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Anti Yeast Activity of *Streptomyces olivaceus* Strain 115 Against *Candida albicans*

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Abstract: Emergence of Azole-resistant *Candida albicans* is a growing problem worldwide. Searching for new principles is a global need with high priority. With this aim, *in vitro* anticandidal activity of pure cultures of soil Actinomycetes isolated from Kerman Province were screened against a registered isolate of *C. albicans* (PTCC No. 5027). In comparison with Clotrimazole, the drug of choice of the pathogen, from 50 tested Actinomycetes, crude sap of *Streptomyces olivaceus* strain 115 showed *in vitro* antagonistic anti-yeast effect on the pathogen. The tests performed aseptically on lawn cultures of the pathogen in well diffusion bioassay system. Inhibitory zones represented complete suppression of *C. albicans*. The nature and further biological activity of the active principle are under investigation.

Key words: Anticandidal activity, actinomycete, *Streptomyces olivaceus*

INTRODUCTION

Oropharyngeal candidiasis (OPC) that is refractory to therapy with fluconazole has been increasingly reported in HIV-infected patients after long-term use of fluconazole^[1]. *Candida albicans* is the cause of mucosal, cutaneous and systemic infections including OPC, the most frequent opportunistic infection among AIDS patients^[2,3]. The azole antifungal agents have proven effective for the management of OPC. The repetition and lengthy duration of therapy for OPC in this patient population has led to an increased incidence of treatment failures leading to the emergence of azole resistance in this pathogenic fungus. It is mentioned that about two decades ago *C. albicans* was commonly regarded as little more than culture contaminant; however, because of developed resistance, in recent years this organism has become a major human pathogen^[4]. Frequency of *C. albicans* infections has risen dramatically and the development of drug-resistant *C. albicans* is a major concern worldwide^[5,6]. Kieren *et al.*^[7] reported that Fluconazole-resistant *C. albicans*, a cause of oropharyngeal candidiasis in patients with human immunodeficiency virus infection has recently emerged as a cause of candidiasis in patients receiving cancer chemotherapy and marrow transplantation. In their studies they found *C. albicans* isolates became resistant to fluconazole and amphotericin B after two weeks of

antifungal drug exposure. Clinically, antimicrobial therapy is going through a crisis due to the rapid development of resistance to existing antimicrobial agents^[8]. In this regard, searching for new principles is a global need with high priority. Actinomycetes are one of the most attractive sources of different kinds of metabolites such as antifungals and antibacterials. The *Streptomyces* spp. have been the source of the majority of antibiotics and their ability to parasitize and degrade spores of fungal plant pathogens is well established^[9,10]. In search for new anticandidal agents, *in vitro* activity of pure soil actinomycetes isolated from Kerman Province were screened against a registered isolate of *C. albicans*. From 50 tested actinomycetes, *Streptomyces olivaceus* strain 115^[11,12] showed *in vitro* antagonistic effect on the pathogen in well method bioassays.

MATERIALS AND METHODS

Preparation of micro-organism: A registered isolate of lyophilized *Candida albicans* (PTCC No. 5027) was obtained from PTCC (Persian Type Culture Collection, Tehran, Iran). It was rejuvenated and maintained on SDA at 4°C for bioassays.

Culture media: Sabouraud dextrose agar (SDA) was used for bioassays and propagation of *C. albicans*. Casein glycerol (or starch) agar (CGA) was used for isolation,

identification and supporting *Streptomyces olivaceus* strain 115^[13]. CGA was composed of: glycerol or soluble starch, 10 g; casein, 0.3 g; KNO₃, 2 g; NaCl, 2 g; K₂HPO₄, 2 g; MgSO₄.7H₂O, 0.05 g; CaCO₃, 0.02 g; FeSO₄.7H₂O, 0.01 g and agar, 18 g in 1 L of distilled H₂O (pH 7.2). In submerged cultures, agar was excluded (CG medium). *Streptomyces* colonies with different morphologies were selected and transferred to CGA slants for further studies.

Screening and bioassay: Screening was performed according to the procedure used by Shahrokhi *et al.*^[11]. For bioassays, a yeast suspension of approximately 1.5x10⁶ cells mL⁻¹ in sterile normal saline was prepared as described by Forbes *et al.*^[14]. An aliquot of 1.5 mL was uniformly seeded on the solid media (15 mL, 4 cm thickness) in Petri dishes, left aside for 15 min and excess was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers^[14]. Concentration of about 50 mg mL⁻¹ of the active crude was prepared in dimethyl sulfoxide (DMSO): methanol (1:1, v/v) solvent (DM solvent) and administered to fullness in each well. The plates were then incubated at 32° C for 48 h. After incubation, bioactivity was determined by measuring the diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Positive control included Clotrimazole (1% topical solution, Pars Daru, Tehran, Iran) and negative control included solvent without active crude, although no anticandidal activity was noted in the solvent employed for the test.

RESULTS AND DISCUSSION

Screening and bioassays: In screening for Actinomycetes having antagonistic activity against *C. albicans*, 50



Fig. 1: Bioassay result of anti-yeast activity of crude sap of *Streptomyces olivaceus* strain 115 in well diffusion method on lawn culture against *Candida albicans* (PTCC No. 5027). Clockwise from top: *S. olivaceus* strain 115; negative (solvent) control; Clotrimazole 1% (positive control) and blank well

isolates of soil actinomycetes from Kerman Province, southeast of Iran, were screened from which one isolate, *Streptomyces olivaceus* strain 115 showed *in vitro* activity against the tested pathogen. Figure 1 shows the well method bioassay result of the active strain and the controls.

The increasing prevalence of drug-resistant *C. albicans* is a major concern worldwide. Search for new antifungals especially from natural origins as plants and micro-organisms is a need for future therapeutic usages. In the present study, the most active isolate was found as *Streptomyces olivaceus* strain 115. Characterization of the biologically active principle is under investigation.

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