

Biocontrol of spoilage in Avocado (*Persea americana* Mill) Using *Pseudomonas fluorescens* and *Bacillus subtilis* BT2

ABSTRACT

The avocado (*Persea americana* Mill.) is a dicotyledonous plant of the *Lauraceae* family. It is a fruit rich in nutrients, which has many benefits for human health. It is especially appreciated for its taste quality. However, post-harvest diseases of avocados due to fungal attacks are one of the main constraints on conservation and marketing in Côte d'Ivoire. The aim is to achieve biological control of avocado spoilage fungal strains during post-harvest conservation using *Pseudomonas fluorescens* and *Bacillus subtilis* BT2. Thus, 200 sampled avocados were used for this study. Identification of the fruit spoilage fungal flora on PDA (Potato Dextrose Agar) medium, pathogenicity tests, antagonism and biocontrol tests using *P. fluorescens* and *B. subtilis* BT2 were carried out. The results obtained made it possible to identify six (6) fungal genera on spoiled avocados including *Colletotrichum*, *Botryodiplodia*, *Absidia*, *Rhizopus*, *Alternaria* and *Aspergillus*. The respective isolation frequencies were 42.03%, 22.70%, 15.46%, 8.21%, 5.8% and 5.8%. *Bacillus subtilis* and *Pseudomonas fluorescens* presented inhibition rates of these fungal genera which vary from 38.72±2.21% to 65.17±0.72% for *B. subtilis* BT2 and from 44.05±3.25% to 66.51±0.71% for *P. fluorescens*. Also, these two bacteria inhibited the growth of these spoilage fungi in vivo. The protection tests of healthy avocado fruits with the supernatant of *P. fluorescens* and *B. subtilis* BT2 made it possible to preserve the avocado fruits for eight days without any alteration of the pulp. The results of this study therefore made it possible to highlight the effectiveness of *P. fluorescens* and *B. subtilis* BT2 as good biocontrol agents for the conservation of avocado fruits in Côte d'Ivoire.

Keywords: Avocado, Spoilage fungi, Bacillus subtilis BT2, *Pseudomonas fluorescens*, Biocontrol.

1. INTRODUCTION

The avocado (*Persea americana* Mill), known as the alligator pear or butter pear, belongs to the *Lauraceae* family [1]. It is a subtropical or tropical fruit native to Mexico and Central America. In Côte d'Ivoire, avocado cultivation began in 1960. At that time, Côte d'Ivoire avocado was exported. From 1980, with the withdrawal of agricultural sectors, avocado cultivation lost intensity. Avocado orchards occupy only 300 ha with production estimated at only 4.6% of African production and the main producing areas are located in the Central and Southern regions of the country [2,3]. As an oilseed fruit, avocado is increasingly consumed, not only for its flavor but also for its high nutritional value. The fruit has gained popularity due to increasing consumers and awareness of its dietary value. Indeed, avocado has a considerable content of monounsaturated fatty acids as well as minerals, vitamins and other antioxidant phytochemicals [4,5]. Despite its nutritional and economic importance, the avocado sector faces several problems of post-harvest spoilage due to the increase in pathogens. Difficulties in applying good agricultural practices and especially inadequate post-harvest handling of fruits are the main causes of these parasitic infections [6]. Contamination of avocado fruits by microorganisms occurs in particular in the fields, during post-harvest packaging and during storage [2]. The development of these species of fungi is favored by the ripening process of the fruits but especially by their composition. These rots affect the organoleptic properties and the market value of the fruits, which causes enormous losses of up to 90% [7]. To date, the preservation methods used are physical and chemical. However, these methods remain insufficient. Furthermore, the plant protection products used have harmful effects on the health of the consumer and can be responsible for public health problems [8]. These chemical control methods used also pose growing resistance constraints for pathogens. These disadvantages of chemical pesticides have led to the search for alternatives for the development of other products that are more respectful of the environment and the health of the consumer in order to fight against phytopathogens. Thus, biological control through the use of bacteria generally recognized as safe has been widely studied (GRAS). *Bacillus subtilis* and

Pseudomonas fluorescens, existing biological control agents are then long used as biopesticides due to their antibacterial and antifungal properties. Thus, several research studies have demonstrated the effectiveness of *Bacillus subtilis* and *Pseudomonas fluorescens* in the biological control of fruit pathogens [9,10]. The work of [11] showed that the use of *Bacillus subtilis* GA1 as a biopesticide allowed the inhibition of various pathogenic microorganisms of mango, thus improving their shelf life. Also, [12] also reported that the *Bacillus subtilis* species isolated from the cocoa rhizosphere had the capacity to inhibit pod deterioration germs but also to induce resistance against fungal and viral phytopathogens in particular swollen shoot virus (CSSV). The work of [13] showed that *Pseudomonas fluorescens* could be used in the reduction of post-harvest spoilage of cashew nuts, namely *Aspergillus*, *Penicillium* and *Rhizopus*. *Pseudomonas fluorescens* was also tested *in vivo* for inhibition of the growth of *Geotrichum* sp, a tomato pathogen [14]. However, there is little data on the use of bacterial biopesticides in the biological control of avocado fungal diseases in Côte d'Ivoire. It is in this context of biocontrol of fungal diseases that this study takes place, the general objective of which is to carry out biocontrol of fungal strains of avocado spoilage during post-harvest conservation using *Pseudomonas fluorescens* and *Bacillus subtilis* BT2.

2. MATERIAL AND METHODS

2.1 Material

The plant material consisted of healthy and spoiled mature avocado fruits of the Mexicolavariety (Fig.1) obtained directly from the markets. Two bacterial strains including *Pseudomonas fluorescens* isolated from the rhizosphere of the cashew tree and *Bacillus subtilis* BT2 isolated from the rhizosphere of the cocoa tree preserved in cryotubes in a freezer (-80°C) were used in this study.



Fig. 1. Healthy (a) and altered (b) avocados

2.2 Methods

2.2.1 Sampling

Samples were collected from 5 sellerstaken at random from the main markets. From each of the 5 sellers, 5 samples of spoiled avocados and 5 samples of healthy avocados were taken at random, making a total of 200 samples in all 4 municipalities. Once collected, the different fruits were individually packaged in Stomacher plastic bags, labeled, sealed then kept in a cooler containing dry ice where the temperature is maintained at 4°C, by cold accumulators. The transport of the samples was carried out on the same day of collection and did not exceed two hours for the analysis of the different test portions.

2.2.2 Isolation of avocado spoilage fungal strains

The microorganisms were isolated by direct contact on Potato Dextrose Agar (PDA) agar [15]. Thus, three spoiled avocados were randomly chosen from each sample, washed with tap water containing 2% bleach for 2 min, rinsed three times with sterile distilled water and

disinfected using household papers soaked in 70% ethanol in order to eliminate exogenous microflora. Using a previously sterilized scalpel and tweezers. Under aseptic conditions, external (skin) and internal (pulp) fragments were collected from each spoiled fruit and inoculated into Petri dishes containing Potato Dextrose Agar (PDA) medium. The plates were then incubated at 28°C for 5 to 7 days. To obtain a pure culture, transfer of colonies to PDA medium according to the method described by [16, 17] was produced. Thus, a mold colony filament was taken using sterile forceps then placed at a single point in the center of a Petri dish containing the PDA medium in order to obtain typical mold development. Incubation is carried out at 28°C for 7 days. This method is repeated until pure colonies are obtained.

2.2.3 Identification of avocado spoilage molds

Identification of molds was carried out on the basis of macroscopic and microscopic observations according to the method of [16, 18]. Macroscopic identification was carried out according to the method of [16] through an examination of culture on PDA. The cultural characteristics determined were the appearance of the colonies (fluffy, woolly, cottony, velvety, powdery or granular), the shape of the colonies (regular or irregular), the relief of the colonies (flat, convex, pleated, etc.), the color of the colonies (white, cream or colored, yellow, orange, brown, green, gray to black), colony size (small, extensive or invasive) and growth (rapid or slow). The isolated fungi were identified on the basis of their morphological characteristics according to the method described by [18]. To carry out the microscopic examination, a drop of methylene blue was placed on a clean slide, a small fragment of the isolate was placed on the drop using forceps and covered with a coverslip then observed under an electron microscope with an objective 40x and the size of the conidiospores (short or long).

2.2.4 Culture of *Pseudomonas fluorescens* and *Bacillus subtilis* BT2

The strains of *P. fluorescens* and *B. subtilis* BT2 were respectively subcultured on KING B agar and on Mossel medium by the streak method from colonies already isolated, and preserved. The plates were incubated at 30°C for 24 hours to obtain young cultures and isolated colonies which were used to carry out the antagonism tests.

2.2.5 Antagonism test

This test was carried out in order to verify the existence of a possible inhibitory action of *P. fluorescens* and *B. subtilis* on one or more isolated molds. The method of [11] was used. In Petri dishes containing YPGA medium, the bacteria *P. fluorescens* and *B. subtilis* (antagonists) were spread by making a longitudinal streak dividing the dish into two equal parts. Subsequently, two discs each having the diameter of the tip of a Pasteur pipette (0.3 cm), obtained with a cookie cutter from a fungal culture are placed on either side of the streak at a distance of 1 cm from the edge of the box in three steps. The control dishes consisted of inoculating the mold by transplanting it to the center of the Petri dish where there was no inoculation with *P. fluorescens* and *B. subtilis*. Incubation is carried out at 30°C for 4 days. Colony growth of pathogens (molds) and antagonistic agents (*P. fluorescens* and *B. subtilis*) is observed every day until the seventh day. After 4 days, the percentage of growth of the fungus in the boxes was determined using the method of [19], then the inhibition rate was deduced according to the following formula:

$$\frac{R - r}{R} \times 100$$

r : Radial growth of the microorganism with antagonism

R : Radial growth of the microorganism without confrontation of antagonism

2.2.6 Biocontrol test

A pre-culture of *P. fluorescens* and *B. subtilis* is carried out by seeding 24-hour colonies in 25 mL of YPG broth for 8 hours at 30°C. The pre-culture was used to inoculate two 100 mL YPG broths and incubated at 30°C for 48 h. After 48 hours of incubation, the culture is centrifuged at 7000 rpm for 10 min [20]. The supernatant is then collected and stored at -4°C for *in vivo* antagonism tests. Healthy avocados were washed in tap water containing 2% bleach, rinsed three times with sterilized distilled water, disinfected using household papers soaked in 90% ethanol and injured with a scalpel (three injuries per lawyer). Then, 50 µL of the supernatant of *P. fluorescens* and *B. subtilis* were applied into the wounds, then each inoculated with 50 µL of the mold spore solution (pathogen) prepared according to the method of [21] as described in paragraph 5.1. Finally, the fruits were kept in sterile jars for 5

days[22]. The controls consisted of inoculating the avocados with suspensions of spores of the pathogenic strains without application of the supernatant of *P. fluorescens* and *B. subtilis*.

2.2.7 Fruit protection test

2.2.7.1 Production and harvesting of biomass and supernatant of biocontrol agents

The production of biomass and supernatant of the two bacterial biocontrol agents was used to carry out the various protection tests on fruits. 250 mL Erlenmeyer flasks containing 65 mL of YPG medium were each inoculated with a 24-hour colony of the biocontrol agents. The pre-cultures were incubated at 30°C with shaking at 105 rpm for 8 hours. These pre-cultures were used to inoculate 2L Erlenmeyer flasks containing 500 ml of YPG medium. Two Erlenmeyer flasks, each containing 500 ml of sterile YPG medium, were each inoculated with 65 ml of the pre-culture. The Erlenmeyer flasks were incubated at 30°C with shaking at 105 rpm for 48 hours. The biomass was harvested after centrifugation of the medium at 3500 rpm for 10 minutes. The pellet which represents the biomass was separated from the supernatant and washed three times with physiological water (9 ‰ NaCl). The biomass and supernatant were stored at -4°C for conservation tests.

2.2.7.1 Preservation of fruits with the supernatant

The protection test on fruits was carried out by immersion as described by [23]. It consisted of immersing the fruits in a container containing the supernatant of the biocontrol agents. You must ensure that the entire surface of the fruit is immersed. Thus, the healthy fruits were carefully washed in tap water containing 2% bleach, rinsed three times with sterile distilled water and disinfected using household paper soaked in 90% ethanol then immersed for 5 minutes in the supernatant and dried in the open air. The controls having undergone the same steps were not treated with the supernatant. The fruits were stored at room temperature (25°C) after immersion.

2.2.8 Statistical analysis

R software version 4.0.4 for Windows, one-way analysis of variance (ANOVA), was performed to compare the biomass inhibition rates of *P. fluorescens* and *Bacillus subtilis* BT2 against fungal isolates.

3. RESULTS

3.1 Avocado spoilage fungal strains

The results of the analysis showed contamination of the avocado samples by fungi which presented in various aspects, textures and colors. A total of 207 fungal isolates were isolated taking into account the resemblance of thalli and spores. The phenotypic identification made it possible to highlight 6 fungal genera, namely *Aspergillus*, *Rhizopus*, *Absidia*, *Colletotrichum*, *Botryodiplodia* and *Alternaria* (Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6 and Fig. 7).

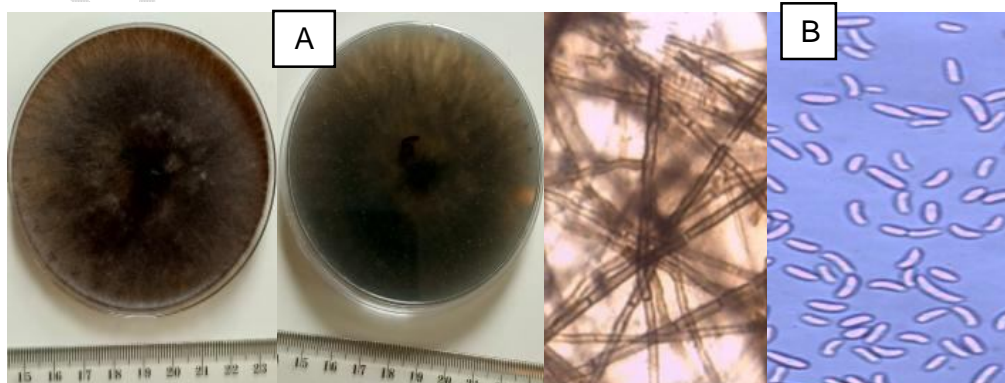


Fig. 2. Macroscopic appearance (A) and microscopic appearance (B) of *Colletotrichum* sp. on PDA medium

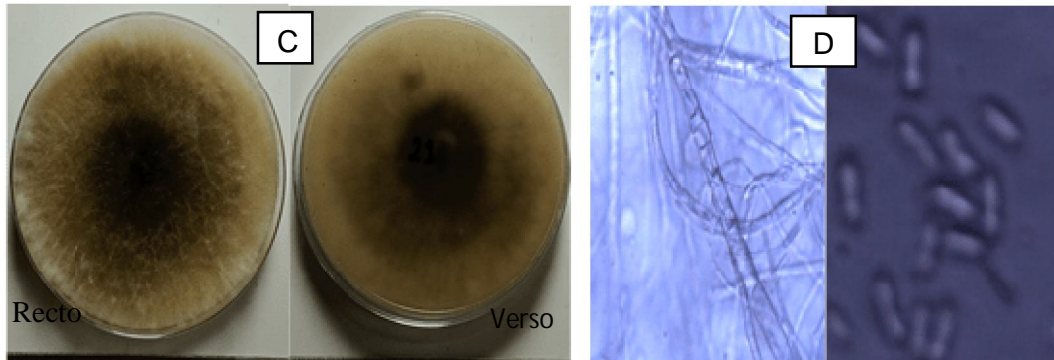


Fig. 3. Macroscopic appearance (C) and microscopic appearance (D) of *Botryodiplodia* sp. on PDA medium

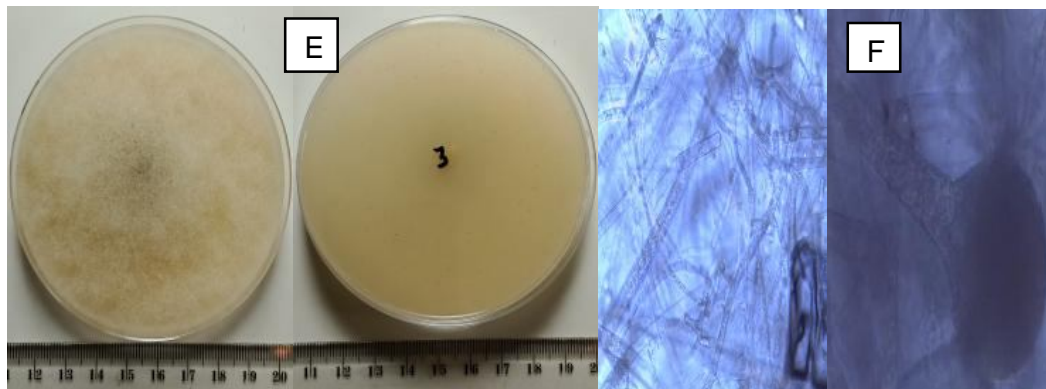


Fig. 4. Macroscopic appearance (E) and microscopic appearance (F) of *Absidia* sp. on PDA medium

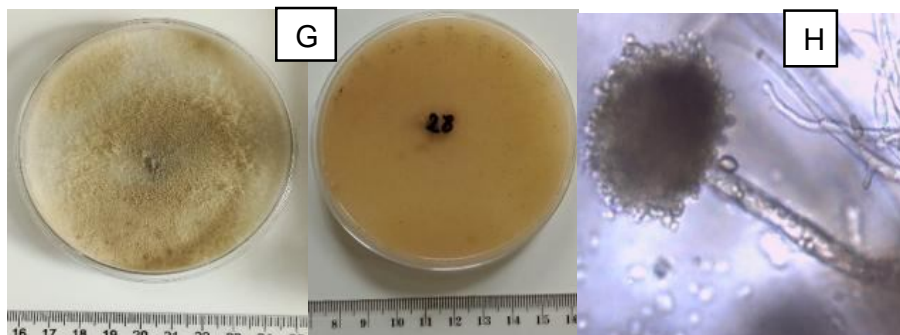


Fig.5. Microscopic appearance (G) and macroscopic appearance (H) of *Aspergillus* sp. on PDA medium

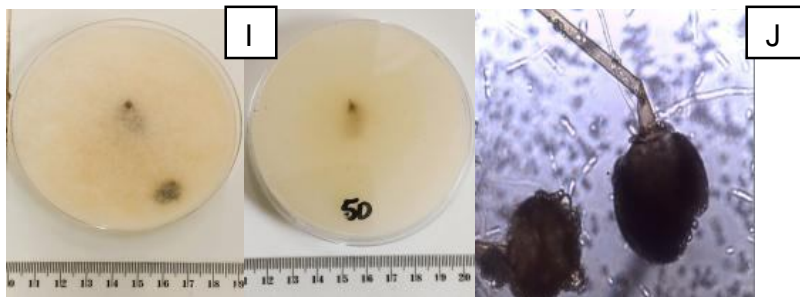


Fig. 6. Macroscopic appearance (I) and microscopic appearance (J) of *Rhizopus* sp. on PDA medium

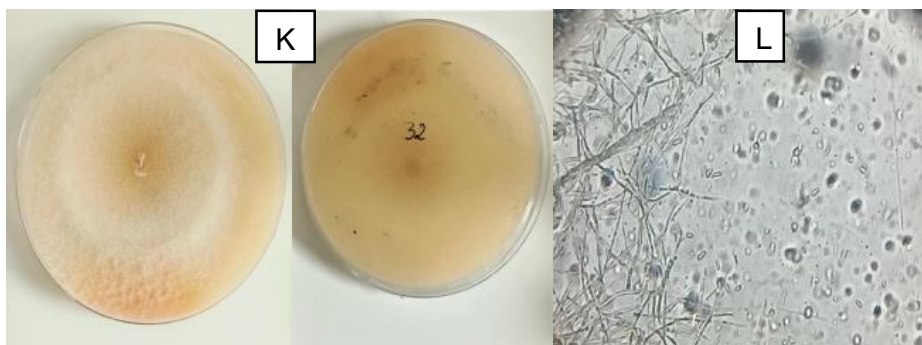


Fig.7. Macroscopic appearance (K) and microscopic appearance (L) of *Alternaria* sp. on PDA medium

3.2 Frequency of isolation of fungal strains

In total, 207 mold isolates were obtained from samples. Among these isolated molds, *Colletotrichum* sp and *Botryodiplodia* sp are the majority with respective isolation frequencies of 42.03% and 22.07%. Concerning the other fungal strains, they were isolated with frequencies of 15.46 and 8.21% respectively for *Absidia* sp and *Rhizopus* sp; As for *Alternaria* sp and *Aspergillus* sp, they presented low isolation frequencies, namely 5.8% for *Alternaria* sp and 5.80 % for *Aspergillus* sp (Table 1.).

Table 1. Frequency of isolation of avocado spoilage fungi

Fungal isolates	Isolation frequency of fungi isolates (%)
<i>Colletotrichum</i> sp	42,03 (87)
<i>Botryodiplodia</i> sp	22,70 (47)
<i>Absidia</i> sp	15,46 (32)
<i>Rhizopus</i> sp	8,21 (17)
<i>Aspergillus</i> sp	5,80 (12)

<i>Alternaria</i> sp	5,8 (12)
Total	100 (207)

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

3.3 *In vitro* antagonistic activity of *P. fluorescens* and *B. subtilis* BT2

The *in vitro* antagonism tests carried out made it possible to demonstrate the rate of inhibition of the growth of fungal isolates by *P. fluorescens* and *B. subtilis* BT2. These tests showed that *P. fluorescens* presents a higher inhibition rate on all the molds isolated than *B. subtilis* BT2 with the highest inhibition rate observed in *Rhizopus* sp 66.51±0.71%. There is no significant difference in the inhibition rates between *P. fluorescens* and *B. subtilis* BT2 at the threshold of 0.05 for all the fungi isolated except for the molds *Colletotrichum* sp and *Absidia* sp in which the inhibition rates were significantly different at the threshold of 0.05. The confrontation of bacteria with *Rhizopus* sp presented the highest inhibition rates 66.51±0.71% for *P. fluorescens* and 65.17±0.72% for *B. subtilis* BT2. On the other hand, the lowest rates of reduction in fungal growth were observed in *Aspergillus* sp 38.72±2.21% for *B. subtilis* BT2 and 44.05±3.25% for *P. fluorescens* (Table 2.). The control consisted of transplanting the mold into the center of the PDA agar without seeding bacteria. The molds grew normally, hence there was no inhibition.

Table 2. Statistical values of *in vitro* comparisons of biopesticides and fungi isolates







Strains	<i>B. subtilis</i> BT2	<i>P. fluorescens</i>
<i>Colletotrichum</i> sp	(52,50±0.80) ^b	(61,47±0.70) ^a
<i>Btryodiplodia</i> sp	(55,82±1,63) ^a	(57,93±0.70) ^a
<i>Absidia</i> sp	(56,69±0.33) ^b	(61,82±1.60) ^a
<i>Rhizopus</i> sp	(65,17±0.72) ^a	(66,51±0.71) ^a
<i>Aspergillus</i> sp	(38,72±2,21) ^a	(44,05±3,25) ^a
<i>Alternaria</i> sp	(53,83±1,65) ^a	(54,15±0.17) ^a

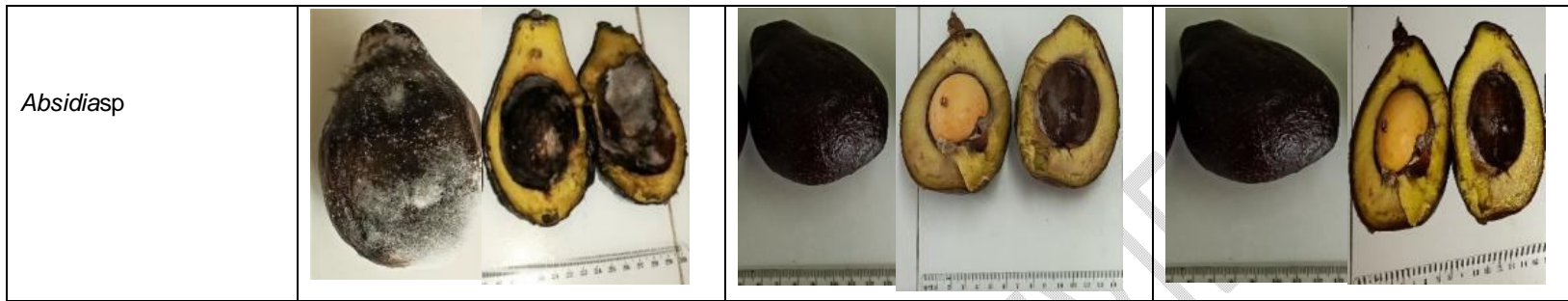
For the same line, the values assigned to the same alphabetical letter are not statistically different and $p < 0.05$ corresponds to the significance threshold.




3.4 Biocontrol

The results obtained showed that after 5 days of storage, the fruits having received an application of the supernatant of the biocontrol agents and inoculated with the suspension of spores of the pathogens showed no growth of mold (Table 3.).

Table 3. Appearance of Avocados 5 days after treatment with biopesticide supernatant and inoculation with pathogen spore suspension

fungal strains	Appearance of avocados		
	Control (untreated) after 5 days	Fruits after 5 days of treatment	
		<i>B.subtilis</i>	<i>P.fluorescens</i>
<i>Colletotrichum</i> sp			
<i>Botrydiplodia</i> sp			






Appearance of avocados			
fungalstrains	Control (untreated) after 5 days	Fruits after 5 days of treatment	
		<i>B.subtilis</i>	<i>P.fluorescens</i>
<i>Alternaria</i>			

Rhizopus sp.



UNDER PEER REVIEW







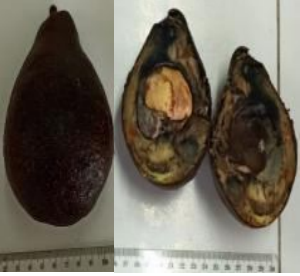


fungalstrains	Appearance of avocados		
	Control (untreated) after 5 days	Fruits after 5 days of treatment	
		<i>B.subtilis</i>	<i>P.fluorescens</i>
<i>Aspergillus</i> sp			

UNDER PEEL

3.5 Fruit storage

Avocados treated with the supernatant of *B. subtilis* BT2 and *P. fluorescens* did not show signs of damage after eight (8) days. The witness was altered after 5 days. (Table 4.).

Table 4. Appearance of fruits 8 days after treatment with biopesticide supernatant

Duration	Control (fruits not treated with supernatant)	Fruits treated with supernatant	
		Supernatant of <i>B. subtilis</i>	Supernatant of <i>P. fluorescens</i>
Three (3) days			
Five (5) days			
Eight (8) days			

4. DISCUSSION

In order to contribute to the fight against post-harvest losses of avocados in Côte d'Ivoire due to fungal contaminants, this study was carried out. The results obtained following the phenotypic identification of fungal strains isolated from avocados showed that a diversity of molds is responsible for the deterioration of avocados in Côte d'Ivoire. Six (6) genera of molds have been identified and they are, *Colletotrichum*, *Botryodiplodia*, *Absidia*, *Rhizopus*, *Alternaria* and *Aspergillus*. These results are in agreement with the work carried out by [24, 25, 26, 27] who isolated and identified the genera *Colletotrichum*, *Aspergillus* and *Botryodiplodia* on avocado and mango fruits. These fungi are known to cause the deterioration of many products such as fruits, seeds, vegetables and tubers, both in the fields and during storage. The

presence of these molds could be explained by several environmental factors, such as aeration, pH, water availability, nutrients and temperature which would favor the growth of molds in preserved agricultural products. The infection of fruits by these molds could have their origin from the fields, and according to [28] fungal attacks occur normally produce when the fruits are in the field but often show no symptoms until storage or transport after harvest and its climacteric ripening mode which is characterized by a high accumulation of ethylene, which stimulates more rapid ripening due to high rate of respiration [29]. Among the 6 genera identified, *Colletotrichum* sp and *Botryodiplodia* sp are in the majority with respective isolation frequencies of 42.03% and 22.07%; These two fungal genera would be the main agents of spoilage of avocados. This corroborates with those of [30] who showed that *Colletotrichum* sp main spoiler of tropical fruits and leafy vegetables has been reported on many fruits such as avocado, almond, coffee, guava, apple, dragon fruit, cassava, mango, sorghum and strawberries. Also, according to [31] *Botryodiplodia* sp was identified as the most isolated pathogen on avocado in Chile, Spain, Mexico, New Zealand, Peru, South Africa and the United States. These strains have been identified as main spoilage agents in South Africa and on the Hass variety in Colombia [32, 33, 34]. Generally, contamination of agricultural products depends on several factors and can occur before, during and after harvest, depending on handling, packaging and storage methods [35]. It could therefore be attributed to post-harvest practices. The use of bacterial biocontrol agents in this study showed the capacity of these agents to inhibit the main fungal strains responsible for spoilage of avocado fruits, in particular isolates of *Colletotrichum* sp, *Absidia* sp, *Botryodiplodia* sp, *Rhizopus* sp, *Aspergillus* sp1, *Aspergillus* sp2 and *Alternaria* sp. This inhibition by *P. fluorescens* and *B. subtilis* BT2 was demonstrated by *in vitro* antagonism and biocontrol tests. The results of the *in vitro* tests presented inhibition rates greater than 50% of the growth of the strains with a high inhibition rate observed in *Rhizopus* sp. This would be due to the fact that these biocontrol agents produce antifungal substances that would inhibit the growth of mold. These results are similar to those obtained by [11] who showed the inhibitory power of *Bacillus subtilis* GA1 against spoilage germs in the conservation of mangoes in Côte d'Ivoire. As part of a similar study of the fight against *Geotrichum* sp on tomato, [14] also showed that *Pseudomonas fluorescens* F19 had the ability to inhibit the growth of *Geotrichum* sp. This reduction in the incidence of microorganisms in the spoilage of avocado fruits could be explained by the fact that these biocontrol agents produce antifungal substances which inhibit the growth of mold through the production of lipopeptide molecules by *B. subtilis* GA1 notably fengycin, surfactins and iturins which manifest themselves by the bursting of the cell wall of fungi [9]. The latter can either activate plant defenses or have a direct antibacterial or antifungal effect [36]. Likewise, *Pseudomonas fluorescens* F19 produces metabolites such as phenazines and pyrrolnitrin and also siderophores which are iron chelating agents preventing its use by pathogens [37]. *Pseudomonas fluorescens* would have acted either by mycoparasitism, which is a trophic relationship established by a microorganism to the detriment of a fungus [38]. This action results from the production of lytic enzymes such as glucanase and chitinase, or from the induction of resistance or even antibiosis. However, biocontrol agents were less effective on *Aspergillus* isolates with inhibition rates below 50%. This could be explained by the very slow growth of these strains during *in vitro* confrontation tests or their resistance to these biocontrol agents. The biocontrol tests showed that the supernatant of the bacterial biocontrol agents has the ability to inhibit the growth of mold over a period of 5 days with fewer signs of fruit spoilage compared to the control. Mold growth had already been observed from the third day of storage. [14] reported that tomato treated for 24 hours with *P. fluorescens* F19 before pathogen inoculation showed a significant reduction in the incidence of rot caused by *Geotrichum* sp. These results reflect the capacity of biocontrol agents to produce antifungal molecules which could protect fruits against fungal damage. Healthy fruit preservation tests revealed that the supernatant of the bacterial biocontrol agents tested exert an inhibitory action on pathogens. Indeed, the colonization of avocado fruits made it possible to prevent the proliferation of pathogenic fungi inside the fruits. This made it possible to preserve the firmness of the fruit. This could mean that the antifungal substances produced by the bacteria would be contained in the supernatant. These results are in accordance with those of the work of [10] who using *B. subtilis* GA1, *Pseudomonas fluorescens* CI and *Pseudomonas fluorescens* F19 were able to preserve pineapple for 14 days.

5. CONCLUSION

This work was carried out with the aim of contributing to the biopreservation of avocado fruits exposed to fungal contaminants responsible for spoilage and post-harvest losses through the use of *Pseudomonas fluorescens* and *Bacillus subtilis* as bacterial biocontrol agents. The fungal profile of the avocado samples was evaluated. Six fungal genera including *Colletotrichum*, *Botryodiplodia*, *Absidia*, *Rhizopus*, *Alternaria* and *Aspergillus* have been identified. The genera *Colletotrichum* and

Botryodiplodia were predominant and caused the most alterations. The different antifungal activities of *Pseudomonas fluorescens* and *Bacillus subtilis* BT2 were evaluated *in vitro* and *in vivo*. In both cases, these two bacteria have shown their ability to reduce or inhibit the fungal strains responsible for spoilage of avocado fruits. The preservation of avocado fruits using these bacterial biocontrol agents made it possible to preserve the fruits for at least eight days compared to the control which was completely spoiled after 5 days. In view of these results, these two bacteria could be used in the formulation of biopesticides for the preservation of avocado fruits 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 manuscripts.

REFERENCES

1. Chanderali AS, Soltis DE, Soltis PS, Wolstenholme BN (2013). Taxonomy and botany. The Avocado: Botany, Production and Uses. 2013 ; 2 : 31-50.
2. Kouadia AMJ, Abo K, Kouadio KT. Evolution des infections naturelles sur les mangues, les avocats et les bananes en Côte d'Ivoire et principaux champignons responsables. Journal of Applied Biosciences. 2019 ; 134(1) : 13710-13721. <https://doi.org/10.4314/jab.v134i1>.
3. FAOSTAT. <http://www.fao.org/faostat/en/#data/QC/visualize>. 2014. Accessed 29 Sept 2017.
4. Villa-Rodríguez JA, Molina-Corral FJ, Ayala-Zavala JF, Olivas GI, González Aguilar NGA. Effect of maturity stage on the content of fatty acids and antioxidant activity of 'Hass' avocado. Food Research International. 2011 ; 44(5) : 1231-1237.
5. Schaffer B, Wolstenholme BN, Whitley AW. The Avocado Botany, Production and Uses, 2nd ed.; CABI Publishing: New York, 2013.
6. Yaoub A, Mpounze EGP. Isolation and pathogenicity evaluation of postharvest fungal of some fruits in Cameroun. International Journal of Environmental Research. 2017; 2(1) : 56-60. <http://dx.doi.org/10.22161/ijeab/2.1.9>.
7. Anonyme. Côte d'Ivoire, étude de collection dans le secteur agricole en Côte d'Ivoire. Rapport final. JICA JAICAF. 2013 ; 19-30. French.
8. Yapi EY, Koffi FY, Ouattara Z, Alloue-Boraud WAM. Biocontrol of fungal diseases of zucchini (*Cucurbitapeposp*) in Côte d'Ivoire by *Pseudomonas fluorescens* F19., Cogent Food & Agriculture. 2021 ; 7: 1942404 <https://doi.org/10.1080/23311932.2021.1942404>.
9. Ongena M. (2014). Biopesticides: Une protection plus naturelle pour les cultures. Université de Liège. 2014 ; 10. Available : <http://reflexions.ulg.ac.be>.
10. Koffi YF, Alloue-Boraud WAM, Koffi LB, Adohi AF, Dje MK, Ongena M. Highlighting *Bacillus subtilis* GA1 antifungal potentialities for pineapple (*Ananas comosus*) conservation in Côte d'Ivoire. International Journal of Agronomy and Agricultural Research. 2016 ; 9(1) : 100-108.
11. Alloue-Boraud M, Ban-Koffi L, Dadie T, Dje K, Ongena M. Utilisation de *Bacillus subtilis* GA1 pour la lutte contre les germes d'altération de la mangue en Côte d'Ivoire. Journal of Animal and Plant Sciences. 2015 ; 3(25) : 3954-3965. French.
12. Koua SH, Coulibaly ND, Alloue-Boraud WM, Konan F, Dje KM. *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. <https://doi.org/10.1007/s00284-020-02027-x>
13. Ake MDF, Tehua AA, Koffi YF, Sanogo YM, Alloue-Boraud WAM. Phenotypical identification of moisture associated with cashew nuts (*Anacardium occidentale* L.) in Côte d'Ivoire and control of

Pseudomonas fluorescens Ci effect. International Journal of Innovative and Applied Research. 2017; 7 (9) : 1-8.

14. Ban Koffi L, Alloue-borand WAM, Assamoi J, Koussémon M, Ongéna M. (2015). Study of protective effect of *Pseudomonas fluorescens* F19 against microorganisms responsible of tomato fruits (*Lycopersicon esculentum*) spoilage in Côte d'Ivoire. Journal of Innovative Research. 2015 ; 3(9) : 9-16.

15. Djossou O. Microflore post-récolte du café robuste et utilisation des bactéries pour le contrôle des moisissures mycotoxigènes et producteurs de l'Ochratoxine A. Thèse de doctorat. Université Paul Cézanne Aix Marseille III. 2011; 123. French.

16. Botton B, Breton A, Fevre M, Gauthier S, Guy PH, Larpent JP, Reymond P, Sarglier JJ, Vayssier Y, Veau P. Usefullmoist and nusable: industrial importance. 2nd ed. Masson: Collection Biotechnologies (Paris), 1990.

17. Fouzia L, Sara C. (2015). Isolement, identification et activité antibactérienne des moisissures d'un sol forestier à Constantine. Thèse de doctorat, Université des Frères Mentouri Constantine. 2015 ; 118. French.

18. Guiraud JP. Microbiologie alimentaire. Dunod. Paris, 1998.

19. Korsten L, Jager ESD. (1995). Mode of action of *Bacillus subtilis* for control of avocado postharvest pathogens. South African Avocado Grower's Association Yearbook. 1995; 18: 124-130.

20. Touré Y, Ongéna M, Jacques P, Guiré 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.

21. Gandomi H, Misaghi A, Basti AA, Bokaei S, Khosravi A, Abbasifa A, Javan AJ. Effect of *Zataria multiflora* Boiss. Essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. Food Chemical Toxicology. 2009 ; 47: 2397-2400.

22. Regnier T, Plooy W, Combrinck S, Botha B. Fungitoxicity of *Lippia scaberrima* essential oil and selected terpenoid components on two mango postharvest spoilage pathogens. Postharvest Biology and Technology. 2008 ; 48: 254-258.

23. Cissé M. Immobilisation d'un système lactoperoxyde dans un enrobage de chitosane dans le but de prolonger la conservation des mangues. Thèse de doctorat, Université Montpellier Supagro. 2012 ; 159. French.

24. Awa OC, Samuel O, Oworu OO, Sosanya O. First report of fruit anthracnose in mango caused by *Colletotrichum gloeosporioides* in Southwestern Nigeria. International Journal of Scientific and Technology Research. 2012 ; 1 : 30-34.

25. Diedhiou PM, M'Baye N, Drame A, Samb PI. Alteration of postharvest diseases of mango *Mangifera indica* through production practices and climatic factor. Full Length Research Paper. African Journal of Biotechnology. 2007 ; 6 (9) : 1087-1094.

26. Djeugap JF, Kuate JR, Fontem DA. Etat sanitaire post-récolte de la mangue commercialisée dans la ville de Dschang et efficacité in vitro des huiles essentielles contre *Colletotrichum gloeosporioides* Penz., agent causal de l'anthracnose. AFPP. 9ème conférence internationale sur les maladies des plantes. 2009 ; 571-578. French.

27. Djeugap JF, Tsopmbeng NG, Keuete KE, Yaouba A, Serferbe S. Isolation and identification of fungi associated with avocado fruit from local markets of the west region of Cameroun. International Journal of Agriculture and Biosciences. 2015 ; 4(2): 64-68.

28. Tagro SG, N'Dri DY, Niamien PM, Koffi RN, Louis BK, Yao MK. Comparison of the degree of fermentation and fungal profiles of raw cocoa beans sourced from three Ivorian main producing regions. African Journal of Food Science. 2008 ; 2: 112-118.

29. Blakey R, Tesfay S, Mathaba N, Bertling I, Bower J. Some initial changes in 'Hass' avocado (*Persea americana* Mill.) physiology due to ethephon. *International Journal of Postharvest Technology and Innovation*. 2012 ; 2 : 334–344.
30. Cannon PF, Damm U, Johnston PR, Weir BS. *Colletotrichum* – current status and future directions. *Studies in Mycology*. 2012 ; 73 (1) :181–213.
31. Valencia AL, Gil PM, Latorre BA, Rosales IM. Characterization and pathogenicity of *Botryosphaeriaceae* species obtained from avocado trees with branch canker and dieback and from avocado fruit with stem end rot in Chile. *Plant Disease*. 2019 ; 103(5) : 996-1005.
32. Giblin FR, Tan YP, Mitchell R, Coates LM, Irwin JAG, Shivas RG. *Colletotrichum* species associated with pre- and post-harvest diseases of avocado and mango in eastern Australia. *Australasian Plant Pathology*. 2018; 47 :269–276.
33. Sharma G, Maymon M, Freeman S. Epidemiology, pathology and identification of *Colletotrichum* including a novel species associated with avocado (*Persea americana* Mill) anthracnose in Israel. *Sci. Reports*. 2017 ; 7 : 15839.
34. Weir BS, Johnston PR, Damm U. The *Colletotrichum gloeosporioides* species complex. *Journal Studies in Mycology*. 2012 ; 73 :115–180.
35. Amadi JE, Nwaokike P, Olan GS, Garuba T. Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava*) in Awka metropolis. *International Journal of Engineering and Applied Sciences*. 2014 ; 4(10) : 7-12.
36. Pérez-García A, Romero D, De Vicente A. Plant protection and growth stimulation by microorganisms: biotechnological applications of *Bacillus* in agriculture. *Current Opinion in Biotechnology*. 2011; 22(2): 187-193.
37. Loper JE, Gross AEH. Genomic analysis of antifungal metabolite production by *Pseudomonas fluorescens* Pf-5. *European Journal of Plant Pathology*. 2007; 119: 265–27.
38. Bartnicki-Garcia S. Cell wall chemistry, morphogenesis, and taxonomy of fungi. *Annual Reviews in Microbiology*. 1968 ; 22(1) : 87-108.