Molecular Identification of 16S rDNA and Polyketide Synthase Genes of Antagonist Bacteria against Xanthomonas oryzae Pathovar oryzae from Rice Phyllosphere

Rivia Kumala Dewi, Suranto, Ari Susilowati and Aris Tri Wahyudi

Department of Bioscience, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, 57126, Central Java, Indonesia

Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Kampus IPB Darmaga, Bogor, 16680, Indonesia

Corresponding Author: Ari Susilowati, Department of Bioscience, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, 57126, Central Java, Indonesia

ABSTRACT

Biocontrol using antagonist agent is one of environment-friendly method of controlling bacterial leaf blight disease in rice field. Eight antagonistic bacteria against Xanthomonas oryzae pathovar oryzae as well as the causal bacterial leaf blight disease in rice, have been isolated from rice phyllosphere of Wonogiri and Sukoharjo Regency, Central Java, Indonesia, using dual plate method. The aims of this study were to identify molecularly of 16S rDNA and polyketide synthase (PKS) genes of antagonist bacteria. The PKS gene is recorded as one of antibiotic compounds class which encode the polyketide biosynthesis. The amplification of 16S rDNA gene was performed using 63f and 1387r primers, while PKS gene detection was performed using degKS2F.gc and degKSR5.gc primers. The nucleotide sequences of 16S rDNA and PKS genes was aligned using GenBank database and BLAST-N program from NCBI site was operated. The results showed that the eight isolates identity of SH2a, MO142, MO22g, MO34h, MO34i, MO34j, MO43a and MO63j were Pseudomonadaceae SH2a, Pantoea sp. MO142, Pantoea sp. MO22g, Erwinia sp. MO34h, Pantoea sp. MO34i, Pantoea sp. MO34j, Pantoea sp. MO43a and Pantoea sp. MO63j, respectively. Bacterial antagonists of PKS genes have similarities with the gene of nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) Lysobacter lactamgenus. This indicates that the antagonist mechanism of antagonist bacteria is antibiosis.

Key words: Antagonist, phyllosphere, PKS gene, 16S rDNA gene, Xanthomonas oryzae pv. oryzae

INTRODUCTION

Xanthomonas oryzae pathovar oryzae are bacteria that caused Bacterial Leaf Blight (BLB) in rice field and causing a significant economic losses in almost all country producing rice in Asia (Nayak et al., 2008). Biocontrol approach using antagonist agent has been considered as one of the environment-friendly method of BLB disease in rice plantation (Donghua et al., 2013).

The identification of antagonist agent needs to be done in order to facilitate its development as a biocontrol agent of Xanthomonas oryzae. The 16S rDNA gene which is believed encode 16S rRNA, is widely used as a genomics marker for the prokaryotic identification (Vinje et al., 2014) due to the
fact that these genes are universally spread among the bacteria. The advantages of using the 16S rDNA gene were have highly conserved regions to construct a universal primer and highly variable regions for species identification (Nossa et al., 2010). The gene has been stored in GenBank and therefore can be used to identify bacteria that cannot be cultured (Drancourt et al., 2000).

The antagonist mechanism which is conducted by antagonist bacteria to phytopathogen microbes include antibiosis, competition and production of lytic enzymes (Bouizgarne, 2013). The Induced Systemic Resistance induction (ISR) (Pal and Gardener, 2006), as well as Plant Growth Promoting Rhizobacteria (PGPR) (Shivalingaiah and Umesha, 2013). The antibiosis is recorded as a mechanism that plays an important role in the suppression of plant diseases by antagonist bacteria (Lo, 1998; Mishra et al., 2013). One of the genes which were involved in many antibiotics biosynthesis is polyketide synthase (PKS) gene, that involved in encoding the polyketide biosynthesis enzymes.

Eight isolates phyllosphere bacteria antagonistic against Xanthomonas oryzae have been sampled and isolated from Wonogiri and Sukoharjo Regency, Central Java Province, Indonesia. Usually searching of biocontrol agent toward Xanthomonas oryzae were mostly done from rhizosphere (Donghua et al., 2013; Shivalingaiah and Umesha, 2013) and only a small portion was conducted from phyllosphere (Gangwar, 2013). The aims of this study were first to identify molecularly of 16S rDNA genes that can be used to determine the identity of antagonist bacteria. Secondly to identify polyketide synthase (PKS) genes molecularly so that it can be used as an early detection of antagonist mechanism which were applied by antagonist bacteria toward Xanthomonas oryzae.

MATERIALS AND METHODS

Media and culture: Xanthomonas oryzae was stored in its agar (which is consisted of calcium carbonate (CaCO₃) 30 g L⁻¹ (Merck), glucose 10 g L⁻¹ (Himedia), yeast extract 5 g L⁻¹ (Conda) and agar 15 g L⁻¹) in the form of stab and slant agar. Antagonist bacteria isolates (SH2a, MO142, MO22g, MO34h, MO34i, MO34j, MO43a and MO63j) were stored in nutrient agar (Himedia). Antagonistic ability of phyllosphere bacteria against Xanthomonas oryzae was tested using a dual plate method (Velusamy et al., 2013) on nutrient agar. Nutrient broth (Merck) was used for culturing antagonist bacteria in order to extract their genomic DNA.

Amplification of 16S rDNA gene: Genomic DNA extraction of antagonist bacteria was performed using Presto™Mini gDNA Bacteria kit (Geneaid) according to the manufacturer’s recommendation. Biophotometer was used to look at concentration and purity of genomic DNA. The 1,300 bp DNA fragment generated from genomic DNA was amplified using 63f forward primer (5'-CAGGCCTAACACATGCAAGTC-3') and 1387r reverse primer (5'-GGGCGGWGTGTACAAGGC-3') (Marchesi et al., 1998). The PCR mixture was consisted of 1.25 μL for each primer 63f and 1387r (10 pmol); 2 μL DNA template <25 ng μL⁻¹; 8 μL ddH₂O and 12.5 μL kit KAPA2G™ Fast Ready Mix (Kapa Biosystems). The PCR conditions were 95°C for 3 min followed by 30 cycles of 95°C for 15 sec, 55°C for 15 sec, 72°C for 30 sec and finally 72°C for 2 min. Amplicon separation was done by horizontal electrophoresis using 1% agarose on 90 V and 400 mA for 60 min. The agarose gel was then stained with ethidium bromide. The PCR product was then sequenced (At 1st Base Singapore).
Detection of polyketide synthase (PKS) gene: The PKS gene detection was done by amplifying the genomic DNA of antagonist bacteria using forward primer degKS2F.gc (5'-GCSATGGAYCCSCARCARCGSVT) and reverse primer degKSR5.gc (5'-GTSCCSSGTSCCRT GSSCYTCSAC) (Schirmer et al., 2005). The PCR mixture was done by mixing of 1.25 μL for each primer degKS2F.gc and degKSR5.gc (10 pmol); 2 μL DNA template; 8 μL ddH2O and 12.5 μL kit KAPA2G™ Fast Ready Mix. The PCR was performed by 30 cycles of 94°C for 40 sec, 55°C for 40 sec and 72°C for 75 sec. Amplicon separation was done by using 1% agarose. After running electrophoresis at 75 constant current for 90 min, the gel was then stained using ethidium bromide. The PCR product was then sequenced (At 1st Base Singapore).

Data analysis: Nucleotide sequences of 16S rDNA and PKS genes was aligned with the GenBank database using BLAST-N program (basic local alignment search tool-nucleotides) from NCBI (National Center for Biotechnology Information) site (www.ncbi.nlm.nih.gov). Percentage of similarities have been obtained from 16S rDNA gene alignment were used to determine antagonist bacteria identity, whereas PKS gene alignment was used to determine the similarities of PKS gene based on the GenBank database. Phylogenetic analysis of KS domain from PKS gene of antagonist bacteria with some other bacteria was conducted with phylogenetic tree construction using Molecular Evolutionary Genetics Analysis (MEGA) 6 software (Tamura et al., 2013).

RESULTS AND DISCUSSION
Identity of antagonist bacteria: Rice phyllosphere bacteria that consistently antagonistic against Xanthomonas oryzae were SH2a isolate was collected from Wonogiri Regency and MO142, MO22g, MO34h, MO34i, MO34j, MO43a, as well MO63j isolates were collected from Sukoharjo Regency. All have capability with inhibition zone ranging 12-20 mm.

Based electrophoregram on Fig. 1, it was noted that the 16S rDNA gene amplicons of all eighth antagonist bacteria showed single band at ±1,300 bp. The similarities were obtained from the alignment using BLAST-N program, which can be used to determine the identity of antagonist bacteria. Determining identity of antagonist bacteria was carried out according
Table 1: Percentage similarity of partial sequence 16S rDNA gene of eight antagonist bacteria to the GenBank database

| Bacteria isolates | Related species             | Accession      | Identity (%) | Antagonist bacteria identity |
|-------------------|-----------------------------|----------------|--------------|-----------------------------|
| SH2a              | Pseudomonas fulva strain 1Y1103 | JQ229796.1     | 93           | Pseudomonadaceae SH2a       |
| MO142             | Pantoee sp. A1059           | KC236721.1     | 98           | Pantoee sp. MO142           |
| MO22g             | Pantoee sp. Ca045           | KJ427829.1     | 96           | Pantoee sp. MO22g           |
| MO34h             | Erwinia sp. ICB408          | HM748066.1     | 95           | Erwinia sp. MO34h           |
| MO34i             | Pantoee sp. Ca045           | KJ427829.1     | 97           | Pantoee sp. MO34i           |
| MO34j             | Pantoee sp. Ca045           | KJ427829.1     | 96           | Pantoee sp. MO34j           |
| MO43a             | Pantoee sp. Ca045           | KJ427829.1     | 95           | Pantoee sp. MO43a           |
| MO63j             | Pantoee sp. CT2             | EF585309.1     | 98           | Pantoee sp. MO63j           |

Bosshard et al. (2003), in which the ≥99% similarity was recorded as the same species, while the similarity in between 95 and 99% was grouped as the same genus and the similarity index bellow <95% included in the same family. Based on these criteria, it was known that antagonist bacteria SH2a isolate was a bacterium from Pseudomonadaceae family. The isolates of MO142, MO22g, MO34i, MO34j, MO43a and MO63j belongs to the Pantoee genus, while MO34h isolate was included to the Erwinia genus (Table 1).

Antagonist bacteria which were isolated from rice phyllosphere dominated by bacteria from Pantoee genus. According to Bulgarelli et al. (2013) that genera of bacteria commonly found in phyllosphere were Pseudomonas, Sphingomonas, Methylobacterium, Bacillus, Massilia, Arthrobacter and Pantoee. Pseudomonas, Pantoee and Erwinia are genus that commonly used as an antagonist bacteria. Based on this research, there were known that the genus has wide inhibitory spectrum against phytopathogen microbes, started by Pseudomonas, Pantoee and Erwinia genera, respectively.

It was recorded that species within the genus Pseudomonas has great potential to be developed into biocontrol agents for various phytopathogen microbes. Several examples that support this statement were the ability of Pseudomonas fluorescens against Xanthomonas oryzae (Shivalingaiah and Umesha, 2013), P. fluorescens 1100-6 against Rhizobium vitis causes tumors in wine (Eastwell et al., 2006), P. fluorescens and P. putida against P. solanacearum causes wilt disease in mulberry (Nuraeni and Fattah, 2007); P. rhizosphaerae JAN against Erwinia amylovora causes fire blight disease (Paternoster et al., 2010) and P. brassicacearum J12 against Ralstonia solanacearum causes bacterial wilt in tomatoes (Zhou et al., 2012).

Related research in using Pantoee as antagonist bacteria against phytopathogen bacteria has been conducted by several researchers including Sammer et al. (2009) using Pantoee agglomerans against Erwinia amylovora and Pseudomonas syringae pv. glycinea (Sammer et al., 2012) and P. ananatis BRT175 against E. amylovora causes fire blight disease on apples and pears (Walterson et al., 2014). Accordingly Pseudomonas spp., Pantoee spp. and Erwinia spp. have capability in preventing ability against some phytopathogen microbes, such as Erwinia persicinus that can inhibit Bemisia tabaci (Ateyyat et al., 2009) and E. chrysanthemi RK-67 in inhibit Aspergillus flavus, causing post harvest diseases on lemon cultivars Meyer and Interdonato (Kotan et al., 2009).

**Detection of polyketide synthase (PKS) gene:** The detection of PKS genes was the first step should be performed in order to detect the antagonist mechanism were applied antagonist bacteria to the Xanthomonas oryzae. This gene was believed involved in polyketide biosynthesis. Polyketide is a class of antibiotic compounds that have various biological activities (Wang et al., 2014). This antibiotic was synthesized by the PKS genes including erythromycin (Faizal et al., 2008), diacetylphloroglucinol (Zha et al., 2006) and pyoluteorin (De Souza and Raaijmakers, 2003).

The detection of PKS gene was done by amplifying ketosynthase (KS) domain from genomic DNA of antagonist bacteria. Schmitt and Lumbsch (2009) recorded that the regions were very
Fig. 2: Electrophoregram of polyketide synthase (PKS) gene amplicons, M: (1) kb marker, 1: Pseudomonadaceae SH2a, 2: Pantoea sp. MO142, 3: Pantoea sp. MO22g, 4: Erwinia sp. MO34h, 5: Pantoea sp. MO34i, 6: Pantoea sp. MO34j, 7: Pantoea sp. MO43a and 8: Pantoea sp. MO63j, bands in the yellow box is the targeted bands

highly conserved in type I PKS, namely a KS and acyltransferase (AT) domains. These two domains which is often used to predict the evolution of PKS gene. The primers used for amplification of KS domain produced approximately 700 bp band (Schirmer et al., 2005).

The electrophoregram of PKS gene amplicons showed that the Pseudomonadaceae SH2a did not produce specific band, accordingly. Pantoea sp. MO142 and Pantoea sp. MO63j generate unspecific bands and the thickest band showed a smaller size than the targeted size, therefore sequencing DNA was not to do. Smaller amplicon size than the targeted size presumably because of the used primers which was constructed from degenerated primer and this could produce less specific that causes primers anneal on more than one place. Pantoea sp. MO22g, Erwinia sp. MO34h, Pantoea sp. MO34i, Pantoea sp. MO34j and Pantoea sp. MO43a produce unspecific bands but the thickest band showed close to targeted size (Fig. 2). The alignment results of fifth antagonist bacteria showed low similarities with nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene from Lysobacter lactamgenus. This occurrence was because there is a lot of bases at the beginning, middle and end of the sequence were different. By contrast conversely, the fifth nucleotide sequence of antagonist bacteria has a specific features and this has been proved in their base similarities, not only on of the beginning nucleotide, but also to middle and end of the sequence (Fig. 3).

The alignment results of query cover showed a low percentage, (12-33%), while the lowest E value was recorded 8×10^{-7} and the similarities was 78-80%. This was realized because the size of the amplicons were only about 700 bp compared to the size of the nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene Lysobacter lactamgenus that have 15,150 bp on size (Table 2).

Nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene from Lysobacter lactamgenus contains three modules nonribosomal peptide synthetase (NRPS), namely module 1 (M1), module 2 (M2), as well as module 3 (M3). The type I PKS consists of ketosynthase
Fig. 3: Comparison of sequence nonribosomal peptide synthetase-polyketide synthase hybrid (cpbl) gene *Lyso bacter lactamgenus* with partial sequences polyketide synthase (PKS) genes antagonist bacteria *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j and *Pantoea* sp. MO43a. A point (.) indicates the similarity between sequences of nucleotide bases, a dash (-) indicates a gap.
Fig. 4: Phylogenetic tree of KS domain antagonist bacteria with some other bacteria, *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j, as well as *Pantoea* sp. MO43a are the antagonist bacteria that were obtained from this study (marked with a triangle). The KS domain sequence of *Xanthomonas euvesicatoria* that was isolated from *Capsicum annuum* cv. Kurtovska kapija leaves from Macedonia was obtained from European Nucleotide Archive (ENA) with accession no. >ENA|KHL65014|KHL65014.1, *Pseudomonas fluorescens* (>ENA|ADQ55965|ADQ55965.1) that was isolated from sponge in Baikal lake Russia, *Streptomyces venezuelae* (>ENA|AB024980|AB024980.1) is one of the actinomycetes and *Serratia marcescens* (>ENA|AFX60305|AFX60305.1) belong to Enterobacteriaceae family.

Table 2: Similarity of partial sequence polyketide synthase (PKS) genes of five antagonist bacteria to the GenBank database

| Bacteria isolates     | Similarity                  | Query cover (%) | E value | Identity (%) | Accession     |
|-----------------------|------------------------------|-----------------|---------|--------------|---------------|
| *Pantoea* sp. MO22g   | Nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene *Lysobacter lactamgenus* | 53              | 5e-10   | 80           | DQ278492.1    |
| *Erwinia* sp. MO34h   | Nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene *Lysobacter lactamgenus* | 12              | 8e-08   | 80           | DQ278492.1    |
| *Pantoea* sp. MO34i   | Nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene *Lysobacter lactamgenus* | 13              | 7e-08   | 80           | DQ278492.1    |
| *Pantoea* sp. MO34j   | Nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene *Lysobacter lactamgenus* | 13              | 7e-08   | 80           | DQ278492.1    |
| *Pantoea* sp. MO43a   | Nonribosomal peptide synthetase-polyketide synthase hybrid (cpbI) gene *Lysobacter lactamgenus* | 21              | 9e-17   | 78           | DQ278492.1    |

(KS), acyltransferase (AT), ketoreductase (KR), acyl carrier protein (ACP) and thioesterase (TE) domains. The NRPS gene is a gene that involved in nonribosomal peptide biosynthesis. Nonribosomal peptides are a group of compounds that have various biological activities such as antibacterial (vancomycin), anticancer (bleomycin), immunosuppressants (cyclosporine) (Tang *et al.*, 2007), siderophores (pyoverdin), toxins (HC toxins) and surfactant (surfactin) (Caboche *et al.*, 2008).

Type I PKS is a multi-domain enzymes that use modular strategy, with each module consisting of at least three domains, namely ketosynthase (KS), acyltransferase (AT) and acyl carrier protein (ACP) domain. This type I PKS module may contain additional domains such as ketoreductase (KR), dehydratase (DH) and enoylreductase (ER) (Ayuso-Sacido and Genilloud, 2005). Based on the detection results of the PKS gene in genomic DNA of *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j and *Pantoea* sp. MO43a, this indicated that antagonist mechanism of antagonist bacteria with antibiotics production (antibiosis).

Genetic relationship of KS domain: Based on the phylogenetic tree in Fig. 4, showed that the KS domain of antagonist bacteria i.e., *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j
and *Pantoea* sp. MO22g have a very closely genetic relationship and clustering in a group. These KS domain of antagonist bacteria have a closely genetic relationship with *Xanthomonas euvesicatoria* that alike as phyllosphere bacteria though in the different plants. Fourth of these KS domain have a far distant compare to the KS domain of *Streptomyces venezuelae* and *Pseudomonas fluorescens*. The KS domain of *Pantoea* sp. MO43a is in a group with KS domain of *Serratia marcescens* and separated from the other group.

ACKNOWLEDGMENT
This study was supported by a Competence Grant Hibah Kompetensi from the Directorate General of Higher Education (DIKTI) of the Republic of Indonesia (2015) to ATW. We are therefore grateful for this funding and support of this research.

REFERENCES
Ateyyat, M.A., M. Shatnawi and M.S. Al-Mazra'awi, 2009. Culturable whitefly associated bacteria and their potential as biological control agents. *Jordan J. Biol. Sci.*, 2: 139-144.
Ayuso-Sacido, A. and O. Genilloud, 2005. New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: Detection and distribution of these biosynthetic gene sequences in major taxonomic groups. *Microb. Ecol.*, 49: 10-24.
Bosshard, P.P., S. Abels, R. Zbinden, E.C. Bottger and M. Altwegg, 2003. Ribosomal DNA sequencing for identification of aerobic gram-positive rods in the clinical laboratory (an 18-Month Evaluation). *J. Clin. Microbiol.*, 41: 4134-4140.
Bouizgarne, B., 2013. Bacteria for Plant Growth Promotion and Disease Management. In: Bacteria in Agrobiology: Disease Management, Maheshwari, D.K. (Ed.). Springer-Verlag, Heidelberg, ISBN: 978-3-642-33638-6, pp: 15-47.
Bulgarelli, D., K. Schlaper, S. Spaepen, E.V.L. van Themaat and P. Schulze-Lefert, 2013. Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.*, 64: 807-838.
Caboche, S., M. Pupin, V. Leclere, A. Fontaine, P. Jacques and G. Kucherov, 2008. NORINE: A database of nonribosomal peptides. *Nucleic Acids Res.*, 36: D326-D331.
De Souza, J.T. and J.M. Raaijmakers, 2003. Polymorphisms within the *prnD* and *pltC* genes from pyrrolnitrin and pyoluteorin-producing *Pseudomonas* and *Burkholderia* spp. *FEMS Microbiol. Ecol.*, 43: 21-34.
Donghua, J., L. Qinying, S. Yiming and J. Hao, 2013. Antimicrobial compound from a novel *Streptomyces termitum* strain ATC-2 against *Xanthomonas oryzae* pv. oryzae. *Res. J. Biotech.*, 8: 66-70.
Drancourt, M., C. Bollet, A. Carlioz, R. Martelin, J.P. Gayral and D. Raoult, 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J. Clin. Microbiol.*, 38: 3623-3630.
Eastwell, K.C., P.L. Sholberg and R.J. Sayler, 2006. Characterizing potential bacterial biocontrol agents for suppression of *Rhizobium vitis*, causal agent of crown gall disease in grapevines. *Crop Protect.*, 25: 1191-1200.
Faizal, I., R. Lestari, F. Kurnia, A. Latif and D. Hadianto *et al.*, 2008. Polymorphism analysis of polyketide synthase gene from Actinomycetes genome DNA of Taman Nasional Gunung Halimun soil by using metagenome method. *J. Biotechnol. Res. Trop. Reg.*, 1: 1-4.
Gangwar, G.P., 2013. Efficacy of different isolates of fluorescent pseudomonads against bacterial leaf blight of rice. Afr. J. Agric. Res., 8: 4588-4591.
Kotan, R., N. Dikbas and H. Bostan, 2009. Biological control of post harvest disease caused by Aspergillus flavus on stored lemon fruits. Afr. J. Biotechnol., 8: 209-214.
Lo, C.T., 1998. General mechanisms of action of microbial biocontrol agents. Plant Pathol. Bull., 7: 155-166.
Marchesi, J.R., T. Sato, A.J. Weightman, T.A. Martin and J.C. Fry et al., 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Applied Environ. Microbiol., 64: 795-799.
Mishra, D.S., A. Kumar, C.R. Prajapati, A.K. Singh and S.D. Sharma, 2013. Identification of compatible bacterial and fungal isolate and their effectiveness against plant disease. J. Environ. Biol., 34: 183-189.
Nayak, D., M.L. Shanti, L.K. Bose, U.D. Singh and P. Nayak, 2008. Pathogenicity association in Xanthomonas oryzae PV. Oryzae-the causal organism of rice bacterial blight disease. ARPN J. Agric. Biol. Sci., 3: 12-27.
Nossa, C.W., W.E. Oberdorf, L. Yang, J.A. Aas and B.J. Paster et al., 2010. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J. Gastroenterol., 16: 4135-4144.
Nuraeni, S. and A. Fattah, 2007. Uji efektivitas bakteri antagonis Pseudomonas fluoescens dan P. putida untuk mengendalikan P. solanacearum penyebab penyakit layu pada tanaman murbei. Jurnal Perennial, 3: 44-48.
Pal, K.K. and B.M. Gardener, 2006. Biological control of plant pathogens. Plant Health Instructor. 10.1094/PHI-A-2006-1117-02
Paternoster, T., G. Defago, B. Duffy, C. Gessler and I. Pertot, 2010. Selection of a biocontrol agent based on a potential mechanism of action: Degradation of nicotinic acid, a growth factor essential for Erwinia amylovora. Int. Microbiol., 13: 195-206.
Sammer, U.F., B. Volksch, U. Mollmann, M. Schmidtkle, P. Spiteller, M. Spiteller and D. Spiteller, 2009. 2-amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine, an effective peptide antibiotic from the epiphyte Pantoea agglomerans 48b/90. Applied Environ. Microbiol., 75: 7710-7717.
Sammer, U.F., K. Reiher, D. Spiteller, A. Wensing and B. Volksch, 2012. Assessment of the relevance of the antibiotic 2-amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine from Pantoea agglomerans biological control strains against bacterial plant pathogens. MicrobiologyOpen, 1: 438-449.
Schirmer, A., R. Gadkari, C.D. Reeves, F. Ibrahim, E.F. DeLong and C.R. Hutchinson, 2005. Metagenomic analysis reveals diverse polyketide synthase gene clusters in microorganisms associated with the marine sponge Discodermia dissoluta. Applied Environ. Microbiol., 71: 4840-4849.
Schmitt, I. and H.T. Lumbsch, 2009. Ancient horizontal gene transfer from bacteria enhances biosynthetic capabilities of fungi. PLoS One, Vol. 4. 10.1371/journal.pone.0004437
Shivalingaiah and S. Umesha, 2013. Pseudomonas fluorescens inhibits the Xanthomonas oryzae pv. oryzae, the bacterial leaf blight pathogen in rice. Can. J. Plant Protect., 1: 147-153.
Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: Molecular evolution genetics analysis version 6.0. Mol. Biol. Evol., 30: 2725-2729.
Tang, G.L., Y.Q. Cheng and B. Shen, 2007. Chain initiation in the leinamycin-producing hybrid nonribosomal peptide/polyketide synthetase from *Streptomyces atroolivaceus* S-140. *J. Biol. Chem.*, 282: 20273-20282.

Velusamy, P., J.E. Immanuel and S.S. Gnanamanickam, 2013. Rhizosphere bacteria for biocontrol of bacterial blight and growth promotion of rice. *Rice Sci.*, 20: 356-362.

Vinje, H., T. Almoy, K.H. Liland and L. Snipen, 2014. A systematic search for discriminating sites in the 16S ribosomal RNA gene. *Microb. Inform. Exp.*, Vol. 4. 10.1186/2042-5783-4-2

Walterson, A.M., D.D.N. Smith and J. Stavrinides, 2014. Identification of a *Pantoea* biosynthetic cluster that directs the synthesis of an antimicrobial natural product. *PLoS One*, Vol. 9. 10.1371/journal.pone.0096208

Wang, H., D.P. Fewer, L. Holm, L. Rouhiainen and K. Sivonen, 2014. Atlas of nonribosomal peptide and polyketide biosynthetic pathways reveals common occurrence of nonmodular enzymes. *Proc. Natl. Acad. Sci.*, 111: 9259-9264.

Zha, W., S.B. Rubin-Pitel and H. Zhao, 2006. Characterization of the substrate specificity of PhlD, a type III polyketide synthase from *Pseudomonas fluorescens*. *J. Biol. Chem.*, 281: 32036-32047.

Zhou, T., D. Chen, C. Li, Q. Sun and L. Li *et al.*, 2012. Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. *Microbiol. Res.*, 167: 388-394.