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# Agricultural sustainability: bacterial bio-inputs to improve the physiological and morphological development of *Urochloa decumbens* cv. Basilisk

## Sustentabilidade agrícola: bioinsumos de bactérias para melhoria do desenvolvimento fisiológico e morfológico de *Urochloa decumbens* cv. Basilisk

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## ABSTRACT

Microorganisms play a crucial role when closely associated with plants and can be considered a new sustainable tool for protecting and promoting pasture growth. The aim of this work was to prospect a microbial consortium (MIX) with the ability to promote the growth and development of *Urochloa decumbens* cv. Basilisk pastures, based on the hypothesis that MIX may have similar potential to chemical fertilization. To achieve the objectives, seven treatments were carried out, five of which were with MIXs (MIX 1, 2, 3, 4, and 5, previously taxonomically and biotechnologically characterized), one treatment with chemical fertilization, and one control treatment — without co-inoculation or chemical fertilization. It was possible to observe that, in general, the MIXs had the potential to increase the chlorophyll content, number of leaves and tillers, root length, and green and dry root mass compared to the control and chemical fertilizer treatments. The MIX 1 specifically, made up of bacteria from the genera *Klebsiella* sp., *Rhizobium* sp., and *Sinomonas* sp. showed a high potential for increase, surpassing the treatment with chemical fertilization, especially in the variables green and dry mass of the root with increases of 46 and 36%, respectively, number of leaves with 36%, and number of tillers with 37%. Microbial consortia could become an ecologically, socially, and economically viable alternative to maintaining pastures.

**Keywords:** bacterial consortia; microorganism-plant interaction; degraded pastures.

## RESUMO

Os microrganismos desempenham papel crucial quando estreitamente associados às plantas e podem ser considerados uma nova ferramenta sustentável para a proteção e promoção de crescimento de pastagens. O objetivo deste trabalho foi prospectar um consórcio microbiano (MIX) com habilidades para a promoção de crescimento e desenvolvimento de pastagens de *Urochloa decumbens* cv. Basilisk, com base na hipótese de que o MIX pode apresentar potencial semelhante à adubação química. Para atingir os objetivos, foram realizados sete tratamentos, cinco deles com MIX's (MIX 1, 2, 3, 4 e 5, previamente caracterizados taxonomicamente e biotecnologicamente), um tratamento com adubação química e um tratamento testemunha — sem co-inoculação e sem adubação química. Foi possível observar que, de forma geral, os MIX's apresentaram potencial de incremento frente às testemunhas e ao tratamento de adubação química para as variáveis teor de clorofila, número de folhas e perfilhos, comprimento da raiz, e massa verde e seca da raiz. Especificamente o MIX 1, composto por bactérias dos gêneros *Klebsiella* sp., *Rhizobium* sp. e *Sinomonas* sp., apresentou elevado potencial de incremento, superando o tratamento com adubação química, principalmente nas variáveis massa verde e seca da raiz com incrementos de 46 e 36%, respectivamente, número de folhas com 36% e número de perfilhos com 37%. Os consórcios microbianos podem se tornar uma alternativa viável, ecológico, social e economicamente, para a manutenção de pastagens.

**Palavras-chave:** consórcios bacterianos; interação microrganismo-planta; pastagens degradadas.

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## Introduction

forage grasses belonging to the genus *Urochloa*, also known as *Brachiaria*, are cultivated all over the world and play an important role in Brazilian livestock farming due to their low production costs and high practicality in supplying animals with pasture (Cheruiyot et al., 2018; Oliveira et al., 2024). According to Suhaimi et al. (2017), more than 80% of pastures planted in tropical regions are made up of *Urochloa decumbens* Stapf. The globalization of the economy has led agriculture to become increasingly efficient and competitive (Wanga et al., 2024). As a result, some of the techniques widely used in pastoral environments, such as incorrect management of fertilization, animal stocking rates, and poor choice of forage cultivar are leading these pasture areas to degradation, and more severely to desertification (Santos et al., 2022). Therefore, they cause various kinds of damage to the ecosystem, especially to arable areas across the globe (Corato et al., 2024).

Thus, a great challenge of pastoral production systems is the use of practices capable of increasing plant productivity (forage mass), and consequently, animal productivity (meat, milk, wool, and others), guaranteeing environmental sustainability (Berça et al., 2021). With this aim in mind, the use of plant growth-promoting bacteria (PGPBs) is gaining prominence (Lima et al., 2018; Oliveira et al., 2018; Sammauria et al., 2020; Oliveira et al., 2022).

PGPBs are microorganisms naturally present in the soil, in symbiosis and/or mutualistic association with the plant, and can be characterized as endophytic (occupying inter- and intracellular spaces) and epiphytic (when found on the external surface of the plant) (Martins et al., 2023). These microorganisms can help plants through direct mechanisms such as biological nitrogen (N) fixation, production of phytohormones such as indoleacetic acid of the auxin class (Khalifa and Alsowayeh, 2023), as well as producing siderophores and extracellular enzymes, thus increasing the systemic resistance of plants to pathogens and pests, an indirect mechanism of plant growth promotion (Oliveira et al., 2018; Cruz et al., 2023).

When PGPBs are multiplied and inoculated into seeds or plant tissues, they aim to colonize the inside and/or outside of the plant and thus contribute to plant development (Dias and Santos, 2022). It has been suggested for decades that these microorganisms, closely associated with plants, are key players in promoting sustainable plant growth and can be used to formulate bioproducts (Kuklinsky-Sobral et al., 2004). Research has therefore been carried out to select bacteria with biotechnological potential, capable of providing greater development to forage plants and stabilizing/increasing production in the field (Oliveira et al., 2022).

Despite all the efforts to bioprospect mono- or co-inoculants capable of promoting plant growth (Nardi et al., 2016), little has been elucidated, as a huge diversity of microorganisms with unknown functions is still described. This lack of knowledge, especially of microbial interactions in an agroecosystem, has led to scientific inconsistency about the production and use of bioinoculants (Trabelsi et al., 2020). In this context, researchers suggest that difficulties related to the successive use of inoculants are due to the effect of the plant/microorganism interaction, such as the stage of plant development, plant genotype, and geographical location of the host (Dos-Santos et al., 2021). Bioprospecting and inoculation of beneficial microorganisms are therefore of crucial importance (Figueredo et al., 2023).

PGPB inoculation in forage grasses has shown benefits, as observed by Heinrichs et al. (2020), who inoculated *Brachiaria brizantha* cv. Marandu seeds with *Azospirillum* sp. strains, identifying an increase in the dry mass of the aerial part in different evaluation periods (with an experimental period of two years). In studies carried out by Pandey et al. (2019), it was evident that inoculants with PGPBs could also be used to help forage plants control physiological stresses, promoting resistance to salinity and low soil fertility.

Despite the great advance of knowledge in the area of bioprospecting and application of beneficial microorganisms to plants, these studies still comprise a tool with a strong impact on sustainable agriculture, reducing environmental damage (Oliveira et al., 2018). Towards enhancing sustainable pasture production with low environmental impact, this work was based on the hypothesis that co-inoculation of bacteria with the potential to promote plant growth and development is a crucial factor in ensuring good physiological and morphological development of *U. decumbens*. Therefore, the main objective of this work was to evaluate the efficiency of co-inoculums formulated with PGPBs on the physiological and morphological development of *U. decumbens* cv. Basilisk.

## Materials and Methods

### Seeds and bacterial strains

The experiment was conducted in a greenhouse at the Federal University of Agreste de Pernambuco, Brazil (8°54’23.7”S 36°29’39.7”C). Commercial seeds of *U. decumbens* cv. Basilisk and 24 PGPB strains previously evaluated by Oliveira et al. (2018), summarized in Supplementary Material 1, were used. The bacterial strains belong to Júlia Kuklinsky-Sobral’s bacterial culture collection at the Forage and Biotechnology Center of the Federal University of Agreste de Pernambuco. Twelve strains were isolated from each plant species *U. decumbens* Stapf., and *U. humidicola* (Rendle) Schweickerdt. Of these 12 strains per plant species, six were isolated from each niche, root (endophytic), and rhizosphere (Table 1).

### Preparation of inoculums and formulation of MIXs

To obtain the inoculum, pure bacterial colonies were incubated in a liquid culture medium, 10% trypcase soy agar, plus 0.05% tryptophan.

**Table 1 – Characterization of origin and biotechnological potential of diazotrophic bacterial isolates from Brachiaria decumbens Stapf. and Brachiaria humidicola (Rendle) Schweick.**

| Isolate code | Species identified* | QS | CE | Enzymes (EI) | | AM | IPS | IAA ($\mu$g mL$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | P-5 | P-8 | | | CLT | SLT |
| *Brachiaria decumbens* Stapf. isolates in the Root Endophytic niche | | | | | | | | | |
| **UAGB69** | *Enterobacter kobei* CPI 105566 | - | 0.000 | 0.000 | 4.736 | 0.000 | 0.000 | 162.100 | 0.000 |
| **UAGB154** | *Klebsiella variicola* F2R9 | - | 0.000 | 0.000 | 1.092 | 0.000 | 2.056 | 100.080 | 14.644 |
| **UAGB156** | *Klebsiella variicola* AT-22 | + | 0.000 | 0.000 | 1.177 | 0.000 | 1.374 | 67.177 | 20.800 |
| **UAGB167** | *Rhizobium hainanense* I66 | + | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 138.641 | 15.940 |
| *Brachiaria decumbens* Stapf. isolates in the Root Endophytic niche | | | | | | | | | |
| **UAGB71** | *Sinomonas atrocyanea* DSM 20127 | - | 0.000 | 4.003 | 3.764 | 2.765 | 0.000 | 113.670 | 0.000 |
| **UAGB80** | *Sphingomonas paucimobilis* DSM 30198 | - | 0.000 | 0.000 | 1.869 | 1.622 | 2.037 | 5.844 | 0.000 |
| **UAGB139** | *Burkholderia cenocepacia* AU 1054 | + | 0.000 | 0.000 | 2.988 | 0.000 | 5.484 | 4.722 | 2.533 |
| **UAGB150** | *Rhizobium cauense* CCBAU 101002 | - | 1.038 | 6.275 | 4.394 | 2.477 | 3.155 | 5.033 | 3.999 |
| Isolates of *Brachiaria humidicola* (Rendle) Schweick. in the Root Endophytic niche | | | | | | | | | |
| **UAGB01** | *Pantoea* sp. | + | 0.000 | 0.000 | 4.256 | 1.807 | 3.827 | 35.521 | 5.469 |
| **UAGB105** | *Burkholderia territorii* LMG 28158 | + | 1.685 | 0.000 | 0.000 | 0.000 | 3.050 | 51.785 | 23.877 |
| **UAGB106** | *Burkholderia lata* 383 | + | 1.653 | 0.000 | 0.000 | 0.000 | 3.493 | 49.231 | 26.333 |
| **UAGB110** | *Enterobacter* sp. | + | 6.715 | 0.000 | 0.000 | 0.000 | 3.733 | 2.090 | 0.855 |
| Isolates of *Brachiaria humidicola* (Rendle) Schweick. in the Rhizosphere niche | | | | | | | | | |
| **UAGB10** | *Bacillus anthracis* str. Ames | + | 1.200 | 0.000 | 4.864 | 1.090 | 0.000 | 13.482 | 9.211 |
| **UAGB60** | *Klebsiella* sp. | + | 0.000 | 0.000 | 2.299 | 2.204 | 0.000 | 4.982 | 0.000 |
| **UAGB119** | *Enterobacter kobei* CIP 105566 | + | 1.238 | 0.000 | 0.000 | 0.000 | 2.793 | 3.733 | 15.600 |
| **UAGB128** | *Ralstonia pickettii* 12J | - | 0.000 | 0.000 | 0.000 | 0.000 | 3.050 | 10.980 | 8.877 |

Source: Oliveira et al. (2018).

+: positive for the evaluated characteristic; -: negative for the evaluated characteristic; QS: production of the quorum sensing molecule; EI: enzymatic Index (relation between the diameter of the hydrolysis halo and the diameter of the bacterial colony); CE: cellulase production; P-5: pectinase production at pH 5.0; P-8: Pectinase production at pH 8.0; AM: amylase production; IPS: inorganic phosphate solubilization index; IAA: indole acetic acid; CLT IAA: production in culture medium with supply of the precursor amino acid L-Tryptophan; SLT IAA: production without L-Tryptophan.

The bacterial cultures were then diluted in phosphate buffered saline and the optical density was adjusted to 0.095 in a Metash V-5000 Invisible Spectrophotometer at 630 nm, corresponding to $10^6$ colony forming units per mL$^{-1}$. The seeds were superficially disinfected with a 1% sodium hypochlorite (NaOCl) solution for 5 min, washed in distilled water, and immersed in the inoculum for 30 min under gentle agitation (Lima et al., 2018).

## Pre-implantation experimental design

The experiment was set up in a randomized block design with seven treatments: five bacterial co-inoculums (called MIXs) (Table 2), one with chemical fertilization, and a control — without co-inoculums or chemical fertilization. The microcosm was set up in pots containing 7.5 L of soil, with each treatment containing 30 pots. Each MIX was formulated by a consortium of bacterial strains, considering the plant species, except for MIX 1, which was formulated with five bacterial strains that stood out in the work by Oliveira et al. (2022).

**Table 2 – Formulation of potential plant growth-promoting bacteria co-inoculants (MIXs) used on Urochloa decumbens cv. Basilisk seeds.**

| Co-inoculants | Code of bacterial isolates | Origin of bacterial strains |
|---|---|---|
| **MIX 1** | UAGB 60 - UAGB 156 - UAGB 154<br>UAGB 167- UAGB 71 | - - |
| **MIX 2** | UAGB 10 - UAGB 39 - UAGB 60<br>UAGB 94 - UAGB 106 - UAGB 132 | *Urochloa humidicola* (Rendle) Schweickerdt |
| **MIX 3** | UAGB 68 -UAGB 71 - UAGB 80<br>UAGB 96 - UAGB 147 - UAGB 167 | *Urochloa decumbens* Stapf. |
| **MIX 4** | UAGB 01 - UAGB 93 - UAGB 105<br>UAGB 110 - UAGB 119 - UAGB 128 | *Urochloa humidicola* (Rendle) Schweickerdt |
| **MIX 5** | UAGB 69 - UAGB 70 - UAGB 139<br>UAGB 150 - UAGB 154 - UAGB 156 | *Urochloa decumbens* Stapf. |

MIX 1, which was formulated with the five bacterial strains that best increased the germination and initial development of *Brachiaria decumbens* cv. Basilisk seedlings (UAGB 60, UAGB 156, UAGB 154, UAGB 167, UAGB 71), according to Oliveira et al. (2022).

## Soil characterization and preparation

The soil used was classified as Yellow Latosol (Embrapa, 2006), and the chemical analysis indicated: pH 5.22; 5.22 mg dm$^3$ of phosphorus (P); 0.24 cmolc dm$^3$ of K; 4.70 cmolc dm$^3$ of calcium (Ca); 2.80 cmolc dm$^3$ of magnesium (Mg); 0.30 cmolc dm$^3$ of aluminium$^3$+ (Al$^3$+); 0.65 cmolc dm$^3$ of potential acidity (H+Al). Initially, the soil in the whole experiment was corrected using the base saturation method. For the treatment with chemical fertilization, nitrogen-phosphorus-potassium (NPK) was used in the 30-60-30 formulation, triple superphosphate, and potassium chloride for the foundation fertilization, and ammonium sulphate was applied in three installments, with the first application after the uniformization cut and on the 70th and 105th days after sowing. All fertilizations were based on the recommendations for the state of Pernambuco (Cavalcanti, 2008). To control the humidity of the pots, their capacity was determined and the water was replaced by weighing the pots daily and replacing the evaporated water.

## Inoculation procedure

Initially, 15 seeds co-inoculated with the MIXs (described in Table 2) were added to each pot. On the 15th day after sowing, thinning took place, leaving two seedlings per pot. On the 35th day after sowing, the uniformization cut took place. All plants in all treatments were cut 10 cm above the ground, and the experimental period began. Three evaluations were carried out every 35 days (on the 70th, 105th, and 140th days after sowing). At each evaluation, ten pots were randomly assessed and discarded (10 repetitions per evaluation), and the remaining plants were pruned to 10 cm above the ground. After each pruning, there was a new incorporation of chemical fertilizer and a new re-inoculation for the treatments co-inoculated with bacteria.

## Parameters evaluated

During each evaluation cycle, every 35 days, assessments were made of the effect of plant growth promotion on the variables chlorophyll content (CLO), using the soil plant analysis development (SPAD)-502 portable chlorophyll meter, based on the middle portion of the first fully expanded leaf of the forage canopy. The leaf length (LL) and leaf width (LW) of three fully expanded leaves of the forage canopy were measured, and the leaf area (LA) was determined according to Bianco et al. (2000). It should be noted that a fully-expanded leaf was considered to be the leaf with the ligule exposed.

Plant height (PH) was also assessed, taking into account the distance between the ground and the tip of the tallest leaf. The number of leaves (NF) and number of tillers (NT) in each pot were then divided by two to represent just one plant. To assess root length (RL), the soil in each bucket was washed in running water, and the roots were measured from the meristem of the largest root to the neck of the plant.

To obtain the aerial part green mass (APGM) and root part green mass (RPGM), the parts were separated and weighed. The plant material was then taken to a Tecnal TE-393/2 forced-air circulation oven at 55°C for 72 hours and weighed to obtain the dry mass of the aerial part (APDM) and root (RDM). Subsequently, these values were divided by two to represent just one plant.

In the statistical analysis, the differences between the groups were compared by orthogonal contrast using the t-test at 5% probability, and considering the evaluation cycles as repetitions. Next, the means of all the treatments per variable on the 70th, 105th, and 140th days after germination were compared with the control, using the Dunnett's test at 5%. The treatments that stood out were compared using the Scott-Knott test at 5%, using the statistical software Sisvar®, version 5.6. The treatment groups were evaluated by principal component analysis using Past® statistical software, version 4.0.

## Results

The practice of co-inoculating bacteria with the potential to promote plant growth is effective in positively influencing the physiological and morphological development of *U. decumbens* cv. Basilisk plants, with significant potential ($p<0.050$%). In addition, it is worth noting that the co-inoculations provided significantly higher values ($p<0.050$) than the control treatment (without co-inoculum and chemical fertilization), for the variables CLO, NL, NT, RL, RGM, and RDM (Table 3). This shows the differentiation between the MIXs and the control, and the closeness of the MIXs to the treatment with chemical fertilizer, when assessed together (Figure 1). Specifically looking for the potential increase of PGPB co-inoculation compared to treatment with chemical fertilizer, there were discrete numerical benefits in the variables LL, LW, LA, PH, and RDM. However, it should be noted that these discrete numerical benefits are valuable biological benefits for the plants. Compared to the control treatment, the MIX's promoted an increase of 10 to 36% for the variables CLO, NL, NT, RL, RGM, and RDM (Table 4), considering these same characteristics, which showed statistical significance in the orthogonal contrast test ($p<0.05$) (Table 3).

Among the co-inoculums, MIX 1 composed of the strains UAGB 60 — *Klebsiella* spp.; UAGB156 — *Klebsiella* spp.; UAGB 154 — *Klebsiella spp.*; UAGB 167 — *Rhizobium spp.*, and UAGB 71 — *Sinomonas spp.* (Tables 1 and 2) stood out, with the highest number of variables superior to the control treatment (Table 5). Besides, MIX 1 provided the greatest increases (Table 6) and sometimes resembled and surpassed the treatment with chemical fertilization (Table 7). Among the other MIXs, MIX 3 and MIX 4 had seven variables with higher values than the control treatment, followed by MIX 5 with six variables, and MIX 2 with five variables (Table 5). It should be noted that among the variables with the highest percentage increases for all the MIXs, APGM, APDM, RL, RGM, and RDM stand out (Table 6). The plant species from which the bacterial isolates originated had little influence. The MIXs with the strains isolated from both plants (Table 2) were able to promote the development of the physiological and morphological characteristics of *U. decumbens* cv. Basilisk (Tables 6 and 7).

**Table 3 – Comparison between groups of means by orthogonal contrasts for the physiological and morphological characteristics (plant$^{-1}$) of brachiaria grass (Urochloa decumbens cv. Basilisk) co-inoculated with potentially growth-promoting bacteria, in relation to the chemical fertilization and control treatments. The brachiaria grass plants were grown in 7.5 L pots and the averages of three evaluation cycles were presented, with an interval of 35 days between evaluations.**

| Average | CLO | LL | LW | LA | PH | NL | NT | APGM | APDM | RL | RGM | RDM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - - | - - - - - - - cm - - - - - - - - | | | | - - | - - | - - - g - - - | | cm | - - - g - - - | |
| **MIXs** | 32.234 | 24.242 | 1.467 | 35.268 | 48.963 | 111.518 | 29.993 | 51.152 | 17.382 | 92.684 | 100.513 | 27.767 |
| **CF** | 34.650 | 23.227 | 1.450 | 33.663 | 48.073 | 133.467 | 37.133 | 59.410 | 20.181 | 93.248 | 104.664 | 26.947 |
| **TEST** | 29.332 | 21.790 | 1.377 | 29.578 | 44.392 | 90.450 | 24.678 | 42.225 | 15.013 | 73.511 | 74.065 | 21.690 |
| **General** | 32.072 | 23.086 | 1.431 | 32.836 | 47.143 | 111.812 | 30.601 | 50.929 | 17.525 | 86.481 | 93.081 | 25.468 |
| **MIX *vs.* CF** | | | | | | | | | | | | |
| **T-test** | -3.976* | 0.517 | 0.337 | 0.429 | -0.029 | -2.928* | -2.910* | -1.909 | -2.766 | -0.284* | -0.736* | 0.310* |
| **MIX *vs.* TEST** | | | | | | | | | | | | |
| **T-test** | 4.525* | 1.707 | 2.422 | 2.303 | 0.920 | 3.618* | 2.975* | 1.815 | 2.196 | 7.492* | 6.040* | 3.119* |

MIXs: averages of all the bacterial co-inoculums used in the work; CF: chemical fertilization treatment; TEST: control treatment (without bacterial co-inoculums and chemical fertilization); CLO: chlorophyll; LL: leaf length; LW: leaf width; LA: leaf area; PH: plant height; NL: number of leaves; NT: Number of tillers; APGM: aerial part green mass; APDM: aerial part dry mass; RL: root length; RGM: root green matter; RDM: root dry matter. *Significant at 5% probability using the t-test.

**Table 4 – Performance of co-inoculums of potentially plant growth-promoting bacteria (MIXs) related to the chemical fertilization and control treatments, for the physiological and morphological characteristics (plant$^{-1}$) of brachiaria grass (Urochloa decumbens cv. Basilisk), grown in 7.5 L pots, with presentation of the averages of three evaluation cycles, with an interval of 35 days between evaluations.**

| Average | CLO | LL | LW | LA | PH | NL | NT | APGM | APDM | RL | RGM | RDM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - - | - - - - - - - cm - - - - - - - - | | | | - - | - - | - - - g - - - | | cm | - - - g - - - | |
| MIX's | 32.234 | 24.242 | 1.467 | 35.268 | 48.963 | 111.518 | 29.993 | 51.152 | 17.382 | 92.684 | 100.513 | 27.767 |
| **CF** | 34.650 | 23.227 | 1.450 | 33.663 | 48.073 | 133.467 | 37.133 | 59.410 | 20.181 | 93.248 | 104.664 | 26.947 |
| **TEST** | 29.332 | 21.790 | 1.377 | 29.578 | 44.392 | 90.450 | 24.678 | 42.225 | 15.013 | 73.511 | 74.065 | 21.690 |
| **General** | 32.072 | 23.086 | 1.431 | 32.836 | 47.143 | 111.812 | 30.601 | 50.929 | 17.525 | 86.481 | 93.081 | 25.468 |
| **Percentage gain of MIX over CF** | | | | | | | | | | | | |
| | 0% | 4% | 1% | 5% | 2% | 0% | 0% | 0% | 0% | 0% | 0% | 3% |
| **Percentage gain of MIX over TEST** | | | | | | | | | | | | |
| | 10% | 11% | 7% | 19% | 10% | 23% | 22% | 21% | 16% | 26% | 36% | 28% |

MIXs: averages of all the bacterial co-inoculums used in the work; CF: chemical fertilization treatment; TEST: control treatment (without bacterial co-inoculums and chemical fertilization); CLO: chlorophyll; LL: leaf length; LW: leaf width; LA: leaf area; PH: plant Height; NF: number of leaves; NP: number of tillers; APGM: aerial part green mass; APDM: aerial part dry mass; RL: root length; RGM: root green matter; RDM: root dry matter.

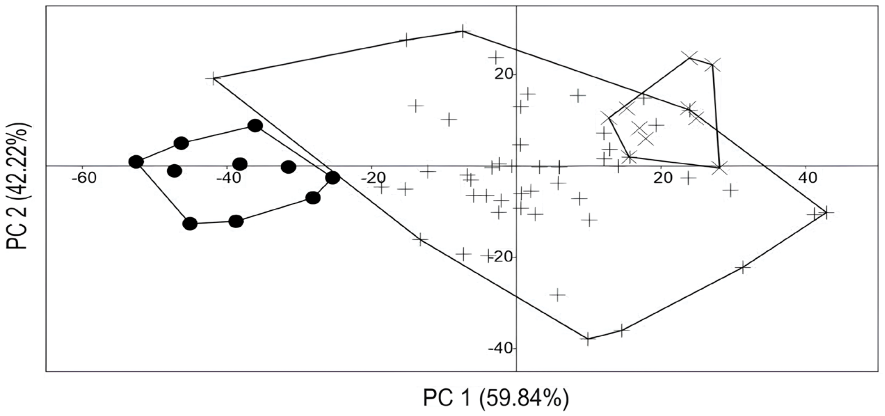

**Figure 1 – Principal component analysis of the physiological and morphological characteristics of brachiaria grass plants (Urochloa decumbens cv. Basilisk) co-inoculated with potentially plant growth-promoting bacteria (+), compared to the chemical fertilization (×) and control treatments (●). The plants were grown in 7.5 L pots (two plants per pot) and the averages of three evaluation cycles were presented, with an interval of 35 days between evaluations.**

**Table 5 – Comparison by Dunnett's test between co-inoculums of potentially plant growth-promoting bacteria (MIXs) and control treatment, for the physiological and morphological characteristics of (plant$^{-1}$) brachiaria grass (Urochloa decumbens cv. Basilisk), grown in 7.5 L pots, showing the averages of three evaluation cycles, with an interval of 35 days between evaluations.**

| Average | CLO | LL | LW | LA | PH | NL | NT | APGM | APDM | RL | RGM | RDM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - - | - - - - - - - cm - - - - - - - - | | | | - - | - - | - - - g - - - | | cm | - - - g - - - | |
| **MIX 1** | 32.954* | 24.020* | 1.435 | 34.096* | 52.228* | 122.633* | 33.844* | 54.328* | 17.658* | 96.019* | 108.140* | 29.560* |
| **MIX 2** | 31.414 | 24.090* | 1.463 | 35.173* | 47.326 | 112.248* | 30.496 | 47.874 | 17.444 | 93.714* | 91.858* | 26.031 |
| **MIX 3** | 32.040* | 23.603 | 1.432 | 33.615 | 47.679 | 122.167* | 32.567* | 52.595* | 16.988 | 93.663* | 103.392* | 28.500* |
| **MIX 4** | 32.374* | 24.281* | 1.512* | 36.273* | 47.084 | 101.842 | 27.708 | 47.858 | 17.636 | 88.870* | 107.723* | 28.057* |
| **MIX 5** | 32.390* | 25.217* | 1.494* | 37.181* | 50.497* | 98.700 | 25.350 | 53.107* | 17.183 | 91.152* | 91.454* | 26.686 |
| **CF** | 34.650* | 23.227 | 1.450 | 33.663 | 48.073 | 133.466* | 37.133* | 59.410 | 20.181* | 93.248* | 104.664* | 26.947 |
| **TEST** | 29.332 | 21.790 | 1.377 | 29.578 | 44.392 | 90.450 | 24.678 | 42.225 | 15.013 | 73.511 | 74.065 | 21.690 |

MIXs: averages of all the bacterial co-inoculums used in the work; CF: chemical fertilization treatment; TEST: control treatment (without bacterial co-inoculums and chemical fertilization); CLO: chlorophyll; LL: leaf length; LW: leaf width; LA: leaf area; PH: plant Height; NL: number of leaves; NT: number of tillers; APGM: aerial part green mass; APDM: aerial part dry mass; RL: root length; RGM: root green matter; RDM: root dry matter.

**Table 6 – Performance of co-inoculums of potentially plant growth-promoting bacteria (MIXs) compared to the control treatment, for the physiological and morphological variables (plant$^{-1}$) of brachiaria grass (Urochloa decumbens cv. Basilisk), grown in 7.5 L pots, showing the averages of three evaluation cycles, with an interval of 35 days between evaluations.**

| Average | CLO | LL | LW | LA | PH | NL | NT | APGM | APDM | RL | RGM | RDM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - - | - - - - - - - cm - - - - - - - - | | | | - - | - - | - - - g - - - | | cm | - - - g - - - | |
| **MIX 1** | 32.954 | 24.020 | 1.435 | 34.096 | 52.228 | 122.633 | 33.844 | 54.328 | 17.658 | 96.019 | 108.140 | 29.560 |
| **MIX 2** | 31.414 | 24.090 | 1.463 | 35.173 | 47.326 | 112.248 | 30.496 | 47.874 | 17.444 | 93.714 | 91.858 | 26.031 |
| **MIX 3** | 32.040 | 23.603 | 1.432 | 33.615 | 47.679 | 122.167 | 32.567 | 52.595 | 16.988 | 93.663 | 103.392 | 28.500 |
| **MIX 4** | 32.374 | 24.281 | 1.512 | 36.273 | 47.084 | 101.842 | 27.708 | 47.858 | 17.636 | 88.870 | 107.723 | 28.057 |
| **MIX 5** | 32.390 | 25.217 | 1.494 | 37.181 | 50.497 | 98.700 | 25.350 | 53.107 | 17.183 | 91.152 | 91.454 | 26.686 |
| **TEST** | 29.332 | 21.790 | 1.377 | 29.578 | 44.392 | 90.450 | 24.678 | 42.225 | 15.013 | 73.511 | 74.065 | 21.690 |
| **Percentage gain of MIX 1 over TEST** | | | | | | | | | | | | |
| | 12% | 10% | 4% | 15% | 18% | 36% | 37% | 29% | 18% | 31% | 46% | 36% |
| **Percentage gain of MIX 2 over TEST** | | | | | | | | | | | | |
| | 7% | 11% | 6% | 19% | 7% | 24% | 24% | 13% | 16% | 27% | 24% | 20% |
| **Percentage gain of MIX 3 over TEST** | | | | | | | | | | | | |
| | 9% | 8% | 4% | 14% | 7% | 35% | 32% | 25% | 13% | 27% | 40% | 31% |
| **Percentage gain of MIX 4 over TEST** | | | | | | | | | | | | |
| | 10% | 11% | 10% | 23% | 6% | 13% | 12% | 13% | 17% | 21% | 45% | 29% |
| **Percentage gain of MIX 5 over TEST** | | | | | | | | | | | | |
| | 10% | 16% | 8% | 26% | 14% | 9% | 3% | 26% | 14% | 24% | 23% | 23% |

MIX: bacterial co-inoculums; TEST: control treatment; CLO: chlorophyll; LL: leaf length; LW: leaf width; LA: leaf area; PH: plant height; NL: number of leaves; NT: number of tillers; APGM: aerial part green mass; APDM: aerial part dry mass; RL: root length; RGM: root green matter; RDM: root dry matter.

**Table 7 – Comparison of means of the treatments with co-inocula of potentially plant growth-promoting bacteria (MIXs) and the treatment with chemical fertilization for the physiological and morphological variables (plant$^{-1}$) of brachiaria grass (Urochloa decumbens cv. Basilisk), grown in 7.5 L pots, showing three evaluation cycles, with an interval of 35 days between evaluations.**

| Average | CLO | LL | LW | LA | PH | NL | NT | APGM | APDM | RL | RGM | RDM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - - | - - - - - - - cm - - - - - - - - | | | | - - | - - | - - - g - - - | | cm | - - - g - - - | |
| **MIX 1** | 32.954b | 24.020a | - | 34.096a | 52.228a | 122.633b | 33.844b | 54.328a | 17.658b | 96.016a | 108.140a | 29.559a |
| **MIX 2** | - | 24.090a | - | 35.173a | - | 112.248c | - | - | - | 93.714b | 91.857c | - |
| **MIX 3** | 32.040b | - | - | - | - | 122.167b | 32.567b | 52.595b | - | 93.663b | 103.392b | 28.500a |
| **MIX 4** | 32.374b | 24.281a | 1.512a | 36.273a | - | - | - | - | - | 88.870c | 107.722a | 28.057a |
| **MIX 5** | 32.390b | 25.217a | 1.494a | 37.181a | 50.497b | - | - | 53.107b | - | 91.152c | 91.595c | - |
| **CF** | 34.650a | - | - | - | - | 133.466a | 37.133a | - | 20.180a | 93.248b | 104.663b | - |

MIX: bacterial co-inoculums used; CF: chemical fertilization treatment; CLO: chlorophyll; LL: leaf length; LW: leaf width; LA: leaf area; PH: plant height; NL: number of leaves; NT: number of tillers; APGM: aerial part green mass; APDM: aerial part dry mass; RL: root length; RGM: root green matter; RDM: root dry matter. Different letters in the same column differ statistically by the Skott-Knott test at 5% probability.

## Discussion

Currently, there are approximately 200 million hectares of pastures in Brazil, both natural and planted (Hungria et al., 2021). Of this total, a high percentage shows some signs of degradation, with estimates of 89% of the total planted area (Roque et al., 2022). Degraded pastures are a major liability in Brazilian agriculture, as the lack of restoration and recovery, and intensive occupation of pastoral areas are the main factors for desertification (Berça et al., 2021), and consequently the increase in environmental impacts related to deforestation and occupation of new forest areas for cattle farming (Mendes et al., 2011). Despite this obstacle, the country stands out as the largest producer and exporter of beef, with approximately 70% of production taking place extensively on pasture, thanks to its large land area (Delevatti et al., 2019).

With the globalization of the economy and the growth of the world's population, it is still necessary to increase animal production (Berça et al., 2021), requiring an exponential rise in the quantity and quality of pastures, mitigating the impacts on the environment related to the intensive use of chemical fertilizers for the restoration or recovery of such areas, or the exploration of new areas (Feltran-Barbieri et al., 2021). In this context, recent studies are bioprospecting methodologies or alternatives to understand how to manipulate and maintain a balance between intensive plant and animal production, and environmental sustainability (Guimarães et al., 2023).

Schematically, and based on this information, this work has shown that the use of microbial consortia (co-inoculation or MIX) can be considered as an alternative biorational way of improving pastures. The management of co-inoculation of bacterial strains in *U. decumbens* cv. Basilisk, proposed in this work, has generally been shown to promote an increase in the physiological and morphological characteristics of plants, thus possibly increasing the productive capacity of pastures.

By mimicking the control as pastures with a low degree of productivity and reduced vigor (Hungria et al., 2016; Bono et al., 2019), we can suggest that the MIX tested treatments were crucial in ensuring the promotion of some plant characteristics, providing compounds or secondary metabolites to induce an increase of 10, 23, and over 26% for the variables CLO and NL, in the root growth parameters RL, RDM, and APGM (Table 4). These results suggest that the inoculated microbiota could interact or colonize the plant's tissues and in this mutualistic interaction, the microorganisms promoted the plant's development through direct and indirect mechanisms (Kuklinsky-Sobral et al., 2004). Meanwhile, the plant provides carbonaceous compounds to sustain the synthetic bacterial community (Azevedo et al., 2000), and thus, both develop.

In addition, if we consider the growth promotion results of the plants inoculated with MIXs and the results of the treatment with chemical fertilization, we can also suggest that inoculation can be an ecologically correct alternative, and is directly related to the reduction of environmental impacts caused by the intensive use of chemical inputs (Batista et al., 2018). This is because there was equal potential for promoting plant growth (Tables 3 and 7).

Globally, the use of microbial inoculants has increased significantly in recent years. However, their application is still modest in pastures, which are generally degraded and require special use of microbial inoculants for recovery. Efforts to increase the use of PGPBs in pastures could have a profound positive impact on economic, social, and environmental ecosystems around the world (Bashan et al., 2013; Oliveira et al., 2022).

Previous comprehensive studies have demonstrated the potential of growth-promoting microorganisms when inoculated into forage plants (Oliveira et al., 2018; Oliveira et al., 2022; Guimarães et al., 2023). For example, Itzigsohn et al. (2000) showed that the inoculation of *Azospirillum brasiliense* and *A. brasiliense*+phosphate fertilizer had the same potential for pasture development at 35 and 105 days after inoculation. Furthermore, these treatments were twice as good as those with phosphate fertilization and the control treatment without inoculation, elucidating that inoculation of plant growth-promoting microorganisms combined with controlled and reduced doses of fertilizers can provide a solution with less impact on the environment.

In this same context, Da Costa et al. (2022), studying the growth promotion of *U. brizantha* cv. Marandu, demonstrated in greenhouse experiments, the synergistic effect of co-inoculation of *Bacillus subtilis* with *Trichoderma asperellum* in improving growth, biomass, and absorption of N, P, and K in Marandu grass, resulting in better efficient nutrient utilization. Furthermore, it is suggested that the synergistic interactions that favored plant growth occurred from the formation of a biofilm between *Bacillus* ssp. and *Trichoderma* spp. due to the intracellular increase, growth, or colonization of the bacteria in the fungal hyphae. Thus, it corroborates the hypothesis of co-inoculation of plant growth-promoting microorganisms. This suggestion and previous results corroborate the results shown in Tables 3 and 4.

As contemplated in this study with *U. decumbens* cv. Basilisk and in other studies using grasses such as *U. brizantha* cv. Marandu, BRS Paiaguás and Xaraés (Hungria et al., 2021), and *Megathyrsus maximus* cv. Tamani, Mombaça, Tanzânia-1, and Quênia (Guimarães et al., 2023), it was observed that growth-promoting bacteria, when inoculated, promoted the development of the cultivars evaluated with or without the presence of biostimulants. According to Santana et al. (2020), microbial inoculants are a suitable economic technology to help plants withstand environmental adversities, by means of chemical compounds, reducing the factors that affect their development (Dias and Santos, 2022).

In addition, this work tested five bacterial consortia, containing five bacterial strains for MIX 1, and six strains each for the other MIXs 2, 3, 4, and 5. The results showed that MIX 1, made up of strains belonging to the genera UAGB 60 — *Klebsiella* sp.; UAGB156 — *Klebsiella* sp.; UAGB 154 — *Klebsiella* sp.; UAGB 167 — *Rhizobium* sp., and UAGB 71 — *Sinomonas* sp., from the roots and rhizosphere of *U. decumbens* and *U. humidicola* (Oliveira et al., 2022), showed greater efficiency in promoting an increase in the physiological and morphological characteristics of *U. decumbens* cv. Basilisk plants, compared to the other treatments with bacterial co-inoculations.

Similarly, Liu and Wirén (2022), aiming to understand the dynamics of the maize microbiome, built a synthetic community containing seven bacterial strains (*Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Ochrobactrum pituitosum*, *Herbaspirillum frisingense*, *Pseudomonas putida*, *Curtobacterium pusillum*, and *Chryseobacterium indologenes*), which was inoculated into axenic maize plants. The authors observed that the presence of these seven strains enabled the control of the *Fusarium verticiliodes* fungus. In addition, when these same authors removed the *E. cloacae* bacterium from the system, they observed symptoms of the fungus (Baldwin et al., 2014). This information corroborates the results found in this study, suggesting the search for an ideal gnotobiotic community, in which microorganisms interact with each other to promote plant development and protection (Agler et al., 2016).

Nevertheless, it is suggested that in these constructed communities, the microorganisms must have characteristics of communication between them (van der Heijden and Hartmann, 2016). Specifically, it has been demonstrated for the strains UAGB 60 — *Klebsiella*; UAGB156 — *Klebsiella*; and UAGB 167 — *Rhizobium*, which can produce the *quorum sensing* molecule (Table 1), that is described as a bacterial communication molecule (Nievas et al., 2012). In this context, these microorganisms with the ability to produce the molecule *N-acylhomoserine lactone*, can modulate the abundance of other microorganisms in the plant microbiome for the benefit of the host (Poudel et al., 2016; Hartman et al., 2017).

A good bio-inoculant is able to promote the modulation of the autochthonous microbial community of the environment, whether plant or soil, and not just have a transient effect on the microbial community, as this will be quickly suppressed by the resilience of the ecosystem (Trabelsi and Mhamdi, 2013). Therefore, these factors make studies of the molecular mechanisms that determine the interaction between microbial communities and the insertion of these bio-inoculants an important target to be achieved in order to manipulate the plant microbiome in favor of agricultural production (Ramakrishna et al., 2019; Rilling et al. 2019).

Even so, recent ecological theories suggest that plant-associated communities are organized into microbial conglomerates "Microbial Hubs", and in the middle of these, there is one or a few central or key microorganisms, "Keystone Species". In these plant-microorganism interaction networks, the plant genotype plays a fundamental role in the selection of these microorganisms. These in turn, through communication mechanisms, have the potential to recruit other "helper" strains to better assist in promoting plant growth (Agler et al., 2016).

Over time, studies have suggested that knowledge of the molecular processes involving microorganisms that occur in nature to ensure the resistance and resilience of forests can be manipulated and transmitted to agriculture to be absorbed as a sustainable form of plant growth promotion. In fact, these species, present in the rhizosphere or in the endophytic environment, interact with each other and with the plant to protect it and ensure its survival (Hardoin et al., 2015).

In addition, it was possible to observe that the values of root development parameters (RL, RGM, and RDM) were statistically higher than the treatment with CF. These results may corroborate the potential for producing indoleacetic acid, which is related to plant growth and apical development (Figueredo et al., 2023), as observed by the strains evaluated in this study (Table 1). Despite the lack of detailed information in this study on the relationship between the production of these phytohormones and pasture root growth, this potential can only be speculated.

Similar recent works support our results, suggesting that the root growth of *B. decumbens* cv. Basilisk seedlings was positively affected by the amount of indoleacetic acid produced by a bacterial strain (Oliveira et al., 2018). Thus, corroborating the entire hypothesis of the work, even knowing the microbial potential in the improvement of degraded pastures, further detailed research into molecular aspects that relate to the taxonomy-function binomial is necessary to precisely identify which microorganisms perform key communication functions and growth promotion functions within this synthetic community (Mendes et al., 2011).

Finally, it has been proposed that it is of crucial importance to elucidate or bioprospect new microorganisms capable of promoting an increase in the physiological and morphological characteristics of pastures *in vivo* (Abdelaal et al., 2021). In general, these concepts support this work's hypothesis to use MIX to improve the quality and productivity of pastures, thus, ensuring a lower proportion of synthetic inputs applied in pastoral areas, and greater productivity and environmental sustainability.

## Conclusion

The co-inoculums formulated with bacteria with biotechnological potential were able to increase the physiological and morphological development of *U. decumbens* cv. Basilisk plants, with the highest percentages of increase observed in the CLO, NL, NT, RL, RGM, and RDM variables. With regard to co-inoculums, MIX 1 composed of the strains UAGB 60 - *Klebsiella* spp., UAGB156 - *Klebsiella* spp., UAGB 154 - *Klebsiella* spp., UAGB 167 - *RhizobiuPPm* spp., and UAGB 71 - *Sinomonas* spp. stood out, with the highest number of variables superior to the control treatment (without co-inoculums and chemical fertilization), as well as being similar to the treatment with chemical fertilization.

There are few studies on PGPB co-inoculation applied to *U. decumbens* cv. Basilisk, highlighting the contribution of this work to the scientific community. The choice of PGPBs with high symbiotic power, phytohormone production, biological N fixation, and P solubilization helps plant development, possibly reducing chemical fertilization and contributing to the environmental quality of pastoral areas.

## Authors' Contributions

SILVA, L.S.: conceptualization; data curation; formal analysis; writing – original draft. ESPÍNDOLA, N.L.: writing – original draft; writing – review & editing. TAVARES, B.M.S.: writing – original draft; writing – review & editing. SANTOS, J.M.G.: writing – original draft; writing – review & editing. BARBOSA, V.M.S.: writing – original draft; writing – review & editing. ANDRADE, P.A.M.: writing – original draft; writing – review & editing. OLIVEIRA, J.T.C.: data curation; formal analysis; writing – original draft; writing – review & editing.

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