Identification of actinomycetes that stimulate eggplant (*Solanum melongena*) growth in Mali

ABSTRACT

Aims: This study aims to formulate at least one efficient biostimulant based on actinomycetes that promotes good eggplant growth in Mali.
Study design: The experimental design was a completely randomized block design with three replications and four treatments randomly distributed. The treatments were three Actinomycete strains (Act, S1 and St1) and a non inoculated control and the mode of application (inoculation or spraying) of these treatments were considered as blocking factor.
Place and Duration of Study: The trials were conducted in Bamako, in the Greenhouse of the laboREM-Biotech situated on the colline of Badalabougou.
Methodology:Sorghum seeds inoculated or not with*Actinomyces* sp.Ts1, *Actinomyces* sp. S1 and *Actinomyces* sp. Act; were randomly sown in plastic pots filled with approximately 2.50 kg of air-dried soil. The first block sown with coated seeds and the second with non-inoculated seeds and seedlings from them pulverized. Leaf length, leaf area, Leaf diameter, leaf area, leaf fresh weight, leaf dry weight, root fresh weight, root dry weight, plant height and Root length were determined.
Results:
Eggplant seed inoculation with all the tested Actinomycetes strains significantly enhanced leaf length, Leaf diameter, leaf area, leaves fresh weight. The highest results were obtained with the strain Act, followed by strain St1. Sprayed treatment improved eggplant leaf length, Leaf diameter, leaf area, leaves fresh weight, leaves dry weight, root fresh weight, root dry weight; compared to the coated treatment
Conclusion:fieldsapplication, which may reduce production cost, by increasing eggplant growth and production while reducing production cost.The present work specifythat,Act *and* Ts1and spray method were suitable for inoculating on eggplant. *Actinomyces*sp. Act produced the highest leaf length, Leaf diameter, leaf area, leaves fresh weight. Therefore, *Actinomyces* sp. Actis suggested for eggplant bioinoculant.

1. INTRODUCTION

Eggplant (*Solanum melongena* L.) is a dicotyledonous plant of the Solanaceae family, cultivated for its vegetable-fruit. It is native to Asia where it was domesticated in prehistoric times and constitutes with African eggplants (*Solanum aethiopicum* and *Solanum macrocarpon*), the three species of cultivated eggplants. Eggplant is rich in phenolic compounds and alkaloids, antioxidants favorable to metabolic syndrome [1]. It is traditionally used to lower blood cholesterol levels. Eggplant contains small amounts of phytosterols (compounds that limit the absorption of dietary cholesterol) and soluble fibers (pectin) known to reduce cholesterol levels by trapping part of the food's fats in its filaments. The fibers and polyphenols contained in eggplant, by preventing the action of a digestive enzyme, reduce the glycemic index of the meal and reduce the rise in blood sugar. Polyphenols are again effective in blocking the proliferation of cancer cells [2]. Despite all these benefits of eggplant, its production is reduced in Mali and the gardeners who grow it encounter many constraints of various nature that greatly reduce their yield.

These include the deficiency of agricultural soils in nutrients such as nitrogen, potassium and especially soluble phosphorus due to their overexploitation, the abusive use of agricultural inputs which are becoming increasingly expensive and dangerous for the environment and the multiple damage caused by insects and diseases that can destroy up to 100% of production [3]. Faced with the multiple constraints on eggplant crops, the use of growth-promoting actinomycetes appears to be reliable alternatives to generally shake up the level of market garden production and in particular eggplant production.

Furthermore, plant growth does not rely solely on the availability and absorption of nutrients. Some growth mechanisms are controlled by substances other than nutrients. These substances are produced by microorganisms better known by their English name Plant Growth Promoting Bacteria (PGPB) of which growth-promoting actinomycetes are included. Some bacteria with PGPR activity are used as inoculants to improve root development via the production of certain

phytohormones [4]. Many other bacteria are capable of improving plant well-being by limiting the growth of phytopathogenic microorganisms. Thus, actinomycetal bacteria are the best candidates to be applied in the form of living cells due to their capacity to produce bioactive metabolites with antibiotic properties [5], [6], [7]. Despite the importance of these bacteria and their successful use to improve the production of several crops around the world; stimulatory actinomycetes are an alternative to chemicals, inexpensive and environmentally friendly, remain very little used in market gardening in Mali. This is why this study is part of the framework of identifying strains of actinomycetes capable of stimulating the growth of eggplant in a more environmentally friendly sustainable development approach.

2. MATERIAL AND METHODS

2.1 Study site

The trials were conducted in Bamako, in the Greenhouse of the LaboREM-Biotech situated on the colline of Badalabougou.

2.2 Material
2.2.1 Biological material
2.2.1.1 Plant material

Eggplant seed of the long violet variety was used during the test

2.2.1.2 Microorganisms
Three Actinomycetes strains: Actinomycete sp. S1, Actinomycete sp. St1 and Actinomycete sp. Act from the LaboREM-Biotech collection, were used as biocontrol agents.

2.3 Methods
2.3.1 Biochemical characteristics of the isolates:
The catalase [9] (Venkateshwarran et al., 1999, oxydase [9] (Caridis et *al.,* 1991)and pectinase [8] [10] (Delarasse, 2014; Benkahoul et al., 2017) tests were done according to methods described by the cited authors. In each case, growth of the isolates was recorded by visual observation (Hossain et *al.,* 2015). Bergey's Manual of Determinative Bacteriology (1994) was used to identify the tested isolates.

2.3.2 Plant growth promoting characteristics:
To determine the plant growth promoting characteristics, the followings tests have been performed: the fixation of atmospheric nitrogen and the production of ammonia (NH_3) was carried out according to the method of [11]. The indole acetic acid production was determined according to the method described by Guardado-Fierros et al. [12]. The siderophore production test was performed on solid King-B medium according to the Hafsa method [13]. HCN production was assessed using the Khan method [14].

2.3.3 Experiment 1. Determination of seed germination and seedlings vigor activities
To evaluate the role of the studied Actinomycetes strains (St1, Act abd S1) in the growth of Eggland seedlings, each Actinomycete strain was cultured in 250 ml flasks containing 150 ml of Bennett medium without agar. Then, they were shaken at 28±2^{o}C for one week. After incubation, the different cultures were centrifuged at 10.000 rpm for 15 minutes to separate the pellet from the supernatant. For the treatments of each strain, the inoculum was adjusted to 1.10^{8} bacteria/ml using spectrophotometry by adjusting the optical density (OD) to 0.5 for a wavelength of 600 nm. To inoculate eggplant seeds, twenty grams (20 g) of seeds were sanitized with 0.024% sodium hypochlorite solution for 2 minutes and then rinsed thoroughly with sterile distilled water according to the method described by Noumavo et al. [15]. After treatment, the seeds were placed in a sterile beaker containing 20 ml of 2% methylcellulose solution as an adhesive and 20 ml of the bacterial inoculum of interest. Two grams (2 g) of sterile activated carbon powder were added to this mixture. The seeds were coated in the mixture until a uniform layer was formed. The coated seeds were dried under a laminar flow hood for four(4) hours. To determine the effect of the Actinomycete strains on eggplant seedlings vigor, germination tests were carried out on semi-solid agar using the method described by Pacome et al. [16]. Ten (10) days after sowing, the number of germinated seeds was counted and the germination rate was determined. The roots and leaves lengths of each seedling was measured to determine the vigor index which is given by the following formula:

$$\textbf{Vigor index} = \frac{\text{Root length} - \text{Shoot lengh}}{\text{Shoot lengh}} \text{x Germination rate}$$

2.3.4 Experiment 2: Determination of the effect Actinomycete strains on the growth of eggplants

Efficiency of Actinomycete strains on plant growth and nutrient uptake in eggplant was evaluated under greenhouse conditions by seed bacterization. Bacterization of surface sterilized seeds was performed by coating the seeds in the mixture composed with 20 ml of 2% methylcellulose solution as an adhesive, 20 ml of the inoculum of the bacteria of interest and 2g of sterile activated carbon powder until a uniform layer was formed. The coated seeds were dried under a laminar flow hood for four hours. Eggplant seeds treated with sterile distilled water alone were considered as control. Seeds either inoculated with Actinomycete strains or treated only with sterile distilled water were sown in plastic pots (100 mm × 75 mm × 85 mm) filled with approximately 2.50 kg of air-dried soil from LaboREM-Biotech test site. The pots were held on racks in a complete randomized block design with Actnomycete strains and sterile distilled water as treatments, and mode of application (Seed coating and seedling pulverization) as blocking factor. Each treatment was replicated three times. The pots were maintained under greenhouse conditions and watered regularly. The leaf area was determined according to the formula described by [17]: Leaf area = K x leaf length x leaf diameter, with k = 0.75. Leaf length, Leaf diameter, leaf area, leaf fresh weight, leaf dry weight, root fresh weight, root dry weight, plant height and Root length were determined.

Statistical analysis of data

The Bartlett test was performed to verify the homogeneity of the variance of the means for the different measured parameters. Treatments with non-homogeneous means were log-transformed before analysis. A two-factor analysis of variance (Block and treatment) for each parameter was performed using the general linear models procedure of SAS [14]. The Least Significant Difference (LSD) test at probability level 0.05 was used to separate the means when the ANOVA F-test indicated a significant effect of the treatments [18].

Ethical considerations

This study focuses on the production and formulation of a biostimulant for eggplant growth and production. The laboratory, greenhouse and fiel manipulations were carried out in accordance with good practices in this area. The transfer of results will be carried out on the basis of a “win-win” partnership with market gardeners in Mali.

3. RESULTS AND DISCUSSION

Tested Actinomycetes characteristics

Colony aspects of isolates St1, Act and S1 on ISP2 are presented in Figure 1, while Gram and vegetative cells of the isolate S1 is presented in Figure 2. Data collected on morphological and biochemical characteristics of these three Actinomycete isolates used in this work showed that: All the selected strains appeared dry, rough and adhered to the agar and had a vegetative and aerial mycelium. The Act strain was white in color, with large and round colonies. The St1 strain had small, round colonies and white in color. The S1 strain, on the other hand, had gray, round colonies and small in size. The results clearly showed that the ISP2 medium is the most favorable for the purification of the selected actinomycetes strains. Like the S1 isolate (Figure 2), the St1 and Act isolates are Gram positive and present in filamentous form.

Figure 1: Appearance of aerial mycelium on ISP2 medium of actinomycete strains after 7 days of incubation. Act (A); St1 (B); S1 (C).

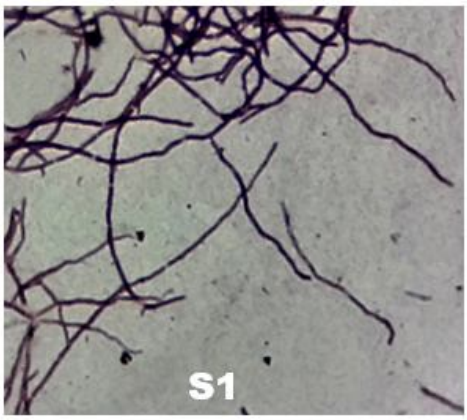

Figure 2. Microscopic observation of strain S1 after Gram staining

The tested Actinomycete isolates produced Ammonia, pectinase, indoleacetic acid, siderophores and cyanhydric acid (Table 1).

Table 1. test for the production of ammonia (NH_3), pectinase, indoleacetic acid (IAA) and siderophores

Actinomyces strains	Ammonia production	Pectinase	Indol acetic acid (IAA)	Siderophores	Cyanhydric acid
St1	-	+	++++	++	+++
Act	-	+	+++	+++	-
S1	-	+	++	++++	++
Témoins	-	-	-	-	-

+ : Positive, **-** : Négative, **++** : High production, **+++** : Very high production, ++++ : Excellent production

The analysis of results in table 1 showed that all the tested strains produce pectinase, indoleacetic acid (IAA) and siderophores at different level. St1 produce the highest IAA level, while the highest siderophore level was produced by S1 (Figure 3). The Actinomycete isolates Act and S1 produce no cyanhydric acid (Figure 4), and no strain revealed the production of ammonia.

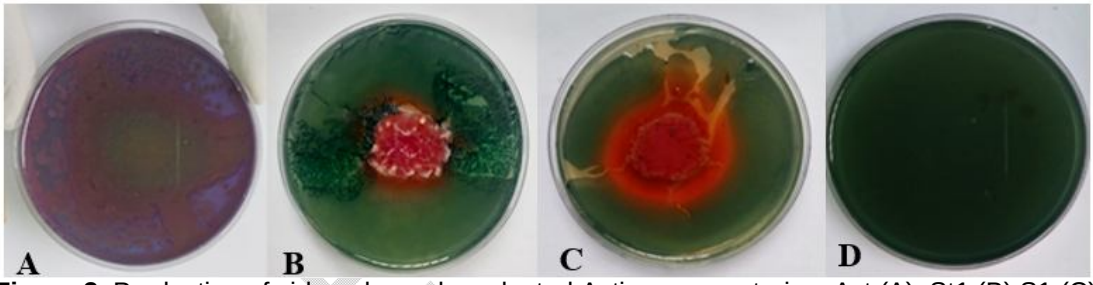

Figure 3. Production of siderophores by selected Actinomyces strains: Act (A), St1 (B) S1 (C) and the Control (D).

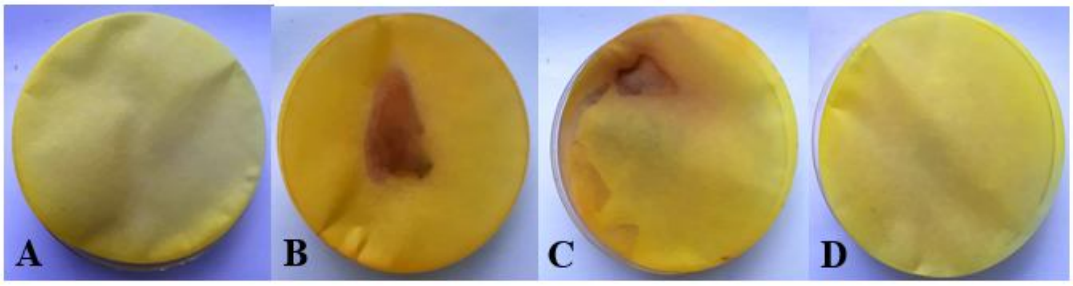

Figure 4. Hydrocyanic acid production by Actinomycetes strains: Act (A), St1 (B) S1 (C) and the Control (D).

Effects of tested actinomyces strains on eggplant seeds germination rate and vigor index

Table 2. Effect of Actinomycetes strains on Eggplant leaf lengh (cm), root lengh (cm), germination rate (%) and vigor index

Actinomyces strains	Plant height (cm)	Root length (cm)	Germination rate (%)	Vigor index
St1	4,17	2,13	85	535,5
Act	4,43	2,70	90	641,7
S1	4,17	2,27	75	483
TE	4,17	1,60	30	173,1

Inoculation with the isolated Actinomyces strains significantly increased seed germination, plant height and root lengh of eggplant (*Solanum melongena*) (Table 2). Only *Actinomyces* sp. Act increased plant height, compared to the non-inoculated control. The highest percentage of seed germination rate, plant height, and root length were recorded with Actinomyces sp. Act (Table 2). All the tested Actinomycetes strains significantly improved plant vigor compared to the control. The highest vigor index was recorded for the strain Act, followed by the strain St1 (Table 2).

Table 3. Summary from the analyses of variance for eggplant leaf length (LF), Leaf diameter (LD), leaf area (LA), leaves fresh weight (LFW), leaves dry weight (LDW), root fresh weight (RFW), root dry weight (RDW), plant heigth (PH) and Root length (RL).

	Measured parameters							
	DF	LL	LD	LA	LFW	LDW	RFW	RDW
Blocs	1	0.20	0.52	0.54	21.88***	9.50*	43.25***	12.40**
Treatments	3	2.81*	3.32*	3.45*	3.28*	2.92	1.65	2.51

*, **, ***Significant at P<0.05, P<0.01 and P<0.001; respectively. NS: Statistically not significant.

Inoculation with Actinomyces strains significantly affected leaves lengths, Leaf diameter length, plant aerial fresh weight (Table 4). Plant aerial fresh weight, dry weight and root fresh and dry weights were significantly affected by the treatments' application method (table 4).

Table 4. Effect mode of application of the treatments on eggplant leaf length (LF), Leaf diameter (LD), leaf area (LA), leaves fresh weight (LFW), leaves dry weight (LDW), root fresh weight (RFW), root dry weight (RDW), plant heigth (PH) and Root length (RL).

	Measured plant growth parameters								
Mode of application	LL	LD	SF	APFr	APSec	RPFr	RPSec	LPA	LPR
Sprayed	15.51	7.40	89.17a	52.00a	8.63a	6.77a	1.31a	43.16	31.63
Coated	14.97	7.02	80.94b	34.76b	6.19b	3.09b	0.92b	43.23	19.00

Regarding the effect of the mode of inoculation, sprayed treatment improved eggplant leaf length, Leaf diameter, leaf area, leaves fresh weight, leaves dry weight, root fresh weight, root dry weight; compared to the coated treatment (Table 4). No differences were observed between the two treatment modes in their effects on leaf

Table 5. Effect of Actinomyces strain on maize leaves number, stem length, number of grains/pot and Weight of grains produced (g)/pot.

	Measured plant growth parameters									
Actinomyces strains	LF	DF	SF	APFr	APSec	RPFr	RPSec	TEAR	LPA	LPR
St1	16.83a	7.58a	96.06a	43.5ab	7.45a	4.10a	1.01a	321.8a	46.12a	20.30a
Act	16.45a	8.01a	100.44a	52.77a	9.12a	5.84a	1.35[a]	327.1a	43.33a	21.83a
S1	15.3 3ab	7.47a	89.19a	38.23b	5.18a	5.01a	0 .94a	456.4a	43.80a	20.72a
Control	12.36b	5.77b	54.53b	39.04b	5.82a	4.77a	1.14[a]	301.8a	39.33a	20.30a

Eggplant seed inoculation with all the tested Actinomycetes strains significantly enhanced leaf length, Leaf diameter, leaf area, leaves fresh weight (Table 5). The highest results were obtained with the strain Act, followed by strain St1 (Table 5).

Discussion

Results obtained in this study showed that: the three selected actinomyces strains produced compounds with plant growth promoting (PGP) activities. The main compounds produced were: pectinase, indole acetic acid (IAA), hydrocyanic acid (HCN) and siderophores. This result was consistent with that obtained by Kassogue et al. [9] who also obtained during these Master's activities, twenty (20) endophytic strains of rice that had PGP activity in the in-vitro test. This in-vitro test showed that 55% of the selected local strains produced Indole Acetic Acid (IAA). Dicko et al. [19] also obtained 12 indigenous actinomycete strains with PGPRs activity, capable of producing siderophores to improve corn growth. *Bacillus thuringiensisand Azospirillium* sp. and Actinomycete strains; isolated in agricultural soils in Mali produced enzymes (cellulase and pectinase), indole acetic acid and siderophores [9, Mallé, Dicko]. Greenhouse tests showed that siderophore and IAA-producing strains significantly improved the growth and production of maize plants by improving plant nutrition and protection against insect pests and pathogens through the activation of plant defense systems and promotion of plant mineral uptake [9]. These results confirm former research results which showed that: IAA production by microorganisms improves plant growth by promoting cell elongation, cell division, root initiation, and modification of specific gene expression under stress conditions [20] [21]. These results are in agreement with other research results which showed that rhizosphere bacteria, mainly those of the genus *Bacillus* with high amount siderophore production improved significantly maize, sorghum and tomato growth and production [22], [23].

The eggplant seeds inoculated with the three selected actinomyces strains respectively showed an improved vigor index compared to the control non-inoculate, and the inoculated seeds showed faster germination with a rate of 83% in average against 30% for the control. This improvement in the germination rate observed in the study may be due to an increase in the synthesis of phytohormones such as indole acetic acid and gibberellin that triggers the activity of α-amylase and other specific seed germination enzymes (protease and nuclease) involved in starch hydrolysis and assimilation [24]. These results were in agreement with those obtained by Dicko et al. [19] who obtain germination rate ranging from 84 to 100% for maize seeds using Actinomyces with high plant growth promotion activities. In fact, these actinomyces produce high levels of siderophores and indole acetic acid, besides high antimicrobial and enzymes production. Noumavo et al. [15] obtained a 100% germination rate after seed inoculation with different PGPRs, which is in line with the results obtained and confirms that using bacteria to inoculate plant seeds can be an effective way to improve agricultural production.

In greenhouse tests, sprayed mode of treatment improved eggplant leaf length, Leaf diameter, leaf area, leaves fresh weight, leaves dry weight, root fresh weight, root dry weight; compared to the coated treatment. Regarding the PGPR used as treatment, they all significantly enhanced leaf length, Leaf diameter, leaf area, leaves fresh weight compared to control. These results complement several research results out of which those of [25], [26], [27] and [28]

showed the ability of rhizobacteria and endophytes as potential PGP able to be used to develop biofertilizer or biopesticide for application in eggplant production. Mokabel et al. [25] working on the role of Plant Growth Promoting Rhizosphere Microbiome as alternative biofertilizer in boosting *Solanum melongena* L. adaptation to salinity Stress, showed that addition of bioinoculum to salt-treated plants increased pigment content. Similarly, K+, K+/Na+, Ca2+, P, and N contents were significantly enhanced; while spermine, spermidine, and puterscine increased in root and shoot. RAPD/PCR showed gene expression upregulation of photosystem II D2protein, glutathione reductase, glutathione-S-transferase, protease I, and protease II [25]. Attia et al. [26] working on Application of *Rhizopus microsporus* and *Aspergillus oryzae* to enhance the defense capacity of eggplant seedlings seedlings against *M aeloidogyne incognita* showed the capacity of plant growth promoting fungi to increase plant growth and production through the production of compounds which can improve plant growth and activate plant defense capacity against plant pathogens. Li et al. [27] working on the effect of plant-growth-promoting fungi on eggplant (*Solanum melongena* L.) in New reclamation land obtained four isolates (HZ123, HZ23, HZ10, and HZ06) with P solubilization, siderophore production, and indole acetic acid (IAA) production capacities. From these bacteria, isolate HZ123 showed the highest ability to solubilize P and produce siderophores and IAA. But, results of in vivo PGP assays demonstrated that isolate HZ123 has a minimal negative effect on the growth of eggplant; however, the other three isolates, particularly isolate HZ06, caused the greatest increase in eggplant biomasses. Achari and Ramesh [28] working on the diversity, biocontrol, and plant growth promoting abilities of xylem sesiding bacteria from solanaceous crop Antagonistic isolated twenty-eight strains inhibited growth of *R. solanacearum* and produced volatile and diffusible antagonistic compounds and plant growth promoting substances *in vitro.* Out of thes isolates strains XB86, XB169, XB177, and XB200 recorded a biocontrol efficacy greater than 85% against BW and exhibited 12%–22 % increase in shoot length in eggplant in the greenhouse screening. Theses Xylem residizing bacteria (XRB) with high biocontrol and plant growth promoting activities were identified as strains of Staphylococcus sp., Bacillus sp., Streptomyces sp., Enterobacter sp., and Agrobacterium sp.

4. CONCLUSION

Fields application of biostimulant may reduce production cost, by increasing eggplant growth and production while reducing production cost. The present work specifies that, *Actinomyces* sp. Act *and Actinomyces* sp. Ts1 and spray method were suitable for inoculating on eggplant. *Actinomyces* sp. Act produced the highest leaf length, Leaf diameter, leaf area, leaves fresh weight. Therefore, *Actinomyces* sp. Act is suggested for eggplant bioinoculant.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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