



INVESTIGATING THE USE OF INDIGENOUS MICROBIAL ISOLATES FOR BIOCONTROL OF LEAFSPOT DISEASE IN SOLO- GOLD SWEET POTATO

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ABSTRACT

The study explored the use of indigenous microbial isolates for biocontrol of leafspot disease and growth promotion in Solo-gold sweet potato by isolating microorganisms from tissues of a healthy sweet potato variety and assessing the effectiveness of the isolates in suppressing leafspot disease on Solo-gold sweet potato. The treatment consisted of leaf, stem and root parts of a healthy sweet potato variety (Ex-igbariam), collected for fungal and bacterial isolation in the laboratory. The isolates were prepared into suspensions and inoculated into leafspot infected Solo-gold plant in the greenhouse. Isolates inoculation was carried out singly and, in all combinations. Data on the disease incidence, severity, and other growth parameters were collected at 6, 8, 10, and 12 weeks after planting. Data collected were subjected to analysis of variance (ANOVA) and significant means were compared using Fisher's LSD at 5% level of probability. Five pure cultures (two bacteria and two fungi) were isolated from the healthy sweet potato parts. The results showed that, isolates inoculation effect on Solo-gold differed significantly ($P < 0.01$). While some isolates inhibited leafspot expression on Solo-gold, certain isolates significantly suppressed the disease and enhanced the plant growth compared to the controls. The findings of this study underscore the potential of indigenous microbial isolates as biocontrol agents for managing leafspot disease and improving the growth of Solo-gold sweet potato variety, contributing to the development of sustainable and environmentally friendly strategies for managing diseases in agricultural production.

Keywords: Solo-gold, Sweet-potato, Microbial, Isolate, Biocontrol.

INTRODUCTION

Conventionally, the management of foliar diseases in plants relies heavily on inorganic chemical products, which can lead to environmental pollution and the development of resistant strains of pathogens (Sharma *et al.*, 2017). This calls for an urgent need to explore sustainable and eco-friendly alternatives for managing plant diseases while ensuring the productivity and quality of plant products. In the quest for food security and ecosystem sustainability, a promising avenue is the utilization of biocontrol agents against plant diseases. Endophytes, the microorganisms residing within the tissues of plants, have emerged as promising biocontrol agents in combating plant diseases (Zhang, 2009). While endophytes usually rely on their host for nutrition and safety, they also benefit the plants in several ways such as accelerating seedling emergence, promoting plant growth, enhancing yield, helping in bioremediation, improving nutrient cycling and reducing the proliferation of pathogens (Velmourougane *et al.*, 2017).

Among the orange-fleshed sweet potato (OFSP) varieties in Nigeria, Solo-gold sweet potato stands out for its high beta-carotene content, high dry matter, tolerance to sweet potato weevil and resistance to the devastating sweet potato viral disease (SPVD) (Maru, 2020). However, the susceptibility of Solo-gold sweet potato to leafspot pathogens may threaten its sustainable production and compromises food security in Nigeria.

Leveraging on the antagonistic interactions between endophytic microbes and pathogenic microbes, biocontrol strategies offer a sustainable approach to disease management in plant production. Hence, this study aimed to investigate the biocontrol potential of indigenous microbial isolates derived from healthy sweet potato variety (Ex-igbariam) against leafspot pathogens affecting Solo-gold variety. The objectives were to isolate microorganisms (bacteria and fungi) from healthy sweet potato tissues;



to assess the effectiveness of the isolates in suppressing leafspot disease on Solo-gold sweet potato; and to evaluate impact of the microbial treatments in promoting Solo-gold vine growth.

MATERIALS AND METHODS

Plant Tissue Collection and Microbial Isolation

Samples of leaves, stems and un-tuberized roots of healthy Ex-igbariam sweet potato were aseptically collected in separate sterile bottles from a sweet potato plot in Osogbo. Collected plant samples were transferred immediately to the Microbiology Laboratory of Osun State University, Osogbo, Nigeria for microbial isolation.

Potato Dextrose Agar (PDA) medium prepared at 39 g per liter of distilled water and Nutrient Agar (NA) medium prepared at 28 g per liter of distilled water were used for fungal and bacterial isolation respectively. The media were autoclaved at about 121°C for 15 minutes, allowed to cool down and poured into petri-dishes at about 20ml per plate to solidify. Prior to pouring, cooled PDA medium was supplemented with streptomycin sulphate powder (anti-bacterial) at 50g/liter and mixed thoroughly for bacterial growth inhibition.

Each plant sample was surface sterilized by soaking the tissue in ethanol (70%, v/v) for 30 seconds, then in hypochlorite (1%, v/v) for two minutes, followed by soaking in ethanol (70%, v/v) for 30 seconds and finally rinsed in sterile distilled water three times and blot-dried on clean sterile paper to remove excess water (Tan *et al.*, 2012).

With the aid of sterilized dissecting scalpel, the surface sterilized leaf, stem and root tissues were sectioned into about 2 mm sizes and sample pieces were aseptically inoculated separately on PDA and NA plates.

Continuous subculturing and incubation (21-25°C for 3 days for fungus; 38°C for 24 hours for bacterium) were carried out from distinct colonies on separate freshly prepared growth media till pure cultures were achieved (Tan *et al.*, 2012; Shazia *et al.*, 2022).

To establish the morphological characteristics of the isolates, active colonies of the pure cultures were examined using bright-field and differential interference contrast light microscopy at 40x objective magnification.

Screenhouse Experiment

The experiment was conducted at the screenhouse of National Root Crops Research Institute, Iresi Sub-Station, Boluwaduro Local Government Area of Osun State, Nigeria. Top soil was collected and heat sterilized with metal drum till the uppermost part of the soil maintained 80°C for 30 minutes. Cooled sterilized soil was dispensed in pots of black polythene bags (30 cm x 29 cm) and arranged in the screen house. The pots were punctured at the bottom, adequately watered till excess water drained out and planted with clean foundation stock of Solo-gold sweet potato.

Fungal isolates causing leafspot disease on Solo-gold was obtained from the National Root Crops Research Institute, Iresi Sub-Station. At one week after planting (WAP), the pathogen was sub-cultured and prepared into 10⁵ spores/ml suspensions which inoculated into the Solo- gold plants by punching the base (soil level) of the stem with the aid of sterile injection needle and dispensing 0.5 ml at the spot. At two 2 WAP, suspensions of the microbial isolates were also prepared from their active plates at 10¹⁰ colony forming unit/ml and 10⁵ spores/ml bacterial and fungal isolates respectively, and inoculated separately into the plant. 0.5 ml of each microbial suspension was also intermixed in all combinations and inoculated separately into the plant. Two control plants were prepared– Solo-gold plants inoculated with only sterile distilled water, and with only pathogenic suspension, and all inoculations were made up to 5 ml with sterile distilled water.

Experimental Design

The experiment contained 30 treatments of leafspot infected Solo-gold plant inoculated with the microbial isolate suspensions in three replications. The experiment was laid out in a Completely Randomized Design (CRD). The isolates treatments consisted of 28 combinations of five different isolates obtained and two controls (Solo-gold plant inoculated with only sterile distilled water and with only pathogenic suspension).



Plant Management and Data Collection

Pots were carefully watered to avoid splashing at 0.5 liter per pot at 2 day-intervals from the day of planting. N.P.K 15:15:15 fertilizer was applied at 2 gm per pot at 4 weeks after planting and data on leafspot disease incidence, leafspot disease severity, vine length (cm), number of leaves and average leaf area (cm²) were collected at 6, 8, 10 and 12 WAP.

Disease incidence and severity were calculated by:

$$\text{Disease Incidence} = \frac{n}{N} \times 100\% \quad (\text{Muhammad } et al., 2018)$$

$$\text{Disease Severity} = \frac{\sum(n \times v) \times 100\%}{N \times Z} \quad (\text{Muhammad } et al., 2018)$$

Where :
n = Number of leafspot infected plant within the treatment
N = Number of plant sample observed within the treatment
v = Number of leafspots of an infected plant
Z = Highest number of leafspots observed

The result was translated into rating scale 0-5 for both incidence (ranging from no infection to >80% foliage of the infected plant) and severity (ranging from no infection to >80% symptomatic portion of the infected foliage).

Leaf area was calculated by:

$$\text{Leaf Area} = L \times W \times 0.58 \quad (\text{Schrader } et al., 2021).$$

Where: L = Length of the leaf
W = Width of the leaf

Statistical Analysis

Data collected on disease incidence and severity were subjected to logarithm transformation before analysis of variance (ANOVA). ANOVA was carried out using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA). Significant means were compared using Fisher's LSD at 5% level of probability. Transformed analyzed data was back-transformed for interpretation.

RESULTS

Characteristics of Fungal and Bacterial Isolates

In this study, isolation and culturing from healthy Ex-igbariam yielded two bacterial and three fungal pure cultures. The morphological characteristics of the isolates are presented on Plate 1 and Tables 1 and 2.

The morphological characteristics of the fungi ranged differently. It was observed that all fungi isolated were septate in their conidiophores despite differences in mycelia pigmentation of the pure cultures (Table 1). Bacteria morphological characteristics also ranged differently (Table 2) and in spite of the differences in other morphological characteristics, they were all bacilli in cellular shape. Differences in morphological characteristics of the isolated organisms proved each of the isolate was different from the other.

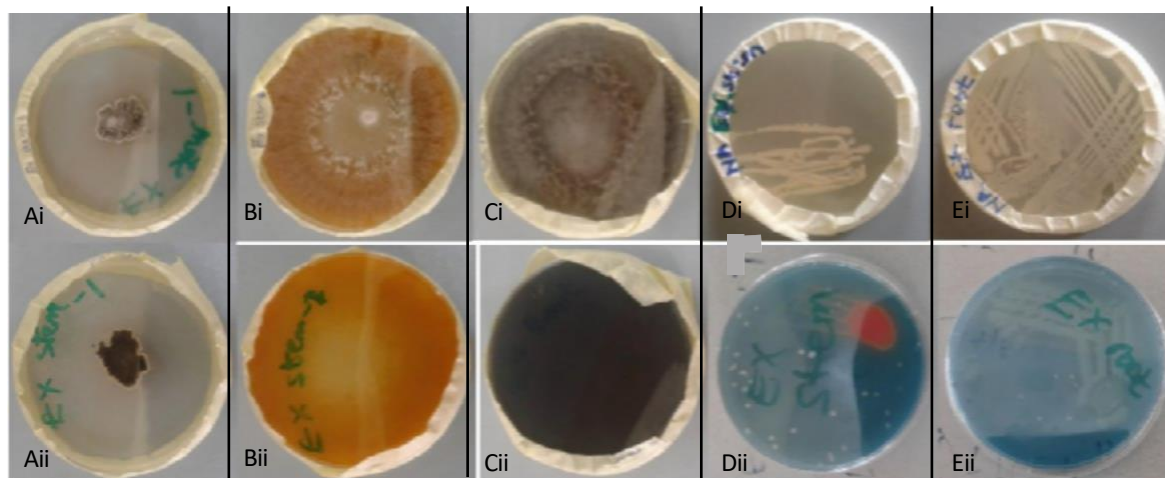


Plate 1: Microbial Isolates from Healthy Ex-igbariam Sweet Potato

(A) fungus from the stem showing (i) obverse and (ii) reverse view (B) fungus from the stem showing (i) obverse and (ii) reverse view (C) fungus from the root showing (i) obverse and (ii) reverse view (D) bacterium from the stem showing growth on (i) nutrient agar and (ii) cleg agar (E) bacterium from the root showing growth on (i) nutrient agar and (ii) cleg agar.

Table 1: Characteristics of Fungal Isolates from Healthy Sweet Potato

Isolate code	Source from Plant	Obverse Colour on Plate	Reverse Colour on Plate	Surface Texture	Form	Growth on Plate	Conidiphore
A	Stem	Pale	Pale black	Woolly	Irregular	Restricted	Septate
B	Stem	Pale yellow	Yellow	Woolly	Circular	Full	Septate
C	Root	Pale	Black	Woolly	Circular	Full	Septate

Table 2: Characteristics of Bacterial Isolates from Healthy Sweet Potato

Isolate Code	Source from Plant	Colour on Nutrient Agar	Colour on Cleg Agar	Gram Reaction	Shape of Bacterium
D	Stem	Pale	Deep Orange	Negative	Bacilli
E	Root	Pale	Pale	Negative	Bacilli

Leaf Spot Incidence and Severity

Out of the 30 treatments of the leafspot infected Solo-gold plant, 7 treatments of the microbial isolates were lost by 12 WAP. The mean square values from the analysis of variance for leafspot incidence and severity at 6, 8, 10 and 12 WAP of leafspot infected Solo-gold sweet potato inoculated with indigenous microbial isolates is presented in Tables 4 and 5 respectively. The result showed low rating (< 1) for average leafspot incidence and severity across WAP. The result revealed that there were high significant ($P \leq 0.01$) differences in the treatment effects for leafspot disease incidence and severity on Solo-gold sweet potato. There were no occurrences of leafspot disease on Solo-gold plant at 10 and 12 WAP.

Table 3: Leafspot Disease Incidence at 6, 8, 10 and 12 WAP of Infected Solo-gold Plants Inoculated with Indigenous Microbial Isolates

Source of Variation	DF	6 WAP	8 WAP	10 WAP	12 WAP
Isolate	22	0.0802**	0.2623**	0.0000	0.0000
Error	46	0.0078	0.0092	0.0000	0.0000
R ² (%)		83	92	-	-
CV (%)		143.6	47.4	-	-
Average		0.10	0.36	0.00	0.00

** : significance at $P \leq 0.01$; DF: degree of Freedom; R²: Coefficient of Determination; CV: Coefficient of Variation

Table 4: Leafspot Disease Severities at 6, 8, 10 and 12 WAP of Leafspot Infected Solo-gold Plants Inoculated with Indigenous Microbial Isolates

Source of Variation	DF	6 WAP	8 WAP	10 WAP	12 WAP
Isolate	22	0.0886**	0.2023**	0.0000	0.0000
Error	46	0.0010	0.0131	0.0000	0.0000
R ² (%)		98	87	-	-
CV (%)		50.7	60.4	-	-
Average		0.10	0.33	0.00	0.00

** : significance at $P \leq 0.01$; DF: degree of Freedom; R²: Coefficient of Determination; CV: Coefficient of Variation

The average leafspot disease incidence and disease severity of leafspot infected Solo-gold plants inoculated with the microbial isolates is presented in Table 5. The post hoc result revealed that there is a significant different between the control plants and 12 treatments of the isolates completely inhibited expression of leafspot disease on Solo-gold plant.\

Table 5: Average Leafspot Disease Incidence and Disease Severity of Leafspot Infected Solo-gold Plants Inoculated with Indigenous Microbial Isolates

Treatment Code	Incidence	Severity
Control 1 (Distilled water)	0.00	0.00
Control 2 (Pathogens)	0.92	0.86
A	0.35	0.27
B	0.00	0.00
C	0.33	0.33
D	0.00	0.00
AC	0.00	0.00
AE	0.00	0.00
CD	0.00	0.00
CE	0.19	0.19
DE	0.23	0.23
ABC	0.19	0.19
ABD	0.00	0.00
ABE	0.23	0.19
ACD	0.00	0.00
BCD	0.00	0.00
BCE	0.00	0.00
CDE	0.00	0.00
ABCD	0.00	0.00
ABCE	0.19	0.23
BCDE	0.00	0.00
ABCDE	0.12	0.16
LSD_{0.05}	0.10	0.09

The analysis of variance result (Table 6) revealed high significant ($P \leq 0.01$) treatment effect for all the measured growth traits (vine length, number of leaves and leaf area). The post hoc result (Figure 1) revealed that fungal isolate treatments A and C produced significantly higher vine length and number of leaves in Solo-gold sweet potato than which was produced by the controls across WAP.

Table 6: Effects of Inoculation on Vine length, Number of Leaves and Leaf Area of Leafspot Infected Solo-gold Sweet Potato Inoculated with Microbial Isolates

Source of Variation	DF	Vine length (cm)	No of leaves (count)	Leaf area (cm ²)
Isolate	22	35184.621**	3811.067**	1375.326**
Error	253	1154.843	212.454	217.963
R ² (%)		73	61	35
CV (%)		34.8	40.2	33.0
Grand Mean		97.49	36	45

** : significance at $P \leq 0.01$; DF: degree of Freedom; R²: Coefficient of Determination; CV: Coefficient of Variation

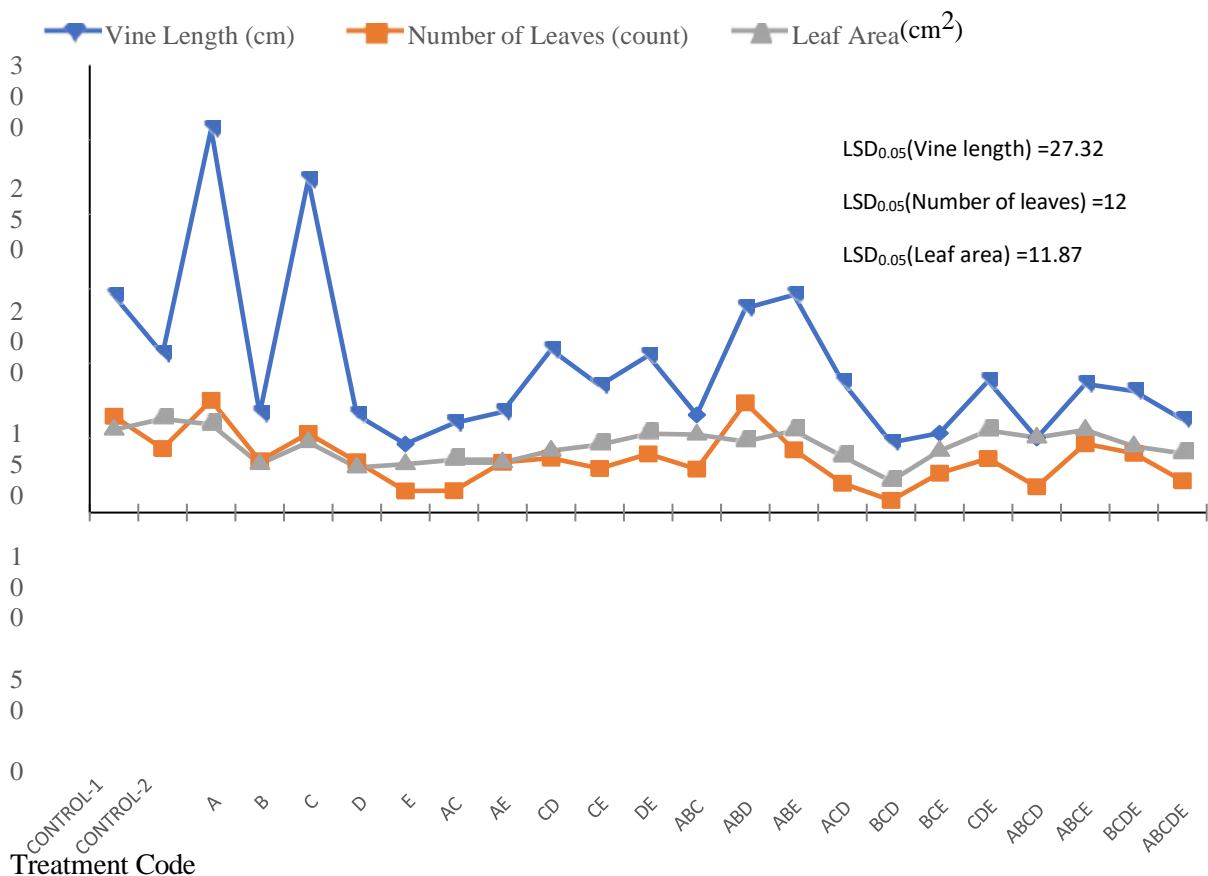


Figure 1: Average Vine Length, Number of Leaves and Leaf Area of Leafspot Infected Solo-gold Plant Inoculated with Indigenous Microbial Isolates

DISCUSSION

Sweet potato is predominantly propagated through vegetative vine cuttings, making planting materials an important pathway for the transmission of plant pathogens. Consequently, seed-borne and vegetative propagule-borne pathogens represent persistent challenges in crop production because they facilitate the recurrence and spread of diseases within cropping systems (Gitaitis and Walcott, 2007). In recent years, increasing concerns regarding the environmental and health impacts of synthetic



agrochemicals have stimulated interest in biological alternatives for plant disease management. Among these alternatives, endophytic microorganisms have received considerable attention due to their capacity to enhance plant growth and suppress pathogens while maintaining ecological sustainability (Eid *et al.*, 2021).

Isolation of Microbial Isolates

In the present study, microbial isolation from healthy Ex-igbariam sweet potato tissues yielded five pure cultures consisting of three fungal isolates and two bacterial isolates. The isolates exhibited distinct morphological characteristics with respect to colony appearance, pigmentation, and growth patterns, indicating the presence of diverse endophytic microorganisms within the plant tissues. The fungal isolates were characterized by septate conidiophores, while the bacterial isolates exhibited Gram-negative bacilli morphology.

The occurrence of diverse endophytic microbial communities in sweet potato tissues has been documented in previous studies. For instance, Jiang *et al.* (2019) reported that sweet potato plants harbor a wide range of culturable endophytes, although only a few taxa dominate the microbial community. They identified 75 culturable microbes that were associated with field-grown sweet potato plants. The successful recovery of both bacterial and fungal endophytes in the present study therefore supports the concept that healthy plant tissues serve as reservoirs of beneficial microorganisms that may contribute to plant health and resilience.

Suppression of Leafspot Disease

The greenhouse experiment demonstrated that inoculation with indigenous microbial isolates significantly influenced the expression of leafspot disease in Solo-gold sweet potato. Highly significant treatment effects were observed for both disease incidence and severity during the early stages of plant development. While the pathogen-inoculated control exhibited the highest levels of disease expression, several microbial treatments markedly reduced disease incidence and severity. Notably, twelve microbial treatments completely inhibited the expression of leafspot symptoms. Furthermore, leafspot symptoms were absent across treatments at 10 and 12 weeks after planting.

These findings indicate that certain indigenous microbial isolates possess strong antagonistic potential against the leafspot pathogen. Biological control by endophytic microorganisms may occur through multiple mechanisms, including competition for ecological niches and nutrients, production of antimicrobial metabolites, secretion of hydrolytic enzymes that degrade pathogen structures, and induction of systemic resistance in the host plant (Compant *et al.*, 2005). In their study, Jiang *et al.*, (2019) demonstrated similar pathogen suppression by endophytic bacteria such as *Bacillus* and *Pantoea*, which are capable of producing antifungal compounds and eliciting plant defense responses. Therefore, the suppression of leafspot disease observed in the present study is consistent with previous reports highlighting the role of endophytic microorganisms as effective biological control agents in crop protection.

Plant Growth Promotion

In addition to disease suppression, microbial inoculation significantly influenced vegetative growth parameters of Solo-gold sweet potato, including vine length, number of leaves, and leaf area. The analysis of variance revealed highly significant treatment effects for all measured growth traits. Among the isolates, fungal treatments A and C produced significantly greater vine length and higher leaf numbers compared with the uninoculated control plants, indicating that these isolates possess plant growth-promoting properties.

The growth-promoting effects observed in this study are consistent with previous findings demonstrating that endophytic microorganisms can enhance plant growth through a variety of physiological mechanisms. Many plant growth promoting microorganisms synthesize phytohormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which influence cell division, elongation, and overall plant development (Eid *et al.*, 2021). Additionally, some endophytes improve nutrient availability through nitrogen fixation, phosphate solubilization, or siderophore production, thereby enhancing nutrient uptake by plants (Velmourougane *et al.*, 2017).

Endophytes may also stimulate plant defense pathways, thereby enabling plants to allocate resources more efficiently toward growth and development under pathogen pressure (Compant *et al.*,



2005). The improved vegetative growth observed in the present study may therefore be attributed to one or a combination of these mechanisms. However, further studies involving molecular or biochemical characterization of the isolates would be necessary to elucidate the specific mechanisms responsible for the observed plant growth promotion.

Implications for Sustainable Sweet Potato Production

Overall, the findings of this study demonstrate that indigenous microbial isolates derived from healthy sweet potato tissues can suppress leafspot disease and enhance vegetative growth in the susceptible Solo-gold variety. These results highlight the potential of exploiting naturally occurring endophytic microorganisms as biological control agents and plant growth promoters in sweet potato production systems.

The integration of such microbial inoculants into crop management strategies could contribute to reducing dependence on chemical pesticides while promoting environmentally sustainable agricultural practices. However, additional investigations are required to identify the isolates at the molecular level, evaluate their stability and efficacy under field conditions, and determine their compatibility with existing crop management practices. Such studies would be essential for translating these findings into practical biocontrol applications for sweet potato cultivation.

CONCLUSION AND RECOMMENDATION

This study spotted fungal and bacterial isolates, as potential endophytes, resident within tissues of healthy Ex-igbariam sweet potato. The study also demonstrated biocontrol potency of some of the microbial isolates in inhibiting or suppressing leafspot disease in Solo-gold sweet potato. In addition to the biocontrol potential, it was evinced from this study that fungal and bacterial microorganisms from healthy sweet potato variety possessed plant growth promotion ability in Solo-gold sweet potato production.

As our understanding of plant-microbe interactions continues to evolve, so too do the prospects for leveraging microbial inoculants in sustainable agriculture. The success of biocontrol strategies in managing leafspot disease in Solo-gold sweet potato through indigenous inoculant underscores the immense potential of harnessing nature's bioresources for crop protection. Moving forward, concerted efforts are needed to unravel the complexities of microbial communities and optimize their application in agricultural settings. By integrating cutting-edge technologies such as metagenomics and synthetic biology, we can unlock novel avenues for enhancing plant health and resilience in the face of evolving environmental challenges.

In conclusion, this research endeavored to contribute to the development of sustainable and environmentally friendly strategies for managing leafspot diseases in Solo-gold sweet potato cultivation. By harnessing the inherent biodiversity of agroecosystems and promoting the ecological balance between beneficial microbes and plant pathogens, we aspire to foster resilience and sustainability in sweet potato production systems, ultimately ensuring food security and agricultural sustainability in regions reliant on this vital crop.

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