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In vitro Activity of *Pseudomonas fluorescens* Strains against *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune*, which Cause Diseases in Sugarcane in Côte d'Ivoire

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Abstract— *Sugar cane (Saccharum officinarum L.) is a key crop for the economy of Côte d'Ivoire. However, domestic production remains insufficient to meet domestic demand, resulting in a sugar deficit for the country. This low productivity is largely due to fungal diseases, which cause considerable economic losses. Whilst several control methods have been used, some pose risks to human health. It is against this backdrop that this study was conducted, the objective of which was to evaluate the in vitro effect of Pseudomonas fluorescens strains against Fusarium verticillioides, Fusarium subglutinans and Schizophyllum commune, which cause diseases in sugarcane in Côte d'Ivoire. To this end, 150 soil samples were collected from the rhizosphere of healthy and diseased sugarcane plants for the isolation and phenotypic and molecular identification of Pseudomonas fluorescens strains. In vitro antifungal tests were then carried out. The results showed that strains of Pseudomonas fluorescens were isolated and identified from the rhizosphere of both healthy and diseased sugarcane plants. In vitro tests showed that 95 per cent of the bacterial strains inhibited the mycelial growth of the three fungal pathogens, with inhibition rates ranging from 40.16 ± 6.9 per cent to 80.65 ± 6.5 per cent. These results suggest that Pseudomonas fluorescens, when tested under field conditions, could be used effectively as a biocontrol agent against fungal diseases in sugarcane crops in Côte d'Ivoire.*

Keywords— Sugar cane, Pathogenic fungus, Pseudomonas fluorescens, Côte d'Ivoire.

I. INTRODUCTION

Sugar cane (*Saccharum officinarum* L.) is a perennial plant of the Poaceae family. It is native to New Guinea and is cultivated for its stems, which contain a sweet sap (Meslien, 2009). In 2022, global sugarcane production reached approximately 1.92 billion tonnes, accounting for 79% of total global sugar production (ChenhanTech, 2022). This sector constitutes a strategic agro-industrial sector, both for the production of sugar and for that of ethanol or energy co-products. In 2023–2024, the main producing countries were Brazil and India, with annual production ranging from 753 to 783 million tonnes and from 405 to 490 million tonnes respectively (Financial Times, 2023). Sugar cane cultivation generates economic value amounting to tens of billions of US dollars (USD). For example, the Brazilian group Raízen recorded a turnover of 47 billion USD in 2023

(Financial Times, 2023). The Illovo Sugar group (South Africa) is the leading player in Africa, exporting to the European Union, the United States and West Africa. Côte d'Ivoire is a medium-sized producer in Africa. In 2018, the country's annual sugarcane production was estimated at 1.9 million tonnes, ranking it 53rd globally and 16th in Africa (REPERCI, 2024). Within the country, sugarcane is mainly used for direct consumption or in the sugar industry. It is also used in the cosmetics and pharmaceutical sectors. Furthermore, it represents the main source of economic activity for the population in the northern part of the country (Kouamé et al., 2010). Despite the importance of sugarcane, Côte d'Ivoire continues to face a sugar deficit. This sugar deficit is generally caused by diseases affecting sugarcane cultivation, including bacterial diseases such as leaf blight caused by *Xanthomonas albineans*, and fungal diseases such as smut, red mould and pokkah boeng. These fungal diseases result in losses estimated at between 10 and 30 per cent of the national yield (Yao et al., 2020). Although there are no comprehensive official national data, fungal diseases cause economic losses ranging from 20 to 40 per cent of the national yield. Thus, based on an estimated national sugarcane production of around 330,000 tonnes, a 20 per cent loss equates to 66,000 tonnes, representing nearly 13 million USD in annual economic losses (Kouamé et al., 2010). These fungal diseases are characterised by symptoms such as leaf chlorosis and green mottling of varying severity. The most susceptible varieties can be literally stunted to the point of resembling tufts of grass. Sometimes the stems are covered in cankers and the internal tissues are necrotic. This deterioration of the stems leads to a reduction in their sugar content and losses in yield. These diseases have caused crop losses of up to 40 per cent in the Borotou-Koro and Zuénoula sugar complexes (Yao et al., 2020). In order to combat these pathogens effectively, pesticides – particularly synthetic fungicides from the benzimidazole, triazole and strobilurin families – are primarily used. However, the overuse of these synthetic fungicides in sugarcane plantations could lead to the emergence and rapid development of resistant strains, which would result in a resurgence of diseases (Cawoy et al., 2011). In view of these adverse consequences, it is important to find alternative solutions that will enable the continued control of plant pathogens whilst reducing the use of chemicals and also protecting the environment. These may involve the rationalisation of agricultural practices, the use of resistant plant varieties and/or the use of biopesticides (Thakore, 2006). It is against this backdrop that this study is set, the overall objective of which is to evaluate the in vitro effect of *Pseudomonas fluorescens* strains against *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune*, which cause diseases in sugarcane in Côte d'Ivoire.

II. MATERIALS AND METHODS

2.1 Material

The materials used in this study consisted, on the one hand, of samples collected from two major sugarcane-producing areas in Côte d'Ivoire, namely Zuenoula and Borotoukoro. These soils, taken from the rhizosphere of sugarcane, were used to isolate bacterial biocontrol agents. In addition, samples of apparently healthy sugarcane cuttings were used to carry out biocontrol tests on pathogens in a greenhouse. Furthermore, pathogenic fungal strains of *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune*, which cause diseases in sugarcane in Côte d'Ivoire, were used. These fungal strains were obtained from the Laboratory of Food Biotechnology and Microbiology at the Food Science and Technology Training and Research Unit (UFR-STA) of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). These strains were identified from symptomatic parts of sugarcane in Côte d'Ivoire, in accordance with the work of Okoue et al. (2025).

2.2 Methods

2.2.1 Sampling area

Soil samples, as well as apparently healthy sugarcane cuttings, were selected from plots in the two sugarcane production areas, namely the Zuenoula Integrated Agricultural Unit (UAI), situated between 7°30' and 7°40' north latitude, and between 6°50' and 6°15' west longitude, and the Integrated Agricultural Unit (UAI) at Borotou-Koro, situated between 8°31' north latitude and 7°17' west longitude.

2.2.2 Identification of bacterial biocontrol agents

2.2.2.1 Sampling

Soil samples were taken from the rhizosphere of apparently healthy sugarcane plants and those showing symptoms of disease, located at least 50 m apart, at a depth of 0 to 30 cm, with a view to identifying biocontrol agents. A total of 150 soil samples were collected from the Borotou-Koro and Zuénoula sugar complexes, of which 75 were taken from the rhizosphere of apparently healthy sugarcane plants and 75 from those showing symptoms of disease. These samples were placed in sterile

Stomacher bags, labelled, sealed and then stored in a cool box containing dry ice at 4°C, before being transported to the laboratory for various analyses.

2.2.2.2 Isolation of *Pseudomonas fluorescens* isolates

The isolation of presumptive *Pseudomonas fluorescens* was carried out using the suspension-dilution method and by plating dilutions of soil suspensions onto King B agar (Vidhyasekaran et al., 1997). To this end, 1 g of soil was sampled using a sterile spatula and suspended in 9 ml of sterile distilled water. After vortexing the suspension for 10 minutes, decimal dilutions ranging from 10^{-1} to 10^{-7} were prepared. Next, 0.1 ml of each suspension was spread onto Petri dishes, each containing 20 ml of King B agar medium. The Petri dishes were incubated at 30 °C in the dark. After 48 hours of incubation, the characteristic colonies of putative *Pseudomonas fluorescens* were counted and the bacterial load per soil sampling site was determined using equation (2) in accordance with ISO 7218 (2007). After counting, the putative *Pseudomonas fluorescens* isolates were subcultured onto King B agar until pure colonies were obtained. The pure colonies were preserved in 50% glycerol in Eppendorf tubes at -20°C and in tubes containing King B agar poured into slants at 4°C (Bossis et al., 2000).

$$N\left(\frac{ufc}{g}\right) = \frac{\sum C}{V \times (n_1 + 0{,}1\ n_2) \times d} \quad (1)$$

Where

N (ufc/g): Microbial load

C: Number of colonies across all Petri dishes;

V (ml): Volume of the soil solution inoculum;

n1: Number of Petri dishes inoculated with the first dilution under consideration;

n2: Numbers of Petri dishes inoculated with the second dilution under consideration;

d: Dilution selected.

2.2.2.3 Phenotypic identification of putative *Pseudomonas fluorescens* isolates

The phenotypic identification of putative *P. fluorescens* isolates was carried out using various methods based on morphological and physiological criteria. Following purification, the colonies obtained on King B agar were examined with the naked eye. The macroscopic examination was carried out by assessing the size, shape and lustre of the colonies (Benzina et al., 2016). The physiological examination, meanwhile, focused on the production of yellow-green pigment on King B agar at 4 °C. The microscopic examination was based on Gram staining, which involved preparing a bacterial smear on a heat-fixed microscope slide. The smear was then examined under the microscope at magnifications of ×40 and ×100 (Bossis et al., 2000). Presumed *Pseudomonas fluorescens* species appear as pink-stained bacilli, i.e. Gram-negative bacilli (Bossis et al., 2000).

2.2.3 Molecular identification of putative *Pseudomonas fluorescens* isolates

2.2.3.1 DNA extraction

To extract DNA, pre-cultures were prepared by inoculating the strains into a nutrient broth and incubated at 30 °C for 24 hours. A 1.5 ml volume of bacterial suspension was transferred to sterile Eppendorf tubes. The bacterial biomass was recovered by centrifugation at 4000 rpm for 10 minutes. The biomass was then mixed with 500 µl of H_2O. The thermal shock DNA extraction technique, based on the disruption of bacterial membranes by treating the pre-cultures at 100 °C followed by -20 °C, was used to recover genomic DNA (Queipo et al., 2007).

2.2.3.2 16S rRNA gene amplification reaction

The gene encoding the 16S rRNA subunit is a fundamental tool for the genetic identification and phylogenetic tracing of bacteria (Emmanuel et al., 2009). In order to amplify the genes encoding the 16S rRNA subunit, universal primers specific to the bacterial kingdom will be used. The template strand primer is 27F and the complementary strand primer is 1492R (reverse primer). A PCR master mix with a final volume of 25 µl was prepared, consisting of nuclease, Taq buffer, dimethyl sulphoxide (DMSO), $MgCl_2$, deoxyribonucleotides, primer F, primer R, Taq polymerase and the DNA to be analysed (Table I). Amplification was carried out in a thermal cycler (Techne® Prime Thermal Cycler Range, USA) according to the programme described by Arzu et al. (2011). The first stage was the initial denaturation. The second stage comprised 35 cycles, each

consisting of a denaturation phase, a hybridisation phase and an elongation phase. Finally, the third stage was the final elongation (Table II).

TABLE 1
REAGENT USED IN THE PCR REACTION MIXTURE FOR THE MOLECULAR IDENTIFICATION OF BACTERIAL ISOLATES

Reactive	Volume per reaction (µl)
Nuclease Free Water	11.825
Tampon Taq	5
Diméthylsulfoxyde (DMSO)	0.125
$MgCl_2$	4
DNTP	0.6
ADN	2
Amorce F	0.625
Amorce R	0.625
Taq polymérase	0.2
Total	**25**

TABLE 2
PCR PROTOCOL FOR THE MOLECULAR IDENTIFICATION OF BACTERIAL ISOLATES

Stage	Temperature (°C)	Time	Number of cycles
Initial denaturation	94	7 min	
Amplification	94	1 min	35
	53	43 s	
	72	45 s	
Final elongation	72	7 min	

2.2.3.3 Agarose gel electrophoresis

Agarose gel electrophoresis is a widely used method in molecular biology that enables the separation of DNA fragments according to their molecular weight. It is based on the migration of negatively charged nucleic acids in the presence of an electric field. The migration of DNA fragments takes place within the agarose gel matrix, with smaller molecules moving more rapidly than larger ones. Electrophoresis provides essential information, notably the molecular weight of the amplified gene, by comparison with size markers. Following amplification, the PCR products were analysed by electrophoresis on a 1.5% agarose gel. The gel was immersed in an electrophoresis tank containing 0.5× TBE. Aliquots of 10 µl of amplified fragments were mixed with 2 µl of loading buffer (50% TE, pH 8; 50% glycerol; bromophenol blue) before being loaded into the gel wells. The gel was visualised using a transilluminator under UV light following electrophoresis at 90 volts for 90 minutes. The size of the DNA fragments was determined using the Reddy Run Super Ladder-Low 1000 bp molecular weight marker as a reference. The PCR products were subsequently stored at –20 °C for further use.

2.2.4 Evaluation of the in vitro antifungal activity of bacterial biocontrol agents against sugarcane pathogenic fungi

2.2.4.1 Preparation of culture media and direct comparison between pathogens and antagonist

The *P. fluorescens* bacteria were first re-inoculated 48 hours prior to the preparation of the YPGA culture media used. These purified bacteria were then streaked onto the pre-prepared YPGA agar in a straight line dividing the Petri dish into two equal parts. Next, two mycelial discs, each 1 mm in diameter, from seven (7)-day-old pathogenic fungi were placed on either side of the streak, 1 cm from the edge of the Petri dish (Korsten and Jager, 1995). Control plates containing no bacterial biocontrol agent were prepared by inoculating a mycelial fragment of the pathogenic fungus in the centre of the Petri dish. Antifungal activity was assessed by measuring the diameter of the mycelial growth of the pathogenic fungi using a ruler graduated in centimetres (cm) in each of the Petri dishes comprising the treatments. This measurement was taken daily until the pathogenic fungus had filled the control dishes. At the end of these measurements, the rate of inhibition of the pathogen's mycelial growth was calculated using the following formula (2) by Korsten and Jager (1995):

$$TI(\%) = (\frac{R-r}{R}) \times 100 \quad (2)$$

Where

TI (%) = Inhibition rate

r: Radial growth of the fungus in the presence of the bacterial strain

R: Radial growth of the fungus in the absence of the bacterial strain

All in vitro antifungal tests were performed with three replicates per treatment, and each experiment was conducted twice to ensure reproducibility.

2.3 Statistical Analyses

The data collected were analysed using analysis of variance (ANOVA I and II) with Statistica software, version 7.1. One-way analysis of variance (ANOVA I) was used to investigate the variability of bacterial strains across different sugarcane-growing regions. Two-way analysis of variance (ANOVA II) was used to screen for antagonistic bacteria and the concentrations at which they significantly reduce or completely inhibit the in vitro growth of pathogens. Where a significant difference was found, the means were compared using the Newman-Keul test at a 5 per cent significance level. The significance of the test is determined by comparing the probability (P) associated with the test statistic to the value $\alpha = 0.05$. Thus, when $P \geq 0.05$, it is concluded that there is no difference between the means. However, when $P < 0.05$, there is a significant difference between the means.

III. RESULTS

3.1 Potential of Bacteria with Biopesticidal Activity Isolated from the Sugarcane Rhizosphere

3.1.1 Abundance and morphological characteristics of the putative *Pseudomonas fluorescens* isolates

The average concentration of putative *Pseudomonas fluorescens* ranged from 2.5×10^6 to 1.4×10^6 CFU/g of soil for the Zuenoula and Borotou-koro complexes, respectively, in soil samples taken from the rhizosphere of apparently healthy sugarcane plants. Those from symptomatic sugarcane plants ranged from 9.1×10^4 to 3.5×10^4 CFU/g of soil. Regardless of the sampling area, the levels of putative *Pseudomonas fluorescens* in soils from symptomatic sugarcane plants were lower than those in soils from apparently healthy plants. Statistical analyses revealed a significant difference in the load of presumptive *Pseudomonas fluorescens* isolated from the rhizosphere of symptomatic and healthy sugarcane plants (Figure 1). Following purification, a total of 407 bacterial isolates presumed to belong to the genus *Pseudomonas fluorescens* were isolated from soil samples taken from the rhizosphere of apparently healthy and symptomatic sugarcane plants in both study areas,

namely Borotou-koro and Zuénoula. The characterisation of presumptive *Pseudomonas fluorescens* isolates using macroscopic characteristics following purification on King B agar revealed the formation of distinct colonies visible to the naked eye, exhibiting the specific morphological criteria of presumptive *Pseudomonas fluorescens*. On King B agar, presumptive *Pseudomonas fluorescens* isolates are characterised by rapid growth, producing small, creamy, overlapping colonies that are circular, smooth, regular in appearance and invasive, with yellow-green pigment production in the agar (Figure 2A). Identification by microscopic examination reveals straight or slightly curved bacilli with rounded ends. The cells occur singly or in pairs. They are asporangiate. Following Gram staining, the presumptive *Pseudomonas fluorescens* isolates appear as pink rods, indicating that they are Gram-negative bacilli. Indeed, bacteria belonging to the genus *Pseudomonas* are small rods with polar cilia and are Gram-negative (Figure 2B). Following phenotypic identification, a total of 103 bacterial isolates presumed to be *Pseudomonas fluorescens* were selected and coded using letters of the alphabet, followed by an Arabic numeral and a letter, or not. The first letter, 'P', stands for *Pseudomonas*, and the other letters and numbers are arranged at random.

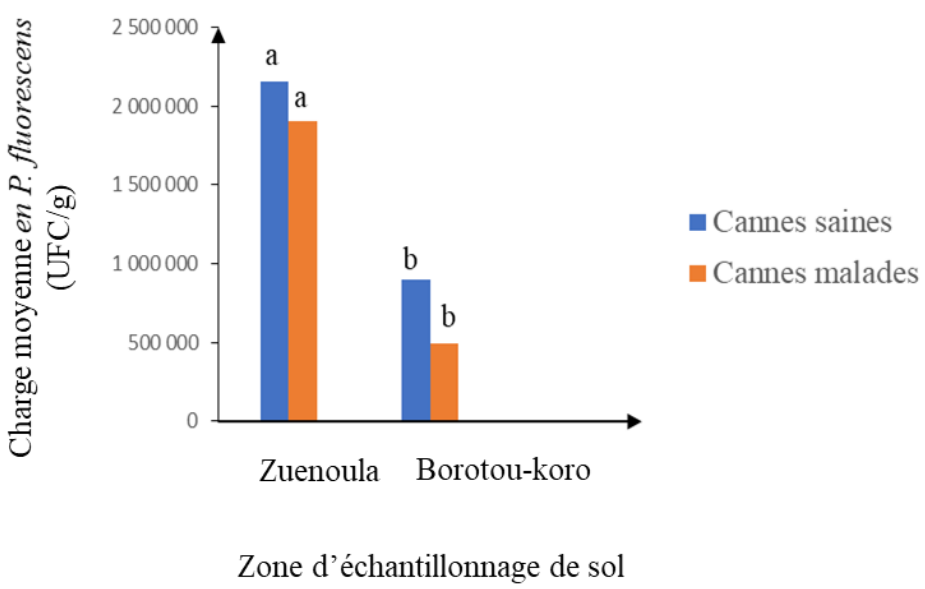

FIGURE 1: Bacterial load of putative *P. fluorescens* isolates from soil samples taken from the two sugar complexes. Bands with the same letters are statistically identical at the 5 per cent significance level, and those with different letters are statistically different at the 5 per cent significance level.

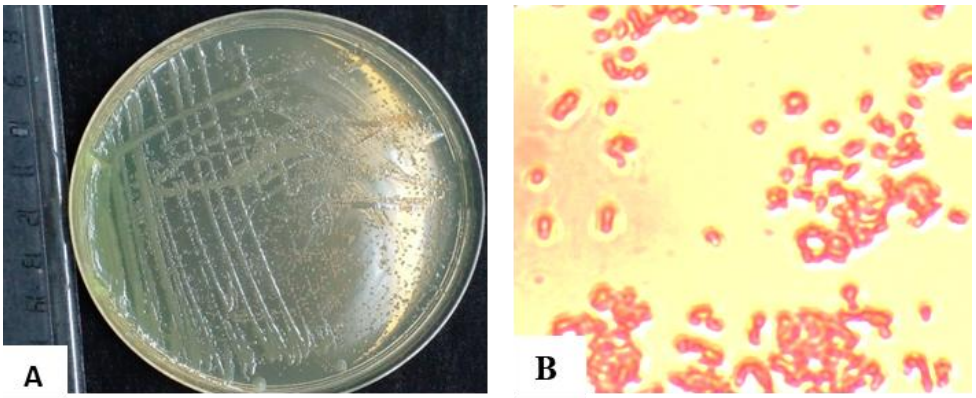

FIGURE 2: Macroscopic and microscopic appearance of putative *P. fluorescens* isolates from the rhizosphere soil of the Zuenoula and Borotou-Koro sugar complexes. A - Macroscopic appearance of putative *Pseudomonas fluorescens*; B - Microscopic appearance of putative *Pseudomonas fluorescens*.

3.1.2 *Pseudomonas fluorescens* strain identified using molecular tests

The search for *Pseudomonas fluorescens* isolates through the analysis of soil samples resulted in the isolation of rhizobacteria distributed across the various wells formed in the agarose gel. The molecular characterisation method, involving amplification of the 16S rDNA gene, enabled the identification of *Pseudomonas fluorescens*. The size of the amplicons was approximately 1,500 bp (Figure 3).

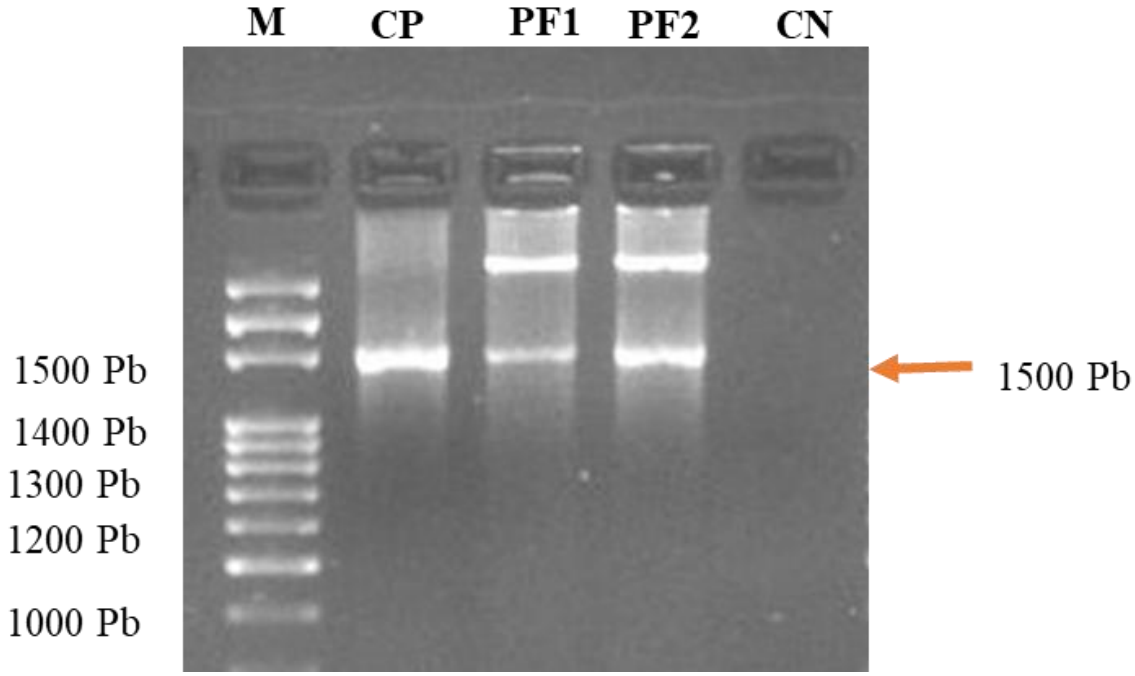

FIGURE 3: Electrophoretic profile of *Pseudomonas* sp. isolates from the sugarcane rhizosphere. M: Molecular weight marker; PF1: *Pseudomonas fluorescens* 1; PF2: *Pseudomonas fluorescens*, CP: *Pseudomonas fluorescens* CI and CN: H_2O.

3.2 In vitro Antagonistic Activity of *Pseudomonas fluorescens* Strains against the Mycelial Growth of Sugarcane Pathogenic Fungi

The results of the antagonism tests carried out showed that the isolates of *Bacillus* sp. and presumptive *Pseudomonas fluorescens* were capable of inhibiting the mycelial growth of sugarcane pathogenic fungi (Figures 4, 5 and 6). The results showing the average rates of inhibition of mycelial growth of pathogenic fungi by the genus *Bacillus* are presented in Table III. Average inhibition rates exceeding 50 per cent were observed for the majority of the identified isolates. These average inhibition rates ranged from 44.12 to 80.47 per cent for the *Pseudomonas* isolates PCI and PZ13, respectively, on the growth of *Schizophyllum commune*, and from 46 to 79.24 per cent respectively for the *Pseudomonas* PE91 and PZ13 isolates on the mycelial growth of *Fusarium verticillioides*, and from 48.68 to 66.34 per cent respectively for the *Pseudomonas* PF26 and PEZ83 isolates on the mycelial growth of *Fusarium subglutinans*. Statistical analyses revealed a significant difference at the 5 per cent significance level between the inhibition rates. The best inhibitory effect was observed for the *Pseudomonas* isolates PZ13 and PEZ83, with mean inhibition rates of 80.47 per cent, 79.24 per cent and 79.52 per cent respectively. These mean inhibition rates are statistically equivalent at the 5 per cent significance level (Table III).

TABLE 3
RATES OF INHIBITION OF MYCELIAL GROWTH IN SUGARCANE PATHOGENIC FUNGI BY STRAINS OF *PSEUDOMONAS FLUORESCENS*

Strains	*Schizophyllum commune*	*Fusarium subglutinans*	*Fusarium verticillioides*
PE75	(53.45±4.40)^{cdef}	(55.30±2.26)^{cd}	(53.71±7.30)^{bc}
PCE	(50.60±3.79)^{def}	(51.12±1.02)^{de}	(54.81±4.34)^{bc}
PE6Z51	(65.07±1.37)^{bc}	(57.09±2.43)^{bcd}	(60.13±2.04)^{ab}
PEZ1	(58.56±5.15)^{cde}	(44.65±1.91)^{e}	(54.70±1.46)^{bc}
PQ94	(72.97±1.62)^{ab}	(61.32±1.43)^{bc}	(59.45±1.90)^{ab}
PEZ2	(61.36±3.74)^{bcd}	(58.11±2.00)^{bcd}	(52.94±3.46)^{bc}
PEZ4	(44.60±3.99)^{f}	(51.12±1.02)^{de}	(57.65±4.60)^{abc}
PCI	(44.12±7.47)^{f}	(54.87±2.00)^{cd}	(61.17±1.25)^{ab}
PEZ83	(78.09±2.18)^{a}	(75.63±2.67)^{a}	(66.34±2.57)^{a}
P65Z9	(53.49±3.57)^{cdef}	(54.87±2.00)^{cd}	(57.65±4.60)^{abc}
PE5	(59.99±1.42)^{cde}	(58.50±1.53)^{bc}	(56.80±4.04)^{abc}
PZ20	(54.76±5.01)^{cdef}	(62.70±1.48)^{b}	(54.27±1.71)^{bc}
PEZ13	(80.47±2.17)^{a}	(79.24±0.71)^{a}	(61.64±2.87)^{ab}
PEZ181	(57.52±6.29)^{cde}	(61.33±2.55)^{bc}	(55.09±4.30)^{bc}
PE91	(48.31±3.50)^{ef}	(46.00±3.24)^{e}	(55.12±2.14)^{bc}
PO12	(59.99±4.28)^{cde}	(59.89±1.27)^{bc}	(60.31±1.43)^{ab}
PE1	(79.52±0.82)^{a}	(54.87±2.00)^{cd}	(58.96±3.97)^{abc}
PZ26	(59.87±4.72)^{cde}	(55.34±1.91)^{cd}	(57.65±4.60)^{abc}
PE60	(55.23±5.01)^{cdef}	(54.31±3.76)^{cd}	(56.04±1.40)^{abc}
PF26	(49.68±3.82)^{def}	(54.25±5.16)^{cd}	(48.68±1.14)^{c}

On the same row, values with the same letters are statistically identical at the 5 per cent significance level.

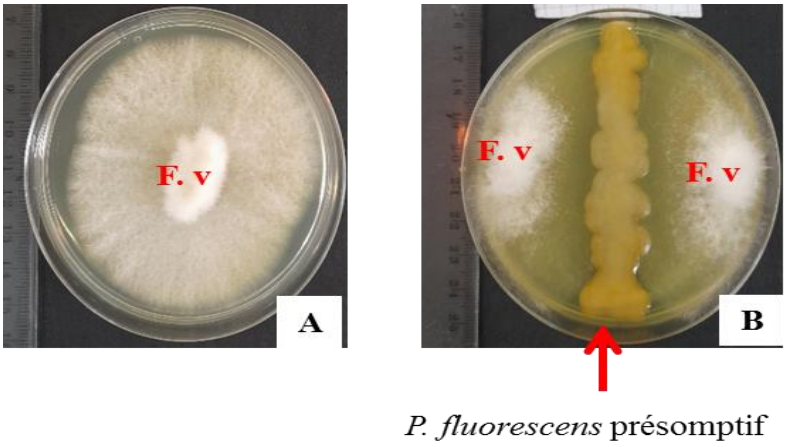

FIGURE 4: Inhibition of *Fusarium verticillioides* mycelial growth by putative *Pseudomonas fluorescens* isolates from the sugarcane rhizosphere. A – *Fusarium verticillioides* (F. v) on YPGA medium without bacterial challenge; B – Co-culture of *Fusarium verticillioides* (F. v) and putative *P. fluorescens* on YPGA medium.

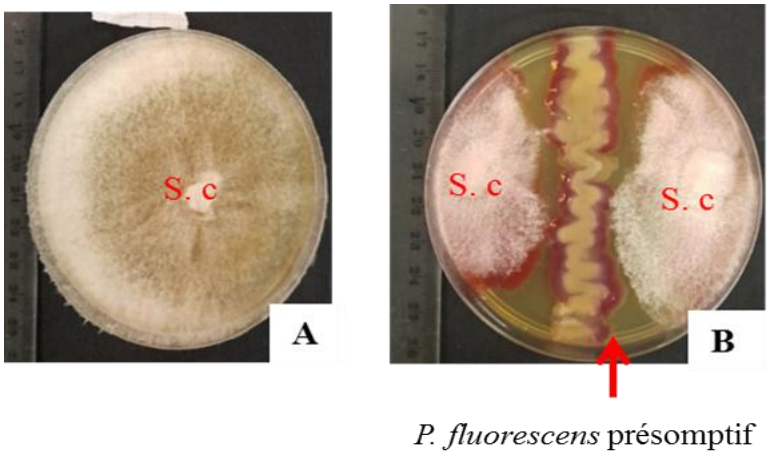

FIGURE 5: Inhibition of mycelial growth of *Schizophyllum commune* by isolates of putative *Pseudomonas fluorescens* isolated from the rhizosphere of sugarcane. A – *Schizophyllum commune* (S. c) on YPGA medium without bacterial challenge; B – Co-culture of *Schizophyllum commune* (S. c) and presumptive *P. fluorescens* on YPGA medium.

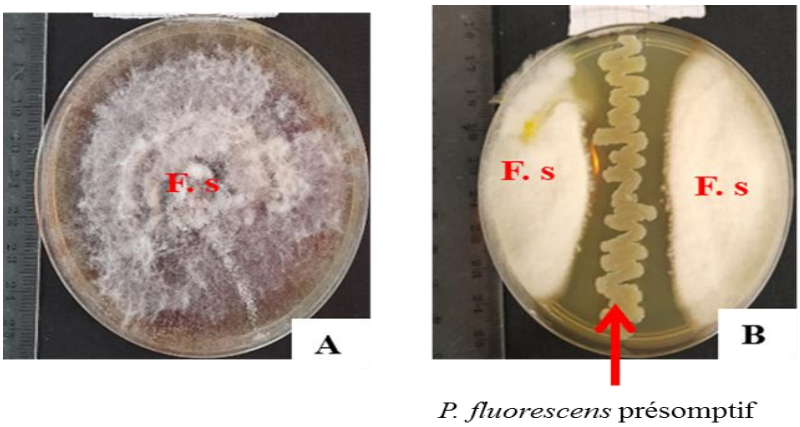

FIGURE 6: Inhibition of *Fusarium subglutinans* mycelial growth by putative *Pseudomonas fluorescens* strains isolated from the sugarcane rhizosphere. A – *Fusarium subglutinans* (F. s) on YPGA medium without bacterial challenge; B – Co-culture of *Fusarium subglutinans* (F. s) and putative *P. fluorescens* on YPGA medium.

IV. DISCUSSION

The aim of this chapter was to identify the bacterium *Pseudomonas fluorescens*, which has biopesticidal potential, from the rhizosphere of sugarcane. The results of the isolation and identification of putative *Pseudomonas fluorescens* from the sugarcane rhizosphere demonstrated the presence of *Pseudomonas fluorescens* in the rhizosphere of sugarcane in the localities of Zuenoula and Borotou-Koro. The *Pseudomonas fluorescens* strains isolated from the rhizosphere of asymptomatic sugarcane plants constituted a significant proportion of the soil microbial community. The isolation of these strains suggests that the sugarcane plantations in the localities of Borotou-Koro and Zuenoula are naturally rich in bacteria of the genera *Pseudomonas*. The low abundance of *Pseudomonas fluorescens* observed in soil samples from the rhizosphere of diseased sugarcane plants was statistically different from that found in soil samples from the rhizosphere of healthy sugarcane plants. This difference could indicate that the microbial population influences the development or absence of sugarcane diseases. Indeed, according to Ongena (2014), better plant protection would require a microbial population of at least 10^6 cfu/g of soil. Recent studies have also shown that strains of *Pseudomonas putida* and *Stenotrophomonas* sp. have been isolated from the rhizosphere of carrots and coffee beans, respectively (Van et al., 2004). The rhizosphere of plants is a zone containing a diverse range of rhizobacteria. Species such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Arthrobacter*, *Bacillus*, *Serratia*, *Xanthomonas* and *Erwinia* are abundant there (Suman et al., 2010). Frietas and Germida (1990) isolated strains of *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and *Pseudomonas putida* from the rhizosphere

of millet. Watanabe and Hayano (1993), for their part, isolated strains of *Bacillus licheniformis*, *Bacillus mycoides* and *Bacillus megaterium* from the rhizosphere of rice.

The results showed that all isolates produced inhibition percentages ranging from 50 to 81 per cent. Indeed, the selected isolates were capable of inhibiting the growth of all the fungi they were tested against. These results could be explained by the fact that the selected strains were capable of producing a variety of antimicrobial and antifungal compounds, namely lytic enzymes, siderophores and lipopeptides, thereby enabling them to reduce or completely inhibit the growth of pathogenic strains. Indeed, most microorganisms possessing antagonistic activity are isolated either from fruit, from plants or from the rhizosphere (Janisiewicz and Korsten, 2002). Silva et al. (2015) isolated a variety of bacterial isolates (*Enterobacter aerogenes*, *Lysinibacillus fusiformis*, *Klebsiella oxytoca* and *Hafnia alvei*) from a fruit native to Brazil (*Psychotria hoffmannseggiana*), all of which exhibited antagonistic activity against the pathogenic strain *Aspergillus carbonarius*. These authors stated that the antagonistic activity observed in these different strains was due to their production of cellulase and pectinase. In their research, Oztopuz et al. (2018) demonstrated that the *Bacillus subtilis* strain Ege-B-1.19, isolated from soil, was capable of secreting a variety of hydrolytic enzymes. These enzymes comprised chitinase, chitosanase, N-acetyl-β-hexosaminidase and protease. These enzymes thus enable it to inhibit the growth of a range of phytopathogenic fungi, including *Aspergillus niger* EGE-K-213, *Aspergillus foetidus* EGE-K-211, *Aspergillus ochraceus* EGE-K-217, *Fusarium solani* KCTC6328, *Rhizoctonia solani* KACC 40111 and *Colletotrichum gloeosporioides* KACC 40689. Previous studies have also demonstrated the biocontrol potential of certain strains of *Bacillus subtilis* against various plant-pathogenic fungi. *Bacillus subtilis* was able to inhibit *Aspergillus niger*, *Fusarium verticillioides*, *Aspergillus flavus* and *Penicillium digitatum* (Waewthongrak et al., 2015). These results suggest that *Bacillus subtilis* is effective as a biological control agent against pathogenic fungi. Indeed, strains of *P. fluorescens* possess broad-spectrum antagonistic activity against plant pathogens. These activities include, amongst others, direct antibiosis, spatial competition or nutritional status and the production of siderophores (Showkat et al., 2012). Thus, during the stationary phase of growth, *P. fluorescens* species produce several metabolites with potent antifungal properties, such as phenazines, pyrrolnitrin, pyoluteorin and 2,4-diacetylphloroglucinol (2,4-DAPG), which are reported to inhibit, in vitro, the growth of pathogenic fungi (Haass and Defago, 2005). These metabolites act by impairing the pathogen's germination, growth and/or sporulation, causing distortion of the pathogen's hyphae, altering the appearance of colonies and inducing the production of specific structures such as pseudoparenchymata (Campbell, 1989). These results may also stem from the secretion of lytic enzymes such as β-1,3-glucanase and chitinase, whose mechanism of action involves parasitism through the degradation of the pathogenic fungus's cell walls (Ban Koffi et al., 2015). Showkat et al. (2012) demonstrated that *P. fluorescens* isolates collected from the wheat rhizosphere can inhibit, in vitro, the growth of *Fusarium oxysporum* and species of the genus *Aspergillus* that infect wheat crops in Kashmir.

The mechanisms by which *P. fluorescens* exerts its antagonistic activity can be categorized into several modes of action. First, direct antibiosis involves the production of diffusible antibiotics such as phenazines, pyrrolnitrin, and 2,4-DAPG, which directly inhibit fungal growth by interfering with cellular processes. Second, siderophore production sequesters iron from the environment, making it unavailable to pathogens and thereby limiting their growth. Third, competition for nutrients and space on the root surface prevents pathogen colonization. Fourth, the production of lytic enzymes such as chitinase and β-1,3-glucanase degrades fungal cell walls, leading to lysis of hyphae. Fifth, induced systemic resistance (ISR) can be triggered in the host plant, priming its defense responses against subsequent pathogen attack. The relative contribution of each mechanism may vary depending on the specific strain, the pathogen, and environmental conditions. Future studies should investigate the specific mechanisms operating in the most effective strains identified in this study (PZ13 and PEZ83) to better understand their biocontrol potential.

The results of this study are particularly promising for the development of sustainable disease management strategies in Côte d'Ivoire's sugarcane sector. The high inhibition rates observed (up to 80.47%) demonstrate the potential of these *Pseudomonas fluorescens* strains as effective biocontrol agents. However, several challenges must be addressed before field application. These include: formulation and delivery methods to ensure bacterial survival and establishment in the field; compatibility with existing agricultural practices; potential effects on non-target organisms; and regulatory approval for commercial use. Future research should include greenhouse and field trials to validate the efficacy of these strains under realistic conditions, as well as studies on formulation optimization and application timing.

V. CONCLUSION

This study enabled the isolation and identification of a variety of bacterial isolates from the sugarcane rhizosphere. The strains were selected on the basis of their potential antagonistic activity. The identification of bacterial isolates from the sugarcane

rhizosphere revealed the species *Pseudomonas fluorescens*. All these bacterial strains were capable of inhibiting the three fungi they were tested against, with high inhibition rates. In view of the various results obtained, these bacterial strains could be effective biocontrol agents against fungal diseases of sugarcane in Côte d'Ivoire.

Limitations of the Study: The study was conducted under in vitro conditions, which may not fully reflect field conditions where environmental factors, microbial competition, and plant-pathogen interactions can influence biocontrol efficacy. The specific mechanisms of antagonism were not investigated directly. Field validation of the most promising strains is needed to confirm their efficacy under realistic conditions. Future research should include greenhouse and field trials, formulation development, and investigation of the mechanisms of action.

Future Research Directions: Future studies should focus on: (1) evaluating the most effective strains (PZ13 and PEZ83) under greenhouse and field conditions; (2) investigating the specific mechanisms of antagonism (antibiosis, siderophore production, lytic enzyme activity, induced systemic resistance); (3) developing effective formulation and delivery methods for field application; (4) assessing the compatibility of these strains with other disease management practices; and (5) evaluating the effects of these strains on non-target organisms and soil health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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