Original Research Article

MORPHOLOGICAL DIVERSITY OF FUNGAL STRAINS RESPONSIBLE FOR DESSERT BANANA ROT, MUSA SAPIENTUM (MUSACEAE) PRODUCED IN THE SOUTH COMOE REGION ON IVORY COAST

ABSTRACT

Aims: The banana dessert grown in different regions of the world, encounters important difficulties that cause it to lose its marketability especially because of the diseases related to conservation. The objective here is to identify the strains responsible for fungal diseases of bananas when it is stored.

Study design: This study was undertaken in order to ensure the competitiveness of the dessert banana from Ivory Coast on the international market which is threatened by the recurrent problem of post-harvest rot.

Place and Duration of Study: Agrovalorisation Laboratory, Agroforestry Training and Research Unit, Université Jean Lorougnon GUEDE Daloa Côte d'Ivoire, between February 2021 and March 2022.

Methodology: The study involved 120 bananas divided into two batches of 10 hands each, one with signs of necrosis and the other with no signs. Banana fragments (crown, epicarp and explant) were deposited on the growing media at several distinct points and slightly embedded in the agar. The resulting colonies were transplanted successively until a pure strain was obtained from a single mushroom colony per petri dish.

Results: A total of 11 different genera were identified from 105 isolates. 57 are from necrotic bananas and 36 from bananas with no signs of necrosis. Fungal strains isolated there are: *Trichoderma* sp. (15%), *Fusarium* sp. (1%), *Scytalidium* sp. (39%), *Mucor* sp. (1%), *Scopulariopsis* sp. (1%), *Alternaria* sp. (4%), *Aureobasidium* sp. (1%), *Aspergillus* of the *Glaucus* group (10%), *Cladosporium* sp. (2%), *Pseudallescheria* sp. (6%) and *Chrysosporium* sp. (20%). No strains of the genus *Colletotrichum musea* responsible for anthracnose that can develop on both green and ripe fruit have been isolated. However, morphological characterization has not among to identify several other species (12) especially those not sporulating.

Conclusion: This diversity of isolated strains in this work is identical to that most frequently isolated and cited in the literature.

Keywords: *Banane dessert, Souches fongiques, Isolats, Ivory Coast.*

1. INTRODUCTION

Banana dessert (Musa Sapientum) is one of the most consumed fruits in the world. It is produced in tropical and subtropical areas [1]. It is grown in more than 150 countries and is the second largest fruit producer in the world, behind orange and ahead of grapes [2]. Most of Côte d'Ivoire's production is export-oriented. The exported banana dessert belongs exclusively to the Cavendish sub-group [3]. This constitutes a capital contribution to the country's economy. The banana sector accounts for 8% of agricultural GDP, 2% of national GDP and provides employment for nearly 8,000 to 10,000 people [4]. In Côte d'Ivoire, banana dessert is grown on an estimated 6000 hectares [5] and has undeniable agronomic and economic value as it has 30 become one of the most important agricultural export products. Thanks to the success recorded in recent years in the restructuring of the banana sector in Côte d'Ivoire, the volume of sales of bananas has continued to increase and the country is considered to be the leading African supplier of bananas dessert (Grande Naine and William, Cavendish subgroup, AAA genomics group) on the European Union market [6]. In addition, bananas are Côte d'Ivoire's first fresh fruit export (about 95% of its production). So it's a very important culture. However, like any cultivated plant, bananas face multiple parasitic threats including bacterial diseases such as *Xanthomona* wilt [7], viral diseases [8], and fungal diseases [9]. In Côte d'Ivoire it should be noted that there is a recurrent problem of post-harvest rot of fruit intended for export. Thus, in order to be competitive in the international market, players in the sector place particular emphasis on the management of all factors that can deteriorate the quality of the product [4]. With a view to developing a biological control method based on microbial antagonism, previous work has made it possible to evaluate the fungal diversity of the banana phyllosphere in and around Daloa, and then look for fungi that can control the phytopathogen *Fusarium oxyspoum*. An in-depth knowledge of the microbial community, in particular fungal, associated with the fruits of bananas is crucial to understand the influence of this flora on the development, health, productivity and especially the conservation of this plant of interest. The present study was therefore undertaken to help identify the strains responsible for fungal diseases of dessert bananas during its preservation. Specifically, it concerns:

- isolate new fungal strains from dessert bananas before and after onset of disease symptoms;

- identify fungal strains isolated from dessert bananas when it is stored.

2. MATERIAL AND METHODS

2.1. Materials

2.1.1. Equipment

2.1.1.1. Biological Material

The biological material used in this study consists of various fragments (the crown, the skin or the epicarp and the distal or explant end) from the 'Great Dwarf and William Dessert' banana.

2.1.1.2. Laboratory Equipment

The growing media used in this work are Patatos Dextrose Agar (PDA) and Sabouraud au Chloramphenicol, the most commonly used for isolation and macroscopic identification of fungal strains. The preservation of the strains required the use of the Czapeck Yeast Extrat Agar medium.

2.2. Methods

The work of isolation and identification of pathogenic fungal strains was carried out at the Agrovalorisation laboratory of the University Jean Lorougnon GUÉDÉ (UJLoG) of Daloa.

The identification of these genera is based primarily on the identification keys described in the literature [10,11], based on the macroscopic characters of colonies (appearance, colour, shape, contour, etc.) and on microscopic characters of mycelium and conidia or spores (partitioning of mycelium, form of spores, form of fruiting organs, etc.). The detection of fungal diseases in dessert bananas was based on samples taken before any post-harvest treatment on symptomatic (with signs of necrosis) and asymptomatic (with no signs of necrosis) fruit (Figure 1).

A: Fruits without necrosis B: Necrotic fruits

Figure 1: Asymptomatic and symptomatic fruits of banana dessert

2.2.1. Sampling and sampling of dessert bananas

After the selection of the intervention sites, diagonal sampling was carried out at the plantation level. Two batches of 10 banana hands (Figure 2) each were collected prior to any post-harvest treatment. The bananas collected were taken to the laboratory in a box for analysis (Figure 2).

Figure 2: Batch of 10 banana hands

2.2.2. Preparation of dessert banana samples

The preparation of the dessert banana samples is done by careful cleaning. With tap water, we washed the bananas before disinfecting the fragments (crown, epicarp, explant) to use. First, with sodium hypochlorite (bleach) at 5%, then in 70% ethanol for 5 minutes and finally, we rinsed them three times with distilled water [12].

2.2.3. Isolation and purification of fungal strains

The isolation of the fungal strains responsible for banana rot during storage was based on the determination keys described in the literature [10, 11], based on the macroscopic characteristics of the colonies and on the microscopic characteristics of the mycelium and conidia or spores.

After seeding the banana fragments on the Patatos Dextrose Agar (PDA) and Sabouraud media with chloramphenicol, the fungi that appeared were transplanted to new PDA and Sabouraud media with Chloramphenicol solidified in petri dishes. Previously isolated colonies were purified by successive transplants [13]. A mycelial fragment approximately 1 cm in diameter was collected from the growing front of the crop and transferred to Petri dishes on PDA and Sabouraud media with new chloramphenicol until pure strain was obtained (only one mushroom colony per petri dish) using the Huguenin and Beccas [14] method (Figure 3).

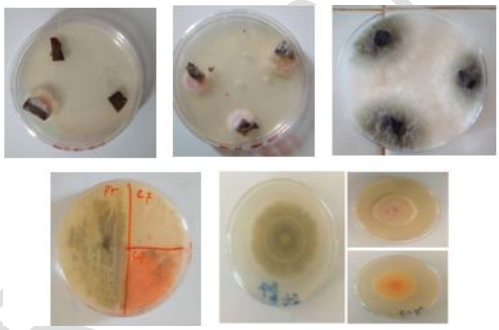


Figure 3: Isolation process of a homogeneous fungal strain

2.2.4. Identification of Individual Isolates

2.2.4.1. Macroscopic Study

Morphological and cultural characteristics are determined after seeding pure strains on specific crop media. Identification is done with the naked eye, it is based mainly on the characters such as: the texture of the thallus (velvety, woolly, powdery, cottony, flaky, etc.), the growth speed, the appearance of the airborne mycelium, the color (face and back) colony, odour, thallus colour and growth contour [15].

2.2.4.2. Microscopic Study

A finer classification was made using the Optika brand optical microscope at different magnifications (GX10, GX40) as well as immersion (GX100) according to the identification

keys of Bush [16]. It takes into account the type of mycelium (presence or absence of partitions), the shape and colour of the conidia or spore and finally its presence or not. Microscopic identification of fungi is based on several methods. Those used in this work are those of the adhesive tape and the blue cotton lactophenol method [17]. These two methods are described below:

- Adhesive tape: a small piece of adhesive tape was applied from the sticky side to the colony and then placed on an object-holder blade;

- Cotton blue lactophenol: A fragment of the colony was removed with a platinum loop and placed on an object-holder blade in a drop of dye, then the whole was covered with a cover sheet that crushes the preparation.

2.2.5. Preservation of strains

The pure strains obtained were retained on the Czapek Yeast Extrat Agar medium tilted in cryotubes at +4°C.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Diversity of mushrooms isolated on dessert bananas

Out of 105 fungi isolated, 93 were identified, of which 57 strains originated from dessert bananas showing symptoms of necrosis and 36 other strains from dessert bananas showing no sign of necrosis. Morphological characterization failed to identify several other species (12), particularly those that do not sporulate. The mushrooms identified belong to 11 genera listed in Table I below:

Table 1: Diversity of isolated mushrooms on dessert bananas

GENUS	PHYLUM	CLASS	ORDER	FAMILY
Trichoderma sp.	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
***Fusarium* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)

***Scytalidium* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
***Mucor* sp.**	Zygomycotina	Zygomycetes	Mucorales	Mucoraceae
***Scopulariopsis* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
***Alternaria* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Dematiaceae (phaéohyphomycetes)
***Aureobasidium* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Dematiaceae (phaéohyphomycetes)
***Aspergillus* of *Glaucus* group**	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
***Cladosporium* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Dematiaceae (phaéohyphomycetes)
***Pseudallescheria* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
***Chrysosporium* sp.**	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)

3.2. Macroscopic and microscopic description of isolated genera

This study made it possible to highlight different macroscopic characters (appearance, color, shape, contour, etc.) of the colonies. Some genera have widely varying colonies while others have a single type of colony. The microscopic characters of the mycelium and of the conidia or spores observed show a difference in the partitioning of the mycelia, in the shape of the spores, in the shape of the fruiting bodies, etc.

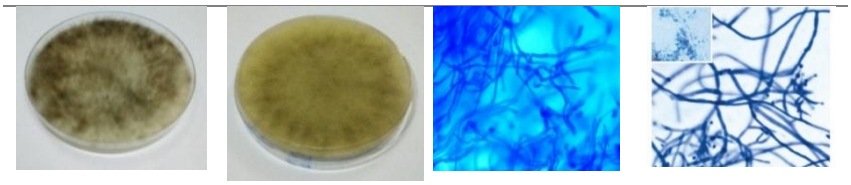
3.2.1. ***Trichoderma* sp.**

This fungus produces woolly colonies, initially white in colour, then appear as they age isolated greenish tufts or concentric rings on the growing medium.

The quill-shaped phials are arranged in whorls on conidiophores with an acute angle, the whole having a pyramidal appearance (Table 2).

Table 2: Macroscopic and microscopic appearance of *Trichoderma* sp.

Macroscopic appearance		**Microscopic appearance**	
Front	**Reverse**	**Photo**	**Reference**

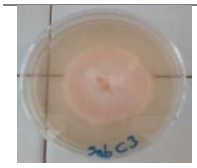
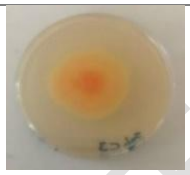
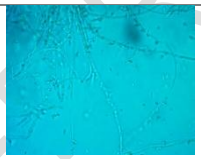
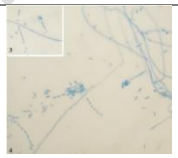


3.2.2. ***Fusarium* sp.**

The colonies are cottony in appearance, sometimes fluffy, white at first, then pink to violet. The verso is dark purple.

Conidiophores are simple or verticillate, short. The microconides are numerous, ovoid or claviform, arranged in pseudo-heads or constituting long chains at the top of the phialids (Table 3).

Table 3: Macroscopic and microscopic appearance of *Fusarium* sp.

Macroscopic appearance		**Microscopic appearance**	
Front	**Reverse**	**Photo**	**Reference**
			

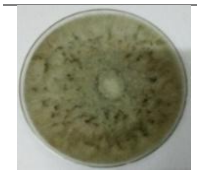
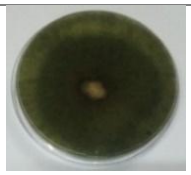
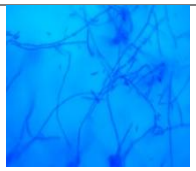
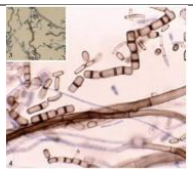
3.2.3. ***Scytalidium* sp.**

This fungus produces extensive, fluffy or flocculent colonies, aerial, grey initially becoming blackish afterwards. On the back, the colonies are dark with a black diffusible pigment.

Hyphae, septate, of two types: some are hyaline, narrow, 2-3 µm in diameter, while others, wider, have a thick and pigmented wall (Table 4).

Table 4: Macroscopic and microscopic appearance of *Scytalidium* sp.

Macroscopic appearance		**Microscopic appearance**	
Front	**Reverse**	**Photo**	**Reference**

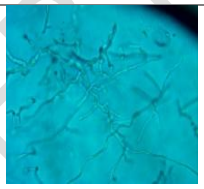
			

3.2.4. ***Mucor* sp.**

Colonies very fast growing and extensive, have a woolly texture. The colour varies from grey to brown on the surface, the verso is colourless. The optimum growth temperature of these fungi is 25ºC.

Sporocystophore carrying a globular sporocyst without process. Broad filaments little or not septate. They end in an ovoid columella without a process and often have a narrowing under the columella (Table 5).

Table 5: Macroscopic and microscopic appearance of *Mucor* sp.

Macroscopic appearance		**Microscopic appearance**	
Front	**Reverse**	**Photo**	**Reference**
			

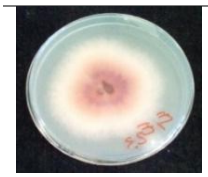
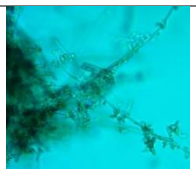
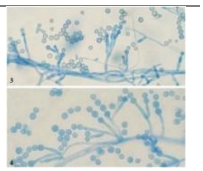
3.2.5. ***Scopulariopsis* sp.**

In the absence of cycloheximide, the colonies, velvety, quickly becoming powdery or granular. Initially whitish, they then turn beige to hazelnut-brown (light milk coffee). The underside is cream to brownish.

Conidiogenic cells (annelids), cylindrical, more or less swollen at the base, are isolated or grouped at the end of conidiophores short, septate and hyaline (Table 6).

Table 6: Macroscopic and microscopic appearance of *Scopulariopsis* sp.

Macroscopic appearance		**Microscopic appearance**	
Front	**Reverse**	**Photo**	**Reference**

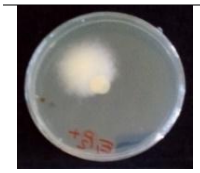
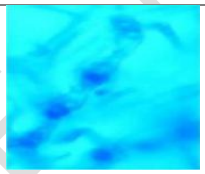
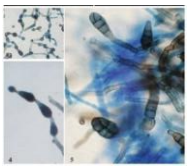
			

3.2.6. ***Alternaria* sp.**

The colony, initially white-grey, quickly becomes dark (dark green to black) on both sides. The texture is fluffy to woolly.

They are arranged in short chains and have a more or less marked beak. Some filaments are pigmented brown. Conidiophores are partitioned, brown, septate, single or branched (Table 7).

Table 7: Macroscopic and microscopic appearance of *Alternaria* sp.

Macroscopic appearance		**Microscopic appearance**	
Front	**Reverse**	**Photo**	**Reference**
			

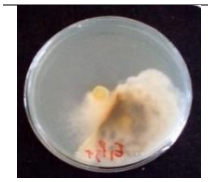
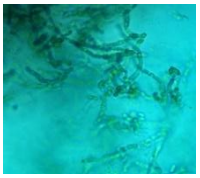
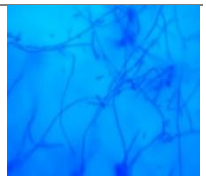
3.2.7. ***Aureobasidium* sp.**

The texture of the colonies is mucoid; they are pale pink at first becoming brown to black with age. The reverse is colourless.

Hyaline filament, becoming dark brown and producing arthroconidia or chlamydospores. Single-celled, hyaline conidia formed from conidiogenic cells in filaments (Table 8).

Table 8: Macroscopic and microscopic appearance of *Aureobasidium* sp.

Macroscopic appearance	**Microscopic appearance**

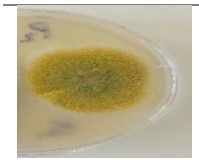
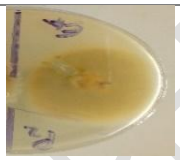
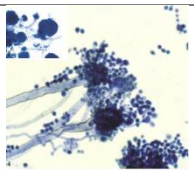
Front	Reverse	Photo	Reference
			

3.2.8. ***Aspergillus* of *Glaucus* group**

Colonies not very extensive, flat, powdery, green with rapid growth. The verso changes from yellow orange to dark brown. Bright yellow spots may appear when cleistotheties are produced in large numbers.

Aspergillar head associated with cleistotheca. Phialids are inserted directly on the hemispherical vesicle and produce large conidia, globular or oval (Table 9).

Table 9: Macroscopic and microscopic appearance of *Aspergillus* of *Glaucus* group

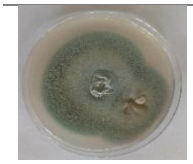
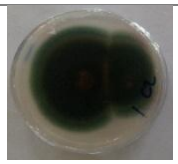
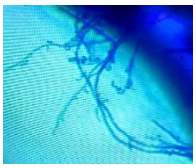
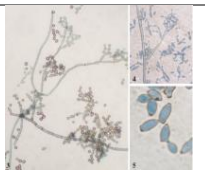
Macroscopic appearance		Microscopic appearance	
Front	**Reverse**	**Photo**	**Reference**
			

3.2.9. ***Cladosporium* sp**.

The colonies have a velvety or flaky texture, sometimes powdery. The colour ranges from olive green to very dark black brown, and the reverse is black brown.

Single or pluricellular blastospores arranged in acropenic chains. Some spores have colouring reinforcements at their extremities corresponding to the scars of budding or release (Table 10).

Table 10: Macroscopic and microscopic appearance of *Cladosporium* sp.

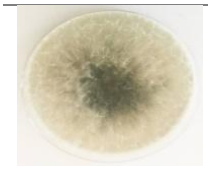
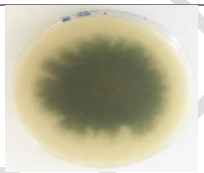
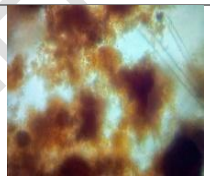
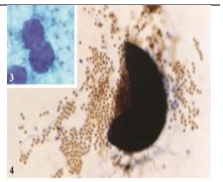
Macroscopic appearance		Microscopic appearance	
Front	**Reverse**	**Photo**	**Reference**
			

3.2.10. ***Pseudallescheria* sp.**

Early cottony, woolly colonies, whitish in colour, becoming grey as they age. The verso is dark, almost black.

Graphium shape characterized by the presence of annelids at the top of conidiophores grouped in coremics. Cleistotheces first hyalins and brown ascospores (Table 11).

Table 11: Macroscopic and microscopic appearance of *Pseudallescheria* sp.

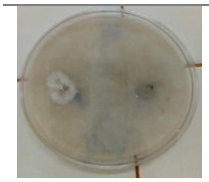
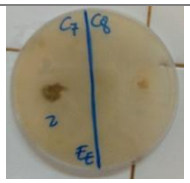
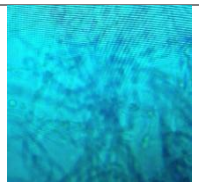
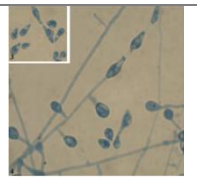
Macroscopic appearance		Microscopic appearance	
Front	**Reverse**	**Photo**	**Reference**
			

3.2.11. ***Chrysosporium* sp.**

Growth is rapid, leading to fluffy, flaky or powdery colonies. They are white, with a light brown verso.

Mycelium gives terminal or lateral aleurias. Aleurias are unicellular, ovoid or ampliform. We also observe interlayer, cylindrical or barrel-shaped, and truncated aleurias (Table 12).

Table 12: Macroscopic and microscopic appearance of *Chrysosporium* sp.

Macroscopic appearance		Microscopic appearance	
Front	**Reverse**	**Photo**	**Reference**
			

3.3. Frequency of isolation of fungi from dessert bananas

In total, of the 105 isolates, 93 were identified, i.e. 88.57%. The morphologically unidentified species were also important (12 or 11.43%). Fusarium have one of the lowest isolation rates in this study (Figure 4).

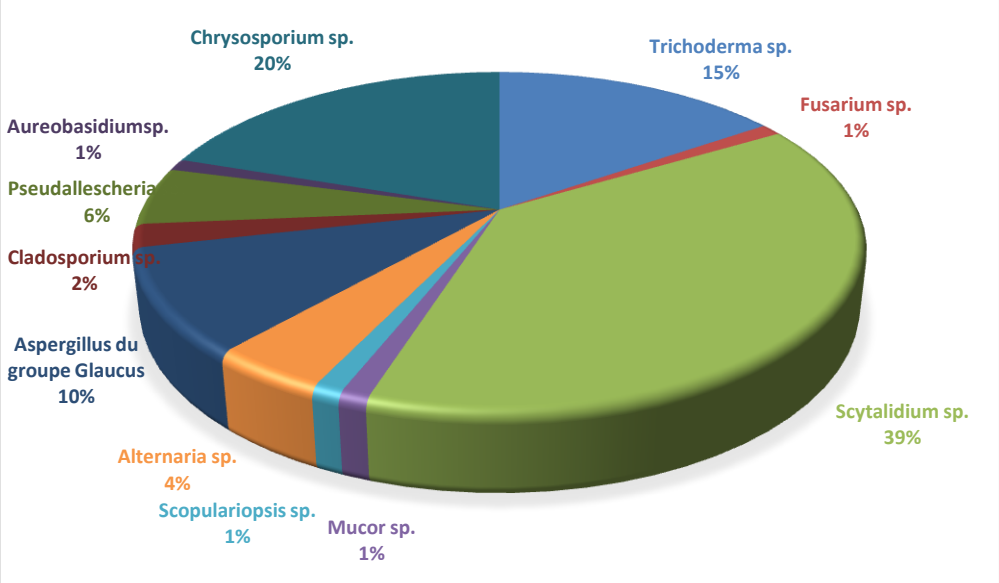


Figure 4: Mushroom isolation rates

3.3.1. Frequency of isolation of fungi before the development of post-harvest diseases

The first isolation carried out on the batch of bananas before the development of post-harvest diseases revealed the following (9) nine genera: *Trichoderma* sp. (3), *Mucor* sp. (1), *Scopulariopsis* (1), *Alternaria* sp. (1), *Aureobasidium* (1), *Aspergillus* of the *Glaucus* group (6), *Cladosporium* sp. (2). *Pseudallescheria* sp. (4), and *Scytaldium* sp. (17).

38.71% of the fungal strains identified in this work come from bananas even before the appearance of necrosis.

3.3.2. Frequency of isolation of fungi after the development of post-harvest diseases

After the development of post-harvest symptoms on the second batch of bananas, a second isolation carried out from infected explants showed (57) fifty-seven isolates identified in (7) seven fungal genera. This is *Fusarium* sp. (01), *Scytaldium* sp. (18), *Alternaria* sp. (03), *Trichoderma* sp. (12), *Pseudallescheria* sp. (2), *Aspergillus* of the *Glaucus* group (3) and *Chrysosporium* sp. (18). A large number of the strains obtained were isolated from bananas after the appearance of symptoms of post-harvest diseases.

3.2. Discussion

In this study, the identification of fungi on untreated bananas revealed the actual presence of fungi, better revealed that the dessert banana is a rich reservoir of a large number of fungal strains. This great diversity would explain the significant development of microorganisms that cause the appearance of necrosis on bananas and the rotting of their crowns. Remember that these fungal strains were isolated from bananas sampled before any post-harvest treatment. This shows that these pathogens isolated from bananas come from the fields during production. These results are therefore no different from those obtained by Ewané [3] during his work which revealed the appearance of necrosis on the crowns inoculated with sterile water, thus confirming the existence within them of infections. pre-established. Indeed, the fruits remained covered on the banana trees by plastic bags to avoid the bites of harmful insects of the fields, are constantly wet because of the heat. This shows that fungi could find the favorable conditions necessary for their development there. Also, infections in the crown of bananas appear to be caused by wounds from machetes or other tools used for harvesting. Cutting the hands of bananas from the stem and making bunches exposes the injured crown walls to fungal infection and development of crown rots. Previous studies [18] have confirmed that the pathogens implicated in crown rots being essentially wound parasites, development of these fungi on undamaged green parts of the bunch cannot occur.

In addition, a large number of the strains obtained were isolated from bananas after the appearance of symptoms of post-harvest diseases. The appearance of these symptoms (necrosis, softening, mummification, etc.) is therefore proof of an evolution in the development of fungi on bananas. This is probably why the work of Ewané [3] showed that keeping the fruits at room temperature would be favorable to the germination of spores deposited on the surface of freshly cut crowns.

Our results clearly show that dessert bananas are teeming with several fungal strains. Those identified in this work are the same as those generally isolated from bananas and cited in the literature, particularly in those of Demoulin [19] and Ewané [3] carried out in Cameroon on the study of the biodiversity of the microflora of banana washing water in the packing station. On the other hand, among the strains identified in this work, the fungus Colletotrichum musea responsible for banana anthracnose does not appear. It is one of the most feared mushrooms by farmers, because it can develop both on green fruit and on ripe fruit. On the green fruit, it causes dark brown or black wounds [20].

The constitution of different colonies for the same species was done by taking into account the variations of colors and the structure of the mycelium, the growth rate of the colony, the form of the culture. Some species have widely varying colonies while others have only one type of colonies. Sabouraud with chloramphenicol is the medium on which the fastest growth of fungi has been observed. The difference in colony growth rate on the two culture media used is surely due to the fact of the presence of chloramphenicol in the composition of the Sabouraud medium, unlike the PDA medium. Fusarium had one of the lowest isolation rates in this study. These results do not agree with the work carried out on the diversity of banana microflora from which the information drawn is that Fusarium are pathogens frequently isolated from crown rots [3]. The reasons why the morphological identification did not make it

possible to classify certain strains could be the fact that there is variability between the different isolates (the shape, the size of the conidia, and the color of the colony) and because the characteristics (desired traits) required are often not well developed (absence of macro-conidia in certain isolates after culture). This can be seen with the most often difficult identification of Fusarium species [3].

4. CONCLUSION

This study showed the presence of fungal strains on bananas produced in the South-East of Côte d'Ivoire. Identification of strains of Trichoderma sp., Fusarium sp., Scytalidium sp., Mucor sp., Scopulariopsis sp., Alternaria sp., Aureobasidium sp., Aspergillus of the Glaucus group, Cladosporium sp., from Chrysosporium sp. and Pseudallescheria sp. on the basis of cultural and morphological characters showed that different fungi are responsible for the symptoms observed on dessert bananas. These strains showed great variability in terms of the characters studied. They were characterized by different colorations (white, grey, brown, black brown, olive green, etc.) with cylindrical conidia of variable lengths and diameters. These results show that banana trees are subject to many parasitic constraints including fungal diseases. Fungi affecting banana fruits cause numerous losses, both quantitative and qualitative; because the attacked fruits can no longer be sold since they lose their market value on both local and international markets. Morphological characterization failed to identify several other species, particularly those that do not sporulate. It would therefore be interesting in future work to use molecular characterization to identify and confirm the morphological characterization of the strains studied.

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