European Journal of Biological Sciences 5 (2): 59-63, 2013
ISSN 2079-2085

DOI: 10.5829/idosi.ejbs.2013.5.2.73113

The Streptomyces Flora of Coimbatore Region and its Potential as a Source of Antibiotics Against Fungal Pathogens

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Abstract: Soil actinomycetes particularly Streptomyces spp are the source of most active ingredients of medicine. While screening of actinomycetes for antifungal activity, Streptomyces were isolated from soil sample. An attempt was made to screen for the production of antifungal metabolite. Secondary metabolites from the selected strain was active against fungus like *Candida albicans*, *Aspergillus flavus* and *Aspergillus fumigatus*. Mycelial growth of fungi was inversely proportional to the concentration of extract and results indicated the effectiveness of extract against fungal pathogens.

Key words: Actinomycetes · Secondary metabolites · Bioactive compounds · Fungi · Antifungal activity.

INTRODUCTION

Fungal pathogens pose serious problems worldwide and cause a number of plants and animal diseases such as ringworm, athlete's foot, and several more serious diseases [1]. Fungi able to use almost any surface for growth. Unfortunately, they also are proficient at colonizing and using plants, humans and animals as substrates and causing diseases as well as spoilage in food and pharmaceutical products [2]. In addition they cause great economic losses and public health problems, especially involving immune-compromised patients. Fungal infections in humans range from the superficial and common, such as dermatophytosis and onychomycosis, to deeply invasive and disseminated such as candidiasis and aspergillosis.

The actinomycetes are noteworthy as antibiotic producers, making three quarters of all known antibiotics. The Streptomyces are especially prolific and can produce a great antibiotics and other class of biologically active secondary metabolites [3]. For the fungal disease control, a serious search is needed to identify active antifungal compound which are less dependent on chemicals and are more environmentally friendly [4]. Investigation can possibly reveal actinomycetes species that produce novel antibiotics. It is anticipated that the isolation, characterization and the study on actinomycetes can be useful in the discovery of antibiotics and novel species of actinomycetes [5]. This study investigated the antifungal activity of the cell-free culture filtrate of this antagonist to determine antifungal compounds. The antifungal potential of extracellular metabolites produced by soil-borne actinomycetes could be exploited in future as an antifungal compounds.

MATERIALS AND METHODS

Isolation of Actinomycetes: Soil samples were collected from different locations in Coimbatore. The soil samples air dried for 7 days at room temperature. 5 g soil sample was suspended in 50 ml of sterilized water in a 250ml Erlenmeyer flask and then shaked at 150 rpm for 30 min. The soil suspension 0.5 ml was spread on the Starch casein agar medium for actinomycetes isolation. After incubation for 4 week at 30°C, actinomycete colonies appearing on the Starch casein agar were transferred to the Yeast malt extract agar for colony maintenance [6].

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Characterization of Actinomycetes: Morphological observations were made with a light microscope [7]. The morphology of spore bearing, hyphae with entire spore chain and structure of spore chain with the actinomycetes morphologies were compared as described by Bergey's manual [8]. This was done by using cover slip method [9] in which individual cultures was transferred to the base of cover slips buried in ISP 4 medium.

Carbon utilization was determined on plates containing ISP basal medium 9 to which carbon sources were added to a final concentration of 1.0%. The plates were incubated at 27°C and growth was read after 15 days using glucoses as positive control. The ability to utilize nitrogen sources was determined in a basal medium containing different nitrogen sources. Results were observed after 15 days. [10].

Fermentation and Extraction of Secondary Metabolite: A few blocks of ISP2 containing promising Streptomyces were inoculated into 30 ml of SMK broth and incubated at room temperature 28°C for 7 days and eventually transferred on to the 300 ml krasilnikovs broth and incubated in rotary shaker for 21 days at room temperature [11]. Culture filtrates were extracted with three half volume of ethylacetate. The organic phase was pooled followed by evaporation at 40°C. The crude extract was used for bioactivity against test fungi.

Test Pathogens: Three pathogenic fungi namely *Candida albicans*, *Aspergillus flavus* and *Aspergillus fumigatus* were collected from Department of Microbiology in Karpagam University and sub cultured and used throughout the study.

Antifungal Activity of Actinomycetes: Agar well diffusion method was used for the antifungal activity [12]. Mueller Hinton Agar was prepared and the fungal cultures were swabbed on the plates (*Candida albicans, Aspergillus flavus* and *Aspergillus fumigatus*). Three wells of 6 mm diameter were prepared with the help of sterile borer and loaded with 50 µl of solvent extracts. Zone of inhibition was measured after 3 days at 28°C.

Effect of Medium Composition on Antifungal Activity: The strain was cultivated on the different media including Starch nitrate broth, Starch casein broth, Glycerol asparagine broth and Oat meal extract broth to understand its growth and metabolite production [13]. Biomass was analysed as cell dry weight per 100 ml of culture medium. Production of antifungal metabolites by the strain was expressed in terms of inhibition zone against pathogenic fungus exerted by 50 ppm of solvent extract obtained from different media respectively.

RESULT AND DISCUSSION

A total of 34 different actinomycetes were recovered from soil samples collected from Coimbatore using starch casein agar which is specific for the actinomycetes isolation. All isolates grew on an agar media showing morphology of Streptomyces. The colour of the substrate mycelium and aerial spore mass were varied. The vegetative hyphae of the isolates were branched. Some produced diffusible pigments. Melanin was produced on peptone yeast extract agar and tyrosine agar. The utilization of the carbohydrates and nitrogen source and other characteristics are summarized in Table 1.

The crude extract of antifungal compounds isolated from Streptomyces spp were used to check the antifungal activity against test pathogens namely *Candida albicans, Aspergillus flavus* and *Aspergillus fumigates*. On Mullerhinton medium, the isolates showed different zone of inhibition against three pathogens. Crude extract extracted from Streptomyces species proved the ability of fungal inhibition. Above all the extract of KMA16 showed better antifungal efficiency in all tested fungal pathogens than other isolates. It has been observed that the extracts of all five active isolates have inhibited the growth of nearly all the test pathogen (Table 2).

Similar results have been observed by various authors including Khamna (2009) who reported that the crude extract of antifungal compounds were active against *R. stolonifer*, *A. flavus*, *F. oxysporum* and *Alternaria* [14]. Lim *et al*. (2000) selected 32 Actinomycetes isolates, which showed the inhibitory activity against mycelial growth of plant pathogenic fungi like *Alternaria mali*, *Colletotrichum gloeosporides*, *F. oxysporum*, *cucumerinum*, *Magnaporthe grisea*, *Phytophthora capsici,* and *Rhizoctonia solani* [15]. Similar results have been found by Gebreel *et al*. (2008), Anitha and Rebeeth (2009) and Kavitha *et al*. (2010) [16,17,18,].

Table 1: Morphological and physiological characteristics of five selected isolates

	Isolates				
Characteristics	KMA08	KMA16	KMA27	KMA29	KMA32
Aerial mycelium	+	-	+	-	+
Substrate mycelium	-	+	-	+	-
Spore chain morphology					
Rectiflexibles	-	-	+	-	-
Spirales	-	+	+	+	+
Verticillat	-	-	-	-	-
Sporemass colour					
Red					
	-	-	-	+	-
Grey	+	+	+	-	-
Mycelium pigment red orange	-	-	-	-	-
Diffusible pigment produced	-				
	+	-	-	-	
Melanin production					
Peptone Yeast					
	-	-	-	-	-
Yeast extract agar	-	-	+	-	-
Tyrosine Agar	+	+	-	-	-
Utilisation of Nitrogen and carbon source					
L-Valine	+	+	+	+	-
L-Phenylalanine	+	+	+	-	-
L-Histidine	+	+	+	+	+
L-Lysine	+	+	+	+	+
L-Tyrosine	+	-	+	-	-
Glucose	+	+	+	+	+
Mannitol	+	+	+	+	+
Xylose	+	+	+	+	-
Galatose	+	+	+	+	+

+ = positive - = negative

Table 2: Antifungal activity of solvent extracts of the isolates

	ZONE OF INHIBITION (mm)				
Test pathogens	*KMA08*	*KMA16*	*KMA27*	*KMA29*	*KMA32*
C. albicans	15	17	12	11	10
A. flavus	18	19	11	12	7
A. fumigates	14	15	10	10	9

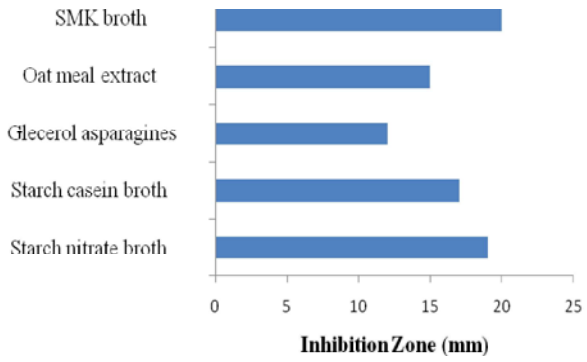

Fig. 1: Antifungal compound production by using different media

Among different media tested, Krasilnikovs (SMK) media supported good growth of the strain as well as antifungal metabolite production (Fig. 1). Starch nitrate and Starch casein broth were also found suitable for bioactive metabolite production. Antifungal antibiotic was reported from *S. purpeofuscus* CM 1261 indicating the variation in metabolite production by different strains [19].

Many important bioactive compounds are provided by Actinomycetes with high commercial value and continue to be routinely screened for new bioactive compounds. The activities of bioactive compounds from Streptomyces are categorized as pharmacologically agrobiologically active agents. Thus, it is obvious that the activity profile of Streptomyces products is very broad [20]. In search for soil Actinomycetes having antifungal activity against plant fungal pathogens, 110 isolates were screened by Aghighi *et al*. (2004), from which 14 isolates were found active against *A. solani*, *A. alternate*, *Fusarium solani*, *Phytophthora megasperma*, *V. Dahlia* and *Sacchromyces cerevisiae* [21]. In this present investigation, the results indicated that soil is a good source for the isolation of actinomycetes and found that Streptomyces species were presented predominant. Finding of this study suggested that the antifungal substance present in actinomycetes isolate could be used to inhibit wide range of pathogen. Further studies needed to identify the bioactive compounds of the selected isolates and their principle behaviour as antifungal agents.

CONCLUSION

Among microorganisms actinomycetes are the important sources for bioactive metabolites especially antibiotics. Based on the results Crude extract extracted from Streptomyces species proved the ability of fungal inhibition. It has been observed that the extracts of all five active isolates have inhibited the growth of nearly all the test pathogen. It is expected that the current attempt of isolation, characterization and the study on soil actinomycetes will be useful for identification of new antibiotics effective against challenging pathogens.

ACKNOWLEDGEMENTS

All the authors are grateful to our management, Karpagam University for providing facilities and encouragement.

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