Current Journal of Applied Science and Technology

Volume 43, Issue 9, Page 43-56, 2024; Article no.CJAST.122618
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Use of *Bacillus subtilis* Strains BK1 and BT2 in the Biocontrol of Fungi Spoilage in Plaintain (*Musa paradisiaca* L. Corn1 Variety)

Yao Fulgence Koffi [a*], Andrée Emmanuelle Sika-Kadji [b], Amoa Armist Tehua [c], Famatié Sangaré [b] and Waze Aimée Mireille Alloue-Boraud [b]

[a] Département de Biochimie-Génétique, UFR Sciences Biologiques, Université Peleforo GON COULIBALY de Korhogo, BP 1328 Korhogo, Côte d'Ivoire.
[b] Laboratoire de Biotechnologie et Microbiologie des Aliments, Unité de Formation et de Recherche en Sciences et Technologie des Aliments (UFR-STA), Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Abidjan, Côte d'Ivoire.
[c] Département de Biologie Végetale, UFR Sciences Biologiques, Université Peleforo GON COULIBALY de Korhogo, BP 1328 Korhogo, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/cjast/2024/v43i94429

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122618

Original Research Article

Received: 02/07/2024
Accepted: 05/09/2024
Published: 13/09/2024

ABSTRACT

The plantain occupies fourth place in terms of live production in Côte d'Ivoire. However, it is subject to fungal attacks during its post-harvest conservation, leading to a depreciation of its marketability leading to countless losses. The aim of this work was to evaluate the antagonistic properties of two

**Corresponding author: E-mail: fulgencekoffi85@yahoo.fr;*

Cite as: *Koffi, Yao Fulgence, Andrée Emmanuelle Sika-Kadji, Amoa Armist Tehua, Famatié Sangaré, and Waze Aimée Mireille Alloue-Boraud. 2024. "Use of Bacillus Subtilis Strains BK1 and BT2 in the Biocontrol of Fungi Spoilage in Plaintain (Musa Paradisiaca L. Corn1 Variety)". Current Journal of Applied Science and Technology 43 (9):43-56. https://doi.org/10.9734/cjast/2024/v43i94429.*

strains of *Bacillus subtilis* (BK1 and BT2) against the fungi responsible for spoilage of plantain variety Corn 1 in Côte d'Ivoire. To do this, a total of 180 samples composed of healthy and spoiled bananas were used for fungal isolations, carrying out biocontrol and fruit protection tests. The results of this study showed that nine (8) fungal isolates: *Aspergillus* sp, *Rhizopus* sp, *Rhizomucor* sp, *Colletotrichum* sp, *Rhizoctonia* sp, *Fusarium* sp, *Lasiodiplodia* sp and *Geotrichum* sp were identified as responsible for the alterations of plantain. *In vitro* antagonism and biocontrol tests showed that the two strains of *Bacillus subtilis* BK1 and BT2 have inhibition capacities on all 8 fungal isolates. Thus the use of the supernatant of BK1 and BT2 allowed the reduction of alterations of plantains between 80.06±2.81% and 58.44±4.50%. The two biocontrol agents tested were able to extend the shelf life of bananas over a period of 12 days. *Bacillus subtilis* BK1 and BT2 could therefore be used as biocontrol agents in the fight against fungal damage to plantain in Côte d'Ivoire.

Keywords: Plantain; spoilage fungi; antagonistic activity; conservation; Bacillus subtilis.

1. INTRODUCTION

Plantains (*Musa paradisiaca* L.) constitute a large-scale food crop food and economic importance in tropical and intertropical humid forest zones. Around 85% of production is self-consumed and/or sold locally in different countries in Africa, Latin America and Asia [1,2]. In terms of world production, plantain is the fourth agricultural product after rice, wheat and corn. In Côte d'Ivoire, plantain is the fourth largest food crop after yams, cassava and rice, with a production of 1,600,000 tonnes/year [3]. In Africa, Côte d'Ivoire is the leading plantain exporting country [4]. It constitutes the basis of food security for many populations [5,6]. From a culinary point of view, two types of bananas are distinguished, namely dessert bananas and plantains [7]. Bananas offer multiple uses. They are consumed mainly as a cooked or fried vegetable but are also the subject of numerous transformations: chips, fries, donuts, mash, jam and beer [6]. Despite its capital importance, from a food and economic point of view, this seasonal and highly perishable crop is subject to post-harvest conservation problems which cause enormous losses [8,9]. In fact, the conservation of plantain is limited by poor conditions harvesting, transport, storage and marketing. These conditions contribute to the development of plantain spoilage germs after harvest [10]. Microscopic fungi are the greatest agents of spoilage of plantain after harvest. They alter the organoleptic quality of the plantain causing enormous economic losses. These microscopic fungi, often present on plantains from the field, attack bananas through wounds caused during harvesting, transport, storage and sale [11,12]. Chemical control with the use of chemical fungicides is the main route used by farmers against these germs. These chemical pesticides, although effective, have effects devastating effects on the environment and biodiversity and present risks for consumers. From farm to fork, these pesticides are often found, after treatment, in plant products [13]. Research and development of new techniques is therefore judicious and justified. Plantain conservation techniques that are more concerned with consumer well-being, biodiversity and the environment have been implemented according to several studies. Among the latter, the most studied are cold preservation, preservation in controlled atmosphere, ionizing radiation, polyethylene films [8,9]. In addition to the effectiveness of these techniques, they are expensive and rigorous for farmers because they are meticulous and require special attention with qualified labor. Thus, in addition to these techniques, the use of microorganisms as bio-preservatives presents itself as the most suitable alternative solution to combat post-harvest losses caused by microorganisms. Indeed, scientific studies have demonstrated the ability of *Bacillus subtilis* to inhibit the growth of pathogenic fungi fruits and vegetables in Côte d'Ivoire. The use of *Bacillus subtilis* GA1 as a biopesticide made it possible to inhibit various microorganisms such as *Colletotrichum* sp, *Candida* sp (a), *Penicillium* sp, *Candida* sp (s), *Pseudomonas* sp and a lactic acid bacteria isolated from mangoes and made it possible to store mangoes for more than ten days. Thus, *Bacillus subtilis* GA1 could be used as a biopesticide for the conservation of mangoes in Côte d'Ivoire [14,15]. These various works thus make it possible to justify the choice made on *Bacillus subtilis* in order to fight against plantain spoilage fungi and to extend the shelf life of this foodstuff. It is within this framework that this study falls, the general objective of which was to evaluate the antagonistic properties of *Bacillus*

subtilis BK1 and BT2 against plantain spoilage fungi in the city of Abidjan (Côte d'Ivoire).

2. MATERIALS AND METHODS

2.1 Materials

The plant material used for this study consisted of healthy plantains and spoiled plantains of Corn 1 variety (Fig.1). Two strains of *Bacillus*, *Bacillus subtilis* BT2 and *Bacillus subtilis* BK1 isolated from the rhizosphere of the cocoa tree and preserved in cryotubes in a freezer (-80°C) were used as bacterial biopesticides.

Fig. 1. Plantains Corn 1 variety: healthy (A) and spoiled (B)

2.2 Methods

2.2.1 Sampling

One hundred and eighty (180) samples of plantains were taken directly from the 3 plantain unloading platforms in the city of Abidjan (Côte d'Ivoire) at a rate of 60 plantains per site. These 180 bananas were made up of 45 spoiled plantains for fungal isolations and 135 healthy plantains for biocontrol and conservation tests collected from 5 sellers. Once collected, the different fruits were packaged individually in bags plastic stomachers, labeled, sealed then kept in a cooler containing dry ice where the temperature is maintained at 4°C. Microbiological analyzes were carried out 2 to 3 hours after sampling.

2.2.2 Isolation of plantain spoilage fungi

The direct contact isolation technique on PDA agar as described by [15] was used to isolate plantain spoilage fungi. Three spoiled plantains were chosen at random from each sample then disinfected with 2% bleach for 2 min in order to eliminate the exogenous microflora then rinsed twice with sterile distilled water in order to eliminate the bleach residue. The bananas are dried in sterile trays then disinfected with hydrophilic cotton soaked in 70% ethanol before sampling. Three fragments of banana peels and banana flesh will be removed using a sterile scalpel. These fragments will be placed separately on Petri dishes containing Potato Dextrose Agar (PDA). The plates will be incubated at 28°C for 5 to 7 days. To obtain a pure strain, several subcultures on PDA medium are carried out. The choice of species to be sampled is made by eye, taking into account the resemblance of thalli and spores. Once these are isolated in pure culture, they are subcultured on PDA medium to measure their apical growth speed and observe the macroscopic and microscopic characteristics.

2.2.3 Identification of isolated fungi

The macroscopic identification of the isolated fungi was carried out according to the method of [16] by examining the culture on PDA agar. Cultural characteristics determined were the appearance of the colonies (fluffy, woolly, cottony, velvety, powdery or granular), the relief of the colonies (flat, rounded, wrinkled, etc.), the color of the colonies (white, cream or colored, yellow, orange, brown , green, gray up to black), the size of the colonies (small, extensive or invasive), the speed of growth (fast or slow) as well as the color of the back of the Petri dish and the diffusion of the mycelium in the agar. The method described by Guiraud (1998) was used by removing a filament using forceps then placed in a drop of methylene blue, placed on a slide and covered with a coverslip then observed under an optical microscope LEICA DM750 with X40 and X100 lens. The characteristics observed were the appearance of the mycelium (compartmentalized or not), the presence and shape of the spores (oval, spherical, round, etc.), the shape of the conidial heads and the size of the conidiospores (short or long). The frequency of mushroom isolation was calculated according to the method of [17].

$$\textbf{IF (\%) = NI/TNI ×100}$$

IF : Isolation frequency in percentage
NI : Number of isolations of a fungal genus
TNI : Total number of fungal genera isolations

2.2.4 Culture of *Bacillus subtilis* strains BK1 and BT2

Bacillus subtilis strains were subcultured using the streak method on YPGA agar from colonies already isolated, identified and stored at -80°C. The plates were incubated at 30°C for 24 hours to obtain young cultures. The isolated colonies were used for carrying out fruit antagonism and protection tests.

2.2.5 Antagonism test

This test was carried out with the aim of verifying the existence of an antifungal potential of *Bacillus subtilis* BT2, *Bacillus subtilis* BK1 on each of the isolated molds. The *in vitro* antifungal activity of *Bacillus subtilis* strains was carried out with the supernatant of the two strains of *Bacillus subtilis*. This test was carried out according to the method described by [14]. The two strains of *Bacillus subtilis*, BK1 and BT2, were each spread on Petri dishes containing YPGA medium by making a longitudinal streak dividing the dish into two equal parts using a sterile platinum loop. Subsequently, a fragment of the mycelium of the same mushroom was deposited on either side of the streak at a distance of 1 cm from the edge of the box. This operation was carried out with all fungal isolates. The control dishes consisted of inoculating the mold by transplanting it to the center of the Petri dish where there was no inoculation with Bacillus subtilis. Incubation was carried out at 30°C for 7 days. The evolution of the confrontation of the colonies of pathogenic molds and strains of Bacillus subtilis were observed every day until the seventh day. After 7 days, the percentage of growth of the fungus in the boxes was determined using the method of [18], then the inhibition rate was deduced according to the following formula:

$$\text{Inhibition rate} = [(R - r) / R] \times 100$$

r : Radial growth of the microorganism with antagonism
R : Radial growth of the microorganism without confrontation of antagonism

2.2.6 Biocontrol test

This test was carried out to evaluate the ability of *Bacillus subtilis* to reduce or stabilize a alteration due to isolated fungi. Healthy plantains without alterations have been used. The bananas were washed in tap water, rinsed three times with distilled water sterile and disinfected using paper towels soaked in 70% ethanol. Using a sterile micropipette tip, two wells of 3 mm in diameter and 5 mm in depth were made on each banana [19]. A total of three treatments were performed. The first treatment consisted of treating the bananas with the supernatant of *Bacillus subtilis* BT2 by adding to each well a volume of 50 µL of suspension loading 10^5 CFU/mL of each of the isolated fungi. The fungi were added individually. Another treatment was carried out with all isolates. The second treatment was carried out by adding 50 µL of *Bacillus subtilis* BK1 supernatant. The third treatment was carried out by replacing Bacillus subtilis with 50 µL of sterile distilled water as a control to evaluate the antifungal activity of Bacillus subtilis. Infection with the isolates was carried out at the same time as the treatment with a 10^6 CFU/mL load suspension of *Bacillus subtilis* BT2 and BK1 by adding the same volume. The treated bananas were incubated at room temperature (25°C) for 7 days. The results were expressed as a percentage reduction in infection according to the formula [19].

$$P = ((Dt-De) / (Dt-K)) \times 100$$

P : percentage reduction in infection.
K : diameter of the well.
De et **Dt** represent respectively the lesion diameter measured on the spoilage control and on the control plantains.

2.2.7 Fruit protection test

This protection test on fruits was carried out using the immersion technique described by [20]. This technique involves immersing healthy plantains in a container containing the supernatant of Bacillus subtilis. The immersion time is not important but you must ensure that the surface of the plantains has been completely wet. A preculture of each strain of *Bacillus subtilis*, BT2 and BK1, was carried out by subculture in 50 mL of YPG broth using a sterile loop. It was incubated at 30°C with shaking at 105 rpm for 8 hours. The preculture obtained was used to inoculate, in duplicate, 500 ml of YPG broth contained in an Erlenmeyer flask with a capacity of 2 L. The inoculated broth was incubated at 30°C with stirring at 105 rpm for 72 hours. After 72 hours of incubation, the biomass solution obtained was centrifuged using a centrifuge at 3000 rpm for 10 min as described by [14] in order to recover the supernatant.

Healthy plantains were carefully washed in tap water and rinsed three times with sterile distilled water and disinfected using paper towels soaked in 70% ethanol. They are then immersed in two types of *Bacillus subtilis* solutions. The first containing the BT2 supernatant, the second containing the BK1 supernatant. The control was not immersed in the biomass solution. The bananas were then dried in the open air and finally stored at room temperature (25°C) for seven days. Treated plantains are compared to controls that have not received any treatment in order to assess the effectiveness of the biopesticide.

2.3 Statistical Analysis

R software version 4.0.4 for Windows was used for statistical analysis. The analysis of variance (ANOVA) was carried out to first compare the average diameters of spoilage observed after treatment of plantains. Secondly, it was used to compare the inhibition rates of fungi isolated by *Bacillus subtilis* strains. The Tukey test was carried out to determine the different classes of homogeneity with a significance threshold of 5%.

3. RESULTS

3.1 Microscopic Spoilage Fungi Isolated from Plantain

Plantain samples are contaminated with molds exhibiting colonies of various appearances, textures and colors. Subsequently, 244 fungal isolates were isolated taking into account the resemblance of thalli and spores. Phenotypic identification according to the macroscopic characteristics of the colonies (appearance, color, shape, contour, etc.) and on the basis of the microscopic characteristics of the mycelium and conidia or spores (compartmentalization of the mycelium, shape of the spores, shape of the reproductive organs , etc.) made it possible to highlight fungi belonging to 8 genera, namely *Aspergillus, Rhizopus, Rhizomucor, Colletotrichum, Rhizoctonia, Fusarium, Lasiodiplodia* and a yeast including *Geotrichum* (Figs. 2-9).

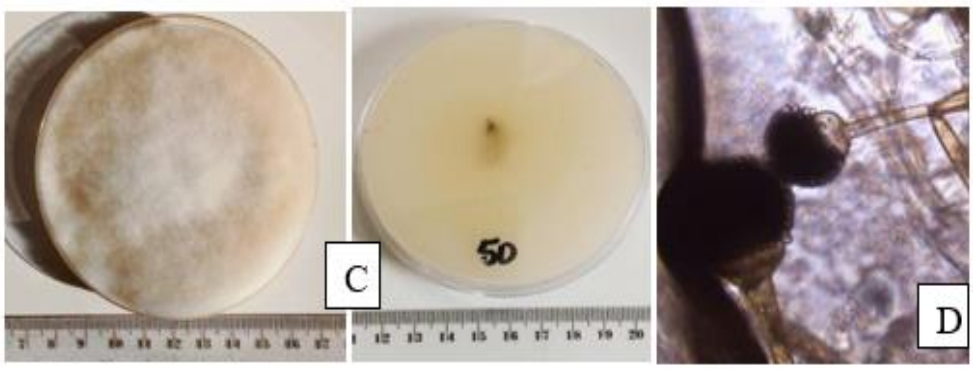

Fig. 2. Culture of *Rhizopus sp* on PDA medium: macroscopic appearance (C), microscopic appearance (D)

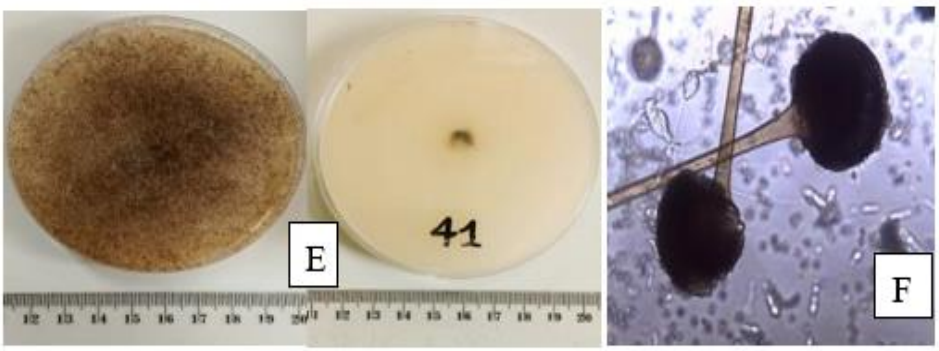

Fig. 3. Culture of *Rhizomucor sp* on PDA medium: macroscopic appearance (E), microscopic appearance (F)

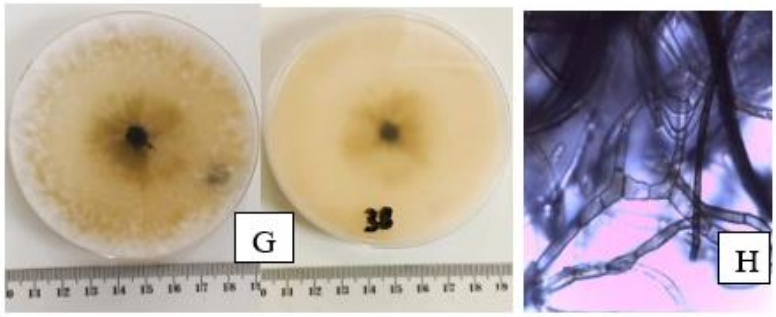

Fig. 4. Culture of *Rhizoctonia sp* on PDA medium: macroscopic appearance (G), microscopic appearance (H)

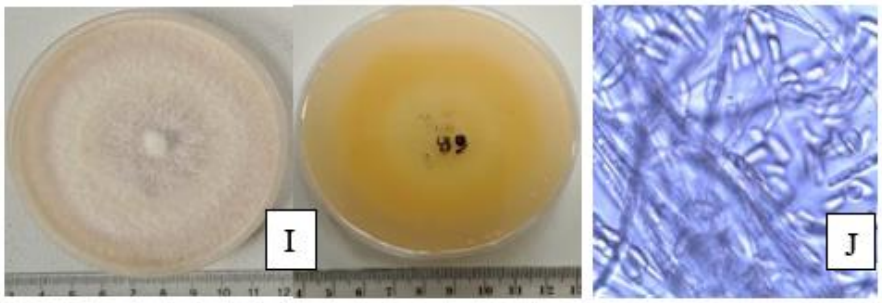

Fig. 5. Culture of *Colletotrichum sp* on PDA medium: macroscopic appearance (I), microscopic appearance (J)

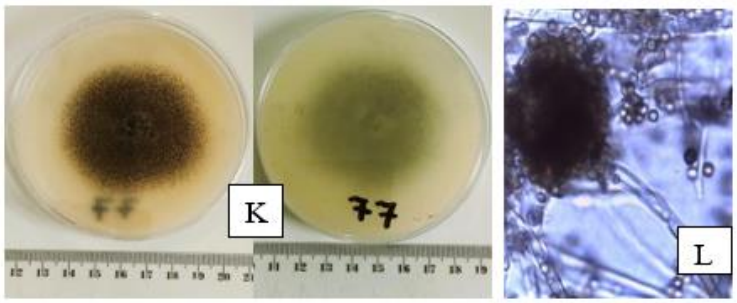

Fig. 6. Culture of *Aspergillus sp* on PDA medium: macroscopic appearance (K), microscopic appearance (L)

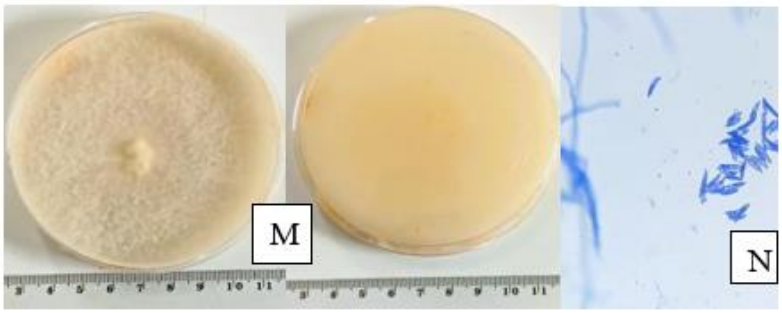

Fig. 7. Culture of *Fusarium* sp on PDA medium: macroscopic appearance (M), microscopic appearance (N)

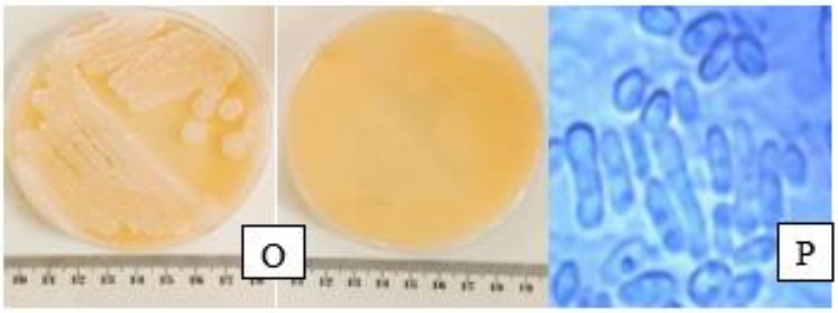

Fig. 8. Culture of *Geotrichum sp* on PDA medium: macroscopic appearance (O), microscopic appearance (P)

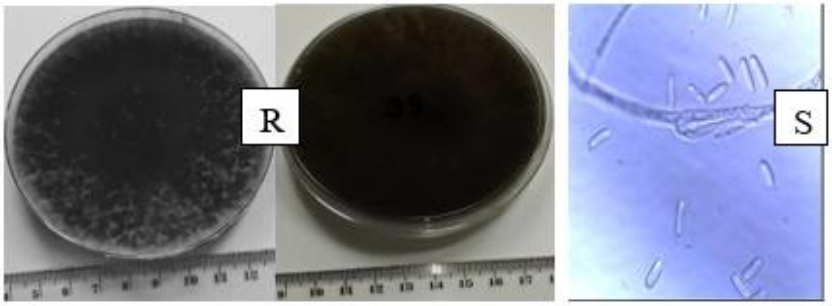

Fig. 9. Culture of *Lasiodiplodia sp* on PDA medium: macroscopic appearance (R), microscopic appearance (S)

3.2 Frequency of Isolation of Fungal Strains

In total, 244 fungal isolates including eight (08) genera of molds and one (1) genus of yeast were isolated from spoiled plantains. These eight (08) genera were found in all samples of spoiled bananas. Among these isolated fungi, *Aspergillus* sp, *Fusarium* sp, *Lasiodiplodia* sp and *Colletotrichum* sp are in the majority with respective frequencies of 19.68%, 14.34%, 13.93% and 13.93% (Table 1). The minority fungi were *Geotrichum* sp and *Rhizomucor* sp with respective frequencies of 8.20% and 7.79%.

Table 1. Isolation frequency of fungal

Fungal isolates	Frequency (%)
Fusarium sp	14,34 (35)
Lasiodiplodia sp	13,93 (34)
Colletotrichum sp	13,93 (34)
Rhizoctonia sp	11,88 (29)
Aspergillus sp	19,68 (52)
Rhizopus sp	10,25 (25)
Geotrichum sp	8,20 (20)
Rhizomucor sp	7,79 (19)
Total	100 (244)

Numbers in parentheses represent the number of isolates of each fungal species.

3.3 Antagonism *In vitro*

The two strains of *Bacillus subtilis*, BK1 and BT2, have inhibition capacities on all 9 fungal isolates (Table 2). Table 4 illustrates the inhibition rates of plantain spoilage fungi by *Bacillus subtilis* BK1 and BT2 strains. Both strains inhibited the growth of all fungal isolates. The BK1 and BT2 strains reacted in the same way against the fungal isolates except the mold *Colletotrichum* sp where the BT2 strain proved to be more effective than the BK1 strain with a respective inhibition rate of 75.67±0.69 and 73.29±0.34. The highest inhibition rates were observed with *Colletotrichum* sp, *Aspergillus* sp1 and sp2 with more than 70% inhibition with the two strains of *Bacillus subtilis*. Rhizomucor sp presented the lowest inhibition rate with both biopesticides (52.04±1.59 for BK1 and 53.86±0.09 for BT2).

3.4 Biocontrol

Treatments of bananas with *Bacillus subtilis* BT2 and *Bacillus subtilis* BK1 strains led to a reduction in the spoilage of plantains by pathogenic isolates (Tables 3 and 4). The alterations caused by the strains *Colletotrichum* sp, *Lasiodiplodia* sp, *Aspergillus* sp1 and sp2 were the most reduced at more than 70%. The

maximum reduction in infection was achieved with *Lasiodiplodia* sp with 78.84±0.57 by BK1 and 80.06±2.81 by BT2. The alterations caused by *Rhizomucor* sp were the least reduced with 58.44±4.50 by BK1 and 60.01±1.59 by BT2. The alterations made by other pathogens were inhibited by more than 60%.

3.5 Fruit Protection

For carrying out plantain protection tests, mature plantains of apparently healthy Corn 1 variety were used (Fig.10). Symptoms of spoilage were observed on plantains that had not received treatment from the fifth day (Fig.11) while the treated plantains remained intact (Fig.12 and Fig.13). On the tenth day the control bananas were completely damaged and wilted with unpleasant odors. They had yellowish and whitish mycelium and spores on their surface, while the pulp had black spots. On the tenth day, the plantains treated with the BK1 and BT2 supernatants remained apparently intact, both the surface and the pulp. Signs of spoilage were observed on treated plantains only on the twelfth day after treatment with black spots on the fruit. However, the inside of his bananas showed no signs of deterioration, nor the release of unpleasant odors, nor change in color of the pulp (Fig.12 and Fig.13). The two *Bacillus subtilis* strains BK1 and BT2 showed similar results in plantain protection tests.

Table 2. Rate of inhibition of plantain spoilage fungi by *Bacillus subtilis* BK1 and BT2 strains

Strains	BK1	BT2
Rhizopus sp	(62,65±1,20)[a]	(62,26±0,92)[a]
Rhizomucor sp	(52,04±1,59)[a]	(53,86±0,09)[a]
Rhizoctonia sp	(60,63±0,85)[a]	(60,58±0,11)[a]
Colletotrichum sp	(73,29±0,34)[b]	(75,67±0,69)[a]
Aspergillus sp	(76,90±0,92)[a]	(76,30±1,25)[a]
Fusarium sp	(56,74±1,43)[a]	(56,16±1,42)[a]
Geotrichum sp	(65,25±1,20)[a]	(64,26±0,92)[a]
Lasiodiplodia sp	(65,17±0.72)[a]	(66,51±2.31)[a]

On the same line, values with the same letters are statistically identical at the 5% threshold and those with different letters are statistically different at the 5% threshold.

Table 3. Percentage (%) reduction in damage by the supernatant of *Bacillus subtilis* BK1 and BT2

Souches	BK1	BT2
Rhizopus sp	(67,15±2,33)[a]	(69,09±0,50)[a]
Rhizomucor sp	(58,44±4,50)[a]	(60,01±1,59)[a]
Rhizoctonia sp	(64,22±2,05)[a]	(62,44±0,90)[a]
Colletotrichum sp	(72,36±1,18)[a]	(70,41±2,11)[a]
Aspergillus sp	(74,44±3,50)[a]	(72,44±2,44)[a]
Fusarium sp	(64,13±4,50)[b]	(70,44±1,12)[a]
Geotrichum sp	(64,44±4,50)[a]	(70,44±4,50)[a]
Lasiodiplodia sp	(78,84±0,57)[a]	(80,06±2,81)[a]

Table 4. Appearance of plantains after the confrontation between fungal pathogens and the supernatant of biopesticides

Strains	BK1	Control	BT2	Control
Rhizopus sp				

Strains	BK1	Control	BT2	Control
Aspergillus sp				
Colletotrichum sp				
Rhizoctonia sp				
Fusarium sp				
Lasiodiplodia sp				
Rhizomucor sp				
Geotrichum sp				

Fig. 10. Appearance of plantains on the first day of protection test

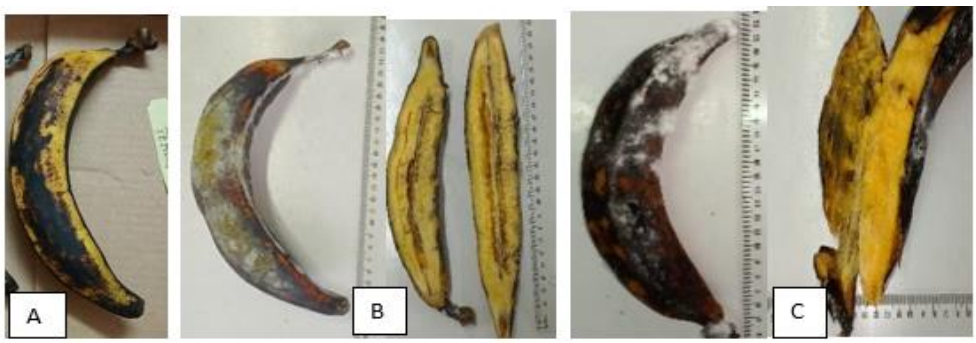

Fig. 11. Appearance of untreated plantains after 5 days (A), 10 days (B) and 12 days (C)

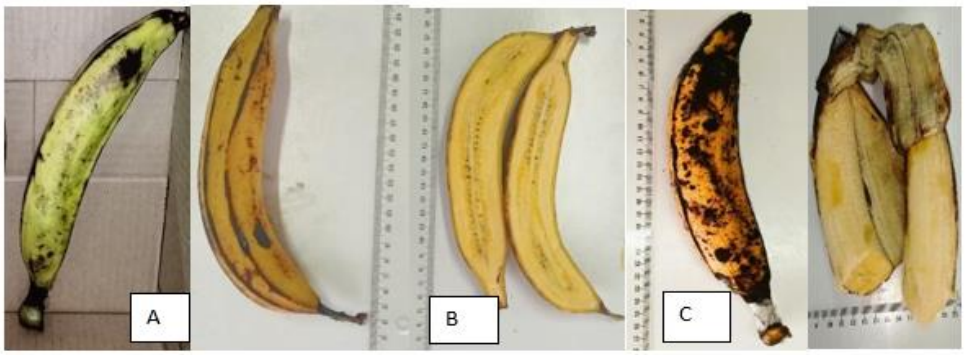

Fig. 12. Appearance of plantains treated with the supernatant of *Bacillus subtilis* BT2 after 5 days (A), 10 days (B) and 12 days (C)

Fig. 13. Appearance of plantains treated with *Bacillus subtilis* BK1 supernatant after 5 (A), 10 (B) and 12 days (C)

4. DISCUSSION

Microscopic fungi are real agents of post-harvest alteration of the plantain. The general objective of this work was to evaluate the antagonistic properties of two strains of *Bacillus subtilis* (BK1 and BT2) against the fungi responsible for spoilage of plantain variety Corn 1 in Côte d'Ivoire. The results obtained following the phenotypic identification of the fungal strains isolated plantains have shown that 8 fungal genera are responsible for Post-harvest alterations of plantains in Côte d'Ivoire These 8 genera are *Aspergillus, Rhizopus, Rhizomucor, Colletotrichum, Rhizoctonia, Fusarium, Lasiodiplodia* and a yeast including *Geotrichum*. The results of pathogenicity tests carried out on plantains have showed that all these isolated fungi were agents responsible for fruit deterioration. These tests thus made it possible to demonstrate that *Fusarium* sp *Rhizopus* sp, *Rhizomucor* sp, *Colletotrichum* sp *Lasiodiplodia* sp and *Rhizoctonia* sp were responsible for a more significant deterioration of the plantain. These results, similar to those of [21] who isolated the fungi *Botryodiplodia theobromae*, *Colletotrichum musae*, *Aspergillus niger*, *Fusarium moniliforme*, *Geotrichum candidum*, *Fusarium semitectum* and *Aspergillus flavus* from dessert bananas in Côte d'Ivoire [10] isolated the genera *Colletotrichum, Fusarium, Aspergillus, Phoma, Penicillium, Curvularia, Botryodiplodia* and *Rhizoctonia* from banana, mango and avocado in Côte d'Ivoire. The presence of these fungi and their diversity could be explained by factors such as the environment, the degree of sanitation, harvesting, transport and storage methods [22].

According to [23] these fungi are known to cause the deterioration of many products such as fruits, seeds, vegetables and tubers both in the field and during storage. Among these 8 isolated genera, *Colletotrichum* sp, *Lasiodiplodia* sp and *Fusarium* sp were the most isolated. These three (3) genera were identified as those having caused the most serious alterations of plantain. This could be explained by the fact that these three genera are the main agents of deterioration of the plantain. As a result, these molds would easily proliferate in plantains. These results are in agreement with those of [24], those of [21,25] who identified these genera as the most often encountered and most important pathogens on bananas during conservation. The work of [21] showed that *Lasiodiplodia* sp is the main causal agent of post-harvest banana rot. He was also identified as the main yam adulterer in Côte d'Ivoire by [26] The high isolation rate of *Colletotrichum* sp could be explained by its ease of colonizing plantains. These results are consistent with the work of [10] who recorded a high prevalence of *Colletotrichum* on avocado, mango and banana in Côte d'Ivoire. The high prevalence of *Fusarium* sp could be explained by the fact that the plantain is a preferred host for this gender. It very easily colonizes the surface of fruits and its proliferation would lead to the degradation of the fruit's nutrients. These results are consistent with those of [25] who showed a prevalence of the *Fusarium* genus on fruits. This genus represents 53% of the spoilage fungal flora of tropical fruits in Madagascar. The presence of *Rhizoctonia* sp could be explained by the fact that it is telluric. Indeed, investigations have shown that during sale, plantains are exposed to the ground, which would encourage their contamination by *Rhizoctonia* and storage conditions would be the cause of its proliferation. These results corroborate those of [27] who showed that one of the most pathogenic species of this genus, *Rhizoctonia solani*, attacks a very wide range of horticultural, cereal or ornamental crops and is present in most soils. [28] also showed that *Rhizoctonia solani* is the main pathogen responsible for brown rhizoctonia of potatoes in Algeria. Cool, moist soil conditions at the time of sowing are favorable for its growth. The presence of the *Rhizopus* genus in plantains is confirmed with the work of [29] and those of [30]. Indeed, [29] showed that fungi of the genus *Rhizopus* are the second cause of fungal contamination of seeds and fruits sold in Kenyan markets. The presence of *Aspergillus* sp on plantain could be due to the fact that it has a mode of saprophytic life which would favor their associations with stored agricultural products. These results corroborate those of [21] and those of [10] who isolated the genus *Aspergillus* from dessert bananas in Côte d'Ivoire. The presence of *Rhizomucor* sp and *Geotrichum* sp would have originated in markets. In fact, the type *Rhizomucor* is renowned for the damage it causes to tomatoes. The genus *Geotrichum* sp is the major alterator of citrus fruits. Since banana and tomato traders are nearby, these types could contaminate plantains through hand-borne contamination and wind. These results are consistent with those of [30] who identified *Rhizomucor miehei* as a fungal spoilage agent of tomato in Algeria. According to [31] the genus *Geotrichum*, is a microorganism commonly associated with the spoilage of several fruits and vegetables such as tomatoes, cucumbers,

carrots and many other fruits and vegetables. The *in vitro* and *in vivo* comparison of the two strains of *Bacillus subtilis* BK1 and BT2 and the spoilage germs revealed that the strains of *Bacillus subtilis* have the capacity to inhibit the growth of the main fungal strains responsible for spoilage of plantain. . The two strains of *Bacillus subtilis* BK1 and BT2 had similar actions on the fungal isolates. This could be explained by the fact that these two strains produce the same antifungal compound. The effectiveness of these two strains of *Bacillus* would be linked to their capacity for this bacterium to produce, during its stationary growth phase, metabolites of antifungal nature such as fengycins, iturins and surfatins. These results are consistent with those of [2] and those of [32] who showed that the biocontrol potential of *Bacillus subtilis* strains depended on fengycin and iturin biosynthesis genes in conservation. post harvesting cocoa pods. The percentages of reduction in plantain spoilage were between 80.06±2.81% and 58.44±4.50%. These results are close to those obtained by [14], during their work on mango conservation. They found that Bacillus subtilis GA1 inhibited major spoilage fungal strains at rates that were between 59.37% and 84.78%. Also, the work of [33] showed the effectiveness of *Bacillus subtilis* GA1 in inhibiting the growth of *Aspergillus* sp by 81.42% during pineapple conservation. During in vitro and in vivo confrontations, the alterations caused by *Rhizomucor* sp were least reduced. This could be explained by the fact that Rhizomucor grows very quickly. As a result, it would develop before the strains of *Bacillus subtilis* have time to produce the antifungal compound. The weather that the strains of *Bacillus subtilis* take would therefore be sufficient for *Rhizomucor* sp to proliferate. This would explain the major difference between the in vitro and in vivo confrontations concerning this mold. Plantain protection tests revealed that the two biocontrol agents tested exert an inhibitory action on pathogens. Indeed, immersing plantains in the supernatant of Bacillus subtilis BK1 and BT2 made it possible to prevent the proliferation of pathogens on the surface and inside of the fruits and to preserve the firmness of the fruit as well as its organoleptic characteristics. The appearance of black spots on the twelfth day could be due to the normal ripening process of the plantain. The plantains could therefore be preserved thanks to the metabolites of the two biocontrol agents for more than 12 days without being spoiled, unlike the controls which showed signs of spoilage from the fifth day. These results corroborate those of [14] and those [33] who respectively preserved mangoes for 10 days and pineapples for 14 days in Côte d'Ivoire using *Bacillus subtilis* GA1.

5. CONCLUSION

This present study made it possible to contribute to the biological fight against fungi plantain spoilage. From this study, eight (08) genera of fungi namely *Aspergillus, Rhizopus, Rhizomucor, Colletotrichum, Rhizoctonia, Fusarium, Lasiodiplodia* and *Geotrichum* have been identified as the spoilage flora of plantain. Most listed and most incriminated in the alteration of plantain were *Colletotrichum* sp, *Lasiodiplodia* sp and *Fusarium* sp. The use of *Bacillus subtilis* BK1 and BT2 as biopesticides made it possible to inhibit different micro-organisms and preserve bananas plantains for more than 12 days. Thus, these two bacteria could serve as a good biopesticide for the conservation of plantains in Côte d'Ivoire.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gandonou G, Ahanhanzo C, Agbangla C, Agbidinoukoun A, Doussoh A, Cacai G, Dossoukpevi R. In vitro micropropagation of the local variety "Aloga" of plantain (*Musa x paradisiaca* L.) in Benin. International Journal of Biological and Chemical Sciences. 2012; 6(3).
2. Koua SH, N'golo DC, Alloue-Boraud WM, Konan F. Bacillus subtilis Strains Isolated from Cocoa Trees (*Theobroma cacao* L.) Rhizosphere for their use as Potential Plant Growth Promoting Rhizobacteria in Côte d'Ivoire. Current Microbiology. 2020; 77(9): 2258-2264.
3. PNIA. National Agricultural Investment Program. 2014 Review. 2014. http://www.gouv.ci/_actualite-article.php?d=1&recordID=5309

4. FAO. Banana Market Review, Preliminary Results 2019. 2020.
5. Lassoudière A. The banana tree and its cultivation. Quae, Versailles, France. 2017.
6. Lassois L, Busogoro JL Jijakli H. The banana: from its origin to its marketing. Biotechnology Agronomy Society Environment. 2009; 13 (4): 575-586.
7. Odah O, Aziadekey M, Tozo K, Akpavi S, Koukouma R., Guelly A, Gbeassor M. The genetic diversity of plantains cultivated in the western zone of the Plateaux Region of Togo. International Journal of Biological and Chemical Sciences. 2014; 7(5):1910.
8. Lepengue A, Mouaragadja I, Dick E, Mbatchi B, Ake S. Improving the shelf life of plantains at ambient temperatures. International Journal of Biological and Chemical Sciences. 2010; 4(3). French.
9. Yao AK, Koffi DM, Irié ZB, Niamke SL. Preservation of plantains (Musa AAB) in the green state using polyethylene films of different thicknesses. Journal of Animal & Plant Sciences. 2014; 23(3): 3677-3690. French.
10. Kouadia AMJ, Kouabenan A, Kouadio T K. Evolution of natural infections on mangoes, avocados and bananas in Côte d'Ivoire and the main fungi responsible. Journal of Applied Biosciences. 2019; 134: 13710 – 13721. French.
11. Kwa M, Temple L. The Plantain Banana. Socio-economic and Technical Issues, Experiences in Intertropical Africa. (eds.). 2019. CTA: Wageningen. 199p. French.
12. Cirad. Fungal Disease Meetings. 2021. www.IT2: Tropical Technical Institute. French.
13. FAO. Pesticide Management in the Banana Sector. World Banana Forum collection of good practices. 2017. 5p. French.
14. Alloue-Boraud W A M, Ban-Koffi L, Dadie AT, Dje KM, Ongena M. Use of Bacillus subtilis GA1 for the control of mango spoilage germs in Côte D'Ivoire. Journal of Animal and Plant Sciences. 2015; 3(25): 3954-3965. French.
15. Djossou O. Post-harvest microflora of robusta coffee and use of bacteria for the control of mycotoxynogenic molds and producers of Ochratoxin A. Doctoral thesis. Paul Cezanne University AIX Marseille III. 2011; 123 p. French.
16. Botton B, Breton A, Fevre M, Gauthier S, Guy PH, Larpent JP, Reymond P, Sarglier JJ, Vayssier Y, Veau P. 2nd edition. Useful moist and nusible: industrial importance. Paris. Milan. Barcelona: Masson. « Collection Biotechnologies (Paris) ». 1990; 52 p.
17. Walder M. 7th edition. Statistics and probability calculus. Dalloz Paris. 1996; 225-227.French
18. Korsten L, Jager ESD. Mode of action of Bacillus subtilis for control of avocado postharvest pathogens. South African Avocado Grower's Association Yearbook. 1995; 18: 124-130.
19. Touré Y, Ongéna M, Jacques P, Guiro A, Thonart P. Role of lipopeptides produced by Bacillus subtilis GA1 in the reduction of grey mould disease caused by Botrytis cinerea on apple. Journal of Applied Microbiology. 2004; 96: 1151–1160.
20. Cissé M. Immobilization of a lactoperoxide system in a chitosan coating to extend the conservation of mangoes. Doctoral thesis, University of Montpellier
1. Supagro (France). 2012; 159 p. French.
21. Kouadio KT, Thérèse AA., Jean P, Eric-Olivier T, N'sou ILVO. "Inventory of Post-Harvest Pathogenic Fungi of Dessert Banana "Cavendish" and Evaluation of Their Sensitivity to Azoxystrobin in Côte d'Ivoire". European Scientific Journal. 2017; 13 (21): 26-26.
22. Djeugap JF, Tsopmbeng NG, Keuete KE, Yaouba A, Serferbe S. (2015). Isolation and identification of fungi associated with avocado fruit from local markets of the west region of Cameroon. International Journal of Agriculture and Biosciences. 2015; 4 (2): 64-68.
23. Dongmo GZ, Djeugap JF, Fenohi N, Kenfack ND, Takuete R, Teguefouet P. Contribution to the identification of post-harvest fungi associated with *Ricinodendron heudelotii* and *Garcinia kola* kernels collected in the Western Highlands Cameroon. International Journal of Biologycal and Chemical Science. 2017; 11 (4): 1840-1850. French.
24. Abd-Alla MA, El-Gamal NG, El-Mougy NS, Abdel-Kader MM. Post-harvest treatments for controlling crown rot disease of Williams banana fruits (*Musa acuminata* L.) in Egypt. Plant Pathology & Quarantine. 2014; 4(1): 1-12.
25. Jean-Michel LPT. Identification of the fungal flora of tropical fruits in Madagascar. 7th Indian Ocean Agri-Food Meetings, November 16-21, 2018 – Moroni – Comoros. 2018.1-13. French.

26. Assiri KP, Koutoua S, Assi ST. Fungi responsible for post-harvest rot in yam Dioscorea cayenensis-rotundata variety kponan. Journal of Applied Biosciences. 2017; 111: 10957-10968. French.
27. Leblanc K, Edith P, Hogue R. Detection of anastomotic groups of *Rhizoctonia solani*, reducing the use of biopesticides. IRDA, Canada. 2008. French.
28. Imane T, Zellagui C. Rhizoctonia solani a phytopathogen for potatoes. Bibliographic synthesis on biological control methods. University of Brothers Mentouri Constantine Faculty of Natural and Life Sciences. Algeria. 2020. 100p. French.
29. Nyirahakizimana H, Mwamburi L, Wakhisi J, Mutegi CK, Christie ME, Wagacha JM. Occurrence of Aspergillus Species and Aflatoxin Contamination in Raw and Roasted Peanuts from Formal and Informal Markets in Eldoret and Kericho Towns, Kenya. Advances in Microbiology. 2013; 3: 333-342.
30. Benselimane S. Boureghda A. Improvement of the preservation of fresh tomatoes against fungal alterations using aqueous extracts of some medicinal plants. Applied Microbiology and Food Sciences. 2019; 51p. French.
31. Palou L, Smilanick JL, Crisosto CH. Evaluation of food additives as alternative or complementary chemicals to conventional fungicides for the control of major postharvest diseases of stone fruit. Journal of Food Protection. 2009; 174 (72): 1037-1046.
32. Ongena M. Biopesticides: a more natural protection for crops. University of Liège - http://reflexions.ulg.ac.be. 2014, 10p.
33. Koffi YF, Traore M, Yapi YE, Ban-Koffi L, Diguta C, Alloue-Boraud MWA, Matei F. Biocontrol of Post-harvest Fungal Diseases of Pineapple (*Ananas comosus* L.) Using Bacterial Biopesticides. American Journal of Microbiological Research. 2021; 9 (2): 34-43.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/122618