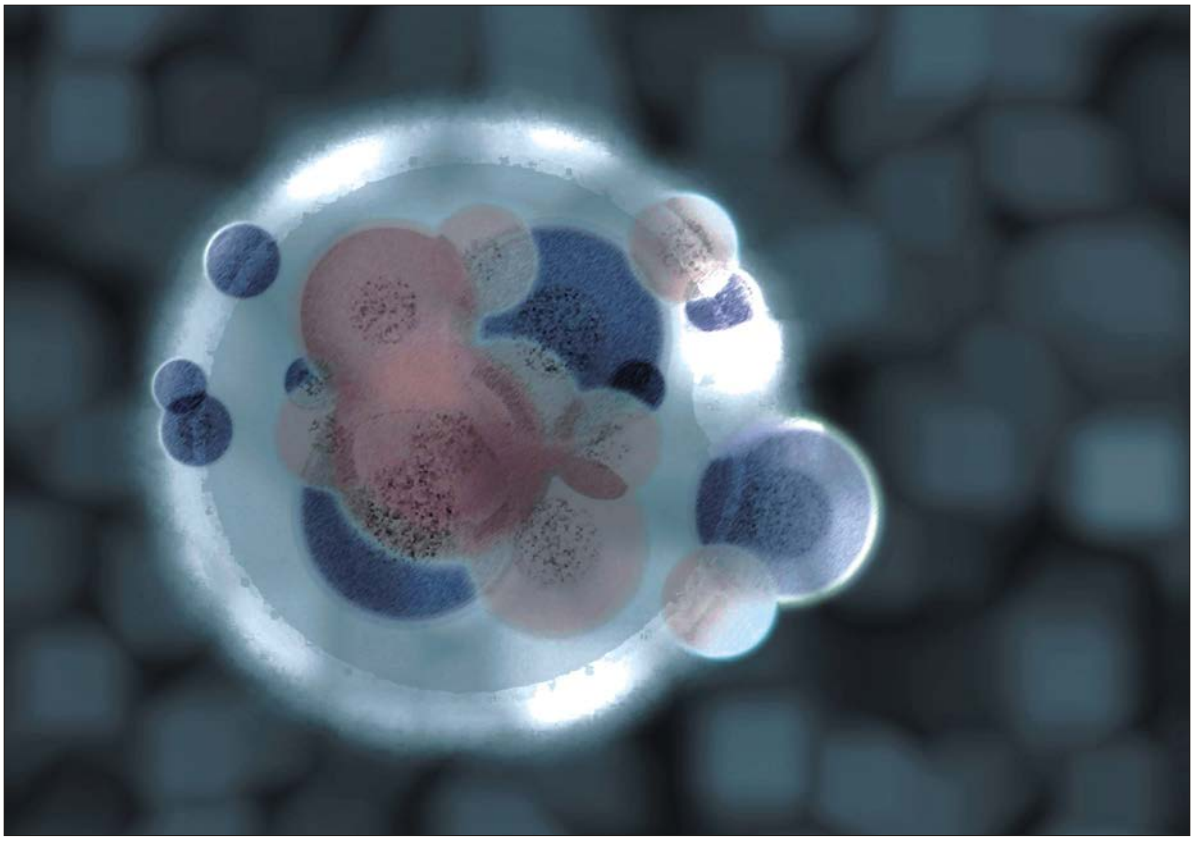

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Antibacterial Activity of Iranian *Streptomyces coralus* Strain 63 Against *Ralstonia solanacearum*

[1]G.H. Shahidi Bonjar, [2]S. Zamanian, [3]S. Aghighi,
[4]P. Rashid Farrokhi, [5]M.J. Mahdavi and [6]I. Saadoun
[1-3]Department of Plant Pathology, [4]Department of Agronomy and Plant Breeding,
College of Agricultural Sciences, Bahonar University of Kerman, Iran
[5]Iranian Academic Centre for Education, Culture and Research, Bahonar University Branch, Kerman, Iran
[6]Department of Applied Biology, Jordan University of Science and Technology, Irbid, Jordan

Abstract: *Ralstonia solanacearum* has worldwide economical importance because of its destructive and soil-borne nature. Clearly chemical measures have lost their attractiveness because of development of resistant strains and undesirable effects on our environment. Consequently, biological control of pathogens is gaining great importance worldwide. To investigate for proper biocontrol agents and to obtain antibacterial antagonists from Iranian soil Actinomycetes, a vast survey was performed. Over 170 isolates of soil Actinomycetes were isolated and screened among which one isolate showed high level of activity in Agar disk and Well diffusion methods against *R. solanacearum*. It was identified as *Streptomyces coralus* strain 63. High concentration of antibacterial agent was detected at 8-11th day in shake cultures. Longevity *in vitro* of the active crude in soluble state determined about 40 days at room temperature. In thermal inactivation point studies, active crude retained activity up to 93°C. Antibacterial activity of the antagonists found in this study highlights their importance as candidates for further investigation in biological control of tested pathogenic bacteria.

Key words: Antibacterial activity, *Ralstonia solanacearum, Streptomyces coralus*, longevity *in vitro*, thermal inactivation point, biological control

INTRODUCTION

Use of agrochemicals is falling into disfavor because of environmental pollution and detrimental effects on a variety of nontarget organisms. Potential use of natural products based biocontrol agents as soil supplements or replacement for agrochemicals has been addressed in many recent reports[1-6]. In search for biocontrol agents, bacteria as *Pseudomonas flourescens, Bacillus subtilis* and *Erwinia herbicola* Eh252 have shown activity against *Erwinia carotovora* subsp. *carotovora*[7-9]. Actinomycetes, by virtue of their wide distribution and filamentous growth in soil, colonize the root surface and the rhizosphere and exert inhibitory effect on pathogenic microorganisms via production of biologically active secondary-metabolites. *Streptomyces* is a well known genus of the Actinomycete family. They usually inhabit soil and commonly enhance soil fertility. These prokaryotes have characteristics which make them useful as biocontrol agents against bacterial plant pathogens[10]. Several investigators have reported that *in vivo* studies have documented satisfactory results in use of *Streptomyces* against some root pathogens. *Streptomyces* sp. strain 5406 has been used in China for the last 35 years to protect cotton crops against soil-borne pathogens[11]. At the present research, 200 isolates of Actinomycetes were isolated from different localities of fertile soils of Kerman Province, Iran and screened against *Ralstonia solanacearum*. Some biological properties of the main antagonist are presented. The active strain, identified as *Streptomyces coralus* strain 63, is under *in vivo* evaluation for further characterization of its active metabolites and use in development of biofertilizers and biobactericides.

MATERIALS AND METHODS

Pathogenic bacterium and culture media: *R. solanacearum* was kind gift of Prof. Rahimian, Department of Plant Pathology, College of Agricultural Sciences, University of Sari, Iran. This bacterium was rejuvenated on Nutrient Agar (NA) (Difco) at 27-29°C. Stock cultures stored at 4°C and sub cultured as needed. Casein Glycerin (or starch) Agar (CGA) was used for

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

screening and isolating of Actinomycetes as described by Kuster and Williams[12].

Isolation of actinomycetes: Soil samples were collected from grasslands, orchards and vegetable fields in different localities of Kerman Province, Iran. Details about preparation of soil cultures, isolation and purification of Actinomycetes is gathered and used by Zamanian[13].

Antibacterial bioassays and mode of action: Bioassays of the isolated Actinomycetes were performed in two methods: agar well-diffusion and agar disk methods and mode of actions were elucidated as performed by Zamanian[13].

Preparation of crude extract from shaked cultures: Active strain was grown in shaked cultures of CG medium under 130 rpm at 30°C. To monitor the activity, aseptically small aliquots of culture media were taken every 24 h for 37 days and the activity was evaluated by well diffusion-method[14,15]. To prepare crude extracts at 8 to 11th days of post inoculation which the activity reached maximum, the cultures were harvested and then 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 before use.

Taxonomy of active strain: The bacterial antagonist with strong activity was characterized following the direction mentioned in the methods manual of international cooperative project for description and deposition of cultures of *Streptomyces* (ISP)[16].

Thermal inactivation point: To detect the effect of temperature on antibacterial activity, small aliquots (10 mg mL^{-1}) of soluble crude were exposed to 30-140°C with five increments increase for 10 min at each and cooled on ice afterwards. Bioactivity of treated samples was evaluated using Well diffusion method. Control included incubation of an untreated sample at 26°C.

Shelf life of active crude: To measure the stability of the active crude in soluble state, 5 mg mL^{-1} samples were prepared in distilled water and placed in small vials. These samples were kept at room temperature and tested using Agar diffusion-method against the target bacterium at 14 days intervals throughout the period which activity persisted.

RESULTS

Isolation of actinomycetes: From tested soil samples, 170 pure cultures of Actinomycetes were isolated on CGA medium which were then used in antibacterial screening surveys.

Antibacterial bioassays and mode of action: In both bioassay methods, Agar well-diffusion and Agar disk methods, antibacterial activity of strain 63 was well revealed against *R. solanacearum* and mode of action was determined as bactericidal.

Taxonomy of actinomycetes: Active isolate was identified as *Streptomyces coralus* strain 63 as indicated in Table 1.

Thermal inactivation points: Active crude of *S. coralus* strain 63 retained antibacterial activity up to 93°C.

Shelf life of active crude: Stability of the active crude in distilled water at room temperature (12-30°C) was about 40 days for *S. coralus* strain 63 as assayed by using Agar diffusion-method against the pathogen.

DISCUSSION

It is well investigated that genetic engineering provides an opportunity to protect plants from bacterial diseases leading to reduction or limiting the use of synthetic bactericides. Clearly, the genes for antibacterial metabolites can be engineered into plants to increase the resistance of crop plants to pathogens attack and decrease the use of environmentally unfriendly hazardous chemicals. The major factor limiting the application of this technology is the identification and isolation of useful genes that code for antibacterial metabolites. Soil- driven Actinomycetes do not have adverse effect or alter the biological buffering of soils as chemical measures do, hence, they should receive higher attention in research for biological controls worldwide. In ideal biological control

Table 1: Taxonomic criteria of *Streptomyces coralus* strain 63

Morphological criteria							Sugar utilization							
Aerial mass color	Melanoid pigment	Reverse side pigment	Soluble pigment	Spore Chain	Spore surface	Reverse side color	Arabinose	Xylose	Inositol	Mannitol	Fructose	Rhamnose	Sucrose	Raffinose
R	+	+	+	RF	ND	Dark brown	-	+	+	+	+	+	+	+

R: red; -: Negative; +: Positive; RF: Rectus-Flixibilis; ND: Not determined

measures, proper microorganisms are those having well adaptation in soil and rhizosphere exerting effective antagonistic activity against soil pathogens persistently. In this regard, one possible approach to biological control of *R. solanacearum* is to inoculate soil with selected antagonists[17-20]. We believe that our research findings can be considered for further studies of Actinomycete microflora in native Iranian soils with the goal to find new agents in biocontrol of plant diseases. Antibacterial activity of the isolate found in this study highlights its importance as candidate for further investigation in biological controls.

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REFERENCES

1. Saadoun, I. and F. Al-Momani, 1997. Streptomycetes from Jordan soils active against *Agrobacterium tumefaciens*. Actinomycetes, 8: 24-36.
2. Saadoun, I., F. Al-momani, H. Malkaawi and M.J. Mohammad, 1999. Isolation, identification and analysis of antibacterial activity of soil Streptomycetes isolates from North Jordan. Microbios, 100: 41-46.
3. Shimizu, M., Y. Nakagawa, Y. Sato, T. Furumai, Y. Igaroshi, H. Onaka, R. Yoshida and H. Kunoh, 2000. Studies on endophytic Actinomycetes (I) *Streptomyces* sp. isolated from Rododendron and its antifungal activity. J. Gen. Plant Pathol., 66: 360-366.
4. 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.
5. Shahidi Bonjar, G.H., 2004a. Screening for antibacterial properties of some Iranian plants against two strains of *Escherichia coli*. Asian J. Plant Sci., 3: 310-314.
6. Shahidi Bonjar, G.H., 2004b. New approaches in screening for antibacterials in plants. Asian J. Plant Sci., 3: 55-60.
7. Vanneste, J.L. and D.A. Cornish, 1995. Development of a quantitative bioassay to study the interaction between biological control agents and soft rot Erwinia. http://www.hornet co.nz/publications/ nzpps/proceedings/95/95-34.htm
8. Vanneste, J.L., J.H. Perry, L.J. Perry-Meyer and R.J. Bedford, 1995. *Erwinia herbicola* Eh252 as a biological control agent of bacterial soft rot on potatoes. http://www.hortnet.con.nz/publications/ nzpps/proceedings/94/94-198.htm
9 Vanneste, J.L., J. Yu and D.A. Cornish, 1996. Biological control of bacterial soft rot. http://www. actohort.org
10. Keiser, T., M.J. Bibb, M.J. Buttner, K.F. Chater and D.A. Hopwood, 2000. General introduction to actinomycete biology. In: Practical *Streptomyces* Genetics'. The John Innes Foundation, Crowes, Norwich, England, pp: 1- 21.
11. Valois, D., K. Fayad, T. Barasubiye, M. Garon, C. Dery, R. Brzezinski and C. Beaulieu, 1996. Glucanolytic actinomycetes antagonistic to *Phytophthora fragariae* var. *rubi*, the causal agent of raspberry root rot. Applied Environ. Microbiol., 62: 1630-1635.
12. Kuster, E. and S.T. Williams, 1964. Selection of media for isolation of *Streptomyces*. Nature, 202: 928-929.
13. Zamanian, S., 2004. Investigation of antibacterial activity of several soil Actinomycetes of Kerman Province of Iran against *Ralstonia solanacearum* and *Erwinia carotovora* subsp. *carotovora*. M.Sc. Thesis, College of Agriculture, Bahonar University of Kerman, pp: 318.
14. Dhingra, O.D. and J.B. Sinclair, 1995. Basic plant pathology methods. CRC Press: USA., pp: 287-296, 390-391.
15. 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.
16. Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Intl. J. Sys. Bacteriol., 16: 313-340.
17. Roberts, M.A., 2002. Actinomycetes, biocontrol, questions and answers. http://www.palouse.net/ibs/ micro2.htm
18. Zamanian, S., G.H. Shahidi Bonjar and I. Saadoun, 2005. First Report of Antibacterial Properties of a New Strain of *Streptomyces plicatus* (strain101) against *Erwinia carotovora* subsp. *carotovora* from Iran. Biotechnology, 4: 114- 120.
19. Shahrokhi, S., G.H. Shahidi Bonjar and I. Saadoun , 2005. Biological Control of Potato Isolate of *Rhizoctonia solani* by *Streptomyces olivaceus* strain 115. Biotechnology, 4: 132- 138.
20. Shahidi Bonjar, G.H. and S. Aghighi, 2005. Chitinolytic and Microsclerostatic Activity of Iranian Strains of *Streptomyces plicatus* and *Frankia* sp. on Olive Isolate of *Verticillium dahliae*. Biotechnology, 4: 108- 113.