Biocontrol of Avocado(*Percea americana*Mill)SpoilageUsing*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 manybenefits for humanhealth. It isespeciallyappreciated for its taste quality. However, post-harvestdiseases of avocados due to fungalattacks are one of the main constraints on conservation and marketing in Côte d'Ivoire. The aimis to achievebiological control of avocadospoilagefungalstrainsduring post-harvest conservation using*Pseudomonas fluorescens* and *Bacillus subtilis* BT2. Thus, 200 sampledlawyerswereused for thisstudy. Identification of the fruit spoilagefungal flora on PDA (Potato Dextrose Agar) medium, pathogenicity tests, antagonism and biocontrol tests using*P. fluorescens* and *B.subtilis* BT2 werecarried out. The resultsobtained made it possible to identify six (6) fungalgenera on spoiledavocadosincluding*Colletotrichum*, *Botryodiplodia*, *Absidia*, *Rhizopus*, *Alternaria* and *Aspergillus*. The respective isolation frequencieswere 42.03%, 22.70%, 15.46%, 8.21%, 5.8% and 5.8%. *Bacillus subtilis* and *Pseudomonas fluorescens*presented inhibition rates of thesefungalgenerawhichvaryfrom38.72±2.21% to 65.17±0.72% for *B. subtilis* BT2 and from44.05±3.25 % at 66.51±0.71% for *P. fluorescens.*Also, thesetwobacteriainhibited the growth of thesespoilage fungi in vivo. The protection tests of healthyavocado fruits with the supernatant of *P. fluorescens* and *B.subtilis*BT2 made it possible to preserve the avocado fruits for eightdayswithoutanyalteration of the pulp. The results of thisstudytherefore 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 fluorenscens, Biocontrol.

1. INTRODUCTION

The avocado (Percea americana Mill), known as the alligator pear or butter pear, belongs to the Lauraceaefamily[1]. It is a subtropical or tropical fruit native to Mexico and Central America. In Côte d'Ivoire, avocado cultivation beganin 1960. At that time, Côte d'Ivoireavocadowasexported. From 1980, with the withdrawal of agricultural sectors, avocado cultivation lostintensity. Avocadoorchardsoccupyonly 300 ha with production estimated at only 4.6% of African production and the main producing areas are located in the Central and Southernregions of the country [2,3]. As an oilseed fruit, avocadoisincreasinglyconsumed, not only for itsflavor but also for its high nutritional value. The fruit has gainedpopularity due to increasingconsumers and awareness of itsdietary value. Indeed, avocado has a considerable content of monounsaturatedfattyacids as well as minerals, vitamins and otherantioxidantphytochemicals[4,5]. Despiteitsnutritional and economic importance, the avocadosector faces severalproblems of post-harvestspoilage due to the increase in pathogens. Difficulties in applying good agricultural practices and especiallyinadequate post-harvest handling of fruits are the main causes of theseparasitic infections [6]. Contamination of avocado fruits by microorganismsoccurs in particular in the fields, during post-harvest packaging and duringstorage[2]. The development of thesespecies fungi isfavored by the ripening process of the fruits but especially by their composition. These rots affect the organolepticproperties and the market value of the fruits, which causes enormouslosses of up to 90% [7]. To date, the preservationmethodsused are physical and chemical. However, thesemethodsremaininsufficient. Furthermore, the plant protection productsused have harmfuleffects on the health of the consumer and can beresponsible for public healthproblems[8]. Thesechemical control methodsusedalso pose growingresistanceconstraints for pathogens.Thesedisadvantages of chemical pesticides have led to the search for alternatives for the development of otherproductsthat are more respectful of the environment and the health of the consumer in order to fightagainstphytopathogens. Thus, biological control through the use of bacteriagenerallyrecognized as safe has been widelystudied (GRAS). *Bacillus subtilis* and

Pseudomonas fluorescens, existingbiological control agents are then long used as biopesticides due to theirantibacterial and antifungalproperties. Thus, severalresearchstudieshave demonstrated the effectiveness of *Bacillus subtilis* and *Pseudomonas fluorescens* in the biological control of fruit pathogens[9,10].Thework of [11]showedthat the use of *Bacillus subtilis* GA1 as a biopesticide allowed the inhibition of variouspathogenicmicroorganisms of mango, thusimprovingtheir shelf life. Also, [12] alsoreportedthat the *Bacillus subtilis*speciesisolatedfrom the cocoarhizospherehad the capacity to inhibitpoddeteriorationgerms but also to induceresistanceagainstfungal and viral phytopathogens in particularswollen shoot virus (CSSV). The work of [13] showedthat*Pseudomonas fluorescens*couldbeused in the reduction of post-harvestspoilage of cashew nuts, namely*Aspergillus*, *Penicillium* and *Rhizopus*. *Pseudomonas fluorescens*wasalsotested*in vivo* for inhibition of the growth of *Geotrichum*sp, a tomatopathogen[14]. However, thereislittle data on the use of bacterial biopesticides in the biological control of avocadofungaldiseases in Côte d'Ivoire. It is in thiscontext of biocontrol of fungaldiseasesthatthisstudytakes place, the general objective of whichis to carry out biocontrol of fungalstrains of avocadospoilageduring post-harvest conservation using*Pseudomonas fluorescens*and *Bacillus subtilis* BT2.

2. MATERIAL AND METHODS

2.1 Material

The plant materialconsisted of healthy and spoiled mature avocado fruits of the Mexicolavariety(Fig.1) obtaineddirectlyfrom the markets. Twobacterialstrainsincluding*Pseudomonas fluorescens*isolatedfrom the rhizosphere of the cashew tree and *Bacillus subtilis* BT2 isolatedfrom the rhizosphere of the cocoatreepreserved in cryotubes in a freezer (-80°C) wereused in thisstudy.

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

2.2 Methods

2.2.1 Sampling

Sampleswerecollectedfrom 5 sellerstaken at randomfrom the main markets. Fromeach of the 5 sellers, 5 samples of spoiledavocados and 5 samples of healthyavocadosweretaken at random, making a total of 200 samples in all 4 municipalities. Once collected, the different fruits wereindividuallypackaged in Stomacher plastic bags, labeled, sealedthenkept in a coolercontaining dry icewhere the temperatureismaintained at 4°C, by cold accumulators. The transport of the sampleswascarried out on the sameday of collection and did not exceedtwohours for the analysis of the different test portions.

2.2.2 Isolation of avocadospoilagefungalstrains

The microorganismswereisolated by direct contact on Potato Dextrose Agar (PDA) agar [15].Thus, threespoiledavocadoswererandomlychosenfromeachsample, washedwithtap water containing 2% bleach for 2 min, rinsedthree times withsteriledistilled water and

disinfectedusinghouseholdpapersoakedin 70% ethanol in order to eliminateexogenousmicroflora. Using a previouslysterilized scalpel and tweezers. Under aseptic conditions, external (skin) and internal (pulp) fragments werecollectedfromeachspoiled fruit and inoculatedinto Petri dishescontaining Potato Dextrose Agar (PDA) medium. The plates werethenincubated at 28°C for 5 to 7 days. To obtain a pure culture, transfer of colonies to PDA medium according to the methoddescribed by [16, 17] wasproduced. Thus, a moldcolony filament wastakenusingsterile forceps thenplaced at a single point in the center of a Petri dishcontaining the PDA medium in order to obtaintypicalmolddevelopment. Incubation iscarried out at 28°C for 7 days. This methodisrepeateduntil pure colonies are obtained.

2.2.3 Identification of avocadospoilagemolds

Identification of moldswascarried out on the basis of macroscopic and microscopic observations according to the method of [16, 18].Macroscopic Identification wascarried out according to the methodof [16]through an examination of culture on PDA. The cultural characteristicsdeterminedwere 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 wereidentified on the basis of theirmorphologicalcharacteristicsaccording to the methoddescribed by [18]. To carry out the microscopicexamination, a drop of methylenebluewasplaced on a clean slide, a small fragment of the isolatewasplaced on the drop using forceps and coveredwith a coverslipthenobservedunder an electron microscope with an objective x 40 and 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 wererespectivelysubcultured on KING B agar and on Mossel medium by the streakmethodfrom colonies alreadyisolated, and preserved. The plates wereincubated at 30°C for 24 hours to obtainyoung cultures and isolated colonies whichwereused to carry out the antagonism tests.

2.2.5 Antagonism test

This test wascarried out in order to verify the existence of a possible inhibitory action of *P. fluorescens* and *B subtilis* on one or more isolatedmolds. The methodof [11]wasused. In Petri dishescontaining YPGA medium, the bacteria*P. fluorescens* and *B. subtilis* (antagonists) were spread by making a longitudinal streakdividing the dishintotwoequal parts. Subsequently, two discs eachhaving the diameter of the tip of a Pasteur pipette (0.3 cm), obtainedwith a cookie cutter from afungal culture are placed on eitherside of the streak at a distance of 1 cm from the edge of the box in threesteps. The control dishesconsisted of inoculating the mold by transplantingit to the center of the Petri dishwheretherewas no inoculation with*P. fluorescens* and *B subtilis*. Incubation iscarried out at 30°C for 4 days. Colonygrowth of pathogens (molds) and antagonistic agents (*P. fluorescens* and *B. subtilis*) isobservedeverydayuntil the seventhday. After 4 days, the percentage of growth of the fungus in the boxes wasdeterminedusing the method of [19],then the inhibition rate wasdeducedaccording to the followingformula:

r : Radial growth of the microorganismwithantagonism

R : Radial growth of the microorganismwithout confrontation of antagonism

2.2.6 Biocontrol test

A pre-culture of *P. fluorescens* and *B. subtilis*iscarried out by seeding 24-hour colonies in 25 mL of YPG broth for 8 hours at 30°C. The pre-culture wasused to inoculatetwo 100 mL YPG broths and incubated at 30°C for 48 h. After 48 hours of incubation, the culture iscentrifuged at 7000 rpm for 10 min [20]. The supernatantisthencollected and stored at -4°C for *in vivo*antagonism tests. Healthyavocadoswerewashed in tap water containing 2% bleach, rinsedthree times withsteriledistilled water, disinfectedusinghouseholdpapersoakedin 90% ethanoland injuredwith a scalpel (three injuries per lawyer). Then, 50 µL of the supernatant of *P. fluorescens*and *B. subtilis*wereappliedinto the wounds, theneachinoculatedwith 50 µL of the mold spore solution (pathogen) preparedaccording to the method of [21] as described in paragraph 5.1. Finally, the fruits werekept in sterile jars for 5

days[22]. The controlsconsisted of inoculating the avocadoswith suspensions of spores of the pathogenicstrainswithout 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 twobacterialbiocontrol agents wasused to carry out the various protection tests on fruits. 250 mL Erlenmeyer flaskscontaining 65 mL of YPG medium wereeachinoculatedwith a 24-hour colony of the biocontrol agents. The pre-cultures wereincubated at 30°C withshaking at 105 rpm for 8 hours. Thesepre-cultures wereused to inoculate 2L Erlenmeyer flaskscontaining 500 ml of YPG medium. Two Erlenmeyer flasks, eachcontaining 500 ml of sterile YPG medium, wereeachinoculatedwith 65 ml of the pre-culture. The Erlenmeyer flaskswereincubated at 30°C withshaking at 105 rpm for 48 hours. The biomasswasharvestedafter centrifugation of the medium at 3500 rpm for 10 minutes. The pellet whichrepresents the biomasswasseparatedfrom the supernatant and washedthree times withphysiological water (9 ‰ NaCl). The biomass and supernatantwerestored at -4°C for conservation tests.

2.2.7.1 Preservation of fruits with the supernatant

The protection test on fruits wascarried 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 ensurethat the entire surface of the fruit isimmersed. Thus, the healthy fruits werecarefullywashed in tap water containing 2% bleach, rinsedthree times withsteriledistilled water and disinfectedusinghouseholdpapersoakedin 90% ethanolthenimmersed for 5 minutes in the supernatant and dried in the open air. The controlshavingundergone the samestepswere not treatedwith the supernatant. The fruits werestored at room temperature (25^{o}C) after immersion.

2.2.8 Statisticalanalysis

R software version 4.0.4 for Windows, one-wayanalysis of variance (ANOVA), wasperformed to compare the biomass inhibition rates of *P. fluorescens* and *Bacillus subtilis* BT2 againstfungalisolates.

3. RESULTS

3.1 Avocadospoilagefungalstrains

The results of the analysisshowed contamination of the avocadosamples by fungi whichpresented in various aspects, textures and colors. A total of 207 fungalisolateswereisolatedtakingintoaccount the resemblance of thalli and spores. The phenotypic identification made it possible to highlight 6 fungalgenera, namely*Aspergillus*, *Rhizopus*, *Absidia*, *Colletotrichum*, *Botryodiplodia*and *Alternaria*(Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6 and Fig.7).

Fig. 2. Macroscopicappearance (A) and microscopicappearance (B) of *Colletotrichum*sp on PDA medium

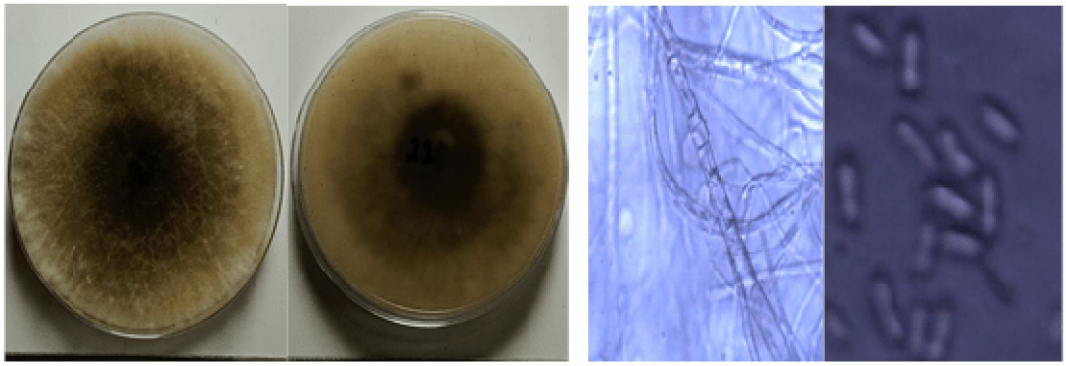


Fig. 3. Macroscopicappearance (C) and microscopicappearance (D) of *Botryodiplodia*sp on PDAmedium

Fig. 4. Macroscopicappearance (E) and microscopicappearance (F) of *Absidia*sp on PDA medium

Fig.5. Microscopicappearance (G) and macroscopicappearance (H) of *Aspergillus*sp on PDA medium

Fig. 6. Macroscopicappearance (I) and microscopicappearance (J) of *Rhizopus*sp on PDA medium

Fig.7. Macroscopicappearance (K) and microscopicappearance (L) of *Alternaria*sp on PDA medium

3.2 Frequency of isolation of fungalstrains

In total, 207 moldisolateswereobtainedfromsamples.Amongtheseisolatedmolds, *Colletotrichum*sp and *Botryodiplodia*sp are the majoritywith respective isolation frequencies of 42.03% and 22.07%. Concerning the otherfungalstrains, theywereisolatedwithfrequencies of 15.46 and 8.21% respectively for *Absisia*sp and *Rhizopus*sp; As for *Alternaria*spand *Aspergillus*sp, theypresentedlow isolation frequencies, namely 5.8% for *Alternaria*sp and 5.80 % for *Aspergillus* sp (Table 1.).

Table 1. Frequency of isolation of avocadospoilagefungi

Fungalisolates	Isolation frequency of fungiisolates (%)
*Colletotrichum*sp	42,03 (87)
*Botryodiplodia*sp	22,70 (47)
*Absidia*sp	15,46 (32)
*Rhizopus*sp	8,21 (17)
*AspergilLus*sp	5,80 (12)

*Aternaria*sp	5,8 (12)
Total	100 (207)

Numbers in parenthesesrepresent the number of isolates of eachfungalspecies

3.3*In vitro*antagonisticactivity 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 fungalisolates by *P. fluorescens* and *B. subtilis* BT2. These tests showedthat*P. fluorescens*presents a higher inhibition rate on all the moldsisolatedthan*B. subtilis* BT2 with the highest inhibition rate observed in *Rhizopus*sp 66.51±0.71%. There is no significantdifference in the inhibition rates between*P. fluorescens* and *B. subtilis* BT2 at the threshold of 0.05 for all the fungi isolatedexcept for the molds*Colletotrichum*sp and *Absidia*sp in which the inhibition rates inhibition weresignificantlydifferent at the threshold of 0.05. The confrontation of bacteriawith*Rhizopus*sppresented 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 fungalgrowthwereobserved in *Aspergillus*sp38.72±2.21% for *B.subtilis* BT2 and 44.05±3.25% for *P. fluorescens*(Table 2.). The control consisted of transplanting the moldinto the center of the PDA agar withoutseedingbacteria. The moldsgrewnormally, hencetherewas no inhibition.

TableI. Statistical values of *in vitro*comparisons of biopesticides and fungiisolates

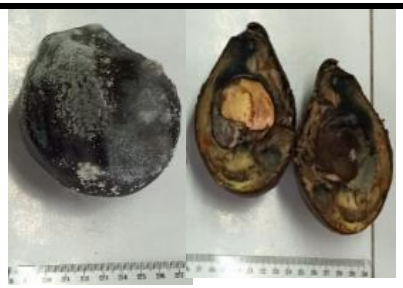
Strains	*B. subtilis BT2*	*P.fluorescens*
***Colletotrichum*sp**	(52,50±0.80)[b]	(61,47±0.70)[a]
***Btryodiplodia*sp**	(55,82±1,63)[a]	(57,93±0.70)[a]
***Absidia*sp**	(56,69±0.33)[b]	(61,82±1.60)[a]
***Rhizopus*sp**	(65,17±0.72)[a]	(66,51±0.71)[a]
***Aspergillus* sp**	(38,72±2,21)[a]	(44,05±3,25)[a]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

For the same line, the values assigned to the samealphabeticalletter are not statisticallydifferent and p<0.05 corresponds to the significancethreshold.

3.4 Biocontrol

The resultsobtainedshowedthatafter 5 days of storage, the fruits havingreceived an application of the supernatant of the biocontrol agents and inoculatedwith the suspension of spores of the pathogenshowed no growth of mold(Table 3.).

Table 3. Appearance of Avocados 5 daysaftertreatmentwith biopesticide supernatant and inoculation withpathogen spore suspension

fungalstrains	Appearance of avocados		
	Control (untreated) after 5 days	Fruits after 5 days of treatment	
		B.subtilis	***P.fluorescens***
*Colletotrichum*sp			
*Botryodiplodia*sp			

*Absidia*sp			

fungalstrains	**Appearance of avocados**		
	Control (untreated) after 5 days	**Fruits after 5 days of treatment**	
		B.subtilis	***P.fluorescens***
*Alternaria*sp			

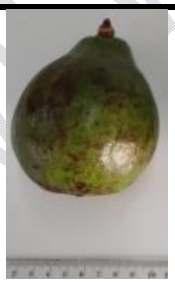
Rhizopus sp			

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

3.5 Fruit storage

Avocadostreatedwith the supernatant of *B. subtilis* BT2 and *P. fluorescens*did not show signs of damage aftereight (8) days. The witnesswasalteredafter 5 days. (Table 4.).

Table 4. Appearance of fruits 8 daysaftertreatmentwith biopesticide supernatant

Duration	Control (fruits not treatedwithsupernatant)	Fruits treatedwithsupernatant	
		Supernatantof *B.subtilis*	Supernatant of*P.fluorescens*
Three(3) days			
Five (5) days			
Eight (8) days			

4. DISCUSSION

In order to contribute to the fightagainst post-harvestlosses of avocados in Côte d'Ivoire due to fungal contaminants, thisstudywascarried out. The resultsobtainedfollowing the phenotypic identification of fungalstrainsisolatedfromavocadosshowedthat a diversity of moldsisresponsible 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.*Theseresults are in agreement with the workcarried out by [24, 25, 26, 27] whoisolated and identified the genera*Colletotrichum*, *Aspergillus* and *Botryodiplodia* on avocado and mango fruits. These fungi are known to cause the deterioration of manyproductssuch as fruits, seeds, vegetables and tubers, both in the fields and duringstorage. The

presence of thesemoldscouldbeexplained by severalenvironmentalfactors, such as aeration, pH, water availability, nutrients and temperaturewhichwouldfavor the growth of molds in preserved agricultural products.The infection of fruits by thesemoldscould have theiroriginfrom the fields, and according to [28]fungalattacksoccurnormallyproducewhen the fruits are in the field but often show no symptomsuntilstorage or transport afterharvest and itsclimactericripening mode whichischaracterized by a high accumulation of ethylene, whichstimulates more rapidripening due high rate of respiration [29]. Among the 6 generaidentified, *Colletotrichum*sp and *Botryodiplodia*sp are in the majoritywith respective isolation frequencies of 42.03% and 22.07%;Thesetwofungalgenerawouldbe the main agents of spoilage of avocados. This corroborateswiththose of [30] whoshowedthat*Colletotrichum*sp main spoiler of tropical fruits and leafyvegetables 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*spwasidentified as the mostisolatedpathogen on avocado in Chile, Spain, Mexico, New Zealand, Peru, South Africa and the United States. Thesestrains have been identified as main spoilage agents in South Africa and on the Hassvariety in Colombia [32, 33, 34]. Generally, contamination of agricultural productsdepends on severalfactors and can occurbefore, during and afterharvest, depending on handling, packaging and storagemethods[35]. It couldthereforebeattributed to post-harvest practices. The use of bacterialbiocontrol agents in thisstudyshowed the capacity of these agents to inhibit the main fungalstrainsresponsible for spoilage of avocado fruits, in particularisolates of *Colletotrichum*sp, *Absisia*sp, *Botryodiplodia*sp, *Rhizopus*sp ,*Aspergillus* sp1, *Aspergillus* sp2 and *Alternaria*sp. This inhibition by *P. fluorescens* and *B. subtilis BT2*wasdemonstrated by *in vitro*antagonism and biocontrol tests. The results of the in vitro tests presented inhibition rates greaterthan 50% of the growth of the strainswith a high inhibition rate observed in *Rhizopus*sp. This wouldbe due to the factthatthesebiocontrol agents produceantifungal substances thatwouldinhibit the growth of mold. Theseresults are similar to thoseobtained by [11]whoshowed the inhibitory power of *Bacillus subtilis* GA1 againstspoilagegerms in the conservation of mangoes in Côte d'Ivoire. As part of a similarstudy of the fightagainst*Geotrichum*sp on tomato, [14]alsoshowedthat*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 couldbeexplained by the factthatthesebiocontrol agents produceantifungal substances whichinhibit the growth of moldsthrough the production of lipopeptide molecules by *B. subtilis* GA1 notablyfengycin, surfactins and iturinswhichmanifestthemselves by the bursting of the cellwall of fungi [9]. The latter can eitheractivate plant defenses or have a direct antibacterial or antifungaleffect[36]. Likewise, *Pseudomonas fluorescens* F19 producesmetabolitessuch as Phenazines and Pyrrolnitrin and alsosiderophoreswhich are ironchelating agents preventingits use by pathogens[37]. *Pseudomonas fluorescens*would have actedeither by mycoparasitism, whichis a trophicrelationshipestablished by a microorganism to the detriment of a fungus [38]. This action resultsfrom the production of lytic enzymes such as glucanase and chitinase, or from the induction of resistance or evenantibiosis. However, biocontrol agents wereless effective on Aspergillus isolateswith inhibition rates below 50%. This couldbeexplained by the very slow growth of thesestrainsduring in vitro confrontation tests or theirresistance to thesebiocontrol agents. The biocontrol tests showedthat the supernatant of the bacterialbiocontrol agents has the ability to inhibit the growth of mold over a period of 5 dayswithfewersigns of fruit spoilagecompared to the control. Mold growthhadalready been observedfrom the thirdday of storage. [14] reportedthattomatoestreated for 24 hourswith*P. fluorescens* F19 beforepathogen inoculation showed a significantreduction in the incidence of rot caused by *Geotrichum*sp. Theseresultsreflect the capacity of biocontrol agents to produceantifungalmoleculeswhichcouldprotect fruits againstfungal damage. Healthy fruit preservation tests revealedthat the supernatant of the bacterialbiocontrol agents testedexert 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 couldmeanthat the antifungal substances produced by the bacteriawouldbecontained in the supernatant. Theseresults are in accordance withthose of the work of [10]whousing*B. subtilis* GA1, *Pseudomonas fluorescens* CI and *Pseudomonas fluorescens* F19 were able to preservepineapple for 14 days.

5. CONCLUSION

This workwascarried out with the aim of contributing to the biopreservation of avocado fruits exposed to fungal contaminants responsible for spoilage and post-harvestlossesthrough the use of *Pseudomonas fluorescens* and *Bacillus subtilis* as bacterialbiocontrol agents. The fungal profile of the avocadosampleswasevaluated. Six fungalgeneraincluding*Colletotrichum, Botryodiplodia, Absidia, Rhizopus, Alternaria*and *Aspergillus* have been identified. The genera*Colletotrichum*and

*Botryodiplodia*werepredominant and caused the mostalterations. The differentantifungalactivities of *Pseudomonas fluorescens* and *Bacillus subtilis* BT2 wereevaluated*in vitro* and *in vivo*. In both cases, thesetwobacteria have showntheirability to reduce or inhibit the fungalstrainsresponsible for spoilage of avocado fruits. The preservation of avocado fruits usingthesebacterialbiocontrol agents made it possible to preserve the fruits for at least eightdayscompared to the control whichwascompletelyspoiledafter 5 days. In view of theseresults, thesetwobacteriacouldbeused in the formulation of biopesticides for the preservation of avocado fruits in Côte d'Ivoire.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) herebydeclarethat NO generativeAI technologies such as Large LanguageModels (ChatGPT, COPILOT, etc) and text-to-image generators have been usedduringwriting or editingof manuscripts.

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