

Tropical Journal of Pharmaceutical Research August 2010; 9 (4): 373-377

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## Research Article

# Production of Antibiotics from Soil-Isolated Actinomycetes and Evaluation of their Antimicrobial Activities

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## Abstract

**Purpose:** To investigate the production of antibiotic from actinomycetes isolated from soil and evaluate its antimicrobial activities.

**Methods:** In a medium formulation study, A-4 and A-4 actinomycete mutant strains (out of the six strains selected from the nine actinomycetes that were screened) were evaluated for maximum antibiotic production using various carbon and nitrogen sources. Zone of inhibition and packed cell volume were the parameters used for the evaluation. Various fermentation conditions such as pH, temperature and dissolved oxygen were also optimized for maximal production of antibiotic from both A-4 and A-4 mutant.

**Results:** Some actinomycetes strains showed promising antimicrobial activity against different strains of bacteria and fungi. Out of the six strains selected, one strain, designated A-4, showed maximum antimicrobial property against Gram positive and Gram negative strains as well as against various fungi.

**Conclusion:** Findings from this investigation reveal that strain A-4 and A-4 mutant strains, in that order, exhibited superior antimicrobial activities to other soil isolates of actinomycetes.

**Keywords:** Actinomycete, Antibiotic, MIC, fermentation.

Received: 30 November 2009

Revised accepted: 14 July 2010

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## INTRODUCTION

The continuing success of a biotechnologist in the search for microbial metabolites for use as antimicrobial compounds (antibiotics) is critical to combating human, animal and plant diseases. Microorganisms constitute an inexhaustible reservoir of compounds with pharmacological, physiological, medical or agricultural applications [1-3]. Antibiotics continue to play a crucial role in the development of tissue culture techniques and basic screenings, primarily in biochemistry, molecular biology, microbiology and genetics (including genetic engineering) and to a lesser extent, pharmacology and organic chemistry.

The actinomycetes are Gram positive, organisms that tend to grow slowly as branching filaments. Many actinomycetes will grow on the common bacteriological media used in the laboratory, such as nutrient agar, trypticase soy agar, blood agar, and even brain-heart infusion agar. Actinomycetes encompass a wide range of bacteria. They have universal occurrence and play an active part in the cycle of nature [4,5]. Antibiotics are the best known products of actinomycete. The morphology of an actinomycete growing on agar can provide useful and rapid clues to its identity, but viewing isolated colonies can give little worthwhile information. Morphological characters are still widely used for characterizing genera, for example, the presence or absence of spores on the substrate mycelium or the formation of zoospores in specialized spore vesicles or sporangia. The ability to produce motile spores is more widespread in the actinomycetes. [6].

The present study is an attempt to produce antibiotics from actinomycetes, isolated from soil, by fermentation and the determination of their antimicrobial activity.

## EXPERIMENTAL

### Materials

Dextrose IP, NaCl, soyabean meal, n-butanol, silicon oil, dimethyl sulphoxide (DMSO), n-hexane, tryptone, lactose, maltose, fructose, yeast extract powder, nutrient agar, agar powder, International Streptomyces Project (ISP) 2 to 7 media, actinomycetes agar, Bennett's agar, nutrient broth, sucrose, silica gel 60-120 mesh, silicagel powder, chloroform, ethyl acetate, and starch were obtained from Nice Chemicals Ltd, Mumbai. Magnesium sulphate, potassium dihydrogen phosphate, methanol, silica gel (TLC grade), peptone (Loba Chemicals Ltd, Mumbai), skimmed milk and glucose were also used in this study. They were all of analytical/pharmacopoeial grade and used as received without further purification.

### Optimization of fermentation parameters

The following fermentation parameters were found to be ideal for maximum antibiotic productivity using A-4 strain of actinomycete [10]: (a) temperature, 28 °C; (b) pH, 7.0 (c); dissolved oxygen (DO<sub>2</sub>) concentration, 60 %; (d) harvesting time (fermentation duration), 96 h ; and (e) inoculum concentration, 10 %v/v.

### Optimization of fermentation duration

The duration of the fermentation process was optimized to obtain the harvesting time for maximum antibiotic productivity. Using the foregoing media formulation and fermentation parameters and conditions, the media were prepared and sterilized, inoculated with 10 % of a 24-h incubated inoculum of A-4 and then incubated for 0 to 144 h. The harvesting time for maximum antibiotic productivity was determined by packed cell volume (PCV) and antimicrobial activity using cup-plate method [10].

## Downstream process for fermentation

Fermentation was carried out for 4 days at the optimized fermentation parameters indicated above. Following fermentation, the medium was centrifuged at 10,000 rpm for half an hour at 4 °C to remove biomass and cell debris and the supernatant was separated. The cell pellet of the biomass was triturated with sterile sand to disrupt the cell wall, washed with Tris buffer (pH 7.4) filtered and centrifuged, and the supernatant, which contained all the intracellular components, was separated out. Both sets of supernatant were assessed for antimicrobial activity [12].

## Determination of antimicrobial activity

Antimicrobial activity was determined by cup plate technique. The test bacteria were grown on nutrient agar while the test fungi were grown on Sabouraud dextrose agar. The extracts, i.e., supernatant liquid from fermentation media, were dissolved in 200 ml of ethyl acetate and 50 µL of the solution was placed in the agar cup. Growth inhibition was measured after incubation for 24 h at 37 °C for bacteria and 72 h at 27 °C for fungi. Antimicrobial activity was estimated by measuring the inhibition zone diameters [11,12].

## Structural identification of antimicrobial compound

The structure of the isolated compound was characterized using nuclear magnetic resonance (NMR) spectroscopy [13].

## RESULTS

### Fermentation process

The optimization data for optimum growth and antibiotic production with regard to the maximum duration of fermentation are shown in Table 1.

**Table 1:** Optimization data for duration of fermentation for antibiotic production

Time (h)	A-4		A-4 mutant	
	PCV (%)	Zone of inhibition (cm)	PCV (%)	Zone of inhibition (cm)
0	--	--	--	--
12	0.1	--	0.02	--
24	0.2	2.0	0.1	--
36	0.25	2.3	0.3	2.1
48	0.4	2.2	0.4	2.4
60	0.4	2.4	0.6	2.7
72	0.7	2.5	0.9	2.8
84	0.9	2.7	1.5	3.2
96	1.5	2.7	3.3	3.6

Well diameter = 0.9 cm; test organism = *Klebsiella pneumoniae*; PCV = packed cell volume

### Antimicrobial activity

Table 2 shows the minimum inhibitory concentration (MIC) of the isolated antibiotics from A-4 and A-4 mutant fermentation broth. The antibiotics showed broad spectrum of activity against Gram-positive and Gram-negative organisms with MIC in the range of 100 - 125 and 62.5 - 100 µg/ml, for A-4 and A-4 mutant respectively. The isolated compounds also showed significant antifungal activity against fungal strains with MIC of 125 and 62.5 -125 µg/ml for A-4 and A-4 mutant, respectively.

**Table 2:** MIC of the isolated antibiotics against bacteria and fungi

Test microorganism	MIC (µg/ml)	
	A-4	A-4 mutant
Gram-positive organisms		
<i>Staphylococcus aureus</i>	125	100
<i>Bacillus subtilis</i>	125	100
Gram negative organisms		
<i>Escherichia coli</i>	125	100
<i>Pseudomonas aeruginosa</i>	100	62.5
<i>Klebsiella pneumoniae</i>	100	62.5
Fungi		
<i>Candida albicans</i>	125	125
<i>Saccharomyces cerviciae</i>	125	62.5

## Structure of antibiotic

NMR spectroscopic data and the functional groups of the antibiotic are shown in Table 3.

**Table 3:** NMR spectroscopic data for the isolated antibiotic

$\delta$ value	Functional group
1.24	Methyl H-shift
2.86	Methyl H-shift
3.29	Aldehyde
3.48	Alcohols
3.7	Esters
5.2	Alkene

## DISCUSSION

Medium formulation is necessary for each fermentation process. It is necessary to optimize each and every component of fermentation media by varying the concentration of media constituents in order to achieve maximum antibiotic production. The purpose of media optimization is to support efficient growth of microorganisms. Different combinations of medium constituents and sequences of optimized fermentation conditions need to be investigated to determine growth conditions that produce biomass that is physiologically best suited for antibiotic production.

For fermentation process optimization, a basal medium containing 0.3 % yeast extract, 0.35 % CaCO<sub>3</sub> and 0.5 % NaCl was used for antibiotic production as indicated in the literature [10,12]. In order to achieve maximum antibiotic production, experiments were conducted to optimize the various parameters such as carbon source, nitrogen source, temperature, pH, DO<sub>2</sub>, and micronutrients etc. It was observed that rapid utilizable carbon source did not support the antibiotic production as antibiotic synthesis does not totally depend on the growth of the microorganism; rather, it depends upon the adverse conditions imposed by the medium or surroundings.

The antibiotics isolated from A-4 and A-4 mutant fermentation broth showed broad spectrum of activity against both Gram-positive and Gram-negative organisms (MIC, 100-125 µg/ml) as well as antifungal activity (MIC, 62.5 - 125 µg/ml). They demonstrated superior antimicrobial activity to other soil isolates of actinomycetes that were investigated. The A-4 mutant showed higher antibiotic potency. NMR studies showed that the antibiotic, A-4 strain, belongs to the macrolide group.

## CONCLUSION

The findings from this investigation reveal that the strains, A-4 and A-4 mutant, which were soil isolates of actinomycetes, exhibited superior antimicrobial activity to other soil isolates of actinomycetes.

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