Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 5 (1): 5-8, 2006
ISSN 1682-3974

Antagonistic Potential of Two Native *Streptomyces* Strains in Biocontrol of the Major Causals of Common Scab of Potato in Iran

[1]M. Kalantar Zadeh, [2]G.H. Shahidi Bonjar, [3]P. Rashid Farrokhi, [4]A. Ghasemi, [5]S. Aghighi and [6]M.J. Mahdavi
[1,2,5]Department of Plant Pathology, College of Agricultural Sciences, Bahonar University of Kerman,
[3]Department of Agronomy and Plant Breeding, College of Agricultural Sciences,
Bahonar University of Kerman, [4]Plant Pests and Diseases Research Institute, Tehran,
[6]Iranian Academic Centre for Education, Culture and Research, Bahonar University Branch, Kerman

Abstract: *Streptomyces scabies* and *S. acidiscabies*, the two major phytopathogens induce potato common scab in potato growing areas of Iran. Soil Actinomycetes including 174 isolates were assayed for assessing antagonistic activity against *Streptomyces scabies* and *S. acidiscabies*. From tested isolates, *S. olivaceus*, strain 115 and *S. plicatus*, strain 101 showed high anti-scab activity revealed by bioassays in agar disk and well diffusion methods. For further biological characterizations, the active strains were grown in submerged cultures to determine growth curve and prepare crude extracts. Preliminary greenhouse studies indicated that amending soil with the *S. olivaceus*, strain 115 and *S. plicatus* strain 101 reduce crop losses due to the pathogens. Antibacterial activities of both antagonists were of bactericidal type on both pathogens with complete inhibitory effects.

Key words: *Streptomyces scabies, Streptomyces acidiscabies, Streptomyces olivaceus, Streptomyces plicatus*

INTRODUCTION

Common scab diseases of potato are of the important limitations of yield and quality in growing potato arias in infected fields of the world. Causal agents, several species of *Streptomyces*, that reduce yield and shorten storage durability of tubers have tuber and soil-borne nature. They cause more severe losses in monoculture farms mainly with clay soil and improper agricultural management. Many of management efforts have focused on using chemicals or fertilizers to alter the soil pH[1-3]. The classical control measures have not succeeded in eliminating the disease. The persistence of *Streptomyces* sp. in soil for years makes potato scab difficult to control. Many non-pathogenic *Streptomyces* demonstrate antagonistic characteristics against the pathogens. These criteria include production of different kinds of secondary metabolites and biologically active substances which some have high commercial values such as enzymes and antibiotics[4,5]. With the respect to their role in biological control of soil-borne pathogens, at the present research, 174 isolates of Actinomycetes were isolated from agricultural soils of kerman Province, Iran and screened against the two major *Streptomyces* strains inducing potato scab in Iran[6]. From tested isolates, 13 showed antagonistic effects against the pathogens. Two *Streptomyces* strains, 101 and 115, were the most actives which were revealed by *in vitro* studies.

MATERIALS AND METHODS

Microorganisms and culture media: *S. scabies and S. acidiscabies* the causal agents of potato scab disease in major potato growing areas of Iran were kind gifts from Plant Pests and Diseases Research Institute, Tehran Iran. The Pathogens were grown at 25-31 and maintained on Yeast Malt Extract Agar (YMEA) composed of Yeast extract, 4 g; Malt extract, 10 g; Glucose, 4 g and Agar, 20 g in 1 L[7]. All cultures stored at 4°C and sub-cultured as needed. Casein glycerol (or starch) agar (CGA) was used for screening and isolating of Actinomycetes which composed of: glycerol or soluble starch, 10 g; casein, 0.3 g; KNO_3, 2 g; NaCl, 2 g; K_2HPO_4, 2 g; $MgSO_4.7H_2O$, 0.05 g; $CaCO_3$, 0.02 g; $FeSO_4.7H_2O$, 0.01 g and Agar, 18 g in 1 L of distilled H_2O (pH 7.2)[8]. In submerged cultures, agar was excluded (CG medium). Actinomycete colonies with different morphologies were selected and transferred to CGA slants for further studies.

Soil culture procedure and isolation of streptomycete antagonists: For isolation of Actinomycetes, soil samples were collected from grasslands, orchards and vegetable

Corresponding Author: G.H. Shahidi Bonjar, Department of Plant Pathology, College of Agricultural Sciences, Bahonar University of Kerman, Iran Tel: 0913 342 0125

fields in different localities of Kerman province, Iran. Several samples randomly were selected from mentioned localities using an open-end soil borer (20 cm in depth, 2.5 cm in diameter) as described by Lee and Hwang[4]. Soil samples were taken from a depth of 10-20 cm below the soil surface. The soil of the top region (10 cm from the surface) was excluded. Samples were air-dried at room temperature for 7-10 days and then passed through a 0.8 mm mesh sieve and were preserved in polyethylene bags at room temperature before use. Samples (10 g) of air-dried soil were mixed with sterile distilled water (100 mL). The mixtures were shaken vigorously for 1 h and then allowed to settle for 1 h. Portions (1 mL) of soil suspensions (diluted 10^{-1}) were transferred to 9 mL of sterile distilled water and subsequently diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Inocula consisted of adding aliquots of 10^{-3} to 10^{-6} soil dilutions to autoclaved CGA (1 mL in 25 mL CGA) at 50°C before pouring the plates and solidification. Three replicates were considered for each dilution. Plates were incubated at 30°C for up to 20 days. From day 7 after, Actinomycete colonies were isolated on CGA, incubated at 28°C for one week and stored refrigerated as pure cultures before use. For screening studies, 174 pure Actinomycete isolates were collected and maintained refrigerated in stock.

***In vitro* bioassay for anti-*Streptomyces* activity:** From the refrigerated stocks, each Actinomycete isolate was smeared on CGA medium as a single streak and after incubation at 28°C for 4- 6 days, from well-grown streaks 6 mm agar disks of Actinomycete colony mass was prepared by using sterile cork borers with 6 mm in diameter. Disks were then aseptically transferred to YMEA plates having fresh lawn culture of *S. scabies* and *S. acidiscabies*. Controls included using plain disks from CGA medium. Plates were incubated at 29-31°C for 4-6 days and bioactivity was evaluated by measuring the diameter of inhibition zones (DIZ, mm)[8,9].

Classification of the active antagonists: From 13 active Actinomycete isolates two showed high antagonistic activity and their colonies were characterized morphologically and physiologically to the genus level following the direction mentioned in the methods manual of international cooperative project for description and deposition of cultures of *Streptomyces* (ISP)[10]. Identification procedures of the active isolates were done by Saadoun *et al.*, Department of Biological Sciences, University of Science and Technology, Irbid, Jordan as described by Saadoun and Gharaibeh[5].

Bioassays: To evaluate the antibacterial activity of *S. olivaceus* and *S. plicatus* against the pathogens, bioassays were performed in two ways: agar disk and well methods as used by Shahidi Bonjar[11] and Aghighi *et al.*[12]. Antibacterial activity around the *S. olivaceus* and *S. plicatus* agar disks or wells was close to the ratings applied by Lee and Hwang[4] and El-Tarabily *et al.*[13].

Monitoring activity: The antagonists *S. olivaceus* strain 115 and *S. plicatus* strain 101 were grown in CG medium on rotary shakers under 130 rpm at 30°C in submerged cultures. To monitor the activity, aseptically small aliquots of culture media were taken every 24 h for 25 days and the activity was evaluated by well diffusion-method[8] against lawn cultures of *S. scabies* and *S. acidiscabies* and antibacterial activity was measured as described. In solid cultures, active *S. olivaceus* strain 115 and *S. plicatus* strain 101 were grown in CGA as streaks and to monitor the activity, aseptically 6 mm agar disks were taken by sterile cork borer every day for 25 days and the activity was evaluated by agar disk-method[9] against lawn cultures of the pathogens and antibacterial activity was measured as mentioned.

Preparation of crude extracts: In submerged cultures, when the activity reached maximum, the cultures were harvested; spores and mycelia were excluded by filtration through two layers of cheese cloth. The clarified sap was then dried to dark crude under reduced air at 50°C and kept refrigerated for further studies.

Detection of bactericidal and/or bacteriostatic activity: Small blocks of inhibition zones (1 mm^3) of *S. olivaceus* strain 115 and *S. plicatus* strain 101 against *S. scabies* and *S. acidiscabies* were transferred to fresh YMEA plates and incubated for 7 days at 30°C. During incubation, growth or lack of growth of the two pathogens were investigated both visually and microscopically.

RESULTS

Screening and bioassays: In screening for Actinomycetes being antagonists of *S. scabies* and *S. acidiscabies*, 174 isolates of soil Actinomycetes of Kerman were screened from which 13 isolates showed antibacterial activity against the tested pathogens (Table 1).

Identification of active *Streptomyces* species: The isolates were determined as *S. olivaceus* strain 115 and *S. plicatus* strain 101. They had prominent activity against *S. scabies* and *S. acidiscabies* the causal agents of potato scab disease in potato growing areas in Iran.

Table 1: Screening results of active actinomycetes isolated from soils of Kerman Province with anti-bactericidal effects on *Streptomyces acidiscabies* and *S. scabies*

Actinomycete isolates No.	Inhibition Intensity on [a/b]	Inhibition Intensity on [a/b]	Actinomycete isolates No.
44	+/–	161	++/–
55	+/–	169	++/+
101	+++/+++	171	++/++
115	+++/+++	172	++/++
113	++/+	174	++/+
148	++/+	157	++/+
156	++/++	Control	–

[a]*Streptomyces acidiscabies*; [b] *S. scabies*

Scaling rates of inhibition intensities are as: no inhibition or pathogen growth not different from control (-); weak inhibition or partial inhibition of 5– 10 mm (+); moderate inhibition of 10-30 mm (+ +) and strong inhibition of > 30 mm (+ + +). Control includes plain agar disk in each bioassay.

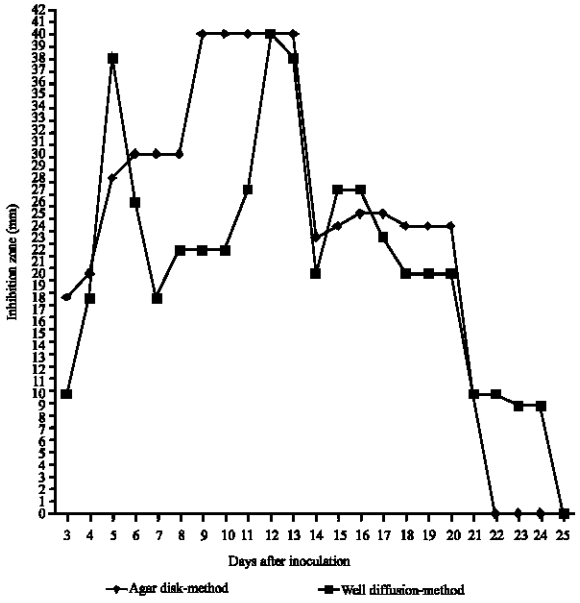

Fig. 1: Activity versus time in submerged and solid media cultures of *Streptomyces plicatus* strain 101 against *S. acidiscabies*

Monitoring activity: Activity versus time in submerged and solid media cultures of *Streptomyces plicatus* strain 101 against *S. acidiscabies* is indicated in Fig. 1. In solid media, the activity of strain 101 reached maximum on 5th and 12th days in tests against *S. acidiscabies* and maximized on 5th day against *S. scabies*. In rotary submerged cultures activity of strain 101 reached maximum at 5th, 12th and 13th days against *S. acidiscabies* and maximized at 5th and 9th days against *S. scabies*. In solid media, the activity of strain 115 reached maximum on 9th to 13th days against *S. acidiscabies* and maximized on 5th day against *S. scabies*. In rotary submerged cultures, activity of strain 115 reached maximum after 5th and 12th days against *S. acidiscabies* and maximized at 5th and 12th days against *S. scabies*. The submerged cultures at 5th day were harvested for preparation of crude extract for use for future investigations (Fig. 1).

Detection of bactericidal and/or bacteriostatic activity: After transfer of small blocks from inhibition zones to fresh medium, lack of growth of pathogens represented bactericidal properties of the antagonists.

DISCUSSION

S. scabies and *S. acidiscabies* are the principal casual agents of potato scab disease. Symptoms of disease range from superficial lesions to deep pits in the tubers that can eliminate tuber quality and marketability. Application of antagonist Streptomyces isolates to potato scab infected fields can effectively control reduction of crop values and could be significant in terms of safety and economy. The results may be considered for further studies of Actinomycete microflora in native Iranian soils with the goal to find new agents in biocontrol of potato scab disease[11]. An ideal and environmentally safe measure in control of potato scab in Iran is to amend the soil mix with selected antagonists. However, this requires investigation of conditions which favor the survival of the antagonists, because soil is very complex substrate in which numerous factors influence the number of microorganisms as well as the qualitative composition of its microflora. In this study, we attempted to isolate and study a preliminary screening of Actinomycetes in restricted area of Kerman Province, southeast of Iran and believe that the results of these findings can form the avenue for production of resistant transgenic-potato plants with recombinant DNA having anti-scab genes cloned from biologically active *S. olivaceus* strain 115 and *S. plicatus* strain 101.

ACKNOWLEDGMENTS

This research is dedicated to Mr. Ali Reza Afzalipour and Mrs. Fakhereh Saba, the founders of Universities in Kerman.

REFERENCES

1. Muncie, J.H., H.C. Moore, J. Tyson and E.J. Wheeler, 1994. The effect of sulphur and acid fertilizer on incidence of potato scab. Am. Potato J., 21: 293-304.
2. Loria, R., R.A. Bukhalid, B.A. Fry and R.R. King, 1997. Plant pathogenicity in the genus *Streptomyces*. Plant Dis., 81: 836-846.

3. Lapwood, D.H. and T.F. Hering, 1970. Soil moisture and the infection of young potato tubers by Streptomyces scabies (common scab). Potato Res., 13: 296-304.
4. Lee, J.Y. and B.K. Hwang, 2002. Diversity of antifungal actinomycetes in various vegetative soils of Korea. Can. J. Microbiol., 48: 407-417.
5. Saadoun, I. and R. Gharaibeh, 2002. The Streptomycetes flora of Jordan and its potential as a source of antibiotics active against antibiotic-resistant Gram-negative bacteria. World J. Microbiol. Biotechnol., 18: 465-470.
6. Fini, O., G.H. Khodakaramian and H. Rahimian, 2003. Phenotypic features and host range of the strains of *Streptomyces* inducing potato scab in Iran. Iran J. Plant Pathol., 39: 23- 26.
7. Schaad, N.W., J.B. Johns and W. Chun, 2000. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd Edn., Am. Phytopathol. Soc. Press. St Paul Minnesota, USA., pp: 242.
8. Dhingra, O.D. and J.B. Sinclair, 1995. Basic Plant Pathology Methods. CRC Press: USA., pp: 287- 296, 390- 391.
9. Acar, J.F. and F.W. Goldstein, 1996. Disk Susceptibility Test. In: 'Antibiotics in Laboratory Medicine (4th Edn)'. William and Wilkins Co.: Baltimore, pp: 1-51.
10. Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Intl. J. Syst. Bacteriol., 16: 313-340.
11. Shahidi Bonjar, G.H., 2003. *In vitro* monitoring of antibacterial properties of soil *Streptomyces*. Research Project Report. College of Agriculture, Bahonar University of Kerman, Iran.
12. Aghighi, S., G.H. Shahidi Bonjar and I. Saadoun, 2004. First report of antifungal properties of a new strain of *Streptomyces plicatus* (strainl 01) against four Iranian phytopathogenic isolates of *Verticillium dahliae*, a new horizon in biocontrol agents. Biotechnology, 3: 90-97.
13. El-Tarabily, K.A., M.H. Soliman, A.H. Nassar, H.A. Al-Hassani, K. Sivasithamparam, F. McKenna and G.E.St.J. Hardy, 2000. Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. Plant Pathol., 49: 573-583.