International Journal of Microbiological Research 2 (2): 167-171, 2011
ISSN 2079-2093

Production of Anti-*Candida albicans* by Egyptian *Streptomyces* Isolates

Amal Said Shahat, Ahmed Abouwarda and Wael Mohamed Abu El-Wafa

Department of General Basic Medical Sciences, Microbiology Department,
National Organization for Drug Control and Research (NODCAR), Giza, Egypt

Abstract: *Streptomyces* isolate *SY*1, which exhibited anticandidial activity, was isolated from a soil sample collected from Kalyoubia governorate, Egypt. The isolate was identified as a strain of *Streptomyces griseolus*. The effect of different agar media (starch nitrate, yeast malt extract, oat-meal extract, inorganic salts starch, glycerol asparagine and fish-meal extract medium) on anticandidial activity by selected strain was studied. Results showed that *Streptomyces griseolus* strain *SY*1 can produce anticandidial metabolites in different tested media. However, starch nitrate agar medium was the most suitable medium for maximum anticandidial activity, which gave 20 mm inhibition zone followed by fish-meal extract, inorganic salts starch, oat-meal extract, yeast malt extract and glycerol asparagine, which gave 16, 15, 15, 13 and 12 mm inhibition zone, respectively. It was concluded that starch nitrate is the best medium for production of anticandidial metabolites.

Key words: Actinomycetes · Antibiotic · Anticandidial

INTRODUCTION

Fungi are able to use almost any surface for growth. Unfortunately, they also are proficient at colonizing and using plants, humans and animal as substrates, causing diseases, as well as spoilage in food and pharmaceutical products. In addition they cause great economic losses and public health problems, especially involving immune-compromised patients. This is in part due to the tremendous advances in medicine that permit the saving of patients with immune-compromising diseases who would otherwise not have survived. Fortunately, it is rare that these patients succumb to fungal infections for which there are few or no drugs available for treatment. Fungal infections in humans range from the superficial and common, such as dermatophytoses and onychomycoses, to deeply invasive and disseminated, such as candidiasis and aspergillosis. The major pathogen has been *candida albicans,* normally a commensal of the oral cavity and gastrointestinal tract of humans [1-5].

The search for new antibiotics continues to be the most importance in research programs around the world because of the increase of resistant pathogens and toxicity of some used antibiotics. Among microorganisms, actinomycetes are among the most investigated groups particularly members of the genus *Streptomyces* from which, a large number of antibiotics was obtained and studied. The vast majority of actinomycetes have originated from soil and their isolation method deal almost exclusively with those suitable for *Streptomyces* species which grow rapidly on soil dilution plates. However, in recent years, the rate of discovery of new antibiotics in the genus *Streptomyces* was declining and isolation of other actinomycete genera, appeared to be necessary to assess the health hazard and to find novel strains producing commercially valuable antibiotics [6].

In the present work, we completely identify up to species of the local *Streptomyces* isolate which produced anticandidial metabolites.

MATERIALS AND METHODS

All the medium ingredients used in this study were obtained from Sigma Chemicals Company (St. Louis, Mo, USA), oat-meal and fish-meal powder were obtained from Central Laboratory of Feed at Agricultural Research Center, Giza, Egypt. Sabouraud's dextrose agar medium was obtained from Difico Company.

Test microorganism - *Candida alibicans* ATCC 10231 was obtained from American Type Culture Collection.

Corresponding Author: Ahmed Abouwarda, Department of General Basic Medical Sciences, Microbiology Department. National Organization for Drug Control and Research (NODCAR). 6, Abu-Hazem st., Pyramids Avenue Giza, Egypt. E-mail: ahmed_mogahed99@hotmail.com

***Streptomyces* Isolation and Screening for Their Anticandidial Activity:** Collection of soil samples (10-20 cm from surface) were obtained from different localities of Kalyoubia governorate, dried at room temperature for 7days, mixed well with $CaCO_3$ (10%, w/w) and incubated at 28 ±2°C for 7 days under saturation condition of humidity. After incubation, 10 g of soil sample were transferred into 500 ml Erlenmeyer flask contained 100 ml sterile distilled water, shacked for 15 min at room temperature on rotary shaker (200 rpm) and then diluted with sterile distilled water to 10^{-6}. Each of sterile starch nitrate plates was surfed with 1ml of diluted soil suspension and then incubated for 7 days at 28 ±2°C.

Streptomyces colonies were preliminary identified according to the method described by Bergey's Manual [7,8]. *Streptomyces* isolates were picked up, re-cultivated several times (by streaking technique) on starch nitrate agar medium and study their anticandidial activity on sterile agar plates of Sabouraud′*s* medium, previously inoculated with loopful of fresh culture of *Candida alibicans* ATCC 10231.

One promising *Streptomyces* isolate *SY1* was selected and streaked on different agar media (starch nitrate [9], yeast malt extract, oat-meal extract, inorganic salts starch, glycerol asparagine [10] and fish meal extract [11]) and incubated at 28 ±2°C for 15 days. After growth, agar discs of *Streptomyces SY1* cultures were made with a sterile crock poorer (0.6 mm) and placed on sterile Sabouraud′s (Defico) plates, previously inoculated with *Candida alibicans* ATCC 10231, then, the plates were incubated at 28 ±2°C for 2 days. The anticandidial activity was recorded by measuring the inhibition zone (mm) around the cultural discs.

Characterization of *Streptomyces* Isolate *SY*1: Cultural and morphological characteristics of the selected isolate *SY1* were studied on yeast malt extract, oat-meal extract, inorganic salts starch and glycerol asparagine media according to the method described by Shirling and Gottlieb [10]. Physiological characteristics: producing of melanoid pigments, tolerant to different concentrations (4, 7, 10 and 13%, w/v) of NaCl, sensitivity to streptomycin antibiotic (50 µg ml^{-1}, w/v), antimicrobial activity and utilization of different carbon sources were studied according to the method described by Shirling and Gottlieb [10]. The selected isolate was completely identified up to species according to the keys proposed by Shirling and Gottlieb [12] and by Bergey's Manual [7].

RESULTS AND DISCUSSION

Results revealed that 187 actinomycete isolates were isolated from a collection of different soil samples obtained from Kalyoubia, Egypt. In addition, all of them were belonged to the genus *Streptomyces* as they form well developed branching, non-septate, non-fragmented aerial mycelia bearing a long spore chains and non-motile spore which not borne in verticillate sporophores [8]. The most efficient isolate on anticandidial activity was *Streptomyces* isolate *SY*1 which will be used in subsequence study.

Suitability of Different Agar Media on Anticandidial Activity: Production of anticandidial metabolites by different microorganisms are influenced by the type and concentration of carbon, nitrogen, phosphorus and minerals contained of production medium.

Data illustrated in figure 1 revealed that the selected *Streptomyces* isolate could grow and produce anticandidial metabolite using of different tested medium. While, the most suitable medium for maximum anticandidial activity was starch nitrate agar medium followed by fish-meal extract, inorganic salts starch, oat-meal extract, yeast malt extract and glycerol asparagine, which gave 20, 16, 15, 15, 13 and 12mm inhibition zone, respectively.

The above mentioned results were in agreement with those obtained by many investigators, they found that the antimicrobial activities of *Streptomyces* spp. were increased or completely inhibited according to the composition of production media [13-15]. Prema *et al.* [16] studied the effect of different media compositions on anticandidial activity by *S. aureofaciens* and *S. albidoflavus*. They found that yeast malt extract medium was the most suitable medium for maximum anticandidial activity by the first tested strain, which gave 24 mm inhibition zone followed by starch casein, Sabouraud's potato dextrose, glycerol asparagine and glucose asparagines medium, which gave 18,16,12,11 and 11 mm inhibition zone, respectively. While, the second strain gave the maximum anticandidial activity (16mm inhibition zone) with Sabouraud's medium followed by other tested media which gave 12mm inhibition zone.

Identification of *Streptomyces* Isolate *Sy*1: *Streptomyces* isolate *SY1* could grow on different tested media; yeast malt extract, oat-meal extract and inorganic salts starch,

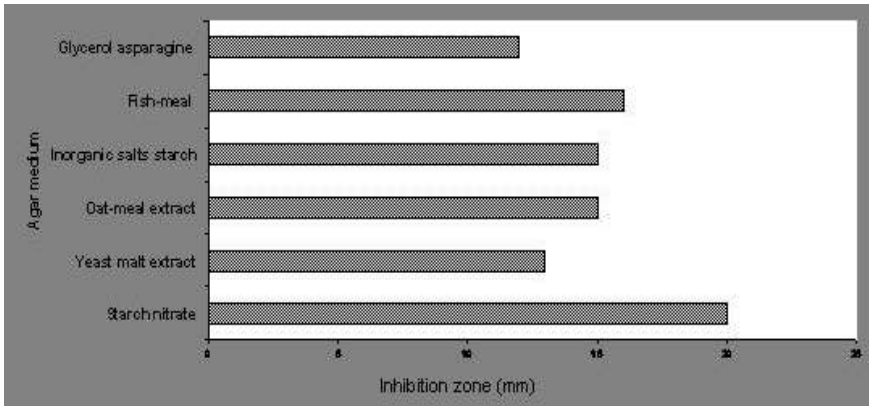

Fig. 1: Effect of different media composition on anticandidial activity of *Streptomyces SY*1

Fig. 2: Electron micrograph of spore surface ornamentation of *Streptomyces* isolate *SY1* (X 25000)

Table 1: Identification of *Streptomyces* isolate SY1

Characteristics	Streptomyces isolate SY1	*S. griseolus*	*S. narbonensis*
Cultural characteristics			
Color of aerial mycelium	Gray	Gray	Gray
Color of substrate mycelium	Brawn	Brawn	Gray- yellow
Diffusible pigments	Colorless	Colorless	Gray- yellow
Morphological characteristics			
spore chain morphology	RF	RF	RF
Spore surface ornamentation	Smooth	Smooth	Smooth
Physiological characteristics			
Melanoid pigment produced	-	-	±
Growth on Czapek`s medium	Poor	Poor	ND
Sodium chloride tolerance	?7	?7	ND
Sensitivity to streptomycin*	+	+	+
Antimicrobail activity	+	+	+
Carbon sources utilization			
No Carbon	-	-	-
D-Glucose	+	+	+
D-Xylose	+	+	+
L-Arabinose	+	+	+
L-Rhamnose	-	±	(+)
D-Fructose	+	+	+
Galactose	+	+	+
Raffinose	-	-	(+)
D-Mannitol	-	-	-
Inositol	-	-	-
Salicin	-	ND	ND
Sucrose	-	±	(+)

*: 50 µg ml-1(w/v), RF: Rectus flexible, ND: Not determined. +: positive; - : negative; ±: doubtful.

glycerol asparagine [10]. The color of aerial mycelium and substrate mycelium were gray and brawn, respectively. Spores chains morphology and spore surface ornamentation were rectus flexible (RF) and smooth type, respectively. In contrast, production of diffusible or milanoids pigments were not observed in various tested media. The experimental isolate made a weakly growth on Czapek`s agar medium [17], grow up to 7% (w/v) NaCl, produced antimicrobial metabolite and could utilize D-glucose, D-xylose, L-arabinose, D-fructose and galactose as a sole carbon sources, but, it could not utilize rhamnose, raffinose, D-mannitol, inositol, salicin or sucrose. While, the growth of the experimental isolate was completely inhibited in Bennet`s agar medium [18], supplemented with 50 μg ml^{-1} streptomycin antibiotic (Table 1 and Figure 2).

The data present in table 1 and figure 2 revealed that the experimental *Streptomyces* isolate *SY1* appeared to resemble *S. griseolus* and *S. narbonensis* [7,12]. However, the later species produced a gray- yellow pigment and could utilize rhamnose, raffinose and sucrose. In contrast, the cultural, morphological and physiological characteristics of the experimental isolate appeared to be in harmony with those of *S. griseolus*. Thus, the experimental isolate *SY1* was identified as a strain of *Streptomyces griseolus.*

In conclusion, local isolate of *Streptomyces* could produce anticandidial metabolite on different agar medium; however, starch nitrate agar medium was the most suitable medium for the maximum production of anti-*Candida* metabolite. The local *Streptomyces* isolate *SY*1 was identified as a strain of *Streptomyces griseolus.*

REFERENCES

1. Fox, J.L., 1993. Fungal infection rates are increasing. ASM News, 59: 515-518.
2. Legan, J.D., 1993. Mould spoilage of bread: the problem and some solutions. International Biodeterioration and Biodegradation, 32: 33-53.
3. Herbrecht, R., 1996. The changing epidemiology of fungal infections: are the lipid-based of amphotericin B and advance?. European J. Haematol., 56: 12-17.
4. Pfaller, M.A., R.N. Jones, S.A. Messer, M.B. Edmond and R.P. Wenzel, 1998. National surveillance of nosocomial blood stream infection due to *Candida albicans:* frequency of occurrence and antifungal susceptibility in the SCOPE program. Diagn. Microbiological of Infectious Disease, 31: 327-332.
5. Selitrennikoff, C.P., 2001. Antifungal Proteins. Applied and Environmental Microbiol., 67: 2883-2894.
6. Okami, B. and A.K. Hotta, 1988. Search and discovery of new antibiotics. In: Goodfellow, M. and Williams, S. T. Mordarski, M. (Eds.), Actinomycetes in Biotechnology. Pergamon Press, Oxford,: pp: 33-67.
7. Bergey's Manual of Determinative Bacteriology, 1974. 8th Ed. Actinomycetes and Related Organisms Family: Streptomycetaceae (Part 17). Buchanan, R. E. and N. E. Gibbons (coeds).Williams and Wilkins. Baltimore, U.S.A. pp: 747-802.
8. Bergey's Manual of Systematic Bacteriology, 1986. Streptomycetes and Related Genera. Williams, S. T. (ed.), Williams and Wilkins. Baltimore, U.S.A., 4: 2451-2469.
9. Waksman, S.A., 1961. The Actinomycetes. Vol. II. Classification, Identification and Description of Genera and Species. Williams and Wilkins Co. Baltimore, U.S.A. pp: 430.
10. Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of streptomycetes species. International J. Systematic Bacteriol., 16: 313-340.
11. Krassilnikov, N.A., 1970. Pigmentation of Actinomycetes and Its Significance in Taxonomy. In: The Actinomycetales. Prauser, H. (ed.), Gustav Ficher Verlag, Jena, pp: 123-131.
12. Shirling, E.B. and D. Gottlieb, 1968. Cooperative description of type strains of *Streptomyces* II. Species description from first study. International J. Systematic Bacteriol., 18: 69-189.
13. Aghighi, S., G.H. Shahidi Bonjar and I. Saadoun, 2004. First report of antifungal properties of a new strain of *Streptomyces plicatus* (strain101) against four Iranian phytopathogenic isolates of *Verticillium dahlia*e, A new horizon in biocontrol agents. Biotechnol., 3: 90-97.
14. Jain P.K. and P.C. Jain, 2006. Isolation, characterization and antifungal activity of *Streptomyces sampsonii* GS 1322. Indian. Journal of Experimental. Biol., 45: 203-206.
15. Nonoh, J.O., W. Lwande, D. Masiga; R. Herrmann, J. K. Presnail, E. Schepers, M. A. Okech, R. Bagine, P. Mungai, A.B. Nyende and H.I. Boga, 2010. Isolation and characterization of *Streptomyces* species with antifungal activity from selected national parks in Kenya. African Journal of Microbiological Res., 4: 856-864.

16. Prema, P., S.P. Audline Suja, S. Subha Ranjani and G. Immanuel, 2009. UV/VIS, FTIR spectrum and anticandidial activity of *Streptomyces* strains. The International J. Microbiol., 7: 115-125.
17. Prauser, H. and R. Folta, 1968. Phagensensibilitat, zellwandzusammensetzung und taxonomie van actinomyceten. Zeitschrift für allgemeine Mikrobiologie, 8: 39-64.
18. Jones, K.L., 1949. Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. J. Bacteriol., 57:141-145.