WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 5, 1338-1347. Research Article ISSN 2277– 7105

OPTIMIZATION OF ANTIMICROBIAL SUBSTANCE PRODUCTION BY *STREPTOMYCES* RW2-3 STRAIN ISOLATED FROM SEA WATER

Aruna V[1] and Rajan S[2*]

[1]Research Scholar, Department of Microbiology, R & D Center, Bharathiar University, Coimbatore.

[2]Research Department of Microbiology, M. R. Government Arts College, Mannargudi – 614 001, Thiruvarur District, Tamilnadu, India.

Article Received on
26 Feb 2015,

Revised on 17 March 2015,
Accepted on 09 April 2015

***Correspondence for Author**
Rajan S
Research Department of Microbiology, M. R. Government Arts College, Mannargudi – 614 001, Thiruvarur District, Tamilnadu, India.

ABSTRACT

Aim: To optimize the process parameters for enhanced production of bioactive metabolites by *Streptomyces* RW2-3. **Materials:** Agar well diffusion assay was employed to study the effect of environmental parameters such as incubation period, pH, temperature and influence of various nutrients such as carbon and nitrogen source and minerals on the bioactive metabolites production by *Streptomyces* RW2-3. **Results:** Optimized and better antibacterial metabolite production was noted when the test actinobacteria was inoculated on M14 medium. The other components like maltose, beef extract and incoculum concentration was needed at 2% concentration. pH of the medium is adjusted with pH 7 and incubated for 7 days at 27°C. As the strain exhibited potent antimicrobial activity it may be explored for biotechnological purposes. **Conclusion:** Result of the present study concluded that *Streptomyces* RW2-3 produced higher quantum of antimicrobial substances.

KEYWORDS: Bioactive metabolite, Streptomyces, Optimization, antimicrobial substance

INTRODUCTION

Marine actinobacteria are the greatest economical and biotechnologically valuable class of prokaryotes producing excellent bioactive secondary metabolites especially antibiotics. Marine actinobacteria are a potential source of novel compounds as the environmental conditions of the sea are entirely different from the terrestrial conditions (Blunt and Prinsep, 2006). Marine actinobacteria have been widely recognized as a potential source of new drug

candidates. They can produce structurally unique metabolites that are not found in their terrestrial counter parts due to their extreme living conditions within the marine environment (Gunasekaran and Thangavel, 2013). Some novel active compounds from marine actinobacteria are reported at high frequency (Usha and Masilamani, 2013). 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 higher quantities of all known antibiotics of microbial origin. Indeed *Streptomyces* sp produces about 75% of commercially and medically useful antibiotics (Hou *et al.*, 2006). The actinobacteria are well adapted to marine environment and able to breakdown complex biological polymers (Baskaran *et al.*, 2011). Enhancement in the growth of actinobacteria is carried out by manipulating the nutritional chemical and physical parameters of the culturing conditions (Gopi *et al.*, 2011). In optimization, media composition plays a remarkable role in the productivity and economics of the crucial process. In the present study, *Streptomyces* RW2-3 strain was screened for its antagonistic activity with reference to the medium composition, and other factors which were optimized for high antibiotic production was tested by agar well diffusion method and the inhibition zones were measured.

MATERIALS AND METHODS

Isolation of antimicrobial substance producing Actinobacteria (Kachhawa *et al.*, 2012) Marine actinobacteria was isolated from Puducherry part of Bay of Bengal. Isolation and enumeration of actinobacteria were done by the serial dilution and pour plate technique in starch casein agar supplemented with the antibiotics cycloheximide (25mg/ml) and nalidixic acid (25mg/ml) to avoid fungal contamination. The isolated Actinobacteria are considered as *Streptomyces* RW2-3 strain.

Screening of Actinobacteria for its antibiotic production (Kachhawa *et al.*, 2012)

Streptomyces RW2-3 strain was grown in yeast extract malt extract broth and incubated at 30^0C in shaker at 200 rpm for 7 days. After incubation, the broth was centrifuged at 5000rpm for 10 minutes with equal volume of ethyl acetate (1:1) in a separation funnel to extract the compounds and the antibacterial study was carried out by agar well diffusion method. 100µl of the supernatant were loaded in the well using micropipette. The zone of inhibition was measured as a total diameter was subtracted from the total diameter.

Test organism used in the study

E. coli isolated from patients infected by UTI used as test organism. The culture were revived in nutrient broth and stored in agar slants for further study.

Optimization of production medium (Kumar and kannabiran, 2010)

Streptomyces RW2-3 strain was selected to study optimized antimicrobial substance production based on the primary screening. Six different media were selected for optimization study. They are nutrient broth, starch casein broth, tryptone yeast extract broth (ISP1), yeast extract malt extract broth (ISP2), M13 and M14. The entire medium were prepared in 250ml conical flasks and sterilized at 121ºC for 15 minutes. Five ml of seed culture was inoculated on 100ml fermentation media and incubated at 28±2ºC on a rotary shaker for 5 days at 150rpm and antibiotic production was assessed. Based on the antibiotic assay, one medium was selected for further optimization study.

Optimization of Temperature, pH and incubation period (Kumar and kannabiran, 2010)

The effect of culture growth and antimicrobial substance production under varying condition such as temperature, pH and different incubation period. Optimum temperature was studied by varying the incubation temperatures at 17^0C, 27^0C, 37^0C, 47^0C and 57^0C. Optimum P^H was studied by varying the incubation P^H at 6, 7, 8, 9, 10. The incubation period varied from 5, 6, 7, 8, 9 and 10 days. *Streptomyces* RW2-3 was inoculated in the optimized media and kept in incubatory shaker at 150 rpm.

Effect of Carbon source (Kumar and kannabiran, 2010)

Various carbon source used in the medium were glucose, fructose, lactose, maltose, mannitol. They were used at 1% concentration (v/v). A flask without any carbon source was kept as control. The fermentation medium with respective carbon source were incubated at optimized condition for optimized days.

Effect of Nitrogen source (Kumar and kannabiran, 2010)

Nitrogen source (1% v/v) used in the medium were Ammonium sulphate, yeast extract, malt extract, beef extract and peptone. A flask without any nitrogen source was kept as a control. The fermentation medium with respective nitrogen source was incubated at optimized condition for optimized days.

RESULTS AND DISCUSSION

Effective antimicrobial substance production was attained at specific environment and cultural condition, which was assessed in the optimization study. *Streptomyces* RW2-3 strain was isolated from the marine water of Pondicherry costal area and it was stored in ISP2 medium for further analysis. The screening results showed that *E. coli* bacterial strains was sensitive to the antimetabolites of *Streptomyces* RW2-3. It produced highest zone of inhibition of 15 mm against *E.coli*51 followed by 14mm for *E.coli*44. The results of the screening results showed that the four other *E.coli* strains were also showed antimicrobial activity but it is comparatively low and only two strains were selected for further assay (Table 1). Marine actinomycetes were more potent than others (Selvakumar, 2010).

Table-1: Screening of *Streptomyces* RW2-3for its antagonistic activity.

S. No	Bacterial pathogens	Zone of inhibition in (mm)
1	*E. coli* - E-51	15
2	*E. coli* - E-44	15
3	*E. coli* - E-71	12
4	*E. coli* - E-52	11
5	*E. coli* - E- 53	12

Streptomyces RW2-3 strain produced antimicrobial substance but effective production needs optimized condition. Six different media were used for better antimicrobial substance production medium. Among the medium tested, M14 medium showed better antimicrobial substance production with maximum activity against *E. coli* (Table 2).

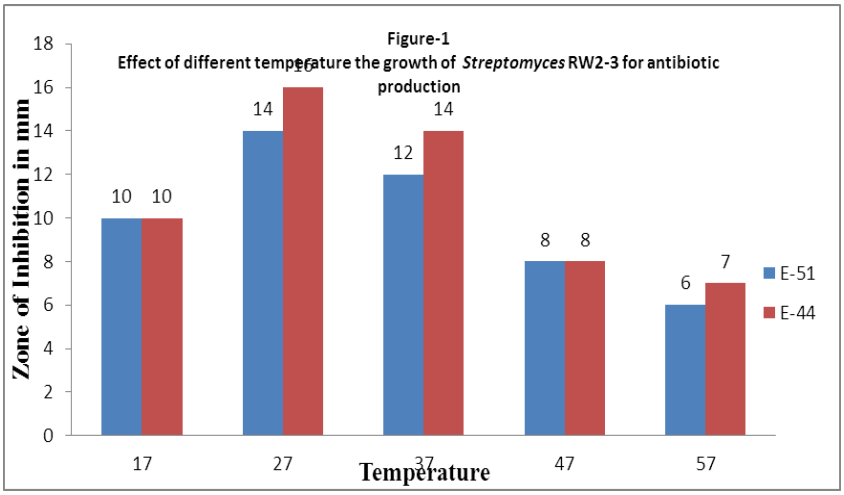
Vijayakumar *et al.*, (2012) reported that Starch casein medium was suitable for effective antimicrobial substance production by *Streptomyces afghaniensis*, whereas in the present study M14 medium is the best. Production may vary depends on the type of strain used for production. The ability of microbes to form antibiotics is not a fixed property but can be greatly increased or completely lost under different conditions of nutrients and or cultivation. M14 was confirmed to be the effective culture media for maximum antibiotic production by showing 14mm and 13mm zone of inhibition for *E.coli*44 and E. coli 51.

Variation in ingredient may influence on growth as well as secondary metabolite production (Sharon *et al.*, 2014).

Table-2: Effect of medium on the metabolite production by *Streptomyces* RW2-3 strain.

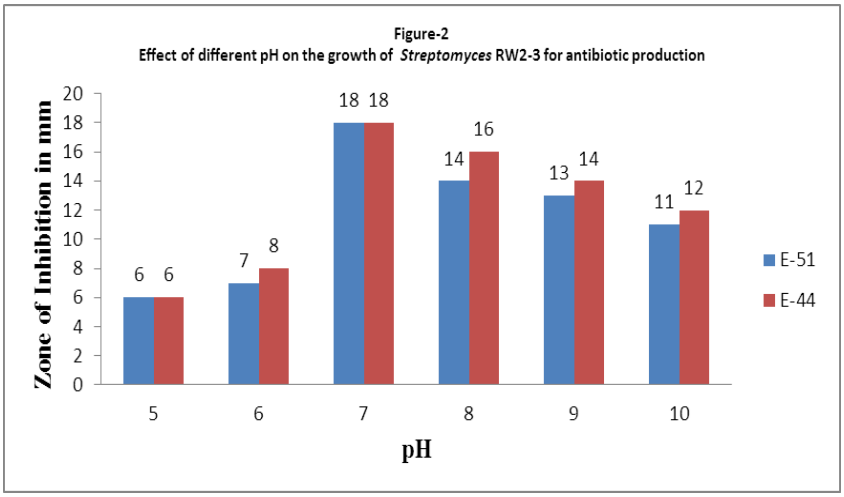
S. No	Medium	Zone of inhibition in (mm)	
		E. coli 44	*E. coli* 51
1	Nutrient broth	9	9
2	Starch casein broth	12	11
3	Tryptone yeast extract broth (ISP1)	11	11
4	Yeast extract malt extract broth (ISP2)	10	12
5	M13	12	12
6	M14	14	13

Temperature played important role in the metabolite production and antimicrobial activity.M14 medium with *Streptomyces* RW2-3 was incubated at different temperature (25°C, 27°C, 32°C, 37°C and 40°C). It was found that 27°C was the optimum temperature for metabolite production (Figure-1). Deviation from optimum temperature affects the efficiency of metabolite. The metabolite obtained at 27°C produced 16 and 14 mm zone of inhibition respectively for *E.coli*44 and *E.coli*51. Our result is in accordance with Singh and Rai (2012). Usha *et al.*, 2014; Saha *et al.*, 2010 reported different results, they reported that *Streptomyces* tritolerans produced higher concentration of antimicrobaial compounds at 35°C. Different incubation temperature optimization was reported by different authors using different strains (Atta *et al.*, 2011; Rajendran *et al.*, 2014; Hanane and Mostefa, 2012).

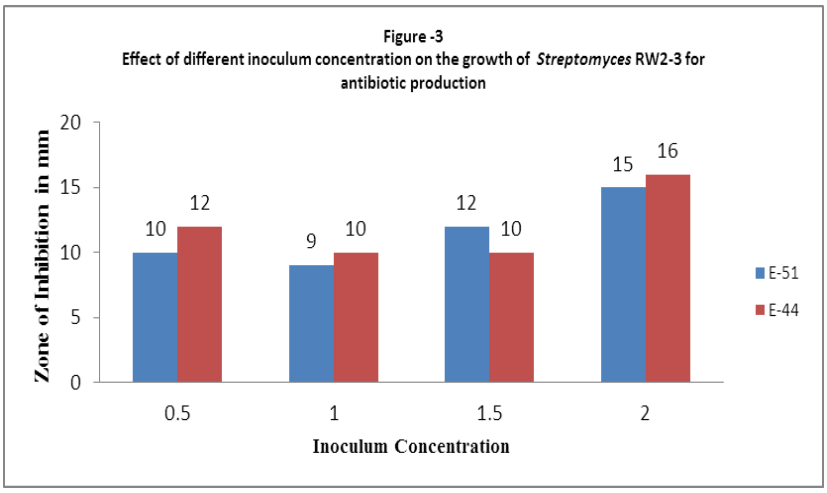


The pH influenced the production of metabolite by *Streptomyces* RW2-3. The initial pH of the M14 media was adjusted to (5, 6, 7, 8, 9, 10) separately and studied pH optimization. *Streptomyces* RW2-3 showed (Figure-2) maximum activity at pH 7. It is also reported that acidic pH inhibited the antibiotic production rather than alkaline pH. The activity of metabolite increased with the increase pH upto pH9 and then dropped down at pH10. pH 7

was confirmed to be the effective pH for maximum antibiotic production by showing 18mm each zone of inhibition for *E.coli*44 and *E.coli*51. Singh and Rai (2012); Atta *et al.*, (2011); Rajendran *et al.*, (2014); Hanane and Mostefa, (2012) indicated similar kind of result.

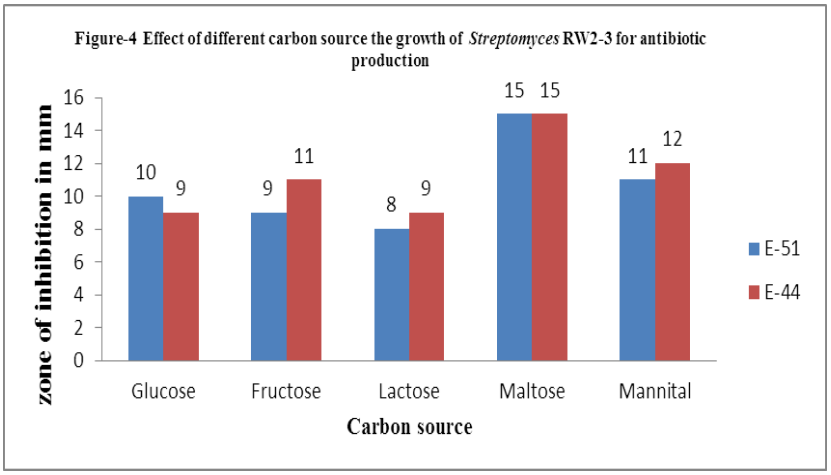


Effect of inoculum concentration on production of metabolite was detected by fermentation media with pH7 and different inoculam concentration (0.5, 1.0, 1.5 and 2.0%). Seed culture was inoculated and incubated at 27°C. The results obtained (Figure-3) demonstrated that the optimal inoculum concentration for better secondary metabolite production was 2% & produced with zone of inhibition at 19mm and 17mm each for *E.coli*44 and *E.coli*51. Inoculum concentration showed direct effect on the growth and metabolite production.

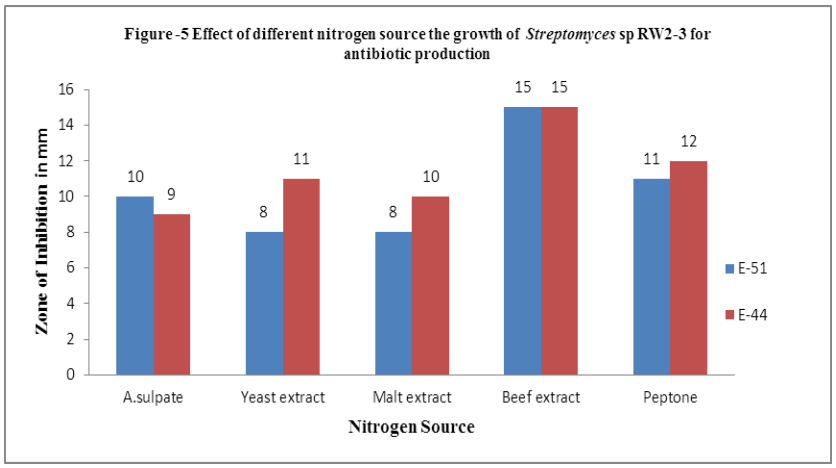


The results indicated that the optimal carbon source for antibiotic production was maltose with 15mm each zone of inhibition for *E.coli*44 and *E.coli*51 (Figure 4). In the present study, maltose is considered as an effective carbon source for better antimetabolite production whereas Singh and Rai (2012) established that glycerol is best for antimicrobial substance

production. Marwick *et al.*, (1999) reported that highest antimicrobial activity of *S. sannanensis* was high when 1% glucose is used as a carbon source. **Ripa *et al.*, 2009** indicated that glucose as a best carbon source. Demain and Fang (1995) described that maltose interferes with the production of secondary metabolites. Similarly recent report from India showed that rice bran influences better antimicrobial substance production (**Lakh, 2014**). Atta *et al.*, (2011) also described the importance of glucose in antimicrobial substance production.



Nitrogen content of the medium influences the production of metabolite. The results of the present study revealed (Figure-5) that the optimal nitrogen source for antibiotic production was beef extract with zone of inhibition at 15mm for *E.coli*44 and *E.coli*51 respectively. The source of nitrogen plays a vital role in secondary metabolite production because nitrogen is a key element found in secondary metabolites (Singh and Rai, 2012). They also reported that soya bean meal directly influences the antimicrobial substance production.



Fermentation media with *Streptomyces* RW2-3 was incubated at 27°C on rotatary shaker for a period of 10 days. After 4 days every 24 hours, the culture broth was analyzed for its

antibacterial metabolite production. It was observed (Figure-6) 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. Rajendran *et al.*, (2014) reported that incubation of 5 days was optimum for better antibacterial metaboilite production. Different report was indicated in this study, which may due to the strain variation.

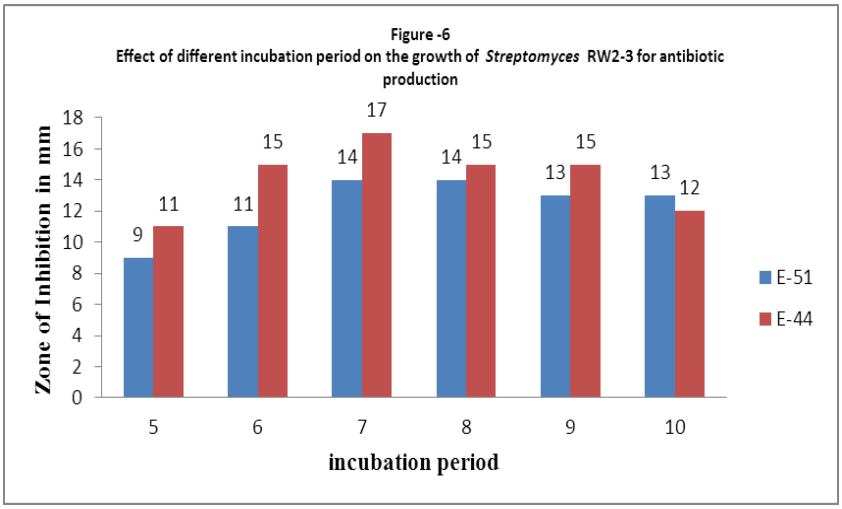


Figure -6
Effect of different incubation period on the growth of *Streptomyces* RW2-3 for antibiotic production

CONCLUSION

The antibacterial metabolite production was carried out using *Streptomyces* RW2-3 strain isolated from marine water and it was found to inhibit the growth of *E.coli*44 and *E.coli*51. Optimized and better antibacterial metabolite production was noted when the test actinobacteria was inoculated on M14 medium. The other components like maltose, beef extract and incoculum concentration was needed at 2% concentration. pH of the medium is adjusted with pH 7 and incubated for 7 days at 27°C.

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