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the Effect of Varied Culture Conditions and Nutritional Requirements in the Production of Antimicrobial Metabolite by *Streptomyces*

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ABSTRACT

Background: This study evaluated the effect of varied culture conditions and nutritional requirements in producing antimicrobial metabolite by *Streptomyces*.
Methods: *Streptomyces* species were isolated from soil samples using the spread plate method on Streptomyces agar. In addition, the yeast-malt broth was used for fermentation and optimization. The different culture conditions were pH, mineral salt concentrations, and agitation rate. The tested nutritional components were Carbon and Nitrogen sources.
Results: We observed the maximum production of the antimicrobial metabolite using the medium containing Galactose anhydride showing high inhibition zone of 32 mm (*Staphylococcus aureus*) and 23 mm (*Pseudomonas aeruginosa*), respectively. Maximum secondary metabolite productivity was also achieved with the medium containing malt extracts *Staphylococcus aureus* (30 mm) and *Pseudomonas aeruginosa* (27 mm). For pH, maximum metabolite production was obtained in the medium adjusted to 10 (30 mm, 33 mm). The best metabolite production was obtained at 0.30 mol/ml (30 mm, 27 mm) with the medium incorporated with additional sodium chloride. Good antimicrobial metabolite production was also observed with a medium and maximum agitation speed of 250 rpm.
Conclusion: For efficient production of antimicrobial metabolite by *Streptomyces* species, Galactose anhydride, malt extract, sodium chloride concentration of 0.30 mol/ml, pH of 10, and agitation speed of 250 rpm should be used.

1. Introduction

The discovery and development of antibiotics have played a significant role in clinical medicine, agricultural research, and generally all parts of life [1]. A few genera of microorganisms produce antibiotics: *Bacillus, Streptomyces,* and *Penicillium* [2]. *Streptomyces* species are gram-positive aerobic acid-fast filamentous bacteria forming massively branched substrate, aerial. They are found abundantly in the soil and branching mycelia [3]. Species of *Streptomyces* are well-known sources of a wide range of antibiotics. Johardhana (2015) [4] reported that antibiotics are produced as secondary metabolites during the idiophase stage of the organism's growth. Depending on the nature of the secondary metabolite produced, the mechanism of action of these antibiotics differs. Some affect the ribosome, some of the cell wall, some protein synthesis, and the nucleus [5]. The characteristic of *Streptomyces* species being able to produce antimicrobial metabolite is not fixed. This ability can be significantly enhanced by altering and modifying the

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fermentation medium's culture condition and nutritional components [6]. This study would shed more light on understanding the principles behind the effect of cultural conditions on the production of secondary metabolites. Further, it evaluated varied carbon sources, nitrogen sources, mineral salt (sodium chloride) concentrations, pH values, and agitation rates to ascertain their effect on the production of secondary metabolites by *Streptomyces* species.

2. Materials and Methods

2.1 Materials

- *Streptomyces* agar (HIMEDIA, M1352-500G, India).
- Mannitoal salt agar (Oxoid, UK).
- Centrimide agar (Oxoid, UK).
- Muller Hinton agar (Merck KGaA, Germany).

2.2 Isolation of the Organism

The *Streptomyces* species were isolated from Umudike, Abia State, Nigeria soil samples by the Hans-Jorgen et al.'s (2015) [7] method, which involves spreading a sample of mixed culture onto a solid agar medium and then streaking the surface of the agar with a sterile loop to create a series of dilution. The isolated organisms were stored in *Streptomyces* agar slants and refrigerated at 4°C.

2.3 Secondary Metabolite Production and Its Susceptibility Testing

The secondary metabolite was produced from the *Streptomyces* species using sub-merged fermentation in Streptomyces broth. A dense inoculum of the organism was transferred into 10 ml broth in a MacCartney bottle and incubated in a shaker incubator at 24°C and 200 rpm for five days. The Five-day fermentation broth containing the supernatant was tested against two organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) obtained from clinical and environmental samples. We confirmed this by culturing in a selective media (Mannitol salt agar for *S. aureus* and Centrimide agar for *P. aeruginosa*), catalase test, coagulase test, and oxidase test.

2.4 Optimization of Culturing Condition and Nutritional Components

2.4.1 Carbon sources

About 10 ml of the production medium (Streptomyces broth) was prepared and dispensed into different MacCartney bottles. 1g of each of the carbon sources (Galactose anhydride, fructose, lactose, maltose, rice starch, corn starch, sucrose, glycerol, glucose) was added into the medium, respectively. A loopful of *Streptomyces* species was inoculated into each bottle. The bottles were incubated in a shaker incubator at 24°C and 200 rpm for five days. An antimicrobial assay using agar well diffusion was carried out. Small holes were punctured on the Muller Hinton agar using a sterile pipette. The supernatants were added to the agar and incubated at 37 °C for 24h. The diameters of the zones of inhibition were noted [8].

2.4.2 Nitrogen sources

About 10 ml of the production medium was prepared and dispensed into different MacCartney bottles. Approximately 1g of the carbon source that showed the best activity was incorporated into each bottle then 1g of the various nitrogen sources (peptone, yeast, malt, ammonium sulphate, casein) was added. The antimicrobial assay was done following the procedure described above on the effect of carbon sources on the production of secondary metabolites by *Streptomyces* [8].

2.4.3 pH

The secondary metabolite production by *Streptomyces* was estimated as described above. It only differed because the individual bottles were first incorporated with the best carbon and nitrogen sources before the pH levels were adjusted from 6 to 11. The broths were also incubated at 24°C and 200 rpm for five days. Their supernatants were also subjected to antimicrobial tests using the test organisms following the procedure described above on the effect of carbon sources on the production of secondary metabolites by *Streptomyces* [8].

2.4.4 Effect of sodium chloride concentration

The bottles containing the optimized media (carbon, nitrogen, pH) were incorporated with different concentrations of sodium chloride (0.10, 0.15, 0.20, 0.25, 0.30, 0.35 mol/ml). The resulting broths were incubated at 24°C and 200 rpm for five days. The antimicrobial assay was carried out following the procedure described above on the effect of carbon sources on the production of secondary metabolites by *Streptomyces* [8].

2.4.5 Rate of Agitation

About 10 ml of the optimized medium (carbon, nitrogen, pH, mineral salt) was dispensed into each MacCartney bottle. A dense inoculum of *Streptomyces* species was inoculated into each bottle. The bottles were then subjected to a different agitation rate (150, 200, 250, 300 rpm) and incubated at 24°C for five days. The supernatants were obtained and subjected to antimicrobial assays against the test organisms following the procedure described above on the effect of carbon sources on the production of secondary metabolites by *Streptomyces* [9].

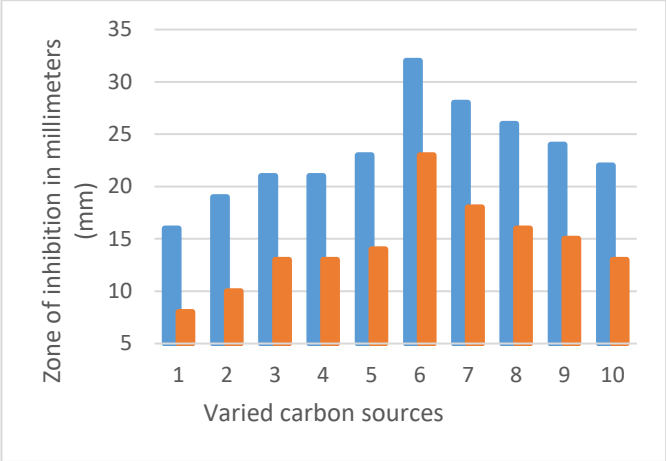
2.5 Statistical analysis

The data was statistically analyzed. ANOVA on ranks was

carried out, and the P value was determined [10].

3. Results and Discussion

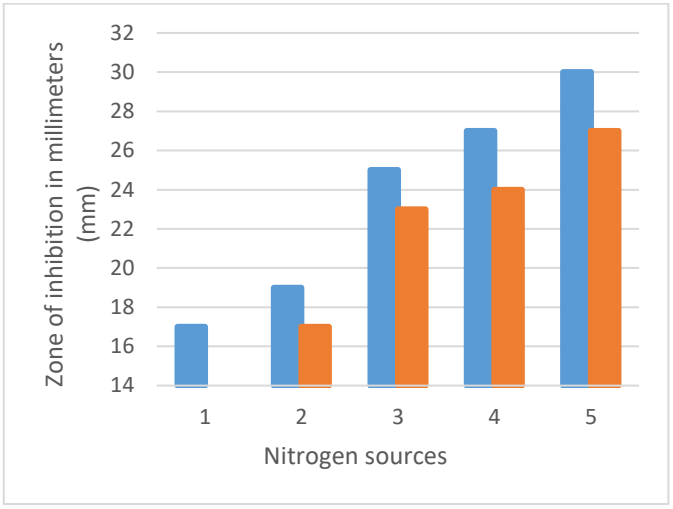
The effect of varied carbon sources in producing secondary metabolite by *Streptomyces* is shown in Figure 1. The highest diameter zone of inhibition was observed in galactose anhydride (32 and 23 mm for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively), followed by glycerol and glucose with zone diameters of 28 mm and 26 mm against *Staphylococcus aureus.* The least diameter zone of inhibition was observed in maltose (16 mm and 8 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively). Using ANOVA on ranks, galactose anhydride had a significant effect on the growth of the test organisms compared to other carbon sources at a P value of 0.05.



Keys 1: maltose 4: rice starch 7: glycerol 9: sucrose
2: fructose 5: lactose 8: glucose 10: corn starch
3: neutral 6: galactose anhydride
Staphylococcus aureus
Pseudomonas aeruginosa

Figure 1: The effects of varied carbon sources on the production of secondary metabolite by *Streptomyces* species. $P \leq 0.05$

The effects of varied nitrogen sources in the production of secondary metabolites by *Streptomyces* species are shown in Figure 2. The production of secondary metabolite was primarily promoted by malt extract, which showed the highest zone of inhibition of 30 and 27 mm for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. This was followed by yeast extract and ammonium sulphate with zone diameters of 27 mm and 25 mm against *Staphylococcus aureus.* The least zone of inhibition of 17 mm and 14 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, was observed in peptone. Statistically, malt extract had a significant effect on the growth of the test compared to other nitrogen sources used at a P value of 0.05. The production broth at pH 10 had the highest zone of inhibition of 30 mm and 33 mm for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, followed by pH 9 and 11 with zone diameter of 28mm and 26mm against *Pseudomonas aeruginosa.* The least was observed at pH 6, which produced 14 mm and 18 mm zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively (Figure 3). ANOVA on ranks was carried out against pH 4, and the optimum pH was found to significantly affect the production broth at a P value of 0.05.



Keys 1: Peptone 3: Casein 5: Malt extract
2: Ammonium sulphate 4: Yeast extract
Staphylococcus aureus
Pseudomonas aeruginosa

Figure 2: The effects of varied nitrogen sources in the production of secondary metabolites by *Streptomyces* species. $P \leq 0.05$

The result of varying salt concentrations in the production of antimicrobial metabolite by *Streptomyces* is shown in Figure 4. The best production was attained in the medium into which 0.30 mol/ml of the salt was incorporated. This was authenticated by the highest zone of inhibition of 30 mm and 27 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. This was followed by the medium incorporated with 0.25 mol/ml of the salt with 29 mm and 25 mm zones of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. In addition, ANOVA was carried out against the control (medium without the carbon and nitrogen sources), and 0.30 mol/ml of the salt was found to significantly affect the production broth at a P value of 0.05. The effect of varying agitation speeds in the production of secondary metabolite by *Streptomyces* species is depicted in Figure 5. Maximum secondary metabolite production was reached in the medium subjected to an agitation speed of 250 rpm. This was proven to be the highest inhibition diameter of 26 mm and 23 mm against

Staphylococcus aureus and *Pseudomonas aeruginosa* obtained from the medium subjected to 250 rpm. The following best was the medium subjected to an agitation speed of 200 rpm. This medium also produced a good amount of antimicrobial metabolite, as shown by the inhibition zone of 20 mm and 17 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

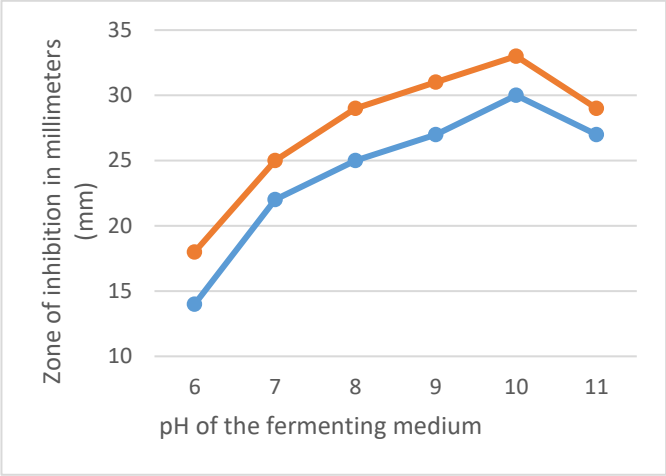


Figure 3: The effects of varied pH conditions in the production of secondary metabolites by *Streptomyces* species. $P \leq 0.05$ (against pH 4).

This study evaluated the effect of varied culture conditions and nutritional requirements in the production of antimicrobial metabolite by *Streptomyces*. The isolated organism (*Streptomyces*) was subjected to its routine incubation period of 5 days. Secondary metabolites were produced on the 4th day of incubation. The result is in agreement with Wolfbang et al. (2015) [3] and Amal et al. (2014) [2], who reported the 4th day of incubation to be the best day for secondary metabolite production by *Streptomyces* species. The zone of inhibition produced was used to correlate the production of antimicrobial (Secondary) metabolite by the *Streptomyces* species. The antimicrobial effect of the secondary metabolites was tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* More significant activity was observed with *Staphylococcus aureus* than with *Pseudomonas aeruginosa.* Generally, *Pseudomonas aeruginosa* is more resistant to antimicrobial agents than *Staphylococcus aureus* [11]. The ability of *Pseudomonas aeruginosa* to display this property is due to the complexity of their cell wall and their unique outer membrane that constitutes an effective barrier to the passage of germicides and for efflux systems [11]. A similar result was observed in a study conducted in Egypt by El-Naggar and Hamouda (2016) [12]. They reported that *Streptomyces* spp. exhibited a broad antimicrobial spectrum against several microorganisms, including multidrug-resistant *E. coli, Pseudomonas aeruginosa, Bipolarisoryzae, Bacillus subtilis*, and *Staphylococcus aureus.* Conversely, Singh et al. (2014) [13] found that *S. sannanensis* exhibits a narrow spectrum antimicrobial activity which could inhibit the growth of only Gram-positive bacteria. Also, the morphological variations between these microorganisms, where Gram-negative bacteria have an outer polysaccharide membrane bearing the structural lipopolysaccharide components, may cause the differential sensitivity between Gram-positive and Gram-negative bacteria [13].

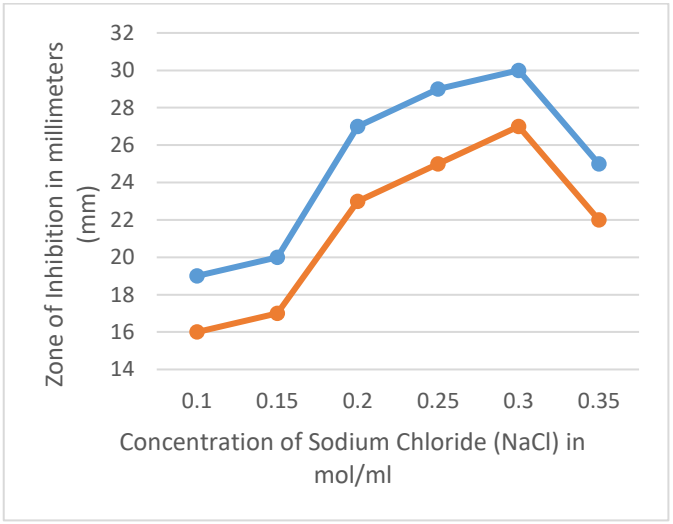


Figure 4: The effects of varied mineral salt concentration in the production of secondary metabolites by *Streptomyces* species. $P \leq 0.05$ (against the control: medium without the carbon and nitrogen sources).

Galactose anhydride was observed to be the best carbon source for producing secondary metabolite by *Streptomyces* species. It produced an inhibition diameter of 32 mm and 23 mm, respectively, against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Similar results showed that antimicrobial agent production was higher in a medium with carbon as its sole source [13]. This indicates that *Streptomyces* effectively used galactose anhydride to produce secondary metabolites [14]. The number of secondary metabolites produced was directly proportional to the zone of inhibition observed with the medium against the tested organisms. The finding agrees with Ateb (2016) [15] and Clarice (2012) [16], who isolated antibiotics-producing *Streptomyces* from Amazonian soils. In a similar work by Ingrid (2012) [17], glycerol was the best carbon source though galactose anhydride was not tested. The high metabolite production by galactose anhydride and glycerol could be attributed to its isomeric nature. Glucose, dextrose, and galactose are isomeric forms of each other and, as such,

are readily isomerized into each other in the presence of hexose isomerase. *Streptomyces* species possess hexose isomerase and can readily reconvert these carbon sources into each other [18]. The most negligible concentration of secondary metabolite production by *Streptomyces* was observed to be maltose, and this was because it showed a minor diameter zone of inhibition. In contrast, Kalaiyarasi et al. (2020) [19] reported in Saudi Arabia that maltose maximized antibiotics production in *Streptomyces.* For the effect of nitrogen sources in the production of secondary metabolite, malt extracts were observed to be the best source, and yeast extracts followed suit. Kalaiyarasi et al. (2020) showed a similar result [19] who observed that yeast extract positively influenced antibiotics production among the nitrogen sources. Malts and yeast extracts are part of the nitrogen sources in the fermentation and optimization medium. Adding additional nitrogen sources into the medium would lead to increased biomass of *Streptomyces* species and, as such, more secondary metabolite production [20]. This finding is consistent with that of Ingrid (2012) [17], who found ammonium sulphate and peptone to be the best nitrogen sources.

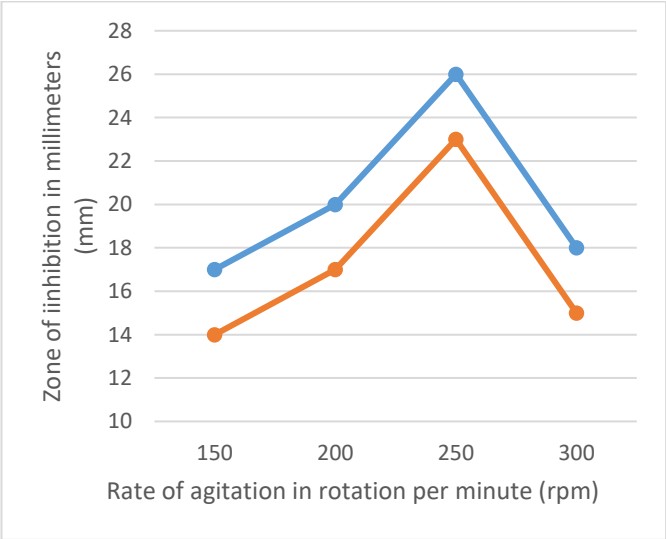


Figure 5: The effects of the varied rate of rotation in the production of antimicrobials by *Streptomyces* species

Furthermore, it was observed from the results that the production of secondary metabolites increased with an increase in pH up to a certain level (pH 10) and then decreased. *Streptomyces* species grow well at a pH of 5.7-7.0 [3], and secondary metabolites are produced in the idiophase phase of growth [19]. The maximum secondary metabolite was observed at pH 9 and 10 and was slightly similar to the results of Singh et al.′s study [13]. They found maximum growth and high antimicrobial activity by *S. sannanensis* was achieved at pH 7. The decline observed at pH 10-11 could be because pH 11 falls outside the optimum range suited for the enzyme of *Streptomyces*, thus, inhibiting the *Streptomyces* species and, as such, reducing the metabolite production. This finding contradicts Amal et al.′s study (2014) [2] that maximum metabolites were produced at pH 8. The secondary metabolite production by *Streptomyces* species was best at 0.30 mol/ml concentration of mineral salt (NaCl). Similarly, Febina (2014) found that *Streptomyces* species showed secondary metabolite production at a NaCl concentration of 2.5% [20]. There has never been any favorable report on the effect of high mineral salt (NaCl) concentration on the production of secondary metabolite by *Streptomyces* species [9][21]. The results from the agitation rate on metabolite production by *Streptomyces* species were observed to increase from 150 rpm to 250 rpm and reduced at 300 rpm steadily. According to Joanne et al. (2013) [22], agitation increases oxygen incorporation into the medium, and therefore, an increase in agitation rate incorporates more oxygen into the medium. *Streptomyces* species are aerobic organisms and, as such, need oxygen to maintain life. More incorporation of oxygen into the medium would invariably lead to an increase in secondary metabolite production.

4. Conclusion

Growth conditions and nutritional components could optimize the secondary metabolite production by *Streptomyces* species. Galactose anhydride, malt extract, 0.30mol/ml of sodium chloride concentration, pH of 9-10, and agitation rate of 250rpm applied in this study showed an increase in secondary metabolite production.

Authors' Contributions

Immaculata Ugochi Nwankwo: Supervision; Methodology; Resources; Funding acquisition; Writing original draft; Validation; Visualization. Chukwuma Great Udensi: Investigation; Data curation; Formal analysis. Chinonye Medline Maduka: Writing-Review and Editing. Appeh Osita Gabriel: Writing-Review and Editing. Nwachukwu Nzubechukwu Prisca: Conceptualization; Investigation; Data curation; Funding acquisition.

Conflicts of Interest

The authors have declared that no competing interests exist.

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References

1. Ingrid RE, Joao LA, Mayra KS, Rudi EL. The Effect of Varying Nutritional Components on the Production of Antibiotics by *Streptomyces* Spp. Isolated from Amazonian Soils. *Fermentation Technology Journal.* 2012; 1(3): 1-4.

2. Ali AM, El-Gammal AA, Rifaat HM. Influence of Nutritional and Culturing Condition on Antimicrobial Metabolite Production by *Streptomyces mesioensis* Isolated from Eygptian Soil. *International Journal of Chemical-TechResearch.* 2014; 6(4): 2552-7.

3. Wolfgang L, Hans-Jorgen B, Jean E, Martha ET, Peter K, William BW. Road Map of the Phylum Actinobacteria. *Bergeys Manual of Systematic Bacteriology Print-Off.* 2015; 5(1): 1-2062.

4. Jonardhana GE, Nooshen KD. Isolation and Evaluation of Antimicrobial Activity of *Streptomyces Flavogriseus* from Soil Samples in India. *Jundishphur Journal of Microbiology.* 2015; 8(2): 15-107.

5. Mariadhas VA, Aldous JH, Savarimuthu IJ, Thankappari S, Vada K. Nutritional Requirment for the Production of Antimicrobial Metabolite from *Streptomyces. African Journal of Microbiological Research.* 2014; 8(8): 750-8.

6. Mustafa O. Antifungal and Antibacterial Compounds from *Streptomyces* strains. *African Journal of Biotechnological Advancements.* 2009; 8(13): 3007-17.

7. Hans-Jorgen B, Jean E, Martha ET, Peter K, William BW, Wolfgang L. Road Map of the Phylum Actinobacteria. *Bergeys Manual of Systematic Bacteriology print-Off.* 2015; 5(1): 1-2062.

8. Saadoun I, Muhana A. Optimal Production Conditions, Extraction, Partial Purification and Characterization of Inhibitory Compound(s) Produced by *Streptomyces* Ds-104 Isolate Against Multi-Drug Resistant *Candida Albicans. Current Trends in Biotechnology and Pharmacy.* 2008; 2: 402-32.

9. Reddy NG, Ramakrishna D, Rajagopal S. Optimization of Culture Conditions of *Streptomyces Rochei* (MTCC 10109) for the Production of Antimicrobial Metabolites. *Egyptian Journal of Biology.* 2011; 13: 21-9.

10. Gao H, Liu M, Liu J, Dai H, Zhou X, Liu X. Medium Optimization for the Production of Avermectin B1a by *Streptomyces Avermitilis* 14-12A Using Response Surface Methodology. *Bioresource Technology.* 2009; 100: 4012-6.

11. Divya G, Vinoth KS, Aarya V, Sankar M, Kirthika HP. Detection of the Most Effective Disinfectant Against Multi Drug Resistant Bacteria in Hospital Infections. *International Journal of Biology, Pharmacy and Allied Sciences.* 2015; 4(4): 1989-99.

12. El-Naggar NA, Hamouda RA. Antimicrobial Potentialities of *Streptomyces Lienomycini* NEAE-31 Against Human Pathogen Multidrug-Resistant *Pseudomonas Aeruginosa. International Journal of Pharmacology.* 2016; 12 (8): 769-88.

13. Singh LS, Sharma H, Talukdar NC. Production of Potent Antimicrobial Agent by Actinomycete, *Streptomyces Sannanensis* Strain SU118 Isolated from Phoomdi in Loktak Lake of Manipur, India. *BMC Microbiology.* 2014; 14: 278.

14. Bundale S, Begde D, Nashikkar N, Kadam T, Upadhyay A. Optimization of Culture Conditions for Production of Bioactive Metabolites by *Streptomyces* Spp. Isolated from Soil. *Advances in Microbiology.* 2015; 5(6): 10.

15. Ateb GO, Bobaji CE. *Streptomyces griseus* ATCC 10971. *International Journal of Microbiology.* 2016; 1(1): 1-5.

16. Clarice MC, Ingrid RE, Joao LA, Mayra KS, Rudi EL. The Effect of Varying Culture Conditions on the Production of Antibiotics by *Streptomyces Spp.* Isolated from Amazonian Soils. *Fermentation Technology Journal.* 2012; 1(3): 1-5.

17. Ingrid RE, Joao LA, Mayra KS, Rudi EL. The Effect of Varying Nutritional Components on the Production of Antibiotics by *Streptomyces* Spp. Isolated from Amazonian Soils. *Fermentation Technology Journal.* 2012; 1(3): 1-4.

18. Shintani T. Food Industrial Production of Monosaccharides Using Microbial, Enzymatic, and Chemical Methods. *Fermentation.* 2019; 5 (47): 1-13.

19. Kalaiyarasi M, Ahmad P, Vijayaraghavan P. Enhanced Production Antibiotics Using Green Gram Husk Medium by *Streptomyces* Sp. SD1 Using Response Surface Methodology. *Journal of King Saud University.* 2020; 32: 2134-41.

20. Febina S, Rachel RD, Shenbagarathai. Optimization of Antibiotic Production by Marine *Actinomycetes: Streptomyces* Sp. KOD10. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2014; 6(2): 506-10.

21. Oskay M. Effects of Some Environmental Conditions on Biomass and Antimicrobial Metabolite Production by *Streptomyces* sp. KGG32. *International Journal of Agriculture and Biology.* 2011; 13: 317-24.

22. Joanne W, Chris W, Linda S. *Prescott's Microbiology* (African Edition, 9th Edition) Mc Graw-Hill and Bam LTD, (Glasglow). 2013: 956-65.