

Isolation of *Fusarium verticillioides* and evaluation of native rhizobacteria for its control in maize

Bastidas-Tirado, Christopher¹; Yáñez-Juárez, Moisés G.¹; Lira-Morales, Juan D.²; Medina-López, Raymundo¹; Hinojosa-Gómez, Jeny²; Zazueta-Torres, Norma D.³; Román-Román, Leonardo¹; Ley-López, Nancy^{1*}

¹ Universidad Autónoma de Sinaloa. Facultad de Agronomía, Doctorado en Ciencias Agropecuarias. Carretera Culiacán-Eldorado km 17.5. Culiacán, Sinaloa, México. Apartado Postal 25, C.P. 80000.

² Centro de Investigación en Alimentación y Desarrollo, A.C. Coordinación Culiacán, Km 5.5 Carretera a Eldorado, Campo El Diez, Culiacán, Sinaloa, México. C.P. 80110.

³ Tecnológico Nacional de México/ITS Eldorado, Av. tecnológico S/N Col. Rubén Jaramillo C.P. 80450. Eldorado, Sinaloa, México.

* Correspondence: nancyley@uas.edu.mx

ABSTRACT

Objective: To isolate and identify phytopathogenic fungi associated with maize cultivation and to evaluate in vitro the antagonistic potential of native rhizobacteria against *F. verticillioides*.

Design/methodology/approach: Phytopathogens were isolated from maize plants exhibiting symptoms of root and stem rot. Their identification was carried out based on morphological characteristics and molecular analyses. In addition, seven native rhizobacterial isolates (CB1-CB7) were obtained, and their antagonistic activity against *Fusarium* strains was determined through *in vitro* dual-culture assays.

Results: The two phytopathogenic strains, FMT08 and FMR13, were identified as *F. verticillioides* and exhibited pathogenic capacity by reducing seed germination and causing root rot. All evaluated rhizobacteria showed inhibitory activity; however, CB4 displayed the most consistent antagonistic effect against both strains, with biological effectiveness values of 53.75% for strain FMT08 and 52.50% for strain FMR13.

Limitations of the study/implications: The native rhizobacterium CB4 evaluated in this study demonstrated the ability to suppress the development of *F. verticillioides*. As a prospective line of research, further studies are required to determine whether this effect is associated with the synthesis of antifungal compounds or with other mechanisms of action.

Findings/conclusions: Two phytopathogenic maize strains identified as *F. verticillioides* and seven native rhizobacterial isolates from Sinaloa were obtained. Among them, CB4 stood out for its stable antagonistic activity against both pathogen strains, positioning it as a promising candidate for biocontrol strategies in maize cultivation.

Keywords: Antagonism, phytopathogen, antifungal, biological effectiveness.

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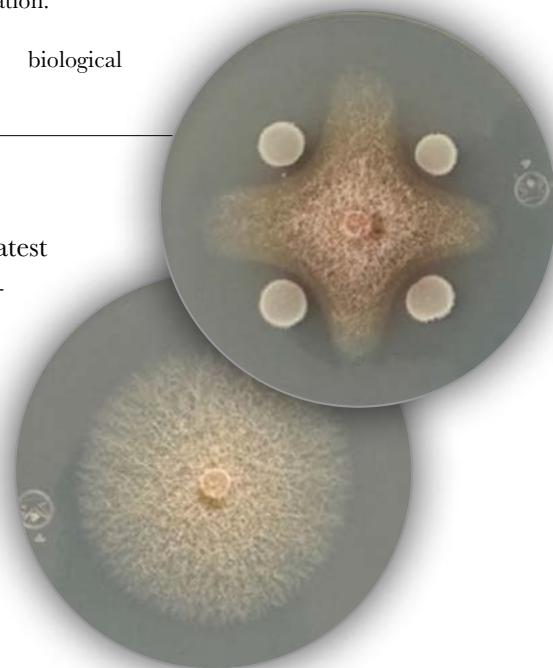
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INTRODUCTION

Maize (*Zea mays* L.) is one of the crops of greatest economic and food importance in Mexico (Vilchis-Granados *et al.*, 2024). In the state of Sinaloa, this crop assumes a particularly strategic role, as it constitutes the foundation of the regional agricultural system (De la Torre-Torres *et al.*, 2025). However, its productivity is constrained by various diseases caused by phytopathogens, including fungi such as



Colletotrichum sp., *Exserohilum* sp., *Ustilago* sp., and *Fusarium* sp., among others (Ramírez-Camejo *et al.*, 2025). The genus *Fusarium* is characterized by its wide distribution and ease of dissemination; consequently, its species rank among the phytopathogenic fungi of greatest economic relevance in maize production (Solórzano-Solórzano *et al.*, 2024). Moreover, in maize, several *Fusarium* species pose a health risk due to their ability to accumulate mycotoxins in the grain, as occurs with *F. verticillioides* (Li *et al.*, 2025). This pathogen may enter the plant through different routes and infect the roots, stem, and ear (De la Torre-Hernández *et al.*, 2014). Among the mycotoxins detected in maize and produced by *Fusarium*, fumonisins are particularly noteworthy, as they are associated with postharvest losses and risks to human and animal health.

The conventional control of *F. verticillioides* relies primarily on the application of agrochemicals (Hu *et al.*, 2025). Although this practice may reduce infection, its continuous use entails several limitations, including the emergence of resistant strains, increased production costs, and adverse effects on the environment and soil microbiota (López-Arellanes *et al.*, 2025). In view of this problem, there is a clear need to explore sustainable and effective alternatives for disease management in maize. Among these alternatives, the use of plant growth-promoting rhizobacteria (PGPR) has gained increasing relevance because of their capacity to antagonize phytopathogens, induce systemic resistance, and improve crop health (Puliga *et al.*, 2024). Native rhizobacteria, adapted to local edaphoclimatic conditions, represent a promising source of biocontrol agents, as they may exhibit greater ecological effectiveness and field persistence (Reddy *et al.*, 2024). Nevertheless, it is necessary to isolate, identify, and evaluate their antagonistic potential against pathogens of regional importance, such as *F. verticillioides*. Therefore, the aim of this study was to isolate and identify fungi associated with maize cultivation in Sinaloa, as well as to evaluate *in vitro* the antagonistic potential of native rhizobacteria against *Fusarium verticillioides*, thereby generating evidence to support the development of sustainable integrated management strategies adapted to local agricultural systems.

MATERIALS AND METHODS

Isolation of rhizobacteria and *Fusarium* sp.

Rhizobacteria were obtained from rhizospheric soil collected from plants established in the municipality of Culiacán, Sinaloa, Mexico. The samples were processed through serial dilutions of 10^{-3} , 10^{-4} , and 10^{-5} , using 50 g of rhizospheric soil as the starting material, and were plated in Petri dishes containing nutrient agar (NA) culture medium (Ogunsola *et al.*, 2025). Subsequently, colonies showing evidence of antagonism against other microorganisms were selected and purified to obtain axenic cultures.

From *Fusarium* spp., two pathogenic isolates associated with maize were obtained from roots and stems of plants exhibiting symptoms characteristic of this phytopathogen. The plant material was collected from growing plants in northern Sinaloa, specifically in the municipalities of El Fuerte (25° 55' 29" N, 108° 56' 2" W) and Ahome (25° 56' 8" N, 109° 11' 59" W), during March and April 2025. The isolates were labeled for identification as isolate FMT08 and FMR13, respectively. Tissue samples were processed according to the method described by Wang and Jeffers (2000). The procedure was carried out in the

Vegetable Diseases Laboratory of the Faculty of Agronomy of the Autonomous University of Sinaloa (FA-UAS), and the pathogenicity of *Fusarium* spp. was confirmed by applying Koch's postulates (Deressa *et al.*, 2025).

Morphological identification of *Fusarium* sp.

From the *Fusarium* spp. isolates, monosporic cultures were obtained on potato dextrose agar (PDA) medium (Velarde-Félix *et al.*, 2018) for each isolate. Characterization was performed by evaluating mycelial growth and observing microconidia, macroconidia, and conidiophores (Leslie & Summerell, 2006).

Molecular identification of *Fusarium* sp. isolates

Molecular identification was performed through amplification of the translation elongation factor 1-alpha (TEF1- α) region and the internal transcribed spacer (ITS). The sequences of the oligonucleotides used are presented in Table 1.

The amplicon was sequenced at the Advanced Genomics Unit of the National Laboratory of Genomics for Biodiversity at CINVESTAV. Sequence editing and consensus sequence assembly were performed using Unipro Gene v.52. Multiple sequence alignments were conducted with Clustal W, and the phylogenetic tree was constructed in MEGA 12 using the Maximum Likelihood algorithm with 1,000 bootstrap replicates. The final tree was edited with iTol v7.

In vitro antagonism of rhizobacteria against *Fusarium* sp. *in vitro* antagonism bioassays were conducted in July 2025 at the Vegetable Diseases Laboratory of the FA-UAS using the dual-culture technique. In each Petri dish containing nutrient agar (NA), a 4 mm mycelial disc of the phytopathogen was placed at the center, and 30 μ L of rhizobacterial cell suspension (3×10^8 CFU mL⁻¹, adjusted according to the McFarland scale) was applied at a distance of 20 mm in the four cardinal directions surrounding the disc. The antagonistic effect was determined by measuring the mycelial growth directed toward the bacterial colony (mm). Biological effectiveness (BE) was calculated using the following formula:

$$BE = \{(C - T) / C\} \times 100$$

where *C* represents the growth of the control and *T* the growth of the treatment (Bolívar-Anillo *et al.*, 2021). Growth measurements were recorded every 24 h from the onset of the experiment.

Table 1. Oligonucleotide sequences used for isolate identification (Montoya *et al.*, 2024).

Name	Sequence	Amplicon
ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	ITS
ITS4	5'-TCCTCCGCTTATTTGATATGC-3'	
Fsp-EF1	5'-ATGGGTAAGGARGACAAGAC-3'	TEF1- α
Fsp-EF2	5'-GGARGTACCAGTSATCATGTT-3'	

The reaction mixture was prepared according to Montoya *et al.* (2024), and the thermocycling conditions are specified in Table 2.

Table 2. Thermocycler conditions for the amplicons evaluated in this study.

Step	ITS		TEF1- α	
	Conditions	Cycles	Conditions	Cycles
Initial denaturation	95 °C for 4 min	1	95 °C for 4 min	1
Denaturation	94 °C for 1 min	30	94 °C for 1 min	35
Annealing	55 °C for 90 s		50.8 °C for 90 s	
Extension	72 °C for 90 s		72 °C for 90 s	
Final extension	72 °C for 5 min	1	72 °C for 5 min	1

Experimental design and statistical analysis. A completely randomized design with 16 replicates per treatment was used for the in vitro assays. The treatments consisted of seven rhizobacterial isolates (CB1 to CB7) and one control without antagonist; each Petri dish was considered an experimental unit. In both experiments, the original data for mycelial growth (mm) and percentage inhibition (biological effectiveness) did not satisfy the assumptions required for parametric analyses. Therefore, the data were transformed into ranks prior to analysis of variance and Tukey's test ($p \leq 0.05$), in accordance with Conover (1999) and Martínez-Garza (2005), using the statistical package SAS Institute Inc. (2023).

RESULTS AND DISCUSSION

Seven rhizobacterial isolates with antagonistic activity were obtained. These isolates were preliminarily characterized to confirm their inhibitory capacity against the evaluated phytopathogens. To facilitate their identification and handling throughout the study, they were assigned the codes CB1 to CB7. This coding system enabled the systematic organization of subsequent analyses and ensured the traceability of the results obtained at each stage of the research. Morphological and molecular identification of the phytopathogens. The pathogenicity test confirmed *Fusarium* sp. in each strain as the causal agent of the disease. In addition, in maize seeds and seedlings, the evaluated strains reduced germination to below 42% and caused necrotic lesions on the primary and secondary roots, as well as a reduced root system. The observed symptoms are consistent with those previously reported for infections caused by species of the genus *Fusarium*. In this regard, Baldwin *et al.* (2014) described similar manifestations associated with the pathogenicity of this genus, whereas Stagnati *et al.* (2020) confirmed the recurrence of these symptoms in different hosts and under diverse environmental conditions. Likewise, Omotayo and Babalola (2023) also reported the presence of comparable signs, thereby supporting the similarity between the present results and those documented in the literature. Preliminary morphological characterization indicated that colonies grown on potato dextrose agar (PDA) for six days initially exhibited a light beige coloration, which gradually darkened to brown and eventually developed violet pigmentation along with dense aerial mycelium. Microscopic examination revealed the production of hyaline, unicellular, ellipsoidal microconidia, as well as falcate, thin-walled macroconidia, which are diagnostic characteristics consistent with species belonging to the genus *Fusarium* (Xi *et al.*, 2021; Leslie & Summerell, 2006). PCR amplification of the ITS and TEF1- α

Antagonism of rhizobacteria. The results obtained from the different sampling times revealed statistically significant differences ($p \leq 0.05$) in the mycelial growth (MG; mm) of *F. verticillioides* strain FMT08 among the rhizobacterial treatments and the control without antagonist (Table 3). At 24 h, all treatments were grouped within the same statistical category, whereas the control showed the greatest growth, with values ranging from 3.88 mm (CB3 and CB4) to 4.19 mm (CB1). At 48 h, the control again recorded the highest growth, reaching 12 mm, while the rhizobacterial treatments significantly reduced fungal development, with growth values ranging from 7.50 to 10.75 mm; notably, treatment with rhizobacterium CB5 produced the lowest fungal growth. At 72 h, the control reached 20 mm of growth, and all bacterial treatments exerted an inhibitory effect, with values ranging from 9.25 mm (CB4) to 9.38 mm (CB5), whereas CB7 reached 12.25 mm, thereby confirming the antagonistic effect of the evaluated rhizobacteria (Table 3; Figure 2).

The analysis of biological effectiveness (%) of the antagonistic bacterial treatments against *F. verticillioides* strain FMT08 also revealed statistically significant differences among treatments (Table 3). At 24 h, the highest biological effectiveness corresponded to treatments CB3 and CB4, which were only statistically superior ($p \geq 0.05$) to that obtained with treatment CB1, with a difference of 5.93% between the maximum and minimum values. At 48 h, the response exhibited greater dispersion: treatment CB5 showed significantly higher biological effectiveness than the remaining treatments, except for CB4; nevertheless, it exceeded CB4 by 6.77 percentage points. In contrast, treatment CB7 recorded the lowest effectiveness, with a difference of 27.1 percentage points relative to CB5. At 72 h, treatments CB4 and CB5 exhibited biological effectiveness values significantly higher than those of the remaining treatments, with differences of 14.4 and 15.0 percentage points, respectively, compared with the treatment showing the lowest effectiveness (CB7) (Table 3; Figure 2).

The rhizobacteria also produced significant differences ($p \leq 0.05$) in the mycelial growth (MG) of *F. verticillioides* strain FMR13 as early as 24 h after the establishment of the experiment (Table 4). In addition, the mean comparison test indicated that treatments CB1 and CB2 were statistically similar and differed from the remaining treatments by exhibiting

Table 3. Antagonistic effect of rhizobacteria against *F. verticillioides* (FMT08), a maize pathogen.

Treatment	24 h MG (mm)	24 h BE (%)	48 h MG (mm)	48 h BE (%)	72 h MG (mm)	72 h BE (%)
CB1	4.19±0.40 B	14.58±7.24 B	9.94±0.85 C	17.19±7.12 C	11.63±1.08 B	41.88±5.44 C
CB2	4.13±0.34 B	15.71±6.13 AB	10.06±0.77 C	16.15±6.43 C	11.88±1.20 B	40.63±6.02 C
CB3	3.88±0.34 B	20.51±7.00 A	10.19±0.40 BC	15.11±3.36 CD	12.06±0.93 B	39.69±4.64 C
CB4	3.88±0.34 B	20.51±7.00 A	8.31±0.48 C	30.73±3.99 AB	9.25±0.68 D	53.75±3.41 A
CB5	3.94±0.25 B	19.23±5.13 AB	7.50±0.52 D	37.50±4.31 A	9.38±0.62 D	53.13±3.09 A
CB6	4.00±0.00 B	17.95±0.00 AB	8.88±0.72 C	26.04±5.99 B	10.38±0.89 C	48.13±4.42 B
CB7	4.00±0.00 B	17.95±0.00 AB	10.75±0.68 B	10.42±5.69 D	12.25±1.29 B	38.75±6.45 C
Control	4.88±0.34 A	—	12.00±0.63 A	—	20.00±0.00 A	—

SD=standard deviation. MG=mycelial growth (mm). BE=biological effectiveness (%). Means that do not share a letter are significantly different according to Tukey's test ($p \leq 0.05$).

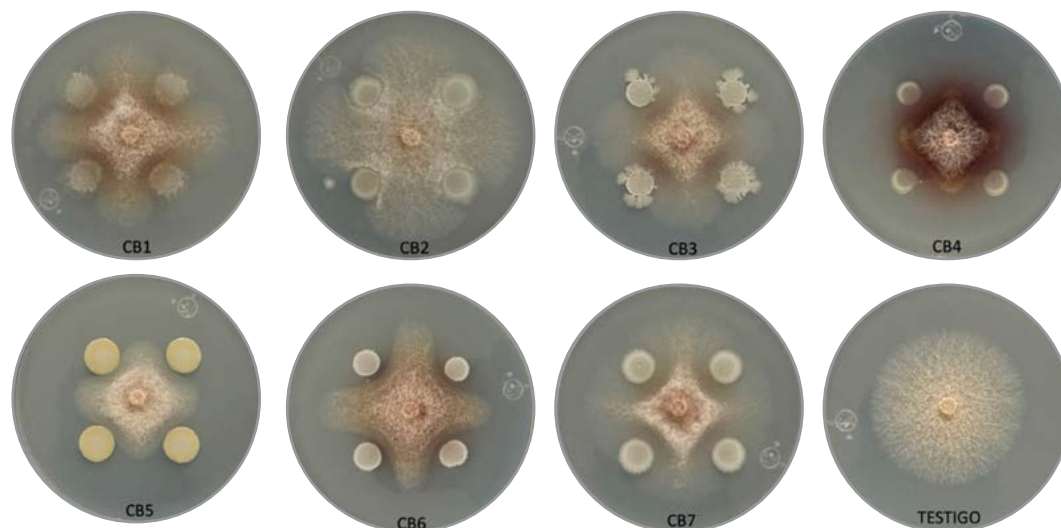


Figure 2. Antagonistic effect of rhizobacteria (CB1 to CB7) on the mycelial growth of *F. verticillioides* (FMT08).

lower MG values, at 3.88 and 2.5 mm, respectively. At 48 h, significant differences among treatments were also observed ($p \leq 0.05$). The control treatment exhibited the greatest mycelial development of the phytopathogen and shared statistical significance with rhizobacterium CB7. In turn, fungal growth under CB7 was statistically similar to that recorded for CB3, which differed from the control. Moreover, the MG recorded for CB1 was statistically similar to that of CB2 and CB6, but different from that observed for CB4 and CB5, which showed the lowest values. Finally, at 72 h after the establishment of the experiment, the results again indicated significant differences among treatments ($p \leq 0.05$). Tukey's test showed that MG under CB4 was the lowest and statistically similar to that recorded for CB6. In contrast, the control without antagonist reached the significantly highest MG value (20 mm).

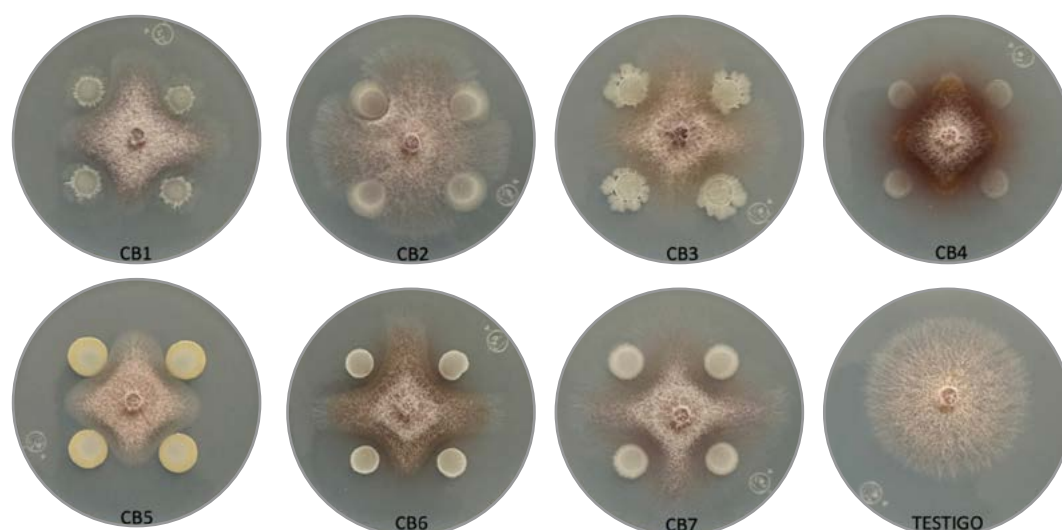
The biological effectiveness (BE) of the rhizobacteria against strain FMR13 showed significant differences ($p \leq 0.05$) at 24 h, following a trend similar to that observed for MG. During this period, treatments CB1 and CB2 were statistically similar to each other and differed from the remaining treatments by presenting the highest BE values. At 48 h, strains CB5, CB4, and CB2 exhibited the highest BE; in contrast, CB3 and CB7 showed the lowest values. At 72 h, the highest BE was recorded for treatments CB4 and CB6, whereas the lowest corresponded to CB3, CB7, and CB2 (Table 4 and Figure 3).

The results obtained from the dual-culture assays between seven native rhizobacteria from Sinaloa and two strains of *F. verticillioides* (FMT08 and FMR13) revealed a differential pattern in biological effectiveness. Rhizobacteria CB4 and CB5 exerted the greatest inhibitory effect on strain FMT08. In contrast, against strain FMR13, CB4 and CB6 showed the highest BE at 72 h. These findings confirm that the antagonistic effect of rhizobacteria depends on the pathogen strain, thereby suggesting a specific interaction associated with the fungal genotype (Mmotla *et al.*, 2025). Likewise, certain microbial agents, such as rhizobacterium CB4, maintained stable performance across different strains of *F. verticillioides*, which positions it as a promising candidate for biocontrol. This

Table 4. Antagonistic effect of rhizobacteria against *F. verticillioides* (FMR13), a maize pathogen.

Treatment	24 h MG (mm)	24 h BE (%)	48 h MG (mm)	48 h BE (%)	72 h MG (mm)	72 h BE (%)
CB1	3.88±0.80 B	22.50±16.12 A	9.50±1.03 C	25.84±8.06 B	11.50±0.97 CD	42.50±4.83 CD
CB2	2.50±1.55 B	50.00±30.98 A	8.69±1.20 CD	32.18±9.33 AB	11.56±1.21 BC	42.19±6.04 CD
CB3	4.88±0.35 A	2.50±6.83 B	11.38±0.62 B	11.20±4.83 C	12.75±1.18 B	36.25±5.92 D
CB4	5.00±0.00 A	0.00±0.00 B	8.44±0.81 D	34.13±6.36 A	9.50±0.73 F	52.50±3.65 A
CB5	4.69±0.48 A	6.25±9.57 B	8.38±0.50 D	34.62±3.90 A	10.56±0.89 DE	47.19±4.46 BC
CB6	4.81±0.40 A	3.75±8.06 B	8.69±0.79 CD	32.18±6.19 AB	10.13±0.72 EF	49.38±3.59 AB
CB7	5.00±0.00 A	0.00±0.00 B	11.56±1.09 AB	9.92±8.29 C	12.38±1.45 BC	38.13±7.27 D
Control	5.00±0.00 A	—	12.81±0.54 A	—	20.00±0.00 A	—

SD=standard deviation. MG=mycelial growth (mm). BE=biological effectiveness (%). Means that do not share a letter are significantly different according to Tukey's test ($p \leq 0.05$).

**Figure 3.** Antagonistic effect of rhizobacteria (CB1 to CB7) on the mycelial growth of *F. verticillioides* (FMR13).

behavior suggests the presence of effective antagonistic mechanisms, thereby supporting its potential application in biological management strategies.

On the other hand, the interaction of the rhizobacteria with *F. verticillioides* strains FMT08 and FMR13 generated differential effects on mycelial growth, with changes in coloration from light beige to dark brown observed at 72 h (Figures 2 and 3). This pattern suggests the presence of structural alterations in the pathogenic strains as a result of this interaction (Ávila-Oviedo *et al.*, 2024; Mmotla *et al.*, 2025). The observed variations indicate that the antagonistic activity of rhizobacteria CB4, CB5, and CB6 may be associated with the synthesis of metabolites with antifungal activity (Ávila-Oviedo *et al.*, 2024). It has been demonstrated that some rhizobacteria act as plant growth-promoting rhizobacteria and influence essential physiological processes, including phosphate solubilization, nitrogen fixation, and phytohormone production (Hasan *et al.*, 2024). The efficacy of these rhizobacteria depends on their ecological compatibility and the environment in which

they are applied (Revillini *et al.*, 2016). In this context, the native rhizobacteria evaluated in this study constitute a particularly effective alternative due to their adaptation to the edaphoclimatic conditions of Sinaloa.

These native rhizobacteria could be integrated into biological management programs for this pathogen, thereby contributing to sustainable strategies for maize cultivation in this region. Nevertheless, additional studies are required to identify the specific antagonistic mechanisms involved and to evaluate their performance under field conditions.

CONCLUSIONS

Two phytopathogenic strains were isolated from maize plants in Sinaloa and identified as *Fusarium verticillioides*, the causal agent of root and stem rot. In addition, seven native rhizobacterial isolates (CB1 to CB7) were obtained, among which CB6, CB5, and CB4 were the most notable, with CB4 exhibiting the greatest antagonistic capacity against the evaluated pathogens. Therefore, CB4 may be considered a potential alternative for the biological management of *F. verticillioides* in maize cultivation. Nevertheless, its efficacy must be validated under field conditions before practical application.

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