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Screening of Antagonistic Bacteria for Biological Control of Rice Diseases

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Preliminary screening for bacteria was done to be used for biological control of leaf spot of rice RD41 causing by *Curvularia* sp. and brown leaf spot of rice var RD41 causing by *Drechslera* sp. *Serratia marcescens* showed ability to inhibit the growth of *Curvularia* sp. and *Drechslera* sp. on NRA medium. Further study will be done to control mechanism of these biocontrol agents against *Curvularia* sp. in pot and field trial.

Keywords: *Serratia marcescent*, biological control, rice disease

Introduction

Rice is divided into *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia (Song and Goodman, 2001). It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize, according to 2014 FAOSTAT data.

State the problems encountered in rice production – insect and diseases used pesticides leading to toxic to human and environment, then the way to solve problem is to reduce or stop toxic chemical pesticides (International Year of Rice, 2004).

Curvularia lunata caused leaf spot for the first time in India, and that symptom showed brown leaf spot and finally blight. Moreover, it has been demonstrated that *C. lunata* caused many symptoms in rice e.g. grain discoloration, leaf spot, black kernel and seedling blight (Groves and Skolko, 1945).

Drechslera oryzae is the causal agent of brown leaf spot disease of rice (*Oryza sativa* L.). Brown spot is one of the important rice diseases in the world and is responsible for significant economic losses (Singh, 2005).

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The objective of this research study was preliminary evaluated *Serratia marcescens* LB01, a gram-negative bacterium classified as a member of the Enterobacteriaceae to control rice disease.

Materials and methods

Identification and characterization of antagonistic bacterium

The strain used in study was obtained from Assoc. Prof. Dr. kasem soytong. The isolate was morphological identified according to Bergey's Manual of Systematic Bacteriology (Grimont and Grimont, 2005).

Enzyme production property

Bacteria strain LB01 was tested for ability to produce extracellular degradative enzymes such as amylase, protease and lipase. Three replicates of each treatment were assayed and non-transferred plates served as negative controls.

Amylase- Starch agar medium was used and streak a drop of bacteria culture onto the starch agar plate, then incubated at 35-37^0 C for 18-24 h. When colonies were visible, it was flooded the plate with Lugol's iodine solution, then observed the clear zone surrounding the colony. If the starch was hydrolyzed by the excreted amylase, a clear zone around the bacterial colony was appeared. A blue or purple zone indicates that starch is not hydrolyzed (Harrigan and McCance, 1976).

Protease- Skim milk agar medium was prepared and streak on the plates, then incubated at 35-37 ℃ for 18-24 h., and observed the clear zone surrounding the colony. When colonies were visible and inspected the plates for clear zones around and below to observe caseinase implies positive reaction (Frazier and Rupp, 1928).

Lipase- Lipase activity test was determined by growing the isolates on Polysorbate 80 agar.Cultural characteristics observed after an incubated at 35-37 ℃ for 18-24 hours. A positive test was appeared as the occurrence of precipitated fatty acid crystals around the colony (Favero, 1967).

Testing property of bacteria against rice pathogen

Bi-culture test: Disease sample of rice was isolated using tissue transplanting technique and seed-borne fungi of rice were isolated by blotter technique by placing 10 seeds for surface sterilized with 0.5% sodium-hypochlorite solution for 3 min. and rinsed twice with sterile distilled water on to sterilized moist filter paper NO 4 in the Petri dish and kept at room temperature, then

periodically observed the fungal structure e.g. fruiting bodies, mycelia etc. under stereo microscope. The mycelia or fruiting body of fungi was transferred on to water agar (WA) for fungal growing for 1-2 days, then transferred onto potato dextrose agar (PDA) until get pure culture, then transfer to PDA slants and keep for further study. The most occurrence pathogen found on rice var RD41 was tested for its pathogenicity.

Antagonism test was performed on NRA on Petri dishes by the dual culture method (Fokkema, 1978). The mycelia plugs (5 mm. diameter) of pathogens from pathogen cultures were placed on the one side of a Pertri dish containing NRA medium, a full loop of bacteria streak at distance of 5 cm. away from the mycelia plug of pathogen isolate on the same dish. Paired culture was incubated at room temperature. The experiment was performed using Completely Randomized Design (CRD) with 4 replications.

Data was collected as colony diameter of pathogen (cm) and number of conidia in paired culture plate and in control plate.

The percent growth inhibition (PGI) was calculated using the formula (Korsten and De-Jager, 1995):

$$PGI\ (\%) = \frac{R1 - R2 \times 100}{R1}$$

R1 = colony or number of conidia in control.

R1 = colony or number of conidia in bi-culture plate.

Data was statistical computed analysis of variance (ANOVA) and treatment mean was be compared using Duncan Multiple's Range Test (DMRT) at P = 0.05 and P = 0.01.

Results

Identification of bacterial isolate

The isolate was short rods, Gram negative, motile and non-spore forming organisms. Colony on nutrient agar was opaque, red in color. *Serratia marcescens* LB01, a gram-negative bacterium classified as a member of the Enterobacteriaceae was confirmed.

Enzyme production property

The Efficacy of isolate LB01 for ability to produce extracellular degradative enzymes was tested. Results showed that LB01 produced amylase and lipase. With this, resulted plates showed clear zones around colonies.

Bi-culture test

Result from the dual culture assay showed LB01 actively against the mycelia growth of *Curvularia* sp. and *Dreschera* sp. in NRA for 13 days.

Discussion

The results showed *Serratia marcescens* LB01 was confirmed by morphologically identification. With this, the bacteria was short-rod shape, motile, gram negative bacteria in their reaction. This was similar to Giri *et al*. (2004).

The research finding was clearly demonstared that *Serratia marcescens* LB01 found to be antagonistic to *Curvularia* sp. and *Drechslera* sp. Several reports are available about the antagonistic effect of *S. marcescens* against various fungal pathogens (Parani *et al*., 2011; Kamensky *et al*., 2003; Jaiganesh *et al*., 2007; Queiroz and Melo, 2006; Khaldi *et al.*, 2015).

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References

Crop/Regions/World list/Production Quantity (pick lists), Rice (paddy). (2014). UN Food and Agriculture Organization, Corporate Statistical Databases (FAOSTST). 2017. Retrieved 11 May 2017. www.fao.org/faostat/en/#data/QC.

Favero M.S., (chm.). (1967). Microbiological sampling of surfaces, Biological Contamination Control Committee, AmericanAsso.For Contamination Control.

Fokkema, N.J. (1978). Fungal antagonism in the phylophere. Ann. Appl. Biol. 89, 115-117.

Frazier WC. and Ripp P. (1928). Studies on the proteolytic bacteria of milk I. a medium for the direct isolation of caseolytic milk bacteria. J Bacteriol. Jul;16(1), 57-63.

Giri AV, Anandkumar N, Muthukumaran G and Pennathur G. (2004). A novel medium for the enhanced cell growth and production of prodigiosin from Serratia marcescens isolated from soil, BMC Microbiol, 4(11): 2-14.

Grimont, F. and Grimont, P.A.D. (2005). Genus XXXIV Serratia Bizio. In: Garrity, G.M., Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds) *Bergey's Manual of Systematic Bacteriology, The Gammaproteobacteria*. Springer, New York, pp. 799-811.

Groves, J.W. and Skolko, A.J. (1945). "Notes on seed borne fungi, III. Curvularia." Can J Res., C. 23:94-104.

Harrigan W. and McCance M. (1976). Laboratory Methods in Food and Dairy Microbiology, Academic Press Inc. (London) Ltd.

International Year of Rice. (2004). "Rice and water: a long and diversified story". (Gramene Reference ID 8372).

Jaiganesh V, Eswaran A anda Balabaskar P, Kannan C. (2007). Antagonistic activity of *Serratia marcescens* against *Pyricularia Oryze*. Not Bot Hort Agrobot Cluj. 35: 48-54.

Kamensky M, Ovadis M, Chet I, Chernin L. (2003). Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. Soil BiolBiochem. 35: 323-331.

Khaldi R. El., Daami-Remadi M., Hamada W., Somai L. and Cherif M. (2015). "The Potential of *Serratia marcescens*: An Indigenous Strain Isolated from Date Palm Compost as Biocontrol Agent of *Rhizoctonia solani* on Potato". J Plant Pathol Microbiol S3: 006.

Korsten, L. and E.S. De Iager. (1995). Mode of action of Bacillus subtilis for control of avocado postharvest pathogens. S. Afr. Avocado Growers Assoc. Yearb, 18, 124-130.

Parani K, Shetty GP, Saha BK. (2011). Isolation of Serratia marcescens SR1 as a source of chitinase having potentiality of using as a biocontrol agent. Ind. J Microbiol. 51(3):247-250.

Queiroz B.P.V., Melo I.S. (2006). Antagonism of *Serratia marcescens* towards *Phytophthora parasitica* and its effects in promoting the growth of citrus. Braz. J. Microbiol. 37:448–450.

Singh R.S. (2005). Plant Disease (8th edition). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 439-444.

Song, F., Goodman, R.M. (2001). Molecular biology of disease resistance in rice. Physiol. Mol. Plant Pathol. 59, 1-11.

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