AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Stenotrophomonas sp. LIMN, *Enterobacter* sp. LCMG, and *Rhizobium* sp. WFRFC: A Bacterial Consortium in the Production of *Zea mays* L. Under Different Agronomic Management Practices

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ABSTRACT

Objective: To evaluate the effect of the bacterial consortium *Enterobacter* sp. LCMG, *Rhizobium* sp. WFRFC, and *Stenotrophomonas* sp. LIMN on three different agronomic management practices in maize (*Zea mays* L.) cultivation, with the question: Does the consortium of bacterial strains have a positive influence on maize production under different cultivation practices in the Ciénega region, Jalisco?

Design/methodology/approach: Treatments evaluated were TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC. A randomized complete block design was established, and agronomic and yield variables were evaluated.

Results: The MT+MA treatment generated a 6.03% increase in grain yield; generated a 10.35% increase in ear height, a 4.87% decrease in plant height, and a 50% decrease in the consumption of synthetic products.

Limitations on study/implications: The agronomic management was carried out according to the practices of the region's farmers.

Findings/conclusions: The bacterial consortium *Enterobacter* sp. LCMG, *Rhizobium* sp. WFRFC, and *Stenotrophomonas* sp. LIMN had a positive effect on maize cultivation for grain production, particularly when combined with agronomic management consisting of 50% traditional management+50% agroecological management. The bacterial consortium could be used as a bio-stimulant in maize production in the Ciénega region, Jalisco.

Keywords: *Zea mays* L., bioinoculants, agronomic management, bacterial consortium.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most nutritionally important cereals and, along with wheat and rice, is among the most widely cultivated and harvested crops worldwide (García

& Laval, 2019). Due to the domestication process and genetic improvement of maize, there is a need to use significant amounts of fertilizers to achieve acceptable yields (Martín & Ribera, 2015). Fertilization, particularly the application of mineral nitrogen to the crop, represents the highest cost in the production process (Zhao *et al.*, 2017). However, the irrational use of this input negatively impacts the agroecosystem (Baez-Rogelio *et al.*, 2017) and even human health (Vejan, 2016). Therefore, authors such as Armenta-Bojórquez *et al.* (2010) recommend optimizing the doses of nitrogen-based fertilizers without negatively affecting plant growth. Aguirre *et al.* (2009) suggest that an alternative to avoid the excessive use of nitrogen fertilizers in maize is the inoculation of seeds with plant growth-promoting bacterial strains (PGPB).

The production and application of inoculants formulated with various microbial species is a well-known practice in agriculture, though it is uncommonly used. Currently, within the framework of sustainability, the search for new microorganisms with diverse plant growth-promoting properties is an emerging research field, as these organisms can partially replace the use of pesticides and chemical fertilizers (Wang *et al.*, 2020). Many of these microorganisms originate from the rhizosphere, a zone of interaction between plant roots and soil, where plant roots exert influence through their exudates, and which harbors the highest population and diversity of microorganisms (Jacoby *et al.*, 2017). Furthermore, those microorganisms capable of enhancing crop development and yield through direct and indirect mechanisms are known as plant growth-promoting microorganisms (PGPM); direct mechanisms improve the nutritional status of the plant by increasing the exploration volume and functionality of roots, water uptake, nutrient availability and absorption, and the overall physiology of the plant (Kumar *et al.*, 2015). On the other hand, indirect mechanisms involve protection against stress caused by abiotic and biotic factors, including biological control against phytopathogens (Saraf *et al.*, 2014).

The application of microorganisms contributes to the ecological and sustainable management of agro-ecosystems. These microorganisms interact beneficially with the resident soil microbiota and enhance its adaptability to local climatic and agroecological conditions, making them suitable as inoculants to improve crop production (Cruz *et al.*, 2021). Pérez-Vázquez *et al.* (2018) consider agroecological production systems to be typically agro-diverse, resilient, energy-efficient, socially just, productive, and based on food sovereignty strategies that promote local production through family farming. These systems integrate innovation processes with a rational or zero use of synthetic inputs (fertilizers, pesticides), GMOs, hormones, and antibiotics in production. In this context, the objective of this study was to evaluate the effect of a bacterial consortium under three different agronomic management practices on maize grain production in the Ciénega region in the state of Jalisco.

MATERIALS AND METHODS

Location and Genetic Material: This research was conducted in a plot located in the community of La Víbora, municipality of Zapotlán del Rey, Jalisco, at coordinates N 20° 24’ 39.5” - W 102° 16’ 06.8”, during the spring/summer 2022 season. The bacterial consortium used consisted of the strains *Stenotrophomonas* sp. LIMN, *Enterobacter* sp.

LCMG, and *Rhizobium* sp. WFRFC, which were previously selected as plant growth-promoting rhizobacteria (Reséndiz *et al.*, 2022). Each strain was cultured on Tryptone Soy Agar (TSA) for 24 h, then grown in Tryptone Soy Broth with agitation at 100 rpm for 24 hours to reach a concentration of 1×10^6 colony-forming units (CFU)$\cdot$ml^{-1}. Seeds of the commercial maize hybrid Pioneer P3095 were sown.

Experimental Design and Agronomic Management

The evaluation was established using a randomized complete block design with four replications. The agronomic management was carried out according to the practices of local farmers, with a row spacing of 0.85 meters. The plant spacing was 12 cm, and the experimental unit consisted of 12 rows of 20 linear meters each. The treatments used were: TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC. The BC was applied three times during the growing season: at planting and two subsequent applications every 15 days. The three cultivation practices were related to nutrition, pest control, diseases, and weeds, in TM, synthetic inputs were used, TM+AM referred to reducing synthetic inputs by 50% and supplementing with 50% bioinputs (solid and liquid). AM utilized 100% bioinputs. The BC was diluted in water for application at a concentration of 1×10^6 CFU$\cdot$ml^{-1} and applied via drench (without nozzle) using a manual pump.

Evaluated Variables

The response variables evaluated were: phenotypic variables including days to male flowering (DMF), days to female flowering (DFF) at the VT-R1 stage, as well as plant height (PH), ear height (EH), stem diameter (SD), ear diameter (ED), ear length (EL), measured in centimeters with data collected from four plants per treatment and block at the R1-R2 stage of the crop. Additionally, physiological readings were taken for maize plant vigor (VIGOR), which was measured five days after planting in each block and treatment. Samples were taken from a total length of three meters and reported as a percentage, and for yield (t ha^{-1}), three samples were taken from a length of three meters, considering three central rows per treatment and block, and adjusted to 14% grain moisture content.

Statistical Analysis

The data obtained for each of the studied variables were analyzed using SAS 9.4. An analysis of variance was performed, and significant statistical differences were assessed using Tukey's test ($P\leq0.05$ and $P\leq0.01$) for mean comparisons.

RESULTS AND DISCUSSION

Results. Under the three different agronomic management practices used for the crop, the phenotypic variables of days to male flowering (DMF), days to female flowering (DFF), plant height (PH), and ear height (EH), as well as the physiological variable of vigor (VIGOR), showed statistically significant differences between treatments and blocks (Table 1). The variables ear length (EL) and yield (YIELD) showed significant differences only between blocks (Table 1). Additionally, there were no significant differences (ns) in stem diameter (SD) and ear diameter (ED) between treatments and blocks (Table 1).

Considering the variables DMF and DFF, the ANOVA showed significant differences between treatments. The numerical differences of 0-2 days both between treatments and between the two flowering stages could have effects on subsequent stages. Notably, synchronization was observed between the two flowering stages in the TM+AM treatment, despite being the latest (Figure 1).

The phenotypic variable EH showed the highest value with the TM+AM treatment (145.75 cm, $P \leq 0.05$), while PH showed the highest value with the TM treatment (291.00 cm, $P \leq 0.05$) (Figure 2). The phenotypic variables EL, ED, and SD did not show differences between treatments (Figure 2).

Regarding plant physiological aspects, the VIGOR variable showed differences between the treatments under study, with the highest values observed in the MA treatment ($P \leq 0.05$) (Figure 3), followed by the TM and TM+AM treatments, with values of 75.33%, 74.33%, and 68.66%, respectively (Figure 3).

Another variable of interest is yield. Although it did not show statistically significant differences between treatments, a 6.03% increase (0.652 t ha^{-1}) was observed in the

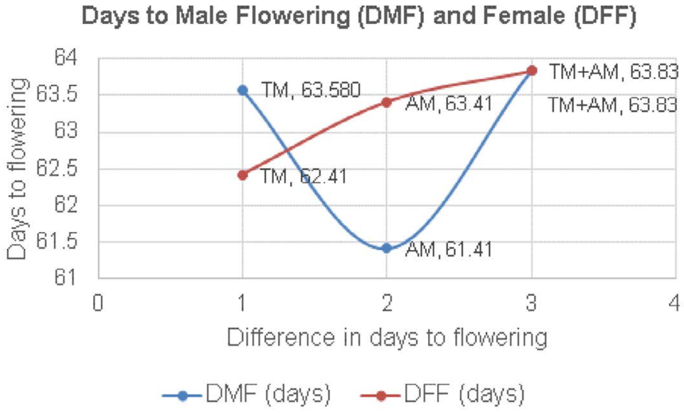

Figure 1. Comparison of means for the variables regarding treatments in yield and agronomic management in maize. TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC. DMF: Days to Male Flowering, DFF: Days to Female Flowering.

Table 1. Mean Squares and Significance of the Analysis of Variance for the Evaluated Variables with Respect to Blocks and Treatments.

S.V.	DF	DMF (days)	DFF (days)	PH (cm)	EH (cm)	SD (cm)	ED (cm)	EL (cm)	VIGOR (%)	YIELD (t ha^{-1})
TREAT	2	7.06*	2.12*	1434.24*	187.06*	0.005ns	0.02ns	0.08ns	51.69**	434281.36ns
BLOCKS	3	5.78*	0.81*	349.62*	53.60*	0.004ns	0.05ns	1.42*	97.76**	1748300.76*
E.E.		2.59	0.48	202.89	30.75	0.007	0.06	0.63	2.91	1291471.57
MEAN		62.94	63.22	273.77	139.08	2.40	3.39	15.78	72.77	11075.39
C.V.		2.55	1.10	5.20	3.98	3.70	7.34	5.05	2.34	10.26

**S.V.: Source of Variation, DF: Degrees of Freedom, TREAT: Treatments, BLOCK: Blocks, E.E.: Experimental Error, C.V.: Coefficient of Variation, DMF: Days to Male Flowering, DFF: Days to Female Flowering, PH: Plant Height, EH: Ear Height, SD: Stem Diameter, ED: Ear Diameter, EL: Ear Length, YIELD: Commercial Grain Yield. ns: Not Significant, *, **: Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

TM+AM treatment (11.43 t ha^{-1}), compared to the TM and AM treatments, with values of 10.78 and 11.02 t ha^{-1}, respectively (Figure 4).

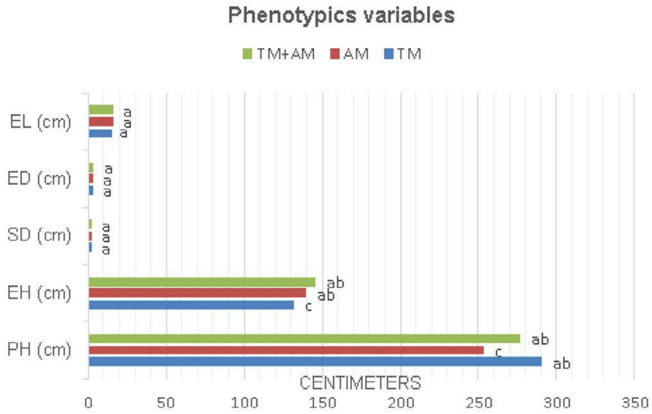

Figure 2. Comparison of means for phenotypic variables regarding agronomic management practices. PH=Plant Height, EH=Ear Height, SD=Stem Diameter, ED=Ear Diameter, EL=Ear Length. MT=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC.

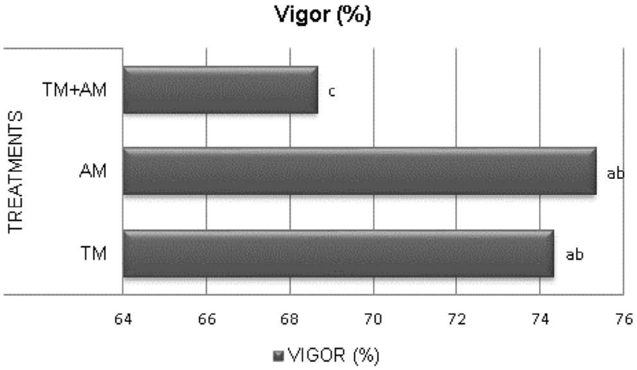

Figure 3. Comparison of mean values for treatments regarding VIGOR in maize. TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC.

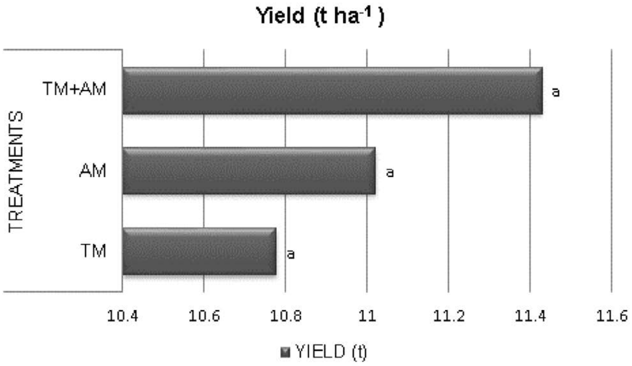

Figure 4. Comparison of mean grain yield across different agronomic management practices and its relation to the bacterial consortium in maize. TM = 100% traditional management + bacterial consortium (CB), TM+AM = 50% traditional management + 50% agroecological management + CB, and AM = 100% agroecological management + CB.

In this study, the effect of a bacterial consortium of three growth-promoting strains on maize grain production was analyzed, under three different agronomic management practices in the La Ciénega region, Jalisco. Previously, Barragán-Nava *et al.* (2022) isolated, identified, and characterized plant growth-promoting rhizobacteria from ten different land uses and planting cycles in the La Frailesca region, Chiapas, from which the strains *Stenotrophomonas* sp. LIMN, *Enterobacter* sp. LCMG, and *Rhizobium* sp. WFRFC were selected. Subsequently, Resendiz-Venado *et al.* (2022) evaluated their effect on maize seedling germination and growth, finding that the bacterial consortium increased the length, dry weight, and fresh weight of the plumule, as well as the number of roots. The field evaluation of the bacterial consortium conducted in this study confirms its potential as a bio-stimulant for maize cultivation.

The application of a bio-stimulant in the early vegetative stages of the crop was reflected in the vigor and reproductive stage; generally, the microorganisms in a bio-stimulant interact with the native microorganisms of the rhizosphere, the soil, and the genetic constitution of the maize type. In this regard, Lopes *et al.* (2016) mention that differences in the structure and composition of the rhizobacterial community are due to the selection performed by the rhizosphere from the microorganisms inhabiting the soil and plant, which modifies the abundance of functional groups according to their ability to adapt to rhizosphere conditions, thus shaping its bacterial community (Mendes *et al.*, 2014). In general, the rhizosphere is an environment regulated by a mixture of complex interactions between plants and microorganisms, where structural and functional diversity, as well as the stability of microbial communities, strongly influence crop quality (Ngullie *et al.*, 2015); that is, the rhizosphere creates a dynamic and nutrient-rich environment around the roots and maintains specific bacterial populations involved in activities that ensure crop stability and productivity (Dennis *et al.*, 2010). The rhizosphere is considered a selective pressure environment for horizontal gene transfer (HGT) events and is regarded as an important factor in increasing genetic diversity and, consequently, in bacterial evolution (Nemergut *et al.*, 2004).

The application of the bacterial consortium under the three agronomic managements favored the yield, showing an increase of 14.80% - 21.73% compared to the average yield reported for the La Ciénega region of 9.39 t·ha^{-1} (SIAP, 2022). Unlike its behavior between treatments where there was no statistically significant effect on this variable; however, a positive effect was observed with an increase of 2.23 and 6.03% in the yield of the AM and TM+AM treatments, respectively. Additionally, considering the reduction of 100% and 50% in the application of synthetic fertilizers, the profitability (data not shown) is improved in the AM and TM+AM agronomic managements. Additionally, considering the reduction of 100% and 50% in the application of synthetic fertilizers, profitability (data not shown) improved in the AM and TM+AM agronomic managements. Similar data were reported by Dotto *et al.* (2010), where the inoculation of *Herbaspirillum seropedicae* in the AS1570 hybrid did not significantly influence its productivity, but it was observed that the hybrid responded positively with an 8.6% increase in grain production. Bio-stimulants are bioproducts developed from one or several microbial strains; currently, their application is considered an appropriate method for introducing probiotics into agricultural soils (Yadav *et al.*, 2017); which release inorganic nutrients for plants from soil minerals, improve the structure of both the subsoil and the topsoil, increase water infiltration, enhance crop quality, and make plants more resistant to various pests and pathogenic organisms (Srivastava and Ngullie, 2009). Biofertilizers also increase soil microbial biodiversity by breaking the dormancy of microbial banks, due to a reinforcement of the relationship between biodiversity and ecosystem functioning (Bhardwaj *et al.*, 2014; Kulasooriya and Seneviratne, 2013). This increase in biodiversity strengthens soil health and enhances tolerance to stress caused by abiotic and biotic factors.

In this context, Santoyo *et al.* (2021) mentions that Plant Growth-Promoting Microorganisms (PGPM) refers to all microorganisms (bacteria, actinomycetes, fungi, or algae) that act through various mechanisms to enhance fertilization, phyto-stimulation, or disease suppression. They play an important role in sustainable agriculture, promote diversity and interaction with other beneficial microorganisms, and generally maintain the sustainability of systems. Various strains of the genera *Stenotrophomonas*, *Enterobacter*, and *Rhizobium* are known as PGPM in different crops, actively participating in biogeochemical nutrient cycles, mainly nitrogen and phosphorus, synthesizing antibiotics, among other characteristics, which support plant establishment, nutrition, and development (Goswami *et al.*, 2016; Shafi *et al.*, 2017). These characteristics exert various effects on the results obtained in the TM+AM and AM treatments, compared to the TM control, both in the phenotypic and physiological variables evaluated. For example, they influenced the variables of vigor, days to both male and female flowering, and the growth and development of the crop, positively affecting the reproductive stage. Sánchez-Yáñez *et al.* (2014) mention that a 50% reduction in nitrogen fertilizer generated a positive response in days to flowering and plant height in maize inoculated with plant growth-promoting bacteria; this suggests that these PGPM genera transformed maize root exudates into plant growth-promoting substances, which in turn induced increased stem growth. João *et al.* (2021) mention that inoculation with a *Bacillus* strain increased plant height and dry weight of shoot and root, changes attributed to the relatively increased abundance of strains from the Burkholderiaceae, Pseudomonadaceae, and Rhizobiaceae families, which are widely described as plant growth-promoting (García-Fraile *et al.*, 2012; Suárez-Moreno *et al.*, 2012; Redondo-Nieto *et al.*, 2013).

The consortium consisting of the *Stenotrophomonas* sp. LIMN, *Enterobacter* sp. LCMG, and *Rhizobium* sp. WFRFC strains also established an association with the evaluated maize hybrid plants, which is why they had a positive effect on the assessed variables. As reported, the plant genotype is a determining factor that directly influences the specificity of the bacteria-plant association, allowing the benefits of the inoculated strains to be obtained (Moreira, 2014).

CONCLUSIONS

The development of biotechnological tools that can be applied in the agroecological management of crops contributes to the sustainable use of genetic resources and the development of sustainable agriculture. Specifically, for maize cultivation, which requires a large amount of inputs for its production, such tools are highly relevant for reducing the environmental, economic, and agronomic impact generated. The application and use of a bacterial consortium consisting of *Enterobacter* sp. LCMG, *Rhizobium* sp. WFRFC, and *Stenotrophomonas* sp. LIMN, in combination with different agronomic management practices in maize cultivation was positive, achieving a 50% reduction in synthetic inputs with the traditional+agroecological management (TM+AM) treatment. Evidence is provided that under agroecological management, a 100% response is obtained compared to traditional (TM) or synthetic management, with a significant impact mainly on the variables DMF, DFF, VIGOR, and YIELD. The use of bacterial consortia and agroecological management reduces the long-term environmental and economic impact on agricultural crops.

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