AGRIVITA Journal of Agricultural Science. 2020. 42(1): 160–167

AGRIVITA
Journal of Agricultural Science
www.agrivita.ub.ac.id

Rhodospirillum centenum, A New Growth Stimulant and Antagonistic Bacteria Against Leaf Spot of Rice Caused by *Curvularia lunata*

Rungrat Vareeket*) and Kasem Soytong

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

ARTICLE INFO

Keywords:
Curvularia lunata
Growth stimulate
Leaf spot of rice
Photosynthesizing bacteria
Rice

Article History:
Received: March 4, 2019
Accepted: January 18, 2020

*) Corresponding author:
E-mail: rungrat.kmitl@gmail.com

ABSTRACT

The research objectives were to find *Rhodospirillum* spp. to promote plant growth and as antagonists to control leaf spot of rice. The results showed that rice var. RD41 and Pitsanulok 2 (PL2) had leaf spot caused by *Curvularia lunata. R. centenum* is gram negative, mobile cell motility and negative of gelatin activity test. SM41 and 61 showed glucose and lactose and/or sucrose fermentation activity, but SM72 and 92 were non-fermented activity. Isolates of *R. centenum* were antagonized *C. lunata*, a leaf spot pathogen. *R. centenum* expressed for ability to produce amylase, protease and lipase. *R. centenum* isolates SM41, SM61, SM72, and SM92 with *C. lunata* causing leaf spot and seed-borne fungus. In addition, the inoculated seeds var. RD 41 and Pitsanulok 2 (PL2) applied *R. centenum* 41, 61, 72 and 79 gave significantly better seed germination, shoot and root length. Isolate SM41 reduced disease incidence in rice var. RD41 which the disease index was on level 2 when the inoculated one was level 5. Isolate SM61 showed the disease index of level 2 but the inoculated control was level 5. It is the first report that *R. centenum* plays the role for growth stimulant and biological control agents against leaf spot of rice caused by *C. lunata*.

INTRODUCTION

Rice (*Oryza sativa* L) is the most widely consumed as staple food for a large part of the world's human population, especially in Asia. It is the food commodity with the third-highest worldwide production, after sugarcane and maize, according to 2015 FAOSTAT data. Rice is the most important crop of human nutrition and caloric intake, providing more than one-fifth of the calories consumed worldwide by human (Smith, 1998). Rice is served to be one of the staple foods to increase in world population, especially in Asia. There are more than 70 diseases caused by pathogene have been recorded on rice (Manandhar, Jørgensen, Mathur, & Smedegaard-Petersen, 1998). The important problem encountered in rice production concerning disease and insect pests, especially diseases caused by *Curvularia lunata* (leaf spot) and *Pyriculria oryzae* (blast) etc., leading to yield loss, low quality and quantity. These diseases are the limiting factors to affect rice cultivation, causing annual yield losses which estimated at 5% (Song & Goodman, 2001).

C. lunata is an invader of monocotyledon plant (Domsch, Gams, & Anderson, 1980), the most common ones are rice, barley, wheat, maize and sorghum (Fakhrunnisa, Hashmi, & Ghaffar, 2006; Pitt & Hocking, 1997). In Thailand, *C. lunata* is one of the most prevalent pathogen in paddy rice fields, where infection of 28% in the fields and 3% of all grains (Pitt et al., 1994). Leaf spot disease becomes the most common symptom of rice diseases caused by phytopathogenic fungi, especially *C. lunata*. The symptoms are recognized by circular necrotic lesion on rice leaves from seedlings until mature stage. Rice infected with leaf spot pathogen can significantly affect yield and productivity. This disease damages rice yield and reduces seed

ISSN: 0126-0537 Accredited First Grade by Ministry of Research, Technology and Higher Education of The Republic of Indonesia, Decree No: 30/E/KPT/2018

Cite this as: Vareeket, R., & Soytong, K. (2020). *Rhodospirillum centenum*, a new growth stimulant and antagonistic bacteria against leaf spot of rice caused by *Curvularia lunata*. *AGRIVITA Journal of Agricultural Science*, *42*(1), 160–167. https://doi.org/10.17503/agrivita.v41i0.2181

quality and usually infects rice plants before and after harvest. The disease severity depends on the surrounding environmental and pathogen race (Thavong, 2002).

The farmers have been applied chemical fungicides for years and faced the pathogens become resistant to those chemical fungicides and much disease epidemic throughout the rice production areas. The effective microbial antagonists are proved to solve these problems. Recently, Tongon & Soytong (2015) stated that nanoparticles obtained from *Chaetomium* sp gave a good control leaf spot of rice caused by *C. lunata*.

Photosynthetic bacteria become widely applied in Asia for various biotechnological applications in agriculture. It is used for water purification, bioremediation of chemical, wastewater purifiers, animal feed and aquaculture supplements, biological hydrogen production and coenzyme Q production, and bio-fertilizers for plant growth etc. The purple nonsulfur bacteria are the most study and being diversed group of the phototrophic bacteria. The purple nonsulfur bacteria are member of *Rhodopseudomonas, Rhodobacter* and *Rhodospirillum* which known to eliminate the H_2S odor nuisance from the facultative ponds of waste ponds by oxidizing to sulfur or sulfate (Kim et al., 2004; Tadesse, Isoaho, Green, & Puhakka, 2003; Veenstra, Al-Nozaily, & Alaerts, 1995). *Rhodospirillum* spp. are gram-negative bacteria, motile, spiral-shaped bacteria. They can be grown in many condition including aerobic or anaerobic environment. Anaerobically, the bacteria grow in fermentation to produce energy as well as photoautotrophic growth. They can be found in marine environments and also in mud soil where light is available for photosynthesis. *Rhodospirillum centenum* can form swarm colonies that rapidly migrate toward or away from light, it depends on the wavelength of excitation which using surface-induced lateral flagella, chemotaxis, and a photosynthetic apparatus (Jiang, Rushing, Bai, Gest, & Bauer, 1998). It is reported to be an anoxygenic photosynthetic bacterium that capable to differentiate to several cell types. When it grows in phototrophically in liquid condition, cells exhibit a vibrioid shape and be a single polar flagellum. But when it grows on a solid surface, *R. centenum* is differentiated to rod-shaped swarm cells that appear numerous lateral flagella (Berleman & Bauer, 2004). *R. centenum* is a photosynthetic non-sulfur purple bacterium that grows well in an anoxygenic, photosynthetic N_2-fixing environment. It is emerging as a genetically amenable model organism for molecular genetic analysis of cyst formation, photosynthesis, phototaxis, and cellular development (Lu et al., 2010). Rana, Meikap, Mondol, Bose, & Mandal (2016) stated that *Rhodospirillum rubrum* is a good mineral solubilizing and plant growth promoting activities on fly-ash. It is the best biofertilizer which has ability in co-operated on fly-ash leading to highest Vigor-Index (990) value. Plant hormones from biomass of the purple non-sulfur bacterium, *R. rubrum* is reported to isolate and found bioactive compounds which expressed high physiological activities (300–330%) in the cytokinin bioassay. Three cytokinins were also found in the cells of *R. rubrum*, one of these identified as 6-(4-hydroxy-3-methyl-2-trans-2-bytenylamino)-9-β-D-ribofuranosylpurine zeatinriboside (Serdyuk, Smolygina, Kobzar, & Gogotov, 1993).

The researcher want to isolate rice leaf spot pathogen and photosynthesizing bacteria, screening for enzyme production and antagonistic activities against leaf spot pathogen, so the objectives of this research were to find *Rhodospirillum* spp. to promote plant growth and as antagonists to control leaf spot of rice.

MATERIALS AND METHODS

Sample Collection, Isolation and Identification

The research was conducted at Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand during 2016-2018. The samples were collected in the rice fields at Chachengsao province, Thailand. The disease samples of leaf spot of rice var. RD41 and Pitsanulok 2 (PL2) were collected and isolated to pure culture using tissue transplanting technique followed the method from Soytong (1992). Pure culture of pathogen was proved pathogenicity in each rice variety by following Koch'Postuate method. The samples were collected from rice fields in Samutprakan, Thailand and brought to the laboratory. The samples were then isolated to be pure culture by dilution plate method. It is diluted and inoculated to soft-agar tubes G-5 medium that consisted of peptone (5.0 g), yeast extract (5.0 g), L-glutamic acid (4.0 g), malic acid (3.5 g), KH_2PO_4 (0.12 g), and K_2HPO_4, (0.18 g) which modified

Copyright © 2020 Universitas Brawijaya

from Kohlmiller Jr. & Gest (1951). The tubes were incubated under anaerobic condition with the illumination level of 1,000 until 1,500 lux at room temperature (28-30°C) for 7-12 days. Then, a full loop of pinkish, reddish or brownish culture both was streaked onto G-5 agar and incubated for 7 days. Each single colony was transferred to new plate and subsequently culture until obtain pure culture.

Species of bacteria were morphological identified by followed *Bergey's Manual of Systematic Bacteriology* (Imhoff & Trüper, 1989). Gram-reaction, mortality, shape and color of colony were determined using light microscopy and scanning electroscopy including gelatin hydrolysis.

Molecular phylogenic identification was done using 16S rDNA amplification. Genomic DNA of the bacterium was extracted using Alkaline Lysis Method according to manufacturer's protocol (Sambrook, Fritsch, & Maniatis, 1989). The primers used for 16S rDNA gene region amplification were 557F (5'-CGCACCTGGACTGGAC-3') and 750R (5'-CCCATGGTCCAGCGCCAGAA-3'). The PCR products were analyzed by electrophoresis with 1% agarose gel in 1xTAE buffer at 100 V for purity and size of approximately 500 bp. Nucleotide sequence and phylogenetic analysis was done by purification. The PCR products were subjected to sequence and compared with those in the National Center for Biotechnology Information (NCBI) Genbank using the BLAST program. The phylogenetic tree was constructed using MEGA6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

Enzyme Production Properties

The bacterial isolates coded as SM41, SM61, SM72, SM92 and SM93 were tested for ability to produce extracellular degradative enzymes such as amylase, protease and lipase. Completely Randomized Design (CRD) was performed with three replication. Each treatment were assayed and non-transferred plates served as negative control. Amylase activity was done as follows: starch agar medium was streaked the bacterial culture by a drop of bacterial suspension onto the starch agar plate, then incubated at 35-37°C for 18-24 h. When colonies were visible, then flooded the plates with Lugol's iodine solution, and observed the clear zone surrounding the colony. If the starch was hydrolyzed by the excreted amylase, a clear zone around the bacterial colony appeared. A blue or purple zone indicated that starch was not hydrolyzed (Harrigan & McCance, 1976). Protease evaluation was used skim milk agar medium, streaked bacterial culture on the plates, incubated at 35-37°C for 18-24 h, then observed the clear zone surrounding the colony. When colonies were visible, then observed and the plates for clear zones around that implies positive reaction. Lipase activity test was determined by growing the isolates on Polysorbate 80 agar. Cultural characteristics were observed after incubated at 35-37°C for 18-24 h. A positive test appeared as the occurrence of precipitated fatty acid crystals around the colony.

Screening of Antagonistic Bacteria Against Leaf Spot Pathogen

Dual culture assay for *in vitro* inhibition of mycelial growth of *Curvularia* sp. was done with antagonistic bacteria isolates. *Curvularia* sp. was cultured on potato dextrose agar plates at 25°C for five days, then a mycelial disc was cut and placed in the center of the petri plate containing nutrient agar medium. Bacterial isolates were transferred to medium at a distance of 1.5 cm from the opposite edge of the same plate, then inhibition zones were measured after incubation at 25°C for 7 days.

Photosythesizing Bacteria for Stimulating Plant Growth and Disease Control

Seeds of RD41 and Pitsanulok 2 (PL2) were surface sterilized with 95% ethanol for 5 minutes, then with 2% sodium hypochlorite for 2 minutes, and rinsed thoroughly in sterilized distilled water, air dried for 1 h, then inoculated with pathogen by soaked into spore suspension of 1 x 10^6 spore/ml for 20 minutes before testing. All bacteria isolates were grown in nutrient (NA) medium. Bacterial cells were washed twice with sterile distilled water, and the inoculation density of the bacterial suspension was adjusted to 1 x 10^6 cells/ml. Seeds were inoculated by overnight (more than 12 h) by soaking with suspension of bacteria (10^7 cfu/ml). The soaked seeds in sterilized distilled water were used as the control. The research was designed as two factors experiment in Completely Randomized Design (CRD) and four replications. Factor A was rice varieties (RD41 and Pitsanulok 2 (PL2)) and Factor B represented bacterial isolates SM41, SM61, SM72, and SM92. Each treatment was ten seeds that put in sterilized Petri dishes containing filter paper (Whatman # 102). Seed germination was examined in 3 days after inoculation, and the root and shoot lengths of the germinated rice

Copyright © 2020 Universitas Brawijaya

seedlings were measured after 7 days incubation at 25°C beginning with a cycle of 10 h dark followed by 14 h of light fluorescent.

Statistical Analysis

The emergence of seedling was calculated with the following formula:

$$\text{Emergence (\%)} = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds grown}} \times 100$$

Data were computed analysis of variance and treatment means were compared using Duncan's Multiple Range Test at P = 0.05 and 0.01 by Statistical Package for Social Sciences (IBM SPSS Statistics, ver. 21.0) software program.

RESULTS AND DISCUSSION

Identification Result of Rice Leaf Spot Pathogen and Photosynthesizing Bacteria

Based on the results showed that leaf spot var. RD41 and Pitsanulok 2 (PL2) caused by *Curvularia lunata* and both isolates were proved to be aggressive isolates causing leaf spot in each rice variety. Similar results were reported by Tann & Soytong (2017) found brown leaf spot caused by *C. lunata* of rice variety IR66 in Cambodia. Four bacterial isolates coded as SM41, SM61, SM72, SM92 were preliminary identify by morphological characters. These isolates were cultured in soft-agar tubes of G-5 medium. The bacterial isolates of SM41, SM61, SM72 and SM92 were morphological and physiological identified as red and orange colonies using *Bergey's Manual of Systematic Bacteriology* (Imhoff & Trüper, 1989) as seen in Fig. 1. Physiological characteristics of cultures SM41, SM61, SM72 and SM92 were studied. All isolates were gram negative, mobile cell motility and negative of gelatin activity test. TSI activity revealed that isolates SM41 and SM61 expressed glucose and lactose and/or sucrose fermentation activity but isolates SM72 and SM92 were non-fermented activity (Imhoff & Trüper, 1989).

Scanning electron microscopy was shown in Fig. 2. The bacterial isolates coded as SM41, SM61, SM72 and SM92 were used for phylogenic analysis. The 16S rRNA gene sequence analysis showed 100% similarity with *Rhodospirillum centenum*. Moreover, the sequences of the SM41, SM61, SM72 and SM92 and closely related isolates were aligned using MEGA6 and phylogenetic analysis exhibited that the isolate SM41, SM61, SM72 and SM92 were also aligned within the same clade with *Rhodospirillum centenum* (Fig. 3) which supported the Blast search result. As result, Berleman & Bauer (2004) reported that *R. centenum* is an anoxygenic photosynthetic bacterium. It can grow in phototrophically liquid, a single polar flagellum expressed heat tolerance and drought resistance.

Enzymatic Ability of Photosynthesizing Bacteria Against Leaf Spot Pathogen

The photosynthesizing bacterium, *Rhodospirillum centenum* for ability to produce extracellular degradative enzymes was evaluated. All isolates were proved to produce amylase, protease and lipase. Anderson & Fuller (1969) stated that *Rhodospirillum rubrum* produced ribulose 1,5-diphosphate carboxylase which subjected to control the facultative photoauthotroph. Rana, Meikap, Mondol, Bose, & Mandal (2016) reported that *R. rubrum* can be used as green fertilizer for agriculture.

In addition, the result test for antagonistic activity against *C. lunata* showed that all isolates of *Rhodospirillum centenum* showed the inhibition zone in dual culture assays after 10 days. Yet in controls were no inhibition zone. It was shown that the number of spore in dual culture plates were higher than in control plates. Similar result was reported by Vareeket & Soytong (2017) which phytosynthesize bacteria expressed to inhibit *C. lunata* causing rice leaf spot.

Photosynthesizing Bacteria for Stimulating Plant Growth and Disease Control

Rhodospirillum centenum isolates SM41, SM61, SM72, and SM92 are photosynthesizing bacteria which were tested to inoculate seed germination of rice var. RD41 with *C. lunata* causing leaf spot and seed-borne fungus of rice var. Pitsanulok 2 (PL2). The results showed that the inoculated seeds var. RD 41 and applied *R. centenum* 41, 61, 72 and 79 gave significant better seed germination shoot and root length than the non-treated control. Similar report by Rana, Meikap, Mondol, Bose, & Mandal (2016) stated that *Rhodospirillum rubrum* increased rice seed germination, sprout growth promoting ability. The inoculated seeds var. RD 41 with *C. lunata* and applied *R. centenum* isolates 41, 61 and 71 gave the highest significance in seed germination, followed by isolate SM92 when compared to the non-treated control.

Copyright © 2020 Universitas Brawijaya

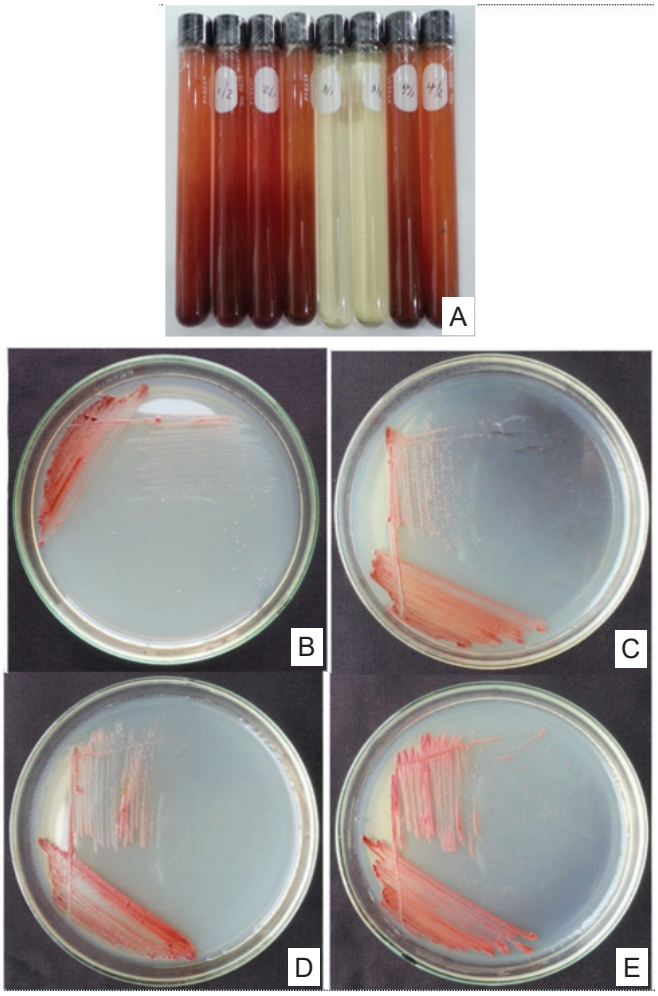

Fig 1. *Rhodospirillum centenum* isolates: A. Bacteria isolates in media slant; B. SM41; C. SM61**;** D. SM72; E. SM92

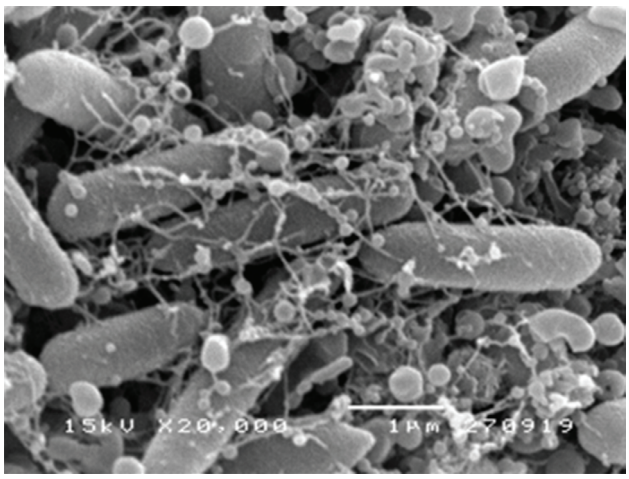

Fig 2. SEM image of *Rhodospirillum centenum*

Copyright © 2020 Universitas Brawijaya

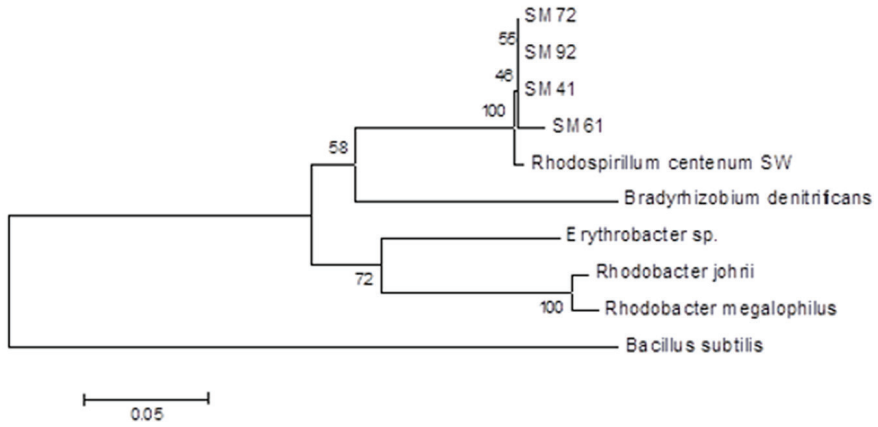

Fig 3. Amplified 16S rRNA gene fragment from the isolated SM41, SM61, SM72 and SM92 were sequenced and blast searched through NCBI database. Closely related sequences were downloaded and aligned using MEGA6. The isolates of SM41, SM61, SM72 and SM92 are presented in the same clade with *Rhodospirillum centenum*

Table 1. Effect of *Rhodospirillum centenum* on plant growth and disease control

Rice variety	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Disease index[2]
RD41	Control	28c[1]	3.63d	3.11d	5
	SM41	45a	4.65a	7.27a	2
	SM61	42a	4.11b	5.96ab	4
	SM72	40ab	3.70 cd	5.31c	4
	SM92	42a	4.34c	6.35b	3
PL2	Control	25c	2.00d	1.70d	5
	SM41	44a	4.83a	7.28a	3
	SM61	42a	4.61a	5.62c	2
	SM72	42a	4.08b	6.58b	4
	SM92	35 ab	4.63ab	5.10c	4

Remarks: [1] Means of four replications, means followed by a common letters are not significantly different by DMRT at P=0.01. [2] Disease index: 1 = 1-20% disease incidence, 2 = 21-40% disease incidence, 3 = 41-60% disease incidence, 4 = 61-80% disease incidence and 5 = 76 -100% disease incidence

The research found that the inoculated seeds var. RD 41 incorporated with *R. centenum* isolates SM41, SM61, SM72, and SM92 significantly gave better shoot and root length than the non-treated control (Table 1). Isolate SM41 incorporated with rice var RD41 and isolate SM61 mixed with rice var. PL2 were the lowest disease incidence when compared to the other isolates and inoculated control. Rana, Meikap, Mondol, Bose, & Mandal (2016) stated that some isolate of *Rhodospirillum rubrum* had ability to decrease disease incidence in rice. The other report for biocontrol of rice leaf spot by Tann & Soytong (2016) who found that nano-metabolites derived from *C. cupreum* gave a good control *C. lunata* causing leaf spot of rice var. Sen Pidao in Cambodia. The research proved that *R. centenum* as a new promising biocontrol agent and bio-stimulant for the first time in rice growth.

CONCLUSION

Leaf spot pathogen (*C. lunata*) of rice var. RD41 and Pitsanulok 2 (PL2) proved to be supressed by *Rhodospirillum centenum*. *R. Centenum* was identified by morphological and molecular phylogeny. It was proved to produce amylase, protease and

Copyright © 2020 Universitas Brawijaya

lipase act as control mechnism. Isolate SM41 and Isolate SM61 gave a good result to reduce leaf spot incidence and promote plant growth. *R. centenum* is a new growth stimulant and antagonistic bacteria against leaf spot of rice caused by *Curvularia lunata.*

ACKNOWLEDGEMENT

It is a part of Ph.D. thesis in Agriculture. I would express my sincerely thank Mr. Boonmee Ruengrat from Strong crop Co. Ltd, Thailand through Association of Agricultural Technology in Southeast Asia (AATSEA) to offer my study for Ph.D. scholarship.

REFERENCES

Anderson, L. E., & Fuller, R. C. (1969). Photosynthesis in *Rhodospirillum rubrum*. IV. Isolation and charactherization of ribulose 1,5-diphosphate carboxylase. *The Journal of Biological Chemistry*, *244*(12), 3105–3109. Retrieved from https://www.jbc.org/content/244/12/3105.full.pdf

Berleman, J. E., & Bauer, C. E. (2004). Characterization of cyst cell formation in the purple photosynthetic bacterium *Rhodospirillum centenum*. *Microbiology*, *150*(2), 383–390. https://doi.org/10.1099/mic.0.26846-0

Domsch, K. H., Gams, W., & Anderson, T.-H. (1980). *Compendium of soil fungi* (Vol. 1). London: Academic Press.

Fakhrunnisa, Hashmi, M. H., & Ghaffar, A. (2006). Seed-borne mycoflora of wheat, sorghum and barley. *Pakistan Journal of Botany*, *38*(1), 185–192. Retrieved from http://www.pakbs.org/pjbot/PDFs/38(1)/PJB38(1)185.pdf

Harrigan, W. F., & McCance, M. E. (1976). *Laboratory methods in food and dairy microbiology*. London: Academic Press Inc.

Imhoff, J. F., & Trüper, H. G. (1989). The purple nonsulfur bacteria. In J. T. Staley, M. P. Bryant, N. Pfennig, & J. G. Holt (Eds.), *Bergey's Manual of Systematic Bacteriology* (Vol. 3) (pp. 1658-1661). Baltimore: Wiiliams and Wilkins.

Jiang, Z.-Y., Rushing, B. G., Bai, Y., Gest, H., & Bauer, C. E. (1998). Isolation of *Rhodospirillum centenum* mutants defective in phototactic colony motility by transposon mutagenesis. *Journal of Bacteriology*, *180*(5), 1248–1255. https://doi.org/10.1128/jb.180.5.1248-1255.1998

Kim, M. K., Choi, K.-M., Yin, C.-R., Lee, K.-Y., Im, W.-T., Lim, J. H., & Lee, S.-T. (2004). Odorous swine wastewater treatment by purple non-sulfur bacteria, *Rhodopseudomonas palustris*, isolated from eutrophicated ponds. *Biotechnology Letters*, *26*, 819–822. https://doi.org/10.1023/B:BILE.0000025884.50198.67

Kohlmiller Jr., E. F., & Gest, H. (1951). A comparative study of the light and dark fermentations of organic acids by *Rhodospirillum rubrum*. *Journal of Bacteriology*, *61*(3), 269–282. https://doi.org/10.1128/jb.61.3.269-282.1951

Lu, Y.-K., Marden, J., Han, M., Swingley, W. D., Mastrian, S. D., Chowdhury, S. R., … Touchman, J. W. (2010). Metabolic flexibility revealed in the genome of the cyst-forming α-1 proteobacterium *Rhodospirillum centenum*. *BMC Genomics*, *11*, 325. https://doi.org/10.1186/1471-2164-11-325

Manandhar, H. K., Jørgensen, H. J. L., Mathur, S. B., & Smedegaard-Petersen, V. (1998). Suppression of rice blast by preinoculation with avirulent *Pyricularia oryzae* and the nonrice pathogen *Bipolaris sorokiniana*. *Phytopathology*, *88*(7), 735–739. https://doi.org/10.1094/PHYTO.1998.88.7.735

Pitt, J. I., & Hocking, A. D. (1997). Primary keys and miscellaneous fungi. In *Fungi and Food Spoilage* (pp. 59–171). Boston, MA: Springer. https://doi.org/10.1007/978-1-4615-6391-4_5

Pitt, J. I., Hocking, A. D., Bhudhasamai, K., Miscamble, B. F., Wheeler, K. A., & Tanboon-Ek, P. (1994). The normal mycoflora of commodities from Thailand. 2. Beans, rice, small grains and other commodities. *International Journal of Food Microbiology*, *23*(1), 35–43. https://doi.org/10.1016/0168-1605(94)90220-8

Rana, G., Meikap, S., Mondol, M., Bose, P. P., & Mandal, T. (2016). Green-fertilizer, *Rhodospirillum rubrum*, for agricultural development on fly-ash without any toxic metal ion release. *Basic Research Journal of Agricultural Science and Review*, *5*(8), 109–117. Retrieved from https://basicresearchjournals.org/agric/abstract/Tanusri et al.html

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: A laboratory manual* (2nd ed.). New York: Cold Spring Harbor Laboratory Press.

Serdyuk, O. P., Smolygina, L. D., Kobzar, E. F., & Gogotov, I. N. (1993). Occurrence of plant hormones in cells of the phototrophic purple bacterium *Rhodospirillum rubrum* 1R. *FEMS Microbiology Letters*, *109*(1), 113–116. https://doi.org/10.1111/j.1574-6968.1993.tb06152.x

Copyright © 2020 Universitas Brawijaya

Smith, B. D. (1998). *The emergence of agriculture*. New York: Scientific American Library.

Song, F., & Goodman, R. M. (2001). Molecular biology of disease resistance in rice. *Physiological and Molecular Plant Pathology*, *59*(1), 1–11. https://doi.org/10.1006/pmpp.2001.0353

Soytong, K. (1992). Antagonism of *Chaetomium cupreum* to *Pyricularia oryzae*. *Journal of Plant Protection in the Tropics*, *9*(1), 17–23. Retrieved from https://eurekamag.com/research/008/165/008165448.php

Tadesse, I., Isoaho, S. A., Green, F. B., & Puhakka, J. A. (2003). Removal of organics and nutrients from tannery effluent by advanced integrated wastewater pond systems technology. *Water Science and Technology*, *48*(2), 307–314. https://doi.org/10.2166/wst.2003.0135

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Tann, H., & Soytong, K. (2016). Bioformulations and nano product from *Chaetomium cupreum* CC3003 to control leaf spot of rice var. Sen Pidoa in Cambodia. *International Journal of Plant Biology*, *7*(1), 59–63. https://doi.org/10.4081/pb.2016.6413

Tann, H., & Soytong, K. (2017). Biological control of brown leaf spot disease caused by *Curvularia lunata* and field application method on rice variety IR66 in Cambodia. *AGRIVITA Journal of Agricultural Science*, *39*(1), 111–117. https://doi.org/10.17503/agrivita.v39i1.768

Thavong, P. (2002). Effect of dirty panicle disease on rice seed vigor. *Thai Agricultural Research Journal*, *20*(2), 111–120. Retrieved from https://li01.tci-thaijo.org/index.php/thaiagriculturalresearch/article/view/219254

Tongon, R., & Soytong, K. (2015). Application of nano-particles from *Chaetomium globosum* to control leaf spot of rice. *Journal of Agricultural Technology*, *11*(8), 1919–1926. Retrieved from http://www.aatsea.org/images/conference_publications/pdf/v11_n8_15_DecemberSpecialissue/031 - Tang-S2.pdf

Vareeket, R., & Soytong, K. (2017). Screening of antagonistic bacteria for biological control of rice diseases. *International Journal of Agricultural Technology*, *13*(7.3), 2569–2573. Retrieved from https://www.semanticscholar.org/paper/Screening-of-Antagonistic-Bacteria-for-Biological-Vareeket/cb68149cb72ddfa5b913269190fbf671c7bd6d93

Veenstra, S., Al-Nozaily, F. A., & Alaerts, G. J. (1995). Purple non-sulfur bacteria and their influence on waste stabilisation pond performance in the Yemen Republic. *Water Science and Technology*, *31*(12), 141–149. https://doi.org/10.1016/0273-1223(95)00501-D

Copyright © 2020 Universitas Brawijaya