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Isolation and Identification of Indigenous Microorganisms of Cocoa Farms in Côte d'Ivoire and Assessment of Their Antagonistic Effects Vis-À-Vis *Phytophthora palmivora*, the Causal Agent of the Black Pod Disease

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1. Introduction

The black pod disease due to *Phytophthora* spp is a destructive disease of cocoa. Worldwide, yield losses have been estimated to 30% (Lass, 1985). Côte d'Ivoire, the first cocoa producing country in the world, with 44% of the world market (ICCO, 2000) is also concerned by this disease. Several species of *Phytophthora* are involved in the disease. In Africa, two species, *P. palmivora* and *P. megakarya*, are the most damaging. The first species, which is the most common, causes damage in all the cocoa producing countries in the world, with yield losses between 20 to 30% ; the second, endemic to central and west Africa, is the most aggressive. This pathogen may cause the loss of the whole pod production in some countries (Flood, 2006). In Côte d'Ivoire, since the discovery of *P. megakarya* in the western region in the 90s, the black pod disease problem became more serious (Koné, 1999; Kouamé, 2006). Yield losses increased from an average of 10% to 35-40% (Kébé *et al*., 1996). Thus, the control of the disease became also a priority.

Although chemical control was developed by the research scientists, the dissemination of this method to the farmers was little successful. The low level of adoption of this technology by the farmers could be explained by the high cost of the fungicides as well as the difficulties related to the provision of water and the application of the fungicides. In addition, the requirements of the international market in terms of bean quality, environmental constrains, health issues for the consumers, and the different moratoriums in this area from the market partners (Anonyme, 2006), are numbers of constraints that do not facilitate the development of the chemical control method.

The strategy adopted in Côte d'Ivoire to control the black pod disease is based on integrated management, which is cost effective and environmentally friendly. This approach combines the use of agronomic practices, resistant cocoa varieties and natural antagonistic microorganisms of *Phytophthora*. Research works continue in order to improve agronomic practices and varietal resistance. The use of natural antagonistic microorganisms of

Phytophthora in the control of black pod disease is a new area of investigation explored by research scientists in several cocoa producing countries. Thus, some species in the genera *Trichoderma* and *bacillus* have been described by several scientific teams as potential biological agent for the control of *Phytophthora* spp. on coca (Bong *et al*., 1996; Krauss *et al*., 2003; Mpika, 2002). On other crops, fungi and bacteria belonging to several genera including *Pseudomonas, Burkholderia*, *Streptomyces*, *Serratia*, *Penicillium*, *Geniculosporiun, Gliocladium, Aspergillus*, *Coniothyrium, Ampelomyces, Phytophthora, Botrytis, Colletotrichum, Pythium, Rhizoctonia, Fusarium, Gaeunannomyces* and *verticillium* have been described as antagonists of many fungi, pathogens of plants. Members of these genera are pathogens of plants such tomato, rice, cucumber, maize, cotton and beans (Hebber *et al*., 1998 ; Benhamoun *et al*., 2000 ; Singh *et al*., 1999 ; de Cal *et al*., 1999 ; Paulitz and Linderman, 1991 ; Gerlagh *et al*., 1999 ; Vidhyasekaran and Muthamilan, 1999 ; Bong and Stephen, 1999 ; Tondje *et al*., 2006a,b).

During this study, the biodiversity was explored in the cocoa ecosystems. Microorganisms, potential antagonists of *Phytophthora* spp., were collected from pods and soils of cocoa farms. A collection of microorganisms was established. The antagonistic effect of these microorganisms on *Phytophthora* was assessed in the laboratory and in the field on the cacao trees.

2. Materials and methods

2.1 Samples and culture media

The microorganisms used in this study were isolated from the cocoa ecosystems, either from soils or pods. The soil samples were collected from cocoa farms in Abengourou in the the Eastern region of the country, and in Divo in the West-central region. In each of the locations, the soil samples were taken in 8 cocoa farms grouped in two categories according to the age of the trees. The first category was made up by 4 young plots with trees being 3 to 5 years old. These newly established plots still have open canopies with little soil litters. The second category was made up by 4 olds farms (25 to 30 years old). These farms, with very mature and fully bearing trees have closed canopies and abundant litters on the soil. In each plot, a bulk sample of 800 g of soil was made-up with 4 samples taken at the base of 4 cacao trees bearing many healthy pods and selected randomly in the plot.

Before taking each soil sample, the litter was totally removed. The samples were then taken in the superficial zones colonized by the fine root system because of fertilizer application. It is in this horizon of 30 to 40 cm deep that the samples were taken (Davet and Rouxel, 1997). Each bulk sample was carefully mixed and divided in two equal parts which were put in plastic containers. In one of them, baits for the antagonists made-up by fragments of pod plugs infected by *Phytophthora palmivora*, were buried in the soil sample (Tim *et al*., 2003). The other was left without any bait. In order to obtain a good colonization of the pod plugs, the soil samples were kept in the laboratory at 20°C for 30 days.

The pods used for the isolation of the microorganisms were collected in the main cocoa producing regions of Côte d'Ivoire. Thus, 390 healthy pods were collected from the 13 cocoa producing regions. In each region, pods were collected in 10 farms, at a rate of 3 pods per farm. The pods were kept in plastic bags labeled with information on the samples and brought to the laboratory for isolation of endophytes.

The diversity of fungi and bacteria in the soil of cocoa farms and in the pods were evaluated on selective culture media. For the isolation of bacteria, two selective media, including the PCAT medium (P. cepacia Azeaic acid tryptamine), specific to *Pseudomonas* and *Burkhoderia*

(Burbage, 1982), and the NYD medium (nutrient yeast dextrose) (Guizzardi and Pratella, 1996), adapted to a larger spectrum of bacteria were used. For the isolation of fungi, the TME (*Trichoderma* medium E), specific to fungi belonging to the genera *Trichoderma* and *Gliocladium* (Papavizas and Lumsden, 1982) were used. The PDA (potato dextrose agar), adapted to a larger spectrum of fungi was used for the isolation of the other fungi.

2.2 Isolation of the microorganisms

2.2.1 Pod endophytes

The surface of the pods was beforehand washed with tap water, and then underwent a series of disinfection in ethanol at 95 % for 30 seconds, in sodium hypochlorite at 10 % for 2 minutes, and again in ethanol at 75 % for 2 minutes, in order to eliminate the microorganisms present on the husk. The pods were rinsed three times in sterile distilled water to eliminate any trace of disinfectant (Arnold, 1999; Evans, et *al*., 2003; Rubini et *al*., 2005).

The sampling zone is chosen and the superficial tissues were removed using a sterile scalpel. Ten cubic shape fragments of 5 to 7 mm were taken per pod in the husk. The samples taken were put in culture on the selective media contained in Petri dishes. The incubation was made in the dark in a steam room, at 26 °C for 2 days for the bacteria and 7 days for the fungi.

2.2.2 Soil microorganisms

The microorganisms were obtained by direct isolation from the soil according to the method described by Davet and Rouxel (1997) and from fragments of pod husk buried in the soil samples. The soil samples were beforehand dried, ground and calibrated by sieving. The fragments of husk were ground in a porcelain mortar to separate each living propagule, because of the gelatinous consistency of the decomposing pod husk.

In both cases, 10 g of ground soil were transferred in 90 ml of sterile distilled water contained in an Erlenmeyer. The mixture is then put in agitation for 30 minutes to obtain a good separation of the particles. To obtain a variable concentration of propagules and facilitate the enumeration of the colonies, a series of dilution was performed from the initial solution whose concentration was 10^{-1} (Rapilly, 1968). To obtain a solution of 10^{-2}, 1 ml of the initial solution was mixed in 9 ml of sterile distilled water. Thus, a series of dilution from 10^{-2} to 10^{-9} was performed in hemolytic tubes. For each dilution, 100µl was pipetted and spread onto the surface of the culture media in Petri dishes. For each dilution and for each medium, 4 Petri dishes were inoculated. The incubation was also made in the dark in a steam room at 26 °C for 2 days for the bacteria and 7 days for the fungi.

2.3 Conservation and identification of the microorganisms

The microorganisms isolated were first purified by two or three successive monospore transplantings on specific culture media. Once purified, each isolate was designated by a code number. For strains of bacteria, the code numbers were preceded by the letter B, followed by an order number. The nomenclature of the fungi isolates begins with one or several first letters of the name of the genus, followed by an order number. The conservation of the microorganisms was then made in a freezer at a temperature of - 80°C for the bacteria, and - 10°C for the fungi. In both cases, agar disks taken near the edge of the purified culture were transferred to 1.5 ml sterile Eppendorf microtubes containing glycerol at 50 %. The identification of the isolated microorgamisms was based on the macroscopic, microscopic, biochemical and molecular characters. The molecular characterization was made by the

method developed by Druzhinina *et al*. (2005) to identify the various species of fungi isolated. This method uses baits specific targeting genes encoding for translation elongation factor 1-alpha (*tef1*) obtained on sequences IST 1 and 2 of the DNAr.

2.4 Evaluation of the antagonistic effect of the microorganisms

The antagonistic effect of the isolated microorganisms against *P. palmivora* was evaluated using three tests. The first test conducted *in vitro*, is a test of direct confrontation between *P. palmivora* and the microorganism in a mixed culture. The second test was realized *in vivo* on leaf disks of cacao tree. The test consists in measuring the leaf susceptibility to *P. palmivora* in the presence of the microorganism according to the scoring scale of Blaha which varies from 0 to 5 (Nyassé *et al*., 1997). The third test was carried out in the field on cacao trees.

The confrontation between *Trichoderma* and *P. palmivora* was realized in Petri dishes containing agar culture media made of potato broth (PDA medium). A fragment of mycelium, 6 mm in diameter, was taken around the edge of the cultures of each fungus. These fragments were transplanted face to face in the same Petri dish, 2 cm from the center of the dishes (Benhanou and Chet, 1996). The controls were monocultures of each of the 2 fungi being confronted. In this case, the fragment of mycelium was placed in the center of the Petri dishes. Each treatment was conducted in 6 replications. The incubation was done in the dark in a cryptogrammic steam room. Twenty four hours after the start of the cultures, the mycelial growth of each fungus was measured daily until the Petri dish was full with the fungal development. Seven days after the mycelial strands of both fungi have met, the survival of the spores of *P. Palmivora* was evaluated by taking fragment of mycelium in the Petri dishes containing the mixed culture according to an axis which passes by the center and the transplanting sites of both fungi. Two consecutive samples were taken 0,5 cm apart. Thus, nine samples were taken in every Petri dish of mixed culture. The fragments of mycelium taken were placed in lesions made on healthy pods collected from trees of the same clone, making sure the pods were not attacked by *Phytophthora* sp. These inoculated pods were placed in crystallizers in which the humidity was maintained by a plug of sterile cotton wool soaked with sterile distilled water. The incubation was done at the ambient temperature of the laboratory. The survival of *Phytophthora* was evaluated by recording the number of brown spots on the surface of the inoculated pods after 15 days of daily observation. The presence of *Phytophthora* in the spots was confirmed by microscopic observations.

The effect of the microorganisms on *P. palmivora* was evaluated through the leaf disk test. This test was performed on disk of cocoa leaves 15 mm in diameter. The disks were beforehand dipped into the bacterial suspension or the suspension of *Trichoderma* for 1 min, and then arranged in containers on a plate of foam soaked with water. Each disk received 10 µl of a suspension of zoospores of *P. palmivora* calibrated to 3.10^5 zoospores / ml. The controls were not dipped in bacterial suspension or suspension of *Trichoderma* before receiving the suspension of zoospores of *P. palmivora.* The leaf disks were obtained from 3 cocoa clones, the reaction of which to *Phytophthora* sp. is known (Tahi et *al*., 2000). Thus the susceptible clone (IFC5), the moderately resistant clone (P7) and the resistant clone (SCA6) were tested. For each clone, 40 leaf disks were inoculated and placed in 4 containers, each representing a replicate. The incubation was done in the dark at 26°C for 7 days. The results were scored according to the scale of Blaha (Nyassé *et al*., 1995).

In the field, the random target method was used to assess the effect of the microorganisms on *P. palmivora*. This method consisted in following the development of the disease on groups of 100 Cacao trees with different treatments. The 100 trees corresponding to each

group were chosen randomly in the field, numbered and marked with the same color. However, care was taken so that each test tree was surrounded by 8 border trees. The *Trichoderma* based biological fungicides were applied using a knapsack sprayer at the concentration of 10^7 conidia ml^{-1}. The entire cocoa tree was treated. Six applications at 21 days interval were made. Over the duration of the trial, weekly count were made for healthy mature pods, rotting pods, wilting pods and pods damaged by squirrels. During the trial, the survival of *Trichoderma* on treated pods and flower cushions was evaluated. Thus, pod samples were taken on the treated trees every 15 days.

2.5 Data analyses

The SAS program (Statistical Analysis System, SAS Institute, Cary, NC) was used for all the statistical analyses. For the isolation of the microorganisms and the leaf disk test, the analyses of variances (ANOVA) were performed on the mean number of microorganisms colonies counted on the culture media and the mean rating score of leaf susceptibility to *Phytophthora* in the presence of bacteria and fungi. The normality of residuals and the homogeneity of the variances were verified. The mean comparisons were realized with the Student Newman and Keuls test at 5%.

3. Results

3.1 Microorganisms isolated

The exploration of the biodiversity of microorganisms obtained from soils of cocoa field and endophytes of pods revealed 2 categories of microorganisms: fungi and bacteria. On the pods, the fungi represented 66.31% of the positive isolation, against 33.04 for the bacteria. In the soil, the two categories of microorganisms were present in inverted proportion: 55.8% for the bacteria and 29.8% for the fungi. In the group of the fungi, the yeast that represented 11.6% of the population on the pods, represented only 3.7% in the soil. Among the bacteria, the Actinomycetes which represented only 0.64% on the pods, reached 10.5% in the soil (Fig.1).

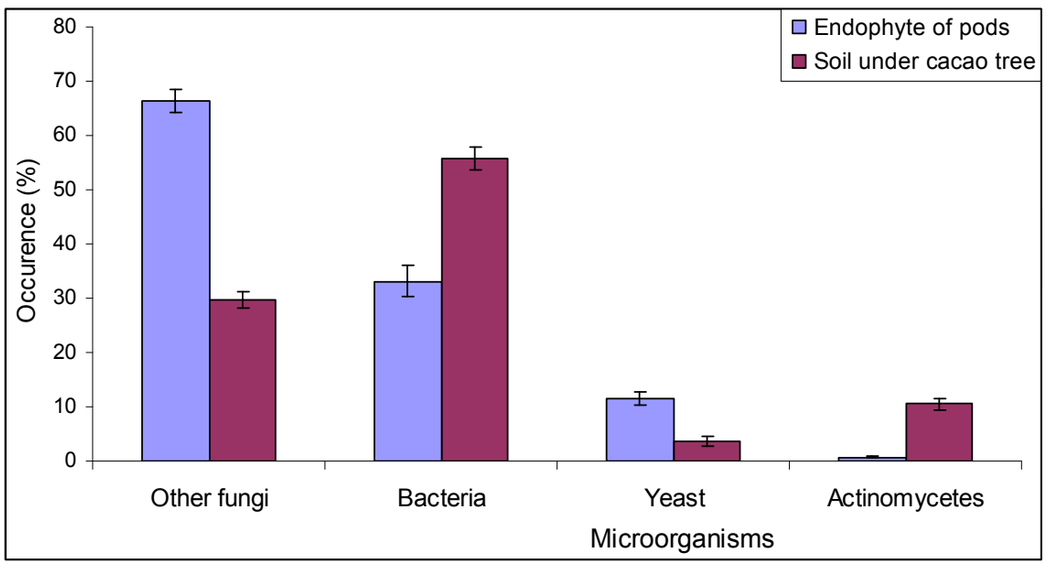

Fig. 1. Group of micro-organisms and their relative importance in the soil under cacao-plantation and the pod cortex

The results of the statistical analyses showed that the mean number of microorganisms colonies counted per gram of soil varied significantly ($P<0.05$) with the isolation method (with baits or no bait). This result revealed that the use of fragment of pods infected by *Phytophthora* as bait, significantly improved the isolation of the microorganisms on PDA, TME and PCAT media (Table 1).

	Culture media			
Isolation methods	NYD	PDA	TME	PCAT
Direct isolation	$3.27\ 10^{11}$ **a** ± $2.14\ 10^{11}$	$4.72\ 10^{6}$ **a** ±$1.42\ 10^{6}$	$4.64\ 10^{5}$ **a** ± $1.60\ 10^{5}$	$5.10\ 10^{5}$ **a** ± $2.50\ 10^{5}$
Baiting isolation	$3.80\ 10^{11}$ **a** ± $2.35\ 10^{10}$	$2.43\ 10^{9}$ **b** ± $1.41\ 10^{9}$	$6.10\ 10^{6}$ **b** ± $1.60\ 10^{6}$	$7.64\ 10^{7}$ **b** ± $3.67\ 10^{7}$

Means within the same column followed by the same letter are not significantly different according to Newman & Keuls's test at 5 % probability

Table 1. Mean numer of colonies for unit (cfu/g) according to the isolation methods and the culture media

For the samples taken in Divo and Abengourou, the statistical analyses did not show any significant differences ($P>0.05$) between regions with regard to the mean number of microorganisms colonies for the different culture media except for the NYD. There was no clear relationship between the density of microorganisms isolated and the age of field except for the bacteria on the NYD medium in Abengourou (Table 2).

		Culture media			
Locations	Age of the farms	NYD	PDA	TME	PCAT
Abengourou	Young farms	$1.9\ 10^{7}$ **a** ± $1.42\ 10^{7}$	$1.75\ 10^{6}$ **a** ± 0	$4.67\ 10^{6}$ **a** ± $2.2\ 10^{6}$	$1.2\ 10^{8}$ **a** ± $6.96\ 10^{8}$
	Old farms	$1.38\ 10^{12}$ **b** ± $4.31\ 10^{10}$	$1.15\ 10^{7}$ **b** ± $1.11\ 10^{6}$	$5.97\ 10^{6}$ **a** ± $3.22\ 10^{6}$	$2.77\ 10^{7}$ **a** ± $1.52\ 10^{7}$
Divo	young farms	$5.81\ 10^{7}$ **a** ± $4.78\ 10^{7}$	$2.05\ 10^{8}$ **a** ± $1.42\ 10^{8}$	$1.42\ 10^{6}$ **a** ± $7.96\ 10^{5}$	$5.75\ 10^{6}$ **a** ± $4\ 10^{4}$
	Old farms	$3.2\ 10^{10}$ **a** ± $2.15\ 10^{10}$	$3.45\ 10^{9}$ **a** ± $1.99\ 10^{9}$	$1\ 10^{6}$ **a** ± $5.76\ 10^{5}$	$5.78\ 10^{4}$ **a** ± $3.77\ 10^{4}$

Means within the same column followed by the same letter are not significantly different according to Newman & Keuls's test at 5 % probability

Table 2. Mean number of colonies for unit (cfu/g) according to the locations and the age of the farms

3.2 Identification of the microorganisms isolated

Regarding the pods, 313 fungi isolates were purified. Among the purified isolates, 58 isolates belonging to 9 genera were identified. These were *Penicillium* sp (6), *Fusarium* sp (7), *Botrytis* sp. (9), *Pestalotia* sp. (24). The remaining isolates identified belong to the genera *Nigrospora* (2), *Physoderma* (1), *Polynema* (1), and *Botryodiplodia* (8). The other isolates (255) belong to diverse species or genera, but the relationship has not been established yet.

One hundred and two (102) colonies of bacterial endophytes of pods were identified. These bacteria belong to two groups based on Gram-coloration response to chemicals: 56 Gram-positive bacteria (45 bacilli and 11 cocci) and 46 gram-negative bacteria (9 bacilli and 37 cocci). The bacterial colonies B105 and B116 were identified as belonging to the genus *Bacillus*. Finally, 55 yeast strains and 2 isolates of actinomycetes were identified.
With regard to the soil, amongst 455 isolates collected and purified, 254 bacteria, 136 fungi, 48 actinomycetes and 17 yeasts were identified. In the group of the fungi, 44 isolates belonging to the genus *Trichoderma* were identified. These were *T. virens* (32 isolates), *T. harzianum* (4 isolates), *T. spirale* (6 isolates) and *T. asperellum* (2 isolates). Three isolates belonging to the genus Clonostachys were also identified. The other isolates have not been identified yet.

3.3 Effect of the microorganisms on *Phytophthora palmivora*

3.3.1 Effect of *Trichoderma* on the mycelial growth of *P. palmivora*

The direct confrontation tests realized *in vitro*, between the isolates of *Trichoderma* sp. and of those of *Phytophthora palmivora* revealed an inhibitory effect of *Trichoderma* on *P. palmivora* in mixed culture. After 3 days of confrontation, the inhibition of the growth speed became very high and the growth of *P. palmivora* practically stops (Fig.2).

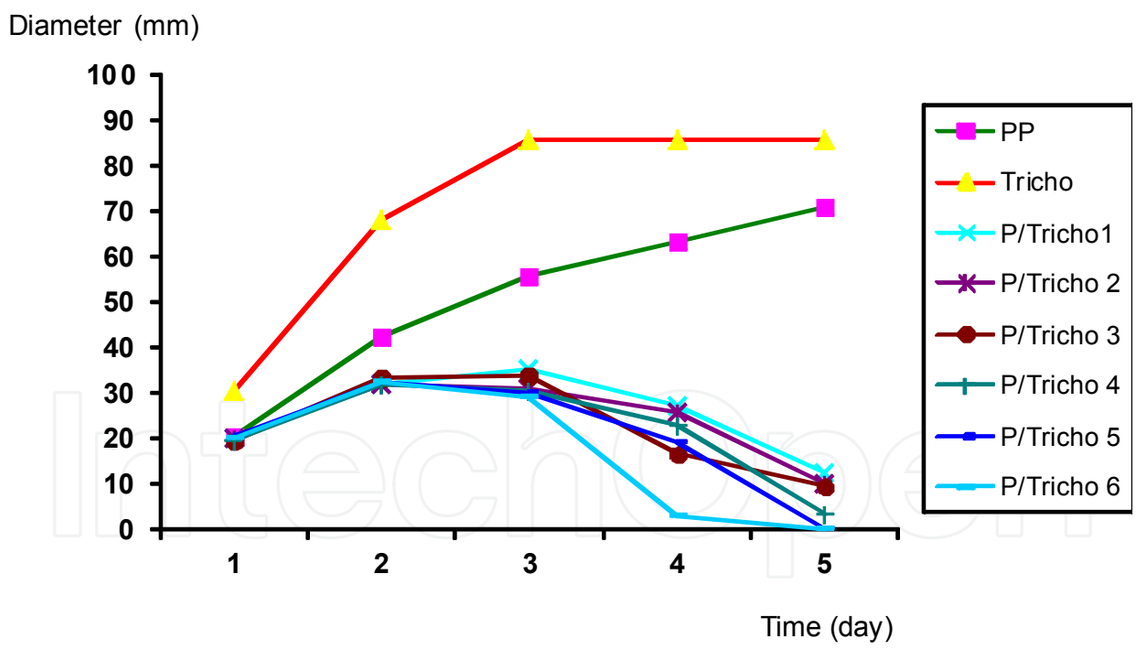

Fig. 2. Influence of *Trichoderma* on the mycelial growth of *Phytophthora palmivora*. Legend. PP : *Phytophthora palmivora*, Tricho: *Trichoderma*, P/Tricho : *Phytophthora/Trichoderma*

The capacity of *Trichoderma* to stop the mycelial growth of *P. palmivora* reveals a deep fungistatic effect. From the fourth day, we note a progressive disappearance of the mycelium of *P. palmivora*. This degradation of the mycelium of *P. palmivora* which is more accentuated at the fifth day, with all the isolates, reveals a mycoparasitic effect of

Trichoderma. After 7 days of confrontation, in mixed culture, the survival of the spores of *P. palmivora* was assessed. The influence of *Trichoderma* sp. varied according to isolates. The percentage of survival of *Phytophthora* varied from 90 to 50 % respectively with *Trichoderma* isolates 3 and 6. This rate falls to 30 to 10 % with *Trichoderma* 2 and 4. Finally, the presence of *Trichoderma* 1 and 5 in mixed culture has a very clear fungicidal effect with a percentage of survival of *P. palmivora* equal to 0.

3.3.2 Influence of the bacteria and *Trichoderma* on the leaf susceptibility to *P. palmivora*

The effect of 37 strains of bacteria on *P. palmivora* was evaluated using the leaf disk test. The results showed that, for the resistant clone (SCA 6) of cacao tree, the rating scores of the leaf susceptibility varied from 3.3 to 0.59, respectively with strains B104 and B105 (Fig. 3).

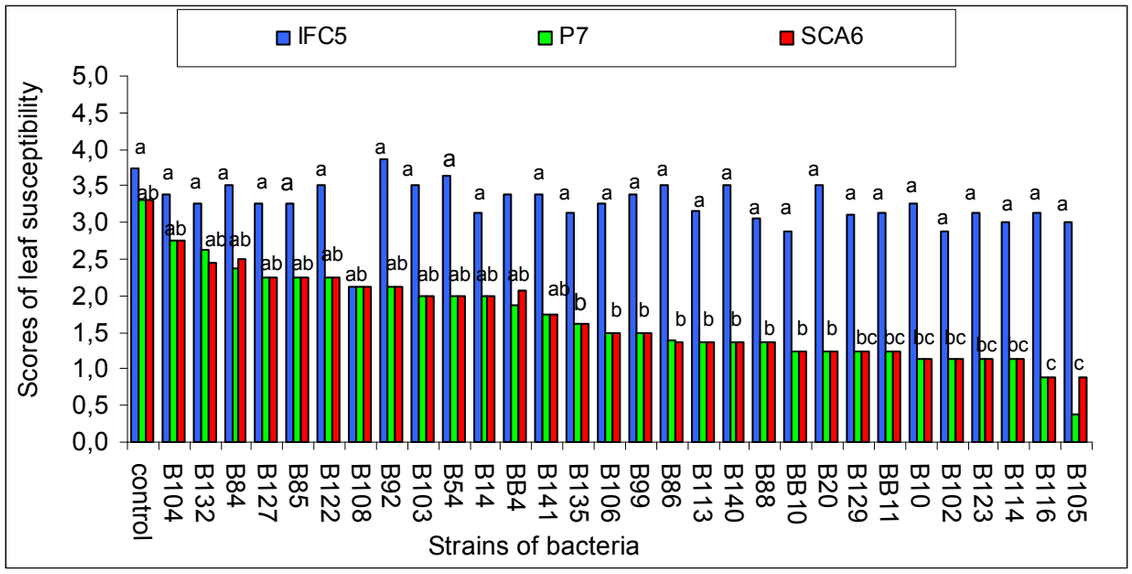

Fig. 3. Effect of the bacterial strains on the leaf discs susceptibility to *P. palmivora* for clones IFC5, P7 and SCA6.

With the moderately resistant clone (P7), the rating scores varied in the same proportions. In both cases, the analysis of variance revealed a significant ($P<0.05$) effect of bacteria and the Student Newman & Keuls test revealed 5 homogeneous groups of bacteria (a, ab, b, bc, and c). The best results were obtained with bacteria BI05 and B116 (group c). With the susceptible clone IFC 5, we note that the reduction of the rating scores of leaf susceptibility is relatively low. The scores varied from 3.8 to 2.8. The statistical analyses did not reveal any significant ($P>0.05$) differences between the treatments and the untreated control.

Similarly, the effect of 57 *Trichoderma* isolates on *P. palmivora* was evaluated using leaf disk of three cocoa clones. For the susceptible clone (IFC5), the scores of leaf susceptibility varied from 2.4 to 0.02 respectively with the isolates T39 of *T. spirale* and T55 of *T. virens*. With the moderately resistant clone (P7), we note a reduction of the scores, which varied from 2.04 to 0.03 respectively with the isolates T39 of *T. spirale* and T28 of *T. virens*. With the resistant clone (SCA6), the scores were less than 1.5 for all the isolates of *Trichoderma* (Fig. 4).

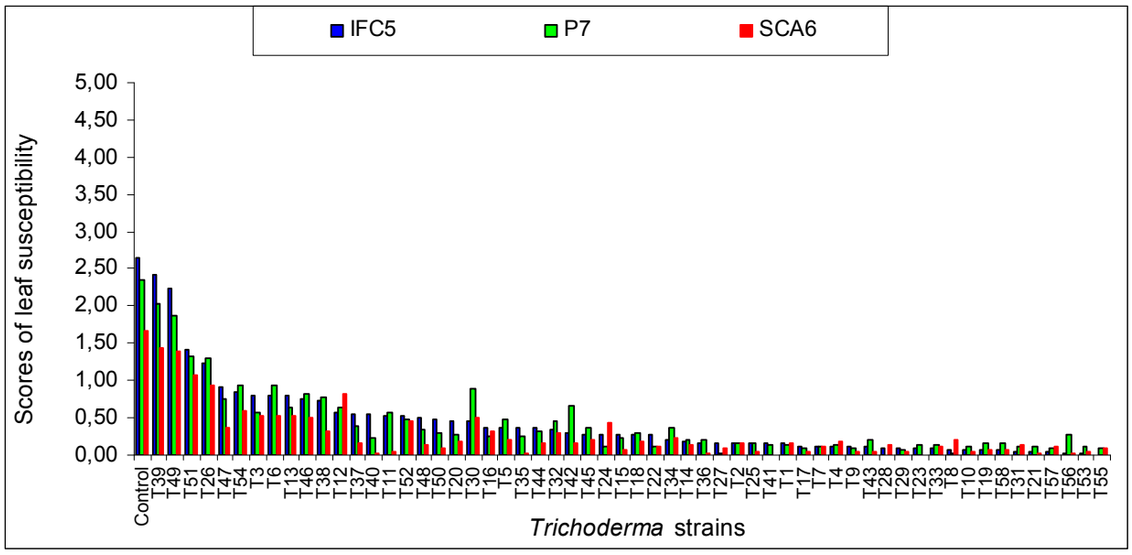

Fig. 4. Effect of *Trichoderma* strains on the leaf discs susceptibility to *P. palmivora* for clones IFC5, P7 and SCA6.

3.3.3 Field efficacy of *Trichoderma* in the control of the black pod disease due to *P. palmivora*

The effects of 4 species of *Trichoderma*, applied to the cacao trees were compared. The results obtained are presented in Table 3. The final percentages of rotten pods for year 1, were 11.75, 8.44, 7.34, and 3.6 respectively with the isolates T4 of *T. spirale*, the isolate T40 of *T. harzianum*, the isolate T7 of *T. virens* and the isolate T54 of *T. asperellum*. These percentages were lower than 18.33 obtained with the untreated control, indicating a reduction of the yield losses from 36 to 80 %. For year 2, the final percentages of diseased pods were lower than that recorded in year 1. For both years of study, the calculated efficacy index was

	Treatments	Rotten pods (%)	Reduction of loss in relation to untreated control (%)	Slope of the epidermic curve	Efficacy index (%)
Year1	Untreated control	18.33	-	15.6	
	T. spirale T4	11.75	36	8.96	42
	T. harzianum T40	8.44	54	8.82	43
	T. virens T7	7.34	60	5.29	66
	T. asperellum T54	3.6	80	3.29	79
Year2	Untreated control	7.46	-	12.04	
	T. harzianum T40	4.16	44	6.73	44
	T. virens T7	4.23	43	6.08	49
	T. spirale T4	2.46	67	4.81	60
	T. asperellum T54	2.21	70	4.68	61

Table 3. Reduction of the final losses due to the black pod disease and efficacy (%) of 4 species of *Trichoderma* during 2 years of field trials after the application

higher than 60 % for the isolate T54 of *T. asperellum*. The analysis of the evolution of the epidemic curves in each treated plot reveals that the isolate T54 of *T. asperellum* and the isolate T7 of *T. virens* substantially reduced the losses due to the black pod disease. Furthermore, these isolates delayed the onset of the epidemic of the black pod disease compared to the untreated control (Fig. 5).

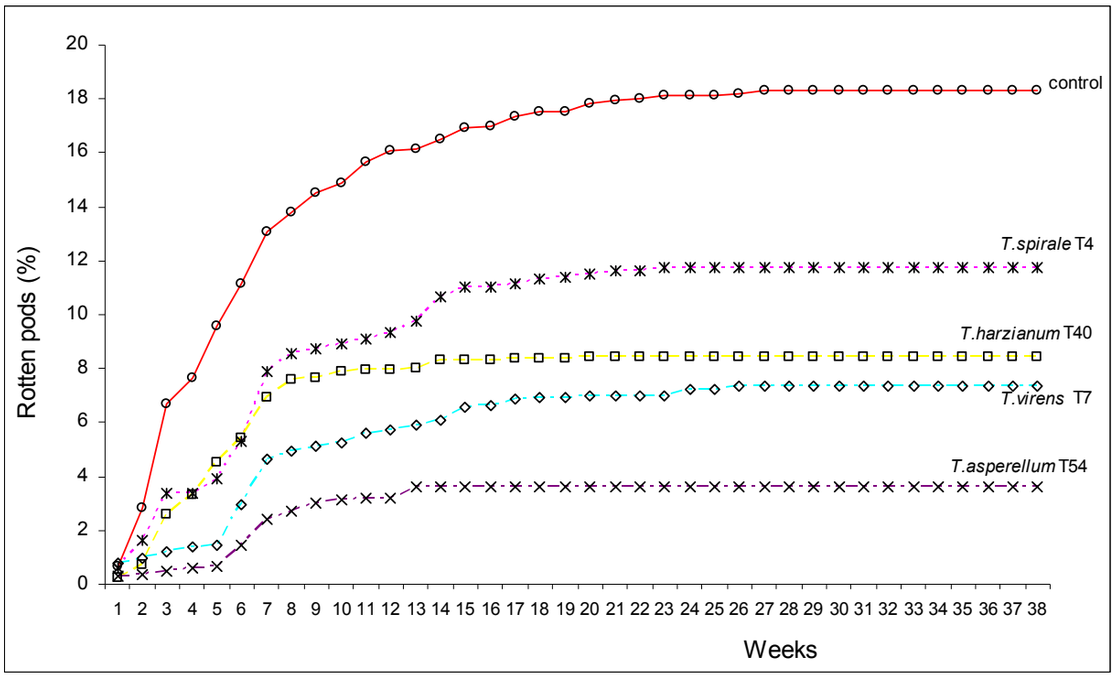

Fig. 5. Effect of the applications of biofungicide containing various species of *Trichoderma* on the evolution of black pod disease in the field.

4. Discussion

This study revealed the existence of a high biodiversity within the microorganisms' populations in the cocoa ecosystem. Regarding the two areas investigated, the soil microorganisms and pod endophytes, the biodiversity varied quantitatively and qualitatively. Endophytes are microorganisms that colonize plant tissues without causing visible symptoms in normal conditions (Carroll, 1998). Since the beginning of the 2000s, these microorganisms are usually studied in the tropical plants for their use in biological control or for the production of substances having pharmacological properties (Azevedo, 2002; Peixoto-Neto, 2002). The isolation carried out on cocoa pods showed a predominance of fungi (66.31%), against 33.04% of bacteria. Similar results were obtained by Evans *et al*. (2003) and Rubini *et al*. (2005). Regarding the soil, the results also showed a high proliferation of both categories of microorganisms with a greater proportion of bacteria. The soils of cocoa field and the pods are therefore the preferred sites of indigenous microorganism, potential antagonists of *P palmivora*, occupying the same ecological niche. The results showed that the use of baits, made by fragments of cocoa pod, infected by *P. palmivora*, significantly improved the isolations of microorganisms in the soil. This improvement could be explained by an affinity between *P. palmivora* and the collected

microorganisms. An analysis of the list of the collected microorganisms revealed the existence of fungi and bacteria identified by several authors as having antagonistic effect against pathogens responsible for plant diseases. This is the case of the genus *Trichoderma* in which several species have been tested for the control of cocoa diseases (Sanogo *et al.,* 2002; Krauss and Soberanis, 2001; Tondje *et al.,* 2007; Samuels, 1996). In the group of the bacteria, the antagonistic effect of the genus *Bacillus* was demonstrated by Shari Fuddin, 2000. The direct confrontation tests carried out *in vitro* between *P. palmivora* and the *Trichoderma* isolates revealed an antagonistic effect going from the inhibition of mycelia growth (fungistatic effect), to the degradation and the disappearance of the mycelium of *P. palmivora* (mycoparasitic and fungicidal effect). Similar results were obtained with *Trichoderma harzianum* on *Fusarium oxysporum* (Hibar *et al.*, 2005). In Central America, similar results were also obtained with *Trichoderma stromaticum* in the control of witch's brooms disease of cacao and with *Trichoderma virens* against the black pod disease (Krauss and Soberanis, 2002). This study thus allowed to isolate and to purify several potential antagonists of *Phytophthora* sp, susceptible to be used in the control the black pod disease.

The evaluation of the effect of the bacterial strains on *P. palmivora* using the leaf disk test revealed a reduction of the leaf susceptibility to *P. palmivora* for resistant clones (SCA 6) and moderately resistant clones (P 7). On the other hand, this effect is less perceptible with the sensitive clone (IFC5). Similar results were obtained by Maurhofer et *al*. (1994) on tobacco, Duijff et *al*. (1997) on tomato and Chen et *al*. (1998) on cucumber. These results could reveal an increase in the level of the intrinsic resistance of the plant by the bacterial strains. The highest effects were obtained with bacterial strains B 105 and B 116, which makes them potential candidates for the biological control against *P. palmivora*. These two bacteria belong to the genus *Bacillus* endowed with an ability to sporulate, suggesting a good ability of dissemination within the framework of a biological control program. Also, on leaf disks, the effect of *Trichoderma* revealed a reduction of the size and the frequency of the necrotic lesions due to *P. palmivora*. Similar results were obtained by Bowers *et al*. (2001b) on leaf disk with *Phytophthora megakarya*. This effect of reducing leaf susceptibility results from the germination of the spores of *Trichoderma,* on the underside of the leaves, which probably inhibits or hampers the germination of the zoospores of *Phytophthora*. This germination would stimulate the mechanisms of defense, and consequently would strengthen the resistance to the penetration and the dissemination of the parasite. Similar results were obtained by Bigirimana et *al*. (1997), Howell et *al*. (2000), Sid Ahmed et *al*., 2000 and Harman et *al*. (2004) on bean, cotton, hot pepper and corn inoculated by *T. virens* and *T. harzianum*, subjected to the attacks of *Rhizoctonia* sp., *Colletrotrichum* sp. and *Phytophthora* sp.

The effects of the isolates T4 of *T. spirale*, T7 of *T. virens* and T40 of *T. harzianum* in the field and the isolate T54 of *T. asperellum* on *P. palmivora*, showed a reduction of the incidence of the black pod disease due to *P. palmivora*. However, this effect is more striking on cacao trees treated with *T. asperellum*. Similar results were obtained by Tondje et *al*. (2007) in Cameroon after evaluating the potential effect of the isolates of *T. asperellum* on the incidence of the black pod disease due to *P. megakarya*. This reduction would be due to the mycoparasitic effect of *T. asperellum* against *Phytophthora* sp. Indeed, *T. asperellum* penetrates and destroys the propagules of *Phytophthora* on the flower cushions, thus reducing the quantity of inoculums of this parasite. Besides the mycoparasitic effect on the propagules (mycelium and sporocystes) of *P. palmivora*, the production of cellulase by *T. asperellum* in the presence of *Phytophthora* sp. would also be determining. The synergy of the modes of action in the

efficicacy of *Trichoderma* to achieve the control of the disease was demonstrated by Chet *et al*. (1997); Howell (2003) and Benitez *et al*. (2004).

5. Conclusion

The exploration of the biodiversity of microorganisms in the soils collected from cocoa farms and in cocoa pods showed that it is possible to know natural populations of microorganisms and beneficial interactions for the cacao tree as well as to identify potential antagonists for *Phytophthora* sp. For the two components investigated (soil and pod), the biodiversity appeared to be highly variable both qualitatively and quantitatively. The results obtained in this study showed that it is possible to exploit the biodiversity for the control of the black pod disease due to *Phytophthora palmivora.* Several isolates of *Trichoderma* and some bacterial strains showed a deep antagonistic effect against *P. palmivora* in the laboratory. The study allowed to demonstrate the efficacy of *Trichoderma asperellum* in the control of the black pod disease. This result arouses a great hope for the cocoa farmers whose revenues constantly decline because of this disease. At national level, appropriate measures have been taken to allow a large scale use of *T. asperellum* for the control of the black pod disease in Côte d'Ivoire.

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Every ecosystem is a complex organization of carefully mixed life forms; a dynamic and particularly sensible system. Consequently, their progressive decline may accelerate climate change and vice versa, influencing flora and fauna composition and distribution, resulting in the loss of biodiversity. Climate changes effects are the principal topics of this volume. Written by internationally renowned contributors, Biodiversity loss in a changing planet offers attractive study cases focused on biodiversity evaluations and provisions in several different ecosystems, analysing the current life condition of many life forms, and covering very different biogeographic zones of the planet.

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