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TOWARDS BIOCONTROL OF POST-HARVEST ANTHRACNOSE BY ANTAGONISTIC BACTERIA AND YEAST ISOLATED FROM FERMENTED MANGO (*MANGIFERA INDICA*, VAR KENT)

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Abstract: *In Côte d'Ivoire, mango fruit plays an important economic role as the third major exported fruit after banana and pineapple. Despite this economic importance, fungal diseases are one of the main issues in the mango value chain with Colletotrichum gloeosporioides causing total postharvest losses estimated up to 20%. The aim of this study was to evaluate the antifungal activity of yeasts, lactic acid bacteria and Bacillus strains isolated from fermented mango fruits as potential biocontrol agents of Colletotrichum gloeosporioides. First, C. gloeosporioides was isolated from contaminated mangoes on potato agar medium, while fermented mangoes were used to isolate yeasts, Bacillus and lactic acid bacteria on DRBC, nutrient and MRS agar, respectively. Afterwards, the in vitro antifungal activity of yeasts, Bacillus and lactic acid bacteria against C. gloeosporioides was tested using dual culture and diffusion methods. The total isolates bateria and yeasts from fermented mango were: 238 yeasts, 214 Bacillus and 252 lactic acid bacteria. The results of antifungal activity of isolated bacteria and yeasts against C. gloeosporioides were as follows: 78 yeasts isolates (33%) were able to inhibit C. gloeosporioides with 11% inhibition more than 40% mycelial growth. The Bacillus isolates were less active (28% inhibition) of C. gloeosporioides with 2% inhibition greater than 60%. As for lactic acid bacteria, they were more active since 58% of isolates showed antifungal activity with 23% of which showed inhibition greater than 60%. Based on the results lactic acid bacteria isolated from fermented mangoes can be used as valuable starters for anthracnose biocontrol.*

Keywords: *antagonistic microorganisms, biocontrol, Colletotrichum gloeosporioides, mango*

1. Introduction

Mango (*Mangifera indica L.*) is one of the most important and commonly eaten fruits in the tropical and subtropical areas. In Côte d'Ivoire, mango fruit plays an important economic role as the third major exported fruit after banana and pineapple. The main producing regions (Korhogo, Ferkessedougou, Sinematiali, Boundiali, Odienne) of mango fruits are in Northern part of Côte d'Ivoire and the most cultivated varieties are Kent, Keitt and Amélie. Despite the economic importance of mango in Côte d'Ivoire, fungal diseases are one of the main issues in the mango value chain, occurring during the production and handling stages [1]. Fungal diseases are known as responsible for postharvest losses in mango production and they also constitute a health risk for consumers due to mycotoxins [2]. Among the fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* is considered as one of the most important that negatively impacts mango production

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causing total losses estimated up to 20% [3,4].
To fight against fungal diseases, chemical fungicides are widely used as treatments to reduce fruit losses from anthracnose [5]. Nevertheless, long-term, and excessive use of chemicals has negative consequences, such as long period of degradation, development of fungicide resistance, harmful effects on human health and the environment [6]. Hence, researchers are working to develop alternative and less toxic strategies for the control of phytopathogens. Among the different methods, biological control appears a promising option for the control of diseases of fungal origin [7,8]. Furthermore, the biological control efficacy of microbial agents has been demonstrated against several postharvest diseases of fruit and vegetables [2].
Biocontrol is essentially based on the use of living microorganisms and/or their metabolites to inhibit the growth or metabolism of phytopathogenic microorganisms [9]. Compared to chemical agents, the use of antagonistic microorganisms has several advantages including specificity of action, absence of toxic and environmentally harmful residues, safer application and more economical production [10]. In addition, biocontrol is known as efficient solution in the short, medium and long term [11]. On the other hand, microbial agents with antagonistic activity used for the control of fungal plant pathogens are from several taxonomic groups such as bacteria, yeasts and filamentous fungi [12]. However, lactic acid bacteria, *Bacillus* and yeasts are often used for food biopreservation [13]. The aim of this study was to evaluate the antifungal activity of yeasts, lactic acid bacteria and *Bacillus* strains isolated from fermented mango fruits as potential biocontrol agents of *Colletotrichum gloeosporioides*.

2. Material and methods

2.1. Sampling of mangoes

Plant material consists of mangoes (*Mangifera indica* var Kent), collected in three (03) producing regions of Northern Côte d'Ivoire: Korhogo (9° 25' 0.001" N 5° 37' 0.001" W), Ferkéssédougou (9° 35' 60" N 5° 12' 0" W), Sinématiali (9° 34' 59.999" N 5° 22' 59.999" W). Mangoes were randomly collected in orchards during harvesting period (mid-May 2022). Two groups of mangoes were collected: healthy mature mangoes for the first group and mangoes with symptoms of anthracnose for the second group (Figure 1). The collected mangoes were put in separated boxes, labeled, and transported to Biotechnology Laboratory, Félix Houphouët-Boigny University) for further analysis.

A B

Fig. 1. Photography of healthy mature Kent mango (A) and contaminated Kent mango with symptoms of anthracnose (B)

2.2. Isolation and morphological identification of *Colletotrichum gloeosporioides*

Isolation of *Colletotrichum gloeosporioides* was carried out according to Pitt and Hocking [14]. Mangoes with symptoms of anthracnose (translucent, brown and blackish lesions spots) were washed with sterile distilled water and dried at ambient temperature. After drying, anthracnose lesions on mangoes surfaces were removed using a sterile scalpel and placed on potato dextrose agar (PDA) medium supplemented with 0.1% chloramphenicol. The Petri dishes were incubated at 30 °C for 3 to 5 days. The fungal isolates were subcultured on PDA medium until pure cultures were obtained. Then, pure cultures were incubated for 7 days at 30 °C and stored at 4 °C on agar slant for identification. Macroscopic and microscopic characters of pure isolates were described by using identification keys of Barnett and Hunter [15] after observation under optical microscope at ×40 magnification (ZEISS, Germany).

2.3. Fermentation of mangoes

Fifteen (15) ripe and healthy mangoes were cut into slices of small pieces of about 5 cm. The slices were crushed in a blender (Moulinex, France) briefly for 5 to 10 seconds and the paste was fermented in sterile and hermetically sealed glass jars for 48 h at laboratory temperature (25 °C).

2.4. Isolation and biochemical identification of lactic acid bacteria and *Bacillus* from fermented mangoes

Twenty-five (25 g) grams of fermented mixture from mangoes slices were mixed in 225 mL sterile buffered peptone water. The whole mixture was stirred for 3 to 5 min for homogenization. Afterwards, serial decimal dilutions (10^{-2} to 10^{-5}) were carried out by using tryptone salt buffer as diluent. For lactic acid bacteria, an inoculum of 100 µL of each dilution was spread on MRS agar supplemented with 100 ppm of nystatin into Petri dishes. Afterwards, the plates were incubated at 30 °C for 24-72 h. Isolation of *Bacillus* sp. was done by inoculation of 100 µL of each dilution on nutient agar [16]**.** The plates were incubated at 30 °C for 24 to 72 h. Biochemical identification of isolates of lactic acid bacteria and *Bacillus* was based on Gram staining, catalase and oxidase tests.

2.5. Isolation and morphological identification of yeasts from fermented mangoes

The isolation of yeasts from fermented mangoes was carried out by spreading 100 µL of each dilution (10^{-3} to 10^{-5}) on Dichloran Rose Bengal Chloramphenicol (DRBC) agar medium and the Petri dishes were incubated at 30 °C for 24 h**.** The morphological identification of yeasts isolates was done by observation under optical microscope at ×40 magnification.

2.6. Screening for antifungal activity of *Bacillus* sp. and yeasts isolates

The *in vitro* antifungal assay for *Bacillus* and yeasts isolates was carried out based on the dual-culture method [16]. Fungal specie (*Colletotrichum gloeosporioides*) plugs of 6 × 6 mm diameter were placed at the center of PDA medium and 5 µL of 24 hours *Bacillus* and yeasts grown in nutrient broth were placed on the opposite four sides of the plates at 1.5 cm away from the fungal disc. Plates containing the fungal plugs without *Bacillus* or yeasts inoculation were used as control. Petri dishes were incubated at 30 °C for 5 days and the growth diameter of *Colletotrichum gloeosporioides* was determined. The percentage of inhibition of the fungal growth was calculated using the following formula:

$$I = (R - r) \times 100/R$$

With *I* - inhibition percentage; *r* - mycelial ray of *Colletotrichum gloeosporioides* in the control plate (mm); *R* - mycelial ray of *Colletotrichum gloeosporioides* in the plate with *Bacillus sp.* or yeasts (mm)

2.7. Screening for antifungal activity of lactic acid bacteria isolates

Lactic acid bacteria isolates were tested for their antifungal activity against *Colletotrichum gloeosporioides* according to agar diffusion method [17]. For this, overnight grown of lactic acid bacteria strains were inoculated on MRS agar by using spot technique. The plates were incubated at 30 °C for 48 hours, and then overlaid with 10 mL of PDA soft agar (PDB and 0.7% agar) containing 10^6 spores/mL of *Colletotrichum gloeosporioides*. After the PDA medium was added, the plates were incubated at 30 °C for 3-4 days. The zones of inhibition around the colonies of lactic acid bacteria were recorded and the percentage of inhibition of the fungal growth was calculated using the following formula:

$$I = d \times 100/D$$

With *d* - diameter of halo around the colonies and *D* - diameter of growth of the control.

2.8. Statistical analysis

Excel 2016 (Microsoft Corporation) was used for statistical analysis.

3. Results and discussion

3.1. Morphological characteristics of isolated *Colletotrichum gloeosporioides*

Two macroscopic characteristics of *Colletotrichum gloeosporioides* were obtained after isolation on potato dextrose agar (PDA) medium (Fig. 2). The first culture showed mycelia with a woolly texture with white color both for face and reverse (Fig. 2.A and 2.B). The second culture highlighted mycelia with a cottony texture, with gray color both for face and reverse (Fig. 2.C and 2.D). In addition, the cultures showed orange conidial mass in the center of plates.

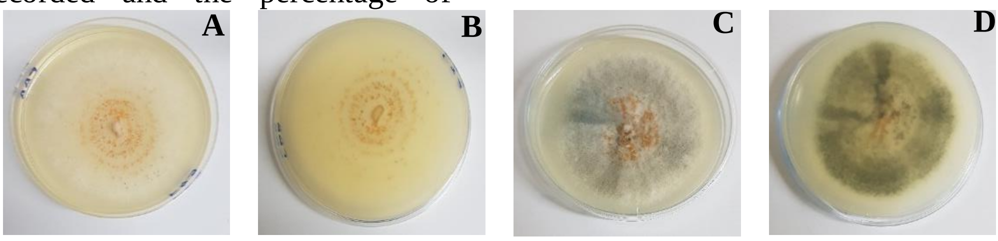

Fig. 2. Photography of macroscopic characteristics of *Colletotrichum gloeosporioides* on potato dextrose agar (PDA) medium.

Microbial spoilage is one of the most important factors in post-harvest fruits losses. Mangoes, like most commercial fruits, are particularly susceptible to many microbial diseases [18]. Among these diseases, anthracnose is one of the most widespread in tropical Africa [19]. The results of our study were in agreement with those of several authors [20,21]. These authors have demonstrated that *Colletotrichum* is the main fungi responsible for anthracnose contamination in mango fruits. Anthracnose contamination is characterized by the development of deep, black rot spots or linear necrotic lesions on the fruit [1]. Particularly, the specie *C. gloeosporioides* is the main causative agent of anthracnose in mango [22].

3.2. Morphological and biochemical characteristics of isolated bacteria and yeasts from fermented mango

The total isolates bateria and yeasts from fermented mango were: 238 yeasts, 214 *Bacillus* and 252 lactic acid bacteria. The lactic acid bacteria isolates were Gram-positive with a diversity of shape (cocci, coccobacillus, or bacillus) and arrangement mode (clump or chain), catalase negative and oxidase negative. As for *Bacillus* strains, they were Gram-positive and exclusively rod-shaped. The yeasts showed a pink color on Dichloran-Rose-Bengal-Chloramphenicol (DRBC) agar medium. The isolation of fermentative microorganisms from mango showed a diversity of microbial groups with occurence of lactic acid bacteria and yeasts. These results were in agreement with those obtained by authors who showed that the surface of mango fruits is colonized by microorganisms mainly composed of Gram-positive and Gram-negative bacteria and yeast [23].

3.3. In vitro antifungal activity of isolated bacteria and yeasts against *Colletotrichum gloeosporioides*

The results of antifungal activity of isolated bacteria and yeasts against *C. gloeosporioides* are shown in Table 1 and Figure 3. For yeasts, 78 isolates (33%) were able to inhibit *C. gloeosporioides* with 11% inhibition more than 40% mycelial growth. The *Bacillus* isolates were less active (28% inhibition) against *C. gloeosporioides* with 2% inhibition greater than 60%. As for lactic acid bacteria, they were more active since 58% of isolates showed antifungal activity, and with 23% of this showed inhibition greater than 60%.

Table 1.

Inhibition percentage of isolated bacteria and yeasts against *C. gloeosporioides*

Isolates	*No inhbition against C. gloeosporioides*	*Inhibition against C. gloeosporioides*		
		I < 40%	**40% < I < 60%**	**I > 60%**
Yeasts	160 (67%)	52 (22%)	26 (11%)	0 (0%)
Bacillus sp.	154 (72%)	37 (17%)	19 (9%)	4 (2%)
Lactic acid bacteria	106 (42%)	10 (4%)	78 (31%)	58 (23%)

Post-harvest diseases are usually controlled by the application of synthetic fungicides [12]. However, the use of synthetic products leads to various issues such as environmental pollution, toxicity, as well as the development of pathogens resistance. Therefeore, it is essential to adopt alternative approaches as biocontrol for plant diseases management [24].

Based on the antagonism tests, the microbial isolates presented inhibition percentage greater than 25%. Moreover, 50% of lactic acid bacteria isolates inhibited *C. gloeosporioides*. This result is higher than that (18%) found by some authors [25]. This result could be explained by the fact that tested isolates were from the same ecological environment as *C. gloeosporioides*.

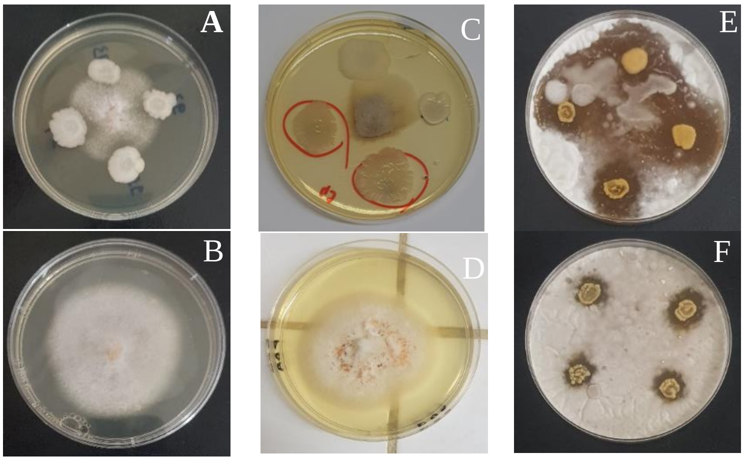

Fig. 3. Photography of *in vitro* inhibition of *C. gloeosporioides* by yeast (A and B), *Bacillus* (C and D), and lactic acid bacteria (E and F) isolates

Indeed, according to some authors [26], antagonistic microorganisms used to control plant diseases should be taken from ecological environment similar to those where the target disease is present.

4. Conclusion

The aim of this study was to evaluate the antifungal activity of yeast, lactic acid bacteria and *Bacillus* strains isolated from fermented mango fruits as potential biocontrol agents of *Colletotrichum gloeosporioides*. The results of the present study showed that yeasts, *Bacillus* and lactic acid bacteria isolates presented inhibition percentage greater than 25% on *Colletotrichum gloeosporioides*. Thus, yeasts, *Bacillus* and lactic acid bacteria isolated from fermented mangoes can be used as valuable starters for anthracnose biocontrol. Before their use as starters, molecular identification of yeasts, *Bacillus* and lactic acid bacteria isolates must be performed.

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