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Research Article

OPTIMIZATION OF ANTIBIOTIC PRODUCTION BY MARINE ACTINOMYCETES *STREPTOMYCES* SP. KOD10

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ABSTRACT

Objective: To improve the productivity of antibacterial compounds of *Streptomyces* sp. KOD10 by optimizing its physical and chemical factors.

Methods: *Streptomyces* sp. KOD10 was screened for its antagonistic activity by agar well diffusion method. In order to improve its efficiency, the effects of medium components, temperature, pH, inoculam concentration, sodium chloride concentration, agitation, fermentation time, carbon and nitrogen sources were optimized and its productivity was examined by agar well diffusion method against four bacterial strains obtained from MTCC, Chandigarh, India namely *Neisseria mucosa*, *Staphylococcus aureus*, *Micrococcus luteus* and *Enterococcus faecalis*.

Results: The bacterial inhibition rate was more in the optimized medium composition (g/L), containing yeast extract 15, malt extract 10, dextrose 15 and sodium chloride 25. The maximum antibiotic activity was observed at the inoculation volume 2% (W/V), rotary speed of 200rpm, fermentation time for 7 days, sodium chloride concentration at 2.5%, temperature 25 to 28^{0}C and initial pH 10.0. The best carbon and nitrogen sources were found to be dextrose and yeast extract at 1.5% (W/V) concentrations respectively.

Conclusion: The results from this study confirmed that the antibacterial substances produced by *Streptomyces* sp. KOD10 were found to be more effective after its optimization.

Keywords: *Streptomyces* sp. KOD10, Optimization, Antibacterial.

INTRODUCTION

Marine actinomycetes are the greatest economical and biotechnologically valuable class of prokaryotes producing excellent bioactive secondary metabolites especially antibiotics [1]. Marine actinomycetes are a potential source of novel compounds as the environmental conditions of the sea are entirely different from the terrestrial conditions [2] [3]. Marine *Streptomyces* are potential organisms for novel natural products and they have a unique metabolic diversity and excellent potential in producing novel compounds. They produce approximately two-thirds of all known antibiotics of microbial origin [4] [5]. Indeed, *Streptomyces* sp. produces about 75% of commercially and medically useful antibiotics [6] [7]. The actinomycetes are well adapted to marine environment and are able to break down complex biological polymers [8]. Enhancement in the growth of actinomycetes is carried out by manipulating the nutritional, chemical and physical parameters of the culturing conditions. In optimization, media composition plays a remarkable role in the productivity and economics of the crucial process [9] [10]. In the present study, *Streptomyces* sp. KOD10 was screened for its antagonistic activity with reference to the media composition, and other factors which were optimized for high antibiotic yield. The efficiency of the antibiotic production was tested by agar well diffusion method and the inhibition zones were measured.

METHODOLOGY

Isolation of *Streptomyces* sp.KOD10

The actinomycete strain *Streptomyces* sp. KOD10 was isolated from the marine soil of Kodiyakarai, Tamil Nadu, India (latitude 10^{0}16'59.55''N, longitude 79^{0}49'56.15''E). The soil was collected in sterile container at 15 cm depth. The actinomycete strain *Streptomyces* sp. KOD10 was isolated by serial dilution plating method in Starch Casein agar medium supplemented with 50mg/liter of Cyclohexamide (Sigma Aldrich) to inhibit fungal contamination. The plates were incubated for 10 days at 28^{0}C.

Screening of *Streptomyces* sp. KOD10 for its antibiotic production

Streptomyces sp. KOD10 was grown in Yeast extract Malt extract broth and incubated at 30^{0}C in a shaker at 200rpm for 7 days. After incubation, the broth was centrifuged at 5000 rpm for 10 minutes with equal volume of Ethyl acetate to extract the compounds [11] and the antibacterial study was carried out by agar well diffusion method. 100µl or 100 microliter of the supernatant were loaded in the well using micropipette. The zone of inhibition was measured as a total diameter of the zone and the well diameter was subtracted from the total diameter.

Test organisms used in the study

Staphylococcus aureus, Micrococcus luteus, Neisseria mucosa and *Enterococcus faecalis* were obtained from MTCC, Chandigarh.

The cultures obtained were in the form of lyophilized powders in sealed vials. The cultures were revived in Nutrient broth and stored in agar slants for further study.

Optimization of antibiotic production by agar well diffusion method

Medium

Streptomyces sp. KOD10 was inoculated in Krasilnikov's synthetic broth (KSB), Nutrient broth (NB), Starch casein broth (SCB), Tryptone yeast extract broth (ISP1), Yeast extract malt extract broth (ISP2) and Inorganic salt starch broth (ISP4) and kept in incubator shaker at 150rpm for 7 days at 25°C.

Temperature

Optimum temperature was studied by varying the incubation temperatures at 25^{0}C, 28^{0}C, 32^{0}C, 37^{0}C and 45^{0}C. *Streptomyces* sp. KOD10 was inoculated in the optimized media and kept in incubator shaker at 150rpm for 7 days.

pH

Optimum pH was studied by varying the medium pH as 5, 6, 7, 8, 9, 10 and 11. The growth media was adjusted to different pH using 1M HCl and 1M NaOH. *Streptomyces* sp. KOD10 was inoculated in the optimized media and kept in incubator shaker at 150rpm for 7 days.

Inoculam concentration

Optimum medium was taken and the *Streptomyces* sp. KOD10 was inoculated at different inoculam concentration such as 0.5%, 1%, 1.5% and 2% (W/V) and kept in incubator shaker at 150rpm for 7 days.

Agitation

Streptomyces sp. KOD10 was inoculated in the optimized media and kept in different agitation speed to achieve high rate of antibiotic production.

The different agitation speeds were 100rpm, 130rpm, 150rpm and 200rpm. A control flask was maintained at 0rpm. All the flasks were incubated for 7days.

Carbon Source

Streptomyces sp. KOD10 was inoculated in the optimized media and kept in incubator shaker at optimized speed and temperature for 7 days.

Various carbon sources used in the medium were Glucose, Dextrose, Sucrose, Sorbitol, Galactose, Glycerol and Maltose. A flask without any carbon source was kept as a control.

Optimized Carbon Concentration

Streptomyces sp. KOD10 was inoculated in the optimized media and kept in incubator shaker at optimized speed and temperature for 7 days.

In the optimized medium, the optimized carbon source was varied in order to get the high rate of antibiotic production. Concentration of the optimized carbon source was 0.5%, 1%, 1.5% and 2% (W/V). A flask without any carbon source was kept as a control.

Nitrogen Source

Streptomyces sp. KOD10 was inoculated in the optimized media and kept in incubator shaker at optimized speed and temperature for 7 days. Nitrogen sources used in the medium were Peptone, Casein, Beef extract, Yeast extract and Malt extract. A flask without any nitrogen source acts as a control.

Optimized Nitrogen Concentration

Streptomyces sp. KOD10 was inoculated in the optimized media and kept in incubator shaker at optimized speed and temperature for 7 days.

In the optimized medium, the optimized nitrogen source was varied in order to get the high rate of antibiotic production. Concentration of the optimized nitrogen source was 0.5%, 1%, 1.5% and 2% (W/V). A flask without any nitrogen source acts as a control.

Sodium chloride concentration

Streptomyces sp. KOD10 was inoculated in the optimized media and kept in incubator shaker at optimized speed and temperature for 7 days. Concentration of the optimized sodium chloride was 0.5%, 1%, 1.5%, 2% and 2.5% (W/V). A flask without any sodium chloride acts as a control.

Incubation Period

Streptomyces sp. KOD10 was inoculated in the optimized media and incubated for different time interval to achieve high rate of antibiotic production. The incubation period varied from 5, 6, 7, 8, 9 and 10 days.

Antibacterial activity of *Streptomyces* sp. KOD10

After the incubation period, the antimetabolites of *Streptomyces* sp. KOD10 were extracted using ethyl acetate and the antibacterial study was carried out by agar well diffusion method. 100µl or 100 microliter of the supernatant were loaded in the well. The zone of inhibition was measured as a total diameter of the zone and the well diameter was subtracted from the total diameter. The experiments were done in triplicates and the results were expressed in Mean ± Standard Deviation.

RESULTS AND DISCUSSION

Isolation and Screening of *Streptomyces* sp.KOD10

Streptomyces sp. KOD10 was isolated from the marine soil of Kodiyakarai and it was stored in ISP1 medium for further analysis. The screening results showed that the four bacterial strains were sensitive to *Streptomyces* sp.KOD10. *Neisseria mucosa* showed highest zone of inhibition of 14mm followed by *Micrococcus luteus* with 12mm. The result of the screening of *Streptomyces* sp.KOD10 for its antagonistic activity was displayed in Table 1.

Table 1: Screening of *Streptomyces* sp. KOD10 for its antagonistic activity

Bacterial pathogens	Zone of inhibition (mm)
Micrococcus luteus	12
Staphylococcus aureus	10
Enterobacter faecalis	8
Neisseria mucosa	14

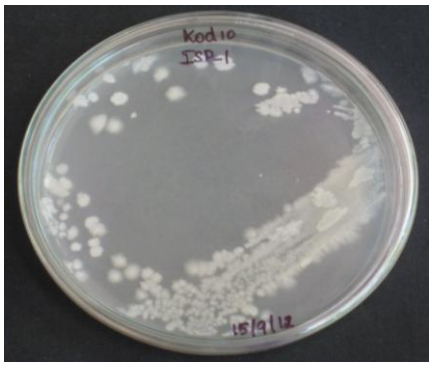

Fig. 1: Subculture plate of *Streptomyces* sp.KOD10 on ISP – 1 medium

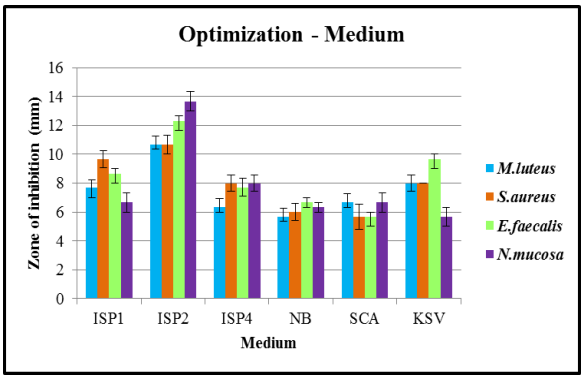

Fig. 2: Effect of different Medium on the growth of *Streptomyces* sp. KOD10 for antibiotic production

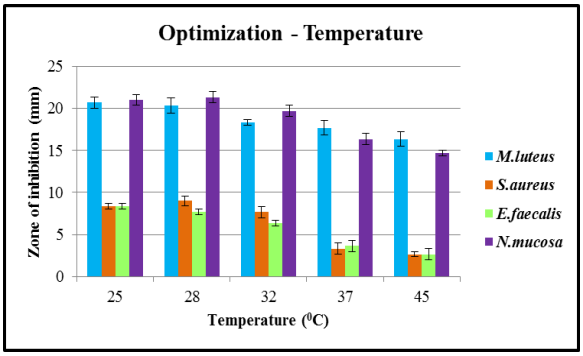

Fig. 3: Effect of different temperature on the growth of *Streptomyces* sp. KOD10 for antibiotic production

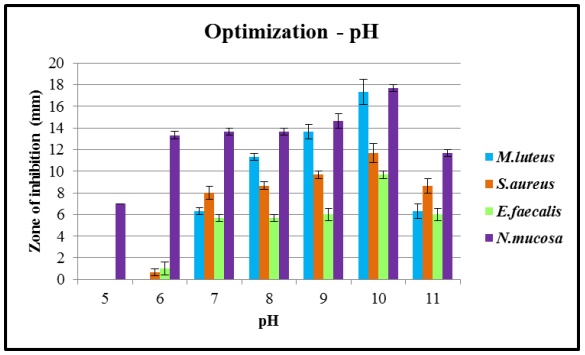

Fig. 4: Effect of different pH on the growth of *Streptomyces* sp. KOD10 for antibiotic production

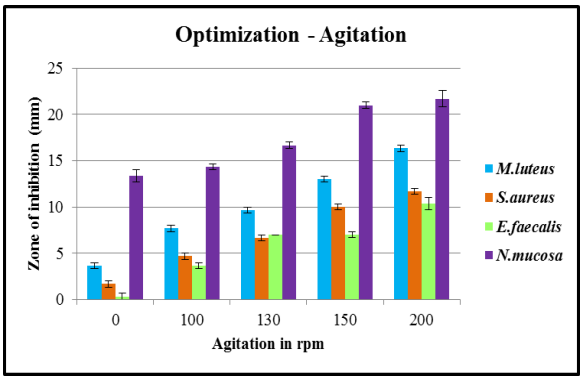

Fig. 5: Effect of different speed on the growth of *Streptomyces* sp. KOD10 for antibiotic production

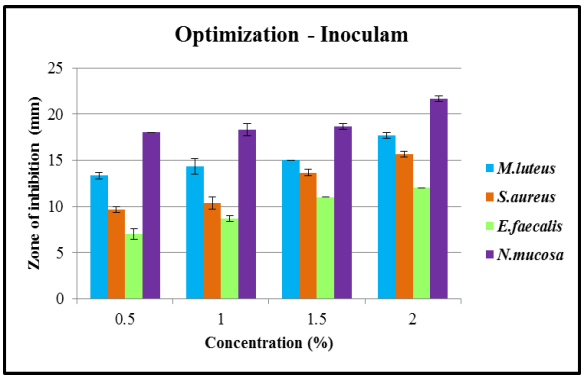

Fig. 6: Effect of different inoculam volume on the growth of *Streptomyces* sp. KOD10 for antibiotic production

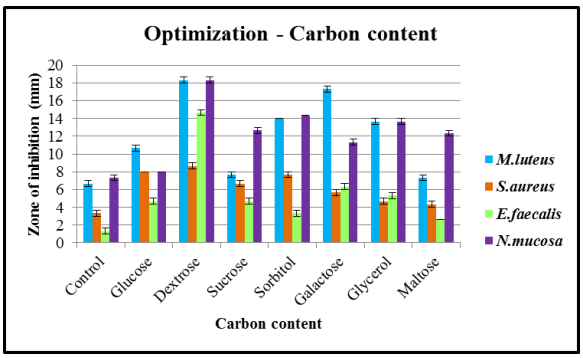

Fig. 7: Effect of different carbon source on the growth of *Streptomyces* sp. KOD10 for antibiotic production

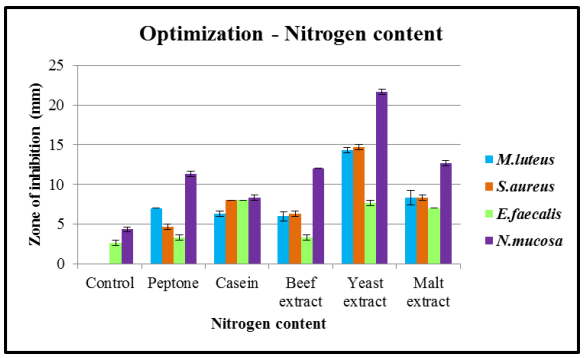

Fig. 8: Effect of different nitrogen source on the growth of *Streptomyces* sp. KOD10 for antibiotic production

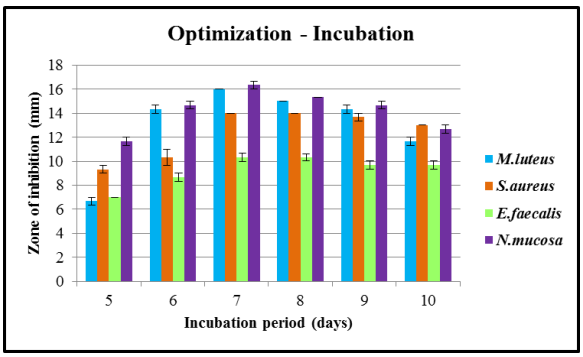

Fig. 9: Effect of different incubation period on the growth of *Streptomyces* sp. KOD10 for antibiotic production

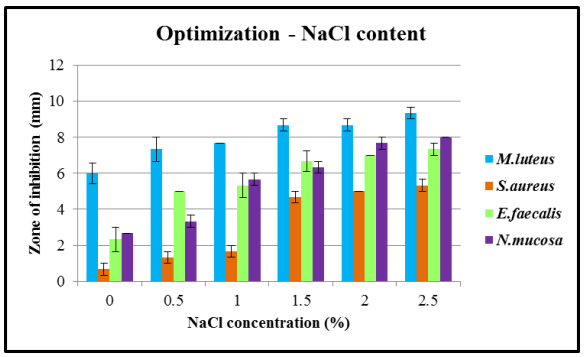

Fig. 10: Effect of different sodium chloride concentration on the growth of *Streptomyces* sp. KOD10 for antibiotic production

Optimization of antibiotic production by agar well diffusion method

Different broth medium, sodium chloride concentration, carbon sources, nitrogen sources and different physical parameters were verified for maximum production of antibiotics. It was found that Yeast extract malt extract broth supports for maximum antibiotic production after 7 days incubation. Maximum yield of antibiotic was observed in the following conditions, NaCl (2.5%); inoculam volume 2% (W/V); incubation temperature of 25 to 28^{0}C and pH value of 10. Dextrose and yeast extract were chosen as the best carbon and nitrogen source respectively.

Medium

Culture media such as Tryptone yeast extract broth (ISP1), Yeast extract malt extract broth (ISP2), Inorganic salt starch broth (ISP4), Nutrient broth, Starch Casein broth, Krasilnikov's synthetic agar were used for the production of desired metabolite from *Streptomyces* sp. KOD10. The ability of microbes to form antibiotics is not a fixed property but can be greatly increased or completely lost under different conditions of nutrients and/or cultivation [12]. Yeast extract malt extract broth (ISP2) was confirmed (Figure 2) to be the effective culture media for maximum antibiotic production by showing 10.67 mm zone of inhibition for *Micrococcus luteus* and *Staphylococcus aureus*. *Streptomyces* sp. KOD10 grown in ISP2 media also inhibited *Enterobacter faecalis* and *Neisseria mucosa* by showing 12.33 mm and 13.67 mm respectively.

Temperature

Temperature played an important role in the metabolite production and activity. ISP2 medium with *Streptomyces* sp. KOD10 were incubated at different temperature (25, 28, 32, 37 and 45^{0}C) on rotary shaker. It was found that 25 to 28^{0}C was the optimum temperature for metabolite production (Figure 3). Deviation from optimum temperature affects the efficiency of metabolite. *Micrococcus luteus* and *Neisseria mucosa* were very sensitive to the extract with 20 mm and 21 mm zone of inhibition respectively.

pH

The pH influenced the production of metabolite by *Streptomyces* sp. KOD10. The initial pH of the ISP2 media was adjusted to (5, 6, 7, 8, 9, 10 and 11) separately. *Streptomyces* sp. KOD10 showed maximum activity at pH 10 (Figure 4). Acidic pH inhibited the antibiotic production rather than high alkaline pH. The activity of metabolite increased with the increase in pH upto pH10 and then dropped down at pH11. pH10 was confirmed to be the effective pH for maximum antibiotic production by showing 17.67 mm zone of inhibition for *Neisseria mucosa.*

Agitation

Effect of agitation on production of metabolite was detected by ISP2 media with *Streptomyces* sp. KOD10 at different agitation conditions (0, 100, 130, 150 and 200 rpm). Agitation showed (Figure 5) direct effect on the growth of *Streptomyces* sp. KOD10 because agitation affects aeration and mixing of the nutrients in the fermentation medium.

Agitation at 200 rpm was found to be most suitable agitation condition for *Streptomyces* sp. KOD10. Agitation at 200 rpm showed greater activity when compared with other lower rpm. [13] reported that agitation at 150 rpm was found to be the most suitable for *Streptomyces* sp. Agitation at 200 rpm was confirmed to be the effective agitation speed for maximum antibiotic production by showing 21.67 mm and 16.33 mm zone of inhibition for *Neisseria mucosa* and *Micrococcus luteus* respectively.

Inoculam concentration

Effect of inoculum concentration on production of metabolite was detected by fermentation media with *Streptomyces* sp. KOD10 at different inoculum concentration (0.5, 1.0, 1.5 and 2.0%). The results obtained (Figure 6) demonstrated that the optimal inoculum concentration for production was 2% with an inhibition zone of 21.67 mm for *Neisseria mucosa*. Inoculum concentration showed direct effect on the production based on varying inoculum level.

Carbon source

Effect of carbon sources on production of metabolite was detected by fermentation media with selected *Streptomyces* sp. KOD10 at different carbon sources (without carbon source, glucose, sucrose, dextrose, maltose, glycerol and sorbitol). The results of the present study revealed (Figure 7) that the optimal carbon source for antibiotic production was dextrose with an inhibition zone of 18.33 mm for *Micrococcus luteus* and *Neisseria mucosa* followed by *Enterococcus faecalis* with 14.67 mm zone of inhibition.

Nitrogen source

Effect of nitrogen sources on production of metabolite was detected by fermentation media with selected *Streptomyces* sp. KOD10 at different carbon sources (without nitrogen source, malt extract, yeast extract, casein, beef extract and peptone). The results obtained (Figure 8) demonstrated that the optimal nitrogen source for antibiotic production was yeast extract with an inhibition zone of 14.67 mm for *Staphylococcus aureus* and 21.67 mm for *Neisseria mucosa*.

Incubation Period

Fermentation media with *Streptomyces* sp. KOD10 were incubated at 28^{0}C on rotary shaker for a period of 10 days. After 4 days, every 24 hours, the culture broth was analyzed for its antibacterial metabolite content. In the present study, the production of the metabolite by *Streptomyces* sp. KOD10 took place during the log phase of growth in the fermentation medium indicating that the metabolite production was directly proportional to the growth rate. It was observed (Figure 9) that the inhibition zone was increased with the incubation period in the production medium and maximum inhibition was achieved for cultures incubated for 7 days. However after 7 days of incubation there was a decline phase in the diameter of inhibition zone.

Sodium chloride concentration

Figure 10 showed the effect of sodium chloride concentration on antibacterial activity, when *Streptomyces* sp. KOD10 was grown in ISP2 media for 7 days. It was observed that the optimal sodium chloride concentration for the production of antibiotic was 2.5% (W/V) with an inhibition zone diameter of 9.33 mm against *Micrococcus luteus*. Sodium chloride concentration in the medium has an intense consequence on the production of antibiotic from microorganisms because of its effect on the osmotic pressure [14] [15].

Yeast extract concentration

The effect of nitrogen concentration on production of active compound by *Streptomyces* sp. KOD10, was studied by varying the concentration of yeast extract (0, 0.5, 1.0, 1.5 and 2%) in W/V. The results obtained (Figure 11) demonstrated that the optimal yeast extract concentration for the production was 1.5 to 2 %.

Dextrose concentration

To study the effect of carbon concentration on production of active compound by *Streptomyces* sp. KOD10, different concentration of dextrose (0, 0.5, 1.0, 1.5 and 2%) were used. The results obtained (Figure 12) demonstrated that the optimal dextrose concentration for the production was 1.5%

CONCLUSION

The antibiotic production was carried out using *Streptomyces* sp. KOD10 and it was found to inhibit the growth of *Neisseria mucosa* better than *Staphylococcus aureus*, *Enterococcus faecalis* and *Micrococcus luteus*. For the maximum production of antimetabolites the suitable factors were optimized experimentally i.e. medium (ISP2), temperature (25 to 28^{0}C), pH (10), incubation period (7), agitation (200rpm), carbon source (dextrose), nitrogen source (yeast extract), inoculam concentration (2%) and sodium chloride concentration (2.5%).

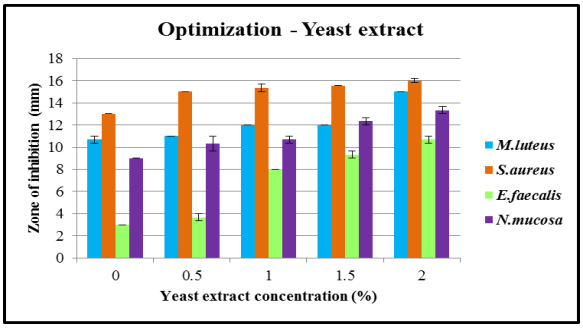

Fig. 11: Effect of different yeast extract concentration on the growth of *Streptomyces* sp. KOD10 for antibiotic production

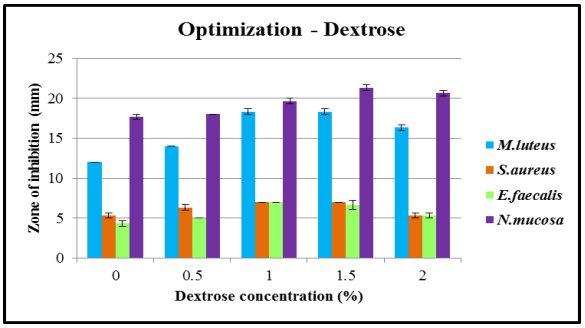

Fig. 12: Effect of different dextrose concentration on the growth of *Streptomyces* sp. KOD10 for antibiotic production

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