Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

STATISTICAL APPROACH TO ACTINOBACTERIAL BIOMASS PRODUCTION WITH SOME PGP TRAITS.

Abderrahmane BENADJILA
PhD student, Oum El Bouaghi University, Faculty of Exact Sciences and Nature and Life Sciences, Department of Nature and Life Sciences Laboratory of Plant Biomolecules and Plant Improvement (LBVAP), e-mail: ab.benadjila@gmail.com

Lamia AOUAR
full professor, Oum El Bouaghi University, Faculty of Exact Sciences and Nature and Life Sciences, Department of Nature and Life Sciences), e-mail: aouarlamia@yahoo.fr

Yacine GOUDJAL
Full professor, Microbial Systems Biology Laboratory (LBSM), Kouba Algiers Higher Normal School, e-mail: goudjal_y@yahoo.fr

Abstract:
Objective: To optimize the growing conditions and the composition of the culture medium in order to produce and optimize actinobacterial biomass.
Material and Methods: From 18 isolates of endophytic and rhizospheric actinobacteria, a preliminary screening in vitro under standard culture conditions on ISP broth was carried out to test the production of PGP traits. Assays for the production of the biomass of Saccharothrix texasensis MB15 strain on wheat agricultural waste medium, an experimental design by the PLACKETT-BURMAN matrix of 11 variation factors was used for the design of the experiments and the analysis of the results.
Results and Discussion: The optimal values obtained for the modulating factors tested for maximum production of biomass are: (12.5 ml leaves extract ; 25 ml/L roots extract ; 1.5 g/L l-tryptophan; 1.5 g/L MgSO4; 5g/l NH4NO3; 7 g/l NaCl; 240 RPM; 10^7 inoculum charge; 7.5 pH; 25 C° temperature and during 7 days). The maximum biomass production of strain MB15 was 100 mg / ml. twelve experiment in the foreground, were necessary to evaluate the effect between the 11 verified factors of the 5 modulating factors of production, the model is considered satisfactory, with $R^2 = 0.97$; Adjusted $R^2 = 0.95$; RMSE of 3.19 and a statistical desirability of 0.89.
Conclusion: the work shows the efficiency of the screening Placket-Burman, by experimentally determining the conditions leading to a very satisfactory biomass produced by the strain *S. texasensis* MB15.

Key words: Optimization; biomass production; Saccharothrix texasensis MB15; Placket-Burman design.

Introduction:

A gram-positive bacterium with filamentous structures that produce asexual spores and branching filaments are known as actinobacteria. From more than 22,000 known secondary metabolites, actinobacteria produce roughly 70% of available secondary metabolites [1]. These bacteria are widely distributed in soil and the aquatic environment, with uncultivable soil microorganisms accounting for 50% of total soil microorganisms. As a

result, they have emerged as the most important and dominant group of soil microbes. Antibiotics, enzymes, nutritional materials, cosmetics, antitumors, immune modulators, and enzyme inhibitors are among the secondary metabolites produced by actinobacteria [2].

Actinobacteria are well recognized as potential biocontrôl agents, and they have shown their efficiency at controlling plant diseases both in vitro and in vivo [3]. The most important biocontrôl mechanisms are hyphae parasitism [2], secondary metabolite production [4], siderophore production [5], and extracellular enzyme production such as cellulases, amylase, and chitinase [6]. Due to the crucial roles they play in producing such PGP compounds and antimicrobial agents, exploration of endophytic microorganisms has been attracting major attention.

Fermentation optimization is necessary to increase product yields while lowering costs. Prior to the 1970s, traditional methods were used to optimize media for fermentation, whereas modern mathematical techniques were used with new technology. Media optimization became more effective, vibrant, and efficient as a result. Physical parameters like pH, temperature, agitation, and aeration, as well as medium components like carbon, nitrogen, and phosphate, must all be identified and optimized accordingly. The use of mathematical methods for media optimization can overcome the limitations of the traditional OFAT method, making it a more effective tool for secondary metabolite production optimization. Changing multiple components in the medium at once can be more effective than changing just one component at a time. [07, 08].

Problematic:

The purpose of this paper was to screen several actinobacteria strains from our laboratory (Laboratory of Biology of Microbial Systems (LBSM), ENS-Kouba, Algiers, Algeria) for selected PGP traits. The best PGP strain was investigated in order to optimize the physicochemical fermentation conditions for actinobacterial biomass production and then to test it for PGP properties after the mathematical optimization process.

Materials and methods:

Actinobacterial strains:

Eighteen strains of 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).

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) [09]. 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 [10].

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

	Strain	Reference	Origin of strains (soil or host plant)
Soil strain		*	Algerian Saharan soil from Ghardaia
	Nocardiopsis dassonvillei strain MB22	*	Algerian Saharan soil from Ghardaia
	Streptosporangium becharense strain SGI	[11]	Algerian Saharan soil from Béchar
	Streptosporangiurn saharense strain SG20	[12]	Algerian Saharan soil from Ghardaia
Endophytic strain	*Streptomyces* sp. strain SN3	[13]	*Aristida pungens*
	Streptomyces cyaneofuscatus strain AR2	[13]	*Astragalus armatus*
	Streptomyces sp. Strain NS 13	[13]	*Cleome arabica*
	Streptomyces mutabilis strain CA2	[13]	*Cleome arabica*
	Streptomyces sp. strain DN4	*	*Phoenix dactylifera*
	Streptomyces sp. strain ML4	*	*Medicago laciniata*
	Streptomyces rochei strain PT2	[13]	*Panicum turgidum*
	Streptomyces asterosporus strain SN2	[14]	*Solatium nigrum*
	Streptomyces neopeptinius strain TL8	[14]	*Terfezia leonis*
	Streptomyces caeruleatus strain ZL2	[10]	*Zizyphus lotus*
	Streptomyces sp. Strain ML2	*	*Medicago laciniata*
	Saceharothrix longispora strain MB29	*	/
	Streptomyces sp. strain AL4	[14]	*Astragallus armatus*
	Streptomyces sp. strain CA12	[13]	*Cleome arabica*

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

Table1. Origin of actinobacterial strains.

Assessment of growth ratio for actinobacterial strains biomass:

The determination of the Dry cell weight (mg/ml) X max of actinobacterial strain was determined after standard culture conditions set on ISP broth media supplemented with 1g L-tryptophan. One milliliter aliquots of actinobacterial spore suspensions (≈10^6 spores/ml) were transferred to a 250 ml conical flask containing 50 ml of ISP broth, 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 10 days. Then the sample was dried at 40 °C for 48 h to a constant weight, aiming at the estimation of the dry cell weight.

Determination of some PGP activities:

IAA production and phosphate solubilization:

All strains were investigated for IAA production. IAA estimation was done using a spectrophotometer and values were expressed in mg/ml, as corresponds to a standard curve of IAA according to Goudjal et al 2013. All selected isolates were screened for their phosphate solubilization potential on PKV liquid media as described by Allali et al 2019. The supernatant cultures were analyzed to assess the phosphate released into the solution (mg/l).

Screening for growth and PGP traits using low cost medium:

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

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

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[NH4NO3: 2.5 g/l; Na2HPO4•2H2O: 1.0 g/l; MgSO4•7H2O: 0.5 g/l; Fe(SO4)3 •5H2O: 0.01g/l; Co(NO3)2•6H2O: 0.005g/l; CaCl2•2H2O: 1.0 mg/l; KH2PO4: 0.5 mg/l; MnSO4•2H2O: 0.1 mg/l; (NH4)6 Mo7O24•4H2O:0.1 mg/l] [9],supplemented with 25 ml of (roots extract ml /leaves extract ml) (Table N°2) ; after incubation on an orbital rotary shaker (200 rpm) at 30 °C for 7 days. In a 250 mL conical flask, 2 mL of the primary inoculum was inoculated into 100 mL of PVK medium and incubated for 3 days at 30 °C and 200 rpm. The PGP traits that were studied were determined as previously noted (Table2).

	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

Table2. Composition in ml of the medium of leaf / root extracts

Screening of cultural conditions by Plackett-Burman design:

Plackett and Burman (1946) [15] mathematical design was used to provide fundamental cultural conditions to enhance S. texasensis strain MB15 growth and for the evaluation of some PGP traits. A total of eleven variables were chosen (variable k = 11, Table 3). Temperature (X1), incubation period (X2), initial pH (X3), inoculum quantity (X4), rotation speed (X5), NaCl concentration (X6), NH4NO3 (X7), MgSO4•7H2O (X8), L-tryptophan concentration (X9) IAA precursor , roots extract concentration (X10) and leaves extract concentration (X11) were the eleven variables to studied by the Plackett-Burman statistical plan.

In these trials, each variable was represented by two levels: high (+) and low (-). (Table 4) Each column represents an independent (assigned) variable, and each row represents a trial. When the sign is positive, the variable's influence on biomass 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 (ρ- value) of each concentration effect was established using the Student's t- test.

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

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 : NH4NO3 (g/l)	2.5	5
X_8 : MgSO4•7H2O (g/l)	0.5	1.5
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

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

Run	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	The maximal cell dry weight (X max) (mg/ml)	
												Experimental	Predicted
1	1	1	-1	-1	-1	1	-1	-1	1	-1	1	66	64,43
2	1	1	1	-1	-1	-1	1	-1	-1	1	-1	67	73,33
3	1	-1	1	1	1	-1	-1	-1	1	-1	-1	64	64,43
4	-1	1	-1	-1	1	-1	1	1	1	-1	-1	68	64.33
5	1	-1	-1	1	-1	1	1	1	-1	-1	-1	70	66,95
6	-1	1	-1	1	1	1	-1	-1	-1	1	-1	100	101,40
7	1	-1	-1	-1	1	-1	-1	1	-1	1	1	59,29	65,21
8	-1	-1	1	-1	1	1	1	-1	-1	-1	1	48	46,67
9	1	1	1	1	1	1	1	1	1	1	1	99	101,67
10	-1	1	1	1	-1	-1	-1	1	-1	-1	1	78	75,21
11	-1	-1	-1	1	-1	-1	1	-1	1	1	1	77	73,33
12	-1	-1	1	-1	-1	1	-1	1	1	1	-1	69,29	64,38

Table 4. Plackett-Burman design matrix in coded values and the Dry cell weight X max (mg/ml).

Statistical analysis:

All assays were carried out in triplicates, and results are expressed as mean ± SD. Results from the Plackett-Burman model, of the biomass 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 was were used to evaluate all the results to draw all the graphs results.

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

Results

Biomass production:

Screening the eighteen actinobacteria for biomass production under standard culture conditions revealed that all strains grew well on ISP2 broth medium supplemented with 1g L-tryptophan (Fig. 1). *Streptomyces asterosporus* strain SN2 had the lowest dry cell weight growth ratio of 22.67±1.53 mg/ml, while *S. texasensis* MB15 had the highest one, 44.33±2.18 mg/ml. *Streptomyces sp* strain CA12, *Streptosporangium becharense* strain SGI, and *Streptomyces caeruleatus* strain ZL2, with 41.66±1.58 mg/ml, 40.33±2.08 mg/ml, and 41.66±2.08 mg/ml, respectively, had a large dry cell weight, implying a high growth ratio.

IAA production and phosphate solubilization:

In the presence of 1g/l tryptophan, quantitative estimation of the PGP hormone indole acetic acid (IAA) revealed that all isolates produced IAA in culture broth ranging from 5.19±0.22 µg/ml to 47.44±0.69 µg/ml. The strain S. texasensis MB15 produced the highest IAA amount with 47.44±0.69 g/ml. *Streptomyces rochei* strain PT2 and *Streptomyces caeruleatus* strain ZL2 had the highest concentrations of IAA, 35.12±0.90 µg/ml, and 33.25±0.24 µg/ml, respectively, followed by Streptomyces sp. strain DN4 28.25±0.22 µg/ml, Streptomyces sp. strain SN3 24.92±0.51 µg/ml. (Fig 1.a).

Except for Streptomyces sp. strains AL4 and ML2, all the studied strains demonstrated a significant ability to solubilize tricalcium phosphate in Pikovskay's liquid medium (Fig 1c.). The amount of liberated phosphorus was calculated, and it was discovered that 5 strains demonstrated significant phosphate-solubilizing activity in vitro, resulting in significant liberated phosphorus amounts. Tricalcium phosphate solubilization capacity ranged from 69.5±0.5 mg/l to 25.1±0.360 mg/ml among the strains, with *Streptomyces asterosporus* strain SN2 having the highest value at 69.5±05 mg/ml, followed by *Streptomyces sp*. strain SN3 67.4±0.57 mg/ml and *S. texasensis* strain MB15 having a value at 64.9±1.14 mg/ml (Fig 1.c).

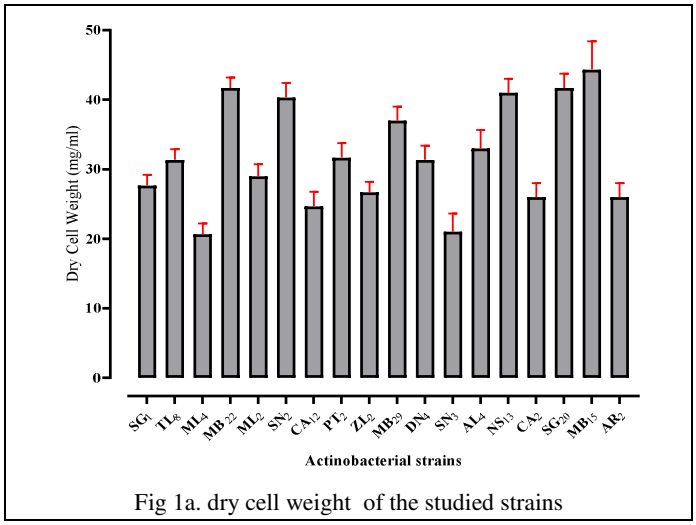


Fig 1a. dry cell weight of the studied strains

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

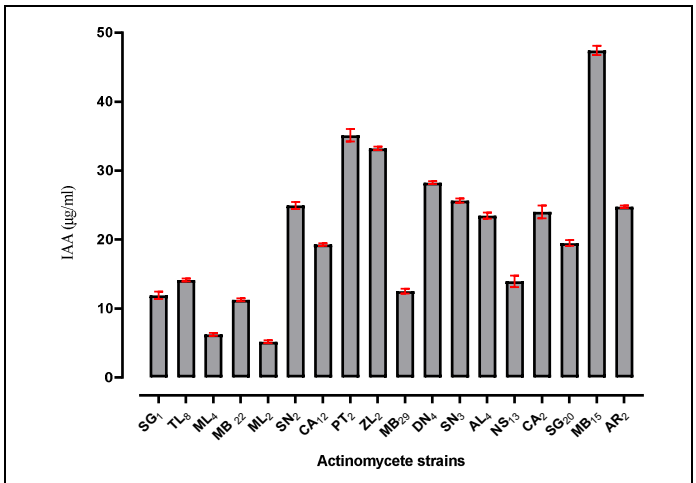


Fig 1b. IAA production ration of the studied strains

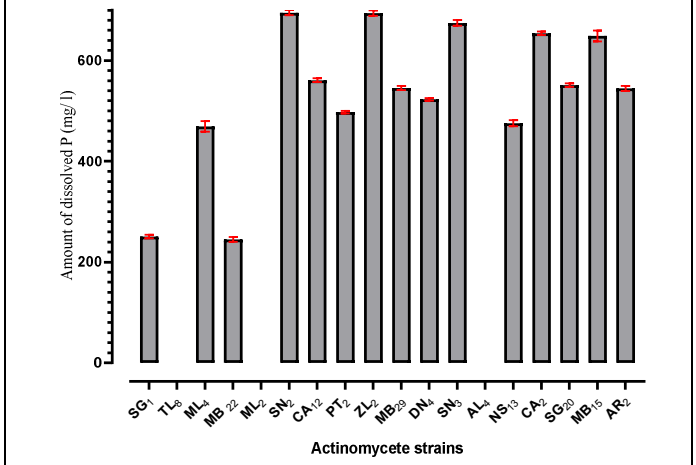


Fig 1c. phosphate solubilization capacity of the studied strains

Fig1. Strains growth IAA production and phosphate solubilization

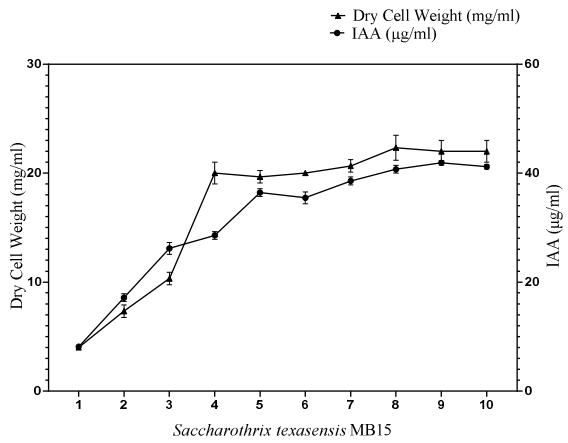
Growth, IAA and phosphate solubilization on low cost medium:

Figure 2 shows the growth, IAA production and phosphate solubilization capacity of S. texasensis MB15 on low-cost media. The effect of medium composition on MB15 strain growth IAA production and phosphate solubilization is clearly visible in these curves and main histogram.

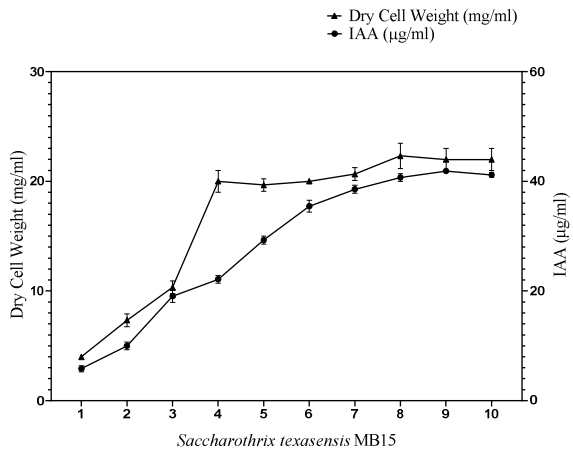
The production of IAA ranges from 8.15±0.38 µg/ml to 42.07±0.12 µg/ml in Fig. 2a, with the maximum amount of IAA production occurring on the eighth day of culture. The IAA production profile in Fig. 2b is very identical, ranging from 6.03±0.29 µg/ml to a maximum of 41.19±0.34 µg/ml. In comparison to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

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

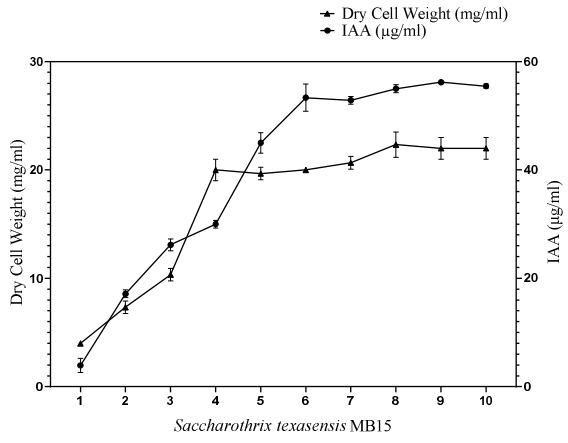
In Fig.2c, it is visible that IAA production began to increase on day 5 and peaked on day 9, with a maximum of 56±0.33 µg/ml. The change of SP2 supplemented with 1g/l of l-tryptophan broth with a basic mineral broth enriched with leaves and/or roots extracts was found to be particularly effective in 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.



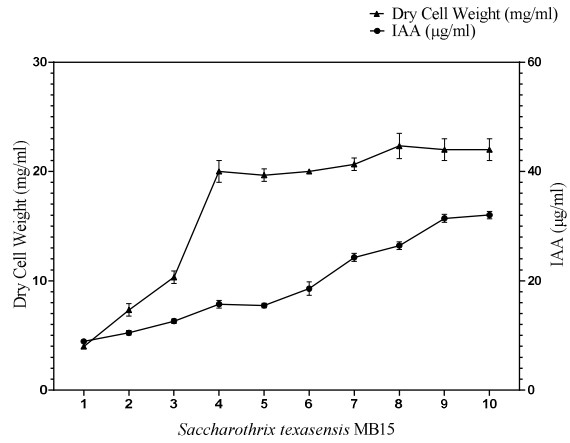
(a) leaf-root extract medium (0% root extract 100% leaf extract)



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

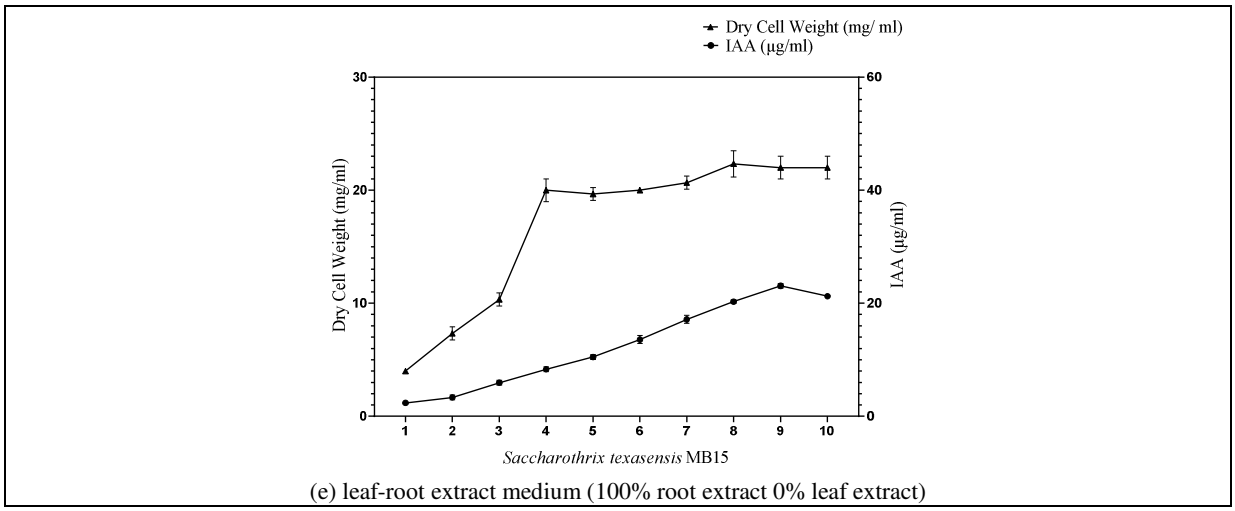


(c) leaf-root extract medium (50% root extract 50% leaf extract)



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

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935



(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

The growth ratio expressed in cell dry weight has a nearly stable yield and the same curve shape. For all growth curves listed above, biomass production ranges from 20±0.45 mg/ml of cell dry weight (CDW) in the 4-day of fermentation to a maximum of 25±0.67 mg/ml on the eighth day of incubation (Fig 2). from (a) to (e).

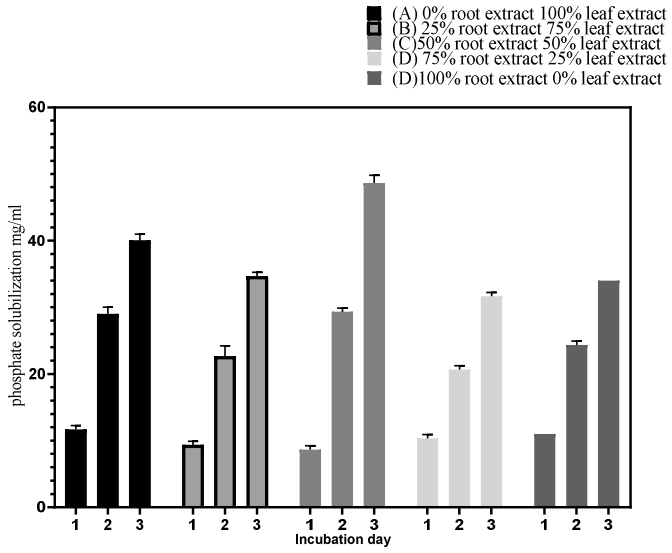


Fig 3. The amount of phosphorus dissolved in a low-cost medium.

The amount of phosphorus dissolved in the different compositions of the low-cost medium from Ca3 (PO4)2 in the supernatant cultures varied from 8.66±0.44 to 48±0.88 mg/ml and the highest amount was achieved by the strain S. texasensis MB15 in the composition of 50:50% roots and leaves extract, which was estimated at 51.44±0.25 mg/ml. (fig 3)

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

Plackett-Burman design for estimating significant variables:

Actual by predicted plot using Plackett-Burman design depicts the positive and negative effects of the selected variables on S. texasensis MB15 biomass production on minimal mineral medium supplemented with wheat leaves and/or roots extracts (Fig 4). Tables 5 and 6 show the most important model values as well as the ANOVA table results.

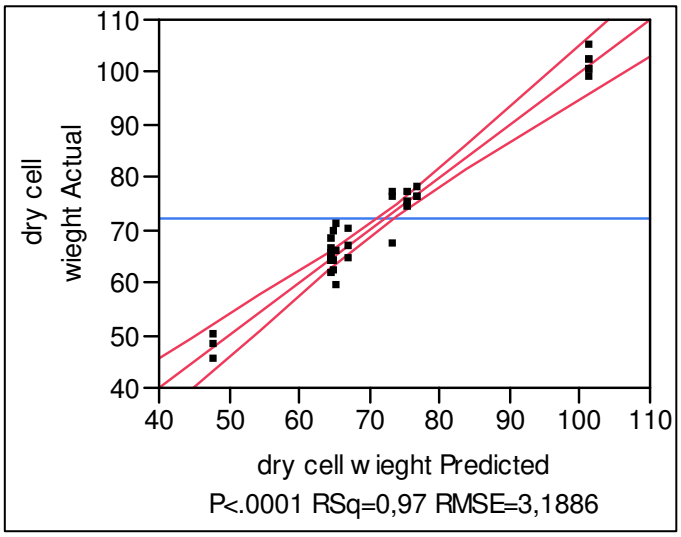


Fig 4. Actual by Predicted Plot

The most relevant medium components affecting S. texasensis MB15 biomass production were investigated using the PB model. Five factors (roots extract, NaCl, rotation speed, incubation time and inoculum quantity) were shown to be the most important factors affecting the cell growth yield, with significant ρ 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 (Table 6). The model probability F 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 = 0.97, on the other hand, agreed with the adjusted one R^2 = 0.96.

RSquare	0,97
RSquare Adj	0,96
Root Mean Square Error	3,19
Mean of Response	72,18
Observations (or Sum Wgts)	36

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	11	7928,70	720,79	70,89

Table 5. Analysis of Variance and Fit for first-order model in Plackett-Burman Design

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

The RMSE ratio is desired, less than 10 (Table 5), and the 72.18 mean response ratio indicates an adequate model design. As a result, this model can be used to explore the design possibilities. As shown in Eq-(1), the final equation was constructed using elements that revealed *S. texasensis* MB15 biomass production as a function of independent variables:

Source	DF	Sum of Squares	*F* value	*p* value>*F*
X1	1	7,63	0,75	0,3950
X2	1	2362,27	232,35	<,0001*
X3	1	22,23	2,19	0,1523
X4	1	2807,32	276,13	<,0001*
X5	1	127,37	12,53	0,0017*
X6	1	189,397	18,63	0,0002*
X7	1	17,297	1,70	0,2045
X8	1	39,717	3,91	0,0597
X9	1	3,63	0,36	0,5559
X10	1	2346,86	230,836	<,0001*
X11	1	5,00	0,49	0,4895

*ρ< .005 significant

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

For the S. texasensis MB15 biomass production, the regression equation for calculating the predicted response in the Plackett-Berman design is as follows: Eq-(1)

$$IAA = 72.18 + 0.46X_1 + 8.11X_2 - 0.80X_3 + 8.83X_4 + 1.88X_5 + 2.30X_6 - 0.70X_7 + 1.05X_8 + 0.32X_9 + 8.07X_{10} + 0.37X_{11}$$

Verification of the models for IAA coproduction and phosphate solubilization:

The cells growing in low cost medium (12.5 ml leaves extract ; 25 ml/L roots extract ; 1.5 g/L l-tryptophan; 1.5 g/L MgSO4; 5g/l NH4NO3; 7 g/l NaCl; 240 RPM; 10^7 inoculum charge; 7.5 pH; 25 C° temprature and durin g 7 days) reached the stationary phase after 48 h of culture. The biomass of culture medium remained relatively stable during *S. texasensis* MB15 growth. The maximum dissolved phosphate produced during *S. texasensis* MB15 was 54.00±0.7 mg/mL, (fig.5) which was observed after 144 h of incubation. The amount of dissolved phosphate go to 46.33±1.33 mg/mL after 144 h, indicating that the sole P source in medium is used during cell growth, and the soluble phosphate precipitated into its insoluble state. Figure 4 shows that the IAA content of the low-cost medium supernatant continued to increase to 58.57±0.48 µg/mL after 168 h of cell growth.

Earth, Life and Health Journal Vol. 1 Nr. 3 2021
ISSN: 2800-0935

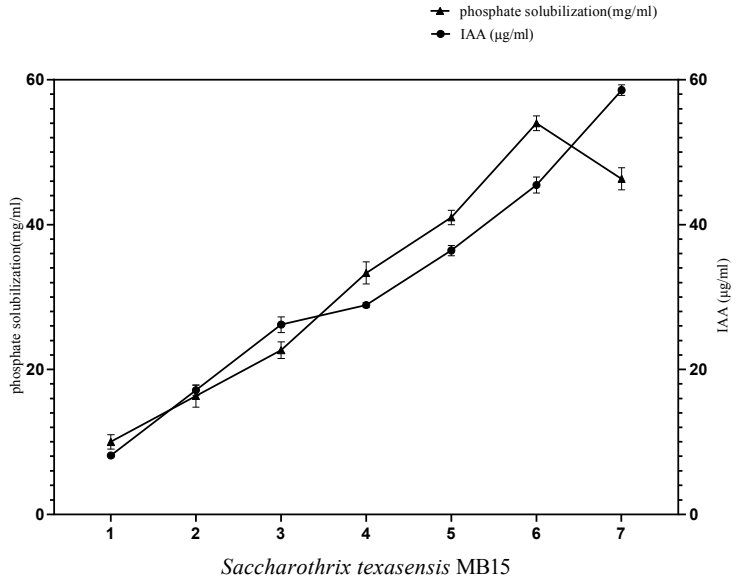


Fig .5 IAA and phosphate solubilization after mathematical optimization

Discussion:

In this study, and under standard cultural conditions for endophytic and rhizospheric actinobacteria, we investigated the actinobacterial kinetics of growth, co-production of IAA, and phosphate solubilization. The maximal cell mass (Xmax), as well as the co-production of IAA and phosphate solubilization capacity, varied depending on the actinobacterial strain. These findings are consistent with previous research on actinobacteria genus biomass production; IAA synthesis and production, as well as phosphate solubilization capacity [16, 17; 10].

Researchers must chose and investigated the S. texasensis MB15 for future testing and large-scale IAA production and biofertilizer production, for example, because of the significant amount of IAA produced and the strain's ability to solubilize phosphate under standard culture conditions. The experiments showed that biomass and IAA can be produced on a low-cost medium using biological waste such as wheat leaf or root extracts. Those were also promising results, which were in connection with Peng et al's findings [18]

High tryptophan levels in roots and leaves stimulate IAA production by rhizospheric or endophytic actinobacteria in the soil [19] which could improve IAA biosynthesis in a basal mineral medium supplemented with waste leaves and roots.

Furthermore, organic wastes are a rich source of tryptophan after being transformed by aerobic bacteria such as actinobacteria [20]. This happens as a result of the cultural conditions of our experience. Actinobacteria are primary decomposers of organic compounds, particularly lignocellulosic wastes, and have been shown in vitro and in vivo to produce a diverse range of hydrolytic enzymes as part of a saprophytic microbial community [21].

We optimized the culture conditions for the selected S. texasensis strain MB15 for biomass and co-production of interest phytohormones using the Plackett-Burman design. The

Plackett-Burman design assisted in highlighting the impact of the 11 variables and optimizing the cultural conditions of S. texasensis MB15 for maximum growth ratio.

As a result, an effective model for explaining the response of experiments involving the strain MB15's cell growth ratio, phosphate solubilization, and IAA production was developed. The experimentally obtained values agree with the predicted values of the model. The model was validated to the optimal level by comparing observed and predicted values. The optimization approach greatly increased the biomass production of S. texasensis MB15.

The mathematical approach to variable optimization using Plackett-Burman design has proven to be a successful system for microbial biotechnology. The Plackett-Burman design for biomass and coproduction of products of interest was discovered to be highly variable due to the presence of eleven variables. Our findings are consistent with those of [22] who reported the Plackett-Burman design's accuracy in determining the most important influence of each cultural condition parameter on biomass production.

By analyzing the significance of each regression model coefficient, the impact of roots extract, NaCl, rotation speed, incubation time, and inoculum quantity on biomass production and enhancement of the production of some PGP traits of S. texasensis MB15 was determined. Several studies such as [23] discovered that NaCl, rotation speed, incubation time, and inoculum quantity all had a moderate to very significant effect on Streptomyces sp. biomass production. However, this is the first study to demonstrate that wheat root extract has a significant effect on biomass cell production, IAA synthesis, and phosphate solubilization by an actinobacterial species. The mathematical established design improves the ability of the system to co-product IAA and to solubilize phosphate under the new conditions of the optimized low-cost medium.

Conclusion

The Plackett-Burman design was used to assess the primary conditions for biomass production; IAA biosynthesis and phosphate solubilization; and to find the ideal cultural conditions for maximum production by S. texasensis strain MB15. According to our findings, statistical design methodology is an effective and efficient strategy for improving cultural conditions. The proposed model quantified the impact of 11 variables on the production of the target molecules. The experimental biomass production, IAA biosynthesis, and phosphate solubilization were estimated as 100 mg/ml of dray cell weight; 58.57±0.48mg/ml, and 54.00±0.7 mg/mL of solubilized phosphorus closely matched the mathematical model's results.

References:

1. Uyeda, M.J.A. (2003), Fattiviracins, antiviral antibiotics produced by an actinomycete. **17(2):** p. 57-66.
2. Rotich, M.C. (2018), Bio-Prospecting for Broad Spectrum Antibiotic Producing Actinomycetes Isolated from Virgin Soils in Kericho County., JKUAT-COHES.

3. Passari AK, Mishra VK, Gupta VK, Yadav MK, Saikia R, Singh BP.(2015), Invitro and in-vivo PGP activities and DNA fingerprinting of antagonistic endophytic actinobacteria associates with medicinal plants. Plos One

4. de Oliveira, M. F., da Silva, M. G., & Van Der Sand, S. T. (2010). Anti-phytopathogen potential of endophytic actinobacteria isolated from tomato plants (Lycopersicon esculentum) in southern Brazil, and characterization of Streptomyces sp. R18(6), a potential biocontrol agent. Research in microbiology, 161(7), 565–572. https://doi.org/10.1016/j.resmic.2010.05.008

5. Khamna S, Yokota A, Lumyong S. (2009), Actinobacteria isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. World J Microbiol Biotechnol;25:649e55.

6. Singh SP, Gaur R.(2016),. Evaluation of antagonistic and plant growth promoting activities of chitinolytic endophytic actinomycetes associated with medicinal plants against Sclerotium rolfsii in chickpea. J Appl Microbiol

07. Elibol, M. (2004), Optimization of medium composition for actinorhodin production by Streptomyces coelicolor A3 (2) with response surface methodology. Process Biochemistry, **39(9):** p. 1057-1062.

08. Wentzel, A., et al. (2012), Optimized submerged batch fermentation strategy for systems scale studies of metabolic switching in Streptomyces coelicolor A3 (2). BMC systems biology, 6(1): p. 59.

09. Atlas, R. (2010), Handbook of Microbiological Media, fourth edition. CRC Press, Boca Raton.

10. Zamoum, M., Goudjal, Y., Sabaou, N., Barakate, M., Mathieu, F., Zitouni, A. (2015), Biocontrol capacities and plant growth–promoting traits of endophytic actinobacteria isolated from native plants of Algerian Sahara. Journal of Plant Diseases and Protection. 122, 215–223.

11. Chaabane-Chaouch. F., Bouras, N., Mokrane. S., Zitouni. A., Schumann. P., Spröer. C., Sabaou. N., Klenk. H.P.(2016a), Streptosporangium saharense sp. nov., an actinobacterium isolated from Saharan soil. International Journal of Systematic and Evolutionary Microbiology. 66,1371–1376.

12. Chaabane-Chaouch. F., Bouras, N., Mokrane. S., Zitouni. A., Schumann. P., Spröer. C., Sabaou. N., Klenk. H.P.(2016b), Streptosporangium becharense sp. nov., an actinobacterium isolated from desert soil. International Journal of Systematic and Evolutionary Microbiology 66,2484–2490.

13. Goudjal. Y., Toumatia, O., Sabaou, N., Barakate, M., Mathieu, F., Zitouni, A.(2013), Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity. World Journal of Microbiology and Biotechnology 29, 1821–1829.

14. Goudjal, Y., Zamoum, M., Meklat, A., Sabaou, N., Mathieu, F., Zitouni, A.,(2016), Plant-growth promotiong potential of endosymbiotic actinobacteria isolated from sand truffles (Terfezia leonis Tul.) of the Algerian Sahara. Annals of Microbiology 66, 91–100.

15. Plackett, R.L., Burmann, J.P. (1946), The Design of optimum multi-factorial experiments. Biometrica 33,305-325.

16. Khamna, S., Yokota, A., Peberdy, J.F., Lumyong, S.(2010), Indole-3-acetic acid production by Streptomyces sp. isolated from some Thai medicinal plant rhizosphere soils. EurAsian. J BioSci. 4, 23–32.

17. Abd-Alla, M.H., El-Sayed, E.S.A., Rasmey, A.H.M.(2013), Indole-3-acetic acid (IAA) production by Streptomyces atrovirens isolated from rhizospheric soil in Egypt. J. Biol. Earth Sci. 3,B182–B193.

18. Peng, Y., He, Y., Lu, J., Li, C.(2014), Screening and optimization of low-cost medium for Pseudomonas putida Rs-198 culture using RSM. Brazilian journal of microbiology 45:1229–37.

19. Gopalakrishnan, S., Vadlamudi, S., Bandikinda, P., Sathya, A., Vijayabharathi, R., Rupela, O., Kudapa, H., Katta, K., Varshney, R.K. (2014), Evaluation of Streptomyces strains isolated from herbal vermi–compost for their plant growth–promotion traits in rice. Microbiol Res. 169, 40–48.

20. Kravchenko, L.V., Azarova, T.S., Makarova, N.M., Tikhonovich, I.(2004), The Effect of Tryptophan Present in Plant Root Exudates on the Phytostimulating Activity of Rhizobacteria. Microbiology. 73, 156-158.

21. Makoi, J., Ndakidemi, P.A. (2008), selected soil enzymes: examples of their potential role in ecosystem. Afr. J Biotechnol. 7, 181–191.

22. Sasirekha, B., Shivakumar, S. (2012), Statistical optimization for improved indole-3-acetic acid (IAA) production by Pseudomonas aeruginosa and demonstration of enhanced plant growth promotion. Journal of soil science and plant nutrition.12, 863-873.

23. Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L.(2018), Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci 9,1473.