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I J N R E

PLACKETT-BURMAN modeling of *Saccharothrix texasensis* MB15 IAA production on agricultural wastes

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Résumé:

L'objectif de ce travail est l'optimisation des conditions de fermentation en valorisant des déchets agricoles (racines et feuilles de blé) pour la production biotechnologique de l'acide indole-3-acétique par des souches d'actinobactéries. A partir de 18 souches d'actinobactéries de la collection du Laboratoire de Biologie des Systèmes Microbiens (LBSM, ENS de Kouba), un screening préliminaire pour la production de l'AIA a été effectué in vitro en conditions standards sur bouillon YT. Un deuxième criblage de l'aptitude de la souche *Saccharothrix texasensis* MB15 choisie pour produire l'AIA sur un milieu de fermentation à base de déchets agricoles (racines et feuilles de blé). Suivi d'un design d'optimisation mathématique et expérimental par la matrice de PLACKETT-BURMAN de 11 facteurs de fermentation in vitro.

Les résultats montrent que la souche *Saccharothrix texasensis* MB15 est la meilleure souche productrice d'AIA. *S. texasensis* MB15 est apte de produire d'AIA sur un milieu à base des racines et feuilles de blé. Les valeurs optimales pour les facteurs modulateurs testés pour la production maximale d'AIA sont : L-tryptophane 1,25 (g/L), charge microbienne initiale 5.106 (spore/L) et l'extrait des feuilles de blé à 50 % m/v. La production maximale l'AIA de la souche MB 15 était de 140,5 µg/ml. Trente-cinq expériences seulement étaient nécessaires pour évaluer l'effet de 11 facteurs. L'adéquation du modèle était très satisfaisante avec un R2=0,94 RMSE=8,798.

Ce travail nous a démontré l'efficacité de l'optimisation par les matrices de PLACKETT-BURMAN en déterminant les conditions optimales d'une production importante d'AIA.

Mots clés : Acide indole-3-acétique (AIA); Plackett-Burman; *Saccharothrix Texasensis*; actinobactéries.

Abstract:

This work aims to the optimization of fermentation conditions by recovering agricultural waste (roots and leaves of wheat) for the biotechnological production of indole-3-acetic acid by strains of actinobacteria. Starting with eighteen actinobacteria strains from the collection of the Laboratory of Biology of Microbial Systems (LBSM, ENS of Kouba); a preliminary screening for the production of AIA was carried out in vitro under standard conditions on YT broth. A second screening of the selected strain *Saccharothrix texasensis* MB15's ability to produce IAA on a fermentation medium based on agricultural waste (wheat roots and leaves) was accomplished. The PLACKETT-BURMAN matrix was then used to build a mathematical and experimental optimization design of 11 in vitro fermentation conditions factors.

The results indicates that *Saccharothrix texasensis* strain MB15 is the best IAA producing strain. The strain MB15 is able to produce IAA on a medium based on the roots and leaves of wheat only. The optimal values for the modulating factors tested for maximum IAA production are: L-tryptophan 1.25 (g / L), initial microbial charge 5.106 (spore / L) and extract of wheat leaves at 50% w/v. The maximum IAA production of strain MB 15 was 140.5 µg / ml. Only thirty-five experiments were needed to assess the effect of 11 factors. The fit of the model was very good with an R2 = 0.94; RMSE = 8.798.

This work has shown us the effectiveness of the optimization by the matrixes of PLACKETT-BURMAN in determining the optimal conditions for a large production of IAA.

Keywords: Indole-3-acetic acid; Plackett-Burman; *Saccharothrix texasensis*; actinobacteria

1. Introduction

Auxins are fundamental phytohormones that regulate the promotion and development of plant growth and differentiation (Halliday et al., 2009). Several strains of rhizospheric and endophytic actinobacteria are able to produce IAA from L-tryptophan (Duca et al., 2020). Actinobacteria are particularly beneficial for the biodegradation of organic wastes in specific biological processes such as postharvest agriculture residue degradation (Rateb et al., 2018 and Rastogi et al., 2020). For this, several mathematical approaches such as the degree of experience (DOE) have been used to optimize the biotechnological processes of degradation of wastes and production of high value fermentation products such as enzymes, phytohormones, pharmaceuticals and vitamins (El-Naggar et al.,2016 and Yun et al., 2018). The Plackett-Burman design is one of the relevant multivariate approaches most appropriate for experimental design, mathematical modeling, and statistical optimization of several biotechnological processes (Karlapudi et al., 2018).

The purpose of this research is to optimize the fermentation conditions for the synthesis of IAA in vitro by the actinobacterial Saccharothrix texasensis MB15 strain using agricultural waste, principally wheat roots and leaves.

2. Materials and Methods

2.1. Actinobacterial strains

Eighteen strains of rhizospheric and endophytic actinobacteria, from the collection of the Laboratory for the Biology of Microbial Systems (LBSM, ENS of Kouba Algiers; Algeria) were selected for this study. Endophytic actinobacterial strains were obtained from native and medicinal plants tissues, whereas soil actinobacteria were isolated from Algerian Saharan environments (Table1).

Microbial suspensions of the 18 strains of actinobacteria were prepared for in vitro production of IAA under standard culture conditions as reported by (Vikram et al., 2018 and Sadeghi et al., 2012).

Each strain was cultured at 30 °C on ISP 2 plates (glucose: 4 g/l; yeast extract: 4 g/l; malt extract: 10 g/l; agar: 20 g/l; pH 7) (Atlas., 2010). Actinobacterial spores were collected after 7 days of culture, using a 0.05 percent Tween-20 solution, and the concentration of the resulting spore suspension was adjusted to 10^6 spores/ml, using the Thoma cell as described by (Zamoum et al., 2015).

2.2. Assessment for IAA production

The production of IAA by actinobacteria was determined under standard culture conditions according to the modified method of Khamna et al., (2010). One milliliter aliquot of actinobacterial spore suspensions (≈10^6 spores/ml) were transferred to a 250 ml conical flask containing 50 ml yeast extract-Tryptone broth (Tryptone: 10 g/l; yeast extract: 5 g/l; NaCl: 5 g/l; L-tryptophan: 1 g/l; pH 7.2). (Atlas., 2010) supernatant cultures were extracted by centrifugation at 5000 rpm for 20 minutes after incubation on an orbital rotary shaker (200 rpm) at 30 °C for 5 days. IAA was revealed by mixing 2 mL supernatant culture with 4 mL Salkowski reagent revealed IAA production. Positive IAA production was shown by the formation of a pink color after 30 minutes in a dark room. A spectrophotometer (JANWAY-6405) was used to measure optical density at 530 nm, and the amount of IAA produced was calculated using a standard IAA graph (Acros Organics) (Ruanpanun et al. 2010).

Table 1. Origin of actinobacterial strains.

	Strain	Reference	Origin of strains (soil or host plant)
Soil strain	*Saceharothrix texasensis* strain MB 15	*	Algerian Saharan soil from Ghardaia
	Nocardiopsis dassonvillei strain MB22	*	Algerian Saharan soil from Ghardaia
	Streptosporangium becharense strain SGI	(Chaabane Chaouch et al. 2016b)	Algerian Saharan soil from Béchar
	Streptosporangiurn saharense strain SG20	(Chaaban Chaouch et al. 2016a)	Algerian Saharan soil from Ghardaia
Endophytic strain	*Streptomyces* sp. strain SN3	(Goudjal et al. 2016)	*Aristida pungens*
	Streptomyces cyaneofuscatus strain AR2	(Goudjal et al. 2013)	*Astragalus armatus*
	Streptomyces sp. Strain NS 13	(Goudjal et al. 2013)	*Cleome arabica*
	Streptomyces mutabilis strain CA2	(Goudjal et al. 2013)	*Cleome arabica*
	Streptomyces sp. strain DN4	*	*Phoenix dactylifera*
	Streptomyces sp. strain ML4	*	*Medicago laciniata*
	Streptomyces rochei strain PT2	(Goudjal et al. 2013)	*Panicum turgidum*
	Streptomyces asterosporus strain SN2	(Goudjal et al. 2016b)	*Solatium nigrum*
	Streptomyces neopeptinius strain TL8	(Goudjal et al. 2016a)	*Terfezia leonis*
	Streptomyces caeruleatus strain ZL2	(Zamoum et al. 2015)	*Zizyphus lotus*
	Streptomyces sp. Strain ML2	*	/
	Saceharothrix longispora strain MB29	*	/
	Streptomyces sp. strain AL4	(Goudjal et al. 2016b)	*Astragallus armatus*
	Streptomyces sp. strain CA12	(Goudjal et al. 2013)	*Cleome arabica*

* Strain from the actinobacterial collection of the LBSM Laboratory (Laboratoire de Biology des Systèmes Microbiens), ENS – Kouba, Algiers, Algeria.

2.3. Screening for growth and IAA production using a mixture of root and leaf extract

Recovering biological waste from durum wheat (Triticum durum Desf.), Leaves and roots were dried at 40 °C for 48 h to constant weight. The dried samples were then ground to a fine powder. In a ratio of 1/2 (powder / distilled water), five hundred grams (500 g) of each powder (leaves or roots) was soaked in 1000 ml of boiling distilled water. The mixture was stirred and kept for 48 h in the refrigerator at 4 ° C. then the resulting mediums were filtered through a filter paper Whatman n°1. one ml of *S. texasensis* MB15 spore suspension (≈10^6 spores/ml) was inoculated In 250 ml flasks containing 25 ml of Basic Mineral Medium [NH_4NO_3: 2.5 g/l; Na_2HPO_4 $2H_2O$: 1.0 g/l; $MgSO_4$ $7H_2O$: 0.5 g/l; $Fe(SO_4)_3$ $5H_2O$: 0.01g/l; $Co(NO_3)_2$ $6H_2O$: 0.005g/l; $CaCl_2$ $2H_2O$: 1.0 mg/l; KH_2PO_4: 0.5 mg/l; $MnSO_4$ $2H_2O$: 0.1 mg/l; $(NH_4)_6$ Mo_7O_{24} $4H_2O$:0.1 mg/l] (Atlas., 2010),., supplemented with 25 ml of (roots extract ml /leaves extract ml) Table 2 ; the IAA concentration was determined as previously described.

Table 2. Composition in ml of the medium of leaf / root extracts.

	1st	2nd	3ed	4th	5th
Leaf extracts ml	0	6.25	12.5	18.75	25
Root extracts ml	25	18.75	12.5	6.25	0

2.4. Screening of cultural conditions for IAA production by Plackett-Burman design

Plackett and Burman (1946) mathematical design was used to provide fundamental cultural conditions to enhance IAA production by S. texasensis strain MB15. A total of eleven variables were chosen (variable k = 11, Table 3). Temperature (X_1), incubation period (X_2), initial pH (X_3), inoculum quantity (X_4), rotation speed (X_5), NaCl concentration (X_6), L-Tryptone concentration (X_7), yeast extract concentration (X8), L-tryptophan concentration (X_9), roots extract concentration (X_{10}), and leaves extract concentration (X_{11}) were the eleven variables to studied by the Plackett-Burman statistical plan.

Table 3. Low and high levels of Plackett-Burman screening design.

Variables	Low level (-1)	High level (+1)
X_1 : Temperature (°C)	25	35
X_2 : Incubation time (day)	4	7
X_3 : Initial pH	6.5	7.5
X_4 : Inoculum quantity (CFU/ml)	10^5	10^7
X_5 : Rotation speed (rpm)	80	240
X_6 : NaCl (g/l)	3	7
X_7 : Tryptone (g/l)	05	15
X_8 : Yeast extract (g/l)	03	07
X_9 : L-tryptophan (g/l)	0.5	1.5
X_{10} : Roots extract (ml)	12.5	25
X_{11} : Leaves extract (ml)	12.5	25

In these trials, each variable was represented by two levels: high (+) and low (-). (Table 3). (K +1)/2 and (k - 2)/2 were the number of positive and negative signs each trial, respectively. Each column represents an independent (assigned) variable, and each row represents a trial. The equation (01) was used to determine the effect of each variable:

$$E(X_i) = \frac{2(XM_i^+ - M_i^-)}{N} \qquad (01)$$

Table 4. Plackett-Burman design matrix in coded values and the responses of IAA production (µg/ml).

Run	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	[IAA] µg ml^{-1}	
												Experimental	Predicted
1	1	-1	1	1	1	-1	-1	-1	1	-1	-1	103.00	103.52
2	-1	1	1	1	-1	-1	-1	1	-1	-1	1	67.00	45.34
3	-1	-1	1	-1	1	1	1	-1	-1	-1	1	104.00	102.76
4	-1	1	-1	-1	1	-1	1	1	1	-1	-1	70.00	72.34
5	1	-1	1	1	1	-1	-1	-1	1	-1	-1	102.00	101.67
6	-1	1	1	1	-1	-1	-1	1	-1	-1	1	100.00	102.76

7	-1	1	-1	-1	1	-1	1	1	1	-1	-1	67.00	65.91
8	-1	-1	1	-1	-1	1	-1	1	1	1	-1	64.00	64.05
9	1	-1	-1	-1	1	-1	-1	1	-1	1	1	107.00	80.34
10	1	1	-1	-1	-1	1	-1	-1	1	-1	1	103.00	101.67
11	1	1	1	1	1	1	1	1	1	1	1	63.00	64.67
12	-1	-1	1	-1	-1	1	-1	1	1	1	-1	68.00	80.34
13	-1	1	1	1	-1	-1	-1	1	-1	-1	1	34.50	45.33
14	-1	-1	-1	1	-1	-1	1	-1	1	1	1	66.00	64.05
15	1	-1	1	1	1	-1	-1	-1	1	-1	-1	104.00	103.52
16	-1	-1	-1	1	-1	-1	1	-1	1	1	1	138.00	137.67
17	1	-1	-1	1	-1	1	1	1	-1	-1	-1	100.00	101.67
18	1	1	-1	-1	-1	1	-1	-1	1	-1	1	66.00	80.34
19	1	-1	-1	-1	1	-1	-1	1	-1	1	1	137.00	137.00
20	-1	-1	1	-1	1	1	1	-1	-1	-1	1	103.00	103.54
21	1	-1	-1	1	-1	1	1	1	-1	-1	-1	135.00	137.67
22	-1	-1	1	-1	1	1	1	-1	-1	-1	1	136.00	137.00
23	-1	1	-1	-1	1	-1	1	1	1	-1	-1	138.00	137.00
24	-1	1	-1	1	1	1	-1	-1	-1	1	-1	69.00	73.25
25	1	1	1	1	1	1	1	1	1	1	1	62.00	64.05
26	-1	-1	1	-1	-1	1	-1	1	1	1	-1	65.00	64.67
27	1	-1	-1	-1	1	-1	-1	1	-1	1	1	104.00	102.76
28	-1	1	-1	1	1	1	-1	-1	-1	1	-1	73.00	72.33
29	1	-1	-1	1	-1	1	1	1	-1	-1	-1	66,00	64.67
30	1	1	-1	-1	-1	1	-1	-1	1	-1	1	34.00	45.33
31	1	1	1	1	1	1	1	1	1	1	1	73.00	72.34
32	-1	-1	-1	1	-1	-1	1	-1	1	1	1	76.00	73.25
33	1	1	1	-1	-1	-1	1	-1	-1	1	-1	140,00	137.67
34	-1	1	-1	1	1	1	-1	-1	-1	1	-1	73.00	73.25
35	1	1	1	-1	-1	-1	1	-1	-1	1	-1	66.00	65.91
36	1	1	1	-1	-1	-1	1	-1	-1	1	-1	64.00	65.90

Where E (X_i) is the concentration effect of the variables being tested. IAA production is represented by M_i^+ and M_i^- in trials where the independent variable (X_i) was measured at high and low concentrations, respectively. The number of trials is N. (equals to 36). When the sign is positive, the variable's influence on IAA production is stronger at higher concentrations, and when the sign is negative, the variable's influence is stronger at lower concentrations. The square root of an effect's variance was used to calculate the standard error (SE), and the significance level (P- value) of each concentration effect was established using the Student's t- test equation (02):

$$t(X_i) = \frac{E(X_i)}{SE} \qquad (02)$$

Where, E (X_i) is the effect of variable X_i.

2.5. Statistical analysis

All assays were carried out in triplicates, and results are expressed as mean ± SD. Results from the Plackett-Burman model, of the IAA production was statistically analyzed using ANOVA for the response factor in order to evaluate prototype significance and fitness. $\rho < 0.05$ was considered to be a significant level. The software packages JMP-SAS 11.0 and The Graph-pad Prism software 8.0.2 were used to evaluate all the results to draw all the graphs results.

3. Results and Discussions

3.1. Indole-3-acetic acid production

Under standard cultured conditions, testing of the eighteen actinobacteria for IAA production revealed that all strains were capable of producing IAA (Fig. 1). Streptomyces sp. strain ML2 produced the lowest amount, 5.13±0.07 µg/ml, whereas S. texasensis MB15 produced the highest amount, 40.10±0.86 µg/ml. A few of the eighteen strains produced large quantities of IAA, including *Streptomyces rochei* strain PT2 and *Streptomyces caeruleatus* strain ZL2, which produced 35.12±0.91 µg/ml and 33.24±0.24 µg/ml of IAA, respectively. To estimate IAA production in the next step of this study and basing on the basic mineral broth supplemented with leaves and/or roots wheat extracts, the highest IAA producing strain *S. texasensis* MB15 was chosen.

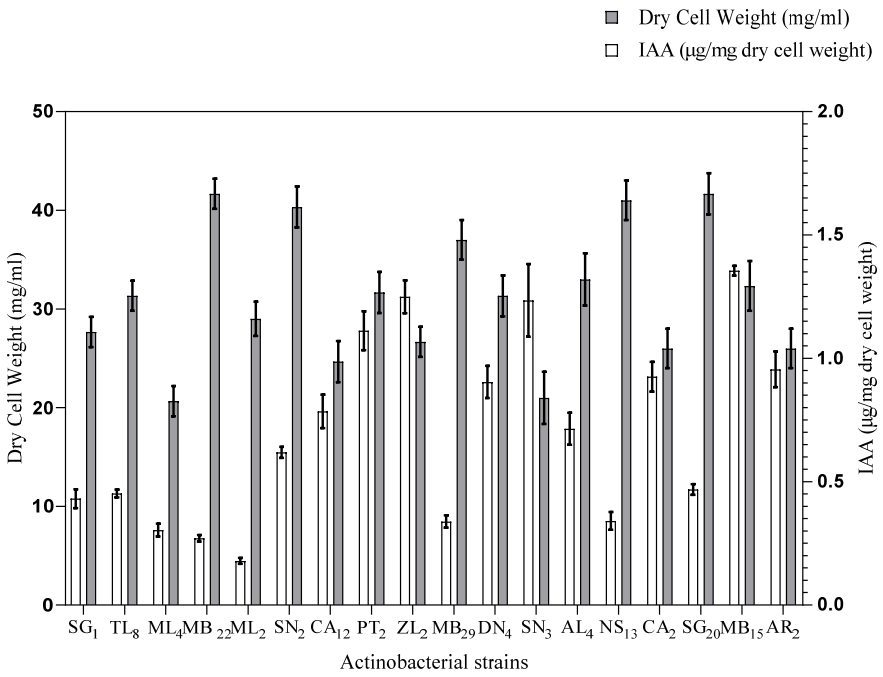
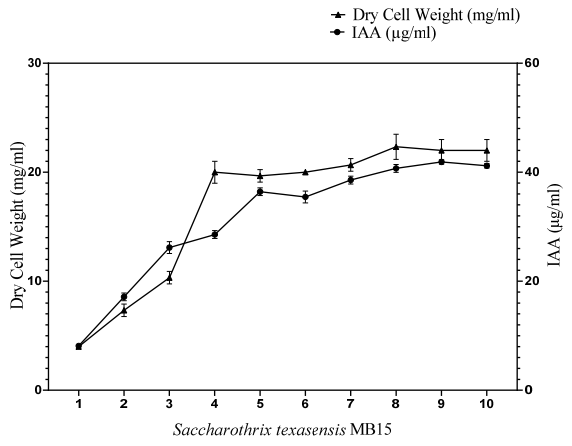
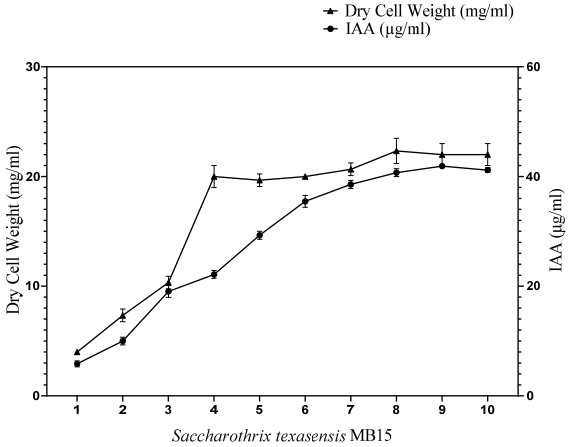


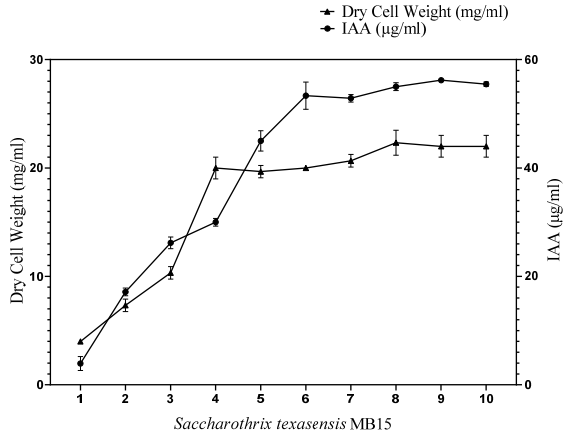
Fig.1. Growth ratio of actinobacterial strains and production of indole 3-acetic acid on Yeast extract-Tryptone broth under standard cultural conditions



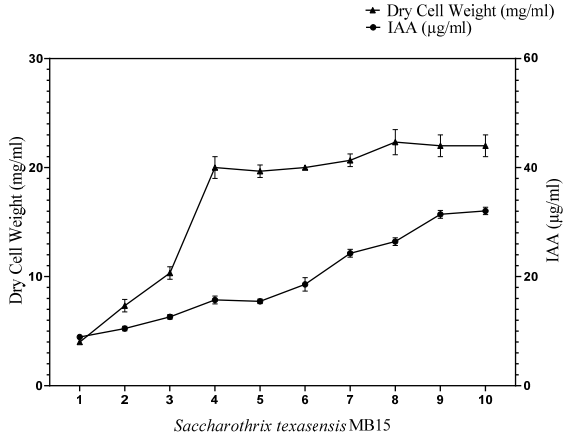
(a) leaf-root extract medium (0% root extract 100% leaf extract)



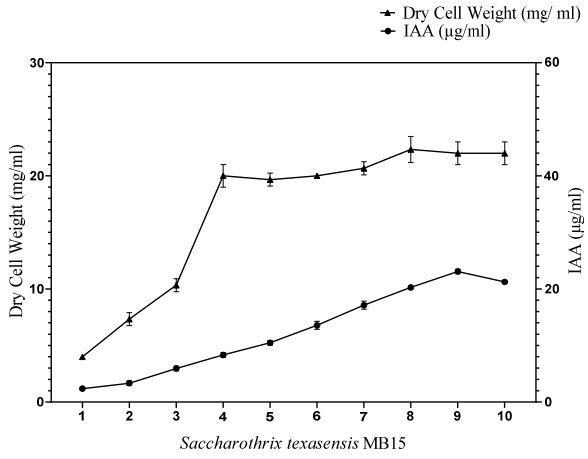
(b) leaf-root extract medium (25% root extract 75% leaf extract)



(c) leaf-root extract medium (50% root extract 50% leaf extract)



(d) leaf-root extract medium (75% root extract 25% leaf extract)



(e) leaf-root extract medium (100% root extract 0% leaf extract)

Fig.2. Growth ratio and production of indole-3-acetic acid on basic mineral media supplemented with leaves and/or roots extracts.

Figure 2 shows the growth and IAA production of *S. texasensis* MB15 on these media. The effect of medium composition on MB15 growth and IAA production is clearly visible in these curves.

The production of IAA ranges from 8.15±0.38 µg/ml to 42.07±0.12 µg/ml in Fig. 2a, with the maximum amount of IAA production occurring on the eighth day of culture. The IAA production profile in Fig. 2b is very identical, ranging from 6.03±0.29 µg/ml to a maximum of 41.19±0.34 µg/ml. In comparison to the results in Fig. 2a and Fig. 2b, distinct profiles of IAA production were found in Fig. 2d and Fig. 2e, revealing low IAA production.

In Fig.2c, it is visible that IAA production began to increase on day 5 and peaked on day 9, with a maximum of 56±0.33 µg/ml. The change of YT broth with a basic mineral broth enriched with leaves and/or roots extracts was found to be particularly effective in increasing IAA production, with a total increase of 1.4 fold. After 9 days of incubation, the medium mixture of 50 percent leaves and 50 percent roots extracts demonstrated the maximum degree of IAA production. In comparison to the results of the previous tests (Fig.2a and Fig.2b), Fig. 2d and 2e indicate different profiles of IAA generation, with a maximum of 32.28±0.23 µg/ml only on the 9th day of culture.

3.2. Plackett-Burman design for estimating significant variables

The positive and negative effects of the selected variables on IAA production by *S. texasensis* MB15 on modified YT supplemented with wheat leaves and/or roots extracts are depicted in a Pareto diagram (Fig. 3) and an Actual by predicted plot using Plackett-Burman design (Fig 4). The most important model values were listed in Tables 5 and 6.

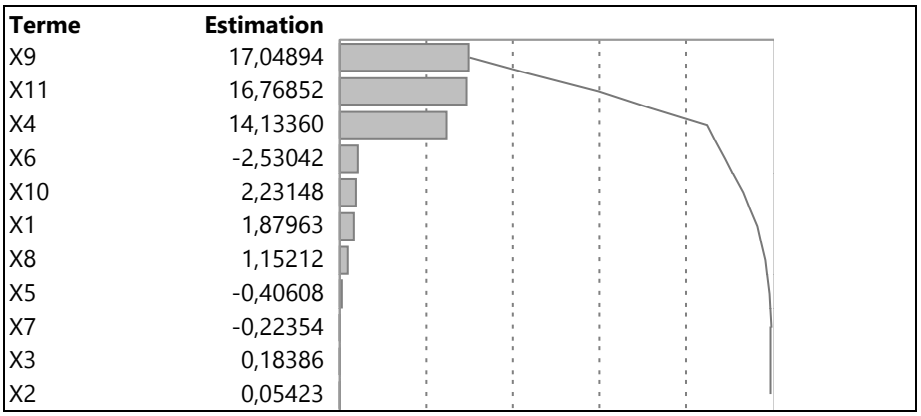


Fig 3. Pareto diagram.

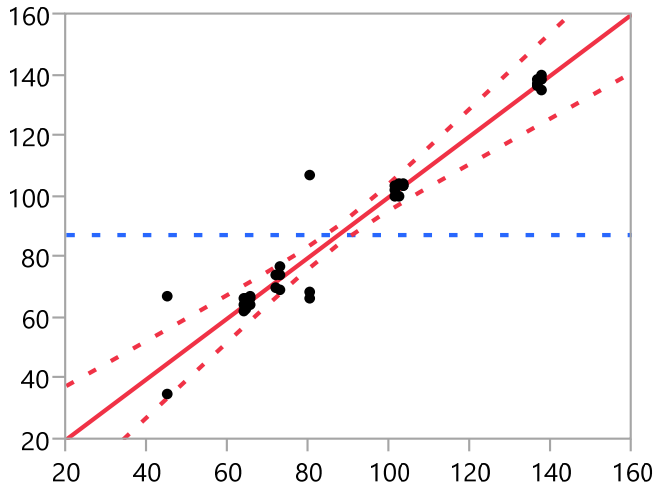


Fig 4. Actual by predicted plot for Plackett-Burman design.

Table 5. Fit for first-order model in Plackett-Burman Design.

R Square	**0.94**
R Square Adjusted	0.91
Root Mean Square Error RMSE	8.79
Mean of Response	87.37

Table 6. Fit for first-order model in Plackett-Burman Design and ANOVA for first-order model in Plackett-Burman Design.

Source	DF	Sum of Squares	Mean Square	*F* value	*p* value>*F*
	11	28371.70	2579.25	33.32	< .0001
X_1	1	127.19	127.19	1.643	0.2121
X_2	1	0.11	0.11	0.001	0.9708
X_3	1	1.22	1.22	0.016	0.9013
X_4	1	7191.31	7191.31	92.91	<0.0001*
X_5	1	5.94	5.94	0.08	0.7842
X_6	1	230.51	230.51	2.98	0.0973
X_7	1	1.80	1.80	0.03	0.8801
X_8	1	47.79	47.79	0.62	0.4397
X_9	1	10464.00	10463.99	135.19	<0.0001*
X_{10}	1	179.26	179.26	2.32	0.1411
X_{11}	1	10122.60	10122.60	130.78	<0.0001*
Residual	24	1857.71	77.40	/	/
Corrected total	35	30229.41	/	/	/

*p <0.005 significant

The most relevant medium components affecting IAA production were investigated using the PB model. Three factors (leaves extract, L-tryptophan, and inoculum quantity) were shown to be the most important in affecting IAA yield production, with significant p values and "Prob< F" values less than 0.05 indicating model terms are significant. The remaining components have p values (0.1) that are significantly higher than the significant level. The main effects of the medium elements, standard variance analysis (ANOVA), and coefficient of regression, F values, and p values of the variables studied in this study are presented in the table below (Table 6). The model probability F = 33.322 indicates that the model is valid

and that there is only a 0.0001% chance that this high result is due to noise. The predicted R^2 = 0.940, on the other hand, agreed with the adjusted R^2 = 0.91.

The signal-to-noise ratio is examined with Adequate precision. The RMSE ratio is desired, less than 10 (Table 5), and the 87.374 mean response ratio indicates an adequate model design. As a result, this model can be used to explore the design possibilities. As shown in equation (3), the final equation was constructed using elements that revealed IAA production as a function of independent variables:

$$IAA = 87.37 + 1.88X_1 + 0.06X_2 + 0.18X_3 + 14.13X_4 + 0.41X_5 + 2.53X_6 + 0.23X_7 + 1.15X_8 + 17.05X_9 + 2.23X_{10} + 16.77X_{11} \quad (03)$$

4. Discussions

In this study, we were focused on examining the production of IAA in standard cultural conditions from endophytic and rhizospheric actinobacteria. Depending on the actinobacterial strain, the amount of IAA produced by the isolates varied. These findings are in accordance research on the IAA synthesis and production by the actinobacteria genus (Khamna et al., 2010; Abd-Alla et al., 2013; Zamoum et al., 2015). The significant amount of IAA produced by *S. texasensis MB15* under standard culture conditions prompted researchers to choose and investigate this promising strain for future testing and large IAA scale production. The findings of the experiments demonstrated that IAA may be produced on a low-cost medium based on a biological waste such as wheat leaf or root extracts. That was also promising results that are in accordance with Peng et al's results. (2014). High tryptophan levels in roots and leaves increase IAA production by rhizospheric or endophytic actinobacteria in the soil (Gopalakrishnan et al., 2014), which could enhance IAA biosynthesis in a basal mineral medium supplemented with leaves and roots wastes. Furthermore, after transformation by aerobic bacteria such as actinobacteria, organic wastes constitute a rich source of tryptophan (Kravchenko et al., 2004). This occurs under the cultural conditions of our experience. Actinobacteria are primary decomposers of organic compounds, notably lignocellulosic wastes, and have been shown to produce a wide spectrum of hydrolytic enzymes in vitro and in vivo as part of a saprophytic microbial community (Makoi et al., 2008).

Using the Plackett-Burman design, we optimized the culture conditions for the selected S. texasensis strain MB15 for IAA production. The Plackett-Burman design helped to highlight the impact of the 11 variables and to optimize *S. texasensis* MB15's cultural conditions for maximum IAA synthesis. As a result, an effective model for explaining the response of experiments involving the production of IAA by the strain MB15 was established. The experimentally obtained values are in agreement with the model's predicted values. By comparing observed and predicted values, the model was validated to the optimal level. *S. texasensis* MB15's IAA production was greatly increased due to the optimization approach. The mathematical approach optimization of variables using Plackett-Burman design has proven to be an effective system for microbial biotechnology. The Plackett-Burman design for IAA production runs was found to be very varied due to large number of variables eleven. Our findings are agreement with those of Sasirekha et al. (2012), who reported the accuracy of the Plackett-Burman design in determining the most important influence of each parameter of cultural conditions on IAA production.

The impact of inoculum quantity, L-tryptophan, and leaves extract on IAA production by *S. texasensis* MB15 was determined by analyzing the significance of each quadratic regression model coefficient.

Rashad et al. (2015) found that the inoculum quantity and amount of L-tryptophan had a substantial effect on the production of IAA by Streptomyces sp. However, to our knowledge, this is the first study to show that wheat leaves extract has a significant effect on IAA synthesis by an actinobacterial species.

Higher or lower L-tryptophan concentrations have a substantial impact on this strain's IAA synthesis. This data suggested that L-tryptophan concentration may be important in S. texasensis MB15 IAA biosynthetic activities, and these findings are consistent with the previous work on actinobacteria strains (Duca and Glick 2020).

5. Conclusion

To assess the primary conditions for IAA production and to find the ideal cultural conditions for maximum IAA production by *S. texasensis* strain MB15, the Plackett-Burman design was used. Our findings revealed that statistical design methodology is an effective and efficient strategy for improving cultural conditions. The suggested model showed the quantitative impact of 11 variables on the production of IAA. The experimental IAA production of 148.0 µg/ml closely matched the mathematical model's predicted value of 146.52 µg/ml, proving the efficacy of this optimization process.

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