"EVALUATION OF THE BIOCONTROL CAPACITY OF NATIVE MICROORGANISMS AGAINST *FUSARIUM OXYSPORUM F. SP.* CUBENSE"

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ABSTRACT

Fusarium Wilt is an economically important disease of bananas caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (FOC). It causes severe losses in the yield and quality of bananas and is extremely difficult to control conventionally using chemical fungicide. Biological control offers an eco-friendly alternative for sustainable plant disease management. The objective of this research was to determine the biocontrol capacity of native microorganisms against *Fusarium oxysporum* f. sp. *cubense*. Isolation of native rhizospheric microorganisms was carried out in Perené and Satipo areas (Junin region) in the central jungle of Peru. Thirty rhizobacterial isolates were screened for antagonistic activity in dual culture. Isolate 27 (JP_27) showed the highest antagonistic activity (81,52% of mycelial growth inhibition) against FOC. The metabolites of isolate 27 inhibited FOC mycelial growth by 90%. Based on the morphological, physiological and phylogeny analysis with 16S rRNA sequence, the isolate 27 was identified as *Burkholderia* sp.

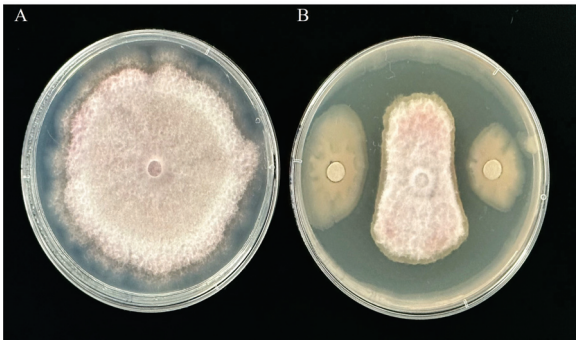
ISOLATION OF THE STRAIN JP_27

A total of 37 isolates with antagonist effect were gotten from the soil of a banana field from the central jungle of Junin Region in Perú. Among them, isolate JP_27 showed a more significant and stronger antagonistic activity against Foc SR4 (Figure 1) and exhibited a broad-spectrum of antifungal activity compare with another isolated (Table 1).

Table 1. Inhibition (%) of Radial Growth of Bacterial Natives Strains with Foc SR4 on PDA plate after 7 days of incubation

Bacterial Natives Strain	Foc TR4 mycelial growth radio (mm)	PIRG*
27	8,7	81,52%
E6	31,4	33,72%
E3	31,4	33,72%
14	33,8	28,78%
Control Foc SR4	47,5	0%

*PIRG: Percentage Inhibition of Radial Growth (R1-R2/R1*100), R1, Radius of Foc SR4 colony in control plate; R2, Radius of Foc SR4 colony in dual culture plate.



A Fig. 1 Antagonistic activity of isolate 27 against Foc SR4 in dual culture on PDA after 7 days of culture at 28°C. (A) Only Foc SR4, (B) Isolated 27 and Foc SR4.

MICELIAL GROWTH INHIBITION OF FOC SR4

For mycelial growth inhibition test, two different culture media were used, Potato Dextrose Broth (PDB) and Trypticase Soy Broth (TSB). For both cases the same treatments were considered. The control treatment, only FOC SR4 was inoculated and for the test treatment, FOC SR4 and the strain JP_27 were inoculated; after 10 days it was observed that growth reduction of the mycelium in both culture media (evaluation of biomass in dry weight) was 95.02 % in PDB and 85.81 % in TSB, as shown in figure 3.

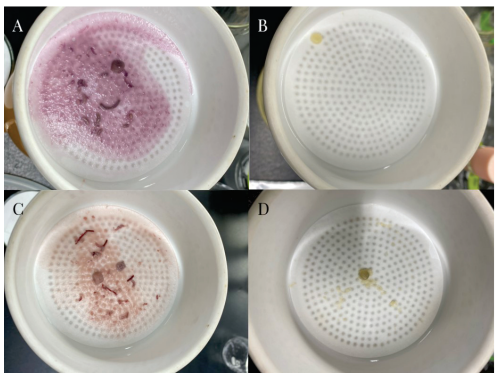


FIG. 3 Micelial inhibition growth of Foc SR4 by Strain JP_27. (A-B) Cultivation in PDB (Potato Dextrose Broth), (A) Only Foc SR4, (B) Isolated 27 and Foc SR4. (C-D) Cultivation in TSB (Tryptic Soy Broth), (C) Only Foc SR4, (D) Isolated 27 and Foc SR4. Incubation period was for 10 days at 28°C.

IDENTIFICATION OF MORPHOLOGICAL, PHYSIOLOGICAL AND ENZYMATIC PROPERTIES OF STRAIN JP_27

The strain JP_27 was slightly yellow shining and smooth in colony appearance on the nutrient Agar (AN) plate. The biochemical tests indicated that it is a gram-negative and shaped bacterium. The enzymatic property carried out showed that strain JP_27 can produce lipase, celluloses, chitinases and proteinases as shown in table 2 and figure 2.

Table 2. Enzymatic properties of Isolate 27

Test	Medium	Result
Lipase (1)	Tween 20	+
Cellulase (2)	CMC	+
Chitinase (3)	Chitin 1%	+
Protease (4)	Skim milk 1%	+

* Each medium contains the specific substrate for the evaluation of each enzyme.

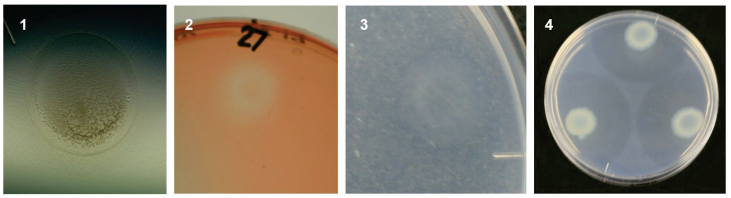


Fig. 2 Lipases, Cellulase and Chitinase was evaluated after 7 days of incubation (30°C). Protease was evaluated after 3 days of incubation (30°C)

EVALUATION OF SECONDARY METABOLITES OF JP_27 STRAIN AGAINST FOC SR4

Secondary metabolites of strain JP_27 were extracted using ethyl acetate, the crude extract was resuspended in 5% Dimethyl Sulfoxide (DMSO) for evaluation. The obtained metabolic extracts were resuspended in 5% DMSO and evaluated using the technique in wells, verifying that the inhibitory activity of FOC SR4 was 90% compared to the absolute control (Table 4).

Likewise, for the observation and verification of the metabolites, a run was carried out on the HPLC equipment where the presence of peaks were observed (Figure 4C), which demonstrates the presence of different metabolic compounds produced by the bacteria. In future work a HPLC-MS analysis will be done for the identification of each one of the metabolites present in the extracts.

Table 4. Inhibition (%) of Foc SR4 Radial Growth by the metabolic extract from Isolate 27 on PDA plate after 7 days of incubation

Bacterial Natives Strain	Foc SR4 mycelial growth radio (mm)	PIRG
7 days	2,5	90%
Control Foc TR4	25,0	0%

*PIRG: Percentage Inhibition of Radial Growth (R1-R2/R1*100), R1, Radius of Foc SR4 colony in control plate; R2, Radius of Foc SR4.

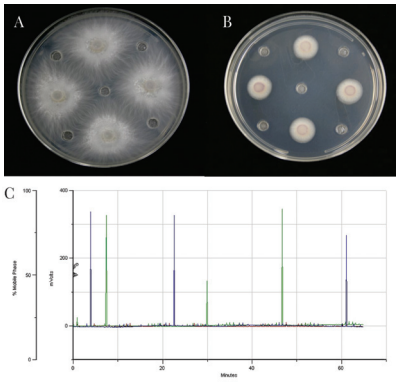


Fig. 4. Bioassay of Isolate 27 metabolite extract against Foc SR4. (A). Control (DMSO 5%), Foc SR4. (B). Zones of inhibition of secondary metabolite extract from isolate 27 against Foc SR4. n = 3 biological replicates. (C). HPLC Chromatography of secondary metabolites extract from Isolate 27.

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