

Screening for Antibiotic Sensitivity of Free and Immobilized Actinomycetes Isolated from India

¹C. Manjula, ¹P. Rajaguru and ²M. Muthuselvam

¹Department of Biotechnology, Bharathidasan Institute of Technology,
Bharathidasan University, Tiruchirappalli-620024, India

²Department of Microbiology, Marudupandiar College, Thanjavur-613403, India

Abstract: In the present study, six actinomycete strains were isolated from soil available in the local area and screened with regard to their potential against gram positive and negative bacteria. Among the six isolated strains (A1, A2, A3, A4, A5 and A6), three isolates A2, A3 and A5 were found to be effective against tested organisms. These 3 strains were further cultivated in fermentation liquid medium for 120 h and immobilized using sodium alginate. A comparative profile on the total antibiotic sensitivity of the free cells and immobilized cells showed that the immobilized strains were found to be effective against the tested microorganism than the free cells. Further, the most potent of the producer strain was selected and identified, based on the cultural and physiological characteristics. From the results, it was concluded that the three isolates showed very promising activities against tested multi-drug resistant bacteria. The immobilized cells of actinomycetes were found to be more efficient for the production of antibiotics with batch fermentation. The possible routes of antibiotic synthesis and its biological efficacy are currently under investigation.

Key words: Actinomycetes • Antibacterial activity • Immobilization • Soil microorganism

INTRODUCTION

Actinomycete species synthesize a numerous natural metabolites with diverse biological activity such as antibiotics. Antibiotics of actinomycetes origin evidence a wide variety of chemical structures, including amino glycosides, anthracyclines, glycopeptides, β -lactams, nucleosides, peptides, polyenes, polyketides, actinomycins and tetracyclins [1]. In general, the antibiotics are widely produced by fermentation using free cell cultures. To enhance the productivity, much attention has been made on the improvement of the cultures employed in the antibiotic production. Among the various strategies adopted, the whole cell immobilization appears to be more effective for antibiotic production [2]. Immobilized cells have also been used in a wide spectrum of applications, such as degradation of phenols [3], production of ethanol [4] and biosensors [5]. The methods of cell immobilization include nonspecific adsorption, covalent attachment and entrapment. Among them, entrapment has been mainly used for the living cells and successively achieved by using natural and synthetic

polymers [6]. Entrapment of living cells with natural polymers such as agar, agarose, alginate etc., is principally carried out by ionotropic or thermal gelation used to increase the yield of antibiotics. Few studies have demonstrated the advantageous use of immobilized growing cells for the continuous production of antibiotics. Chun and Agathos [7] reported that the immobilization of *Tolypocladium inflatum* leads to increase in the production of cyclosporin A up to three-fold higher than the free cells system. Park *et al.* [8] have demonstrated the enhanced production of neomycin by immobilization of *Streptomyces fradiae* on cellulose-immobilized beads. The purpose of the present investigation was to compare the efficacy of immobilized and free actinomycetes for their antibiotic production and its antimicrobial property against human pathogens.

MATERIALS AND METHODS

Isolation of Actinomycetes: Soil samples were collected from 5-15 cm depth into sterile plastic bags from local area and brought to the laboratory in aseptic condition.

Actinomycetes were isolated by soil dilution plate technique using starch-casein-nitrate-rose bengal agar medium. Thus isolated colonies had been preserved in glycerol based media and stored at -20°C. The actinomycetes of which antimicrobial activity should be determined were revived by streaking on Starch-Casein agar and incubated at 30°C for 5 days.

Characterization of Actinomycetes: The potent actinomycetes were characterized by morphological and biochemical methods. Morphological methods consist of macroscopic and microscopic methods. The microscopic characterization was done by cover slip culture method [9]. The mycelium structure, color and arrangement of conidiospore and arthrospore on the mycelium were observed through the oil immersion (100 X). The observed structure was compared with Bergey's manual of Determinative Bacteriology [10] and the organism was identified. Various biochemical tests were performed for the identification of the potent isolates are as follows: Casein hydrolysis, Starch hydrolysis, Urea hydrolysis, Fermentation of citrate, Nitrate reduction etc.

Fermentation Process: The isolates were cultured at 30°C for 120 h in a jar fermentor containing 1 liter of a medium consisting of maltose 4%, sodium glutamate 1.2%, K₂HPO₄ 0.01%, MgSO₄ 0.05%, CaCl₂ 0.01% and FeSO₄ 0.005% with or without sodium alginate beads. Cells were immobilized using sodium alginate by the ionotrophic method. At the end of each fermentation cycle, the sodium alginate beads were aseptically separated from the fermentation broth by filtration using a sterile Büchner funnel.

Isolation of Antibacterial Metabolites: Antibacterial compound was purified from the filtrate by solvent extraction method following the process described by Westley *et al.* [11]. Ethyl acetate was added to the filtrate in the ratio of 1:1(v/v) and shaken vigorously for 1 h for complete extraction. The ethyl acetate phase that contains antibiotic substances was separated from the aqueous phase. It was evaporated to dryness in water bath and the residue obtained was weighed. Thus obtained compound was used to determine the antibacterial activity.

Screening of Actinomycetes for Antibacterial Activity: Antibacterial activity was tested *in vitro* against pathogenic bacteria: *Escherichia coli* ATCC 29998, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 6538. Antibacterial

activities were performed by disc-diffusion assay [12] and effectiveness was measured by zones of inhibition on bacterial culture plates.

RESULTS AND DISCUSSION

Out of 13 actinomycetes subjected for primary screening process, only 6 isolates showed the activity against tested organisms and designated as A1, A2, A3, A4, A5 and A6. The spectrum of antibiotic activity of selected strains was determined using *E. coli*, *P. aeruginosa* and *S. aureus*. It was found that 6 strains suppressed in different degree of the growth of the test microorganisms (Fig. 1). Most of them were not active against *E. coli* but inhibited the growth of *S. aureus* and *P. aeruginosa* in a significant extent. Among the six isolated strains A2, A3 and A5 had shown more inhibitory activity than other three strains. The identification of the potent antibiotic producing strains reveals that all the three strains belong to the genus *Streptomyces*. The results of the biochemical features are presented in Table 1. The optimum growth temperature was found to be 25°C to 30°C, but did not grow at less than 10°C and above 50°C. The isolates failed to grow at acidic pH but grow well at pH 7-8. These three potent isolates were selected for fermentation on the basis of their broad spectrum of activity and largest zone of inhibition. The isolates were cultivated in fermentation liquid medium for 120 h.

The whole cell immobilization technique is an efficient way to overcome the difficulty in producing antibiotics by continuous fermentations with free-cells, since the growth and metabolic production can be uncoupled without affecting metabolite yields. Several attempts have been made to immobilize various microbial species on different support matrices for antibiotic production. Mahmoud *et al.* [13] demonstrated the entrapment of *P. chrysogenum* in calcium alginate and used in bubble

Table 1: Characterization of actinomycetes strains isolated from soil

Test	A1	A2	A3	A4	A5	A6
Indole formation	-	+	-	-	-	-
Methyl red	-	-	-	-	+	-
Catalase	+	+	+	+	+	+
Urease	-	-	+	+	+	+
Lipase activity	-	-	-	-	+	-
Cellulose activity	-	+	+	-	+	-
Hydrolysis of starch	+	-	+	+	-	-
Hydrolysis of casein	-	-	+	+	-	-
Nitrate reduction	+	-	+	+	+	+
Fermentation of citrate	+	+	+	+	+	+
Growth on Mc Conkey	+	+	+	+	+	+

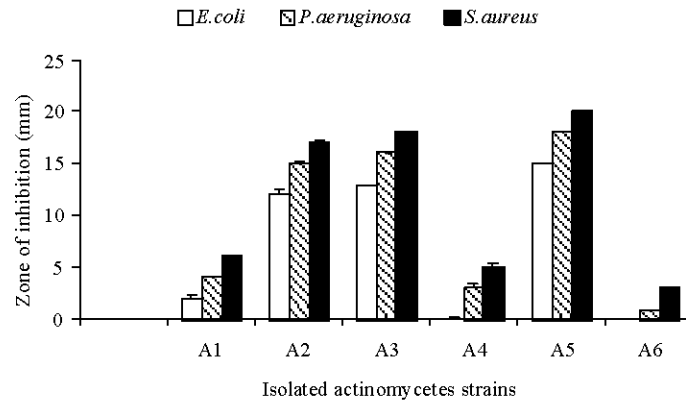


Fig. 1: Antimicrobial activity of actinomycetes strains isolated from soil

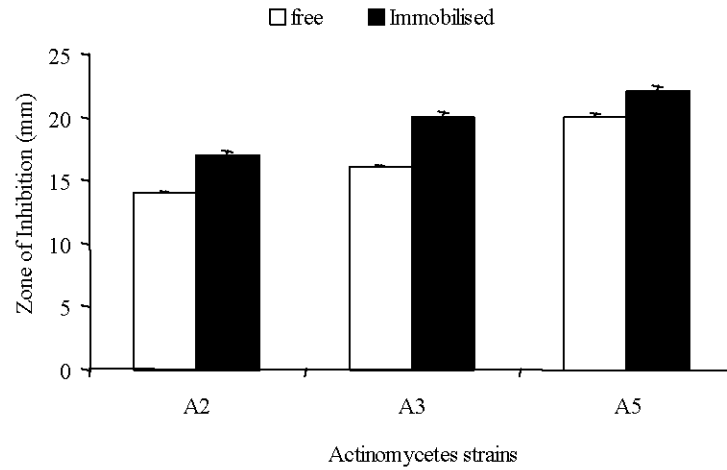


Fig. 2: Antimicrobial activity of actinomycetes against *E. coli*

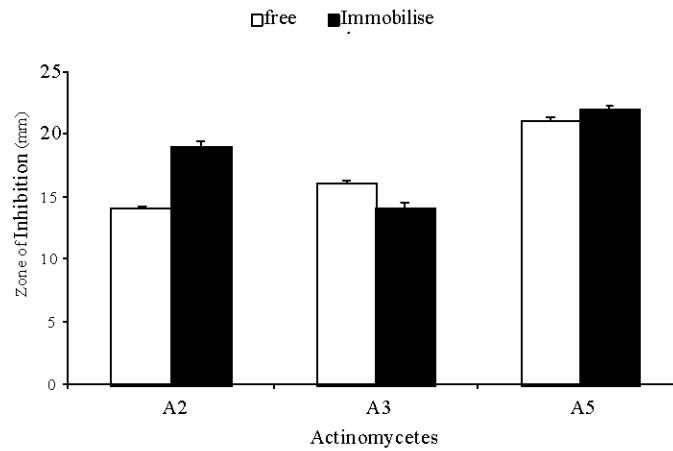


Fig. 3: Antimicrobial activity of actinomycetes against *P. aeruginosa*

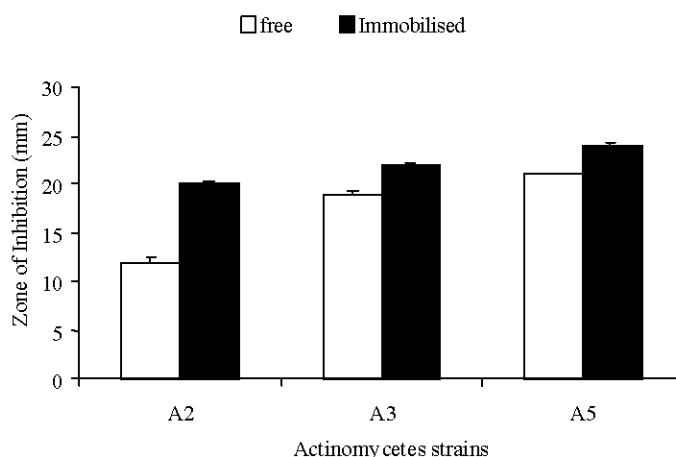


Fig. 4: Antimicrobial activity of actinomycetes against *S. aureus*

column reactors. Ogaki *et al.* [14] demonstrated the use of immobilized cells of *S. rimosus* for the continuous production of oxytetracycline. But Farid *et al.* [15] have attempted entrapment of *S. rimosus* in calcium alginate gels and reported good level of antibiotic production in repeated batch fermentations for a period of 28 days.

Previous studies indicated that the sodium alginate concentration for the immobilization of cells plays a prominent role in the production of antibiotics due to the porosity of the beads which limiting the nutrient supply and oxygen diffusion [16]. Sallam *et al.* [17] studied the application of immobilization technique using alginate for the production of cyclosporin A by a local strain of *Aspergillus terreus*. Alginate at 2% wt/vol was found to be the optimum concentration for formulation of spherical and stable beads with better antibiotic production [18, 19]. The same concentration was used in this study.

Comparative data on the total antibiotic production with free cells and immobilized cells is shown in Fig. 2-4. It was found that the immobilized cells in sodium alginate were more efficient for the production of antibiotics which was confirmed by the zones of inhibition on bacterial culture plates. The antimicrobial activity of immobilized cell culture was up to three-fold higher than that of the free cell system. In addition the isolated A5 was found to be more efficient when compare to other two isolates A2 and A3.

From the results, it is concluded that the immobilized cells of actinomycetes are more efficient for the production of antibiotics. The possible routes of antibiotic synthesis and its biological efficacy are currently under investigation.

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