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Optimization of cultural parameters for production of Antimicrobial Metabolite from Streptomyces sp. FXJ1.449 isolated from Mangrove soil

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***Abstract*— Streptomyces sp. FXJ1.449 was isolated from marine sample collected from mangrove sea- coast, kosamba village, Valsad District, Gujarat and was screened to evaluate its antimicrobial activity by primary screening using cross streak method and by secondary screening using Agar well diffusion method against test bacteria Salmonella typhi (MCC 2901), Shigella spps. (MCC 3209), Staphylococcus aureus (MCC 2043) Bacillus cereus (MCC 2039) ans B. subtilis (MCC 2049). The present study is focused on the study of various physico-chemical parameters such as inoculums size, incubation period, pH, Temperature, Nacl concentration, carbon source, nitrogen sources and trace minerals for medium optimization to enhance production of antimicrobial metabolite by Streptomyces sp. FXJ1.449. This strain was found to be potential antibiotic producer and can be further used commercially for production of valuable products.**

Key Words: Mangrove sea-coast, Streptomyces sp. FXJ1.449, Antimicrobial activity, Optimization.

INTRODUCTION

Marine Actinomycetes are the most biotechnologically and economically valuable prokaryotes. Marine action - mycetes have an incomparable metabolic diversity and are the potential source of novel antimicrobial compounds as the environmental conditions of the sea are completely different from that of terrestrial conditions . Marine streptomyces species are a rich source of several useful bioactive natural products with potential applications. However, The ability of genus Streptomyces to form these bioactive compounds is not a fixed property but can be greatly increased or decreased under different conditions of nutrition and cultivation media. Improvement in the growth and antibiotic production can be carried out by manipulating the nutritional and physical parameters of the culturing conditions. Therefore to design an appropriate frermentation medium is of critical importance in the production of secondary metabolites. (Charu singh et.al.)The present work describes the optimization of various physical and chemical parameters such as inoculums size, incubation period pH,Temperature , Nacl concentration and different carbon sources , nitrogen sources and trace minerals through study of their effects on production of antimicrobial compound produced by an actinomycete, Streptomyces sp. FXJ1.449.

Isolation and screening of Actinomycetes from marine soil sample

Streptomyces species was isolated from Marine soil

sample collected from mangrove ecosystem, Kosamba village (20o37'38.1" N and 72o53'36.1"E) of Valsad District located at south Gujarat. Ten soil samples was collected and subjected to the physical and chemical treatment of heat at 70oC for 30 minutes and CaCO3 treatment respectively. This pretreated soil sample was used for isolation of Actinomycetes on Actinomycetes Isolation Agar (AIA) medium with Rifampicin 5µg/ml and Nystatin 25µg/ml dded to prevent bacterial and fungal growth respectively (Khattab et.al.). Primary screening (cross streak method) and Secondary screening (Agar well diffusion method) was used to evaluate antimicrobial activity of Streptomyces sp. FXJ1.449. against sensitive test organisms such as Salmonella typhi (MCC 2901), Shigella spps. (MCC 3209) , Staphylococcus aureus (MCC 2043) Bacillus cereus (MCC 2039) ans B. subtilis (MCC 2049). During secondary screening Actinomycetes Isolation Broth medium was used to cultivate Streptomyces sp. FXJ1.449 at 150 rpm at 28oC for 7 days.

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Optimization of Physico-Chemical parameters on production of antimicrobial metabolite byAgar well diffusion method:

Basal medium and inoculums preparation:
The Streptomyces sp. FXJ1.449. was grown on Actinomycetes Isolation Agar (AIA) medium for 5-6 days at 28oC . After incubation ,a colony of Streptomyces sp. FXJ1.449. was inoculated into the basal medium. This seed culture was then incubated at 280C on the orbital shaker at 120 rpm for 5-6 days. After Six days, the seed culture was withdrawn and centrifuged. The supernatant obtained was removed and pellets were washed twice with distilled water. This spore suspension was added to sterile 0.05% Tween 20 to form homogenous spore suspension of 0.2 O.D. Inoculum concentration of 2% of this suspension was used for study of optimization of various parameters. (Bunda sunita et.al.). The results of optimization was evaluatedfor its antibacterial activity anainst selected bacteria by Agar well diffusion method.

Determination of effect of Inoculum size:
In order to determine the effect of inoculum size on fermentation process, various concentration of potential actinomycetes was selected such as 1%,2%3%,4% and 5%.

Determination of effect of Incubation period:
To study the effect of incubation period on production of antimicrobial metabolite, a small amount of inoculated basal medium was aseptically withdrawn at every 24 hours for 10 days and centrifuged at 1500 rpm for 10 minutes. After centrifugation supernatant obtained was used for determining the effect of incubation period on production of antimicrobial agent.(S. Satapathy et. al.)

Determination of effect of pH :
To study the effect of pH on production of antimicrobial metabolite, a different range of pH such as pH 6.0, 7.0, 8.0,9.0 and 10.0 was selected.

Determination of effect of Temperature:
To study the effect of temperature on production of antimicrobial metabolite, different temperature range such as 280C, 370C, 450C and 550C was selected.

Determination of effect of Osmotic Pressure:
To study the effect of osmotic pressure on production of antimicrobial metabolite, different concentration of NaCl such as 1%, 2%, 3%, 4% and 5% was selected.

Effect of Carbon sources on production of antimicrobial compound:
To study the effect of different carbon sources on production of antimicrobial metabolites, carbon source in basal medium was replaced by with 1% w/v concentration of different carbon sources such as D-glucose, Lactose, Galactose, Maltose, Mannitol, Mannose, Xylose, Sucrose, Fructose, glycerol and Starch under study. 0.5% w/v Peptone was added as a nitrogen source.

Effect of Nitrogen sources on biosynthesis of antimicrobial compound:
To study the effect of Nitrogen sources on production of antimicrobial metabolites, nitrogen source in basal medium was replaced with 1% w/v concentration of different nitrogen sources such as Peptone, Tryptone, casein, L-aspargine, meat extract, beaf extract, malt extract, yeast extract, Ammonium sulphate, and sodium nitrate under study. D-glucose at 0.5% w/v concentration was added as a carbon source.

Effect of Trace minerals on biosynthesis of antimicrobial compound:
The effect of different trace minerals such as FeSo4, CuSo4, ZnSo4, Mnso4, Mncl2, Mgso4, Mgcl2, Kno3, Caco3, CaCl2, K2HPO4 and Na2HPO4 at 0.001 % w/v concentration was selected on production of antimicrobial metabolite.

II. RESULT AND DISCUSSION

Identification of Potential Actinomycetes isolates:
Based on the morphological, physiological, biochemical and cultural characteristics, the isolate was identified as an actinomycete. Further molecular characterization by 16s rRNA sequencing method confirmed it as an actinomycete, Streptomyces sp. FXJ1.449.

Optimization of physic-chemical parameters for Antimicrobial Metabolite Production from Streptomyces sp. FXJ1.449. :

Effect of Incubation Period and inoculum concentration:
Metabolism of actinomycetes under excess of nutrients avaibility, stimulates the growth of cell biomass; but under nutrition limiting conditions, the cell cycle gets shifted towards stationary phase and initiates the process of secondary metabolite production. In present study, the effect of incubation condition and inoculums size on antimicrobial metabolite production was shown in (Fig.1) and (Fig. 2) respectively. As shown in (Fig. 1) the rate of the production begins to initiate from the 4th day to 10th day with maximum was rate of the production reached at 8th day. The

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inoculums concentration of 5% showed maximum antimicrobial activity against selected five test bacteria as shown in (Fig. 2.)

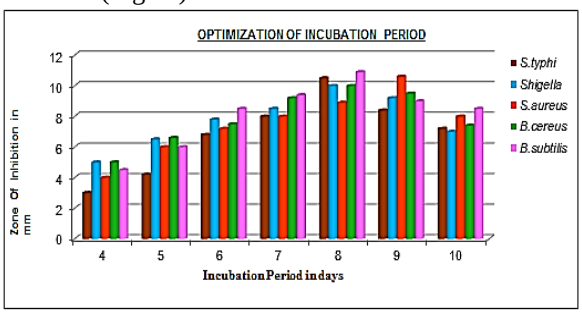

Fig. 1: Effect of incubation period on production of Antimicrobial metabolite.

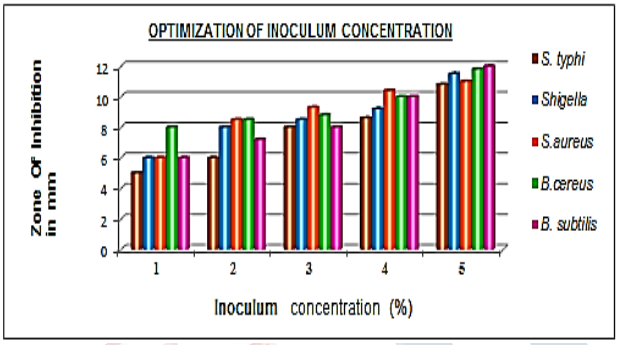

Fig. 2. : Effect of inoculum concentration on production of Antimicrobial metabolite.

Effect of pH and Temperature:
The effect of both pH and temperature on production of antimicrobial activity is shown in (Fig. 3.) and (Fig. 4.) respectively .The highest production was noticed at pH 9.0 and at temperature 28oC.

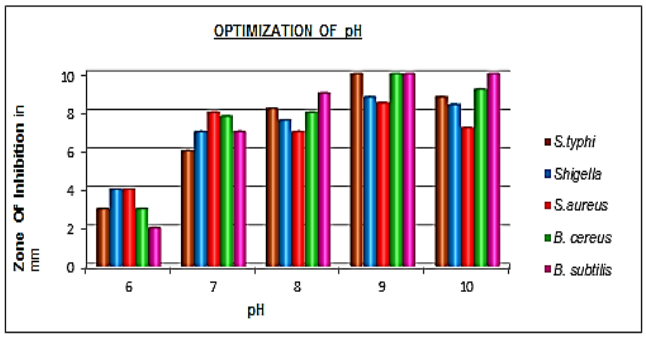

Fig. 3 : Effect of pH on production of Antimicrobial metabolite.

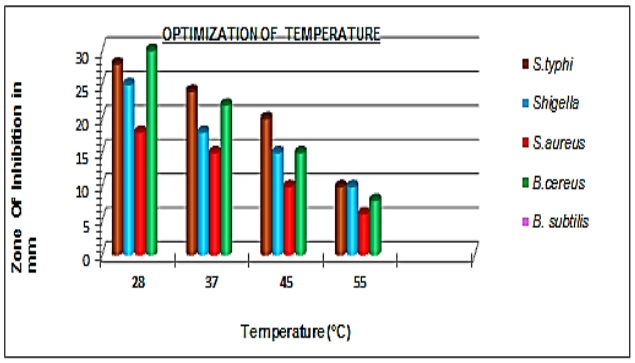

Fig.4: Effect of Temperature on production of Antimicrobial metabolite.

Effect of NaCl concentration (%) :
The effect of NaCl was shown in (Fig. 5). As the Actinomycete isolate under optimization study was isolated from marine source concentration of NaCl greatly influence the production of metabolite. The present study showed that maximum antimicrobial activity was obtained at 2% NaCl concentration.

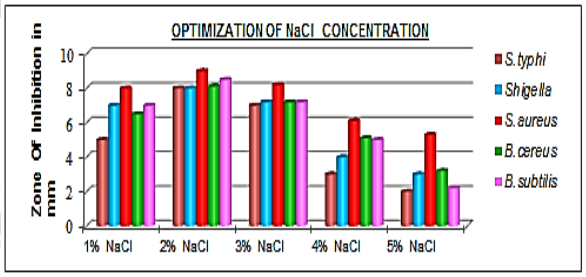

Fig. 5 : Effect of NaCl concentration on production of Antimicrobial metabolite.

Effect of different Carbon source on production of Antimicrobial metabolite.
Effect of several carbon sources selected for study undertaken was shown in (Fig.6). . As shown in (Fig.6) the maximum antimicrobial activity was observed with glucose followed by maltose,glycerol and starch as a carbon source. Maximum activity of antimicrobial metabolite was found when glucose was used as a carbon source.

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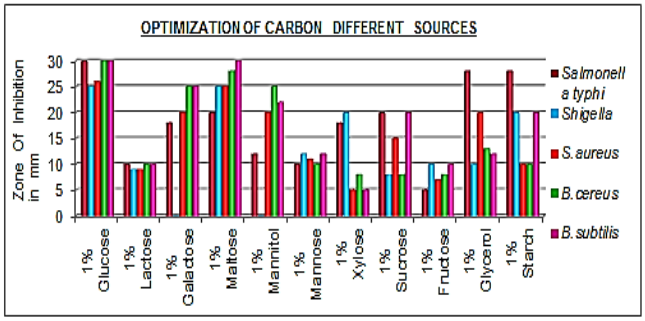

Fig.6: Effect of Carbon source on production of Antimicrobial metabolite.

Effect of Nitrogen source on production of Antimicrobial metabolite:

As shown in (Fig.7), maximum antimicrobial activity is observed with 1% casein as a nitrogen source so, casein was considered as a suitable nitrogen source for production of antimicrobial compound.

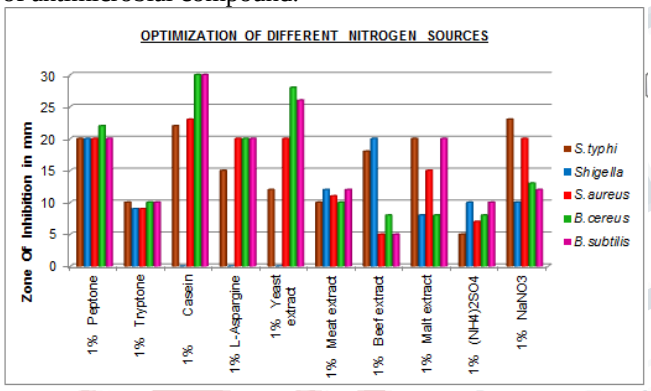

Fig. 7: Effect of Nitrogen source on production of Antimicrobial metabolite.

Effect of Trace minerals on production of antimicrobial metabolite :

The effect of different trace minerals tested on production of antimicrobial metabolite showed that Mgso4 and K2HPO4 are best at 0.01 % (w/v) concentration

III. CONCLUSION

From the present study it was concluded that maximum antimicrobial activity by Streptomyces sp. FXJ1.449 was observed at 5% inoculums concentration and when medium used contains 2% (w/v) D-glucose as a carbon source, 1% (w/v) casein as a nitrogen source, Nacl concentration of 2% , MgSO4 and K2HPO4 at 0.01% (w/v) concentration and pH 9.0 with incubation period of 8 days at 28oC. Streptomyces sp. FXJ1.449 was found to be potential antibiotic producer.

IV. ACKNOWLEDGEMENT

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