Optimization of Indole -3- Acetic Acid Production by *Lysinibacillus sphaericus* strain using Response Surface Methodology

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Abstract: Indole-3-acetic acid (IAA) is an essential plant hormone, produced by various soil-borne bacterial genera that colonize the rhizosphere of plants. IAA influences and facilitates development and growth processes in plants; however, their production is limited by low yield, hence this study. This study therefore, investigated the application of response surface methodology in optimizing the production of IAA by *Lysinibacillus sphaericus*, a plant growth promoting bacterium (PGPB). The bacterium was isolated from the rhizosphere soil of *Zea mays* in a farmland located at Choba, River State, Nigeria. The isolate was identified using its biochemical and molecular characteristics. Sequences from the amplification of the 16S rRNA gene, classified the isolate as *Lysinibacillus sphaericus* (M904987). IAA production was determined by colorimetric technique using Salkowski reagent. Optimization of process parameters using Response Surface Methodology (RSM) based on Box- Behnken Design (BBD) was employed to obtain the best combinations of conditions for IAA production. The best IAA production was obtained at: pH 5.5, incubation temperature 35ºC, inoculum concentration 2.5% and 20µl/mL of L-tryptophan after 24h-incubation period. Under these conditions, maximum amount (151.1 mg/ml) of IAA was obtained. This study has therefore shown that *Lysinibacillus sphaericus* could be utilized as a useful plant growth promoting bacterium (PGPB) to facilitate, stimulate and improve crop yield. Moreover, the deployment of RSM was effective in enhancing the production of IAA by the *Lysinibacillus sphaericus*.
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1.0 Introduction

Indole-3-acetic acid (IAA) is a widely produced phytohormone by bacteria that inhabit the rhizosphere of plants (Patten & Glick, 1996). IAA is an important phytohormone with the capacity to control plant development in both beneficial and deleterious ways (Duca et al., 2014). It is the main auxin in plants, regulating growth and developmental processes such as cell division and elongation, tissue differentiation, apical dominance, and responses to light, gravity, and pathogens (Gomes & Scortecci, 2021).

Indole acetic acid regulates various aspects of plant growth and development. Its diverse functions highlight its importance in shaping the architecture and responses of plants to their environment. IAA as a metabolite has an ever-increasing demand in the global agricultural market and its cost continues to surge due to limitations in the production of a cost-effective plant growth-promoting bacteria (PGPR) (Haldar & Sengupta, 2023). Different studies have reviewed exploitation of PGPR bacteria such as *Bacillus* spp as multispecies bioinoculant, due to their important mechanisms of IAA production and other traits in soil fertilization (Bunsangiam et al., 2021).

Nutritional and other growth parameters such as pH, carbon source, nitrogen source, and supplementation of precursor L-tryptophan influences IAA production (Mohite, 2013). Several studies used response surface methodology (RSM) for the optimization of fermentation parameters for maximum IAA production (Lebrazi *et al*., 2020; Baliyan *et al*., 2021; Waday *et al*., 2022) and enhancement of crop yield in a large-scale microbial production setup, with reported improvement in its yield (Lebrazi *et al*., 2020). RSM is a statistical and mathematical tool for optimizing a response that is influenced by some independent variables. It can simultaneously investigate the effect of different parameters on specific response(s) without the need to carry them out one at a time as in the case of one-variable-at-time (OVAT) method (Boodaeng *et al*., 2023).

This study was therefore designed to optimized nutritional and growth conditions of *Lysinibacillus sphaericus* isolated from *Zey mays rhizosphere* for enhanced IAA production using response surface design-Box Behnken Design (BBD).

2.0 Materials and Methods

2.1 Isolation of *Lysinibacillus spaericus*

The plant growth-promoting bacterium was isolated from the rhizosphere soil of the plant, *Zea mays*. Soil samples were collected following standard methods previously described by Pepper & Gerba (2015). Physicochemical analysis of the soil samples such as soil texture, pH and temperature was carried. Rhizobacterial isolation was carried out as follows: 10g of rhizosphere soil was collected in 250-mL flask. Ninety millilitre (90 mL) of sterile distilled water was added to the soil sample. The flask was incubated on rotary shaker at 120 rpm for 10 min. Thereafter, 1ml of the sample was serially diluted and 0.1 mL of 10^{-4} to 10^{-6} dilutions were spread plated on sterile Nutrient Agar (Himedia, India) and incubated for 3 days at 30 °C. Single colonies were picked up and streaked on freshly prepared nutrient agar plates to obtain pure culture. Colonies of pure bacterial isolates were observed for morphological characteristics and stored for further analysis.

2.2 Screening of *Lysinibacillus spaericus* for IAA production

The bacterium was screened for IAA production by a colorimetric method using the Sakowsky reagent (containing: 50 ml, 35% perchloric acid ($HClO_4$); 1 ml of 0.5 M Iron trichloride ($FeCl_3$)) as described by Gang *et al*. (2019). Salkowski reagent was used as it gives reaction with IAA and does not interact with L-tryptophan. Moreover, this reagent is an important option for qualitative and semiquantitative determination that assures the presence of the hormone in the supernatant of bacterial cultures or liquid formulations of biological inoculants based on the change of colour from yellow to pink, indicative of the presence of Indole Acetic Acid. The screening was carried out as follows: Bacterial culture was inoculated in Nutrient Broth medium containing L-tryptophan (5 g/L), and incubated for 96 h. The culture was then centrifuged at 10,000 rpm for 10 minutes. Two millilitre of the supernatant was taken with 2 drops of Orthophosphoric acid and 4 ml of Sakowski's reagent added to it. The mixture was kept for 30 min in the dark, under room temperature. The presence of IAA was detected by measuring pink colour development after 30 min. Uninoculated medium was used to set the blank and optical density was taken spectrophotometrically at 536 nm using a cuvette.

2.3 Characterization of the bacteria isolate

The bacterial isolate was subjected to several biochemical tests as described by Holt *et al*. (1994) and Madigan *et al*. (2012). 16S rRNA sequencing was also carried out on the isolate to classify the bacterium.

2.4 Experimental design for RSM optimization of IAA production by the bacteria isolate

Four (4) independent variables (temperature (25 to 45 °C), pH (5 to 9), inoculum concentration (2.5 to 7.5 %) and tryptophan concentration (10 to 30 µL/mL)) at three (3) levels, screened through twenty-nine (29) different experimental runs (Tables 1 and 2), with the insignificant ones eliminated to obtain a smaller and more fitting collection of factors were performed. Minimum and maximum values of independent variables investigated in cultivation medium and their centre points are given in Table 1. The BBD comprised eight (24) factorial points and five (5) centre points. The centre point was repeated to obtain a reliable estimate of the experimental error. This ensured adequate estimation of the variation of the response, thereby providing the required number of degrees of freedom for sufficiently testing the model. On establishing the critical factors, the BBD was used to generate a linear model that comprised factorial trials used in estimating linear effects and central points to determine the variability of the pure process with IAA (mg/mL) as the response. Design Expert version 13 was used in designing, analysing and interpreting experimental data obtained through BBD. The system's behaviour is explained by linear equation given in Eq. 1.

Table 1: Factors and their levels in the optimization of IAA production by *Lysinibacillus sphaericus* using BBD-RSM

Symbol	Variables	-1	0	+1
A	Temperature (°C)	25	35	45
B	pH	5	7	9
C	Inoculum concentration (%)	2.5	5	7.5
D	Tryptophan (µL)	10	20	30

The Linear equation is given thus:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \varepsilon; \quad \text{Eq.1}$$

Where:

Y: is the response variable; β_0: is the intercept β_1, β_2, β_3, β_4 are the coefficients representing the linear effects

of factors A, B, C, and D, respectively. A, B, C, D: are the coded values of the independent variables (usually -1, 0, +1 for each factor level) and ε: is the error term.

3.0 Results

3.1 Screening characteristics and identification of the bacterial isolate

The IAA-producing bacterium was isolated from the rhizosphere soil of *Zea Mays*. Production of IAA by the bacterial isolates was evaluated and the data obtained showed high capacity for IAA production. The development of pink colouration was indicative of IAA production capacity. The unoptimized production capacity of the isolate was 81.3 mg/mL. The isolate was identified as *Lysinibacillus sphaericus* based on analysis of the 16S genes sequences.

3.2 Optimisation of IAA production by BBD

The actual and predicted values of IAA obtained through 29 experimental runs based on RSM-BBD is given in Table 2. The Table shows the four independent (temperature, pH, inoculum concentration and L-tryptophan concentration) variables' combinations and their resultant IAA actual and predicted values. The highest actual IAA value was achieved at run 28 with optimal temperature of 35 ^{o}C, pH 5, inoculum concentration of 2.5% and L-tryptophan concentration of 20 µL/mL. However, the model predicted highest IAA production of 111.57 mg/ml at run 24 with optimal temperature of 35 ^{o}C, pH 7, inoculum concentration of 2.5% and L-tryptophan concentration of 30 µL/mL.

Table 2. Box Behnken design employed for independent variables and various experiments' composition

Run	A:Temp., ^{o}C	B:pH	C:Inoculum concentration, %	D:Tryptophan, µL/mL	Predicted IAA (mg/mL)	Actual IAA (mg/mL)
1	45	7	5	30	100.41	70.6
2	35	7	5	20	79.91	88.7
3	35	9	2.5	20	79.94	76.2
4	35	7	5	20	79.91	59.7
5	45	9	5	20	68.75	105.5
6	35	7	5	20	79.91	77.5
7	35	7	5	20	79.91	71.7
8	35	5	5	30	111.57	117.3
9	25	9	5	20	65.73	83.7
10	45	5	5	20	94.10	87.1
11	45	7	5	10	62.44	56.8
12	35	9	7.5	20	54.54	83.7
13	35	7	7.5	10	48.23	47.5
14	35	5	7.5	20	79.89	104.5
15	25	5	5	20	91.08	100.2
16	35	7	7.5	30	86.20	98.3
17	35	9	5	30	86.22	87.6
18	35	5	5	10	73.61	53.9
19	25	7	7.5	20	65.71	25.2
20	25	7	2.5	20	91.11	79.3
21	35	7	5	20	79.91	77.4
22	35	9	5	10	48.26	25.3
23	45	7	7.5	20	68.72	58.3
24	35	7	2.5	30	111.60	91
25	25	7	5	30	97.39	95.3
26	45	7	2.5	20	94.12	95.3
27	35	7	2.5	10	73.63	77
28	35	5	2.5	20	105.29	151.1
29	25	7	5	10	59.42	71.8

3.3 Modelling of the Experimental Variables

Summary of ANOVA for response surface linear model for acetic acid production (mg/mL) is given in Table 3. Acetic acid production by the isolate had a Model F-value of 4.72 and a P-value of 0.0059 (<0.0500), implying that the model is significant. This means that there is only a 0.59% chance that an F-value this large could occur due to noise. In this case B, C, D are significant model terms (Table 4). The Lack of Fit F-value of 4.50 means the Lack of Fit is not significant relative to the pure error. There is a 7.73% chance that a Lack of Fit F-value this large could occur due to noise. Coefficient of determination (R^2) obtained from the model was 0.4404.

Table 3: Summary of ANOVA for IAA production

Response	Parameters	Model	Lack of fit
IAA	p-value	0.0059	0.0773
	F-value	4.72	4.59
	Coefficient of determination	0.4404	
	Adjusted coefficient of determination	0.3471	
	Predicted coefficient of determination	0.1380	

Table 3: Linear model parameters for IAA production

Source	Model values	
	F-value	P-value
Model type	Linear	
Model	4.72	0.0059
A-Temperature (X1)	0.0628	0.8043
B-pH (X2)	4.43	0.0459
C-Inoculum concentration (X3)	4.45	0.0455
L-Tryptophan (X4)	9.94	0.0043
Lack of Fit	4.50	0.0773

3.4 Coefficients of IAA production in terms of coded factors

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the variance inflation factors (VIFs) are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable. All VIFs obtained in this study are orthogonal and are given in Table 5.

Equation in terms of coded factors for the production of indole acetic acid (IAA) by the rhizobacterium *Lysinibacillus spaericus* is given in Eq. 2. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table : Coefficients in terms of coded factors for BBD optimization of IAA production

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	79.91	1	3.87	71.92	87.91	
A-Temperature	1.51	1	6.02	-10.92	13.93	1.0
B-pH	-12.68	1	6.02	-25.10	-0.25	1.0
C-Inoculum concentration	-12.70	1	6.02	-25.13	-0.27	1.0
L-Tryptophan	18.98	1	6.02	6.56	31.41	1.0

Acetic acid (DCC6) = +79.91+1.51A-12.68B-12.70C+18.98D Eq.2

3.5 Effect of experimental parameters on IAA production by the bacterial isolate

Interaction effects of the many variables on acetic acid production studied by plotting 3D curves against any two given independent variables, while keeping others at central level are presented in Figure 1. Figure 2 reveals the plots of predicted values against actual values.

3.5.1 Effect of temperature on indole acetic acid production

Combined effects of temperature-pH, temperature-inoculum concentration and temperature-tryptophan concentration on IAA production revealed optimal temperature of 35 to 45 °C for IAA production.

3.5.2 Effect of pH on indole acetic acid production

For combined effects of pH-temperature, pH-inoculum concentration and pH-tryptophan concentration on IAA production, the optimal pH range is pH 6 to 7.5.

3.5.3 Effect of inoculum concentration on indole acetic acid production by the bacterial isolate

For the combined effects of inoculum concentration-temperature, inoculum concentration-pH and inoculum concentration-tryptophan concentration on acetic acid production, optimal inoculum concentration range is 2.5 to 3.5 %.

3.5.4 Effect of tryptophan concentration on indole acetic acid production

For the combined effects of tryptophan concentration-temperature, tryptophan concentration-pH and tryptophan concentration-inoculum concentration on IAA. The optimal tryptophan concentration range is 28 to 30 µL/mL.

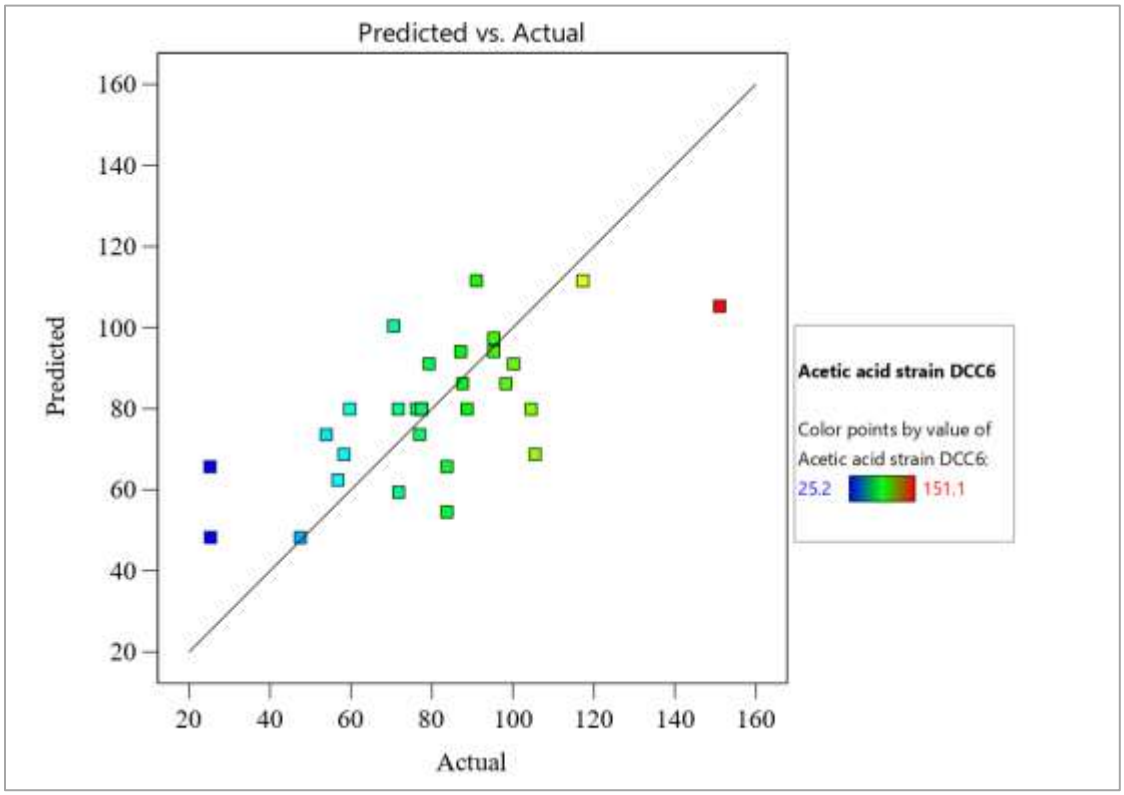

Figure: Plot of actual and predicted values from IAA production based on linear modelling of the interactions between independent factors

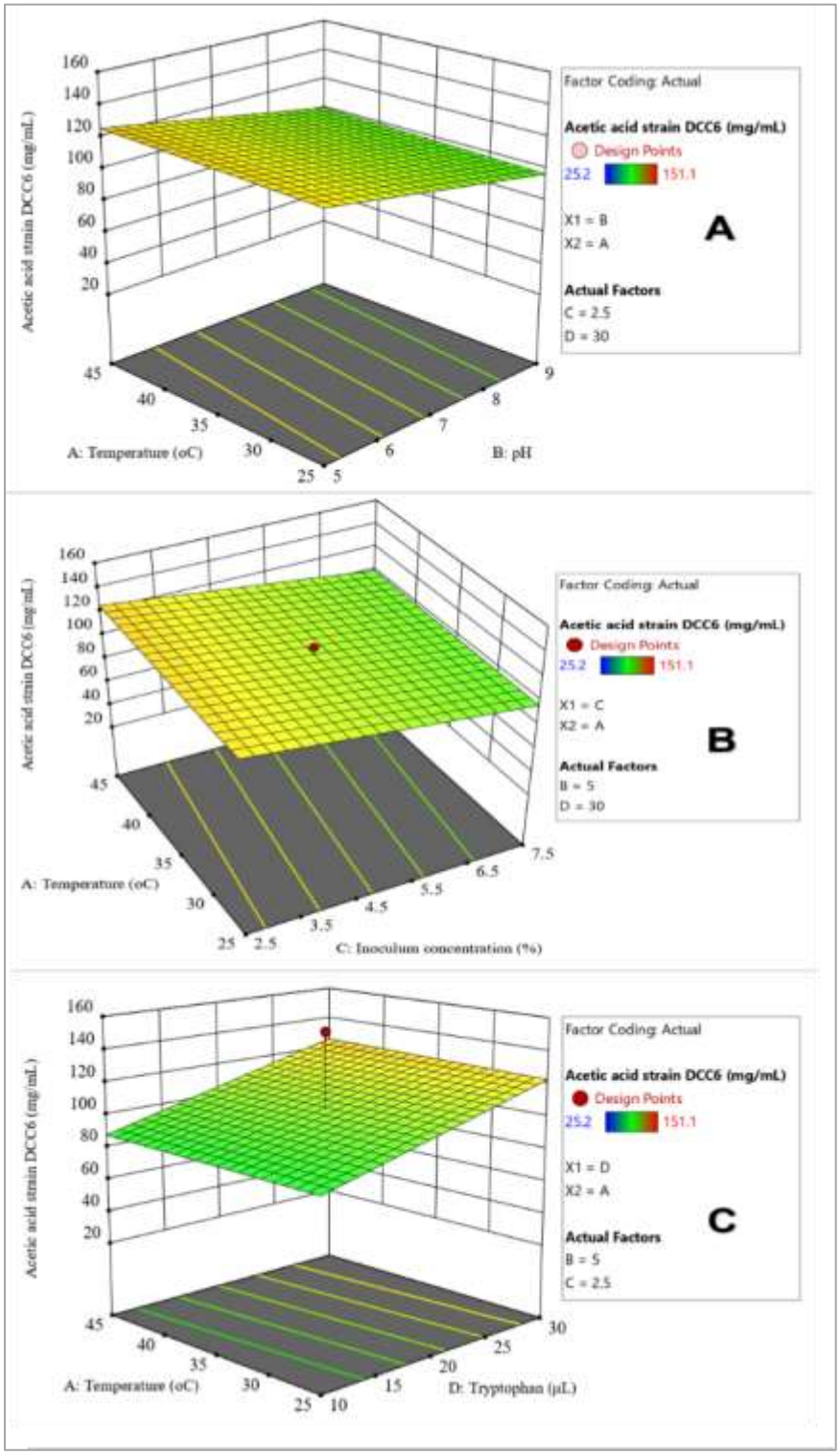

Figure 4.: Response surface 3D for indole acetic acid production *L. sphaericus* in batch fermentation as a function of A: temperature and pH; B: temperature and inoculum concentration and C: temperature and tryptophan concentration.

4. Discussion

This present study attempted to optimize the production of IAA using a rhizobacterium, *Lysinibacillus sphaericus*. *Lysinibacillus sphaericus* used in this study was isolated from the rhizosphere of *Zea mays* (maize). IAA production by the plant was optimized using the Response Surface Methodology (RSM) based on the Box-Behnken Design (BBD). The design generated a linear model that was significant at $p = 0.0059$ with a lack-of-fit that was not significant at $p = 0.0773$. Although the lack-of-fit was low it however was sufficient to navigate the design space. Prior to the optimization, IAA production capacity of the bacterium was 81.3 mg/mL when this is compared to the highest value, 151.1 mg/ml (representing 85.85% increase), obtained after optimization, the optimization techniques therefore significantly enhanced IAA production by the bacterium. Thus, the findings from this study demonstrate the potential capacity of the bacterium to produce IAA and the effectiveness of the RSM technique based on BBD design to optimise the production capacity by altering the cultural conditions (pH, temperature, inoculum concentration and L-tryptophan concentration).

The findings from the application of BBD in the optimization of IAA from *L. sphaericus*, revealed pH 5, 35ºC incubation temperature, 2.5% inoculum, and 20 µl/mL of L-tryptophan as the optimal conditions for IAA production after 24-hour incubation period. Previous studies that utilized RSM technique based on BBD design to optimise secondary metabolite production such as IAA as well as other plant growth-promoting substances have achieved similar results. The study by Malik *et al*. (2011) corroborates the effectiveness of the rhizosphere bacteria in IAA production. They isolated an efficient IAA-producing *Pseudomonas* sp. from the rhizosphere of chickpea (*Cicer arietinum* L.) and green gram (*Vigna radiata*). Similarly, a study by Lebrazi *et al.* (2020) optimised the cultural conditions, including pH temperature and tryptophan concentration, necessary for IAA production, with their result revealing that incubation temperature of 36 °C, a pH of 6.5, an incubation time of 1 day, tryptophan concentration of 1 g/l was optimal for the production of IAA by the isolate. Although there were slight disparities in the optimal values for the independent variables, the findings of their study compared to the values obtained in the present study are quite similar. Their findings underscore the importance of these variables in the enhancement of IAA production and the effectiveness of the RSM-BBD techniques in enhancing IAA production.

In contrast, a more recent study by Arora *et al*. (2024) reported that maximum IAA (121.20 µg/mL) was produced with the following optimal conditions: tryptophan concentration (1 mg/mL) in Potato dextrose broth (48 g/L) under pH 12 and incubation temperature of 35 °C for 7 days. The higher number of days of incubation is understandable considering that they utilised a fungus, for IAA production. However, their study still re-emphasized the importance of these factors in contributing to IAA production.

The choice of the different factors to optimise was based on extensive literature review of significant factors that contribute to IAA production by bacteria. Although most studies are silent on the impact of inoculum concentration, it is noteworthy that inoculum volume can contribute to the amount of IAA produced by an organism. A study by Pantoja-Guerra *et al*. (2023) reported that in most cases IAA production by *Lysinibacillus* spp was optimal at specific inoculum volume of the bacteria. reporting optimal IAA production at 10^8 CFU/mL inoculum concentration. Their findings are consistent with the findings of this study. However, it is noteworthy to state that the specific inoculum concentration that is optimal for IAA production may vary among bacteria depending on the differences in growth kinetics of the different bacteria.

The optimal pH for the production of IAA which fell between pH 5 and 6 as observed in this study showed that IAA was produced more under slightly acidic condition. Different studies have reported different pH for IAA production by different bacteria. Scarcella *et al*. (2017) and Wandira *et al*. (2021) reported optimal IAA production at pH 6, which is similar to the result obtained in this study. However, the report by Chandra et al. (2018) showed that *Stevia rebaudiana* isolated from rhizosphere produced IAA at pH 9. The activity of enzymes responsible for the biosynthesis of IAA can be hindered by pH variation. Extreme pH usually impedes IAA production; thus, IAA production is reduced in either extreme acidic or alkaline pH. However, certain bacteria may display wider pH tolerance depending on their physiological differences. The pH of the medium that favours IAA production may be indicative of the type of agricultural soil on which the bacteria may thrive best. The ability of *Lysinibacillus sphaericus* to thrive and produce IAA under such slight pH conditions suggests that it may be well-suited for application in agricultural soils with slightly acidic pH levels.

Temperature was another critical factor considered in the optimization of IAA production using the *Lysinibacillus sphaericus*. Many studies have reported similar temperature as optimal for IAA production. However, it is noteworthy to emphasize the strain specificity of temperature in secondary metabolite production. The prevailing temperature on which the strain was isolated influences their preference for

incubation for secondary metabolite production including IAA. Since microbial growth and the functionality of biosynthetic enzymes are grossly temperature dependent it is understandable that temperature is a critical factor in IAA production (Chandra *et al*., 2018; Bunsangiam *et al*., 2021).

L-tryptophan in IAA production was also considered as a factor in the present study. L-tryptophan has been described as a precursor to IAA production. An early study by Rekoslavskaya *et al*. (1999) reported the production of IAA was increased several times due to the addition of tryptophan in the production medium. Even more recent studies have confirmed this position. Rekoslavskaya *et al*. (2019) described the distribution of tryptophan-dependent indole-3-acetic acid synthesis pathways in bacteria. This gives credence to the findings of this report on the influence of tryptophan in IAA production.

Conclusions

Optimization of indole acetic acid production by *Lysinibacillus sphaericus* was the focus of this present study. The data obtained revealed that the isolate could be utilized as a useful and sustainable plant growth-promoting bacterium (PGPB) to facilitate, stimulate and improve crop yield. Moreover, the deployment of RSM was effective in enhancing the production of IAA by the *Lysinibacillus sphaericus*. The linear model obtained from the BBD-RSM was significant, indicating that the model can effectively predict the production of IAA by the bacterium. Although the 0.4404 R^2 (Coefficient of determination) shows lower level of accuracy in the predictive capacity of the Model. The derived linear model used in this study is important due to its simplicity, interpretability, and effectiveness in certain situations. The significance of a linear model depends on the specific requirements of the problem at hand and the characteristics of the data. Therefore, more robust model may be required to fully understand the interaction between the different variables involved in IAA production. In all, the factors studied, especially, pH, inoculum concentration and L-tryptophan concentration, significantly influenced IAA production.

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References

1. Arora, P., Tabssum, R., Gupta, A. P., Kumar, S., & Gupta, S. (2024). Optimization of indole acetic acid produced by plant growth promoting fungus, aided by response surface methodology. Heliyon, 10(14), e34356. https://doi.org/10.1016/j.heliyon.2024.e34356
2. Baliyan, N., Dhiman, S., Dheeman, S., Kumar, S., & Maheshwari, D. K. (2021). Optimization of indole-3-acetic acid using response surface methodology and its effect on vegetative growth of chickpea. *Rhizosphere*, *17*, 100321.
3. Bessai, S. A., Bensidhoum, L., & Nabti, E. H. (2022). Optimization of IAA production by telluric bacteria isolated from northern Algeria. Biocatalysis and Agricultural Biotechnology, 41, 102319.
4. Boondaeng, A., Vaithanomsat, P., Apiwatanapiwat, W., Trakunjae, C., Janchai, P., Suriyachai, N., Kreetachat, T., Wongcharee, S., & Imman, S. (2023). Biological Conversion of Agricultural Wastes into Indole-3-acetic Acid by Streptomyces lavenduligriseus BS50-1 Using a Response Surface Methodology (RSM). ACS Omega, (43), 40433-40441. doi: 10.1021/acsomega.3c05004.
5. Bunsangiam, S., Thongpae, N., Limtong, S., & Srisuk, N. (2021). Large scale production of indole-3-acetic acid and evaluation of the inhibitory effect of indole-3-acetic acid on weed growth. Scientific reports, 11(1), 13094.
6. Bunsangiam, S., Thongpae, N., Limtong, S., & Srisuk, N. (2021). Large scale production of indole-3-acetic acid and evaluation of the inhibitory effect of indole-3-acetic acid on weed growth. Scientific reports, 11(1), 13094. https://doi.org/10.1038/s41598-021-92305-w.
7. Chandra, S., Askari, K., & Kumari, M. (2018). Optimization of indole acetic acid production by isolated bacteria from Stevia rebaudiana rhizosphere and its effects on plant growth. Journal, genetic engineering & biotechnology, 16(2), 581–586. https://doi.org/10.1016/j.jgeb.2018.09.001
8. Chandra, S., Askari, K., & Kumari, M. (2018). Optimization of indole acetic acid production by isolated bacteria from Stevia rebaudiana rhizosphere and its effects on plant growth. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 581-586.
9. Duca, D., Lorv, J., Patten, C. L., Rose, D., & Glick, B. R. (2014). Indole-3-acetic acid in plant–microbe interactions. *Antonie Van Leeuwenhoek*, *106*, 85-125.
10. Gang, S., Sharma, S., Saraf, M., Buck, M., & Schumacher, J. (2019). Analysis of Indole-3-acetic Acid (IAA) Production in Klebsiellaby LC-MS/MS and the Salkowski Method. Bio-protocol, 9(9), e3230. https://doi.org/10.21769/BioProtoc.3230

11. Gomes, G. L. B., & Scortecci, K. C. (2021). Auxin and its role in plant development: structure, signalling, regulation and response mechanisms. *Plant Biology*, *23*(6), 894-904.
12. Haldar, S., & Sengupta, S. (2023). An Overview of the Multifaceted Role of Plant Growth-Promoting Microorganisms and Endophytes in Sustainable Agriculture: Developments and Prospects. *Microbial Symbionts and Plant Health: Trends and Applications for Changing Climate*, 179-208.
13. Holt JG, Krieg NR, Sneath PHA (1994) Bergey's manual of determinative bacteriology. Lippincott Williams and Wilkins, USA
14. Lebrazi, S., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production by Rhizobium sp. strain using response surface methodology. Journal of Genetic Engineering and Biotechnology, 18(2): 21. doi:10.1186/s43141-020-00035-9.
15. Lebrazi, S., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production by Rhizobium sp. strain using response surface methodology. *Journal of Genetic Engineering and Biotechnology*, *18*(1), 21.
16. Lebrazi, S., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production by Rhizobium sp. strain using response surface methodology. Journal, genetic engineering & biotechnology, 18(1), 21. https://doi.org/10.1186/s43141-020-00035-9
17. Madigan, M. T., Martinko, J. M., & Parker, J. (1997). Brock biology of microorganisms (Vol. 11). Upper Saddle River, NJ: Prentice hall.
18. Malik, D. K., & Sindhu, S. S. (2011). Production of indole acetic acid by Pseudomonas sp.: effect of coinoculation with Mesorhizobium sp. Cicer on nodulation and plant growth of chickpea (Cicer arietinum). Physiology and molecular biology of plants : an international journal of functional plant biology, 17(1), 25–32. https://doi.org/10.1007/s12298-010-0041-7
19. Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of soil science and plant nutrition*, *13*(3), 638-649.
20. Pantoja-Guerra, M., Burkett-Cadena, M., Cadena, J., Dunlap, C. A., & Ramírez, C. A. (2023). Lysinibacillus spp.: an IAA-producing endospore forming-bacteria that promotes plant growth. Antonie van Leeuwenhoek, 116(7), 615–630. https://doi.org/10.1007/s10482-023-01828-x
21. Pepper, I. L., & Gerba, C. P. (2015). Environmental sample collection and processing. In *Environmental microbiology* (pp. 157-175). Academic Press.
22. Rekoslavskaya, N. I., Yurjeva, O. V., Salyaev, R. K., Mapelli, S., & Kopytina, T. V. (1999). L-tryptophan as IAA source during wheat germination. Bulgarian Journal of Plant Physiology, 25(1-2), 39-49.
23. Scarcella, A. S. D. A., Bizarria, R., Bastos, R. G., & Magri, M. M. R. (2017). Temperature, pH and carbon source affect drastically indole acetic acid production of plant growth promoting yeasts. Brazilian Journal of Chemical Engineering, 34, 429-438.
24. Waday, Y. A., Aklilu, E. G., Bultum, M. S., & Ancha, V. R. (2022). Optimization of soluble phosphate and IAA production using response surface methodology and ANN approach. *Heliyon*, *8*(12).
25. Wandira, T. A., Aisyah, S. N., Oktavioni, M., Fatiah, R., & Jamsari, J. (2021). Different pH levels medium effects in IAA production of phylloplane bacterium Serratia plymuthica strain UBCF_13. In IOP Conference Series: Earth and Environmental Science (Vol. 741, No. 1, p. 012003). IOP Publishing.
26. Wei, J. Y., Chen, H. W., Liu, Y. Y., Lu, H. Y., & Chou, J. Y. (2015). Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signaling & Behavior, 10(8), e1048052.
27. Zhang, P., Jin, T., Kumar Sahu, S., Xu, J., Shi, Q., Liu, H., & Wang, Y. (2019). The distribution of tryptophan-dependent indole-3-acetic acid synthesis pathways in bacteria unraveled by large-scale genomic analysis. Molecules, 24(7), 1411.

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