Saleem TSM et al /J. Pharm. Sci. & Res. Vol.1(2), 2009, 57-60

Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

MICROBIAL PRODUCTION AND OSMAC STUDY OF *STREPTOMYCES* SPECIES CODED AS G-16

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Abstract
The previously isolated *Streptomyces* species coded as G16 was used in the present study. The test media selected were starch casein broth (SCB), GMYP broth and Soya been meal broth (SOB). The seed inoculums were transferred in conical flask were kept on rotary shaker at room temperature (175 rpm) for 5 days. The test was carried out in triplicate. The antibiotic was produced by shake flask fermentation. Starch casein and GMYP broth showed more production at the end of 2 days of fermentation. Compared to starch casein and GMYP broth the production of antibiotic was more using Soya been meal broth. The zone of inhibition was found in all medium by cup plate method, the zone of inhibition were remained same even at the end five days of fermentation in soya bean meal broth. The culture filtrate was extracted with ethyl acetate and tested for activity by bioautography. However, the bioautography showed zone of inhibition equivalent to Rf of the extract from SCB as standard and the spots were close to each other. In addition, the TLC profile of the extract collected from GMYP and SOB medium shows three spots when compared with SCB. From the results, it has been concluded that the OSMAC approach definitely changed the number of components produced in one media to the other. Any way, further confirmation has to be done by individually collecting each component and analyze their chemical structure. The study can be continued in this aspect near future, which may be beyond the scope at present.

Key words
Stereptomyces species, Antibiotic, Fermentation, OSMAC Study

Introduction

Microorganisms play an important role in producing various bioactive substances. The diversity of the pathways that exist may be exploited to get these bioactive substances. Depending on the phase in which these products are produced, they are termed as either primary or secondary metabolites. They are usually formed as a mixture of closely related members of a chemical family [1]. Among the amazing variety of bioactive substances produced by microbes, antibiotics – the "wonder drugs" which are useful in treating various infectious diseases play prominent role in day-to-day life. The volume sales of clinically useful antibiotics emphasize this fact [2, 3]. Among the *Actinomycetes*, *Streptomycetes* are very potent producers of antibiotics. According to in depth analysis by Berdy (1995) around 11,900 antibiotics had been discovered by 1994 of which around 6600 (55%) were produced by *Streptomyces* whereas filamentous fungi produced 2600 (22%), bacteria produced 1400 (12%) and non-*Streptomycete* strains of *Actinomycete* produced 1300 (11%) [1-4]. As per OSMAC approach [5] a systematic alteration of easily accessible cultivation parameters like media composition, aeration, culture vessel and addition of enzyme inhibitors would increase the number of secondary metabolites available from one microbial source. Because of this, it is possible to get more number of compounds with different structures. It is in this backdrop, the present work was initiated. The present investigation made to investigate the time course of antibiotic production in different media, and to analyze whether OSMAC approach is applicable by changing the media employed for shake flask fermentation of previously

isolated *Streptomyces* species coded as G-16 [6].

Materials *& Methods*

The spore suspension of *Bacillus subtilis* (MTCC 619) was prepared by using fresh nutrient agar slant. For stock solution of Streptomycin sulphate 100 mg of sample dissolved in 100 ml of sterile distilled water. To prepare secondary stock solution of 200-units/ml, 25.6 ml of primary stock solution was made to 100 ml with sterile distilled water in a sterile volumetric flask. Secondary stock of 1 to 2 ml in an increment of 0.25 ml was taken and made to 10 ml with sterile distilled water in a series of sterile test tubes to obtain standard solutions of 20, 25, 30, 35 & 40 units/ml labeled as S1, S2, S3, S4 & S5. These standard solutions were subjected to microbiological assay against overnight culture of *Bacillus subtilis.*

The test media selected were starch casein broth (SCB), GMYP broth & soya bean meal broth (SBM). Seed inoculum was transferred at 10% level in to 10 ml of medium in 250 ml conical flask. The seeded flasks were kept on GFL gyratory flask at room temperature (175 rpm) for 5 days.

The test was carried out in triplicate. Samples of 1 ml were withdrawn in sterile tubes at the interval of 24 h for 5 days. Samples were centrifuged at 4000 rpm for 20 minutes and supernatents were subjected to microbiological assay against overnight culture of *Bacillus subtilis.* Based on average zone of inhibition, the time of maximum production of antibiotic in each medium was recorded. The flask showing the presence of antibiotic principle were pooled together individually for each type of media used. This is filtered and extracted by using ethyl acetate and evaporated to dryness.

The collected antibiotic was subjected to TLC by using suitable mobile phase Chloroform: Ethyl acetate: Methanol (70: 27.5: 2.5). The spots in the developed silicagel coated TLC F_{254} plates (E.Merck India Ltd., Mumbai) were detectable under UV illumination, by spraying with Iodine or Methanolic solution of Vanillin Sulphuric acid. The air dried TLC plates were subjected to bioautography technique to find out the inhibitory action of crude extracts.

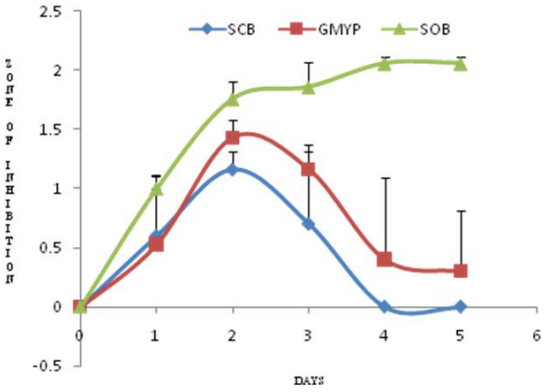
Results

All the selected media showed (Table 1) the antibiotic production at the end of 1st day of fermentation itself. The production of antibiotic by G16 was expressed in terms of Streptomycin sulfate (30 Units/ml). GMYP & SCB showed more production at the end of second days of fermentation. The zones of inhibition were remained same even at the end of 5th days of fermentation in SOB broth and compare to that of other two medium (Graph 1, Fig 1). Through the TLC & Bio-autography (Fig 2) showed zone of

Table 1. Zones of inhibition shown by shake flask samples of G-16 using different media

Name of the medium	Sampling time intervals	Zone of inhibition (cm)	Average Zone of Inhibition Mean ± SD
Starch Casein Broth	0 h	0, 0, 0	0 ± 0
	24 h	0.9, 0, 0.9	0.6 ± 0.52
	48 h	1.2, 1, 1.3	1.16 ± 0.15
	72 h	1.1, 0, 1	0.7 ± 0.61
	96 h	0, 0, 0	0 ± 0
	120 h	0, 0, 0	0 ± 0
GMYP Broth	0 h	0, 0, 0	0 ± 0
	24 h	0, 0.9, 0.7	0.53 ± 0.47
	48 h	1.4, 1.6, 1.3	1.43 ± 0.15
	72 h	1.1, 1.4, 1	1.16 ± 0.21
	96 h	0, 1.2, 0	0.4 ± 0.69
	120 h	0, 0.9, 0	0.3 ± 0.52
Soya Bean Meal Broth	0 h	0, 0, 0	0 ± 0
	24 h	1, 0.9, 1.1	1 ± 0.1
	48 h	1.8, 1.6, 1.9	1.76 ± 0.15
	72 h	1.8, 1.7, 2.1	1.86 ± 0.21
	96 h	2, 2.1, 2.1	2.06 ± 0.06
	120 h	2, 2.1, 2.1	2.06 ± 0.06

Graph 1. Zone of inhibition of antibiotic produced by different media from G16

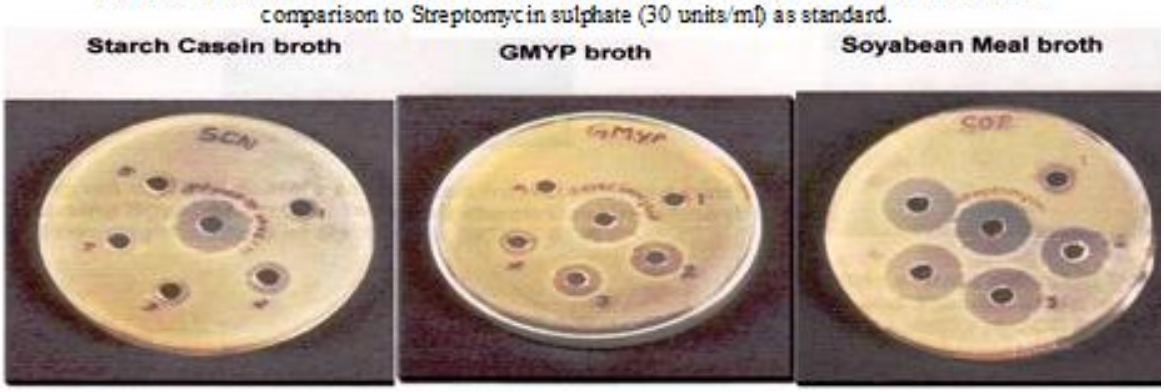


inhibition equivalent to Rf (0.567) of the extract from SCH and as the spots were close to each other.

fermentation. None of the flask of SCB showed activity at the end of 4th and 5th day of fermentation. This may be due to the autolytic enzymes, which would have cleaved the antibiotic produced.

Based on TLC profile, the spot observed in UV illumination at R_f = 0.2 was responsible for the zone of inhibition collected from the fermented media of SCB [6]. The TLC profile of the extract collected from GMYP and SBM were different from SCB at R_f = 0.567. The developed spots were much closed to each other. Though the bioautography showed inhibition equivalent to extract from SCB and as the spots were close to each other.

Fig 1. Antibacterial activity of G-16 fermented broth against over night culture of *B. subtilis* on comparison to Streptomycin sulphate (30 units/ml) as standard.



Discussion

All the selected media showed the antibiotic production at the end of 1st day of fermentation itself. GMYP and SCB showed more production at the end of 2 days of fermentation. Compared to GMYP and SCB, the production of antibiotic was more using SBM broth. The zone of inhibition was found to be more after 4 days of fermentation in seeded SBM broth. It remained same even at the end of 5 days of

Fig 2. Bio-Autography

Conclusion

From the results and discussion it has been concluded that OSMAC approach has definitely changed the number of components produced in one medium to other medium. Any way, further confirmation has to be done by individually collecting each component and analyze their chemical structure. The study can be continued in this aspect near future, which may be beyond the scope at present.

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