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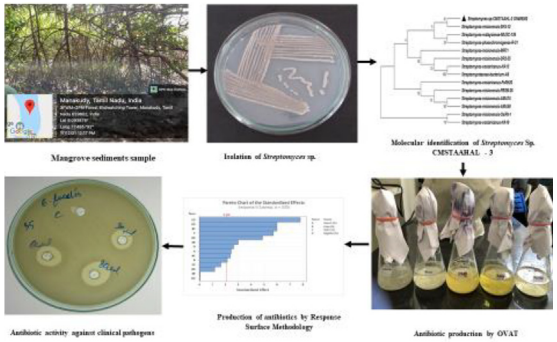
Research Article

Statistical optimization of media components for antibiotic production in *Streptomyces* sp. CMSTAAHAL-3

Jeraldin Nisha Selvaraj [a], Uma Ganapathi [a], Samuel Gnana Prakash Vincent [a], Sathishkumar Ramamoorthy [b], Citarasu Thavasimuthu [a,*]

[a] Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, 629 502 Tamil Nadu, India
[b] Centre for Aquaculture, Sathyabama Institute of Science and Technology, Chennai 600 119, Tamil Nadu, India

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Actinomycetes particularly the *Streptomyces* sp. derived from marine and coastal habitats are regarded as the main source of antibiotics. In the current study, to isolate the antibiotic-producing actinomycetes sediment samples were collected from Manakudy estuary, Kanyakumari District, Tamil Nadu.
Results: The isolates were selected based on the antagonistic activity; the highly active isolate was identified as *Streptomyces* sp. CMSTAAHL-3. The culture media for the antibiotic production was optimized by one variable at a time and confirmed by the zone of inhibition against tested pathogens such as Staphylococcus aureus, *Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli,* and *Pseudomonas aeruginosa.* Then by altering the four factors starch, urea, $MgSO_4$ and NaCl at five levels, statistical optimization of the media components was explored using Response Surface Methodology-Central Composite Design and found that the predicted antibiotic response was closely correlated with the experimentally one and was confirmed by the zone of inhibition.
Conclusions: The *Streptomyces* sp. CMSTAAHL-3 and its antibiotics may be of great use in the treatment of a variety of pathogens, and the ideal culture medium discovered in this experiment will be helpful for further research involving large-scale fermentation for the effective production of antibiotics.
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* Corresponding author.
E-mail address: citarasu@msuniv.ac.in (C. Thavasimuthu).

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1. Introduction

The mangrove forest intertidal zone is a huge ecosystem that includes both marine and terrestrial habitats. Mangrove microorganisms vary from terrestrial microorganisms in that they can develop unique metabolites due to special environmental conditions such as high salt and high wetness. The mangrove ecosystem is a productive and underutilized habitat with an infinite variety of bacteria for the identification of novel and chemically varied antimicrobial compounds [1]. The mangrove forest is a one-of-a-kind intertidal zone between the sea and brackish water and have differing salinity levels [2].

Actinomycetes are Gram-positive, unicellular bacteria with 55% guanine and cytosine in their DNA [3,4]. It can be found practically anywhere in nature, including water, air, soil, food, manure, and compost [5]. Antibiotics, enzymes, bioactive metabolites, and plant growth regulators are abundant in actinomycetes [6,7,8,9,10]. Actinomycetes have greater biotechnological and economic value [11]. Terrestrial actinomycetes differ from marine actinomycetes physiologically and phylogenetically [12]. Marine actinomycetes have been found to be a new source of secondary metabolites, and some isolated secondary metabolites have already been shown to be antagonistic [13,14]. Antibiofilm, antifungal, antibacterial, anticancer, antibiotics, immunosuppressive agents and enzyme activities are among the many biomedical applications of secondary metabolites from actinomycetes [15,16,17,18,19].

Actinomycetes that produce valuable secondary metabolites from mangrove environment have become a hot topic. Streptomycetes, a genus of actinomycetes, yielded over 10,000 bioactive compounds [20]. Actinomyces and streptomyces create 70–80% of all antibiotics produced globally, making them the most prolific producers of microbial bioactive secondary metabolites for potential agricultural, food, pharmaceutical, and industrial applications [21,22]. At least 5,000 bioactive compounds were generated by *Streptomyces* sp., including β-lactams, polyenes, aminoglycosides, tetracyclines, peptides, antibiotics, acrolides, and polyethers [23]. Secondary metabolites generated by *Streptomyces* sp. have been utilized successfully as antibiotics in the treatment of drug-resistant diseases in people such as antitumor and antibacterial activity against MRSA and anti-inflammatory activities etc. [24].

The production of pharmaceutically important secondary metabolites can be impacted by carbon, nitrogen, and sodium chloride sources due to the necessity for optimal growth conditions and nutrient requirements [25,26]. The technique of one component at a time for the production of secondary metabolites claims a large number of experiments and is timeconsuming [27]. Numerous studies on the use of Response Surface Method (RSM) in biochemical and biotechnological processes have been published [28]. RSM is a group of statistically validated experimental techniques for forecasting the outcome, estimating the coefficients in a mathematical model, and determining the suitability of the model [29,30,31,32]. As a result, the RSM and statistical experimental design method are frequently used to identify the most important elements impacting production of secondary metabolites [27,33]. In order to minimize the need for extra culture medium components, RSM can be used to optimize fermentation conditions such that they suit the nutritional needs of specific bacteria [34]. In comparison to other optimization approaches, RSM requires fewer trials to calculate the many variables and their interactions [32]. The quality and quantity of antibiotics can be impacted by small changes in the culture media's composition. A novel substrate in media components might promotes microbial growth [34,35]. Major environmental factors that affect antibiotic production include pH, fermentation time, temperature, and culture media composition. These factors can all be statistically controlled using RSM [36]. Several *Streptomyces* species have employed the RSM approach to improve antibiotic production, including *Streptomyces* sp. HJC-D1 [31], *Streptomyces nogalater* NIIST A30 [37], *Streptomyces* sp. SY-BS5 [38], and *Streptomyces* sp. SYYLWH. With this in mind, we have isolated mangroveassociated actinomycetes from the Manakudy estuary and using one variable at a time (OVAT) and Central Composite Design and Response Surface Methodology (CCD- RSM) to optimize the media components for antibiotic production for the selected isolate.

2. Materials and methods

2.1. Isolation of mangrove-associated actinomycetes

Sediment samples from microbial diversity-rich mangrove environment were collected from Manakudy estuary (Latitude: 8°088′N, Longitude: 77°486′E), Kanyakumari District, Tamil Nadu. The samples were collected in a Ziploc bag, kept briefly on ice, and then transported straightaway to the laboratory for temporary storage at 4°C until the analysis. The samples were serially diluted up to a 10^{-6} dilution for the purpose of isolating actinomycetes, and then plated on various media, including starch casein, actinomycetes isolation agar (AIA), streptomyces isolation agar (SIA), and Sato A' media [39]. The suspected colonies were selected from the nutritive media plates, streaked, and cultured at 28°C for 3 to 5 d. The selected pure culture of actinomycetes colonies was maintained in the starch casein agar media at 4°C.

2.2. In vitro antagonistic activity of the isolated actinomycetes

Four strains were selected and tested against clinical pathogens such as Gram-positive (*S. aureus*, *E. faecalis*) and Gram-negative (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) bacterial pathogens. Spot inoculations of actinomycetes were made in starch casein agar and incubated for two days at 28°C. After 2 d, 5 ml of sloppy agar (0.6%) layer was applied to the actinomycetes plates, which had already been seeded with the tested bacteria and was then incubated at 30°C for 18 to 24 h. The inhibitory zones' diameter was measured. For further investigation, the highly antagonistic active strain was selected [40,41].

2.3. Morphological identification of the selected actinomycetes

The selected isolate's aerial and substrate mycelium was analyzed and compared to Bergey's Manual of Determinative Bacteriology. In order to examine the culture's spore chain and the color of its aerial and substrate mycelia, the culture was allowed to grow on Actinomycetes Isolation Agar (AIA), Streptomyces Isolation Agar (SIA), Sato 'A', and Starch Casein Agar (SCA) medium and incubated for 7 d at 28°C [42].

2.4. Molecular characterization of the selected actinomycetes

The potential strain was selected for molecular identification. The genomic DNA was extracted using the phenol-chloroform technique [43,44]. The 16S rRNA gene fragment was amplified using universal primers (forward primer 5′-AGAGTTTGATCCTGGCAG-3′ and reverse primer 5′-TACGGC TACCTTGTTACGACTT-3′) after [13]. Initial denaturation of the PCR was carried out at 95°C for 5 min, followed by 35 cycles of amplification at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The sequenced bases were aligned by basic local alignment search techniques (BLAST) software [45].

2.5. Preparation of inoculum and culture media for secondary metabolite production

Fresh culture of *Streptomyces* sp CMSTAAHL-3 was inoculated into the 50 ml of basal medium (2.9 g Na_2HPO_4; 2.3 g KH_2PO_4; 1.0 g NH_4Cl; 0.5 g $MgSO_4 \times 7H_20$; 0.002 g $FeSO_4$; 0.5 g $CaCO_3$) and a 5.0 ml trace element salt solution from a stock solution (0.1 g $ZnSO_4 \times 7H_20$; 0.3 g H_3BO_4; 0.2 g $CoCl_2 \times 6H_20$; 0.03 g $MnCl_2 \times 4H_20$; 0.03 g $Na_2B_4O_7 \times 4H_2O$; 0.02 g $NiCl_2 \times 6H_2O$; 0.01 g $CuCl_2 \times 2H_20$ in 1 L water) used as a seed culture. The inoculated flask was kept at 180 rpm at 28°C for 5 to 7 d.

2.6. Antibiotic production by OVAT approach

Initial screening of OVAT was performed by varying the pH, temperature, incubation days, carbon, nitrogen, mineral sources and NaCl concentrations, to analyze the optimum factors influencing the antibiotic production.

In this study, different pH (3, 5, 7, 9 and 11 with 0.2 unit interval); temperature (26, 30, 35, 40°C and 45°C); NaCl concentration (3, 5, 7, 9 and 11%); incubation days (3, 5, 7, 9 and 11 d), 1% carbon sources (starch, glucose, fructose, maltose and sucrose); 1% nitrogen sources (yeast extract, beef extract, urea, tryptone and peptone), 0.1% mineral sources (KH_2PO_4, NH_4Cl, MgSO4, Na_2HPO_4, $CaCO_3$, and $FeSO_4$) were added separately, and the antibacterial activity against human pathogens *S. aureus, E. coli, K. pneumoniae, E. faecalis,* and *P. aeruginosa* was determined. The conclusion of the OVAT approach was utilized to optimize statistical media [46,47].

2.7. Media optimization for antibiotic production by CCD-RSM

A central composite design (CCD) with four factors and five levels was adopted in the current study, requiring 31 trials. The fractional factorial design comprised nine factorial points, fourteen center points, and nine axial points with four parameters. A: Starch, B: Urea, C: Sodium chloride, D: $MgSO_4$ were used to improve the fermentation broth that was used to make antibiotics (Table S1). MINITAB 19 is the software response optimizer tool used for the RSM analysis. Response surface regression was applied to fit the experimental RSM data. Coded values representing the variables according to Equation 1:

$$x_i = X_i - \frac{X_0}{\delta X_i}, i = 0; 1; 2; 3; \cdots; n \qquad \text{Equation 1}$$

where x_i – denotes selected independent variables for coded value, X_i – original value of the selected variable, X_0 – center point value of the actual value of the independent variable, and $-X_i$ step change value.

To optimize the process variables, central composite design and response surface methodology were used. To examine the behavior of a quadratic equation, the quadratic model Equation 2 is helpful.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2 \qquad \text{Equation 2}$$

Where Y = predicted response, β_0 = intercept term, β_i = linear coefficient, β_{ij} = quadratic coefficient, β = interaction coefficient, X_i-X_i = independent variables. The variable X_iX_j represents the first-order interaction between X_i and X_j (i < j).

In order to validate the created model, analysis of variance (ANOVA) was applied. Using the F-test, the significance of the designed model was assessed, and a significant constructed CCD model was one with a *P*-value of less than 0.05. ANOVA, which includes the Fisher's F-test, associated probability P (F), determination coefficient R^2, and correlation coefficient R to assess the goodness of fit of the regression model, was used for the statistical analysis of the model. The calculated coefficients and related probabilities were also included in the analysis along with Student's t-values, P (t) [48,49].

2.8. Extraction of antibiotics for OFT and RSM

The supernatant was combined with an equal amount of (1:1) ethyl acetate after the mycelia biomass was removed by centrifugation at 10,000 rpm for 15 min. The mixture was then stirred for 45 min and maintained in a separating funnel. The solvent layer was collected separately, evaporated and the crude antibiotics collected was dissolved in methanol for the antibacterial analysis against 5 different pathogens *S. aureus, E. coli, K. pneumoniae, E. faecalis* and *P. aeruginosa,* and their zone of inhibition is determined using methanol as control [50,51].

3. Results

3.1. Screening of antibacterial activity

Totally, 4 mangrove isolates (CMSTAAHL-6, CMSTAAHL-7, CMSTAAHL-3, and CMSTAAHL-8) were selected and their antagonistic activity was performed. The *Streptomycetes* sp. CMSTAAHL3 strain outperformed the other three tested strains in terms of effectiveness against the bacterial pathogens. *E. faecalis* and *S. aureus* showed the highest zones of inhibition (0.7 cm and 0.5 cm) (Fig. 1).

3.2. Cultural characteristics of Streptomyces sp. CMSTAAHL – 3 on different media

The cultural and morphological characteristics of the isolate *Streptomyces* sp. CMSTAAHL – 3 on different media was given in Table S2. The aerial mycelia of *Streptomyces* sp. CMSTAAHL – 3 were brownish ash, and substrate mycelia were white with ash color when grown in AIA media. The CMSTAAHL – 3 showed light brown aerial mycelia and brown substrate mycelia when grown in Sato 'A' media. When grown in SCA, the aerial mycelia were brown color with dots and the substrate mycelia was brown in color (Fig. 2).

3.3. Molecular characterization of Streptomyces sp. CMSTAAHL-3

Based on 16S rRNA sequencing, the isolate was identified as *Streptomyces* sp. CMSTAAHL – 3. The 16S rRNA sequence of the strain *Streptomyces* sp. CMSTAAHL – 3 was deposited in Gen Bank (NCBI) database with an accession number: ON406302.

3.4. Screening of nutrient factors for antibiotic production by OVAT approach

The antibiotic production of *Streptomyces* sp. CMSTAAHL – 3 against pathogens like *S. aureus*, *E. faecalis*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa* was optimized by OVAT approach in the current study using seven parameters, including carbon sources, nitrogen sources, minerals, NaCl (Fig. 3), pH, temperature and incubation period (Fig. 4). The results demonstrated that the production of antibiotics was improved by starch (1%), urea (1%), $MgSO_4$, pH 7, 3% NaCl, 7 d of incubation, and 30°C.

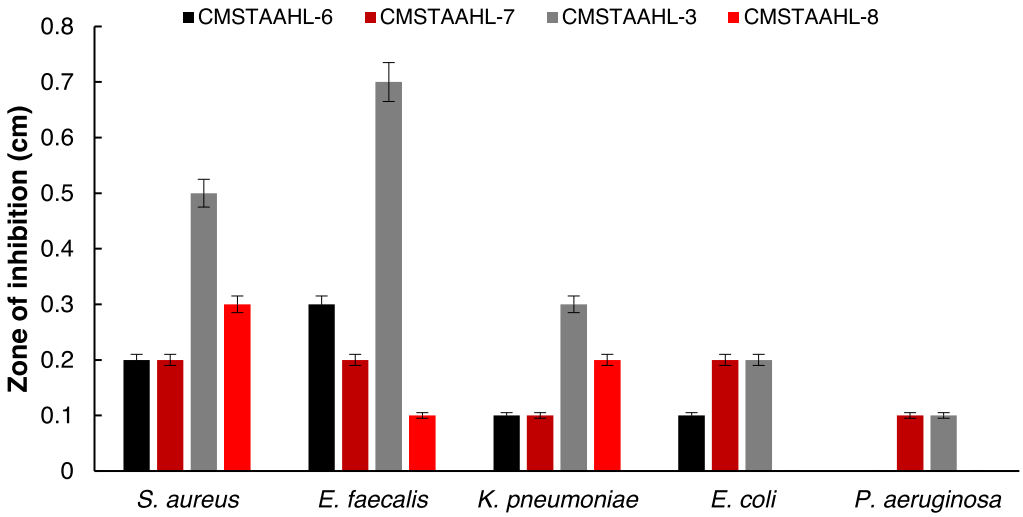

Fig. 1. *In vitro* antagonistic activity for selected active strain by agar overlay method.

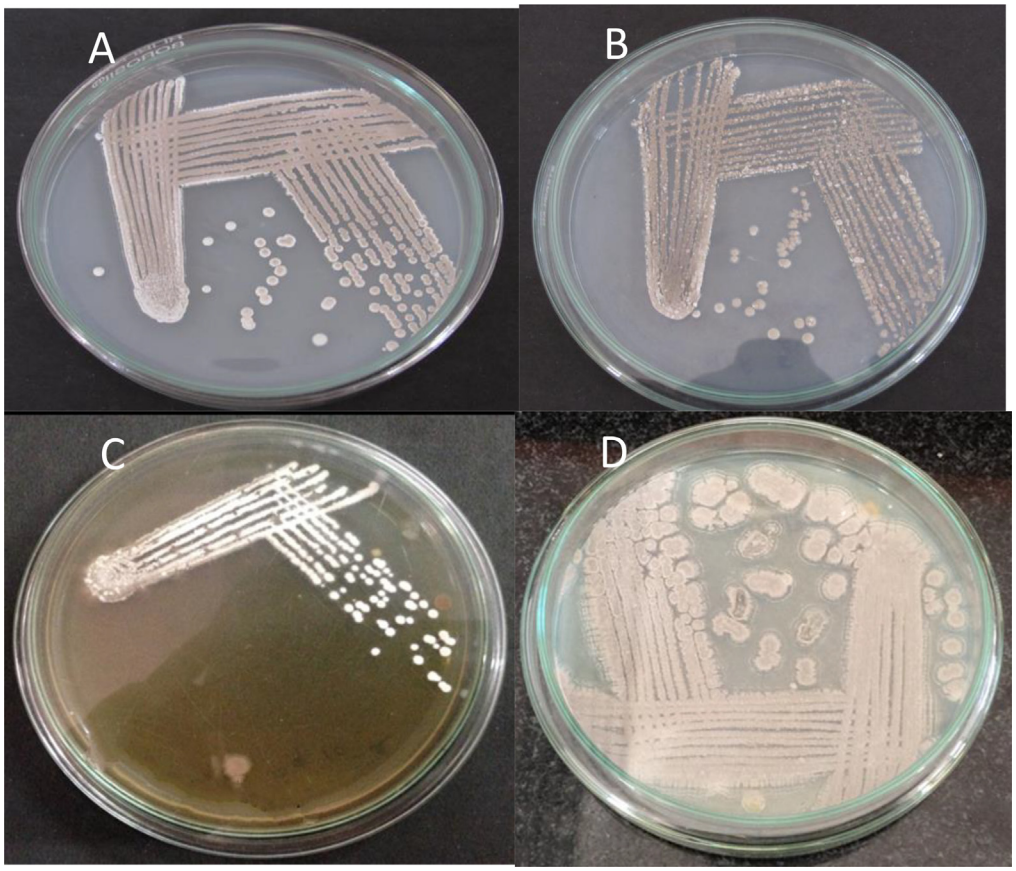

Fig. 2. Growth and morphology of *Streptomyces* sp. CMSTAAHL – 3 on different media (A) Actinomycetes isolation agar; (B) Streptomycetes isolation agar; (C) Sato 'A' media; (D) Starch casein agar.

3.5. Optimization of fermentation medium for antibiotic production through CCD

The CCD of response surface methodology was used to maximize the antibiotic generated by *Streptomyces* sp. CMSTAAHL – 3 by optimizing the medium components. A total of 31 experiments were carried out using various concentrations of starch, urea, NaCl, and $MgSO_4$ (Table 1).

The experimental data were put through a model adequacy test to see if the model would produce inaccurate or approximate findings. In order to ascertain the precise link between the response and the variables chosen for the investigation, linear, interactive,

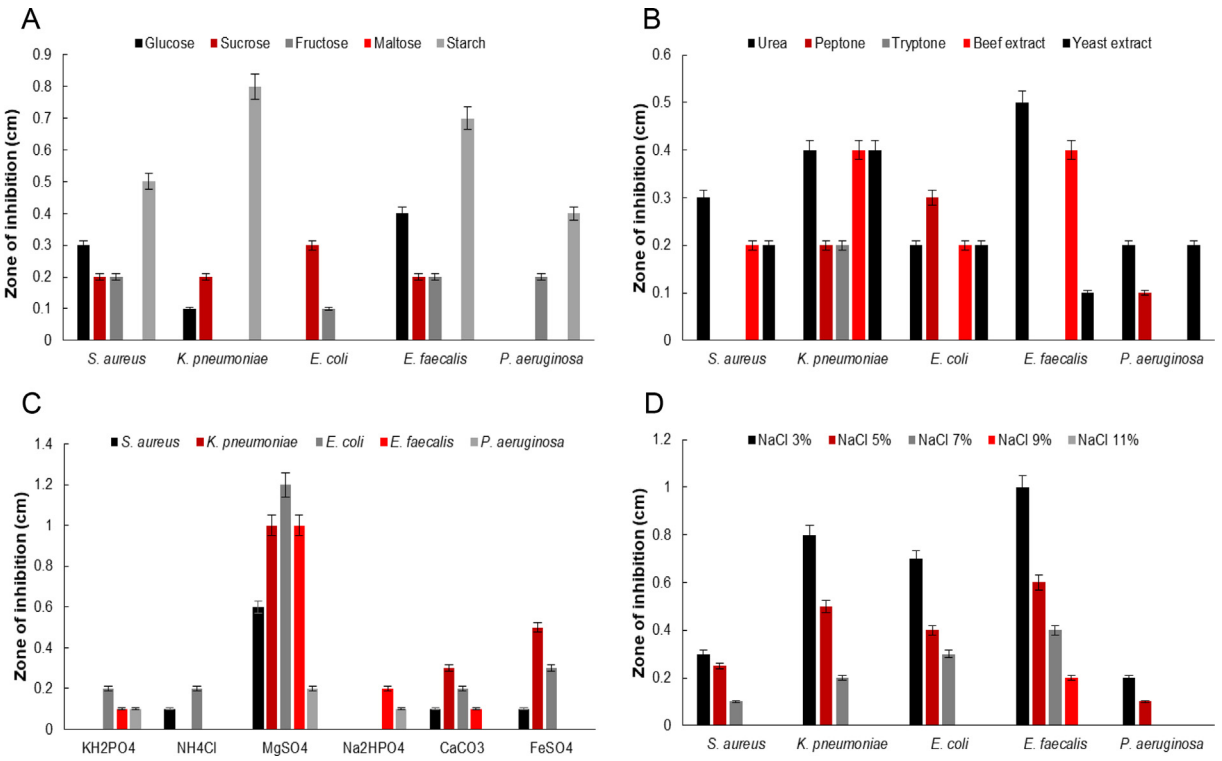

Fig. 3. Effect of antibiotic production by different nutritional factors. (A) Carbon sources, (B) Nitrogen sources, (C) Mineral sources, (D) NaCl concentrations.

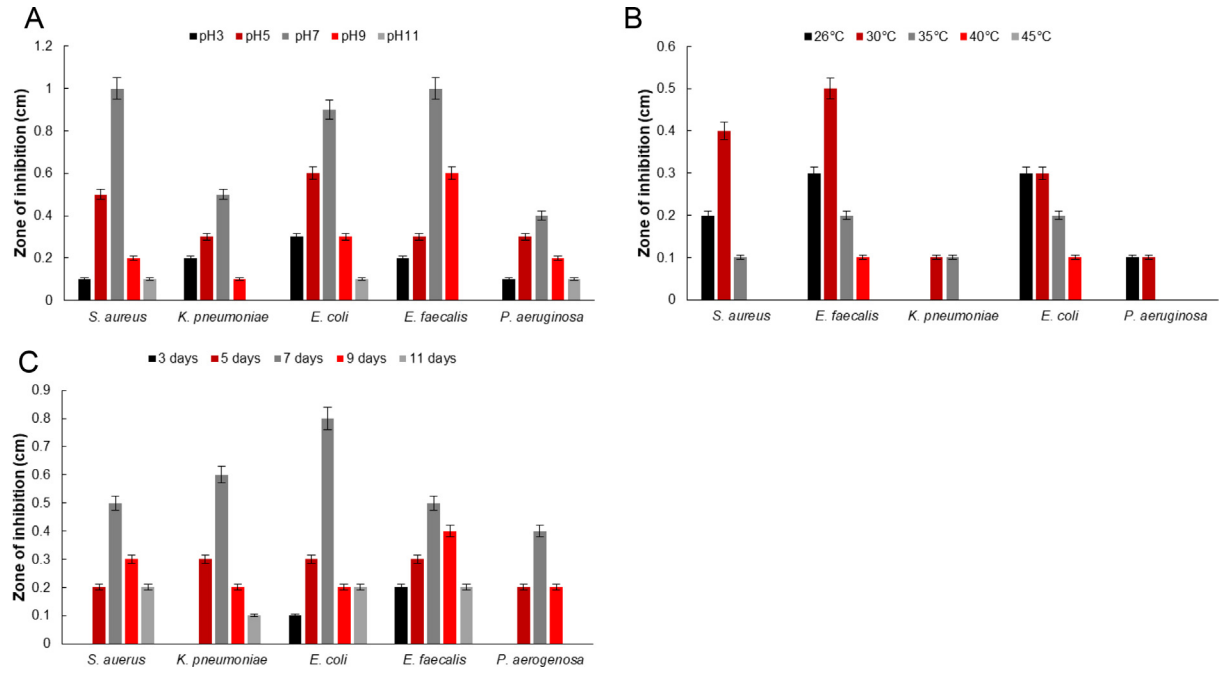

Fig. 4. Effect of antibiotic production by different physical factors. (A) pH, (B) Incubation temperatures, (C) Incubation periods.

and quadratic models were fitted to the experimental data. The best outcome quadratic model for all five responses was identified using the suggested sequential model sum of squares and lack of fit tests (showing degrees of freedom; mean square, *F* value, and *P* value), as well as model summary statistics (showing standard deviation, R^2, adjusted R^2, and predicted R^2).

The results of experiments using a central composite design were determined using second-order polynomial multiple regression, and the results are shown in Table 2. In accordance with regression analysis, the system under a given experimental domain is significant based on the coefficient of determination (R_2) values of the four responses, and high values of R_2 indicated that the full quadratic model equation was capable of representing that the system under a given experimental domain is significant. Additionally, adjusted coefficient of determination (R^2 adj) values also indicated a good agreement between experimental and predicted values (Table 2).

The adequacy of the chosen model was demonstrated by the R^2 coefficient of determination. The trail parameters, their interaction,

Table 1
Experiment design and results of optimization of nutrient medium for the production of antibiotic from *Streptomyces* CMSTAAHL – 3 by the central composite design.

S. NO	(A) (%)	(B) (%)	(C) (%)	(D) (%)	Zone of inhibition of *S. aureus* (cm)		Zone of inhibition of *E. faecalis* (cm)		Zone of inhibition of *K. pneumoniae* (cm)		Zone of inhibition of *E. coli* (cm)		Zone of inhibition for *P. aeruginosa*(cm)	
					Observed values	Predicted values	Observed values	Predicted values	Observed values	Predicted values	Observed values	Predicted values	Observed values	Predicted values
1	0.50	1	2.5	0.025	0.4	0.4	1.2	1.2	0.5	0.4	1.6	1.6	0.7	0.7
2	1	0.50	2.5	0.025	0.9	0.8	1.4	1.2	1.0	1.1	1.8	1.8	0.5	0.4
3	0.75	0.75	3	0.05	1.2	1.1	1.4	1.4	0.9	0.9	1.5	1.5	0.5	0.5
4	0.50	0.50	3.5	0.075	0.9	0.9	1.0	1.0	1.0	1.0	1.5	1.5	0.4	0.4
5	1.25	0.75	3	0.05	1.0	1.1	1.6	1.6	0.2	0.05	1.7	1.7	0.1	0.1
6	0.75	1.25	3	0.05	0.7	0.6	1.2	1.2	0.3	0.3	1.9	1.9	0.4	0.4
7	1	0.50	3.5	0.025	1.4	1.3	1.4	1.5	0.2	0.2	1.2	1.2	0.1	0.1
8	0.75	0.75	2	0.05	0.8	0.8	1.5	1.6	0.6	0.6	1.3	1.3	1.2	1.2
9	0.75	0.25	3	0.05	1.2	1.3	1.4	1.4	0.2	0.1	1.5	1.5	0.5	0.5
10	0.50	1	2.5	0.075	0.5	0.5	1.9	1.8	0.9	0.9	1.8	1.8	0.3	0.3
11	1	1	3.5	0.075	0.9	0.9	1.2	1.1	0.9	1.0	1.5	1.5	0.5	0.5
12	0.75	0.75	3	0.05	1.2	1.2	1.4	1.4	0.9	0.9	1.5	1.5	0.5	0.5
13	1	1	2.5	0.025	0.8	0.7	0.6	0.6	0.4	0.4	1.6	1.6	0.2	0.2
14	0.50	0.50	2.5	0.075	0.6	0.6	1.3	1.3	0.8	0.8	0.8	0.8	0.4	0.4
15	0.50	0.50	3.5	0.025	1.7	1.7	1.2	1.2	0.3	0.3	1.3	1.3	0.1	0.1
16	0.75	0.75	3	0.05	1.2	1.2	1.4	1.4	0.9	0.9	1.5	1.5	0.5	0.5
17	0.50	1	3.5	0.025	0.9	0.8	1.0	1.0	0.7	0.7	1.3	1.3	0.7	0.7
18	0.75	0.75	3	0.05	1.2	1.2	1.4	1.4	0.9	0.9	1.5	1.5	0.5	0.4
19	1	0.50	2.5	0.075	1.3	1.2	1.1	1.1	0.5	0.5	1.8	1.7	0.8	0.8
20	0.75	0.75	4	0.05	1.2	1.2	1.5	1.4	0.7	0.7	1.0	0.9	1.2	1.2
21	1	1	2.5	0.075	1.4	1.4	1.2	1.2	0.3	0.3	2.0	2.0	0.2	0.1
22	0.75	0.75	3	0.05	1.2	1.2	1.4	1.4	1.0	0.9	1.5	1.5	0.5	0.5
23	0.25	0.75	3	0.05	0.5	0.5	2.0	2.0	0.5	0.6	1.6	1.6	0.3	0.3
24	0.50	0.50	2.5	0.025	1.0	0.9	1.2	1.3	0.8	0.8	1.1	1.1	0.5	0.5
25	0.75	0.75	3	0.05	1.2	1.2	1.4	1.4	0.9	0.9	1.5	1.5	0.5	0.5
26	0.75	0.75	3	0.05	1.2	1.2	1.4	1.4	0.9	0.9	1.5	1.5	0.5	0.5
27	0.50	1	3.5	0.075	0.4	0.4	1.3	1.4	2.0	1.9	2.1	2.1	0.6	0.6
28	0.75	0.75	3	0.05	1.2	1.2	1.2	1.4	1.0	0.9	1.5	1.5	0.5	0.5
29	1	0.50	3.5	0.075	1.3	1.2	1.2	1.2	0.4	0.4	1.7	1.7	0.8	0.8
30	1	1	3.5	0.025	0.8	0.8	0.9	0.8	0.3	0.3	0.5	0.5	0.2	0.2
31	0.75	0.75	3	0.01	0.9	1.0	0.4	0.4	1.1	1.1	1.2	1.2	0.0	0.0

Table 2
The second-order quadratic model equations and regression coefficients (%) of responses.

Response	**Second-order quadratic model equation (in coded factors)**	**S**	**Regression coefficient (%)**	
			R^2	R^2adj
S. aureus	$Y = -4.24 + 1.712 \times A + 1.512 \times B + 2.429 \times C + 19.4 \times D + -1.219$ X AB + $-0.019 \times AC + -0.1048 \times AD + 1.9 \times BC + 1 \times BD + -0.4 \times CD + 20 \times A^2 + -1 \times B^2 + 4 \times C^2 + -13x\ D^2$	0.0834414	94.89%	90.43%
E. faecalis	$Y = 2.916 + -3.518 \times A + 0.848 \times B + -1.11 \times C + 52.07 \times D + 1.751 \times AB + -0.249 \times AC + 0.1378 \times AD + -501.8 \times BC + -1.9 \times BD + 0.75x\ CD + -5 \times A^2 + -0.15 \times B^2 + 25 \times C^2 + -4.5 \times D^2$	0.0894816	95.53%	77.48%
K. pneumoniae	$Y = -0.995 + 7.167 \times A + -0.033 \times B + 0.433 \times C + -60.52 \times D + -2.344 \times AB + -2.744 \times AC + -0.2861 \times AD + 248.9 \times BC + -1.4 \times BD + -0.7 \times CD + -22 \times A^2 + 1.5 \times B^2 + 18 \times C^2 + 15 \times D^2$	0.0811533	97.52%	95.35%
E. coli	$Y = -4.95 + 5.47 \times A + 2.77 \times B + 2.95 \times C + -49 \times D + 0.806 \times AB + 1.006 \times AC + -0.2985 \times AD + 3.8 \times BC + -3 \times BD + -1.6 \times CD + 10 \times A^2 + -0.9 \times B^2 + 20 \times C^2 + 11 \times D^2$	0.120094	93.51%	87.82%
P. aeruginosa	$Y = 6.598 + 2.562 \times A + 0.529 \times B + -4.896 \times C + 6.39 \times D + -1.33 \times AB + -0.33 \times AC + 0.6674 \times AD + -263.3 \times BC + -2 \times BD + 0 \times CD + 16 \times A^2 + 0.7 \times B^2 + -14 \times C^2 + 7 \times D^2$	0.073589	96.45%	93.35%

Y: response; A: Starch; B: Urea; C: NaCl; D: $MgSO_4$.

and the unpredictable nature of the reaction were also governed by R^2 values. R^2 value of 94.89% in this investigation for *S. aureus* suggested that around 5.11% of variance were not predicted by the model. *S. aureus* had an adjusted determination co-efficient R^2 of 90.43%, indicating that the model was highly significant (Table 2). For *E. faecalis*, R^2 95.53% meant that 4.47% of changes were not predicted by the model. The model was highly significant for *E. faecalis*, as indicated by the corrected determination co-efficient R^2, which was 91.63 (Table 2). The R^2 score for *K. pneumoniae* was 97.52%, meaning that 2.48% of changes were not predicted by the model. *K. pneumoniae*'s adjusted determination co-efficient R^2 95.35% demonstrated the model's high significance (Table 2). The R^2 score for *E. coli* was 93.51%, meaning that around 6.49% of changes were not predicted by the model. The model was highly significant (*E. coli*) as indicated by the adjusted determination co-efficient R^2 of 87.82% (Table 2). According to the adjusted determination co-efficient R^2 value of 93.35% and the R^2 value of 96.45% for *P. aeruginosa*, approximately 3.55% deviations were not predicted by the model (Table 2).

Since the prob > F value, it was determined that the model was statistically significant with a 95% confidence level. The model value for all five responses was determined to be 21.24, 24.45, 44.92, 16.46, and 31.07 (in terms of inhibition of growth of the pathogenic microorganisms are *S. aureus*, *E. coli*, *E. faecalis*, *K. pneumoniae*, and *P. aeruginosa* respectively, and is represented in cm) and suggested that the model is significant. The most important

variables impacting the growth suppression of pathogenic bacteria by antibiotic compounds produced by *Streptomyces* sp CMSTAAHL-3 for the variables starch, urea, NaCl, and $MgSO_4$ were shown in Table 3, Table 4, Table 5, Table 6 and Table 7 according to an ANOVA analysis. The linear quadratic and interactive term coefficients showed a greater impact on the antibiotic activity against the tested pathogens and were confirmed through their significance against the corresponding *P* values.

For *S. aureus*, the linear terms such as starch ($P > 0.026$), urea ($P > 0.046$), NaCl ($P > 0.001$) and $MgSO_4$ ($P > 0.018$) showed a greater effect on the antibiotic activity. Quadric coefficients such as starch*starch (0.001) were found to be significant and played an important role in antibiotic production. The interactive coefficients such as starch*urea ($P > 0.009$), starch*NaCl ($P > 0.029$), urea*NaCl ($P > 0.001$), starch*$MgSO_4$ ($P > 0.001$), NaCl*$MgSO_4$ ($P > 0.001$) were significant and played a greater impact on antibiotic production (Table 3).

For *E. faecalis*, the linear terms such as starch ($P > 0.001$), NaCl ($P > 0.027$) and $MgSO_4$ ($P > 0.001$) showed their greater effect for the antibiotic activity. Quadric coefficients such as starch*starch ($P > 0.001$), $MgSO_4$*$MgSO_4$ ($P > 0.001$) were significant and played an important role in antibiotic production. The interactive term coefficients such as starch*urea ($P > 0.001$), starch*NaCl ($P > 0,001$) and urea*$MgSO_4$ ($P > 0.023$) were significant and played a greater impact on antibiotic production (Table 4).

For *K. pneumoniae*, the linear terms such as starch ($P > 0.001$) and $MgSO_4$ ($P > 0.001$) showed a greater effect for the antibiotic activity. Quadric coefficients such as starch*starch ($P > 0.001$) urea*urea ($P > 0.001$), NaCl*NaCl ($P > 0.001$) and $MgSO_4$*$MgSO_4$ were significant and played an important role in antibiotic production. The interactive term coefficients such as starch*urea ($P > 0.001$), starch*NaCl ($P > 0.001$), starch*$MgSO_4$ ($P > 0.001$), urea*NaCl ($P > 0.001$), urea*$MgSO_4$ ($P > 0.001$) were significant and played a greater impact on antibiotic production (Table 5).

Table 3
ANOVA result for the production of antibiotics against *S. aureus* response surface quadratic model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	2.07054	0.147895	21.24	0
Linear	4	0.26828	0.06707	9.63	0
Starch (%)	1	0.04188	0.041881	6.02	0.026
Urea (%)	1	0.03267	0.032667	4.69	0.046
NaCl (%)	1	0.22699	0.226987	32.6	0
$MgSO_4$ (%)	1	0.04882	0.048822	7.01	0.018
Square	4	0.17878	0.044696	6.42	0.003
Starch (%)*Starch (%)	1	0.16458	0.164576	23.64	0
Urea (%)*Urea (%)	1	0.00004	0.000041	0.01	0.94
NaCl (%)*NaCl (%)	1	0.01945	0.019453	2.79	0.114
$MgSO_4$ (%)*$MgSO_4$ (%)	1	0.00001	0.000014	0	0.965
2-Way Interaction	6	1.035	0.1725	24.78	0
Starch (%)*Urea (%)	1	0.0625	0.0625	8.98	0.009
Starch (%)*NaCl (%)	1	0.04	0.04	5.75	0.029
Starch (%)*$MgSO_4$(%)	1	0.25	0.25	35.91	0
Urea (%)*NaCl (%)	1	0.25	0.25	35.91	0
Urea (%)*$MgSO_4$(%)	1	0.01	0.01	1.44	0.248
NaCl (%)*$MgSO_4$(%)	1	0.4225	0.4225	60.68	0
Error	16	0.1114	0.006962		
Lack-of-Fit	9	0.03265	0.003628	0.32	0.941
Pure Error	7	0.07875	0.01125		
Total	30	2.18194			

Table 4
ANOVA result for the production of antibiotics against *E. faecalis* response surface quadratic model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	2.74028	0.19573	24.45	0
Linear	4	0.61271	0.15318	19.13	0
Starch (%)	1	0.17687	0.17687	22.09	0
Urea (%)	1	0.01028	0.01028	1.28	0.274
NaCl (%)	1	0.04741	0.04741	5.92	0.027
$MgSO_4$ (%)	1	0.3517	0.3517	43.92	0
Square	4	1.30976	0.32744	40.89	0
Starch (%)*Starch (%)	1	0.33953	0.33953	42.4	0
Urea (%)*Urea (%)	1	0.00686	0.00686	0.86	0.368
NaCl (%)*NaCl (%)	1	0.03363	0.03363	4.2	0.057
$MgSO_4$(%)*$MgSO_4$(%)	1	1.03117	1.03117	128.78	0
2-Way Interaction	6	0.82875	0.13812	17.25	0
Starch (%)*Urea (%)	1	0.22562	0.22562	28.18	0
Starch (%)*NaCl (%)	1	0.14062	0.14062	17.56	0.001
Starch (%)*$MgSO_4$(%)	1	0.01562	0.01562	1.95	0.182
Urea (%)*NaCl (%)	1	0.00563	0.00563	0.7	0.414
Urea (%)*$MgSO_4$(%)	1	0.39062	0.39062	48.79	0
NaCl (%)*$MgSO_4$(%)	1	0.05063	0.05063	6.32	0.023
Error	16	0.12811	0.00801		
Lack-of-Fit	9	0.09311	0.01035	2.07	0.175
Pure Error	7	0.035	0.005		
Total	30	2.86839			

Table 5
ANOVA result for the production of antibiotics against *K. pneumoniae* response surface quadratic model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	4.14172	0.295837	44.92	0
Linear	4	1.28798	0.321994	48.89	0
Starch (%)	1	0.73387	0.733873	111.43	0
Urea (%)	1	0.00002	0.000016	0	0.961
NaCl (%)	1	0.00723	0.007227	1.1	0.31
$MgSO_4$ (%)	1	0.4751	0.475103	72.14	0
Square	4	1.42885	0.357213	54.24	0
Starch (%)*Starch (%)	1	0.60861	0.608613	92.41	0
Urea (%)*Urea (%)	1	0.83401	0.834006	126.64	0
NaCl (%)*NaCl (%)	1	0.14503	0.14503	22.02	0
$MgSO_4$(%)*$MgSO_4$(%)	1	0.25382	0.253816	38.54	0
2-Way Interaction	6	1.875	0.3125	47.45	0
Starch (%)*Urea (%)	1	0.1225	0.1225	18.6	0.001
Starch (%)*NaCl (%)	1	0.1225	0.1225	18.6	0.001
Starch (%)*$MgSO_4$(%)	1	0.3025	0.3025	45.93	0
Urea (%)*NaCl (%)	1	0.5625	0.5625	85.41	0
Urea (%)*$MgSO_4$(%)	1	0.2025	0.2025	30.75	0
NaCl (%)*$MgSO_4$(%)	1	0.5625	0.5625	85.41	0
Error	16	0.10537	0.006586		
Lack-of-Fit	9	0.09037	0.010042	4.69	0.027
Pure Error	7	0.015	0.002143		
Total	30	4.2471			

Table 6
ANOVA result for the production of antibiotics against *E. coli* response surface quadratic model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	3.32279	0.237342	16.46	0
Linear	4	1.0137	0.253424	17.57	0
Starch (%)	1	0.42823	0.42823	29.69	0
Urea (%)	1	0.10999	0.109992	7.63	0.014
NaCl (%)	1	0.33477	0.334773	23.21	0
$MgSO_4$(%)	1	0.3115	0.311499	21.6	0
Square	4	0.38232	0.095581	6.63	0.002
Starch (%)*Starch (%)	1	0.07191	0.07191	4.99	0.04
Urea (%)*Urea (%)	1	0.11203	0.112032	7.77	0.013
NaCl (%)*NaCl (%)	1	0.15789	0.157891	10.95	0.004
$MgSO_4$ (%)*$MgSO_4$(%)	1	0.00006	0.000059	0	0.95
2-Way Interaction	6	2.02	0.336667	23.34	0
Starch (%)*Urea (%)	1	0.5625	0.5625	39	0
Starch (%)*NaCl (%)	1	0.64	0.64	44.38	0
Starch (%)*$MgSO_4$(%)	1	0.0625	0.0625	4.33	0.054
Urea (%)*NaCl (%)	1	0.2025	0.2025	14.04	0.002
Urea (%)*$MgSO_4$(%)	1	0.25	0.25	17.33	0.001
NaCl (%)*$MgSO_4$(%)	1	0.3025	0.3025	20.97	0
Error	16	0.23076	0.014422		
Lack-of-Fit	9	0.01201	0.001334	0.04	1.00
Pure Error	7	0.21875	0.03125		
Total	30	3.55355			

The linear terms such as starch ($P > 0.001$), urea ($P > 0.014$), NaCl ($P > 0.001$) and $MgSO_4$ showed greater effect for the antibiotic activity against *E. coli*. Quadric coefficients such as starch*starch ($P > 0.004$) and urea*urea ($P > 0.013$) were significant and played an important role in antibiotic production. The interactive term coefficients such as starch*urea ($P > 0.001$), starch*NaCl ($P > 0.001$), urea*NaCl ($P > 0.002$) and urea*$MgSO_4$ ($P > 0.001$) were significant and played a greater impact on antibiotic production (Table 6).

The linear terms, starch ($P > 0.001$), NaCl ($P > 0.001$) showed their greater effect during the antibiotic activity against *P. aeruginosa*. Quadric coefficients such as starch*starch ($P > 0.001$), NaCl*NaCl ($P > 0.001$) and $MgSO_4$*$MgSO_4$ ($P > 0.001$) were significant and played an important role in antibiotic production. The interactive term coefficients such as starch*urea ($P > 0.001$), starch*$MgSO_4$ ($P > 0.001$), urea*NaCl ($P > 0.001$) and NaCl*$MgSO_4$ ($P > 0.002$) were significant and played a greater impact on antibiotic production (Table 7).

The lack of fit test values for four responses was found to be insignificant in the current study. The lack of fit *P* value for five responses were 0.094 (Table 3), 0.175 (Table 4), 0.027 (Table 5), 1.00 (Table 6) and 1.00 (Table 7) and in all the five responses, the lack of fit was greater than 0.05 which indicated that the model is highly insignificant and the model was an accepted one.

To identify the media components that influenced the antibacterial compound (antibiotics) produced by *S. aureus*, *E. faecalis*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, the major effect of each variable, such as starch, urea, NaCl, and $MgSO_4$, on antibiotic activity was calculated. Table S3, Table S4, Table S5, Table S6, and Table S7 showed the co-efficient, standard error co-efficient, t-value, and *P* value of each component from the outcome of an antibiotic assay at a 95% confidence level based on their effects.

The Pareto graph (Fig. S1, Fig. S2, Fig. S3, Fig. S4, and Fig. S5) which showed that stronger effects were shown in the upper portion of the graph and progressed down to the bottom section, verified this. From (Table S3), it was found that antibiotic production

Table 7
ANOVA result for the production of antibiotics against *P. aeruginosa* response surface quadratic model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	2.35529	0.168235	31.07	0
Linear	4	1.35245	0.338113	62.44	0
Starch (%)	1	0.09381	0.093809	17.32	0.001
Urea (%)	1	0.004	0.003998	0.74	0.403
NaCl (%)	1	0.92243	0.922433	170.34	0
$MgSO_4$(%)	1	0.00529	0.005293	0.98	0.338
Square	4	1.37243	0.343108	63.36	0
Starch (%)*Starch (%)	1	0.19599	0.195991	36.19	0
Urea (%)*Urea (%)	1	0.01209	0.012089	2.23	0.155
NaCl (%)*NaCl (%)	1	0.78911	0.789114	145.72	0
$MgSO_4$(%)*$MgSO_4$(%)	1	0.28403	0.284026	52.45	0
2-Way Interaction	6	0.7775	0.129583	23.93	0
Starch (%)*Urea (%)	1	0.25	0.25	46.17	0
Starch (%)*NaCl (%)	1	0	0	0	1.00
Starch (%)*$MgSO_4$(%)	1	0.16	0.16	29.55	0
Urea (%)*NaCl (%)	1	0.1225	0.1225	22.62	0
Urea (%)*$MgSO_4$(%)	1	0.1225	0.1225	22.62	0
NaCl (%)*$MgSO_4$(%)	1	0.1225	0.1225	22.62	0
Error	16	0.08665	0.005415		
Lack-of-Fit	9	0.0079	0.000877	0.08	1.00
Pure Error	7	0.07875	0.01125		
Total	30	2.44194			

of *Streptomyces* sp CMSTAAHL 3 against *S. aureus*, the factors such as starch, urea, NaCl, $MgSO_4$, starch*starch, starch*urea, starch*NaCl, starch*$MgSO_4$, urea*NaCl, NaCl*$MgSO_4$ had a greater influence on antibiotic production. Their *P* values were less than 0.05% which indicated their significant contribution for antibiotic production than those of other components. Fig. S1 directly showed that the most important factors for antibiotic production against *S. aureus* were NaCl*$MgSO_4$ (C, D), starch*$MgSO_4$ (A, D), urea*NaCl (B, C), NaCl (C), starch*starch (A, A), starch*urea (A, B), $MgSO_4$ (D), starch (A), starch*NaCl (A, C), urea (B).

Table S4 showed that the factors such as starch, NaCl, starch*starch, MgSO4*$MgSO_4$, starch*urea, starch*NaCl, urea*$MgSO_4$ and NaCl*$MgSO_4$ had a greater influence on antibiotic production against *E. faecalis*. Their *P* values were less than 0.05% which indicated their significant contribution for antibiotic production than those of other components. The Fig. S2 directly showed that the most important factors for antibiotic production against *E. faecalis* were $MgSO_4$*$MgSO_4$ (D, D), urea*$MgSO_4$ (B, D), $MgSO_4$ (D), starch*starch (A, A), starch*urea (A, B), starch (A), starch*NaCl (A, C), NaCl*$MgSO_4$ (C, D), NaCl (C).

The antibiotic production of CMSTAAHL – 3 against *K. pneumonia* indicated that starch, $MgSO_4$, starch*starch, urea*urea, NaCl*NaCl, $MgSO_4$*$MgSO_4$, starch*urea, starch*NaCl, starch*$MgSO_4$, urea*NaCl, urea*$MgSo_4$, NaCl*$MgSO_4$ had a greater influence on antibiotic production. Their *P* values were less than 0.05% which indicated their significant contribution for antibiotic production than those of other components (Table S5). Fig. S3 directly showed that the most important factors for antibiotic production against *K. pneumoniae* were urea*urea (B, B), starch (A), starch*starch (A, A), NaCl*$MgSO_4$ (C, D), urea*NaCl (B, C), $MgSO_4$ (D), starch*$MgSO_4$ (A, D), $MgSO_4$*$MgSO_4$ (D, D), urea*$MgSO_4$ (B, D), NaCl*NaCl (C, C), starch*urea (A, B), starch*NaCl (A, C).

Table S6 indicated that starch, urea, NaCl, $MgSO_4$, starch*starch, urea*urea, NaCl*NaCl, starch*urea, starch*NaCl, urea*NaCl, urea*$MgSO_4$, NaCl*$MgSO_4$ had greater influence on antibiotic production against *E. coli*. Their *P* values were less than 0.05% which indicated their significant contribution for antibiotic production than those of other components. Fig. S4 directly showed that the most important factors for antibiotic production against *E. coli* were starch*NaCl (A C), starch*urea (A, B), starch (A), NaCl (C), $MgSO_4$ (D), NaCl*MgSO4 (C, D), urea*$MgSO_4$ (B, D), urea*$MgSO_4$ (B, D), urea*NaCl (B, C), NaCl*NaCl (C, C), urea*urea (B, B), urea (B), and starch*starch (A, A).

Table S7 indicated that starch, NaCl, starch*starch, NaCl*NaCl, $MgSO_4$*$MgSO_4$, starch*urea, starch*MgSO4, urea*NaCl, urea*$MgSO_4$, NaCl*$MgSO_4$ had a greater influence on antibiotic production against *P. aeruginosa*. Their *P* values were less than 0.05% which indicated their significant contribution for antibiotic production than those of other components. Fig. S5 showed that the most important factors for antibiotic production against *P. aeruginosa* were NaCl (C), NaCl*NaCl (C, C), $MgSO_4$*$MgSO_4$ (D, D), starch*urea (A, B), starch*starch (A, A), starch*$MgSO_4$ (A, D), urea*NaCl (B, C), NaCl*$MgSO_4$ (C, D), urea*$MgSO_4$ (B, D), starch (A).

The combination of input variable settings that best optimize a single response antibiotic production as expressed by a single desire index, D, were found using the response optimization approach. The function rose linearly toward the intended target values to optimize antibiotic production when D was close to 1, showing that. With a predicted *Streptomyces* sp CMSTHHAL – 3 antibiotic production against *S. aureus*, the optimal values of starch, urea, NaCl, and $MgSO_4$ were calculated to be 0.250, 0.250, 4.0, and 0.010 g/L, respectively (Fig. S6). In order to produce the antibiotic from *Streptomyces* sp. CMSTHHAL-3 against *E. faecalis*, the optimum concentrations of starch, urea, NaCl, and $MgSO_4$ were calculated to be 0.250, 1.250, 2.0, and 0.0730 g/L, respectively (Fig. S7). According to estimates, the optimum concentrations of starch, urea, NaCl, and $MgSO_4$ were 0.250, 1.250, 4.0, and 0.0750 g/L, respectively, with a predicted *Streptomyces* sp CMSTHHAL – 3 antibiotic production against *K. pneumoniae* (Fig. S8). The optimal concentrations of urea, starch, sodium chloride, and magnesium sulfate were calculated to be 0.250, 1.250, 3.7, and 0.0750 g/L, respectively, with a predicted *Streptomyces* sp. CMSTHHAL – 3 antibiotic production against *E. coli* (Fig. S9). In order to produce the antibiotic from *Streptomyces* sp CMSTHHAL-3 against *P. aeruginosa*, the optimum concentrations of starch, urea, NaCl, and $MgSO_4$ were calculated to be 1.1894, 0.250, 2.0, and 0.0717 g/L, respectively (Fig. S10).

4. Discussion

The focus of the current work was the isolation and screening of *Streptomyces* sp from mangrove sediments for the development of

antibiotics. Many *Streptomyces* sp were screened for their antimicrobial activity have been isolated from marine environment and mangroves throughout the world [52,53,54,55,56,57,58,59]. Due to the variable environmental factors, many of the mangrove region's actinomycetes are great producers of antibiotics [54,55,58,60,61] . It was also reported that mangrove environments are becoming very important sources for *Streptomyces* sp. for the production of lead molecules due to their adaption towards varying tidal gradients and varying salinity [62]. In our current work also, we have isolated and identified the antibiotic-producing *Streptomyces* sp CMSTAAHL-3 from the Manakudy mangrove sediments, Kanyakumari District, India.

The primary screening of selected 4 strains against the tested pathogens such as Gram-positive (*S. aureus*, *E. faecalis*) and Gram-negative (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) bacteria found that *Streptomyces* sp CMSTAAHL3 strain was effective against the bacterial pathogens *E. faecalis* and *S. aureus.* Similarly, many researchers have reported the antimicrobial activity of *Streptomyces* sp against Gram-positive and Gram-negative human pathogens [57,63,64,65,66]. Furthermore, from the current study based on the comparison of the inhibition zone sizes against the Gram-positive and Gram-negative test organisms, it can be found that the *Streptomyces* sp isolates were more effective against Gram-positive bacteria. This might be because Gram-positive and Gram-negative cells have different cell morphologies, and it is also possible that the antibiotic molecule was more effective against Gram-positive cell components than Gram-negative cell components [67] due to the double membrane barrier and transmembrane efflux mechanism [68].

On various actinomycetes growing media on SCA, AIA, SIA and Sato-A, the isolated strain *+Streptomyces* sp CMSTAAHL-3′s morphological and cultural traits, such as the color of aerial and substrate mycelia and pigmentation, were noticed. Similarly many researchers have studied the morphological and cultural characteristics of the actinomycetes [63,64,69,70,71,72]. White, yellow, orange, red, green, blue, purple, brown, black, and other colors are present in the substrate mycelia, and some hyphae are capable of producing pigments that are either fat- or water-soluble. The culture medium can absorb the water-soluble pigment, producing a medium that is colored appropriately. The non-water soluble pigment (or fat-soluble) produces the colony of the desired color. While identifying new species, it is crucial to take into consideration the color of the substrate mycelia and whether or not there are any soluble pigments. The properties of the species, dietary requirements, or environmental factors all have a role in the formation of various actinobacterial aerial hyphae [73].

The optimization of antimicrobial compound from *Streptomyces* sp by varying incubation period, temperature, pH, salinity, carbon sources and nitrogen sources through OVAT [55,70,74,75]. In the current work also, the antibiotic-producing capacity of the *Streptomyces* sp CMSTAAHL- 3 was found to be maximum in the conditions such as pH (7), temperature (30°C), incubation period (7 d), nitrogen (urea), mineral source ($MgSO_4$), 3% NaCl and carbon sources (starch) [58,76].

Al Farraj et al. [55], Wang et al. [77], Wang et al. [78], and Srivastava et al. [79] found that antibiotic production was enhanced when starch was used as the carbon source. In the present study also, while studying the OVAT, starch enhanced the antibiotic production and was confirmed by optimization studies. Complex carbon sources such as polysaccharides, e.g., starch, are slowly digested, and frequently induce the synthesis of secondary metabolites. This behavior may be explained by the fact that glucose results in catabolic repression, which is the inhibition of the enzymes needed for secondary metabolite formation [80]. Amino acids and nitrogen sources are regarded as direct precursors for the production of antibiotics based on their type and amount. One of the key elements influencing the development of antibiotics and the proliferation of actinomycetes is temperature. The development of chaperones in response to temperature that enables the high antibiotic production at greater growth and allowable temperature may be the cause of the *Streptomyces* sp CMSTAAHL – 3′s rise in antibiotic production at 30°C [81]. Al Farraj et al. [55], and Thenmozhi and Kannabiran [69] also determined the optimized temperature as 30°C for antibiotic activity in *Streptomyces* sp. The biosynthesis of secondary metabolites (antibiotics) and cellular metabolisms in *Streptomyces* sp. are influenced by pH levels [82]. This study discovered that a pH level close to neutral was ideal for the maximal synthesis of metabolites (pH 7). Similar result of maximum antibiotic production at pH 7 was reported by Al Farraj et al. [55] and Yun et al. [72] for antibiotic activity. In the present study optimum incubation day for antibiotic production for *Streptomyces* sp. CMSTAAHL – 3 was 7 d. Similar report of antibiotic production on 7 d incubation had been in observed on *Streptomyces* sp [83]. $MgSO_4$ acted as mineral sources which enhanced the antibiotic production was confirmed by El-Naggar and El-Shweihy [84].

In the current study, the culture media was optimized by Central Composite Design and Response Surface Methodology (CCD-RSM) by varying the starch (carbon source), urea (nitrogen source), NaCl, $MgSO_4$ (mineral source) concentrations and optimized the antibiotic production from *Streptomyces* sp. CMSTAAHL-3 was confirmed by the zone of inhibition of the pathogenic microorganisms. Similarly, there were many reports of the optimization of media components for antibiotic production [72,75]. To find the best combinations of CCD design with RSM for optimizing the antibacterial production by *Streptomyces* sp CMSTAAL −3 response surface methodology, 31 tests, four parameters, and five levels were conducted in the current study. Similarly, 31 experiments with four factors and five experimental levels were optimized for antibiotic production [70].

The R^2 value in the CCD-RSM must always fall between 0 and 1. The stronger the model and the better it predicts the response, the closer it is to 1, the R^2 value [83,84,85,86]. In this study, R^2 value of 94.89% (0.9489) for *S. aureus*; 95.53% (0.9553) for *E. faecalis;* 97.52% (0.9752) for *K. pneumoniae*; 93.51% (0.9351) for *E. coli* and 96.45% (0.9645) for *P. aeruginosa* showed that the model was highly significant. Similar reports about the R^2 value was given by Rajeswari et al. [63] (0.9730) and Coman and Bahrim [87] (0.9072) and confirmed that the model was a significant one.

The coefficients' significance is indicated by their *P* values, which are also crucial for figuring out how the variables interact with one another. Model terms are significant if the value of *P* is less than 0.05 [88]. Since the model had a low *P*-value, the quadratic regression model's ANOVA showed that it was highly significant [83,85,86,89]. The linear, square, and 2-way interaction quadratic regression models in the current investigation fit well, with *P* values for five ones less than 0.05. This revealed that the model for antibiotic production was extremely significant.

If the *P*-value for the lack of fit was larger than 0.05, it signified a significant lack of fit, demonstrating the model's reliability [90]. Lack of fit *P* values in the current investigation were 0.941 for *S. aureus*, 0.175 for *E. faecalis*, 0.027 for *K. pneumoniae*, 1.00 for *E. coli*, and 1.00 for *P. aeruginosa* for the five responses. The lack of fit was more than 0.05 in four out of three responses, indicating that the models were extremely insignificant and the models were valid one.

In our present study, the antibiotic activity for the *Streptomyces* sp CMSTAAHL – 3 was performed using ethyl acetate extract and the antibacterial activities of the extract were studied against *S. aureus, E. coli, E. faecalis,* and *P. aeruginosa*. Similar reports of antibacterial activity of the *Streptomyces* sp. extract against Gram-positive and Gram-negative human pathogens were con-

firmed by other researchers [30,58,67,75,76,90,91,92,93]. Furthermore, validation of the model demonstrated indisputably the dependability of RSM for optimizing media for antibiotic synthesis.

5. Conclusions

Out of the four isolates isolated from the Mankudy mangrove sediments, the authors of the current study found that CMSTAAHL-3, which was identified as *Streptomyces* sp. CMSTAAHL-3 using 16SrRNA sequencing, has greater antagonistic qualities. By using statistical optimization, the conditions for producing antibiotics were improved and, hence, the antibacterial activity was increased. The result may be important and help the progress of antibiotic cultivation on a large scale. For the purpose of developing new drugs, more research should be done to characterize the biologically active substances, *i.e.*, antibiotics.

Author contributions

- Study conception and design: C Thavasimuthu; U Ganapathi; V. SGP Vincent.- Data collection: JN Selvaraj.
- Analysis and interpretation of results: JN Selvaraj; U Ganapathi; SK Ramamoorthy.
- Draft manuscript preparation: JN Selvaraj; U Ganapathi; C Thavasimuthu.
- Revision of the results and approval of the final version of the manuscript: JN Selvaraj; U Ganapathi; C Thavasimuthu.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary material

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