REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.13.e2132
ISSN 2007-3380

Original article / Artículo original

Long-term shelf-life of a synthetic plant growth promoting bacterial community

Vida de anaquel extendida de una comunidad bacteriana sintética promotora del crecimiento de plantas

Díaz-Rodríguez, A.M.[1], *Parra-Cota, F.I.* [2], *Cira-Chávez, L.A.* [1], *García-Ortega, L.F.* [3], *Estrada-Alvarado, M.I.* [1], *Santoyo, G.* [4], *de los Santos-Villalobos, S.* [1]

[1] *Instituto Tecnológico de Sonora, 85000, Ciudad Obregón, Sonora, México.*
[2] *Campo Experimental Norman E. Borlaug-INIFAP, 85000, Ciudad Obregón, Sonora, México.*
[3] *Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, 36824, Irapuato, Guanajuato, México.*
[4] *Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolas de Hidalgo, 58095 Morelia, Michoacán, México.*

Please cite this article as/Como citar este artículo: Díaz-Rodríguez, A.M., Parra-Cota, F.I., Cira-Chávez, L.A., García-Ortega, L.F., Estrada-Alvarado, M.I., Santoyo, G., de los Santos-Villalobos, S. (2025). Long-term shelf-life of a synthetic plant growth promoting bacterial community. *Revista Bio Ciencias 13(nesp): AgroEvento 2025*, e2132. https://doi.org/10.15741/revbio.13.e2132

Article Info/Información del artículo
Received/Recibido: December 12th 2024.
Accepted/Aceptado: January 10th 2026.
Available on line/Publicado: January 16th 2026.

ABSTRACT

The development of long-term stability microbial formulations is essential for advancing the use of synthetic bacterial communities (*SynComs*) in agricultural biotechnology. In this study, the shelf-life of a synthetic bacterial community composed of *Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, *Bacillus paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8 was evaluated. The cofermented *SynCom* reached a final density of 1.60 × 10^9 CFU/mL with efficient sporulation (100 %). Short-term (30 days) stability assays revealed that trehalose and polyvinylpyrrolidone (PVP) maintained spore viability; however, neither additive significantly improved stability compared with the additive-free formulation. Thus, a long-term (12 months at 23.8 ± 1.7 °C) evaluation showed high stability of the *SynCom*, which maintained high spore viability, 92 %. This finding is supported by the genomes of these bacteria, which reveal conserved mechanisms for oxidative and osmotic stress tolerance, including antioxidant enzymes, compatible solute transporters, and PHB biosynthesis. These intrinsic stress-response traits likely contribute to the extended shelf-life of the cofermented *SynCom*. Overall, this *SynCom* represents a promising strategy for developing liquid microbial formulations with long stability under ambient conditions.

KEY WORDS: Synthetic bacterial community, *Bacillus*, *Priestia*, Cofermentation, Microbial inoculants

***Corresponding Author:**

Sergio de los Santos-Villalobos. Laboratorio de Biotecnología del Recurso Microbiano. Instituto Tecnológico de Sonora. 5 de febrero, 818 sur, Cajeme. 85000, Ciudad Obregón, Sonora, México. Teléfono: (644) 4100900 Ext. 2124.
E-mail: sergio.delossantos@itson.edu.mx

Revista Bio ciencias
ISSN 2007-3380

RESUMEN

El desarrollo de formulaciones microbianas de estabilidad a largo plazo es esencial para avanzar en el uso de comunidades bacterianas sintéticas (*SynComs*) en biotecnología agrícola. En este estudio, se caracterizó la vida útil de una comunidad bacteriana sintética integrada por *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T}, *Bacillus paralicheniformis* TRQ65 y *Priestia megaterium* TRQ8. La *SynCom* cofermentada alcanzó una densidad final de 1.60 × 10^{9} CFU/mL con una esporulación eficiente (100 %). Los ensayos de estabilidad a corto plazo (30 días) revelaron que la trehalosa y la polivinilpirrolidona (PVP) mantuvieron la viabilidad de las esporas; sin embargo, ninguno de estos aditivos mejoró significativamente la estabilidad en comparación con la formulación sin aditivos. Por lo tanto, la evaluación del almacenamiento a largo plazo (12 meses, a 23.8 ± 1.7 °C) mostró una alta estabilidad de la *SynCom*, manteniendo una alta viabilidad de las esporas, 92 %. Este hallazgo se sustenta en los genomas de estas bacterias que revelaron mecanismos conservados de tolerancia al estrés oxidativo y osmótico, incluyendo enzimas antioxidantes, transportadores de solutos compatibles y la biosíntesis de PHB. Estas características intrínsecas de respuesta al estrés probablemente contribuyan a la vida útil extendida de la *SynCom* cofermentada. En general, esta *SynCom* representa una estrategia prometedora para el desarrollo de formulaciones microbianas líquidas con una larga estabilidad en condiciones ambientales.

PALABRAS CLAVE: Comunidad bacteriana sintética, *Bacillus*, *Priestia*, Cofermentación, Inoculantes microbianos

Introduction

Global agricultural production is threatened by the effects of climate change, population growth, pest and disease outbreaks, the high cost of fertilizers, and soil degradation and loss of fertility (Díaz-Rodríguez *et al*., 2021). Previous studies have focused on identifying and analyzing beneficial microorganisms in various crops, offering a sustainable alternative for their agronomic management. Therefore, a wide range of microbial strains with plant growth-promoting properties are currently used as bioinoculants to improve crop performance and development. Recently, the global bioinoculant market reached a value of $11.23 billion in 2025 and is expected to grow at an annual rate of approximately 10.5 % by 2030 (Mordor Intelligence Research & Advisory, 2025). In Mexico, 248 bioinoculants are registered with COFEPRIS, representing only 5 % of the national agrochemical market (COFEPRIS, 2025). Despite this progress, several key challenges must be addressed to fully harness their potential for improving crop productivity, particularly in terms of formulation and stability. The effectiveness of these microbial products depends not only

Revista Bio ciencias
ISSN 2007-3380

on selecting beneficial strains with plant growth-promoting capabilities, but also on their ability to remain viable and metabolically active during storage and under field conditions (Herrmann & Lesueur, 2013).

In recent years, the use of synthetic bacterial communities (*SynComs*) has gained considerable interest. *SynComs* are a small number of bacterial strains designed to mimic, to some extent, the function and structure of natural microbiomes (de Souza *et al*., 2020). Unlike natural consortia, which can be highly diverse and unpredictable, *SynComs* are constructed to maximize functional complementarity among strains, providing reproducible and specific effects on plant growth and health (Niu *et al*., 2017; Vorholt *et al*., 2017). These microbial communities can combine traits such as nitrogen fixation, phosphate solubilization, phytohormone production, and antagonism against phytopathogens, thereby enhancing crop productivity and promoting agroecosystem resilience (Tariq *et al*., 2025; Wang *et al*., 2021).

Recent studies have demonstrated the potential of *SynComs* to promote plant growth and confer stress tolerance under both biotic and abiotic conditions. For example, Schmitz *et al*. (2022) developed a *SynCom* composed of five bacterial strains isolated from the desert legume *Indigofera argentea*, which significantly improved tomato growth and salinity tolerance. Similarly, Liu *et al*. (2022) established a wheat-associated *SynCom* composed of eight bacterial strains, including *Priestia megaterium*, *Bacillus pumilus*, and *Acinetobacter pittii*, which increased wheat biomass by 120 % to 157 %, suppressed the pathogenic fungus *Fusarium pseudograminearum*, and shifted the soil microbiome toward beneficial taxa. Additionally, de Souza *et al*. (2020) highlighted that *SynCom* design based on functional complementarity among strains can enhance plant nutrient acquisition, hormonal balance, and disease suppression.

Thus, the native strains evaluated in the present study, *Bacillus cabrialesii* subsp. *cabrialesii* TE3T, *Bacillus paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8, have been extensively characterized for their plant growth-promoting potential. Strain TE3^{T} was isolated from the wheat endosphere (Valenzuela-Aragon *et al*., 2019), whereas strains TRQ65 and TRQ8 were isolated from the wheat rhizosphere in a commercial field under conventional fertilization management in the Yaqui Valley, Mexico (Ibarra-Villarreal *et al*., 2021). These strains have demonstrated their ability to stimulate wheat growth under greenhouse and field conditions (Robles-Montoya *et al*., 2020; Rojas-Padilla *et al*., 2020). In addition, strains TRQ65 and TE3^{T} exhibit antagonistic activity against *Bipolaris sorokiniana*, *Botrytis cinerea*, and other phytopathogenic fungi (Valenzuela-Ruiz *et al*., 2024; Villa-Rodríguez *et al*., 2021). However, to date, their performance has been evaluated only in single-strain fermentation systems, and their long-term stability has not been explored. In this context, the present work aims to evaluate the growth kinetics and shelf-life of a cofermented *SynCom* composed of *B. cabrialesii* subsp. *cabrialesii* TE3^{T}, *B. paralicheniformis* TRQ65, and *P. megaterium* TRQ8, to determine its potential for the development of stable liquid microbial formulations.

Revista Bio ciencias
ISSN 2007-3380

Material and Methods

Bacterial strains and cofermentation

The synthetic bacterial community (*SynCom*) evaluated in this study was composed of *Bacillus cabrialesii* subsp. *cabrialesii* TE3T, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65, which have been previously reported as compatible in coculture (Rojas-Padilla *et al*., 2020).

Before cofermentation, a compatibility assay was performed using nutrient agar as a culture medium. Individual bacterial suspensions were prepared and adjusted to a concentration of **1 × 10^7 colony-forming units (CFU)/mL**. For each plate, 1 mL of the suspension from each strain was evenly spread, and subsequently, 10 µL of each of the three bacterial strains was inoculated at equidistant points on the agar surface. This procedure was alternated across strains to evaluate potential cross-inhibition. The assay was conducted in triplicate, and the plates were incubated at **30 °C for 48 h.**

Thus, cofermentation of the *SynCom* was initiated by individually growing each strain in a synthetic minimal medium composed of: glucose 20 g/L, $(NH_4)_2SO_4$ 8 g/L, K_2HPO_4 23.44 g/L, $MgSO_4 \cdot 7H_2O$ 0.8 g/L, $MnSO_4 \cdot H_2O$ 0.088 g/L, $CaCl_2$ 0.126 g/L, $FeSO_4 \cdot 7H_2O$ 0.06 g/L y $Na_3C_6H_5O_7$ 10.08 g/L, for 18 h at 37 °C with agitation at 180 rpm. Subsequently, the synthetic consortium inoculum was prepared by adjusting each culture to a concentration of 1 × 10^6 CFU/mL. The combined inoculum (corresponding to 10 % of the total working volume) was directly inoculated into a 5 L bioreactor (working volume of 4 L) containing the same synthetic minimal medium described above.

The cofermentation of the *SynCom* was carried out under controlled conditions: 37 °C, pH 7.0 (automatically regulated), agitation at 400 rpm, and aeration at 0.75 vvm. Foam formation was controlled by the addition of a silicone-based antifoam agent (30 % w/v). During the 45 h fermentation period, the culture was monitored hourly by measuring the optical density at 630 nm; residual glucose was quantified using the DNS method (Miller, 1959). Colony-forming units (CFU/mL) and spore concentrations (spores/mL) were determined by serial dilutions followed by heat treatment at 80 °C for 10 min.

Evaluation of Osmoprotective Additives on Consortium Stability

To evaluate the effect of different stabilizing compounds on spore viability, three additives were tested at different concentrations: glycerol (0.5 %, 1 %, and 2 %), polyvinylpyrrolidone (PVP; 0.5 %, 1 %, and 2 %), and trehalose (5 mM, 10 mM, and 15 mM). Each treatment was evaluated in three independent replicates. Aliquots of the bioformulated cofermentation containing each additive were placed into sterile 15 mL Falcon tubes, which served as experimental units for the initial stability assessment. The tubes were maintained at 23.8 ± 1.6 °C, under a 12 h light/12 h dark photoperiod and 36 % relative humidity for 30 days. Subsequently, spore viability was determined using the serial dilution plating method on nutrient agar. The bioformulation with the highest spore

Revista Bio ciencias
ISSN 2007-3380

survival was selected for a 12-month long-term shelf-life study. For this assay, the formulation was packaged in sterile 130 mL polypropylene (PP) bottles. The containers were stored under the same temperature, photoperiod, and relative humidity conditions described above.

Statistical Analysis

Statistical analyses were performed using STATGRAPHICS Plus version 5.1. Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for multiple comparisons, with a significance level set at $p < 0.05$.

Results and Discussion

The compatibility among the *SynCom* strains was confirmed through a Petri dish assay, in which no inhibition halos were observed in any of the evaluated combinations. This confirms the compatibility among the strains and supports their combined use in cofermentation processes. Cofermentation of the *SynCom* in a 5 L bioreactor reached a cell density of $1.60 \pm 0.05 \times 10^9$ CFU mL^{-1}, with a specific growth rate of 0.42 ± 0.04 h^{-1} and a doubling time of 1.65 ± 0.17 h (Table 1). Besides, during cofermentation, the relative abundance of each strain was 62.5 % for *B. cabrialesii* subsp. *cabrialesii* $TE3^T$ (1.00×10^9 CFU mL^{-1}), 26.3 % for *B. paralicheniformis* TRQ65 (4.21×10^8 CFU mL^{-1}), and 11.2 % for *P. megaterium* TRQ8 (1.79×10^8 CFU mL^{-1}). The differences observed in the relative proportions of each strain during cofermentation can be attributed to intrinsic kinetic parameters of each bacterium, such as variable substrate affinities, distinct growth rates, or differences in nutrient utilization efficiency. Even in the absence of antagonistic interactions, these kinetic differences determine the relative contribution of each microorganism within the synthetic community (Zheng *et al*., 2022).

The glucose yield coefficient was $3.70 \pm 0.18 \times 10^{11}$ CFU g^{-1} glucose, reflecting an efficient conversion of substrate into biomass under aerobic conditions. This value falls within the expected range for fermentations involving synthetic communities of *Bacillus* and related taxa, where metabolic trade-offs contribute to a balance between growth and efficient sporulation (Martínez-Álvarez *et al*., 2016).

The cofermented *SynCom* exhibited 100 % sporulation, reaching a maximum spore density of $1.60 \pm 0.01 \times 10^9$ spores mL^{-1}, with a specific sporulation rate of 0.52 ± 0.04 h^{-1} (Table 1). This high performance is consistent with the behavior of spore-forming bacterial communities cultivated in optimized mineral media supplemented with calcium and phosphate ions, which are essential for spore cortex development (Monteiro *et al*., 2014). Thus, the high sporulation efficiency of the cofermented *SynCom* highlights its potential as a compatible bioinoculant (Berninger *et al*., 2018).

Revista Bio ciencias
ISSN 2007-3380

Table 1. Fermentation parameters and kinetic coefficients of the cofermented synthetic community (*SynCom*) in a 5 L bioreactor.

	X_{max} (CFU mL^{-1})	μ_{cel} (h^{-1})	Td (h)	$Y_{x/glucose}$ (CFU g^{-1} glucose)	X_{max} (spore mL^{-1})	μ_{esp} (h^{-1})	% esp
Cofermented *SynCom*	$1.60 \pm 0.05 \times 10^{9\ a}$	$0.42 \pm 0.04^{\ a}$	$1.65 \pm 0.17^{\ c}$	$3.70 \pm 0.18 \times 10^{11\ a}$	$1.60 \pm 0.01 \times 10^{9\ a}$	$0.52 \pm 0.04^{\ c}$	100 %

Values represent the mean ± standard deviation. Different superscript letters within the same row indicate significant differences according to Tukey's test ($p < 0.05$). Abbreviations: Xmax, maximum cell density; μcel, specific growth rate; Td, doubling time; Yx/glucose, biomass yield coefficient; Xmax spore, maximum spore density; μspore, specific sporulation rate; % spore, sporulation percentage.

Source: own elaboration.

The consumption of glucose during the first 18–20 h, followed by the onset of sporulation, reflects a biphasic fermentation pattern typical of *Bacillus* and related species, in which nutrient limitation and quorum-sensing signals trigger the sporulation cascade (Mathot *et al*., 2016; Piggot & Hilbert, 2004). The depletion of the carbon source marked the transition from exponential growth to the stationary phase, immediately followed by a pronounced increase in spore counts (Figure 1). This behavior is consistent with the observations of Liang *et al*. (2024) in batch cultures, where sporulation began after complete glucose consumption. Complementarily, Cristiano-Fajardo *et al*. (2019), using continuous cultures, demonstrated that a high incidence of sporulation (>40 %) was achieved only under glucose-limiting conditions, whereas at higher dilution rates with excess substrate, sporulation was repressed.

Various stabilizing compounds were evaluated to improve the shelf life of the cofermented *SynCom* during 30 days of storage at 23.8 °C. Glycerol, commonly used as a carrier in liquid formulations (Aninth *et al*., 2016), proved to be markedly detrimental to spore viability at all evaluated concentrations (0.5–2 %), resulting in significantly lower counts than the control treatments without additives (Table 2). In contrast, both polyvinylpyrrolidone (PVP) and trehalose preserved spore viability, showing counts that did not differ significantly from those of the control treatment. Previous studies have demonstrated the effectiveness of these two additives in stabilizing cells by reducing water activity and protecting macromolecules and membranes against oxidative or osmotic stress (Conceição *et al*., 2025; Trivedi *et al*., 2016). However, despite the protective effects of PVP and trehalose, no statistically significant improvement was detected compared with the control formulation.

This finding is consistent with the results reported by Gotor-Vila *et al*. (2017), who observed that liquid formulations of *Bacillus subtilis* CPA-8 remained stable for up to four months at room temperature, regardless of the addition of protectants such as sucrose, skim milk, or magnesium sulfate. Their results showed that all treatments maintained high viability ($1.93–2.98 \times 10^9$ CFU/mL) and that the formulation without protectants performed equally well. Similarly, our cofermented *SynCom* exhibited high spore viability, maintaining the relative abundance of the cells (61.7 % *B.*

Revista Bio ciencias
ISSN 2007-3380

cabrialesii subsp. *cabrialesii* TE3^{T}, 26.5 % *B. paralicheniformis* TRQ65, and 11.8 % *P. megaterium* TRQ8), even in the absence of additives. This suggests that the intrinsic physiological or metabolic traits of the studied strains confer natural resistance to storage-related stress. Therefore, the long-term shelf life of this cofermented *SynCom* was further evaluated without additives.

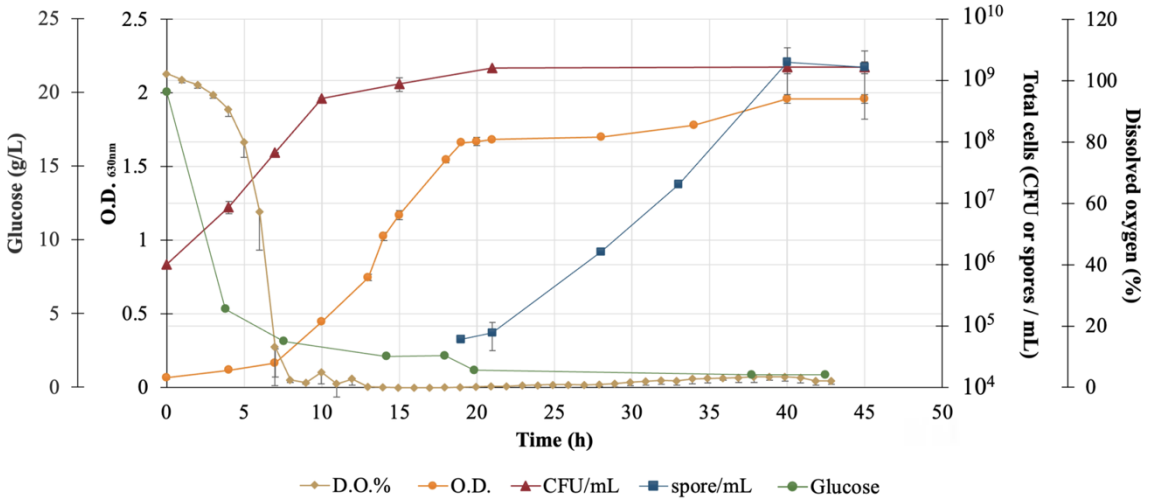

Figure 1. Kinetics of the cofermentation of the studied *SynCom*.

Growth, measured as optical density at 630 nm (O.D. $_{630nm}$, orange circles); residual glucose concentration over time (green circles); dissolved oxygen (%; yellow diamonds); viable cell counts (CFU/mL, red triangles); and spore counts (spores/mL, blue squares) throughout the entire cofermentation process. Data points represent mean values, and error bars indicate the standard deviation.

Source: own elaboration.

Revista Bio ciencias
ISSN 2007-3380

Table 2. Viability of the cofermented *SynCom* with different additives after one month of storage at 23.8 °C.

Treatment	Log (spore/mL)	
	Day 0	Day 30
Without additive	6.1[a]	6.1[ab]
Glycerol 0.5 %		5.6[c]
Glycerol 1 %		5.5[c]
Glycerol 2 %		5.1[d]
PVP 0.5 %		6.2[a]
PVP 1 %		6.1[ab]
PVP 2 %		6.2[a]
Trehalose 5mM		6.0[b]
Trehalose 10mM		6.1[ab]
Trehalose 15mM		6.2[a]

The data are presented as the mean (n = 3). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

Source: own elaboration.

After a new 12-month storage assay at 23.8 ± 1.7 °C, the evaluated co-fermented *SynCom* exhibited a high level of spore viability (92 %) relative to the initial count, decreasing from 9.2 log spores/mL to 8.46 log spores/mL, while maintaining the relative abundance of the strains composing the consortium (60.5 % *B. cabrialesi* subsp. *cabrialesii* TE3^T, 26.7 % *B. paralicheniformis* TRQ65, and 12.8 % *P. megaterium* TRQ8). In contrast, a talc-based formulation of *Bacillus cereus* B25 retained only 53 % viability after 12 months at room temperature (Martínez-Álvarez *et al*., 2016). Comparable results were reported by Rojas-Padilla *et al*. (2022), who developed calcium alginate microencapsulated formulations of the individual bacteria evaluated in this study; after 12 months of storage, all strains exhibited approximately a 45 % reduction in viable counts. A possible explanation for the high long-term cell viability observed in the co-fermented *SynCom* is the accumulation of compatible solutes or stress-response proteins during the stationary phase (Berninger *et al*., 2018). In addition, the genomes of the strains comprising the *SynCom* suggest a high degree of versatility, as they were isolated from the Yaqui Valley, a semi-arid agricultural region characterized by high temperatures, salinity, and water deficit (Ibarra-Villarreal *et al*., 2023; Luers *et al*., 2003), which may confer adaptive stress-response mechanisms.

In this context, genomic analysis revealed a robust genetic repertoire for mitigating oxidative and osmotic stress in the three strains studied (Robles-Montoya *et al*., 2019; Valenzuela Ruiz *et al*., 2023; Valenzuela-Ruiz *et al*., 2019). For oxidative stress defense, all strains encode multiple catalases (e.g., *KatE*, *HPI*, *HPII*), superoxide dismutases (*SodA*, *SodB*, *SodC*), and peroxidases (*AhpC*, *PR*, *GPX*), as well as thioredoxin and glutaredoxin systems that maintain redox homeostasis. Transcriptional regulators such as *PerR*, *OxyR*, *SoxR*, and *Rex*, identified in strains TRQ8 and TRQ65, enable the expression of antioxidant genes (Seixas *et al*., 2022). In addition,

Revista Bio ciencias
ISSN 2007-3380

strain TRQ8 produces polyhydroxybutyrate (PHB), a biopolymer that serves as an intracellular carbon and energy reserve (Aguirre *et al*., 2017). PHB accumulation has been associated with enhanced resistance to oxidative stress, desiccation, and nutrient limitation (Rowaihi *et al*., 2018).

Regarding adaptation to osmotic stress, the three genomes share conserved operons for the transport and synthesis of glycine betaine and proline, including *OpuAA–AC*, *OpuC*, and *ProVWX*, as well as genes involved in choline uptake and conversion (*BetT*, *BetA*, *BetB*, *CodA*). These systems promote the endogenous accumulation of compatible solutes that protect proteins and membranes against dehydration (Rath *et al*., 2020).

Conclusions

The co-fermented *SynCom* evaluated in this study exhibited high cell yield (1.60 ± 0.05 × 10^9 CFU/mL) and high sporulation efficiency (100 %), as well as 92 % stability after 12 months of storage at 23.8 °C without protective additives, while maintaining the relative abundance of the bacterial members. These findings suggest that interactions among the bacteria comprising *SynCom* enhanced metabolic activity, promoting sporulation and resilience, and were supported by their robust genetic repertoire for mitigating oxidative and osmotic stress. Overall, these results support the potential of this plant growth–promoting *SynCom* as a basis for the bioformulation of a stable liquid bioinoculant suitable for agricultural applications.

Author Contributions

Conceptualization, S.d.l.S.-V.; methodology and formal analysis, all authors; investigation, original draft writing, and visualization, A.M.D.R. and S.d.l.S.-V.; writing—review and editing, all authors; supervision, project administration, and funding acquisition, S.d.l.S.-V.

All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) through project CBF-2025-G-562, by PROFAPI ITSON (project 2026-001), and A.M.D.R. received support from the SECIHTI scholarship number 908966.

Acknowledgments

The authors thank all members of the LBRM-COLMENA for their support in developing this research project.

Revista Bio ciencias
ISSN 2007-3380

Conflicts of Interest

The authors declare no conflict of interest.

Referencias

Aguirre, J. B. R., Gómez, L. Z. O., Villalobos, S. D. L. S., & Sánchez, M. L. (2017). Production of polyhydroxybutyrate from milk whey fermentation by *Bacillus megaterium* TRQ8. *Revista Latinoamericana de Recursos Naturales*, *13*(1), 24–31. https://revista.itson.edu.mx/index.php/rlrn/article/view/261/194

Aninth, K. N., Vyshakhi, A., Shilpa, V., & Aswini, S. (2016). Population dynamics and efficiency of coconut water based liquid formulation of *Pseudomonas fluorescens* AMB-8. *Journal of Tropical Agriculture*, *54*(2), 184–189.

Berninger, T., González López, Ó., Bejarano, A., Preininger, C., & Sessitsch, A. (2018). Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. *Microbial Biotechnology*, *11*(2), 277–301. https://doi.org/10.1111/1751-7915.12880

COFEPRIS. (2025, Octubre 25). *Consulta de Registros Sanitarios de Plaguicidas, Nutrientes Vegetales y LMR*. https://siipris03.cofepris.gob.mx/resoluciones/consultas/conwebregplaguicida.asp

Conceição, F. R. S. da, Bessa, L. A., Teixeira, M. B., Cruvinel, B. G., & Vitorino, L. C. (2025). An Evaluation of Inoculant Additives on Cell Viability and Their Effects on the Growth and Physiology of *Glycine max* L. *Agronomy*, *15*(7), 1668. https://doi.org/10.3390/agronomy15071668

Cristiano-Fajardo, S. A., Flores, C., Flores, N., Tinoco-Valencia, R., Serrano-Carreón, L., & Galindo, E. (2019). Glucose limitation and glucose uptake rate determines metabolite production and sporulation in high cell density continuous cultures of *Bacillus amyloliquefaciens* 83. *Journal of Biotechnology*, *299*, 57–65. https://doi.org/10.1016/j.jbiotec.2019.04.027

de Souza, R. S. C., Armanhi, J. S. L., & Arruda, P. (2020). From Microbiome to Traits: Designing Synthetic Microbial Communities for Improved Crop Resiliency. *Frontiers in Plant Science*, *11*, 1179. https://doi.org/10.3389/fpls.2020.01179

Díaz-Rodríguez, A. M., Salcedo Gastelum, L. A., Félix Pablos, C. M., Parra-Cota, F. I., Santoyo, G., Puente, M. L., Bhattacharya, D., Mukherjee, J., & de los Santos-Villalobos, S. (2021). The Current and Future Role of Microbial Culture Collections in Food Security Worldwide. *Frontiers in Sustainable Food Systems*, *4,* 614739. https://doi.org/10.3389/fsufs.2020.614739

Gotor-Vila, A., Usall, J., Torres, R., Abadias, M., & Teixidó, N. (2017). Formulation of the biocontrol agent *Bacillus amyloliquefaciens* CPA-8 using different approaches: liquid, freeze-drying and fluid-bed spray-drying. *BioControl*, *62*(4), 545–555. https://doi.org/10.1007/s10526-017-9802-3

Herrmann, L., & Lesueur, D. (2013). Challenges of formulation and quality of biofertilizers for successful inoculation. *Applied Microbiology and Biotechnology*, *97*(20), 8859–8873. https://doi.org/10.1007/s00253-013-5228-8

Revista Bio ciencias
ISSN 2007-3380

Ibarra-Villarreal, A. L., Gándara-Ledezma, A., Godoy-Flores, A., Díaz-Rodríguez, A. M., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2021). Salt-tolerant *Bacillus* species as a promising strategy to mitigate the salinity stress in wheat (*Triticum turgidum* subsp. *durum*). *Journal of Arid Environments*, *186*, 104399. https://doi.org/10.1016/j.jaridenv.2020.104399

Ibarra-Villarreal, A. L., Rojas-Padilla, J., Chaparro-Encinas, L. A., Díaz-Rodríguez, A. M., Valenzuela-Ruiz, V., Herrera-Sepúlveda, A., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2023). Soil salinity shifts cultivable microbial communities of wheat (*Triticum turgidum* subsp. *durum*) rhizosphere in the Yaqui Valley, Mexico. *Agrociencia*, *57*(5), 882–919. https://doi.org/10.47163/agrociencia.v57i5.2882

Liang, J., Yang, F., Ding, Y., Zhang, X., Feng, D., & Ye, S. (2024). Batch fermentation kinetics study of biosynthesis lipopeptides by *Bacillus altitudinis* Q7 in 5 L fermenter. *Process Biochemistry*, *140*, 96–107. https://doi.org/10.1016/j.procbio.2024.02.007

Liu, H., Qiu, Z., Ye, J., Verma, J. P., Li, J., & Singh, B. K. (2022). Effective colonisation by a bacterial synthetic community promotes plant growth and alters soil microbial community. *Journal of Sustainable Agriculture and Environment*, *1*(1), 30–42. https://doi.org/10.1002/sae2.12008

Luers, A. L., Lobell, D. B., Sklar, L. S., Addams, C. L., & Matson, P. A. (2003). A method for quantifying vulnerability, applied to the agricultural system of the Yaqui Valley, Mexico. *Global Environmental Change*, *13*(4), 255–267. https://doi.org/10.1016/S0959-3780(03)00054-2

Martínez-Álvarez, J. C., Castro-Martínez, C., Sánchez-Peña, P., Gutiérrez-Dorado, R., & Maldonado-Mendoza, I. E. (2016). Development of a powder formulation based on *Bacillus cereus* sensu lato strain B25 spores for biological control of *Fusarium verticillioides* in maize plants. *World Journal of Microbiology and Biotechnology*, *32*(5), 75. https://doi.org/10.1007/s11274-015-2000-5

Mathot, A.-G., Leguérinel, I., Couvert, O., Postollec, F., Broussolle, V., & Coroller, L. (2016). Knowledge of the physiology of spore-forming bacteria can explain the origin of spores in the food environment. *Research in Microbiologoy*, *168*(4), 369–378. https://doi.org/10.1016/j.resmic.2016.10.006

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, 426–428.

Monteiro, S. M. S., Clemente, J. J., Carrondo, M. J. T., & Cunha, A. E. (2014). Enhanced Spore Production of *Bacillus subtilis* Grown in a Chemically Defined Medium. *Advances in Microbiology*, *4*(8), 444–454. https://doi.org/10.4236/aim.2014.48049

Mordor Intelligence Research & Advisory. (2025). *Agricultural Inoculants Market Size & Share Analysis—Growth Trends & Forecasts (2025—2030).* https://www.mordorintelligence.com/industry-reports/agricultural-inoculants-market.

Niu, B., Paulson, J. N., Zheng, X., & Kolter, R. (2017). Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(12), E2450–E2459. https://doi.org/10.1073/pnas.1616148114

Piggot, P. J., & Hilbert, D. W. (2004). Sporulation of *Bacillus subtilis*. *Current Opinion in Microbiology*, *7*(6), 579–586. https://doi.org/10.1016/j.mib.2004.10.001

Rath, H., Sappa, P. K., Hoffmann, T., Gesell Salazar, M., Reder, A., Steil, L., Hecker, M., Bremer, E., Mäder, U., & Völker, U. (2020). Impact of high salinity and the compatible solute glycine betaine on gene expression of *Bacillus subtilis*. *Environmental Microbiology*, *22*(8), 3266–

Revista Bio ciencias
ISSN 2007-3380

3286. https://doi.org/10.1111/1462-2920.15087

Robles-Montoya, R. I., Chaparro-Encinas, L. A., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2020). Improving biometric traits of wheat seedlings with the inoculation of a consortium native of *Bacillus*. *Revista Mexicana Ciencias Agrícolas*, *11*(1), 229–235. https://doi.org/10.29312/remexca.v11i1.2162.

Robles-Montoya, R. I., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2019). Draft genome sequence of *Bacillus megaterium* TRQ8, a plant growth-promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) rhizosphere in the Yaqui Valley, Mexico. *3 Biotech*, *9*(5), 1–5. https://doi.org/10.1007/s13205-019-1726-4

Rojas-Padilla, J., Chaparro-Encinas, L. A., Robles-Montoya, R. I., & de los Santos-Villalobos, S. (2020). Promoción de crecimiento en trigo (*Triticum turgidum* L. subsp. *durum*) por la co-inoculación de cepas nativas de *Bacillus* aisladas del Valle del Yaqui, México. *Nova Scientia*, *12*(24), 1–27.

Rojas-Padilla, J., de-Bashan, L. E., Parra-Cota, F. I., Rocha-Estrada, J., & de los Santos-Villalobos, S. (2022). Microencapsulation of *Bacillus* Strains for Improving Wheat (*Triticum turgidum* subsp. *durum*) Growth and Development. *Plants*, *11*(21), 2920. https://doi.org/10.3390/plants11212920

Rowaihi, I. S. Al, Paillier, A., Rasul, S., Karan, R., Grötzinger, S. W., Takanabe, K., & Eppinger, J. (2018). Poly(3-hydroxybutyrate) production in an integrated electromicrobial setup: Investigation under stress-inducing conditions. *PLoS ONE*, *13*(4). https://doi.org/10.1371/journal.pone.0196079

Schmitz, L., Yan, Z., Schneijderberg, M., de Roij, M., Pijnenburg, R., Zheng, Q., Franken, C., Dechesne, A., Trindade, L. M., van Velzen, R., Bisseling, T., Geurts, R., & Cheng, X. (2022). Synthetic bacterial community derived from a desert rhizosphere confers salt stress resilience to tomato in the presence of a soil microbiome. *ISME Journal*, *16*(8), 1907–1920. https://doi.org/10.1038/s41396-022-01238-3

Seixas, A. F., Quendera, A. P., Sousa, J. P., Silva, A. F. Q., Arraiano, C. M., & Andrade, J. M. (2022). Bacterial Response to Oxidative Stress and RNA Oxidation. *Frontiers in Genetics*, 12. https://doi.org/10.3389/fgene.2021.821535

Tariq, A., Guo, S., Farhat, F., & Shen, X. (2025). Engineering Synthetic Microbial Communities: Diversity and Applications in Soil for Plant Resilience. *Agronomy,* 15(3), 513. https://doi.org/10.3390/agronomy15030513

Trivedi, M., Shanware, A., & Kalkar, S. (2016). Development of Liquid Formulation of native *Rhizobium* sp. for effective plant nourishment. *International Journal of Environmental & Agriculture Research, 2*(9). https://www.researchgate.net/publication/309855468

Valenzuela Ruiz, V., Santoyo, G., Gómez Godínez, L. J., Cira Chávez, L. A., Parra Cota, F. I., & de los Santos Villalobos, S. (2023). Complete genome sequencing of *Bacillus cabrialesii* TE3^{T}: A plant growth-promoting and biological control agent isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley. *Current Research in Microbial Sciences*, *23*(4), 100193. https://doi.org/10.1016/j.crmicr.2023.100193

Valenzuela-Aragon, B., Parra-Cota, F. I., Santoyo, G., Arellano-Wattenbarger, G. L., & de los Santos-Villalobos, S. (2019). Plant-assisted selection: a promising alternative for *in vivo* identification of wheat (*Triticum turgidum* L. subsp. *durum*) growth promoting bacteria. *Plant and Soil*, *435*(1–2), 367–384. https://doi.org/10.1007/s11104-018-03901-1

Revista Bio ciencias
ISSN 2007-3380

Valenzuela-Ruiz, V., Parra-Cota, F. I., Estrada-Alvarado, M. I., Cira-Chávez, L. A., Castro-Longoria, E., & de los Santos-Villalobos, S. (2024). Potenciales mecanismos de control biológico de *Bacillus paralicheniformis* TRQ65 contra hongos fitopatógenos. *Revista Mexicana de Fitopatología*, 48. https://doi.org/10.18781/r.mex.fit.2024-18

Valenzuela-Ruiz, V., Robles-Montoya, R. I., Parra-Cota, F. I., Santoyo, G., Orozco-Mosqueda, M., Rodríguez-Ramírez, R., & de los Santos-Villalobos, S. (2019). Draft genome sequence of *Bacillus paralicheniformis* TRQ65, a biological control agent and plant growth-promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) rhizosphere in the Yaqui Valley, Mexico. *3 Biotech*, *9*(11), 436. https://doi.org/10.1007/s13205-019-1972-5

Villa-Rodríguez, E., Moreno-Ulloa, A., Castro-Longoria, E., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2021). Integrated omics approaches for deciphering antifungal metabolites produced by a novel *Bacillus* species, *B. cabrialesii* TE3^{T}, against the spot blotch disease of wheat (*Triticum turgidum* L. subsp. *durum*). *Microbiological Research*, *251*, 126826. https://doi.org/10.1016/j.micres.2021.126826

Vorholt, J. A., Vogel, C., Carlström, C. I., & Müller, D. B. (2017). Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. *Cell Host and Microbe, 22*(2), 142–155. https://doi.org/10.1016/j.chom.2017.07.004

Wang, J., Liu, L., Gao, X., Hao, J., & Wang, M. (2021). Elucidating the effect of biofertilizers on bacterial diversity in maize rhizosphere soil. *PLoS ONE*, *16*(4), e0249834. https://doi.org/10.1371/journal.pone.0249834

Zheng, Y., Zhao, C., Li, X., Xia, M., Wang, X., Zhang, Q., Yan, Y., Lang, F., Song J. & Wang, M. (2022). Kinetics of predominant microorganisms in the multi-microorganism solid-state fermentation of cereal vinegar. *Lwt*, 159, 113209.