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***In vitro* Susceptibility of *Alternaria solani* to Several Iranian Soil Actinomycetes**

¹F. Sharifi, ²G.H. Shahidi Bonjar, ³S. Shafii Bafti, ⁴B. Barkhordar, ⁵S. Aghighi and ⁶M.J. Mahdavi

¹⁻⁵Department of Plant Pathology, College of Agricultural Sciences, Bahonar University of Kerman

⁶Iranian Academic Centre for Education, Culture and Research, Bahonar University Branch, Kerman, Iran

Abstract: *Streptomyces* sp. are of the most attractive sources of biologically active compounds. In the recent decades, they have attracted high interests as biocontrol agents. In search of finding such principles, *in vitro* suppression of *Alternaria solani* was studied by use of *Streptomyces* antagonists. *A. solani* was sensitive to several species mainly *Streptomyces plicatus*, *S. olivaceus* and two unidentified actinomycetes. Activity versus time was monitored in *S. olivaceus* in solid and shaken cultures. Ongoing goals of this research include isolation, characterization and identification of the active metabolites. Future goals include identification of active genes for use in development of recombinant DNAs in transgenic hosts bearing elevated resistance to infections by *Alternaria* sp.

Key words: *Alternaria solani*, *Streptomyces plicatus*, *Streptomyces olivaceus*, actinomycetes

INTRODUCTION

In near future, biological control of fungal pathogens will be of increasing importance for a more sustainable agriculture^[1]. The definition of biological control includes the plant induced or genetically modified to defend itself. It would be the most significant approach to plant health management in modern biotechnology in the 21st century. *Alternaria solani* is one of economically important plant pathogens with wide host range. Its polyphagous nature and ability of producing carcinogenic metabolites highlight it as potential danger to humans and animals health too^[2,3]. Use of Biocontrol potential of *Streptomyces* spp. against phytopathogenic *Alternaria* sp. has also been reported by other reseachers^[4-6]. Having especial environmental characteristics and being rich in actinomycete population, the microbiology of the Iranian soils has not been explored for active strains of these valuable biocontrol tools. In a preliminary screening, we attempted to isolate and study actinomycetes in restricted area of southeast of Iran. At the present research, *in vitro* suppressive reaction of soil actinomycetes was assayed against *A. solani*. In our screening *A. solani* was the most sensitive phytopathogen to actinomycetes isolates in contrast to others. High sensitivity of *A. solani* to actinomycetes antagonists confirmed our previous bioassays on *A. alternata*^[7]. These results form the avenue for production of agro-bioproducts from biologically active strains of actinomycetes for biocontrol of *Alternaria* infections.

MATERIALS AND METHODS

Culture media and preparation of pathogen: *Alternaria solani* (the tomato isolate, 555C and 557, Khoy, Iran) was kind gift from Dr. Zare, Mycology Laboratory, Institute Research of Plant Pests and Diseases, Tehran. The pathogen was maintained on potato dextrose agar (PDA, Difco- 39 gL⁻¹ PDA L⁻¹ of distilled H₂O, pH 7.2). Casein Glycerol Agar (CGA) was prepared to culture the actinomycetes. It was mixed from basic ingredients as described by Kuster and Williams^[8].

Screening of actinomycetes: Actinomycetes were isolated in pure cultures from soils with high humus and then, screened against the pathogen. The procedures were performed as used by Shafii^[9].

Antifungal bioassays: Antifungal activity of the active strains against *A. solani* and demonstration of mode of actions were performed as used by Shahidi Bonjar and Aghighi^[10]. According to the diameter of inhibitory zone, activity was rated as weak, moderate and strong in each case.

Monitoring activity in solid and shaken cultures: *Streptomyces olivaceus* strain 115 was grown in solid CGA medium and also submerged cultures of CG medium on rotary shakers under 130 rpm at 29°C. To monitor the activity, aseptically 6 mm agar disk plugs and small

aliquots of culture media were taken every 24 h for 30 days and the activity was evaluated by agar disk and well diffusion-methods^[11,12].

RESULTS

Antifungal bioassays: From tested actinomycete isolates, about one third of them were active in dual culture methods. Fig. 1 shows four antagonists and the suppressive reaction of *A. solani*. Fig. 2 shows bioassay results of *Streptomyces olivaceus* strain 115 against *A. solani* measured in well diffusion-method and agar disk-method. In both methods, *S. olivaceus* strain 115 show antifungal inhibitory effects on the pathogen.

Monitoring activity and growth curve: Activity reached maximum after 5 and 9 days in solid and rotary cultures, respectively. In shaken cultures, this interval was used to harvest cultures to prepare crude extract for use in further investigations. Activity versus post seeding time in solid media and rotary cultures is presented in Fig. 3.

DISCUSSION

Actinomycetes produce more than half of the world's antimicrobials and are consequently becoming valuable tools in the field of biological control. Antibacterial activity of the isolate found in this study highlights its importance as candidate for further investigation in



Fig. 1: Suppressive reaction of four actinomycete isolates on *Alternaria solani*. Clockwise from top: the antagonists No. 115, 44, 114 and 101. Center disk is *Alternaria* agar disk which its growth towards the antagonists is inhibited clearly

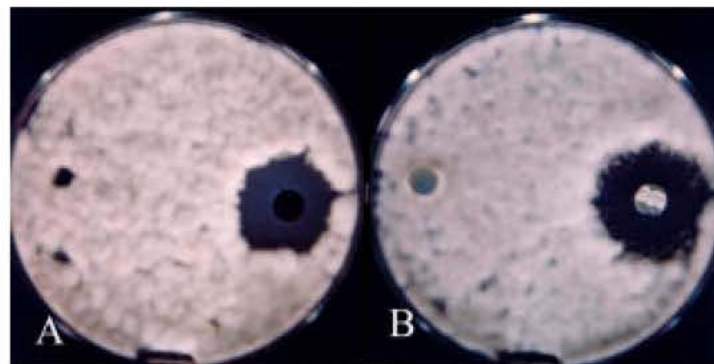


Fig. 2: Bioassay results of *Streptomyces olivaceus* strain 115 against *Alternaria solani* measured in two methods; A) Well Diffusion-Method, left: Solvent well (control, DMSO: Methanol solvent), right: *S. olivaceus* aqueous media at 6th day of growth; B) Agar disk-method, left: blank agar disk (control), right: *S. olivaceus* agar disk 4th day of growth, showing antifungal inhibitory effect on the pathogen

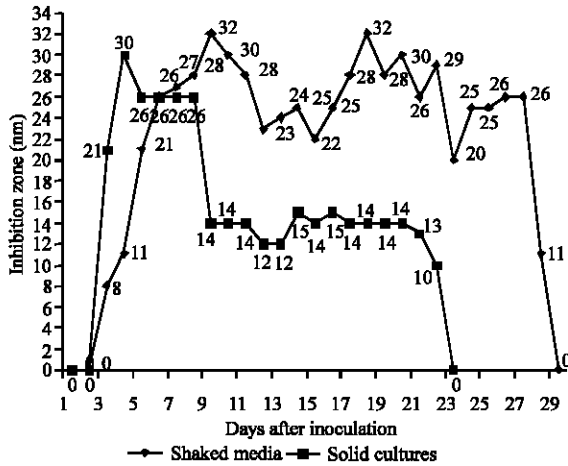


Fig. 3: *In vitro* bioactivity of *Streptomyces olivaceus* strain 115 against *Alternaria solani* in solid and rotary cultures indicative of production time versus inhibition zones

biological control of tested pathogen. In ideal biological control measures, proper microorganisms are those having well adaptation in the applied environment and exert effective antagonistic activity against target pathogens persistently. In this regard, beneficial soil-driven actinomycetes do not have adverse effect or alter the biological buffering of our environment as chemical measures do. They should receive higher attention in research for biological controls worldwide. Our findings represent the presence of potential antifungal metabolite (s) in Actinomycete strains against *A. solani*. Antifungal activity of the isolates found in this study highlights their importance as candidates for further investigation in biological control of tested pathogen. However, it is accepted that all laboratory screenings, such as the present one, can only provide a pre-selection of promising candidates^[13]. The future holds promise for greater emphasis on biocontrol to limit damage from diseases using also genetic engineering^[14]. Techniques are available to design microorganisms that produce the metabolites needed to effectively control pathogens. Successful application of biological controls requires more knowledge and intensive management^[15]. We believe that high susceptibility of *Alternaria* to tested Actinomycetes form the avenue for production of agrobioproducts from biologically active strains.

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