *blaSHV* (14.28% to 18.60%) and *blaIMP* (9.52% to 16.28%) genes were detected in the bacterial strains. *P. aeruginosa* strains (19.05%) harbored the *pslA* and *pelA* genes. The health safety of these biodegradable plant-based packaging contributes to their valorization.

Keywords

Resistance, Biofilm, Genes, *Thaumatococcus daniellii*, *Musa paradisiaca*

1. Introduction

Food packaging, as described by Ojekale *et al.* [1] is designed to preserve and extend the shelf life of foodstuffs and nutrients and to present food to consumers in an ideal state. Among the world's packaging types, non-biodegradable plastic packaging is constantly evolving. The trend towards the systematic use of non-food plastic bags to package products, including hot foods (pasta, sauces, rice), is becoming increasingly apparent, particularly in cities [1]. What's more, despite their ease of use, non-biodegradable plastic packaging poses environmental and health problems, as well as clogging up gutters and farms, particularly in towns and rural areas [2] [3].

Indeed, plastic waste is a major environmental problem because it affects traffic routes, agricultural areas, and clogs water pipes, leading to flooding during the rainy season. This plastic waste is, therefore, a risk factor for soil, water, air and health [4] [5]. It also promotes the proliferation of mosquitoes and other pathogens responsible for diseases such as malaria, typhoid fever and cholera [4] [5]. Despite some considerable efforts in this area, the situation has not shown any significant improvement. In 2019, plastics generated 1.8 billion tonnes of greenhouse gas emissions, or 3.4% of global emissions [6].

In addition, hot-packed foods in plastic bags cause interactions between the contents and the container, often resulting in organoleptic quality defects and toxicological risks [3]. To ensure a healthy environment and improve people's health, several African countries have opted to do away with plastic packaging in favour of biodegradable packaging [3] [7]. In West Africa, various plant organs, in particular leaves, are used either to treat certain diseases or as food or food packaging [3]. This use of biodegradable plant leaves has many advantages, both economically and ecologically. This natural packaging, which has the comparative advantage of being biodegradable, could be a way to reduce pollution [2].

These biodegradable packaging are mainly made of materials that can be decomposed by microorganisms, such as bacteria, fungi and algae. The most commonly used types of biodegradable packaging materials include starch, cane fiber, paper, cardboard, polylactic acid (PLA) bioplastics and parts of plant species [8]-[10].

Many species of plant leaves have been inventoried and characterized in West Africa, and more specifically in Côte d'Ivoire, in order to make the most of this natural packaging [2] [11].

Among these leaves, those of *Thaumatococcus daniellii*, *Musa paradisiaca*, *Zea mays* and *Tectona grandis* are used in Côte d'Ivoire as packaging to protect various foodstuffs [2] [12]. The importance of these packaging sheets lies in their biodegradability, their lower cost and their potential to improve the organoleptic qualities of foodstuffs [11] [13]. Packaging is also an important source of income for those involved in the sector and for the households that use it [11].

Despite all these provisions, the packaging sector used in the agri-food industry in general is faced with major constraints, particularly in developing countries such as Côte d'Ivoire [12] [14]. Among these constraints, microbial contamination is a public health problem [14]. Indeed, this packaging can host multi-resistant bacterial species capable of forming biofilms, including *P. aeruginosa*, *E. coli* and *S. aureus* [14].

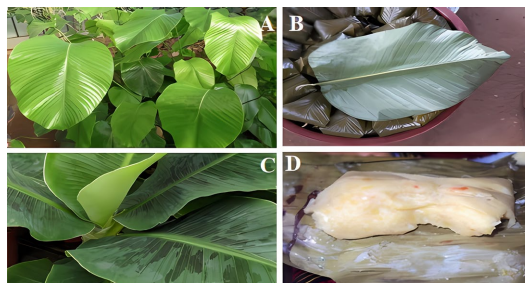
In addition, these packaging are the site of proliferation of fungal species involved in the production of mycotoxins (aflatoxin and ochratoxin) such as *Aspergillus flavus* and *Aspergillus niger* [14].

In Côte d'Ivoire, several studies have been carried out on the diversity of plant-based packaging packaged foods and on the socio-demographic status of households involved in it [2] [12]. Despite this little work carried out in Côte d'Ivoire, data relating to the microbiological characterization of plant leaves used as food packaging are rare, if not non-existent.

The objective of this work was to characterize the diversity of the microbial potential contaminating plant food packaging with a view to improving food preservation in Côte d'Ivoire.

2. Material and Methods

2.1. Plant Material and Sample Size



A: Leaves of *Thaumatococcus daniellii* in the fields; B: Leaves of *Thaumatococcus daniellii* used as food packaging; C: Leaves of *Musa paradisiaca* in the fields; D: Leaves of *Musa paradisiaca* used as food packaging.

Figure 1. Plant species used as food packaging.

The plant material consisted of *Thaumatococcus daniellii* leaves and *Musa*

paradisiaca leaves (Figure 1). In total, two hundred and forty (240) leaf samples including one hundred and twenty (120) leaves of *Thaumatococcus daniellii* and one hundred and twenty (120) other leaves of *Musa paradisiaca* were collected and analyzed.

The sample size was calculated using the equation from Gerville-Réache *et al.* [15]:

$$N = PQ / (E/L)^2$$

With **N**: Minimum sample size, **P**: estimate of the expected proportion (prevalence rate), **Q**: the value of (1-P), **E**: the tolerated margin of error (statistical risk in %), **L**: reduced deviation for the accepted statistical risk.

2.2. Distribution of Samples According to Collection Area

The one hundred and twenty (120) *Thaumatococcus daniellii* leaves were made up of forty (40) samples collected in the harvesting fields, forty (40) others collected in the wholesale markets and finally the last forty (40) samples were taken from packaged foods (Table 1). The one hundred and twenty (120) *Musa paradisiaca* leaves were distributed as previously.

Table 1. Distribution of samples.

Type of plant leaf	<i>Thaumatococcus daniellii</i>			<i>Musa paradisiaca</i>			Total
	Harvest fields	Wholesale Markets	Packaged foods	Harvest fields	Wholesale Markets	Harvest fields	
Origin of the matrix							
Numbers of samples	40	40	40	40	40	40	240
Total		120			120		240

2.3. Sample Selection Criteria

Several species of plant leaves used as food packaging in Côte d'Ivoire, have been identified during previous work [12]. Among these species, the leaves of *Thaumatococcus daniellii* and those of *Musa paradisiaca* are the plant wrappers most commonly used in the agri-food industry in Côte d'Ivoire. In general, consumers prefer food packaged with these two types of plant leaves because they give packaged food good presentation, texture and aroma [11].

2.4. Sampling and Transport

The 25 cm² leaf samples of *Thaumatococcus daniellii* and *Musa paradisiaca* were collected aseptically. These samples taken and introduced into Stomacher bags were stored at +4°C in a cooler containing cold accumulators (dry ice) and transported to the microbiology and molecular biology laboratory. The analyzes were carried out within two (2) hours following collection.

2.5. Microbiological Analysis

2.5.1. Test Sample

The lower and upper surface of each leaf sample was swabbed. Test samples were

obtained by introducing each of the swabs into 10 mL of buffered pepton water. Then, from this mother suspension, successive decimal dilutions were carried out.

2.5.2. Research for Flora Contaminating Plant Packaging

Microbiological analyses included the detection of total aerobic mesophilic flora (FMAT) on PCA (Plate Count Agar), total and faecal coliforms on VRBL (Violet Red Lactose Agar), *E. coli* on TBX (Tryptone Bile X-glucuronide), *S. aureus* on Staph Agar, RSA on TSN (Tryptone Sulphite Neomycin) and *P. aeruginosa* on Cetrimide using standard microbiology methods. The method used for *Salmonella* testing is that specified by ISO 6579 (NF V08-052, 2002) [16]. Buffered Peptone Water and Rappaport-Vassiliadis broth were used as pre-enrichment and enrichment broths respectively. Xylose Lysine-Deoxycholate (XLD) and Hektoen agars were used as isolation media. A non-selective nutrient medium was used to culture suspect colonies for confirmatory biochemical tests. Moulds and yeasts were tested on YGC (Yeast extract Glucose Chloramphenicol).

2.5.3. Resistance Profile of *P. aeruginosa*, *S. aureus* and *E. coli* Strains

The phenotypic resistance profile of *P. aeruginosa*, *S. aureus* and *E. coli* strains was determined by the diffusion method on Müller-Hinton (MH) medium. Antibiotic discs commonly used in human therapy were selected. The antibiotic discs tested belonged to the penicillin, third and fourth-generation cephalosporin, carbapenem, monobactam, aminoglycoside and fluoroquinolone families.

The entire surface of the Müller-Hinton agar was sampled sterilely from the bacterial suspension in a saline solution with turbidity equivalent to 0.5 McFarland. This inoculum of approximately 10^6 CFU/mL was produced from young colonies aged 18 hours taken on ordinary agar. Fifteen minutes after placing the antibiotic-impregnated discs on the surface of the inoculated agar, the plates were turned over and incubated ideally at 37°C for 24 hours.

After 24 hours, the zones of inhibition were determined using a caliper. The results were interpreted in accordance with the rules of the Antibiogram Committee of the French Microbiology Society [17].

2.6. Molecular Characterization of *P. aeruginosa*, *S. aureus* and *E. coli*

2.6.1. Extraction and Purification of Genomic DNA

Strains of *P. aeruginosa*, *S. aureus* and *E. coli* were harvested from an overnight broth culture. The genomic DNA of *P. aeruginosa*, *S. aureus* and *E. coli* was extracted by thermal lysis and purified according to the method described by Guardia *et al.* [18]. After extraction, DNA was diluted and stored at -20°C to serve as a DNA template for polymerase chain reactions (PCR).

2.6.2. Reaction Mixture

The reaction mixture was prepared according to the method described by Pournajaf *et al.* [18]. This 25 µL reaction mixture consisted of 16 µL of sterile Milli-Q water (milli-Q™, Millipore Corporation, USA), 5 µL of 5X concentration loading

buffer, 1.5 μ L of MgCl₂, 2 mM (Promega Corporation, Madison, WI 53711-5399, USA), 0.2 μ L of 10 mM dNTPs, 0.1 μ L of each primer, 10 mM (Intégral DNA Technologie, California, USA), 0, 1 μ L of Go Taq® G2 Flexi DNA polymerase of final concentration 1.5U (Promega Corporation, Madison, WI 53711-5399, USA) and 2 μ L of the DNA template. Sterile Milli-Q water and reference strains were used as negative control and positive control, respectively, for each PCR reaction run.

2.6.3. Amplification of *RhlI* and *RhlR* Genes

The amplification of resistance genes (*blaSHV*, *blaIMP* and *blaTEM*, and biofilm formation genes (*pslA* and *pelA*) was carried out according to the method described by Pournajaf *et al.* [19]. The amplification program included an initial denaturation of 5 min at 95°C followed by 33 cycles of denaturation (95°C for 30 s), annealing (65°C for 60 s) and extension (72°C for 90 s), with a single final extension of 5 min at 72°C. The samples were stored at 4°C until the Thermocycler was stopped. The gene amplification products were revealed on the 1% agarose gel after migration at 120 V for 30 min and visualized by illumination on a UV plate of a lighting device and photographed (Bio-Rad, Gel Doc EZ Imager, USA). The amplification programs and the nucleotide sequence of the primers used are described in (Table 2).

Table 2. Primers for resistance detection and biofilm formation.

Gene	Primer sequence (5'→3')	Amplification program	Size
Resistance			
<i>SHV</i>	F: 5'-TGGTTATGCGTTATATTCGCC-3' R: 5'-GCTTAGCGTTGCCAGTGCT-3'		867 pb
<i>TEM</i>	F: 5'-ATAAAATTCTTGAAGAC-3' R: 5'-TTACCAATGCTTAATCA-3'	95°C, 5 min 33 x [95°C, 30 s; 65°C, 60 s; 72°C, 90 s] 72°C, 5 min;	1075 pb
<i>IMP</i>	F: 5'-TGAGCAAGTTATCTGTATTC-3' R: 5'-TTAGTTGCTTGGTTTTGATG-3'		740 pb
Biofilms			
<i>PelA</i>	F: 5'-CATACCTTCAGCCATCCGTTCTTC-3' R: 5'-CGCATTTCGCCGCACTCAG-3'	95°C, 5 min 33 x [95°C, 30 s; 65°C, 60 s; 72°C, 90s] 72°C, 5 min; 4°C.	656 pb
<i>PslA</i>	F: 5'-TCCCTACCTCAGCAGCAAGC-3' R: 5'-TGTTGTAGCCGTAGCGTTTCTG-3'		786 pb

Pel: pellicle (gene coding for dandruff); *Psl*: polysaccharide synthesis locus, *TEM*: TEMONEIRA-Patient Name, *SHV*: Sulphydryl Variable, *IMP*: M β L Imipenemas.

2.7. Statistical Analysis

Data were entered with SPSS Statistics 20.0 data processing software (IBM Corporation, SPSS Inc, Chicago, USA) and transferred to Excel. Data were entered with Data Gen 5 v. 2.04™. The data were analyzed by t-test at a statistical level of $\alpha < 0.05$ (Excel, MS office).

3. Results

3.1. Diversity of Microbial Potential Contaminating Plant Packaging

The microbial potential mainly showed a macroscopic diversity of germs including strains of *E. coli*, *S. aureus*, *P. aeruginosa* and moulds of the genus *Aspergillus* (Figure 2). Macroscopic identification showed a diversity of moulds including *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*.

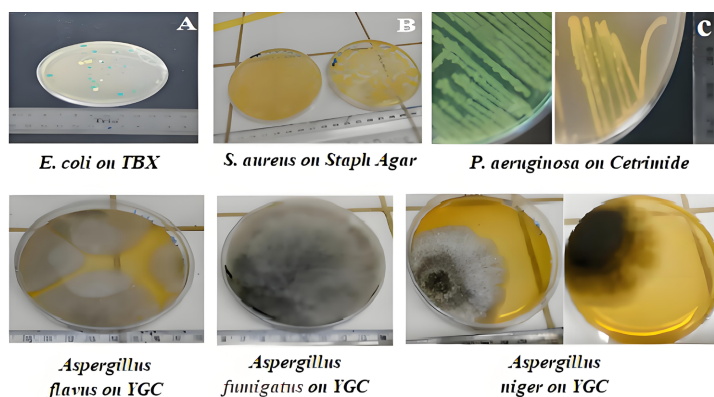


Figure 2. Macroscopic diversity of bacteria and moulds isolated from packaging.

3.2. Vegetable Packaging Contamination Rates

The plant species used as food packaging were contaminated by bacterial (68.7%) and fungal (100%) strains at different rates. In the case of *Thaumatococcus daniellii* and *Musa paradisiaca*, the contamination rate was highest in the packaging taken at the sales market and on the packaged food, respectively (Table 3).

Table 3. Contamination rate of vegetable packaging.

Plant species	Contamination rate of plant packaging (%)						Total
	<i>Thaumatococcus daniellii</i> n = 120			<i>Musa paradisiaca</i> n = 120			
Sampling site	Harvest fields n = 40	Wholesale Markets n = 40	Packaged foods n = 40	Harvest fields n = 40	Wholesale Markets n = 40	Packaged foods n = 40	
Bacterial species	32 (80.0)	35 (87.5)	26 (65.0)	16 (40.0)	20 (50.0)	36 (90.0)	165 (68.7 %)
Fungal species	40 (100)	40 (100)	40 (100)	40 (100)	40 (100)	40 (100)	(240) 100 %

3.3. Average Bacterial and Fungal Load

The average bacterial load varied from 1 log₁₀ cfu/cm² to 7.1 log₁₀ cfu/cm² for *Thaumatococcus daniellii* and from 0.5 log₁₀ cfu/cm² to 4.3 log₁₀ cfu/cm² for *Musa paradisiaca*. Fungal species varied from 1 log₁₀ cfu/cm² to 3.5 log₁₀ cfu/cm² and from 1.1 log₁₀ cfu/cm² to 2.2 log₁₀ cfu/cm² for *Thaumatococcus daniellii* and *Musa paradisiaca* respectively. Extreme bacterial (7.1 log₁₀ cfu/cm²) and fungal (3.5

log₁₀ cfu/cm²) loads were obtained on *Thaumatococcus daniellii* leaves (**Table 4**).

Table 4. Average load of bacterial and fungal species.

Species	Average load of bacterial species (log ₁₀ cfu/cm ²)				Average load of fungal species (log ₁₀ cfu/cm ²)			
	<i>C. Th</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>Candida sp</i>
<i>Thaumatococcus daniellii</i>	6.5	7.1	4.5	1	2.3	2.3	1	3.5
<i>Musa paradisiaca</i>	4.3	5.1	2.3	0.5	1.2	1.1	1.7	2.2

C. Th: Thermotolerant coliform.

3.4. Prevalence of Bacterial and Fungal Species Isolated

The results indicate that the total prevalence of bacterial species in *Thaumatococcus daniellii* and *Musa paradisiaca* is 60.9% and 39.1% respectively. This prevalence is higher for *S. aureus* (45.1%) followed by *E. coli* (38.8%) and lower for *P. aeruginosa* (16.1) (**Table 5**). The total prevalence of fungal species was 32.7% (*Thaumatococcus daniellii*) and 67.3% (*Musa paradisiaca*). In order of increasing importance, the prevalence of fungal species was 41.1% (*A. flavus*), 33.1% (*A. fumigatus*), 13.7% (*A. niger*) and 12.1% (*Candida sp*) (**Table 6**).

Table 5. Prevalence of bacterial species isolated from plant packaging.

Plant species	Prevalence of bacterial species N (%)			Total Prevalence
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	
<i>T. daniellii</i>	117 (58.5)	104 (59.8)	52 (72.2)	273 (60.9)
<i>M. paradisiaca</i>	85 (42.1)	70 (40.2)	20 (27.8)	175 (39.1)
<i>Total</i>	202 (45.1)	174(38.8)	72(16.1)	448(100)

Table 6. Prevalence of fungal species isolated from plant packaging.

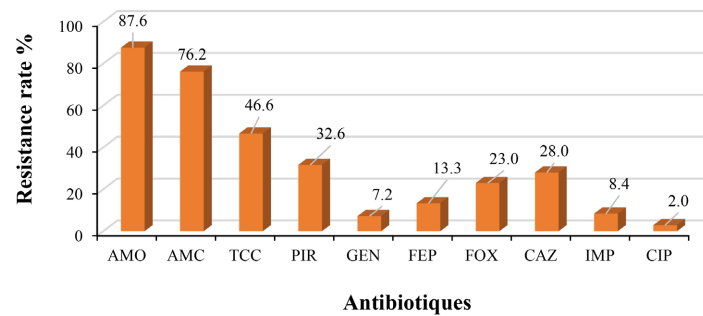
Plant species	Prevalence of fungal species N (%)				Total Prevalence
	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>Candida sp</i>	
<i>T. daniellii</i>	22 (21.6)	31 (38.3)	14 (41.2)	14 (46.7)	81 (32.7)
<i>M. paradisiaca</i>	80 (78.4)	51 (62.2)	20 (58.8)	16 (53.3)	167 (67.3)
<i>Total</i>	102 (41.1)	82 (33.1)	34 (13.7)	30 (12.1)	248 (100)

3.5. Determinants of Resistance and Biofilm Formation

3.5.1. Phenotypic Resistance of Strains

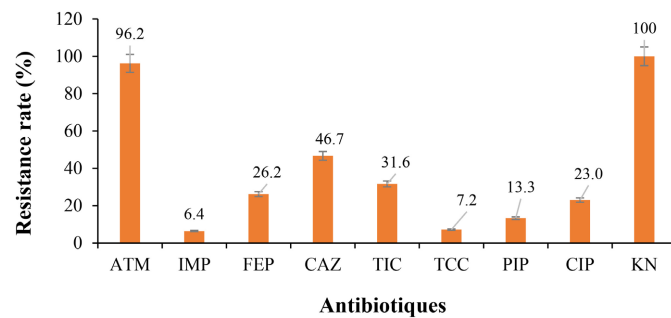
Escherichia coli strains isolated from plant packaging showed resistance to penicillins ranging from 31.6% to 87.3%. Resistance to cephalosporins ranged from 13.3% (Cefepime) to 28% (Ceftazidime) (**Figure 3**). Resistance to carbapenems in *E. coli* strains is 8.4% (imipenem). The majority of *P. aeruginosa* strains were resistant to monobactams, with a rate of 87.6% for aztreonam (**Figure 4**). In addition to resistance to penicillins and cephalosporins, *S. aureus* strains showed resistance to tetracycline (67.6), vancomycin (53), erythromycin (44.6) and levofloxacin

(32.7) (Figure 5).



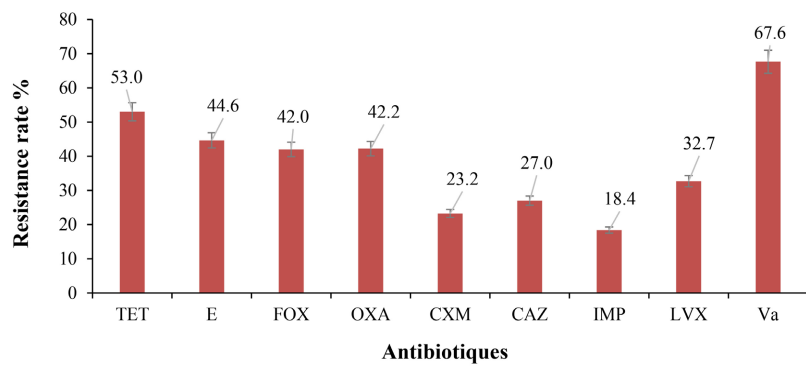
Antibiotiques
Amoxicillin (AMO), Amoxicillin-clavulanic acid (AMC), Ticarcillin-clavulanic acid (TCC), Piperacillin (PIR), Gentamicin (GEN), Cefepime (FEP), Cefoxitin (FOX), Ceftazidime (CAZ), Imipenem (IMP), Ciprofloxacin (CIP).

Figure 3. Level of antibiotic resistance in *E. coli* strains.



Antibiotiques
Aztreonam (ATM), Imipenem (IPM), Cefepime (FEP), Ceftazidime (CAZ), Ticarcillin (TIC), Ticarcillin-Clavulanic acid (TCC), Piperacillin (PIP), Ciprofloxacin (CIP), Kanamycin (KMN).

Figure 4. Level of antibiotic resistance in *P. aeruginosa* strains.



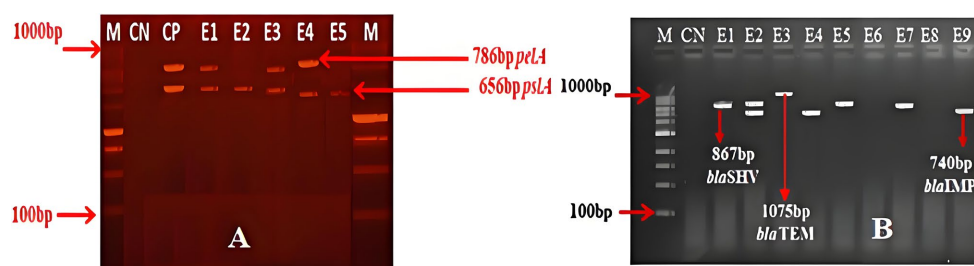
Antibiotiques
Tetracycline (TET), Erythromycin (E), Cefoxitin (FOX), Oxacillin (OXA), Cefuroxime (CXM), Ceftazidime (CAZ), Imipenem (IPM), Levofloxacin (LVX), Vancomycin (VA).

Figure 5. Level of antibiotic resistance in *S. aureus* strains.

3.5.2. Resistance and Biofilm Formation Determinants

Antibiotic-resistant strains of *P. aeruginosa* (21), *E. coli* (43) and *S. aureus* (72)

were tested for resistance and biofilm formation. The *blaSHV* and *blaIMP* resistance genes were detected in the three bacterial strains with prevalences ranging from 14.3% to 18.6% and from 9.5% to 16.3% respectively (Table 7). The *blaTEM* gene was only detected in *P. aeruginosa*. The *pslA* biofilm-forming gene was detected in *P. aeruginosa* (19.1%) and *S. aureus* (8.3%) (Table 7). The *pelA* gene (19.1%) was only detected in *P. aeruginosa*. Figure 6 shows the electrophoretic profile of the resistance and biofilm genes in the different strains studied.



Gel A: Biofilm genes: Line E1, E3, E4 Presence of *pslA* and *pelA* genes in *P. aeruginosa*; Line E2, E5: Presence of *pslA* gene in *S. aureus*; **Gel B:** Resistance genes: Line E1, E2, E5, E7: Presence of *blaSHV* gene; Line E2, E4, E9: Presence of *blaIMP* gene; Line E3: Presence of *blaTEM* gene. CP: positive control; CN: negative control; M: molecular weight marker. *PeI*: pellicle (gene coding for pellicles); *Psl*: polysaccharide synthesis locus; *TEM*: TEMONEIRA-Patient name; *SHV*: Sulfhydryl Variable; *IMP*: M β L Imipenemase.

Figure 6. Electrophoretic profile of resistance and biofilms genes.

Table 7. Gene prevalence by bacterial species.

Bacterial strains	Prevalence of resistance genes (%)			Prevalence of biofilm-forming genes	
	<i>blaSHV</i>	<i>blaTEM</i>	<i>blaIMP</i>	<i>pslA</i>	<i>pelA</i>
<i>P. aeruginosa</i> n = 21	3 (14.3)	2 (9.5)	2 (9.5)	4 (19.1)	4 (19.1)
<i>E. coli</i> n = 43	8 (18.6)	0 (0.0)	7 (16.3)	0 (0.0)	0 (0.0)
<i>S. aureus</i> n = 72	12 (16.6)	0 (0.0)	9 (12.1)	6 (8.3)	0 (0.0)

PeI: pellicle (gene coding for pellicles); *Psl*: polysaccharide synthesis locus; *TEM*: TEMONEIRA-Name of patient; *SHV*: Sulfhydryl Variable; *IMP*: M β L Imipenemase.

4. Discussion

In Africa, several plant leaves are used as food or packaging in the agri-food industry [3] [13]. Among these leaves, those of *Thaumatococcus daniellii*, *Musa paradisiaca*, *Zea mays* and *Tectona grandis* are used in Côte d'Ivoire as packaging to protect various foodstuffs [2] [12]. This use of plant leaves has many advantages, but requires special microbiological provisions to ensure better food safety.

In this study, the results mainly showed a diversity of germs including bacterial strains of the *E. coli* type, *S. aureus*, *P. aeruginosa* and moulds of the *Aspergillus* genus. The same observations were made by Ire *et al.*, and other authors who indicated that bacterial and fungal strains contaminated the leaves of *Thaumatococcus daniellii*, *Musa paradisiaca* and *Tectona grandis* [14] [20] [21].

In fact, in the case of *Thaumatococcus daniellii* and *Musa paradisiaca*, the contamination rate was higher in the plant packaging collected at the sales market and in the packaged food, respectively. This contamination in these two types of matrix could be explained by cross-contamination linked to the handling, conservation and storage of these packages [14] [20]. The results showed that all the samples taken from fields, markets and packaged foods were contaminated by fungal species. This fungal contamination could, on the one hand, be justified by the absolute humidity, which is the quantity of water vapour contained in the air that favours the presence of fungi on plant packaging [21] [22]. On the other hand, this fungal presence could be due to fungal spores in a latent state on food packaging [22].

In addition, this study showed an average bacterial load varying from 1 log₁₀ cfu/cm² to 7.1 log₁₀ cfu/cm² for *Thaumatococcus daniellii* and from 0.5 log₁₀ cfu/cm² to 5.1 log₁₀ cfu/cm² for *Musa paradisiaca*. Fungal species ranged from 1 log₁₀ cfu/cm² to 3.5 log₁₀ cfu/cm² and from 1.1 log₁₀ cfu/cm² to 2.2 log₁₀ cfu/cm² for *Thaumatococcus daniellii* and *Musa paradisiaca* respectively. In these two plant species, the fungal load was higher for *candida sp.*

These different loads observed are justified by the intensity of the activities associated with the sale and use of these two types of leaves [21]. These loads could also be linked to the collection area for these wrappers, their composition, cooking method and the types of food for which they are used [23].

This study also determined the prevalence of bacterial and fungal species identified in the leaves of *Thaumatococcus daniellii* and *Musa paradisiaca* [21] [22]. Thus, the results indicated that the total prevalence of bacterial species in *Thaumatococcus daniellii* and *Musa paradisiaca* was 60.9% and 39.1%, respectively. This prevalence is higher in *S. aureus* followed by *E. coli* and lower in *P. aeruginosa*. The high prevalence of *S. aureus* could be due to the various interactions between packaging, food and handlers [14]. The prevalence of thermotolerant coliforms, mainly *E. coli*, could be due to faecal contamination or to inadequate heat treatment and poor cleaning of packaging before use [14] [23].

In addition to bacterial species, the fungal strains detected in the majority of cases were *A. flavus* (41.1%), *A. fumigattus* (33.1%), *A. niger* (13.7%) and *Candida sp.* (12.1%). This study is in agreement with that carried out by Njoku *et al.*, and Ire *et al.*, who isolated, in addition to these fungal strains, others such as *Fusarium sp.*, *Saccharomyces sp.* and *Penicillium sp.* [14] [22]

Work by Patrignani *et al.* [24] has shown that certain environmental factors make biodegradable plant packaging susceptible to the proliferation of microorganisms. Among these factors, the environment, handling and food could be vectors for contamination of plant packaging [14]. The prevalence of bacterial and fungal species in the leaves of *Thaumatococcus daniellii* and *Musa paradisiaca* shows that these packages could contribute to food poisoning [25].

In addition, the bacterial species detected expressed various antibiotic resistances. The majority of *P. aeruginosa* strains were resistant to monobactams,

with 87.63% resistant to aztreonam. Strains of *E. coli* and *S. aureus* were mainly resistant to penicillins, cephalosporins, carbapenems and fluoroquinolones. The high resistance of strains to aztreonam and penicillins could be due to acquired resistance (plasmids, transposons). This acquired resistance could be explained by increased impermeability of the outer membrane or by the production of inactivating enzymes capable of expelling antibiotics, according to Kumar and Schweizer [26].

However, the resistance of certain strains to cephalosporins, carbapenems and fluoroquinolones can be induced by chromosomal mechanisms and the combination of resistance mechanisms (extended spectrum beta-lactamase (ESBL)) [27]. These results support the hypothesis that packaging can be a reservoir for bacteria that are multi-resistant to antibiotics.

Finally, the study revealed the presence of *blaSHV* and *blaIMP* resistance genes in the three bacterial strains, with prevalences ranging from 14.28% to 18.60% and from 9.52% to 16.28% respectively. These different results could be explained by the fact that *blaSHV* and *blaIMP* determine a high level of resistance to penicillins and carbapenems respectively [28]. These results were observed by Benie *et al.* [29] who indicated in their study that the prevalence of these *blaSHV* genes can also be explained by the fact that most ESBLs belong to the *SHV* beta-lactamase families.

Consequently, these genes detected may be involved in the resistance of strains isolated from food packaging. This study also indicated the presence of the *pslA* biofilm-forming gene in *P. aeruginosa* (19.05%) and *S. aureus* (8.33%). The *pelA* gene (19.05%) was only detected in *P. aeruginosa*. The presence of these *PslA* and *PelA* genes indicates that these strains are involved in polysaccharide and film biosynthesis respectively. The production of polysaccharide and pellicle observed in these strains could favour the mechanisms involved in the virulence and pathogenicity of these strains [30] [31].

This study shows the need to develop these types of biodegradable plant packaging. However, a microbiological challenge and compliance with good hygiene practice measures are still required to guarantee improved food safety.

5. Conclusion

A microbial diversity contaminates plant leaves used as packaging in agro-food crafts. These contaminants were mainly composed of bacterial strains such as *E. coli*, *S. aureus*, *P. aeruginosa* and fungal strains such as *A. flavus*, *A. fumigattus*, *A. niger* and *Candida sp.* Bacterial diversity harbors determinants of resistance and biofilm formation. The physicochemical characteristics and toxicological impact of *Thaumatococcus daniellii* and *Musa paradisiaca* leaves should be evaluated to ensure better food safety.

This research did not receive any specific grant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or

personal relationships that could have appeared to influence the work reported in this article.

Acknowledgements

The authors thank the Institut Pasteur of Côte d'Ivoire, and the Université Félix Houphouët Boigny for their excellent technical assistance.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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