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# Efficient microorganisms increases the yield of four strawberry cultivars

Microrganismos eficientes aumentam o rendimento de quatro cultivares de morangueiro

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Efficient microorganisms (EM) represent a social biotechnology that requires research for validation. Thus, we investigated whether the association between EM and strawberry cultivars in substrate interferes with their agronomic performance. We studied the combination of four cultivars ('Albion', 'Camino Real', 'Monterey', and 'San Andreas') in the absence and presence of EM composed of eight bacteria and three fungi, in a randomized block design, with four repetitions. Fruit production and quality attributes were assessed. Regardless of the cultivar, plants treated with EM had greater production potential. Multivariate analysis revealed the formation of two contrasting groups for 'Camino Real' and 'San Andreas' in relation to EM. 'San Andreas' produced the most strawberries and, together with 'Albion', had the highest fruit yield. Fruits from 'Albion' and 'Camino Real' had the highest total flavonoid content. 'Albion' and 'Monterey' stood out for having the highest sugar content in the fruit. The tastiest strawberries were produced by 'Monterey'. In conclusion, plants treated with EM throughout the cycle have the highest fruit production and produce strawberries with the highest average fresh mass. Thus, the use of this biotechnological tool is viable in horticulture as part of the transition to more sustainable systems that value different genetic resources.
Keywords: *Fragaria* X *ananassa* Duch., phytochemicals, yield.

Microrganismos eficientes (ME) representam uma biotecnologia social que requer pesquisa para validação. Assim, investigamos se a associação entre ME e cultivares de morangueiro em substrato interfere em seu desempenho agronômico. Estudamos a combinação de quatro cultivares ('Albion', 'Camino Real', 'Monterey' e 'San Andreas') na ausência e presença de ME composto por oito bactérias e três fungos, em delineamento de blocos casualizados, com quatro repetições. Avaliou-se a produção e a qualidade de frutos. Independente da cultivar, as plantas tratadas com ME apresentaram maior potencial de produção. A análise multivariada revelou a formação de dois grupos contrastantes para 'Camino Real' e 'San Andreas' em relação ao ME. 'San Andreas' produziu mais morangos e, juntamente com 'Albion', apresentou a maior produtividade de frutos. Frutos de 'Albion' e 'Camino Real' apresentaram o maior teor de flavonoides totais. 'Albion' e 'Monterey' se destacaram por apresentarem o maior teor de açúcares no fruto. Os morangos mais saborosos foram produzidos por 'Monterey'. Em conclusão, as plantas tratadas com EM ao longo do ciclo apresentam a maior produção de frutos e produzem morangos com a maior massa fresca média. Assim, o uso dessa ferramenta biotecnológica é viável na horticultura como parte da transição para sistemas mais sustentáveis que valorizam diferentes recursos genéticos.
Palavras-chave: *Fragaria* X *ananassa* Duch., fitoquímicos, rendimento.

## 1. INTRODUCTION

Despite being appreciated all over the world for the functional and organoleptic characteristics of the fruit, the hydroponic and greenhouse strawberry cultivation (*Fragaria* X *ananassa* Duch.) is still in transition to a less aggressive and more environmentally friendly system. This transition is also a response to consumer demands, who are interested, along with producers, researchers and industry, in ecological and sustainable agricultural practices.

Many advances have been made in recent years through the adoption of agro-sustainable managements, such as the choice of organic-based substrates [1], the use of mycorrhizal inoculants [2] and the application of biostimulants [3]. In the latter case, the focus of research is to isolate, identify and transform fungi [4], bacteria [5], or their combination [6] into bioinputs. However, to intensify their use and reduce their costs, we need to find ways to make these bioinputs available to producers and disseminate them as a social biotechnology. One bio-tool that can contribute to this is efficient microorganisms (EM).

It was in the 1980s that the use of EM in agriculture was discovered, based on research carried out by Professor Teruo Higa at Kyushyu University in Japan, which highlighted the benefits of microorganisms when used in a mixture [7]. Since then, this biotechnology has been applied to a wide variety of crops and agroecosystems [8, 9] with the aim of intensifying long-lasting and dynamic agroecological agriculture. EM is a mixture of natural microorganisms present in plants and soils, with predominant populations of lactic acid bacteria, yeasts, actinomycetes, and photosynthetic bacteria [10]. The principle of EM is to improve the physical, chemical and biological conditions of the growth medium [11]. In addition, these microorganisms have the potential to improve plant development and increase resistance to insects and diseases [12].

These microorganisms are called efficient because they act very quickly and contribute to the balance of the agroecosystem [8]. However, the literature reporting the application of EM to strawberry plants is scarce, with only one study detected [13], which showed that the concentration of 1% and 3% EM in conjunction with 100 kg $N.ha^{-1}$ had positive effects on the morphological characteristics and productivity of strawberry plants, 'Paros' cultivar. Thus, research into the agronomic potential of strawberry cultivars associated with EM enables strawberry growers to choose materials adapted to subtropical growing conditions within a system that is less aggressive to the environment. In addition, the dependence on the large-scale use of chemical inputs in agroecosystems can be reduced.

Therefore, here we investigated whether the association between EM and strawberry cultivars in substrate interferes with their agronomic potential. In addition, to understand the relationship between the use of EM in the four cultivars studied, we explored the data using multivariate analysis. Our findings will help strengthen links among producers, researchers, industry and consumers in the development of sustainable agriculture.

## 2. MATERIAL AND METHODS

### 2.1 Plant material

Strawberry cultivars bare-root daughter plants from the Llahuén nursery in Chilean Patagonia (33º 50' 15.41" S; 70º 40' 03.06" W) formed the plant material for the experiment. The research was carried out in the municipality of Passo Fundo (28º 15' 41" S; 52º 24' 45" W), Rio Grande do Sul (RS) state, Brazil, from June (winter) 2022 to February (summer) 2023, in a greenhouse (430 $m^2$) made of galvanized steel and with a semicircular roof covered with low-density polyethylene film (150 microns thick and with an anti-ultraviolet additive).

### 2.2 Experimental design

The treatments, laid out in a bifactorial design, were four strawberry cultivars ('Albion', 'Camino Real', 'Monterey', and 'San Andreas') in the absence (-) and presence (+) of EM. The experiment was laid out in randomized blocks, with four replications and six plants per plot. 'Camino Real' is classified as short-day (SD) and 'Albion', 'Monterey', and 'San Andreas' as neutral-day (ND) in relation to flowering.

### 2.3 Obtaining and identifying EM

Efficient microorganisms (EM) were captured and subsequently multiplied according to the procedures contained in Agroecological Sheet No. 31, published by the Ministry of Agriculture, Livestock and Supply (MAPA), Brazil [14]. For the process of capturing the EM, cooked rice (*Oryza sativa* L.) was used, which was spread in a bamboo gutter and inserted into a balanced environment (native forest) located in a permanent preservation area on the University of Passo Fundo campus. Approximately seven days after their establishment, the baits were removed from the capture site and the EM colonies that appeared on the rice were separated by color. These colonies were then placed in containers with dechlorinated water and organic brown sugar to ferment. Every two days, the lid of the container was opened to allow gas to be released. When gas formation was no longer observed, EM was ready for use. The material collected and produced was analyzed by Neoprospecta Microbiome Technologies, Brazil, for digital microbiological diagnosis.

Bacteria were identified by high-performance sequencing of the V3/V4 regions of the 16S rRNA gene. Amplification was carried out with primers for the V3-V4 region of the 16S rRNA gene, 341F with sequence (CCTACGGGRSGCAGCAG) and 806R with sequence (GGACTACHVGGGTWTCTAAT).

Fungi were identified by high-performance sequencing of the ITS1 region. Amplification was carried out with ITS1 primer (GAACCWGCGGARGGATCA) and ITS2 primer (GCTGCGTTCTTCATCGATGC).

The libraries of both bacteria and fungi were sequenced using the MiSeq Sequencing System (Illumina Inc., USA). The sequences of both were analyzed using the Sentinel pipeline [15-19]. A total of eleven microorganisms were identified, of which eight were classified as bacteria and three as fungi (Figure 1).

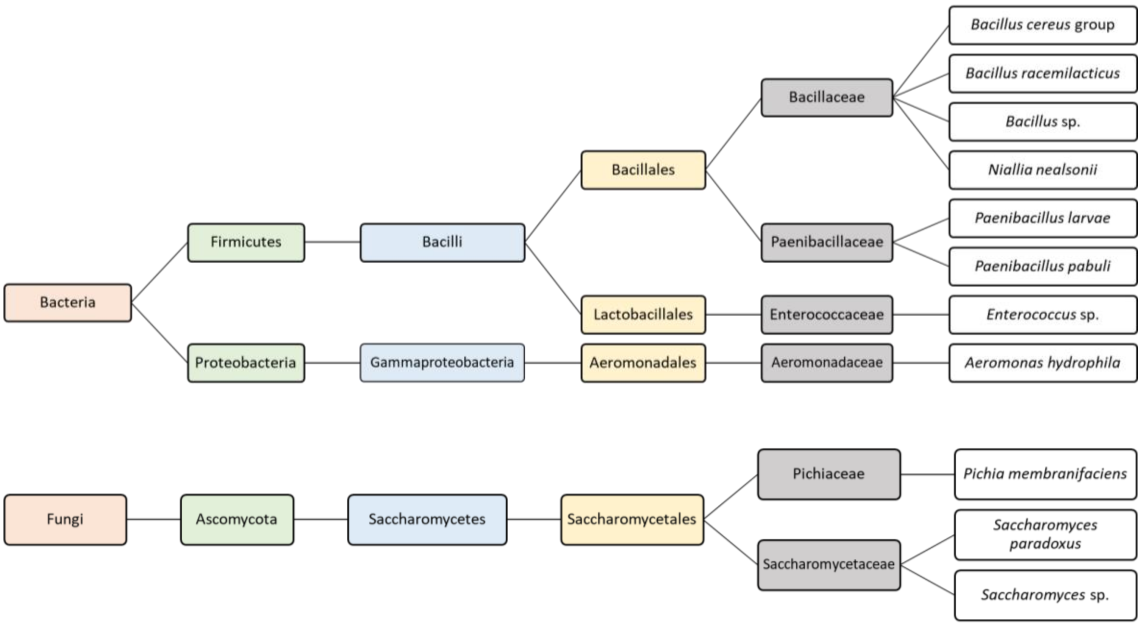


*Figure 1: Community of bacteria and fungi present in the analyzed bioinput, with indication of Kingdom, Phylum, Class, Order, Family, and Species.*

The most abundant bacterium in the sample analyzed was the *Bacillus cereus* group, with 123,507 sequences, while *Pichia membranifaciens* was the most abundant fungus, with 254,230 sequences (Figure 2).

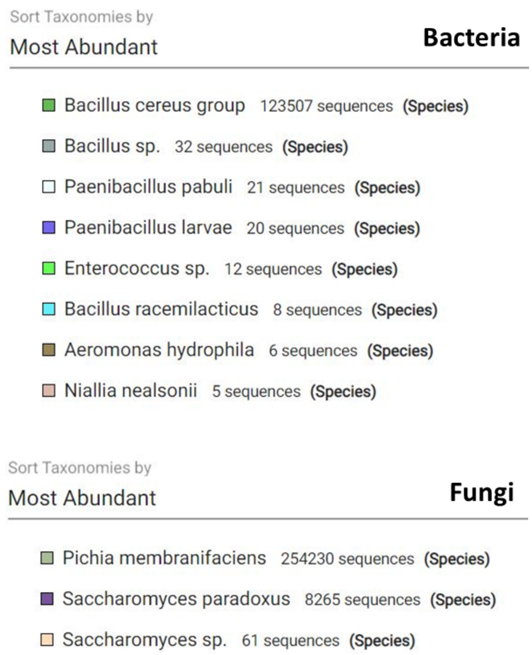


*Figure 2: Species of bacteria and fungi detected in the bioinput sample according to their abundance.*

## 2.4 Strawberry cultivation

The strawberry cultivation system employed was based on substrate. The daughter plants were transplanted in June 2022 into containers measuring 1 m long x 0.3 m wide, filled with the commercial TN Slab® substrate, produced by Agrinobre®. TN Slab® substrate is made up of sphagnum peat, expanded vermiculite, carbonized rice husk, dolomitic limestone, agricultural gypsum, and fertilizers (macro and micronutrients), in quantities not supplied by the manufacturer. The plants were distributed at a spacing of 0.17 m, with one row of plants per container. The physical and chemical characterization of the substrate is shown in Table 1.

*Table 1: Physical and chemical properties of the TN Slab® substrate.*

| Physical properties[1] | | | | | | |
|---|---|---|---|---|---|---|
| D | | TP | AE | RAW | BW | RW |
| (kg m$^{-3}$) | | (m$^3$ m$^{-3}$) | | | | |
| 212 | | 0.885 | 0.502 | 0.144 | 0.017 | 0.222 |
| **Chemical properties[2]** | | | | | | |
| N | $P_2O_5$ | $K_2O$ | OC | pH | EC | C/N |
| % (m m$^{-1}$) | | | | | mS cm$^{-1}$ | ratio |
| 0.82 | 0.58 | <0.25 | 26.10 | 5.6 | 1.05 | 33.42 |

[1]D = density; TP = total porosity; AE = aeration space; RAW = easily available water; BW = buffer water; RW = remaining water. [2]N = nitrogen; $P_2O_5$ = phosphorus pentoxide; $K_2O$ = potassium oxide; OC = organic carbon; pH = hydrogen potential; EC = electrical conductivity; C/N ratio = relationship between carbon and nitrogen.

Two weeks after transplanting the daughter plants, the treatments that received EM were started. For this purpose, a dose of 1 milliliter (mL) of EM was used for each liter (L) of water, always activating the solution with 1 gram of organic brown sugar. The applications were carried out every two weeks using a 20 L manual backpack sprayer (XP Jacto®), with a spray volume of approximately 50 mL plant$^{-1}$. The EM was sprayed on the aerial part (leaves) and on the central base of the plants, so that it would run off into the growing substrate.

The irrigation used in the experiment was localized, using drip strips in an automated system, with a flow rate of 1.41 L.h$^{-1}$ per dripper. The irrigation regime consisted of activating the drip lines seven times a day, with a total wetting time of 14 minutes. The nutrient solutions were fed to the plants weekly [20].

### 2.5 Yield

After fruiting, in August (winter) 2022, total number of fruits per plant (TNF, fruits plant$^{-1}$) and total fruit yield per plant (TP, grams plant$^{-1}$) were evaluated, performed at commercial maturity (>85% visual red color). The fruit was weighed on an electronic digital scale. The average fresh fruit mass (AFFM, grams) was also determined by dividing TP and TNF.

### 2.6 Fruit quality

As for determining the total phytochemical content, in October (spring) 2022, the harvested strawberries were subjected to the extraction procedure [21]. Total flavonoid (TFL) and total polyphenol (TPO) contents were then quantified. TFL content [22], was expressed in milligrams of rutin per 100 grams of fresh fruit (mg rutin 100 g$^{-1}$ FF). TPO content was determined using the Folin-Ciocalteu method [23] and the results were expressed as milligrams of gallic acid equivalent per 100 grams of fresh fruit (mg AGE 100 g$^{-1}$ FF).

Chemical quality analysis was also carried out in October 2022. The characteristics relating to total soluble solids (TSS, %) and total titratable acidity (TTA, % of citric acid) were assessed using 20 fruits from each treatment for each repetition [24]. As an indicator of fruit taste quality, TSS/TTA ratio was determined.

### 2.7 Data analysis

Data obtained was subjected to analysis of variance (ANOVA) and, when significant, the means of the treatments were compared using the Tukey test, at a 5% probability of error, with the aid of R Studio [25].

Data was also subjected to multivariate analysis using principal components analysis (PCA). PCA was carried out after the attributes had been standardized so that each one had a mean of 0 and a variance of 1. We disregarded eigenvalues of less than 1 from the analysis because they did not provide relevant information [26]. We ran the PCA with the *'factoextra'* package in R Studio [25].

## 3. RESULTS

### 3.1 Yield

ANOVA indicated a significant effect of the EM factor for the TP and AFFM attributes and of the cultivars factor for all the production attributes analyzed. 'San Andreas' produced the most strawberries and, together with 'Albion', had the highest fruit production (Table 2). 'Camino Real', on the other hand, produced fruit with the lowest AFFM (Table 2).

Regardless of the cultivar, plants treated with EM throughout the cycle had higher TP and produced strawberries with higher AFFM (Table 2), i.e. the use of EM increased total fruit production by 17%, and these fruits from plants treated with EM were 5.6% larger than those produced by untreated plants.

*Table 2: Yield of strawberry cultivars with EM.*

| Cultivars | TNF[1] (fruits plant$^{-1}$) | TP (grams plant$^{-1}$) | AFFM (grams) |
|---|---|---|---|
| 'Albion' | 25.23±2.87 b | 339.08±18.95 ab | 13.41±0.89 a |
| 'Camino Real' | 24.62±2.78 b | 300.55±17.26 b | 12.08±0.73 b |
| 'Monterey' | 20.43±2.65 b | 279.04±16.48 b | 13.62±0.95 a |
| 'San Andreas' | 31.97±3.04 a | 426.04±19.63 a | 13.28±0.79 a |
| EM[2] | | | |
| With (+) | 26.84±3.04[ns] | 361.33±15.82 a | 13.45±0.92 a |
| Without (-) | 24.29±2.51 | 311.02±17.29 b | 12.74±0.75 b |
| Mean | 25.56 | 336.18 | 13.10 |
| CV (%)[3] | 18.54 | 19.31 | 6.05 |

Data was presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by Tukey's test ($p \leq 0.05$). [1]TNF = total number of fruits; TP = total production; AFFM = average fresh fruit mass. [2]EM = efficient microorganisms. [3]CV = coefficient of variation. [ns]Not significant ($p > 0.05$).

## 3.2 Fruit quality

Regarding the phytochemical quality of the fruit, ANOVA revealed the effect of the cultivars on the TFL attribute. Fruits from 'Camino Real' and 'Albion' had a higher TFL content than 'Monterey' (Table 3).

*Table 3: Phytochemical quality of fruits of strawberry cultivars.*

| Cultivars | TFL (mg rutin 100 g$^{-1}$ FF)[1] |
|---|---|
| 'Albion' | 89.10±12.14 ab |
| 'Camino Real' | 110.48±19.47 a |
| 'Monterey' | 63.02±11.98 c |
| 'San Andreas' | 79.28±12.12 bc |
| Mean | 85.47 |
| CV (%)[2] | 20.21 |

Data was presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by Tukey's test ($p \leq 0.05$). [1]TFL = total flavonoid content. [2]CV = coefficient of variation.

Regarding the fruit postharvest, there were statistical differences among the cultivars in terms of TSS and TSS/TTA. The strawberry cultivars were grouped into three extracts in terms of TSS and TSS/TTA. 'Albion' and 'Monterey' stood out for having the highest sugar content in the fruit, followed by 'Camino Real' and, as the last extract, 'San Andreas' (Table 4). The tastiest strawberries were produced by 'Monterey', followed by 'Albion' and 'Camino Real' and, as the last extract, 'San Andreas' (Table 4).

*Table 4: Postharvest of fruits of strawberry cultivars.*

| Cultivars | TSS (%)[1] | TSS/TTA |
|---|---|---|
| 'Albion' | 10.38±0.86 a | 11.73±0.75 ab |
| 'Camino Real' | 09.41±0.62 ab | 10.90± 0.62 ab |
| 'Monterey' | 10.28±0.59 a | 11.90±0.83 a |
| 'San Andreas' | 07.98±0.49 b | 09.23±0.58 b |
| Mean | 9.51 | 10.94 |
| CV (%)[2] | 14.92 | 16.64 |

Data was presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by Tukey's test ($p \leq 0.05$). [1]TSS = total soluble solids; TSS/TTA = flavor. [2]CV = coefficient of variation.

### 3.3 Multivariate analysis

From the correlation matrix between fruit production and quality attributes, it can be observed that the most significant positive correlations were between TP and TNF (r = 0.96) and TSS and TSS/TTA (r = 0.98) (Figure 3).

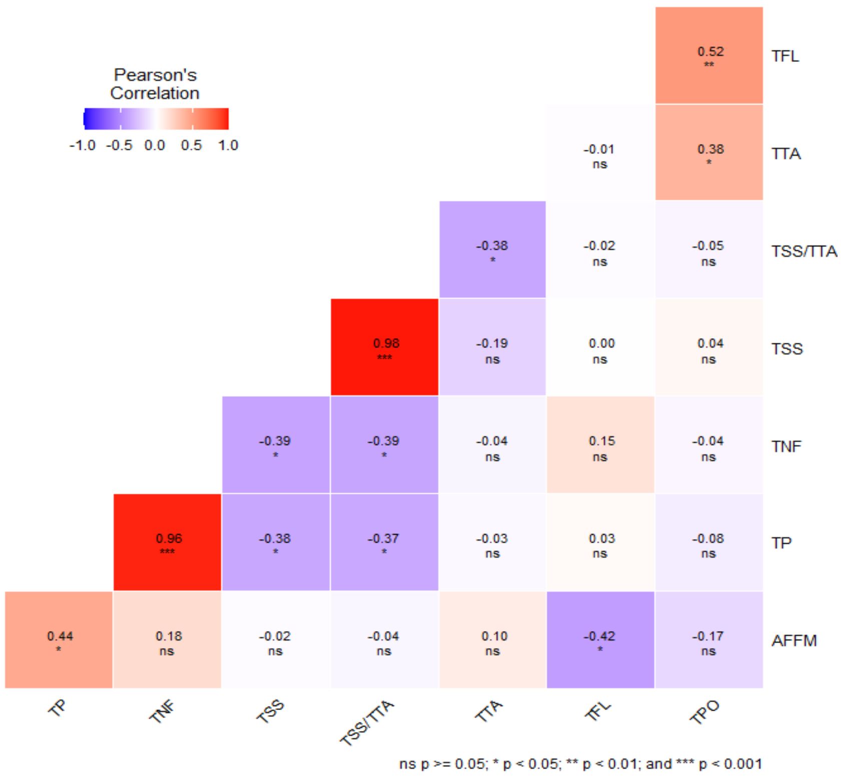


*Figure 3: Correlation matrix among the attributes evaluated in the study. TNF = total number of fruits; TP = total production; AFFM = average fresh fruit mass; TSS = total soluble solids; TTA = titratable total acidity; TSS/TTA = flavor; TPO = total polyphenol content; TFL = total flavonoid content.*

We studied the contribution of fruit production and quality attributes to the first two principal components (PC). TP (33.74%) and TSS (46.20%) were the attributes with the highest contribution to PC1 and PC2, respectively, for 'Albion' (Table 5). For 'Camino Real', AFFM (23.09%) and TPO (32.69%) had the highest contribution to PC1 and PC2, respectively (Table 5). TNF (24.59%) and TSS (38.88%) stood out in their contribution to PC1 and PC2, respectively, in 'Monterey' (Table 5). For 'San Andreas', TP (21.64%) and TFL (25.85%) made the greatest contribution to PC1 and PC2, respectively (Table 5).

*Table 5: Contribution (%) of attributes analyzed in relation to the first two principal components (PC).*

| Attributes | 'Albion' | | 'Camino Real' | | 'Monterey' | | 'San Andreas' | |
|---|---|---|---|---|---|---|---|---|
| | PC1 | PC2 | PC1 | PC2 | PC1 | PC2 | PC1 | PC2 |
| TP[1] | 33.74 | 03.73 | 22.27 | 05.31 | 23.34 | 02.45 | 21.64 | 09.27 |
| TNF | 28.58 | 01.53 | 13.42 | 10.17 | 24.59 | 00.81 | 16.10 | 20.43 |
| AFFM | 12.23 | 05.05 | 23.09 | 00.65 | 02.90 | 18.56 | 13.15 | 07.64 |
| TFL | 00.17 | 21.91 | 15.41 | 00.06 | 22.59 | 00.82 | 11.85 | 25.85 |
| TPO | 07.78 | 19.22 | 04.93 | 32.69 | 18.54 | 01.16 | 10.90 | 18.06 |
| TSS | 06.04 | 46.20 | 15.35 | 18.89 | 00.08 | 38.88 | 15.45 | 01.13 |
| TTA | 11.43 | 02.33 | 05.50 | 32.19 | 07.92 | 37.28 | 10.88 | 17.39 |

[1]TP = total production; TNF = total number of fruits; AFFM = average fresh fruit mass; TFL = total flavonoid content; TPO = total polyphenol content; TSS = total soluble solids; TTA = titratable total acidity.

When we studied the pattern of cultivars in terms of fruit production and quality attributes, we observed the formation of two contrasting groups for ‘Camino Real’ and, above all, ‘San Andreas’ in relation to EM (Figure 4). For ‘Camino Real’ and ‘San Andreas’, the pattern represented by the presence of EM (red color) had a greater influence on fruit production attributes (Figure 4), which confirmed the results of the univariate analysis (Table 2). On the other hand, ‘Albion’ and ‘Monterey’ performed similarly in the presence and absence of EM (Figure 4).

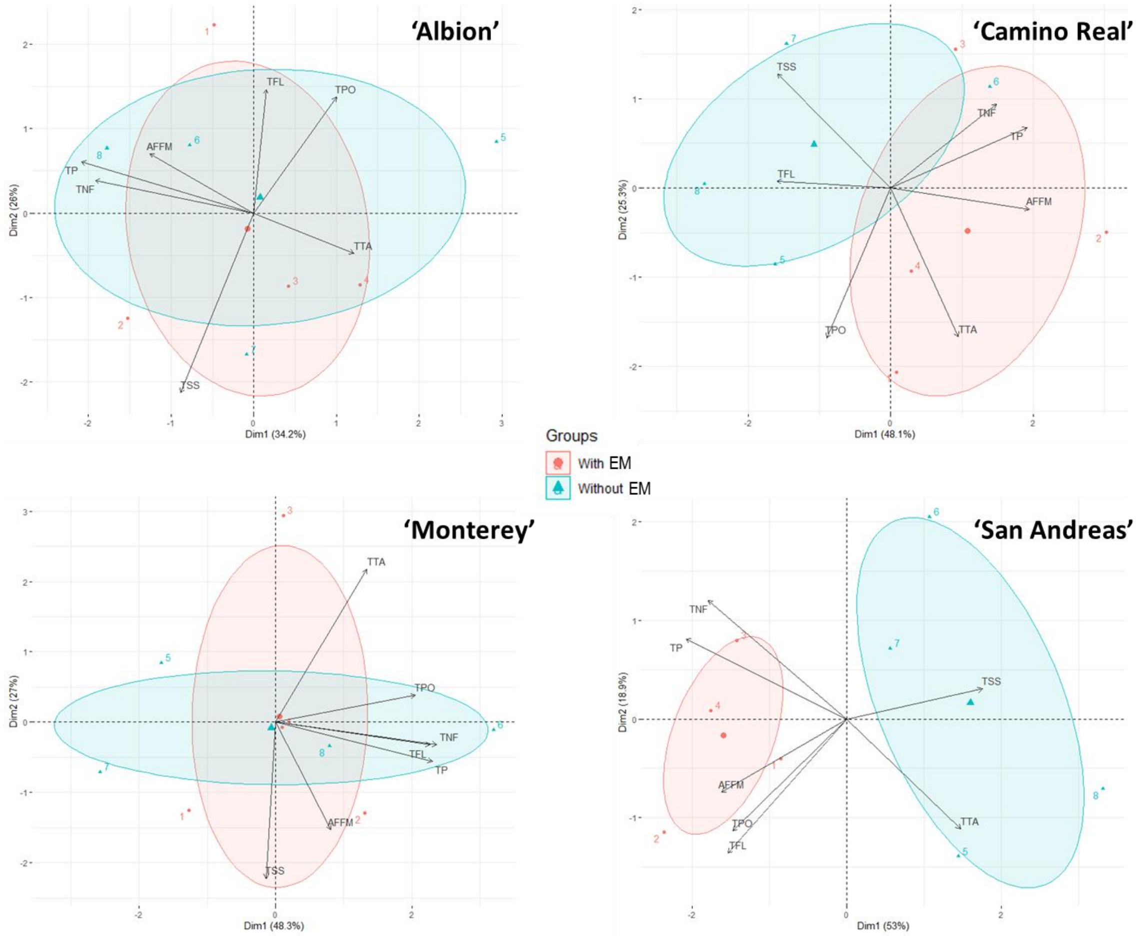


*Figure 4: Principal component analysis of ‘Albion’, ‘Camino Real’, ‘Monterey’, and ‘San Andreas’ in relation to the absence and presence of efficient microorganisms (EM).*

When analyzing EM as the main factor, the PCA showed that in the absence of this bioinput, the strawberry cultivars were distributed in an overlapping way (Figure 5). On the other hand, the use of EM distributed the cultivars more clearly, with the exception of ‘Albion’ (Figure 5). It should be noted that the pattern represented by ‘San Andreas’ did not overlap with the other patterns and had a greater influence on the production attributes, represented by TP and TNF (Figure 5). This indicates that the application of EM to strawberry plants provides more robust and categorical results regarding the horticultural performance of cultivars.

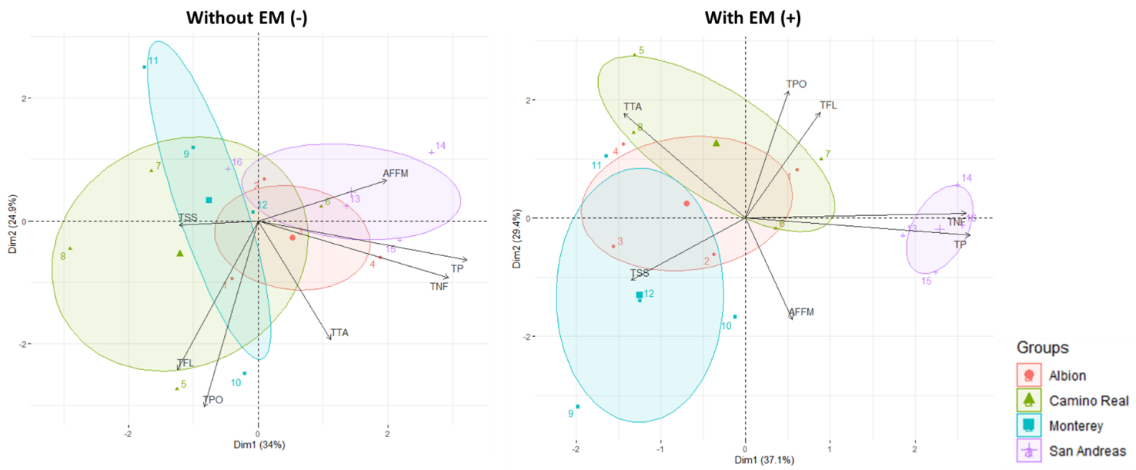


*Figure 5: Principal component analysis in the absence and presence of efficient microorganisms (EM) in relation to strawberry cultivars.*

## 4. DISCUSSION

Here, we report that strawberry plants treated with EM increased their yield throughout the growing cycle. PCA formed heterogeneous groups between cultivars and EM, especially for ‘San Andreas’, which indicated that the use of the bioinput provided more robust and categorical results regarding strawberry performance. In addition, ‘San Andreas’ had the best production potential, ‘Camino Real’ produced fruit rich in flavonoids, ‘Albion’ produced sweeter fruit, and ‘Monterey’ produced tastier strawberries.

EM is a solid alternative to promote safe, sustainable and low-cost production, with the potential to be included in agroecological production systems [27]. However, we still need to understand the agronomic benefits of EM on target plants [28]. Thus, the fact that we did not observe a significant effect on the quality of strawberries produced by plants treated with EM may be associated with its long-term effect. This was confirmed in relation to the production potential, as using the bioinput for longer, from June 2022 to February 2023, improved the crop yield (Table 2). Sousa et al. (2020) [29] showed the benefits of applying EM to lettuce (*Lactuca sativa* L.) only in the crop’s second cycle, by increasing the fresh mass of the aerial part, the number of leaves, the length of the stem and the number of viable plants per plot.

When studying the effect of concentrations of EM on the production of peppers (*Capsicum annuum* L.), Silva et al. (2020) [30] found that the maximum production point occurred with the use of 8.96 $mL.plot^{-1}$. As it is formed by a community of microorganisms found naturally in soils, the authors indicated that the action of EM may suffer interference from the agroecosystem in which it is applied. Thus, there are still doubts about the interactive effects of the environment of use and its relationship with the microbiological composition of EM in various horticultural crops, because knowledge about this bioinput has been improved in recent years [27].

When we searched the literature for information on the species of microorganisms that made up our bioinput (Figure 1), we found that most of them have been identified as beneficial considering their application in agriculture.

Due to its high glycerol production and ability to degrade malic acid, Costantini et al. (2021) [31] showed that *Saccharomyces paradoxos* had important oenological properties to produce low-alcohol wines. On the other hand, the biocidal nature of the killer toxins of *Pichia membranifaciens*, the most abundant fungus in our bioinput (Figure 2), has indicated its use in the agri-food industry [32]. *P. membranifaciens* act as a biocontrol agent for *Penicillium expansum* on peach (*Prunus persica* Batsch) [33] and pear (*Pyrus communis* L.), in the latter case through the production of chitinase and glucanase [34], *Colletotrichum acutatum* on medlar [*Eriobotrya japonica* (Thunb.) Lindl.], by producing chitinase and β-1,3-glucanase [35], and *Botrytis cinerea* on grapes (*Vitis vinifera* L.) and apples (*Malus domestica* Borkh.), by producing the toxins PMKT, exo and endo-β-1,3-glucanases [36-38].

*Paenibacillus pabuli* belongs to the group of plant growth-promoting rhizobacteria (PGPR). In this context, [39] revealed increased expression of key genes regulating anthocyanin biosynthesis pathways in *Arabidopsis thaliana* seedlings treated with *P. pabuli*. They also observed higher expression of phytopathogen-related genes and microbe-associated molecular pattern genes, showing that *P. pabuli* stimulates an induced systemic response in *A. thaliana*.

Recently, *Niallia nealsonii*, another bacterium we detected in EM (Figure 1), was reported to degrade phenol [40], an organic pollutant harmful to humans and agroecosystems due to its mutagenic and/or carcinogenic properties [41]. Thus, *N. nealsonii* has potential as a bioremediation agent for *o*-cresol, *m*-cresol, *p*-cresol, 3,4-dimethyl-1H-pyrazole, ethylbenzene, benzene, toluene, and xylene [40].

*Paenibacillus larvae* is reported as the causative agent of the disease American foulbrood (AFB) in honeybees (*Apis mellifera* L.) [42]. In the lumen of the larval midgut, *P. larvae* spores germinate and, after massive proliferation, the bacterium breaks through the midgut epithelium and invades the hemocele, causing the death of the insect [43]. However, other species of bacteria have antagonistic activity towards *P. larvae*, including multiple species of *Enterococcus*, also present in our bioinput (Figure 1), possibly due to the bacteriocin genes found in their genomes [44]. Here, we emphasize that the multifaceted nature of EM provides interactions that presume equilibrium among microorganisms, so that one inhabitant of the EM can inhibit the action of another. This capacity for synergism and/or antagonism guides the principle of the functioning of the community that forms the EM and generates effects on the plant partner and the agroecosystem in which the bioinput is inserted.

*Bacillus cereus* group, the most abundant bacteria in our bioinput (Figure 2), also known as *B. cereus sensu lato*, is a term widely used to describe a genetically related subdivision of *Bacillus* [45], which comprises *B. cereus sensu stricto*, *B. anthracis*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, *B. weihenstephanensis*, *B. cytotoxicus*, and *B. toyonensis* as the most prominent members [46]. Some strains of *B. cereus* produce bacterial enterotoxins, which can cause vomiting and diarrhea in humans and animals [47]. However, non-pathogenic strains can promote plant growth and be applied as biocontrol agents and biofertilizers.

In addition to promoting plant development through better availability of nutrients and greater production of hormones [27], the application of EM can be a biostrategy for phytosanitary defense. 'Camino Real' is susceptible to leaf spots caused by phytopathogens, which affect plants throughout their growing cycle [48]. However, we found that this cultivar had a lower occurrence of leaf spots when the plants were subjected to EM (Figure 6). This indicates that the microorganisms that made up the EM have potential for use as biofungicides, which has already been reported for *B. cereus* [49], *P. pabuli* [39], and *P. membranifaciens* [35].

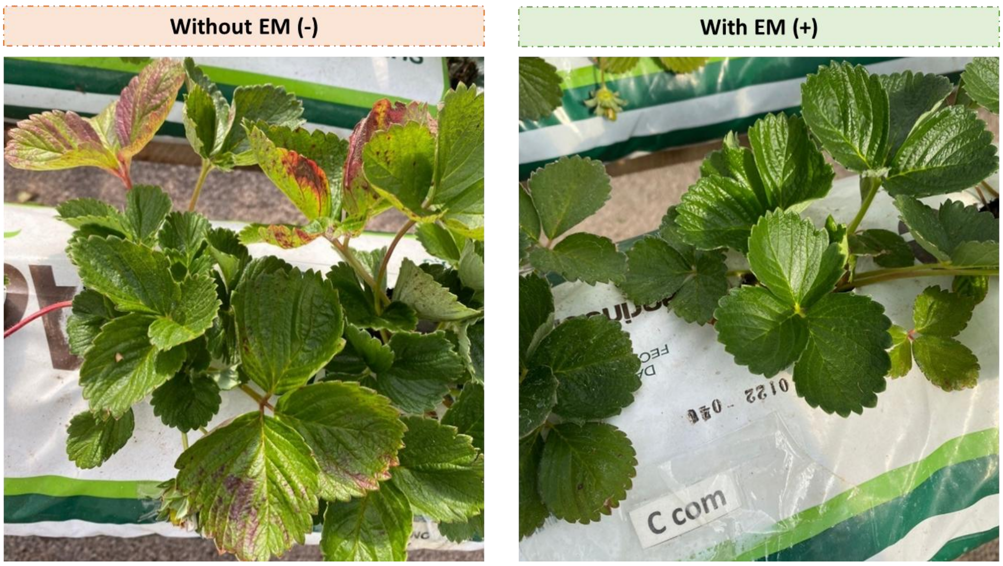


*Figure 6: 'Camino Real' in the absence and presence of efficient microorganisms (EM), evidencing the biofungicide effect of this bioinput.*

Plants and microorganisms work synergistically and their interaction results in the promotion of plant growth and development, which we report through the higher yield of strawberries in plants grown with EM (Table 2). The direct and indirect mechanisms that explain these benefits include: (1) nitrogen fixation; (2) phosphate solubilization; (3) phytohormone synthesis; (4) induction of systemic resistance; (5) production of indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophores, antibiotics, and lytic enzymes [50]. In our study, the joint effect of EM fungi and bacteria (Figure 1) on fruit production (Table 2) may have been caused by one or a combination of these mechanisms.

'Camino Real', classified as SD in terms of flowering, had an enhanced genotypic expression in the EM experiment. Even with the lowest yield and the production of fruit with the lowest AFFM, 'Camino Real' produced fruit with the best phytochemical quality, as well as showing an improved state of plant health. On the other hand, 'San Andreas' and 'Albion' had higher fruit yields but lower berry quality. The multivariate analysis demonstrated the importance of working with different genetic materials, as each one showed maximum expression in different yield components. All this genetic information in this production system has been valued and enhanced using the biotechnological tool EM. Therefore, the use of this bioinput is viable in horticulture in transition processes towards more sustainable systems that value different genetic resources.

## 5. CONCLUSION

Plants treated with efficient microorganisms throughout the cycle have the highest fruit yield and produce strawberries with the highest average fresh mass. Through multivariate analysis, we showed the formation of heterogeneous groups between cultivars and efficient microorganisms and confirmed that the application of efficient microorganisms to strawberry plants provides more robust and categorical results regarding the horticultural performance of cultivars. In addition, the use of efficient microorganisms improves strawberry leaf health. 'San Andreas' stands out in terms of its productive potential and should be an option for strawberry growers looking to improve fruit production. The use of efficient microorganisms does not influence the phytochemical and chemical (post-harvest) quality of strawberries. 'Camino Real' produces fruit with higher levels of total flavonoids. 'Albion' produces sweeter fruit, and 'Monterey', in addition to producing sweeter strawberries, produces tastier fruit.

## 6. REFERENCES

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