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VALORIZATION OF TOMATO PLANT WASTES AND OPTIMIZATION OF GROWTH CONDITIONS FOR INDOLE-3-ACETIC ACID PRODUCTION BY *Streptomyces plicatus* STRAIN PT2

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ABSTRACT

This study focused on the production of the phytohormone indole-3-acetic acid (IAA) using tomato plant tissues as feedstock and on highlighting its growth promotion effect on tomato seedlings. Twelve actinobacterial strains were screened for IAA production under standard growth conditions and the strain *Streptomyces plicatus* PT2 was selected as a promising producer. The establishment of growth conditions to increase IAA production by *S. plicatus* PT2 was conducted using Plackett-Burman mathematical design with seven variables. The variables L-tryptophan quantity and tomato roots-extract rate were the two significant variables influencing IAA production. The most impacting variables were optimized using the surface methodology (RSM) formulated according to the Central Composite Design (CCD). The optimized broth supplemented with 4 mg L^{-1} of L-tryptophan and 25% of tomato roots-extract significantly improved the IAA production from 96.3 µg mL^{-1} within 120h to a maximum of 110.29 µg mL^{-1} within 96h. The *in-planta* application of the produced IAA and a chemical IAA showed a significant increase in the dry weight, the shoot and root lengths of tomato seedlings. This is the first study showing the production of IAA using *Streptomyces plicatus* strain PT2 by exploring tomato plant tissues and its plant-growth promotion efficacy compared to a chemical marketed one. These results support a reasonable approach for the valorization of abundant worldwide tomato plant wastes in the phytohormones production bioprocesses.

Keywords: indole-3-acetic acid; *Streptomyces plicatus* PT2; tomato plant waste; optimization; Plackett-Burman

INTRODUCTION

In recent years, scientists have focused their attention on the potential of beneficial microbes such as actinobacteria for sustainable agriculture (**Myo *et al*., 2019; Benadjila *et al*., 2022; Boubekri *et al*., 2022**). Actinobacteria are also particularly beneficial for biodegradation and waste management in specific biological processes (**Colombo *et al*., 2001; Claessen *et al*., 2002**). Several studies reported that actinobacterial strains actively colonize the plant rhizosphere and promote plant growth by producing phytohormones such as indole-3-acetic acid (**Patten and Glick, 2002; Hamdali *et al*., 2008; El-Tarabily *et al*., 2009; Iqbal and Hasnain, 2013; Zamoum *et al*., 2017**). The IAA is one of the most physiologically active auxins, which can stimulate the cell elongation and proliferation. It also enhances the uptake of minerals and nutrients from the soil (**Teale *et al*., 2006; Goudjal *et al*., 2013**).
Several endophytic and rhizospheric actinobacteria are able to produce IAA from Indole-3-pyruvic acid and L-tryptophan (**Spaepen *et al*., 2007; Duca and Glick, 2020**). These compounds are considered as precursors in the metabolic pathway for IAA release and they are usually added as purified chemical compounds for the explored microorganisms (**Monteiro *et al*., 1988**). As an alternative for the unstable and expensive chemical purified nutrients and precursors, the biotechnological processes involving microorganisms producing IAA could be conceived by exploring plant tissue extracts (**da Rosa *et al*., 2003; Benadjila *et al*., 2022**). Moreover, the recovery of plant wastes as a low-coast feedstock for such microbial processes can remarkably reduce the production fees (**Benadjila *et al*., 2022**).
The valorization of plant tissues in biotechnological processes needs an optimization procedure with the purpose of adjusting the growth conditions and maximizing the bioprotection of microbial metabolites (**Myo *et al*., 2019; Benadjila *et al*., 2022**). Previously, the optimization procedure has been widely used by changing one factor at a time and fixing the others during each run (**Goudjal *et al*., 2013; Sasirekha *et al*., 2012**). The RSM (Response Surface Methodology) approach can currently be applied to study the efficacy of several factors at the same time for the production process (**Karlapudi *et al*., 2018; Bunsangiam *et al*., 2021**). The CCD (Central Composite Design) is used to highlight the interaction between determined factors through Plackett-Burman design and to recognize the optimum conditions for the biological process (**Atefeh *et al*., 2019; Myo *et al*., 2019**). The CCD has a fractional matrix with center points and star points around the center point (**Bunsangiam *et al*., 2021**).
The current study aims at valorizing the tomato plant tissues through a biological process and optimizing the growth conditions for the most productive strain (*Streptomyces plicatus* PT2) to maximize its IAA production compared to standard growth conditions using purified nutrients and precursors. Furthermore, the plant-growth promotion activity of produced IAA will be investigated on tomato seedlings as well as a marketed chemical one. This is the first reasonable approach for the optimization of promising microbial bioprocess with exploring tomato plant wastes as feedstock for the production of IAA by *S. plicatus* PT2.

MATERIALS AND METHODS

Actinobacterial strains

Twelve endophytic and rhizospheric actinobacteria previously isolated and characterized for their plant growth promotion abilities in our research laboratory, were selected in order to be screened for their abilities to produce IAA. Endophytic actinobacteria were isolated from root tissues of Saharan native plants (**Goudjal *et al*., 2013; Goudjal *et al*., 2014; Goudjal *et al*., 2015; Goudjal *et al*., 2016**), and rhizospheric ones from Algerian Saharan soils plants (**Chaabane Chaouch *et al*., 2016a; Chaabane Chaouch *et al*., 2016b; Lahoum *et al*., 2016**) (Table 1). All strains have been listed belong to the microbial collection of our research laboratory (Laboratory of Biology of Microbial Systems (LBSM), ENS - Kouba, Algiers, Algeria).
The actinobacterial strains have been selected on the basis of their plant's promising growth abilities (**Zamoum *et al*., 2017; Goudjal *et al*., 2013**); nevertheless, some of them were selected for being novel species of actinobacteria (**Chaabane Chaouch *et al*., 2016a; Chaabane Chaouch *et al*., 2016b; Lahoum *et al*., 2016**).

Table 1 Origin of actinobacterial strains screened for the IAA production

	Strain	Accession number	Reference	Origin of strains (soil or host plant)
Rhizospheric strain	*Actinomadura adrarensis* strain ACD12	KU356942	**Lahoum *et al*., 2016**	Algerian Saharan soil from Adrar
	Streptosporangium becharense strain SG1	KU593506	**Chaabane Chaouch *et al*., 2016b**	Algerian Saharan soil from Béchar
	Streptosporangium sp. strain SS2	*	**	Algerian Saharan soil from Béchar
	Streptosporangium saharense strain SG20	KT581983	**Chaabane Chaouch *et al*., 2016a**	Algerian Saharan soil from Ghardaïa
	Streptomyces sp. strain RL2	*	**	Algerian Saharan soil from Laghouat
Endophytic strain	*Streptomyces* sp. strain AH1	*	**Goudjal *et al*., 2014**	*Aristida pungens*
	Streptomyces sp. strain DN16	*	**	*Phoenix dactylifera*
	Streptomyces sp. strain ML3	*	**	*Medicago laciniata*
	Streptomyces plicatus strain (PT2)	KC414013	**Goudjal *et al*., 2013**	*Panicum turgidum*
	Streptomyces asterosporus strain SN2	KC414014	**Goudjal *et al*., 2016**	*Solanum nigrum*
	Streptomyces neopeptinius strain TL7	KM891590	**Goudjal *et al*., 2015**	*Terfezia leonis*
	Streptomyces caeruleatus strain ZL2	KP399598	**Zamoum *et al*., 2015**	*Zizyphus lotus*

Legend: (*) Isolate not identified, (**) Not published data.

Screening for indole-3-acetic acid production

The screening for IAA production by actinobacteria under standard growth conditions was determined according to the method used by **Khamna *et al*.** (**2010**). One milliliter aliquot (≈ 10^6 spores mL^{-1}) from each actinobacterial spore suspensions was inoculated into 250 mL-Erlenmeyer flasks containing yeast extract-tryptone (YT) broth (50 mL) amended with L-tryptophan (5mg mL^{-1}). Flasks were then incubated in darkness at 30 °C for 5 days on a rotary shaker (200 rpm). The resulting cultures were then centrifuged for 30 min at 4000 ×g and the production of indole compounds was checked by mixing 1 mL of supernatant culture with 2 mL of Salkowski reagent (**Sadeghi *et al*., 2012**). The production of indole compounds was revealed by the development of a pink colour after 30 min in a dark room. The supernatant cultures showing positive results were separated by centrifugation (5000 rpm; 20 min) and the produced IAA was extracted by ethyl acetate as used by **Goudjal *et al*.** (**2013**). Three replicates per treatment were carried out.

Confirmation of IAA production

The Ethyl acetate extracts were evaporated in a rotary evaporator at 40 °C until dryness. Dry compounds were then re-dissolved in 500 µL methanol for the HPLC analysis (Agilent 1260® Infinity II LC; C-18 column with a reverse-phase and UV-detection at 220 – 280 nm). The mobile phase, a methanol-water linear gradient in two-step of 20 – 50% methanol (0 – 5 min) and 50 – 100% methanol (5 – 35 min) was used at 1 mL min^{-1} flow rate. Final quantization was compared with reference to external calibration curve with standard IAA (Sigma-Aldrich) (**Bunsangiam *et al*., 2021; Benadjila *et al*., 2022**).

Optimization of growth conditions for production of IAA using leaves and roots' extracts

Preparation of leaves and roots' extracts

Besides their composition in bacterial nutrient compounds, tomato plant tissues have been reported for their richness in indole-3-pyruvic acid and L-tryptophan, which are considered as precursors in the biosynthesis pathways of IAA (**Cooney and Nonhebel, 1991**). Additionally, the abundance and low-cost of tomato plant wastes worldwide have suggested their promising valorization as complementary components in a microbial process for the sake of producing IAA. Roots and leaves' extracts were prepared according to a modified method used by (**Benadjila *et al*., 2022**).
Fresh tomato roots and leaves were dried at 30 °C until constant weight, and then blended into a fine powder. Two hundred grams of each powder were soaked for 2h in 1000 mL of boiling distilled water, agitated then stored at +4 °C for 24h. Filtered extracts were then obtained using the filter paper Whatman n◦1.

Screening of culture conditions by Plackett-Burman experimental design

One milliliter of the most productive strains (*S. plicatus* PT2) spore suspension (≈ 10^6 spores mL^{-1}) was inoculated in 250 mL flasks containing 25 mL of the basic mineral medium (**Benadjila *et al*., 2022**) and incubated at 30 °C for 10 days on a rotator shaker (200 rpm). The quantity of IAA produced was evaluated as described above.
Plackett-Burman statistical design was used in order to establish fundamental medium constituents to enhance IAA production (**Plackett and Burmann, 1946**). A total of 7 variables (variable k = 7, Table S1) were retained as follows: Initial pH (X1); concentration of L-tryptophan (X2), rotation speed (X3); NaCl concentration (X4); yeast extract concentration (X5); tomato roots-extract concentration (X6) and tomato leaves-extract concentration (X7). Each variable was represented with two levels, high (+) and low (–) in 13 trials. The number of positive and negative signs per trial was (k+1)/2 and (k-2)/2, respectively.
Each line represents a trial, and each column represents an independent (assigned) variable. The effect of each variable was calculated by the equation (1):

$$E(X_i) = 2(XM_i^+ - M_i^-)/N \quad (1)$$

Where, $E(X_i)$is the concentration effect of the tested variables; $M_i^+ - M_i^-$ represents the production of IAA from the trials, where the independent variable (X_i)measured was present at high and low concentrations, respectively. N is the number of trials.
When the sign is positive, the influence of the variable on the production of IAA is greater at higher concentration and when negative, the influence of the variable is greater at low concentration. The standard error (SE) of concentration effect was the square root of the variance of an effect, and the significance level (*p*-value) of each concentration effect was determined using the Student'st- test:

$$t\ (X_i) = E(X_i)/SE \quad (2)$$

Where, $E(X_i)$ is the effect of variableX_i.

Optimization of the screened variables by RSM

In order to study the correlations and interactions between the selected factors by the Plackett-Burman experimental design, an experimental design was conceived according to the CCD of RSM.
A set of 13 experiments was required with each variable having 5 levels. The factor levels were coded as: +α (+1.41), maximum (+1), central point or middle (0), –α (–1.41), and minimum (−1) (Table 2).
The relationship between the coded values and actual values, independent variable and the response were calculated according to a second order quadratic model (Table 2). The relative effects of two variables on the response were analyzed from surface plots and the three dimensional contour plots (**Dikshit and Tallapragada, 2014**). The mathematical formula was given in equation (3).

$$Y = B_0 + \sum_{i=1}^{k} B_i x_i + \sum_{i=1}^{k} B_{ii} x^2 + \sum_{i>j}^{k} B_{ij} x_i x_j + E \quad (3)$$

Where, Y represents the response function (in our case the IAA concentration in µg mL^{-1}); B_0 is a constant coefficient; $B_i, B_{ii}$and B_{ij} are the coefficients of the linear, quadratic and interactive terms, respectively, while $x_i$and x_j represent the coded independent variables.

Plant growth promoting effect of produced IAA

The plant growth promoting (PGP) properties of the IAA produced and optimized by the strain *Streptomyces plicatus* PT2 were examined on tomato seedlings (*Solanum esculentum* L. *cv*. Aïcha).
Tomato seeds were surface-sterilized by dipping for 3 min in ethanol (70%, v/v), for 5 min in NaClO solution (1%, w/v), and then washing thrice in sterile distilled water (**Dif *et al*., 2021**).
Three different treatments were used in the *in vivo* PGP trials which consisted of: surface-sterilized tomato seeds soaked in sterile distilled water as a control treatment, surface-sterilized tomato seeds treated with 50 µg mL^{-1} of marketed chemical IAA solution (**Shi *et al*., 2009**), the third treatment consisted of soaking the surface-sterilized tomato seeds in the IAA produced by *S. plicatus* PT2 in the same concentration (**Goudjal *et al*., 2013**). The effect of a presoaking period (12, 24, 36 and 48h) on the seedlings growth was studied by measuring the dry weight, shoot and root length of seedlings.
Following a modified method used by **Dif *et al*.** (**2021**), five treated seeds were sown at a depth of 2 cm in plastic pots (12 cm high × 8 cm diameter) filled with autoclaved sandy soil (three times autoclaved for 20 min at 120 °C with an interval of 24h). A completely randomized design was used with 10 replicates for each treatment. The experiment was conducted twice under greenhouse conditions (25 °C, 60% relative humidity and 14h photoperiodicity). Pots were watered daily with 10 mL of sterile tap water. Data for the growth promotion effect were collected after 21 culture days.

Statistical analysis

In order to evaluate prototype significance and fitness, data from the Plackett-Burman model, CCD and RSM design for the IAA production were ANOVA analyzed for the response factor. Where, *p<0.05* was considered as a significant level. The package Minitab 17.1.0 software was used to compare these findings. The data from the *in vivo* PGP of tomato seedlings was statistically analyzed by Student-Newman-Keuls test at *p=0.05* using COSTAT software (V6.400).

RESULTS

Indole-3-acetic acid production

The results of the screening of the twelve actinobacterial strains for IAA production under standard growth conditions are given in Table 3. All strains were able to grow on yeast extract-tryptone broth and showed significant (*p<0.05*) variation in the amounts of IAA and dry cell weight (Table S2). After the screening step, a wide variation was observed in IAA production and dry mass, which varied from 7.5 ± 0.5 to 96.3 ± 0.4 µg mL^{-1} and from 10.0 ± 1.0 to 61.8 ± 0.2 mg mL^{-1}, respectively. The PT2 strain reached the highest amount of dry cell weight (61.8 ± 0.2 mg mL^{-1}) and IAA production (96.3 ± 0.4 µg mL^{-1}) compared to other strains (Table 3).
The supernatant culture of the strain PT2 was used for IAA extraction and HPLC analysis. The HPLC analysis showed that ethyl acetate extract from the culture filtrate of the strain and the corresponding reference of IAA standard revealed peaks at the similar retention time, which confirms the production of IAA molecules by strain PT2.

Table 3 Indole-3-acetic acid production and dry cell weight under standard growth conditions

Strain	IAA production on YT broth	
	IAA (µg mL^{-1})	Dry cell weight (mg mL^{-1})
ACD12	7.5 ± 0.5^{k}	14.9 ± 0.6^{h}
SG1	12.3 ± 0.2^{j}	25.3 ± 0.2^{g}
SS2	35.6 ± 0.9^{f}	32.8 ± 1.2^{f}
SG20	19.8 ± 0.2^{h}	42.2 ± 0.5^{d}
RL2	14.2 ± 0.4^{i}	33.5 ± 0.7^{f}
AH1	47.5 ± 1.1^{d}	42.1 ± 1.8^{d}
DN16	26.1 ± 0.3^{g}	24.0 ± 0.8^{g}
ML3	65.8 ± 0.7^{c}	50.1 ± 0.5^{c}
PT2	96.3 ± 0.4^{a}	61.8 ± 0.2^{a}
SN2	42.5 ± 1.3^{e}	39.6 ± 1.1^{e}
TL7	8.7 ± 1.6^{k}	10.0 ± 1.0^{i}
ZL2	73. 6 ± 0.8^{b}	55.2 ± 0.6^{b}

Legend: ^{m}Average; Values represent means ± standard deviation from three replicates; Means in each column with the same letters are not significantly different according to Student-Newman-Keuls test at *p = 0.05*

Significant factors determined using Plackett-Burman design

The experimental design Plackett-Burman is an effective way to improve IAA production. The strain *S. Plicatus* PT2 producing the highest IAA amount under standard growth conditions was chosen to screen the effects of different variables on IAA production using Plackett-Burman design. All the 7 variables were investigated at two levels (+1, –1) as noted in Table S1. The following polynomial equation expressed the relationship between the response and the screened variables:

$$\boldsymbol{IAA} = 72.33 + 3.03\,\boldsymbol{X_1} + 21.53\,\boldsymbol{X_2} - 1.56\,\boldsymbol{X_3} + 3.37\,\boldsymbol{X_4} + 3.50\,\boldsymbol{X_5} + 11.93\,\boldsymbol{X_6} + 1.40\,\boldsymbol{X_7} \quad (4)$$

The results obtained in Table 4 indicated that there was a wide variation in IAA production, from 24.83 to 102.7 µg mL^{-1}. The negative and positive effects of the variables on the IAA production by strain PT2 are highlighted in a Pareto diagram (Figure 1).

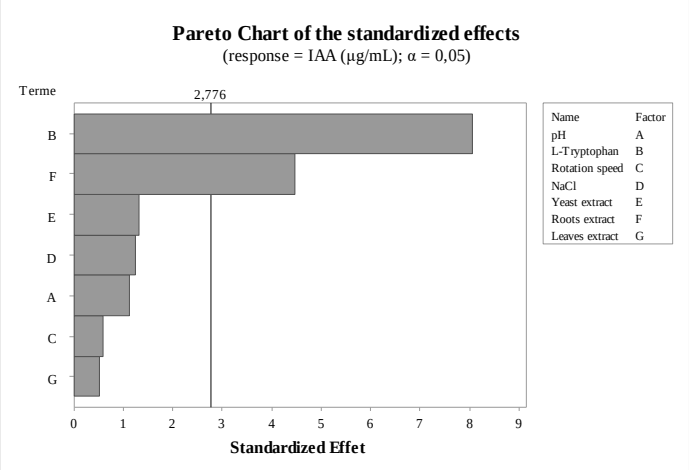


Figure 1 Plackett-Burman design Pareto chart showing the ranking of seven growth factors affecting the IAA production by the strain *Streptomyces plicatus* PT2. The confidence interval of 95% is defined in the chart by the vertical line)

The predicted and actual plots from the Plackett-Burman design of IAA production by this strain are given in Figure 2.

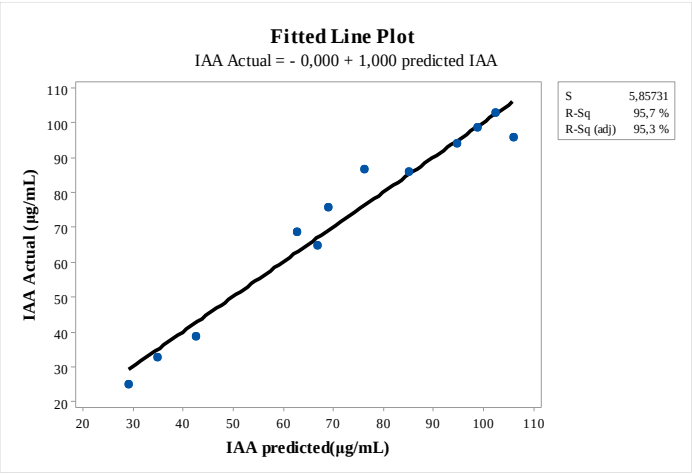


Figure 2 Predicted values for IAA production plot from Plackett-Burman design versus actual IAA production (µg mL^{-1}) by the strain *Streptomyces plicatus* PT2 under optimized growth conditions

Among the 7 variables, the ANOVA analysis showed that, L-tryptophan and roots-extract were found to be the most significant factors (*p<0.05*) (Figure 1 and Table S3). All the predicted values of the Plackett-Burman design were approximately close to the experimental values (Table 4).

Table 4 Plackett-Burman design variables with IAA production by *S. plicatus* PT2 as response

Trials	pH	L-tryptophan	Rotation speed	NaCl	Yeast extract	Roots-extract	Leaves-extract	IAA (µg mL^{-1})	
								Experimental value	Predicted value
1	+1	+1	-1	+1	-1	-1	-1	85.68	84.99
2	+1	+1	-1	+1	+1	-1	+1	94.10	94.79
3	+1	-1	-1	-1	+1	+1	+1	75.65	68.85
4	-1	-1	-1	+1	+1	+1	-1	64.50	66.74
5	+1	-1	+1	-1	-1	-1	+1	32.63	34.87
6	-1	-1	+1	+1	+1	-1	+1	38.68	42.55
7	+1	+1	+1	-1	+1	+1	-1	95.56	105.99
8	+1	-1	+1	+1	-1	+1	-1	68.53	62.66
9	-1	+1	+1	+1	-1	+1	+1	102.70	102.46
10	-1	+1	-1	-1	-1	+1	+1	98.62	98.86
11	-1	-1	-1	-1	-1	-1	-1	24.83	29.15
12	-1	+1	+1	-1	+1	-1	-1	86.52	76.09

Legend: Low level (-1); High level (+1)

This supports that the model (Equation 2) is sufficient to describe the response of the experimental observations of IAA production (Figure 2). The *f*-value of 12.85 ($p<0.05$) confirms the significance of this model (Table S3). The model *f*-value was calculated as a ratio of mean square regression and mean square residual due to the real error. The R^2 = 95.7% indicated that the entire variation was explained by the model. However, the predicted R^2 = 93.83% agreed with the adjusted R^2 = 95.3% (Table S4).

Then, the RSM statistical approach was used to optimize and improve the *in vitro* IAA production by *S. plicatus* PT2.

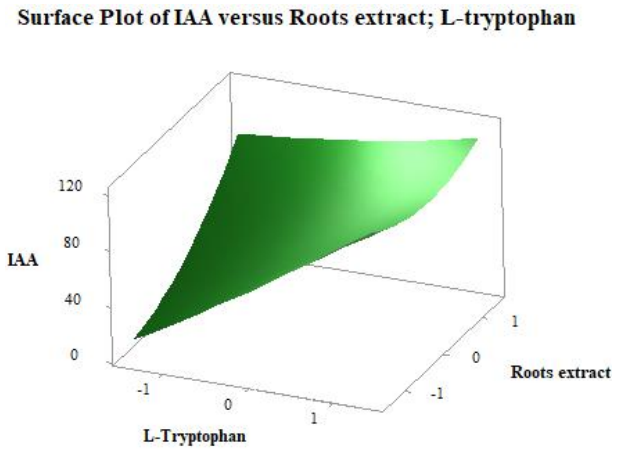
Optimization of IAA production by RSM

A CCD was utilized to study the interactions between the two significant factors: L-tryptophan and root extract quantities. The optimal levels of these factors were also determined through this analysis. The interaction between the two notable variables on the IAA production (response) using RSM is explained in equation (5).

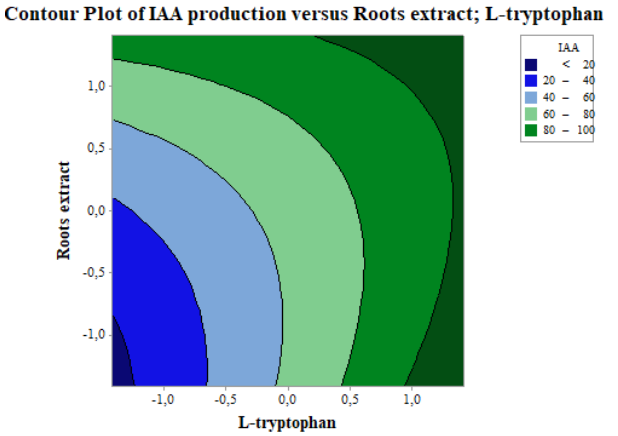
$$IAA = 66.47 + 23.09\ X_1 + 10.59\ X_2 + 0.98\ X_1 \times X_1 + 8.89\ X_2 \times X_2 - 9.96\ X_1 \times X_2 \quad (5)$$

X_1 : L-tryptophan; X_2 : the roots-extract

To study the interaction effects between the variables affecting the IAA production, the response was plotted in the form of three-dimensional (3D) surface (Figure 3A) and two-dimensional (2D) contour plots (Figure 3B) keeping one variable at constant (centralized) level and varying the other independent factor. Figure 3A represents the interaction between the two significant factors: L-tryptophan concentration and roots-extract quantity on the production of IAA. Interactions between these effective variables were considerable (Table S5). The plot showing L-tryptophan levels versus roots-extract indicated that the production of IAA increased with an elevation in L-tryptophan levels and roots-extract (Figure 3A). Concentric circular contour plots were obtained (Figure 3B). This indicated that the interaction between L-tryptophan and roots-extract quantities has a significant positive impact on the IAA production. Nevertheless, the optimum of growth conditions for the maximum of IAA production was estimated by the interpretation of the response surface plots (equation 5).



A



B

Figure 3 Response and contour plots of roots-extract and L-Tryptophan effects on IAA production by *Streptomyces plicatus* strain PT2. The surface response plot (A) represents the IAA production versus interaction of the factors with roots-extract and L-tryptophan. The contour plot (B) shows the IAA production versus the quantities of roots-extract and L-tryptophan

Thus, the maximum of IAA production by strain *S. plicatus* PT2 reached the highest level of 110.29 µg mL^{-1} when the modified yeast extract-tryptone broth was supplemented with 4g L^{-1} of L-tryptophan and 25% of tomato roots-extract (Table 2). The coefficient of determination R^2 = 93.32% was sufficient and gave acceptable compatibility with the experimental and predicted values from the proposed mathematical model (Table S6).

Table 2 Experimental IAA produced by *Streptomyces plicatus* strain PT2 and predicted IAA quantities from the Central Composite Design of factors

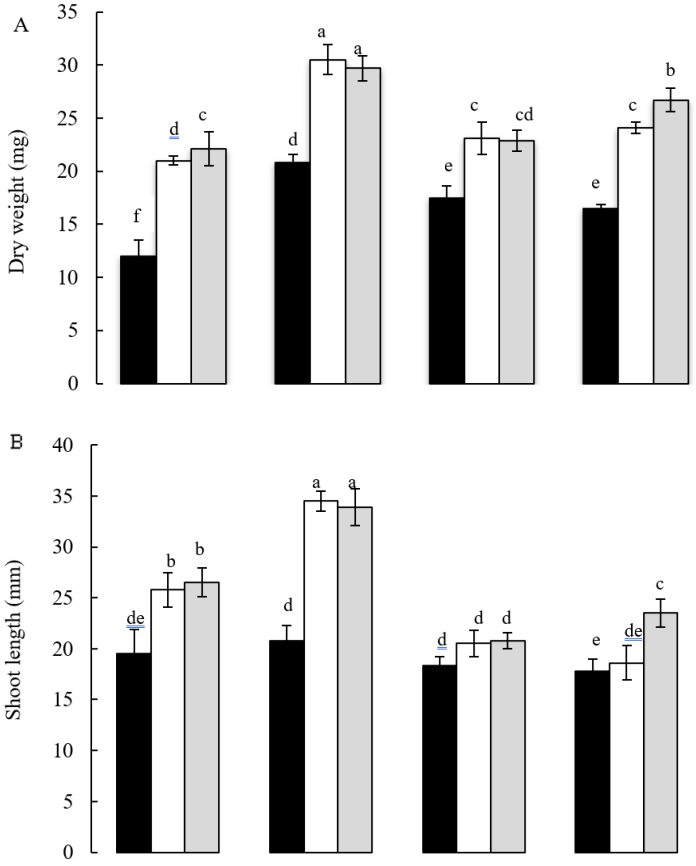
Trials	L-tryptophan	Roots-extract	IAA (µg mL^{-1})	
			Experimental	Predicted
1	0	0	67.5	66.47
2	-1.41	0	34.2	35.79
3	0	0	65.82	66.47
4	+1	-1	95.38	98.81
5	+1	+1	110.29	100.06
6	0	0	65.21	66.47
7	+1.41	0	97.38	101.09
8	0	0	66.77	66.47
9	-1	+1	82.53	73.80
10	0	0	67.07	66.47
11	-1	-1	27.78	32.71
12	0	+1.41	86.92	99.22
13	0	-1.41	76.28	69.28

Legend: Low level (-1); High level (+1); Center point (0); 1.41 (±α)

The IAA compound was experimentally produced by the strain PT2 using optimized yeast extract-tryptone broth and applied for the *in vivo* plant-growth promotion of tomato seedlings as well as the chemical marketed IAA.

Growth promotion effect of IAA treatments on tomato seedlings

The effects of IAA treatments on the *in vivo* PGP of tomato seedlings for different presoaking periods are given in Figure 4. Seeds soaked for 24h in chemical marketed IAA and produced IAA showed greatest efficacy in enhancing growth of tomato seedlings and no significant differences ($p>0.05$) were found between these two treatments. Compared to the control (untreated seeds), treatment of tomato seeds both with chemical IAA and produced IAA for 24h significantly ($p<0.05$) improved the dry weight (Figure 4A), the seedlings shoot (Figure 4B) and root lengths (Figure 4C).



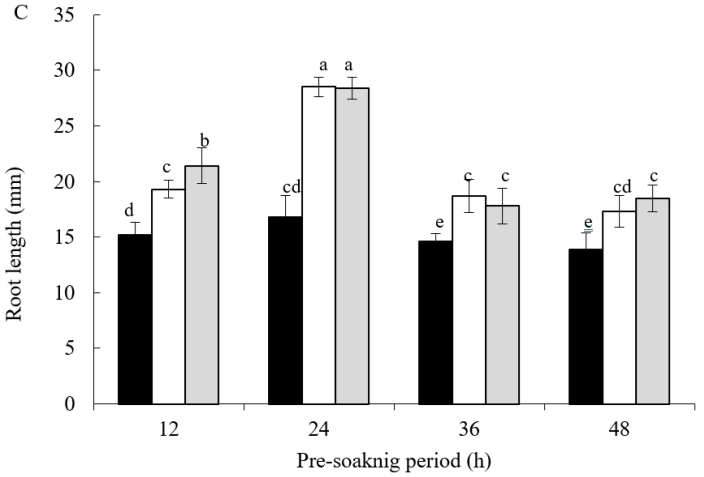


Figure 4 Effect of pre-soaking periods of tomato *cv*. Aïcha seeds in sterile distilled water (black bars), produced IAA (white bars) and chemical marketed IAA (grey bars) on the dry weight (A), shoot length (B) and root length (C). Evaluation was made 21 days after planting in autoclaved sandy soil. Error bars represent the standard deviation from 20 replicates. Bars labeled with different letters indicate significant difference between treatments according to Student-Newman-Keuls test at $p = 0.05$

DISCUSSION

Indole-3-acetic acid, a member of the group of phytohormones, is considered among the most important auxins for plant growth and development (**Teale *et al.*, 2006**). Diverse endophytic and rhizospheric actinobacteria possess the ability to release this phytohormone (**Khamna *et al.*, 2010; Zamoum *et al.*, 2015**). However, most of the bioprocesses involve expensive purified nutrients and precursors to produce IAA (**Benadjila *et al.*, 2022**). The abundance and low-cost of tomato plant tissues harvested at the end of the crop cycle, their richness with nutrients and IAA precursors (**Cooney and Nonhebel, 1991**) open up a promising valorization process by exploring actinobacteria.

In this study, the screening for IAA production showed positive results for all the tested strains under standard growth conditions and the amount of the produced IAA varied depending on the strains. The variation in IAA production depending on the actinobacterial strains and the growth conditions have been reported by **Kang *et al.* (2020); Bunsangiam *et al.* (2021)** and **Benadjila *et al.* (2022)**. Such variation depends on the different physiological pathways that use L-, D-tryptophan and indole-3-pyruvic acid as precursors for IAA biosynthesis (**Cooney and Nonhebel, 1991; Lebrazi *et al.*, 2020**). Among all the 12 actinobacterial strains, the strain *S. plicatus* PT2 produced the highest IAA level. Although several *Streptomyces* spp. have been reported to produce IAA (**El-Tarabily, 2008; Lin and Xu**, **2013**), our study is the first to report a high amount of IAA production by the actinobacterial species *Streptomyces plicatus*. This result led us to explore this promising strain and to optimize the IAA production on a broth supplemented with tomato roots and leaves extracts.

The production of IAA by the strain PT2 was screened and optimized using the statistical methods that combined the Plackett-Burman experimental design and the optimization approach RSM-CCD. The effect of pH, L-tryptophan, rotation speed, NaCl concentration, yeast extract concentration, concentration of tomato roots and leaves extracts were investigated to select the significant variables and to optimize the culture conditions for maximum IAA production. The model was statistically and experimentally validated by comparing the predicted and experimental values. Results from the optimized medium showed high IAA concentrations, which were significantly higher than those provided by the standard medium. These findings are in agreement with those of **Benadjila *et al.* (2022**) reporting a notable improvement of IAA production by *Saccharothrix texasensis* MB15 under optimized growth conditions.

Our findings also showed a wide variation in IAA production within the 12 runs of the Plackett-Burman design. As reported by **Sasirekha *et al.* (2012)** and **Benadjila *et al.* (2022**), this variation indicated the effect of broth composition optimization to maximize IAA production. Moreover, the statistical analyses confirmed that L-tryptophan and roots-extract concentrations in the tested range of variables had remarkable effects on IAA production by strain PT2. Correspondingly, **Patten and Glick** (**2002**) and **Sasirekha *et al.* (2012)** reported that an increase in L-tryptophan concentrations would stimulate higher IAA production. In addition, other researchers noted that the highest IAA productions yields by *Streptomyces* spp. were reached by supplementing media with L-tryptophan (**Goudjal *et al.*, 2013; Rashad *et al.*, 2015**). However, **Khamna *et al.*** (**2010**) deduced a significant decrease in IAA production by *Streptomyces viridis* strain CMU-H009 when supplying with high quantity of L-tryptophan. This compound is the most considerable variable with positive effect on IAA production. Our results are agreed on with **Costacurta and Vanderleyden** (**1995**), reporting L-tryptophan as a precursor in the IAA biosynthesis pathway and its addition to the bacterial culture media can increase the IAA biosynthesis.

On top of that, the roots-extract was also found to be an effective variable for improving IAA production by strain PT2. These results are in agreement with **Gopalakrishnan *et al.* (2014)** and **Benadjila *et al.* (2022**), which reported the enhancement effect of wheat roots-extract on the IAA production by actinobacteria from Saccharothrix and *Streptomyces* genera.

From the RSM-CCD study, it was found that the optimum conditions for maximum yield of IAA from PT2 strain were: 4mg mL-1 of L-tryptophan and 25% of tomato roots-extract. Our results showed a high similarity between predicted and experimental results, which reflect the applicability of RSM statistical approach to maximize IAA production using tomato roots-extract. The R^2 value (0.9332) indicated that 93.32% of the total variation that occurred in the response value could be explained by the proposed model. These findings are supported by **Chen *et al***. (**2009**) reporting the adequacy and the fitness of the suggested mathematical model confirmed by an R^2 value exceeding 0.9. The R^2 value indicates the high correlation index between the experimental value and the predicted value from the model (**Myo *et al.*, 2019**).

In planta trials were done to highlight the plant-growth promoting activity of the produced IAA on tomato seedlings. Compared to untreated seeds, the treatment with the produced IAA notably enhanced the growth of tomato plants as well as the marketed chemical IAA. These results are agreed on by **Goudjal *et al.*** (**2013**), which confirmed the possible application of bacterial produced IAA for the growth improvement of plants. The positive correlation between the plant-growth promotion and amount of IAA produced was previously highlighted by **El-Tarabily** (**2008**); **Hamdali *et al.*** (**2008**); **Goudjal *et al.*** (**2013**) and **Lin and Xu** (**2013**). Nevertheless, this is the first report showing the growth promotion effect of produced IAA under optimized broth supplemented with tomato root extract.

The effect of presoaking period indicated that IAA treatment for 24h had the highest PGP effect. **Shi *et al.*** (**2009**) and **Goudjal *et al.*** (**2013**) reported that the growth promotion of seedlings is significant with a low quantity of IAA. Nonetheless, pre-soaking period of 36 – 48h decreased the dry weight, shoot and root lengths. These findings are in agreement with **Goudjal *et al.*** (**2013**) reporting an adverse effect of long IAA treatments on the seedlings growth.

CONCLUSION

Our study focused on producing and optimizing IAA by actinobacteria using RSM-CCD method. All the screened actinobacteria grew and produced IAA differently under the standard growth conditions. The maximum of IAA production was recorded by the strain PT2 with 96.3 µg mL^{-1}.. Screening of the most influential factors affecting IAA production was adopted using the Plackett-Burman design. L-tryptophan and roots-extract were found to be the significant factors affecting the IAA production. The adoption of such mathematical strategy was highly significant with an R^2 of 95.7%. The use of tomato roots-extract and the optimization of the growth conditions with the RSM-CCD approach was very noteworthy with an increased up to 12.7% in IAA production compared to the standard YT broth with a coefficient of determination R^2 = 93.32%. *In-planta* results indicated that the best seed treatment was achieved after 24h of pre-soaking in the produced IAA with an overall increase of 31.8%, 39.7% and 41.05% in dry weight, shoot and root lengths compared to untreated seeds. At our knowledge, there is no information on medium components optimization for IAA production by *S. plicatus* strain PT2 by a RSM-CDD approach using tomato roots and leaves as cheap agricultural wastes. Furthermore, the findings of the present study highlighted a promoting perspective of application *in-planta* of low-cost produced IAA by eco-friendly optimized fermentation.

These findings could be stated as the potential of these IAA producing strains and the study of optimization of IAA production will promote the growth and ultimately the production of IAA in the field and are urgently needed to reduce environmental pollution due to the current excessive use of agrochemicals harmful to crop fields.

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Authors' contributions: We confirm that this research was conducted in our research laboratory. All authors contributed critically in performing and analyzing the results. YG, AZ and MZ conceived and designed the research. KA, MZ and AB conducted the experiments and analyzed the data. KA YG wrote the manuscript. MZ supervised the research, corresponding author. All authors read and approved the final manuscript.

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Declarations

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SUPPLEMENTARY MATERIALS

Table S1 Experimental range and levels of the independent variables

Variables	Low (-)	High (+)
pH	4.0	8.0
L-tryptophan	0.0	4.0
Rotation speed	150.0	250.0
NaCl	3.5	7.5
Yeast-extract	4.0	8.0
Roots-extract	12.5	25.0
Leaves-extract	12.5	25.0

Table S2 Analysis of variance mean squares of screening of IAA production on yeast extract-tryptone (YT) broth under standard growth conditions

Source of variation	*df*	IAA production (µg mL^{-1})	Dry cell weight (mg mL^{-1})
Strain	11	2465.47***	1063.23***
Coefficient of Variation (%)	/	2.67	2.65

Legend: *** Very highly significant difference (*p<0.001*); *df*- Degree of freedom

Table S3 Analysis of variance mean squares of IAA production using Plackett-Burman design.

Variables	Effect	Coefficient	P-Value
pH	6.05	3.03	0.321
L-tryptophan	43.06	21.53	0.001
Rotation speed	-3.13	-1.56	0.590
NaCl	6.73	3.37	0.277
Yeast-extract	7.00	3.50	0.260
Roots-extract	23.85	11.93	0.011
Leaves-extract	2.79	1.40	0.629
Model (F-Value = 12.85)			0.013

Legend: No significant difference (*p>0.05*); Highly significant difference (*p<0.01*); Very highly significant difference (*p<0.001*)

Table S4 Fit for first-order model in Plackett-Burman Design.

R Square	0.96
R Square Adjusted	0.95
Root Mean Square Error RMSE	5.86
Mean of Response	72.33

Table S5 Analysis of variance mean squares of IAA production using RSM.

Variables	Effect	Coefficient	P-Value
L-tryptophan	46.18	23.09	0.000
Roots extract	21.18	10.59	0.007
L-tryptophan*L-tryptophan	1.97	0.98	0.752
Roots extract*Roots extract	17.78	8.89	0.021
L-tryptophan*Roots extract	-19.92	-9.96	0.040
Model (F-Value = 19.57)			0.001

Legend: No significant difference (*p>0.05*); Highly significant difference (*p< 0.01*); Very highly significant difference (*p<0.001*)

Table S6 Fit for second-order model in central composite design/response surface methodology design.

R Square	0.93
R Square Adjusted	0.88
Root Mean Square Error RMSE	7.90
Mean of Response	66.47