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Effect of critical medium components on antimicrobial compound production from marine *Streptomyces species* (A2) by one factor at a time method

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ABSTRACT

The present study reports effect of critical medium components on antimicrobial compound production from Streptomyces species (A2) isolated from Andaman Coastal area. Antimicrobial activity of strain A2 was confirmed by cross streak method. Culture filtrate of strain A2 showed good activity against gram positive and gram negative bacteria, after 72 hours of fermentation. The ethyl acetate extract of cell free culture filtrate showed 15mm, 16 mm and 13 mm inhibition against S. aureus, B. subtilis and E. coli, respectively. Effect of medium components such as carbon source, nitrogen source, minerals and pH on antimicrobial compound production was tested by classical one factor at a time method using Staphylococcus aureus, Bacillus subtilis and Escherichia coli as test organisms. Of the various variables tested, glucose, glycerol, peptone, soybean meal, sodium chloride, magnesium chloride, manganese chloride, calcium carbonate, and pH 6 and 7 showed significant effect. Further statistical based optimization is needed to prove the effect of interaction of the above variables on antimicrobial compound production from Streptomyces sp A2.

Key word: Actinomycetes, antimicrobial activity, classical one factor at a time.

INTRODUCTION

Nowadays, antibiotic resistant pathogens pose an enormous threat in the treatment of infectious diseases. Appearance of antibiotic resistance in bacteria causes reduction in effectiveness of drug in curing disease or improving a patient's symptoms. Since spread of antibiotic resistance in

microorganism is very fast and exponential emergence of antibiotic resistance, a periodic replacement of the existing broad spectrum antibiotic is necessary [1]. Development of novel drugs having broad spectrum mode of action against pathogens, is the need of the hour. Actinomycetes are aerobic gram positive filamentous bacteria which form asexual spores and mycelia with high guanine + cytosine (G+C) containing DNA. Actinomycetes are the most economically and biotechnologically valuable prokaryote with the unparalleled ability to produce bioactive secondary metabolites. They are the responsible for the production of half of the discovered bioactive secondary metabolite, notably antibiotics [2]. Recently, the rate of discovery of new compounds from terrestrial actinomycetes has decreased, whereas the rate of re isolation of known compound has increased. As marine environmental conditions are extremely different from terrestrial ones, it is surmised marine derived actinomycetes are newly added source for bioactive compounds in particular antibiotics. Streptomycetes are the most common actinomycetes genera exploited from marine sources in terms of biodiversity and bioproducts [3]. Effect of culture conditions on antibiotic production from terrestrial *Streptomyces* is well documented. But report on optimization of antimicrobial compounds production from marine *Streptomyces* is relatively few only. The present study reports classical method based optimization of antimicrobial compound production from one *Streptomyces species* (A2) isolated from Andaman marine sediments.

EXPERIMENTAL SECTION

Description of actinomycetes

Actinomycetes strain *Streptomyces* species (A2) used in this study was previously isolated from Andaman marine sediments using Starch casein agar medium and maintained as slant culture using yeast extract malt extract (YEME) agar (yeast extract 0.4%; malt extract 1.0%; glucose 0.4% and pH 7.0) prepared in 50% seawater. Strain A2 showed good activity against gram positive and gram negative bacteria in preliminary screening by cross streak method.

Antimicrobial compound production and activity testing

Production of antimicrobial compound from *Streptomyces species* (A2) was carriedout by adopting submerged fermentation. Components of production medium include glucose 1.0%; peptone 1%; NaCl 0.1% and pH 7.0. About 10 % inoculum prepared in YEME broth was transferred in to 100 ml production medium in 500 ml Erlenmeyer flask and kept in rotary shaker at 28^0C with 120 rpm. Five ml of culture broth was collected for every 24 hours and separated by centrifugation at 10000 rpm for 10 minutes. Culture supernatant was tested for antimicrobial activity against *S. aureus, B. subtilis* and *E. coli* by agar well diffusion method. Further crude bioactive compound from cell free supernatant was extracted through liquid-liquid extraction method using ethyl acetate and concentrated by evaporation. Antimicrobial activity of crude extract was tested by disc diffusion method at 0.25mg/disc concentration.

Effect of medium components on antimicrobial compound production

Basal medium was prepared and supplemented with different variables in respective concentration (table 2). About 10% of bacterial inoculum was transferred in to all the media and incubated in rotary shaker at 28^0C with 120 rpm for 120 hours. After fermentation, culture medium was separated by centrifugation at 10000 rpm for 10 minutes. The culture supernatant

was tested for antimicrobial activity by agar well diffusion method. Dry weight of *Streptomyces* biomass was calculated [4].

RESULTS AND DISCUSSION

Out of 22,500 microbial bioactive metabolites, 10,100 metabolites are produced by Actinomycetes [5]. *Streptomyces* are especially prolific, producing around 80% of total antibiotic products [6]. Present study focused on antimicrobial substance and production from *Streptomyces species* (A2) isolated from Andaman Coastal area. The strain A2 showed good inhibition against *S. aureus*, *B. subtilis* and *E. coli* in cross streak method and good growth was observed on production medium. Culture supernatant obtained after 72 hours of fermentation showed activity and maximum activity was observed after 120 hours of fermentation (table 1). The ethyl acetate extract of cell free culture filtrate showed 15mm, 16 mm and 13 mm inhibition against *S. aureus, B. subtilis* and *E. coli*, respectively.

Many microorganisms have been evaluated for the production of antimicrobial substance. However the high cost and low yields have been the main problem for its industrial production [4]. Therefore there is a great need to optimize with different substrates that provides maximum production of antimicrobial substance. It is well known that 30-40% of the production cost of antibiotic is taken up by the cost of growth medium [7]. Genus *Streptomyces* is a heterotrophic feeder that can utilize both simple and complex molecules as nutrient and energy sources and hence the effect of critical medium components on antimicrobial compound production from strain A2 was performed in the present study (table 2).

To screen for a suitable substrates and environmental conditions the strain A2 was incubated in basal medium containing different carbon, nitrogen, mineral sources, and in various pH. Of the five different carbon sources tested, glucose and glycerol were found to influence the production of antimicrobial compound. In contrast to our result Islam *et al.,* 2009 [8] reported maltose and sucrose resulted in high antibiotic production. When compared to inorganic nitrogen sources, organic nitrogen sources such as Peptone and soybean meal influenced antimicrobial compound production. Similar to our study Vastrad *et al.,* 2011 [4] reported the organic nitrogen source led to further increase in antibiotic reproduction than inorganic nitrogen source from *Streptomyces fradiae*. Minerals present in the fermentation medium playing critical role in the metabolic processes of all microorganisms. In the present study, among the different salts tested, except potassium chloride all other salts showed effect on antimicrobial compound production. Islam *et al.,* 2009 [8] reveled the maximum production was at pH 6.5 similarly in our study pH 6 and 7 influenced antimicrobial compound production.

Table 1. Antimicrobial activity of culture supernatant of strain A2

Test organisms	Antimicrobial activity (zone of inhibition measured in millimeter in diameter)				
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**	**120 hrs**
S. aureus	-	-	10	12	16
B. subtilis	-	-	8	9	10
E. coli	-	-	7	9	12

Table 2. Effect of critical medium components on antimicrobial compounds production by strain A2

Factors and variables	**Antimicrobial activity***		
Carbon source	***S. aureus***	***B. subtilis***	***E. coli***
Glucose	9	12	10
Glycerol	12	12	17
Sucrose	-	-	-
Starch	-	-	-
Wheat bran	-	-	-
Nitrogen source			
Yeast extract	-	-	-
Peptone	10	12	12
Soybean meal	11	11	12
Sodium nitrate	-	-	-
Casein	-	10	-
Minerals			
Sodium chloride	-	12	12
Magnesium chloride	-	10	11
Manganese chloride	12	12	13
Potassium chloride	-	-	-
Calcium carbonate	10	11	12
pH			
5	-	-	-
6	-	10	10
7	8	12	13
8	-	-	-
9	-	-	-

**zone of inhibition expressed in millimeter in diameter*

In conclusion, the strain A2 isolated from Andaman coastal area was found to produce potential antimicrobial agent and the optimal media, nutrients and culture conditions promoted the effectiveness of the antimicrobial agent.

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