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Streptomyces strain P42 as a potent biological control against chilli anthracnose disease caused by *Colletotrichum* spp.

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ABSTRACT

A total of 130 actinomycetes was isolated from rhizospheres of healthy and diseased (*Colletotrichum*-infected) chilli plants and tested for their inhibitory activity against *Colletotrichum acutatum, Colletotrichum gloeosporioides* and *Colletotrichum truncatum*. Twenty-six isolates were active against at least one of the *Colletotrichum* species and 12 were active against all three anthracnose fungi. Four bioactive isolates, P8, P39, P42 and P115, that exerted high inhibitory activity against all three anthracnose fungi species, were identified using molecular methods. Phylogenetic analysis of the 16S rRNA gene sequences revealed that all four isolates belonged to the genus *Streptomyces*. Strain P42, which showed the highest inhibitory activity, was further tested as biological control agent in a greenhouse study. This strain successfully controlled chilli anthracnose disease by significantly reducing the disease severity. The results of the current study not only revealed that rhizosphere of *Colletotrichum*-infected chilli plants contained more bioactive streptomycete which are bioactive against *Colletotrichum* spp. than rhizosphere of healthy chilli plants, but also demonstrated the efficacy of biological control through soil suppression.

Key words : Anthracnose disease, chilli pepper, *Colletotrichum*, inhibitory activity, *Streptomyces*

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REFERENCES

Ahmad, F., Ahmad, I. and Khan, M. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.* **163** : 173-81.

Atlas, R. M. (2004). *Handbook of Microbiological Media.* CRC Press, United States.

Avin, F. A., Bhassu, S., Shin, T. Y. and Sabaratnam, V. (2012). Molecular classification and phylogenetic relationships of selected edible Basidiomycetes species. *Mole. Biol. Reports*. pp. 1-10.

Bakker, M. G., Glover, J. D., Mai, J. G. and Kinkel, L. L. (2010). Plant community effects on the diversity and pathogen suppressive activity of soil streptomycetes. *Appl. Soil Ecol.* **46** : 35-42.

Baltz, R. H. (2006). Marcel Faber Roundtable : Is our antibiotic pipeline unproductive because of starvation, constipation or lack of inspiration? *J. Indust. Microbiol. & Biotechnol.* **33** : 507-13.

Cai, L., Hyde, K., Taylor, P., Weir, B., Waller, J., Abang, M., Zhang, J., Yang, Y., Phoulivong, S. and Liu, Z. (2009). A polyphasic approach for studying *Colletotrichum. Fungal Diversity* **39** : 183-204.

Cao, L., Qiu, Z., You, J., Tan, H. and Zhou, S. (2005). Isolation and characterization of endophytic streptomycete antagonists of fusarium wilt pathogen from surface-sterilized banana roots. *FEMS Microbiol. Lett.* **247** : 147-52.

Crawford, D. L., Lynch, J. M., Whipps, J. M. and Ousley, M. A. (1993). Isolation and characterization of actinomycete antagonists of a fungal root pathogen. *Appl. and Environ. Microbiol.* **59** : 3899.

De, S. and Chandra, A. L. (1983). A-19, a new antifungal antibiotic. *Indian J. Exp. Biol.* **21** : 31-33.

Ezziyyani, M., Requena, M. E., Egea-Gilabert, C. and Candela, M. E. (2007). Biological control of *Phytophthora* root rot of pepper using *Trichoderma harzianum* and *Streptomyces rochei* in combination. *J. Phytopathol.* **155** : 342-49.

Getha, K. and Vikineswary, S. (2002). Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4 : Indirect evidence for the role of antibiosis in the antagonistic process. *J. Indust. Microbiol. and Biotechnol.* **28** : 303-10.

Hermosa, M., Grondona, I., Iturriaga, E. A., Diaz-Minguez, J., Castro, C., Monte, E. and Garcia-Acha, I. (2000). Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. *Appl. and Environ. Microbiol.* **66** : 1890-98.

Holt, J., Krieg, N., Sneath, P. and Staley, J. (1994). *Bergey's Manual of Determinative Bacteriology*. Williams and Wilkins, Baltimore, Maryland.

Ishikawa, J., Tsuchizaki, N., Yoshida, M., Ishiyama, D. and Hotta, K. (2000). Colony PCR for detection of specific DNA sequences in actinomycetes. *Actinomycetologica* **14** : 1-5.

Jones, C. R. and Samac, D. A. (1996). Biological control of fungi causing alfalfa seedling damping-off with a disease-suppressive strain of *Streptomyces*. *Biol. Control* **7** : 196-204.

Jukes, T. and Cantor, C. (1969). Evolution of protein molecules. In : *Mammalian Protein Metabolism,* Munro, H. (ed.). Academic Press, New York. pp. 21-132.

Khamna, S., Yokota, A. and Lumyong, S. (2009). Actinomycetes isolated from medicinal plant rhizosphere soils : diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. *World J. Microbiol. and Biotechnol.* **25** : 649-55.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. and Yi, H. (2012). Introducing EzTaxon-e : a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Systematic and Evolutionary Microbiol.* **62** : 716-21.

Koehn, F. E. and Carter, G. T. (2005). The evolving role of natural products in drug discovery. *Nature Rev. Drug Discovery* **4** : 206-20.

Labeda, D. and Goodfellow, M., Brown, R., Ward, A., Lanoot, B., Vanncanneyt, M., Swings, J., Kim, S. B., Liu, Z. and Chun, J. (2012). Phylogenetic study of the species within the family Streptomycetaceae. *Antonie van Leeuwenhoek* **101** : 73-104.

Labeda, D. and Shearer, M. (1990). Isolation of actinomycetes for biotechnological applications. In : *Isolation of Biotechnology Organisms from Nature,* Labeda, D. (ed.). McGraw Hill, New York. pp. 1-19.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In : *Nucleic Acid Techniques in Bacterial Systematics,* E. S. and M. G. (eds.). John Wiley & Sons, Chichester, United Kingdom. pp. 115-175.

Links, J., Rombouts, J. and Keulen, P. (1957). The 'Bulging Factor', a fungistatic antibiotic produced by a *Streptomyces* strain, with evidence of an active water-excreting mechanism in fungi. *J. General Microbiol.* **17** : 596.

Mazzola, M. (2002). Mechanisms of natural soil suppressiveness to soil-borne diseases. *Antonie van Leeuwenhoek* **81** : 557-64.

Mohamed, Z. (1982). Physiological and antagonistic activities of *streptomycetes* in rhizosphere of some plants (Egypt). *Egyptian J. Phytopathol.* **14** : 121-28.

Nayaka, S. C., Shankar, A. C. U., Niranjana, S. R., Prakash, H. S. and Mortensen, C. N. (2009). Anthracnose disease of chilli pepper. In : Asian Seed Health Centre (AsSHC) UoM, India & Danish Seed Health Centre for Developing Countries (DSHC), University of Copenhagen, Denmark (ed.). *Technical Bulletin.* Karnataka, India : Asian Seed Health Centre (AsSHC), University of Mysore, India.

Oskay, M. (2009). Comparison of *Streptomyces* diversity between agricultural and non-agricultural soils by using various culture media. *Scientific Res. and Essays* **4** : 997-1005.

Parmar, N. and Dufresne, J. (2011). Beneficial interactions of plant growth promoting rhizosphere microorganisms. *Bioaugmentation, Biostimulation and Biocontrol.* pp. 27-42.

Pelaez, F. (2006). The historical delivery of antibiotics from microbial natural products–Can history repeat? *Biochem. Pharmacol.* **71** : 981-90.

Saitou, N. and Nei, M. (1987). The neighbour-joining method : a new method for reconstructing phylogenetic trees. *Mole. Biol. and Evol.* **4** : 406-25.

Sariah, M. (1989). Detection of benomyl resistance in the anthracnose pathogen, *Colletotrichum capsici. J. Islamic Acad. Sci.* **2** : 168-71.

Satomi, T., Kusakabe, H., Nakamura, G., Nishio, T., Uramoto, M. and Isono, K. (1982). Neopeptins A and B, new antifungal antibiotics. *Agric. and Biol. Chem.* **46** : 2621-23.

Shirling, E. B. and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int. J. Systematic Bacteriol.* **16** : 313-40.

Sousa, C. S., Soares, A. C. F. and Garrido, M. S. (2008). Characterization of streptomycetes with potential to promote plant growth and biocontrol. *Scientia Agricola* **65** : 50-55.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007). MEGA4 : molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mole. Biol. and Evol.* **24** :1596-99.

Than, P. P., Prihastuti, H., Phoulivong, S., Taylor, P. W. J. and Hyde, K. D. (2008). Chilli anthracnose disease caused by *Colletotrichum* species. *J. Zhejiang Univ. Sci.* **B 9** : 764-78.

van Elsas, J. D., Garbeva, P. and Salles, J. (2002). Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. *Biodegradation* **13** : 29-40.

Wang, Y., Zhang, Z., Ruan, J., Wang, Y. and Ali, S. (1999). Investigation of actinomycete diversity in the tropical rainforests of Singapore. *J. Indust. Microbiol. & Biotechnol.* **23** : 178-87.

Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M. S. and Thomashow, L. S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. 1. *Ann. Rev. Phytopathol.* **40** : 309-48.

Xu, L., Li, Q. and Jiang, C. (1996). Diversity of soil actinomycetes in Yunnan, China. *Appl. and Environ. Microbiol.* **62** : 244-48.

Yeo, W. H., Kim, S., Kim, S. S., Yu, S. and Park, E. (1994). Isolation, physico-chemical properties and biological activity of new thiopeptide antibiotics, kimorexins. *J. Microbiol. and Biotechnol.* **4** : 349-53.

Yun, H. K., Ahmad, A. H., Muid, S. and Seelan, J. S. S. (2009). First report of *Colletotrichum* spp. causing diseases on *Capsicum* spp. in Sabah, Borneo, *Malaysia. J. Threatened Taxa* **1** : 419-24.