# Optimization Of Antibiotic Production Of *Streptomyces* Sp Isolated From Mangrove Soil Using Response Surface Methodology

**[1]C. Sweetline* and [2]R. Usha**

[1]Department of Microbiology, New Prince Shri Bhavani Arts and Science College, Chennai, Tamil Nadu, India
[2]Department of Microbiology, School of Life Sciences, Karpagam Academy of Higher Education, Coimbatore 641021, Tamil Nadu, India

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***Abstract***- To improve the antimicrobial compound productivity of *Streptomyces sp* KMA08 by optimizing its physical and chemical parameters. *Streptomyces sp* KMA08 was isolated from mangrove soil of pitchavaram and was screened for its antimicrobial activity by using agar well diffusion method. To improve the production of antimicrobial compound the medium composition and physical parameters were optimized and its productivity was studied against *Staphylococcus aureus Escherichia coli* and *Candida albicans*. Optimum growth of mycelium and antimicrobial compound production occurred at pH 7.2, agitation 180 rpm and temperature 30 ˚ C with glucose 10g/L, soyabean meal 2.5g/L, K2HPO4 2g/L, MgSO4. The optimization of cultural conditions proposed in this paper has effetely improved the antimicrobial compound productivity of *Streptomyces sp* KMA 08

## I. Introduction

Marine Actinomycetes are biotechnologically and economically valuable prokaryotes. They produce special bioactive secondary metabolites particularly antibiotics [1]. Marine actinomycetes are the potential source of novel antimicrobial compounds as the environmental conditions of the sea are completely different from that of terrestrial conditions [2] [3]. Marine *Streptomyces* have an incomparable metabolic diversity and are excellent in producing new natural products. Approximately 2/3 of the well known antibiotics was produced by *Streptomyces* [4] [5]. About 75% of commercially and medically useful antibiotics are produced by the *Streptomyces* spices [6]. *Streptomyces* are filamentous bacteria with a complex life cycle. Aerial growth coincides with the production of many secondary metabolites, including many clinically relevant antibiotics [5]. In the present study, *Streptomyces sp* was screened for its antimicrobial activity and are optimized for the higher yield of antimicrobial compound with reference to medium composition and other factors.

## II. MATERIALS AND METHODS

### Isolation of *Streptomyces sp*

Soil sample were collected from 10-15 cm depth from mangrove regions in local area of Pitchavaram. Samples were taken in a zipped polythene bags and were carried to the laboratory under aseptic conditions for further studies. The actinomycetes strain, *Streptomyces* sp was isolated by serial dilution in starch casein agar medium with rifampicin (5mg/ml) and nystatin (25µg/ml) to inhibit bacterial and fungal contaminations respectively. The plates were incubated at 28 ˚ C for 3 weeks.

### Screening of *Streptomyces sp* for its antimicrobial activity

*Streptomyces sp* was grown in production broth (SS medium), containing (g/L): soluble starch-25, glucose-25, yeast extract-2, CaCO3 and trace elements 1 ml and incubated at 28˚C in a shaker at 180rpm for 4 days. After incubation, the broth was centrifuged for 15 minutes at 5000 rpm and the supernatant was mixed with equal volume of ethyl acetate to extract the antimicrobial compound [11]. The antimicrobial study was carried out by agar well diffusion method [12]. 50µl of the ethyl acetate fraction was loaded in each well and the zone of inhibition was measured in mm.

### Optimization of growth and antimicrobial compound production

### Effect of carbon source

*Streptomyces sp* was inoculated in the inorganic salt medium [13] by varying medium carbon sources. The different carbon sources 1% (w/v) used in the medium were Glucose, Fructose, Galactose, Xylose, Arabinose, Glycerol, Starch, Maltose, Lactose, sucrose, meso-inositol and Mannitol. The flasks were incubated at 180 rpm for 4 days.

### Optimization of best carbon source concentration

*Streptomyces sp* was inoculated in the optimized medium with varying concentrations of carbon source. The concentrations were (g/L) 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20. These flasks were incubated at 180 rpm for 4 days.

### Effect of nitrogen source

The effect of various nitrogen sources on antimicrobial compound production and growth of mycelium was studied by adding organic and inorganic nitrogen sources at 1% (w/v) level into optimization medium.

The organic nitrogen sources employed were soyabean meal, yeast extract, beef extract and peptone. The inorganic nitrogen sources used were ammonium nitrate, ammonium sulfate, potassium nitrate and sodium nitrate.

**Optimization of best nitrogen source concentration**

*Streptomyces sp* was inoculated in the optimized medium with varying concentrations of nitrogen source. The concentrations were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0(g/L) and the flasks were incubated at 180 rpm for 4 days.

**Effect of $K_2HPO_4$**

*Streptomyces sp* was inoculated in optimized medium with varying concentrations of $K_2HPO_4$. The different concentrations employed in the medium were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 g/L

**Effect of $MgSO_4.7H_2O$**

*Streptomyces sp* was inoculated in optimized medium with varying concentrations of $MgSO_4.7H_2O$. The different concentrations used in the medium were 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75 and 2.0g/L

**Effect of Sodium chloride concentration**

*Streptomyces sp* was inoculated in optimized medium with varying concentrations of sodium chloride. The different concentrations used in the medium were 2.5, 5.0, 7.5, 10 and 12.5g/L

**Effect of temperature**

*Streptomyces sp* was inoculated in optimized medium and incubation at different temperatures ranging from 15 – 50˚C at 180 rpm for 4 days.

**Effect of pH**

Optimum pH was studied by adjusting the pH of growth medium at 6, 6.4, 6.8, 7.2, 7.6, 8.0, 8.4 and 8.8. *Streptomyces sp* was inoculated in the optimized medium and incubated at 180 rpm for 4 days.

**Effect of incubation time**

To obtain the high rate of antibiotic production, *Streptomyces sp* was inoculated in optimized medium for different incubation period of 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, 168 hrs and 192 hrs.

**Effect of agitation**

*Streptomyces sp* was inoculated in optimized medium at different agitation rates of 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 rpm. All the optimized flasks were incubated for 7 days.

**Measurement of growth of mycelium**

The growth of *Streptomyces sp* was measured as dry weight of the mycelium (MDW). A whatmann No.1 filter paper was taken and washed twice with distilled water and dried. The weight of the filter paper was noted.

The mycelial content in the culture flask was filtered through the whatmann No.1 filter paper which is previously weighed. The mycelia content along with the filter paper was dried in the hot air oven for 18-24 hrs. The filter paper was cooled and the dry weight of the mycelium was measured.

**Statistical Analysis**

The experiments were carried out in triplicates and the results obtained were expressed in mean ± standard deviation.

**Superior optimization stage: Response surface methodology (RSM)**

Research article having information for highest antibiotic production is scanty. In this respect, the response surface methodology, a combination of mathematical and statistical techniques, which is used to study the effect of several factors influencing the response by varying them simultaneously and carrying out a limited number of experiments. In order to overcome this difficulty and determine the interaction between the studied variables, an experimental factorial design and Response surface methodology (RSM) technique was employed to formulate an optimum medium for mass production.

A central composite design for four independent variables was used to obtain the combination of values that optimizes the response spaces, which allows one to design a minimal number of experiments. For most important factors, i.e. Incubation time, Agitation, Temperature, $MgSo_47H_2O$, $K_2HPO_4$ were believed to play a significant role in improving antibiotic production. RSM using Central composite design was carried out with four factors for antibiotic production.

**Results and discussion**

**Isolation of** *Streptomyces sp*

The actinomycetes strain, *Streptomyces sp* was isolated from the mangrove soil of Pitchavaram sea coast and it was stored in glucose yeast extract malt extract medium for further analysis.

**Test organisms used**

*Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* were obtained from KMCH hospital, Coimbatore.

**Screening of** *Streptomyces* sp **for its antimicrobial activity**

Among the bacterial and fungal strains tested, *Staphylococcus aureus* showed highest zone of inhibition of 22 mm followed by *E. coli* (19 mm) and *C. albicans* showed zone of inhibition of 18 mm.

**Effect of carbon source**

Microorganisms have a capacity to utilize a variety of carbon sources and can adapt to the changes in the osmotic strength nutrients and oxygen limitation and stress conditions [14][15][16]. The presence of carbon source which is easily metabolized, especially glucose, results in a coordinated change of metabolic function in many bacteria [17]. Glucose as a component in the production media provides carbon atoms for the mycelium and produce generations. Maximum antimicrobial compound and mycelium growth was observed with Glucose followed by Glycerol and Starch. In contrast Fructose, Galactose, Xylose, Arabinose, Maltose, Lactose, Sucrose, Meso-inositol and Mannitol showed low antimicrobial compound production and mycelium growth (figure-1).

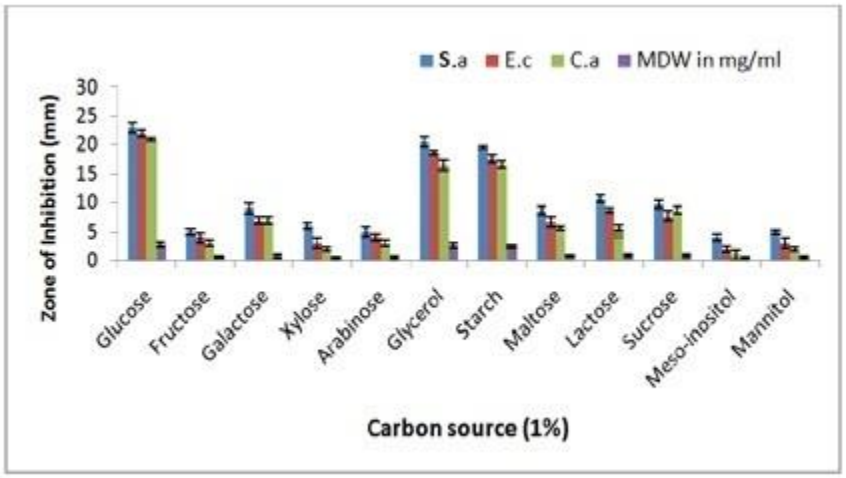



Fig. 1: Effect of different carbon sources on growth and antimicrobial compound production of *Streptomyces sp*

**Optimization of best carbon source concentration**

The growth and antimicrobial compound production of *Streptomyces sp* was increasing continuously from 2.5g/L to 10g/L of glucose concentration. Therefore optimum glucose concentration for the maximum production of antibiotic activity was at 10g/L (figure-2).

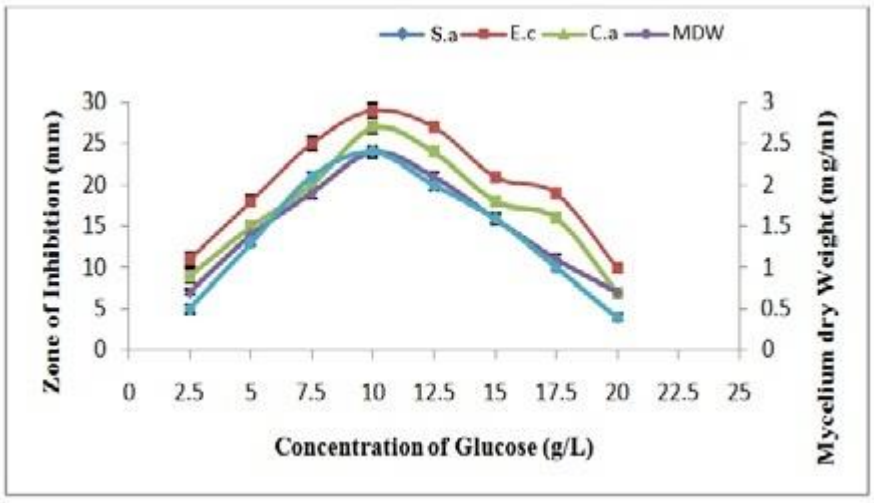



Fig. 2: Effect of glucose concentration on growth and antimicrobial compound production of *Streptomyces sp*

**Effect of nitrogen source**

Maximum antibiotic production and mycelium growth was observed with organic nitrogen source when compared to inorganic nitrogen source. Among organic nitrogen sources, maximum growth and production was shown by soyabean meal followed by peptone and ammonium nitrate (figure-3).

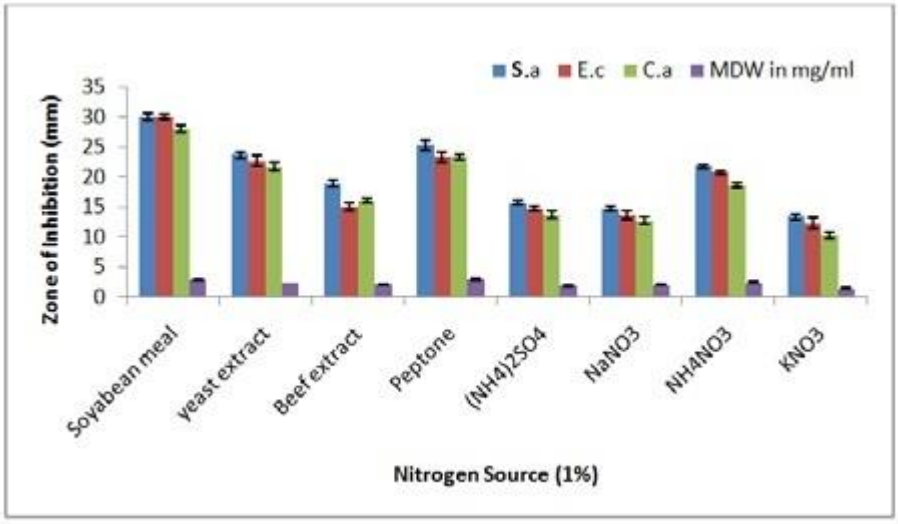



Fig. 3: Effect of different nitrogen sources on growth and antimicrobial compound production of *Streptomyces sp*

Soyabean meal was regarded as appropriate medium component for antibiotic production for the strain *Streptomyces capoamus* [18]. It was also reported that the medium which is supplemented with soyabean meal as nitrogen source was found to produce maximum antimicrobial compound [19].

**Optimization of best nitrogen source concentration**

The antimicrobial compound production and growth of *Streptomyces sp* was increasing continuously from 0.5g/L to 2.5g/L of soyabean meal concentration. Therefore optimum soyabean meal concentration for the maximum production of antimicrobial compound was at 2.5g/L (figure-4).

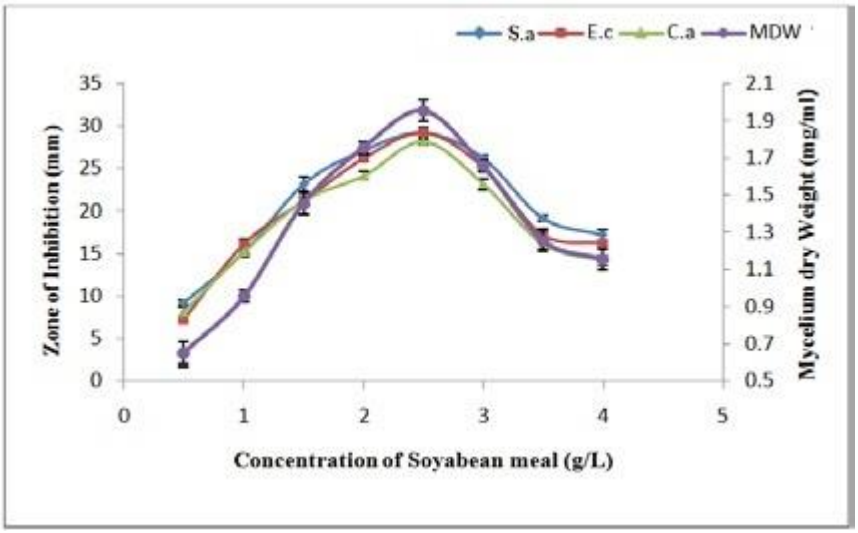



Fig. 4: Effect of Soyabean meal concentration on growth and antimicrobial compound production of *Streptomyces sp*

**Effect of $K_2HPO_4$ concentration**

This results in the good growth of the microorganisms but the antibiotic production is usually reduced. Optimum $K_2HPO_4$ concentration required for the production of antimicrobial compound was 2.0g/L. Further increase in the $K_2HPO_4$ concentration showed a gradual decrease in the production of antimicrobial compound and growth of mycelium (figure-5).

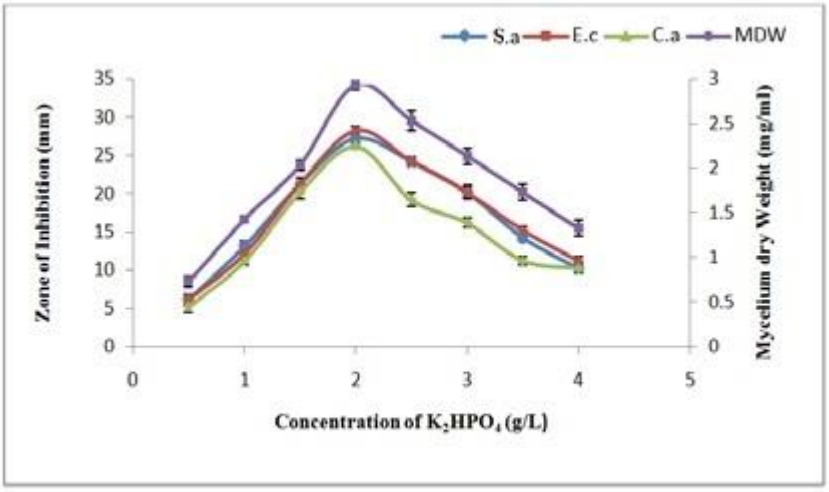



Fig. 5: Effect of $K_2HPO_4$ concentration on growth and antimicrobial compound production of *Streptomyces griseus*

**Effect of MgSO4**

Optimum concentration of MgSO4 concentration required for the production of antimicrobial compound was 1g/L. Further increase in MgSO4 concentration showed a gradual decrease in the production of antimicrobial compound and growth of mycelium (figure-6).The effect of MgSO4 and other metal salts on the antibiotic production was investigated and reported by several authors [20].

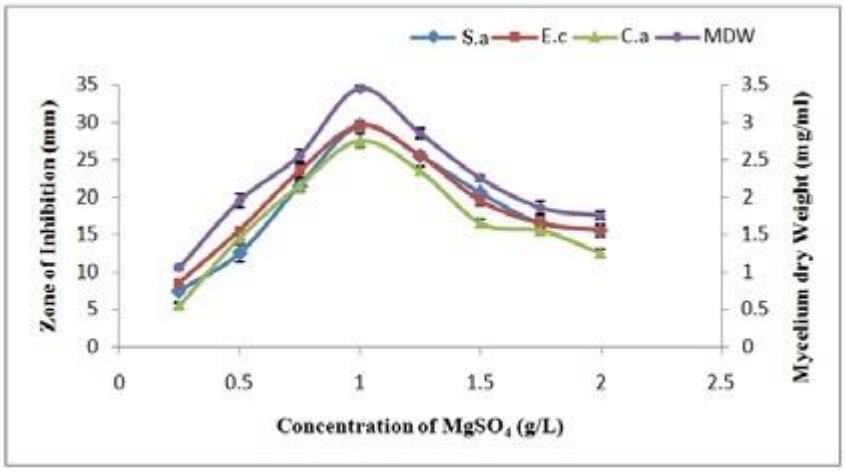



Fig. 6: Effect of MgSO4 concentration on growth and antimicrobial compound production of *Streptomyces griseus*

**Effect of sodium chloride concentration**

Optimum NaCl concentration required for the production of antimicrobial compound was 7.5g/L. Further increase in NaCl concentration showed a drastic decrease in the production of antimicrobial compound and growth of mycelium (figure-7).

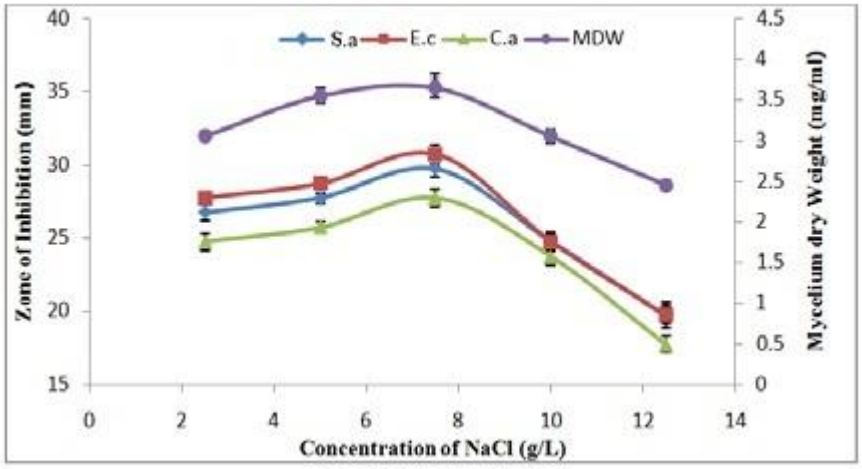



Fig. 7: Effect of NaCl concentration on growth and antimicrobial compound production of *Streptomyces sp*

**Effect of temperature**

The optimum growth and antimicrobial compound production was observed at 30 ˚ C and beyond optimum temperature the growth and antimicrobial metabolite production was decreased. However higher temperature showed adverse effect on both growth and antimicrobial compound production (figure-8).

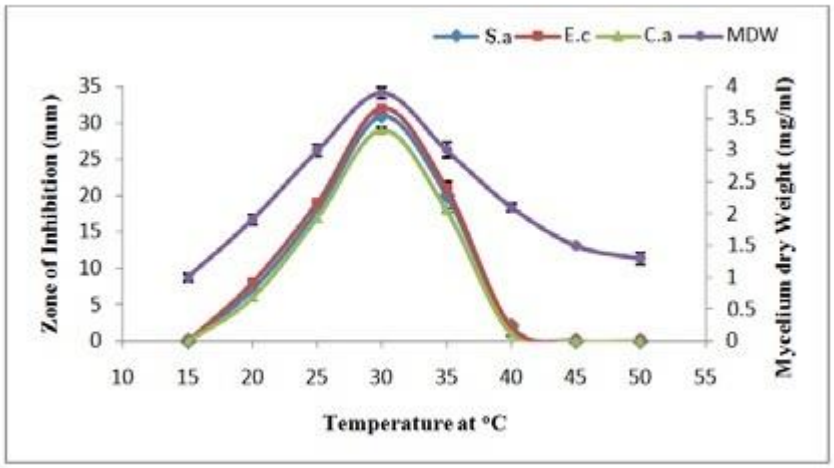



Fig. 8: Effect of temperature on growth and antimicrobial compound production of *Streptomycs griseus*

**Effect of pH**

The changes in pH levels affect the cellular regulation processes and biosynthesis of secondary metabolites in *Streptomyces* species [21]. The growth and antimicrobial metabolite production of *Streptomyces sp* was maximum at pH 7.2 (figure-9).

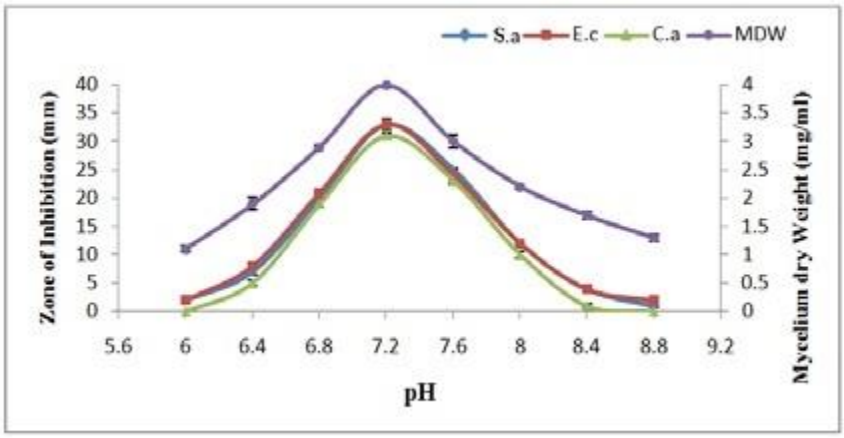



Fig. 9: Effect of pH on growth and antimicrobial compound production of *Streptomyces sp*

**Effect of incubation time**

The antimicrobial compound production and growth of *Streptomyces sp* was increasing continuously from 24 hrs to 120 hrs. Therefore optimum incubation time for the maximum production of antimicrobial metabolite was at 120 hrs (figure-10).

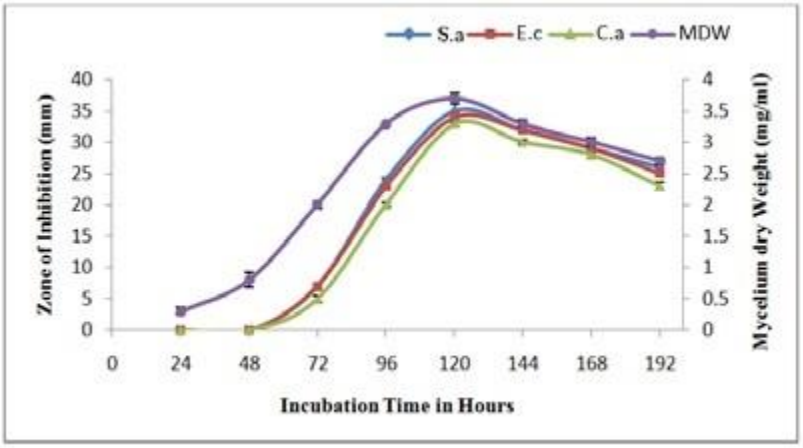



Fig. 10: Effect of incubation time on growth and antimicrobial compound production of *Streptomyces sp*

**Effect of agitation**

Agitation facilitates greater aeration to the cells and provides favourable conditions for the greater availability of the nutrients to the culture. The optimum growth and antimicrobial compound production was observed at 180rpm. Beyond 180 rpm growth and antimicrobial compound production decreased gradually (Fig. 11).

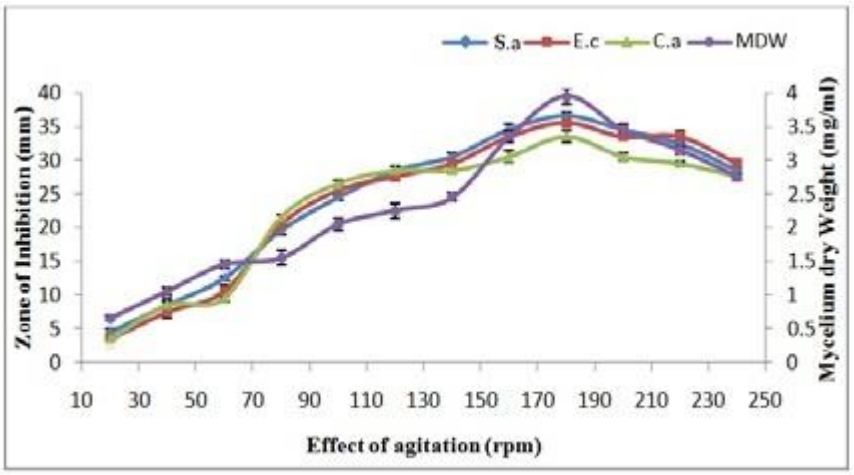



Fig. 11: Effect of agitation on the growth and antimicrobial compound production of *Streptomyces sp*

**Optimization of antibiotic production by response surface methodology (RSM)**

After optimization of various physical and chemical factors, four most important factors, i.e. Incubation time, Agitation, Temperature, $MgSo_47H_2O$, $K_2HPO_4$ were selected for further optimization studies. Taking the above factors into consideration, a response surface methodology using central composite design with four factors was adopted for improving antibiotic production. A total of 30 experiments in central composite design were performed for the media optimization. The statistical software packages Design Expert 5.9, was used to analyze the experimental design. It played a very significant role in enhancing the production of antibiotics[22]. The optimum levels of the key factors and the effect of their interactions on antibiotic production were determined by central composite design of RSM. Many studies have shown the effects of temperature, incubation time, substrate concentration, and inoculum size on antibiotic production. However, the one variable at a time approach and could not explain the mutual interactions among the independent variables and guarantee the determination of optimal condition. Therefore, the interactive effects of these factors selected as key parameters were investigated to maximize the antibiotic production.

To improve the antibiotic production, *Streptomyces sp* was investigated under different culture conditions such as Incubation time, Agitation, Temperature, $MgSo_47H_2O$, $K_2HPO_4$. In the present study, the respective low and high levels with the coded levels in parentheses for the factors were defined as per the explained methodology.

The antibiotic yield (response) of the first experiment central composite design (CCD) for each individual run along with the predicted response. The results obtained after CCD were then analyzed by standard analysis of variance (ANOVA), which gave the following regression equation (in terms of coded factors) of the levels of antibiotic produced as a function of Incubation time, Agitation, Temperature, $MgSo_47H_2O$, $K_2HPO_4$. Following the regression analysis of the experimental data the following quadratic equations were obtained for antibiotic production.

## III. Result and Discussion

The Pareto chart displays the magnitude of each factor estimate and it is a convenient way to view the results of Plackett-Burman experimental design. The main effect was calculated as the difference between the average of measurements made at the high level setting (+) and the average of measurements observed at the low level setting (-) of each factor. Figure 1 shows the Pareto chart for the effect of selected nineteen factors on antibiotic production.

**Figure 1: Pareto chart showing the effect of the selected ten factors on production**

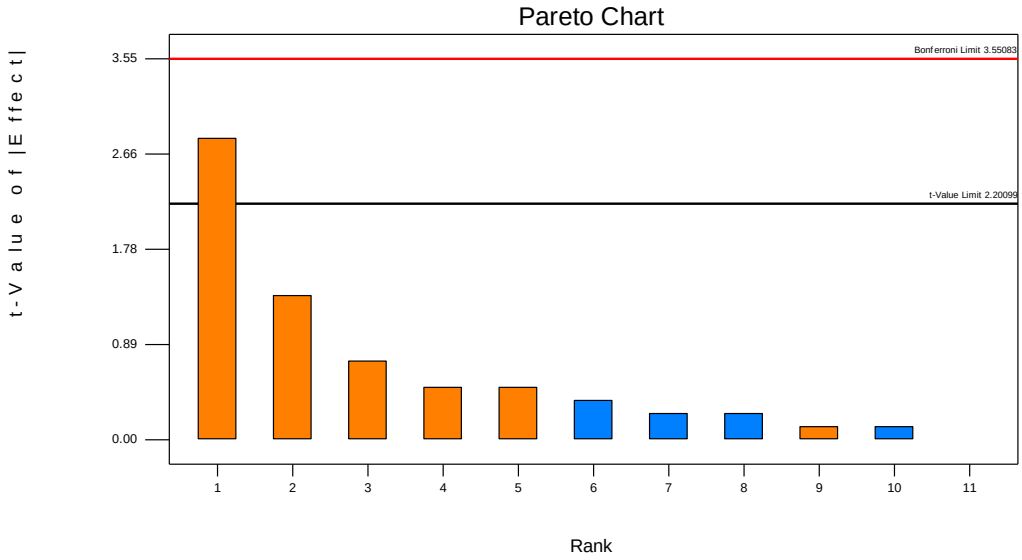



**Table 1: Plackett Burman design for screening variables at different level for the production**

| Code | Variables | Units | | Levels | |
|---|---|---|---|---|---|
| | | | | High | Low |
| A | soy bean meal | % | Numeric | 0.5 | 4 |
| B | Glucose | % | Numeric | 2.5 | 20 |
| C | K2HPO4 | % | Numeric | 0.5 | 4 |
| D | MgSo47H2O | % | Numeric | 0.25 | 2 |
| E | NaCl | % | Numeric | 2.5 | 10 |
| F | Temperature | degree | Numeric | 15 | 50 |
| G | pH | - | Numeric | 6 | 8.8 |
| H | Incubation time | hours | Numeric | 24 | 192 |
| I | Agitation | rpm | Numeric | 20 | 240 |
| J | Dummy1 | - | Numeric | -1 | 1 |
| K | Dummy2 | - | Numeric | -1 | 1 |

J, K is two dummy variables

RSM mainly exploits two designs of experiments for process optimization viz., Central Composite design and Box- Behnken design. Empirical models and statistical analysis are extremely important to elucidate basic mechanisms in complex situations, thus providing better process control and understanding. In most RSM problems, the relationship between the response and independent variables is unknown. According to this program, above mentioned nine factors were chosen and three dummy variables were used to evaluate experimental error. The statistical significance of the regression coefficients was determined by the model equation was determined by Fischer's test and the proportion of variance explained by the model obtained was given by the multiple coefficient of determination. Finally, the physical factors Incubation time, Agitation, Temperature, $MgSo_47H_2O$, $K_2HPO_4$ for each run, the experimental responses along with the predicted response obtained from the regression equation for the 54 combinations are shown in Table 2.

This design contains only a subset of all possible factor-setting combinations and generates information about the main effects of the design variables with the smallest possible number of experiments. The random error variability and test for the statistical significance of the parameter estimates can be determined using the design. The regression coefficient, P value and confidence level were determined and the variables with confidence level greater than 90% were considered to be more significant for antibiotic production.

Analysis of variance (ANOVA) was performed to establish the adequacy and significance of predicted quadratic model as given in Table 2.

Table 2. Results of CCD using four independent variables and three centrepoints showing observed and predicted response

| ***Run*** | ***Incubation time*** | ***Agitation*** | ***temp*** | ***MgSo47H2O*** | ***K2HPO4*** | **Zone of inhibition (mm)** | |
|---|---|---|---|---|---|---|---|
| | | | | | | **Exp.** | **Pred.** |
| 1 | 10 | 130 | 192 | 2.25 | 1.125 | 15 | 22 |
| 2 | 34 | 240 | 108 | 2.25 | 1.125 | 15 | 23 |
| 3 | 29 | 130 | 108 | 0.5 | 1.125 | 15 | 20 |
| 4 | 1 | 20 | 24 | 2.25 | 1.125 | 32.5 | 23 |
| 5 | 33 | 20 | 108 | 2.25 | 1.125 | 15 | 22 |
| 6 | 23 | 130 | 24 | 4 | 1.125 | 32.5 | 23 |
| 7 | 39 | 130 | 24 | 2.25 | 2 | 32.5 | 23 |
| 8 | 40 | 130 | 192 | 2.25 | 2 | 32.5 | 31 |
| 9 | 42 | 130 | 108 | 2.25 | 1.125 | 32.5 | 32 |
| 10 | 46 | 130 | 108 | 2.25 | 1.125 | 32.5 | 33 |
| 11 | 28 | 240 | 108 | 2.25 | 2 | 32.5 | 30 |
| 12 | 3 | 20 | 192 | 2.25 | 1.125 | 32.5 | 28 |
| 13 | 36 | 240 | 108 | 2.25 | 1.125 | 50 | 25 |
| 14 | 22 | 130 | 192 | 0.5 | 1.125 | 32.5 | 28 |
| 15 | 15 | 20 | 108 | 4 | 1.125 | 32.5 | 29 |
| 16 | 32 | 130 | 108 | 4 | 1.125 | 50 | 25 |
| 17 | 14 | 240 | 108 | 0.5 | 1.125 | 32.5 | 29 |
| 18 | 24 | 130 | 192 | 4 | 1.125 | 32.5 | 28 |
| 19 | 2 | 240 | 24 | 2.25 | 1.125 | 32.5 | 24 |
| 20 | 5 | 130 | 108 | 0.5 | 0.25 | 32.5 | 30 |
| 21 | 27 | 20 | 108 | 2.25 | 2 | 32.5 | 24 |
| 22 | 31 | 130 | 108 | 0.5 | 1.125 | 50 | 31 |
| 23 | 7 | 130 | 108 | 0.5 | 2 | 32.5 | 32 |
| 24 | 18 | 130 | 108 | 2.25 | 2 | 15 | 31 |
| 25 | 35 | 20 | 108 | 2.25 | 1.125 | 50 | 25 |
| 26 | 25 | 20 | 108 | 2.25 | 0.25 | 32.5 | 27 |
| 27 | 17 | 130 | 108 | 2.25 | 0.25 | 15 | 26 |
| 28 | 11 | 130 | 24 | 2.25 | 1.125 | 50 | 28 |
| 29 | 12 | 130 | 192 | 2.25 | 1.125 | 50 | 28 |
| 30 | 26 | 240 | 108 | 2.25 | 0.25 | 32.5 | 33 |
| 31 | 44 | 130 | 108 | 2.25 | 1.125 | 32.5 | 31 |
| 32 | 16 | 240 | 108 | 4 | 1.125 | 32.5 | 29 |
| 33 | 19 | 130 | 108 | 2.25 | 0.25 | 50 | 29 |
| 34 | 9 | 130 | 24 | 2.25 | 1.125 | 15 | 26 |
| 35 | 38 | 130 | 192 | 2.25 | 0.25 | 32.5 | 33 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 36 | 30 | 130 | 108 | 4 | 1.125 | 15 | 24 |
| 37 | 41 | 130 | 108 | 2.25 | 1.125 | 32.5 | 32 |
| 38 | 43 | 130 | 108 | 2.25 | 1.125 | 32.5 | 30 |
| 39 | 45 | 130 | 108 | 2.25 | 1.125 | 32.5 | 31 |
| 40 | 20 | 130 | 108 | 2.25 | 2 | 50 | 29 |
| 41 | 37 | 130 | 24 | 2.25 | 0.25 | 32.5 | 27 |
| 42 | 13 | 20 | 108 | 0.5 | 1.125 | 32.5 | 24 |
| 43 | 8 | 130 | 108 | 4 | 2 | 32.5 | 29 |
| 44 | 4 | 240 | 192 | 2.25 | 1.125 | 32.5 | 34 |
| 45 | 6 | 130 | 108 | 4 | 0.25 | 32.5 | 33 |
| 46 | 21 | 130 | 24 | 0.5 | 1.125 | 32.5 | 28 |

The ANOVA showed that the probability value was less than 0.0024 and the model was significant. The coefficient of determination ($R^2$) was 0.58, which ensured a satisfactory adjustment of the quadratic model to the experimental data and indicated that approximately 90% of the variability in the dependent variable (response) could be explained by the model. The adjusted R2 which is more suited for comparing models with different numbers of independent variables, was 0.7292 (Table 3).

The three dimensional response surface and contour presentations were then plotted to study the interaction among various physicochemical factors used and to find out the optimum concentration of each factors for maximum antibiotic production.

Also the result of the analysis showed that the experimental values were significant. The present study provides useful information about the interaction of various physicochemical factors for the production of antibiotic.

**Table 3. ANOVA for the experimental results of the central composite design (quadratic model)**

| | **Sum of** | | **Mean** | **F** | **p-value** | |
|---|---|---|---|---|---|---|
| **Source** | **Squares** | **df** | **Square** | **Value** | **Prob > F** | |
| Model | 425.3 | 20 | 21.27 | 3.37 | 0.0024 | significant |
| *A-agitation* | *39.06* | *1* | *39.06* | *6.18* | *0.0199* | |
| *B-Incubation time* | *56.25* | *1* | *56.25* | *8.91* | *0.0063* | |
| *C-K2HPO4* | *0.25* | *1* | *0.25* | *0.04* | *0.8439* | |
| *D-MgSo47H2O* | *5.06* | *1* | *5.06* | *0.8* | *0.3792* | |
| *E-temp* | *42.25* | *1* | *42.25* | *6.69* | *0.0159* | |
| *AB* | *6.25* | *1* | *6.25* | *0.99* | *0.3294* | |
| *AC* | *6.25* | *1* | *6.25* | *0.99* | *0.3294* | |
| *AD* | *1.14E-13* | *1* | *1.14E-13* | *1.80E-14* | *1* | |
| *AE* | *0.25* | *1* | *0.25* | *0.04* | *0.8439* | |
| *BC* | *6.25* | *1* | *6.25* | *0.99* | *0.3294* | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *BD* | *1* | *1* | *1* | *0.16* | *0.6941* | |
| *BE* | *4* | *1* | *4* | *0.63* | *0.4337* | |
| *CD* | *9* | *1* | *9* | *1.42* | *0.2438* | |
| *CE* | *25* | *1* | *25* | *3.96* | *0.0577* | |
| *DE* | *6.25* | *1* | *6.25* | *0.99* | *0.3294* | |
| *A^2* | *67* | *1* | *67* | *10.61* | *0.0032* | |
| *B^2* | *48.37* | *1* | *48.37* | *7.66* | *0.0105* | |
| *C^2* | *24.85* | *1* | *24.85* | *3.93* | *0.0584* | |
| *D^2* | *1.37* | *1* | *1.37* | *0.22* | *0.6458* | |
| *E^2* | *141.09* | *1* | *141.09* | *22.34* | *< 0.0001* | |
| Residual | 157.92 | 25 | 6.32 | | | |
| *Lack of Fit* | *152.42* | *20* | *7.62* | *6.93* | *0.0205* | *significant* |
| *Pure Error* | *5.5* | *5* | *1.1* | | | |
| Cor Total | 583.22 | 45 | | | | |

**Figure 1: Three dimensional response surface plots showing effects of Incubation time and agitation with corresponding contour plots showing predicted optimal response**

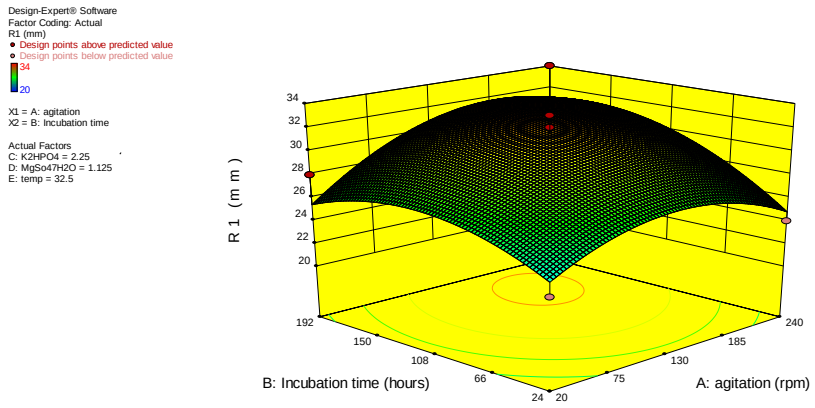



**Figure 2: Three dimensional response surface plots showing effects of K2HPO4 and agitation with corresponding contour plots showing predicted optimal response**

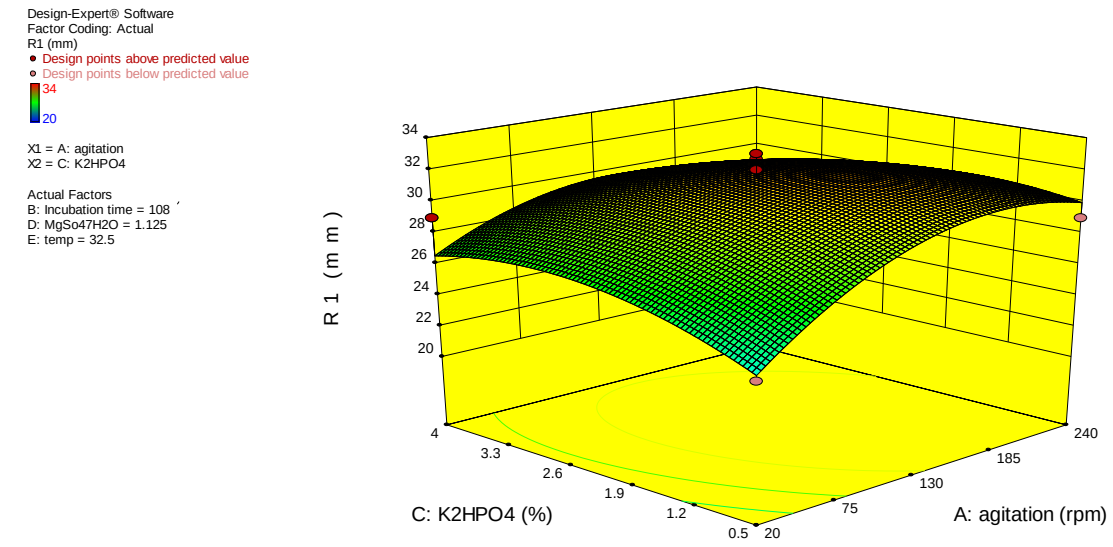



**Figure 3: Three dimensional response surface plots showing effects of $MgSo47H_2O$ and agitation with corresponding contour plots showing predicted optimal response**

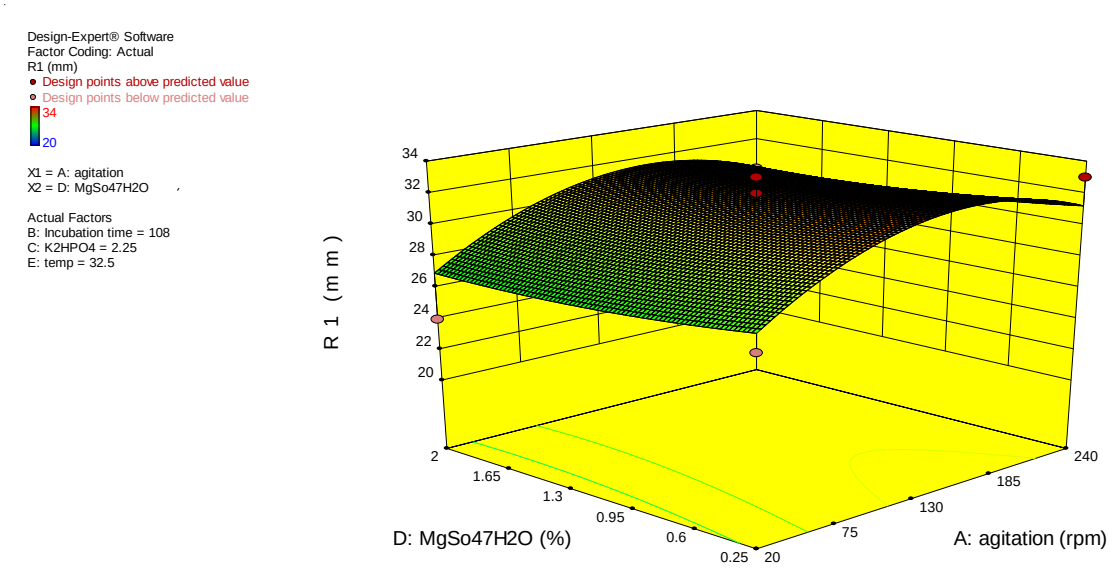



**Figure 4: Three dimensional response surface plots showing effects of Temperature and agitation with corresponding contour plots showing predicted optimal response**

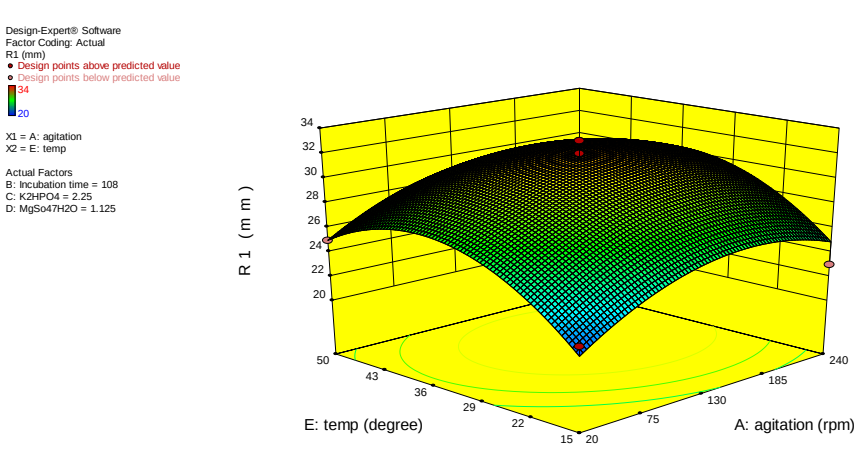



**Figure 5: Three dimensional response surface plots showing effects of K2HPO4 and incubation time with corresponding contour plots showing predicted optimal response**

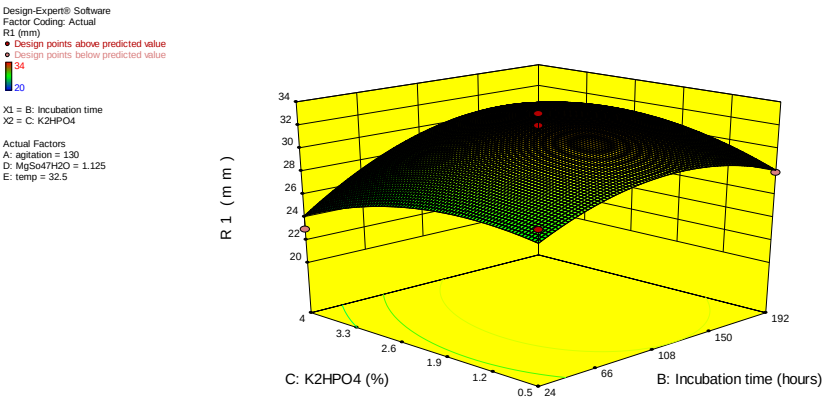



**Figure 6: Three dimensional response surface plots showing effects of MgSo47$H_2O$ and incubation time with corresponding contour plots showing predicted optimal response**

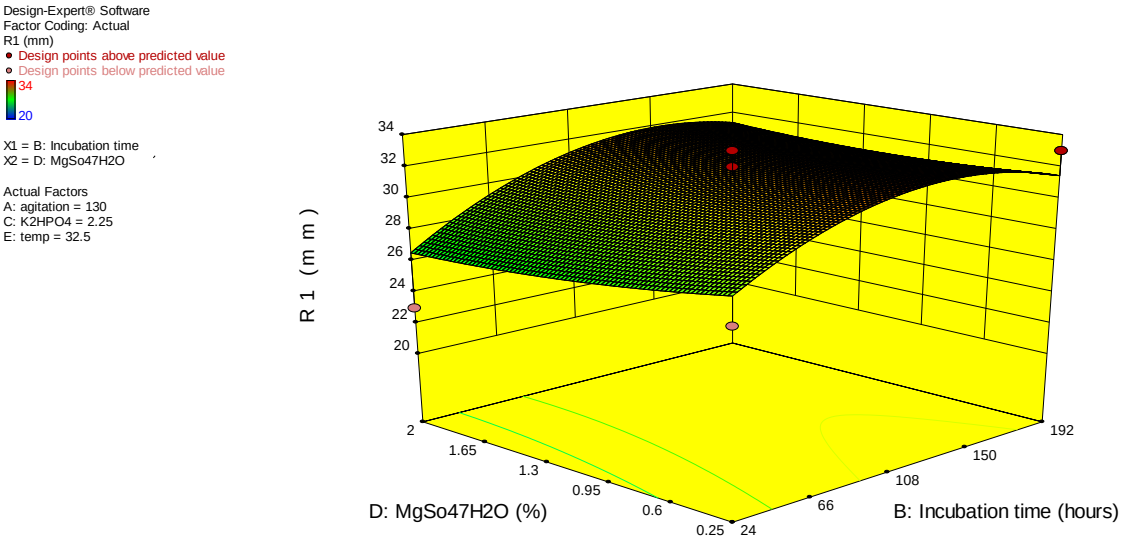



**Figure 7: Three dimensional response surface plots showing effects of temperature and incubation time with corresponding contour plots showing predicted optimal response**

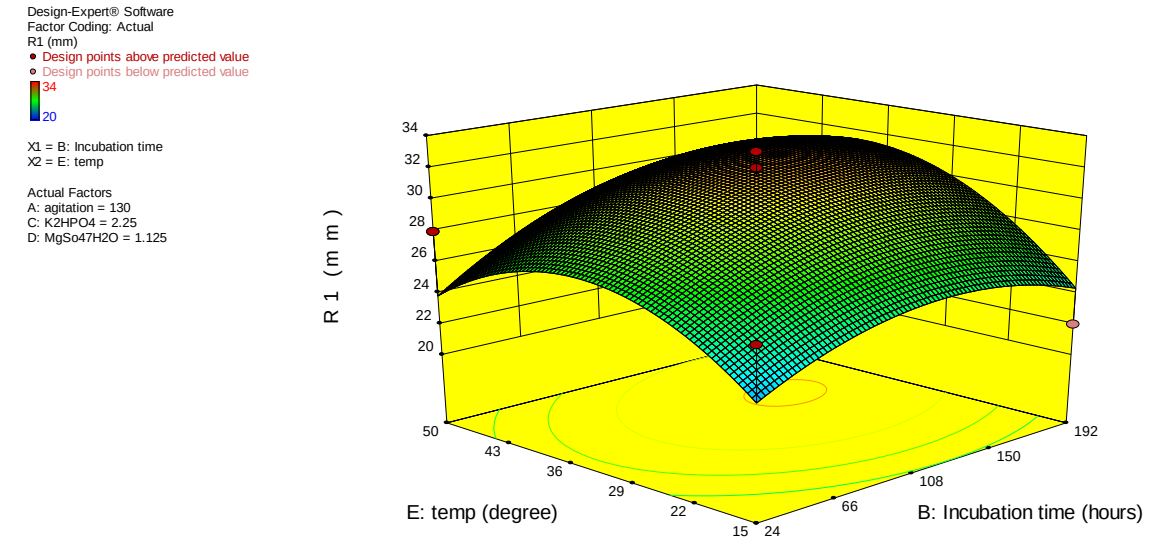



**Figure 8: Three dimensional response surface plots showing effects of temperature and K2HPO4 with corresponding contour plots showing predicted optimal response**

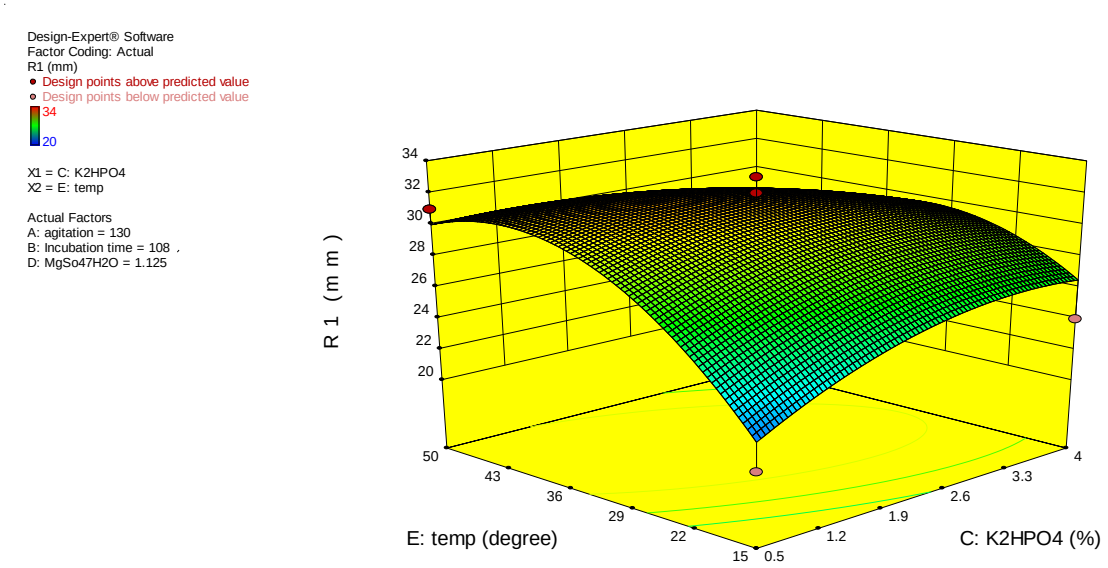
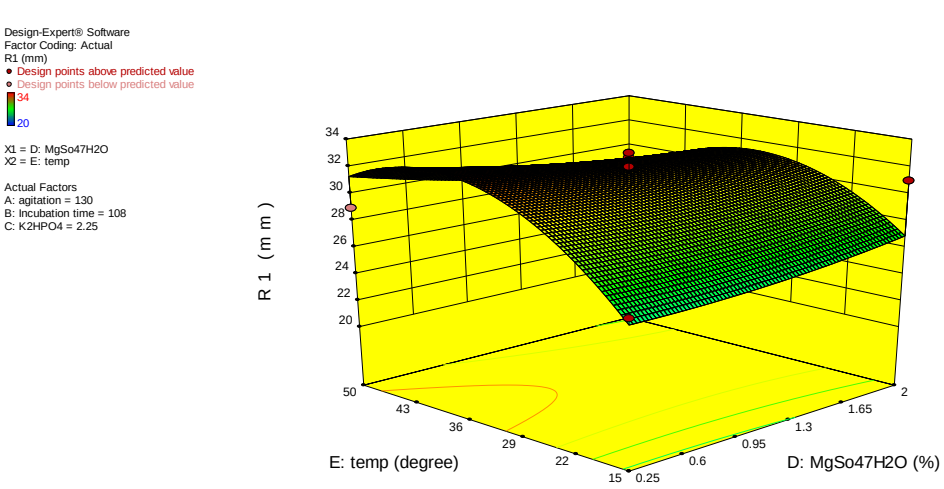



**Figure 9: Three dimensional response surface plots showing effects of temperature and MgSo47H$_2$Owith corresponding contour plots showing predicted optimal response**




Optimization for maximum antibiotic production is extremely important in pharmaceutical industry for economic reason. In the present study production was optimized with four components such as Incubation time, Agitation, Temperature, $MgSo_47H_2O$, $K_2HPO_4$. The antibiotic production found to be greater than the basal medium.

It shows the relative effects of two factors in combinations when all the factors were kept at their central levels. It clearly shows a fairly strong degree of curvature of 3D surface, where the optimum can be easily determined. The RSM approach allowed the determination of the culture conditions that yielded the highest antimicrobial activity by *Streptomyces sp*

Experimental results showed that a maximum antibiotic was obtained, under the condition of Incubation time, Agitation, Temperature, $MgSo_47H_2O$, $K_2HPO_4$ The plots of the quadratic model with two variables kept at constant level and the other two varying with the experimental ranges are showed.

Symmetrical shape indicated that there was a significant interaction between parameters. The results of analysis showed that the experimental values were significantly in agreement with the predicted values and also suggested that the model was satisfactory and accurate.

Statistical optimization methods for fermentation process could overcome the limitations of classical empirical methods and proved to be a powerful tool for the optimization of conditions of production of antibiotics by strain *Streptomyces sp*. The information obtained is considered fundamental and useful for developing a cultivation process for efficient production of antibiotics on a large scale.

## IV. Conclusion

Bioactive compounds produced by *Streptomyces* sp possess enormous efficiency as medicinally important products. We have been successful in designing a economically feasible medium supporting the enhanced growth of *Streptomyces sp* and simultaneously supporting a high yield of medicinally important bioactive compounds. The selected bacterial strain isolated from clinical culture was identified as *Staphylococcus aureus* and *E.coli* fungal culture *Candida albicans* based on the morphological analysis .The optimum condition for production of bioactive compound attained when incubated at 30°C, with a pH of 7.0, at 120 h of incubation inoculum supplemented with glucose, yeast extract best carben and nitrogen source for the strain. The production was higher than the optimized basal medium. An economically cheaper media formulated for production would be used to pharmaceutical industries for large scale production of the medicinally potential drug. Hence, overall data indicates a significant increase in the yield by using newly formulated production medium and optimized cultural conditions. Statistical optimization methods for fermentation process could overcome the limitations of classical empirical methods and proved to be a powerful tool for the optimization of conditions of production of antibiotics by strain *Streptomyces sp*.

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## AUTHORS

**First Author** – C. Sweetline, Department of Microbiology New Prince Shri Bhavani Arts and Science College, Chennai, Tamil Nadu, India

**Second Author** – R. Usha, Department of Microbiology, School of Life Sciences, Karpagam Academy of Higher Education, Coimbatore 641021, Tamil Nadu, India

**Correspondence Author** – jeyasweetline83@gmail.com