Jurnal Perlindungan Tanaman Indonesia, Vol. 23, No. 1, 2019: 148–155
DOI: 10.22146/jpti.38320
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

**Research Article**

# Detection and Identification of Banana-associated Phytoplasma Using Nested-PCR Method

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Received August 27, 2018; revised February 28, 2019; accepted March 29, 2019

## ABSTRACT

Phytoplasma is known to be associated with plant diseases in about 300 plant species from various families. Information on the presence of phytoplasma in bananas as one of the pathogens that can cause disease in bananas in Indonesia has never been reported. This research was conducted with the aim to detect the presence of banana phytoplasma by the nested-PCR method and to identify phytoplasma obtained based on the sequence analysis of the 16S rRNA gene. Standard PCR was carried out using P1/P7 primary pairs, followed by nested-PCR using a pair of R16F2n/R16R2m23SR primers separately that could amplify the target 16S rRNA genes in a row at 1600 bp. BLAST analysis shows that the results of phylogenetic analysis of banana phytoplasmic nucleotide cv. manggala from Tasikmalaya and cv. Raja nangka from Banjar has a genetic relationship that is closer to lethal wilt oil palm Phytoplasma (*Candidatus* Phytoplasma asteris). This phytoplasma belongs to the 16SrI-B group (aster yellows).

Keywords: *Candidatus* Phytoplasma asteris, banana, primer P1/P7, 16SrI-B, nested-PCR

## INTRODUCTION

Phytoplasma is known to be associated with plant diseases in about 300 plant species from various families. Phytoplasma is considered as a potential pathogen that can cause harm to plants. The host range of phytoplasma host plants is quite extensive, including a variety of cultivated and non-cultivated plants, both biennial and annual plants (Lou *et al*., 2013; Al-Saleh & Amer, 2014; Alhudaib & Rezk, 2014).

Diseases caused by phytoplasma are more reported to cause symptoms in some agricultural commodities such as aster yellows, decline peach, the proliferation of apples, coconut lethal yellowing, and yellow grapevine yellowss (Agrios, 2005). Phytoplasma has been found to be associated with disease in a large number of plants throughout the world with symptoms that vary depending on host type, phytoplasma strain, and environmental factors, with symptoms such as yellowing or purple discoloration of leaves and shoots, phyllody, dwarfs, broom diseases, and even plant death (Dickinson *et al.*, 2013).

Indonesia is the sixth largest banana world producer. As one of the banana producer countries, Indonesia had produced 6.20% of the world's total banana production and supplied 50% of Asia production (Suyanti & Supriyadi, 2008). Banana planting cannot be separated from diseases that can decrease both quality and quantity. Researches on banana diseases, especially those caused by phytoplasma has not been widely carried out in Indonesia. Information about phytoplasma banana disease mostly originates from other countries (Semangun, 2007).

Phytoplasma in bananas had been reported by Davis *et al*. (2012) in Papua New Guinea and the Solomon Islands (Davis *et al*., 2015). Symptoms seen in the field are wilting and yellowing leaves, intermittent vascular tissue which is brownish or black color, and necrotic. Information on the presence of phytoplasma in bananas as one of the pathogens that can cause disease in Indonesia had never been reported before. The aim of this study was to detect the presence of phytoplasma in banana with a nested-PCR method using universal primers P1/P7

and specific primers for phytoplasma and to identify and classify phytoplasmas obtained based on sequences of 16S rRNA gene analysis.

## MATERIALS AND METHODS

### *Survey and Collection of Infected Banana Samples in Various Locations*

The sampling of banana pseudostem tissue which was thought to be associated with phytoplasma conducted by doing a survey of infected banana plants and collecting pseudostem samples. Center of banana plantations was selected from each district using purposive random sampling method (Windari *et al.*, 2015; Ismiyatuningsih *et al.*, 2016). The survey of infected banana was carried out in Bogor, Ciamis, Garut, Banjar, Tasikmalaya (West Java), Sleman, Bantul, Kulon Progo, Gunung Kidul (Yogyakarta) and Magelang (Central Java). Observation of infected plant tissue samples based on morphological symptoms seen in the field. Banana pseudostem was cut and the internal pseudostem tissue was observed (Wibowo *et al.*, 2010), then put in a sample paper. Some of the samples were inserted into vial bottles filled with CTAB solution, this was done because the banana pseudostem is easy to damaged (blackening tissue). Each sample was labeled with banana cultivar, sampling location, and its ordinate point.

### *Isolation of Total Plant Tissue DNA*

Suspected phytoplasma infected pseudostem tissues were collected and DNA isolation was carried out. Phytoplasma DNA extraction was carried out using total DNA plant extraction method as described by Dellaporta *et al.* (1983) and Gibb & Padovan (1994) such as follow: pseudostem tissue was cut into small pieces and weighed as much as 0.5 g then put into mortar and added 700 µl of CTAB solution and finely crushed. Then put in a 1.5 ml tube and add 700 µl of CTAB solution. Incubation was conducted in a water bath at 65ºC for 30 minutes and shaken every 10 minutes. Then centrifuged at 10,000 rpm for 5 minutes. The supernatant was taken and put into a new 1.5 ml sterile tube, then added CIAA solution to 1.5 ml (taking CIAA solution to the lower layer) and shaken for 5 minutes then centrifuged at 10,000 rpm for 15 minutes. The supernatant was collected and put into a sterile new 1.5 ml tube and then added 96% cold ethanol solution to 1.5 ml, shaken until it dissolved, stored for one night. Then centrifuged at 10,000 rpm for 15 minutes. After that, the pellet was collected and 70% cold ethanol was added to 1.5 ml, centrifuged 10,000 rpm for 15 minutes. Pellets were taken and seen the purification. Pellets were taken then dried. After the pellets were dried, 50 µl of sterile aquabidest was added and stored at -20ºC.

### *Amplification of DNA by PCR*

The isolated DNA was used as a template in PCR technique using several forward/reverse pairs designed from the 16S-23S rRNA gene region. The primary primer pair consisted of P1 (5'-AAG AGT TTG ATC CTG CAG GAT T-3') (Deng & Hiruki, 1994) and P7 (5'-CGT CCT TCA TCG GCT CTT-=3') (Kirkpatrick *et al.*, 1994). The next pair was R16F2n primers (5'-GAA ACG ACT GCT AAG ACT GG-3') and m23SR (5'-TAG TGC CAG GAC ATC CAC TG-3') (Constable *et al.*, 2003). The PCR reaction was carried out at a total volume of 25 µl, consisted of 12,5 µl GoTaq Green PCR master mix (2x), 1 µl (5 pmol) primary forward primer, 1 µl (5 pmol) reverse primer, 9.5 µl dd$H_2O$ and 1 µl DNA template, which were inserted into the PCR tube in cold condition using a micropipette and then homogenized using vortex. DNA samples from healthy plants were used as control. The initial step was denaturated the target DNA by heating it to 92°C for 1 minute, denaturation at 95°C for 1 minute, annealing at 55°C for 1.5 minutes, extension at 72°C for 1.5 minutes, final extension at 72°C for 5 minutes, and repeated to 35 cycles. DNA amplicon from standard PCR using a pair of universal phytoplasma primers (P1/P7) was then used as a template to be reamplified with nested-PCR using a pair of primers R16F2n/m23SR (Constable *et al.*, 2003). In this nested-PCR, the standard PCR amplicon was first diluted with the TE buffer at a dilution factor of 1:29, then as much as 1 µl of the dilution results were used as a template in nested-PCR. The heating cycle in nested-PCR was not different from standard PCR, except the primary annealing temperature was changed to 58°C (Duduk *et al.*, 2013).

### *Electrophoresis and DNA Visualization*

The DNA from PCR amplification, 5 µl each, and 2 µl loading buffer were load in 2% agarose gel (containing ethidium bromide, in 1x TBE buffer).

Electrophoresis was conducted by horizontal electrophoresis (Bio-Rad), 50 volt DC for 50 minutes. To estimate the size of the DNA, a 1 Kb marker was used. The DNA bands formed by the electrophoresis results were observed above the UV transilluminator and subsequently photographed with digital cameras (Nurjanah *et al.*, 2017; Dwimartina *et al.*, 2017).

### *DNA Sequencing and Phylogenetic Analysis*

The positive result of the DNA amplification product from nested-PCR as much as 40−60 µl were then sequenced at PT First-BASE, Malaysia. The sequence of nucleotides sequencing results was then analyzed using Bioedit 7.1.7. and compared with nucleotide sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) (Mahfut *et al.*, 2016). The phylogenetic between phytoplasma DNA was analyzed using several software, that are Clustal X, Clustal W and Mega version 7.0 (Kumar *et al.*, 2016). Phylogenetic analysis was also carried out on the phytoplasma DNA sequences in GenBank based on Wei *et al.* (2007).

## RESULTS AND DISCUSSION

### *Detection of Phytoplasma*

Early detection of phytoplasma was done by observing the symptoms of the disease on banana plantations (banana plant centers) in Bogor, Garut, Tasikmalaya, Banjar, Ciamis, Bantul, Magelang, Kulon Progo, Gunung Kidul and Sleman (Table 1). Typical symptoms of disease caused by phytoplasma in general that were seen and observed in the field were yellowing leaves, undeveloped fruit and brownish or wet and necrotic black in small discontinuous vascular tissue of the pseudostem part (Figure 1).

Phytoplasma is an obligate pathogenic bacterium from the Mollicute class that does not have cell walls (Lee *et al.*, 2000). Unlike necrotrophic bacteria such as Dickeya and Pectobacterium, or hemibiotrophs such as Xanthomonas which produce extracellular enzymes to break down the host cell walls (Joko *et al*., 2000; 2018), phytoplasma infections can cause disruption of plant hormone balance such as an increase of up to ten times *indole-3acetic acid* (IAA). Phytoplasma can also affect the function of phloem tissue in transporting photosynthesis (Musetti, 2010). The photosynthetic disorder causes lower carbohydrate accumulation and starch level in adult leaves, but that seems only a secondary effect and may be related to inhibition of transportation in phloem tissue. Higher carbohydrate levels in the leaves generally cause chlorosis as feedback from photosynthetic inhibition (Bertaccini, 2007; Maust *et al.*, 2003). However, lower translocation also had been seen in plants with less phytoplasma, this indicated that phytoplasma affects the phloem function and metabolism of host plants (Bertaccini & Duduk, 2009). Davis *et al.* (2012; 2015) reported that banana plants affected by phytoplasma infection showed symptoms of yellowing leaves, necrotic sacs, and discontinuous brownish or black-red vessels.

Detection of a phytoplasma associated with bananas was also carried out through DNA amplification using the nested-PCR method. Amplification using standard PCR with primary primer pair R16F2n / m23SR had successfully amplified the phytoplasma DNA fragments in the internal 16S rRNA gene region from the primary primer site P1/P7. The detection results with nested-PCR successfully amplified DNA bands measuring ± 1600 bp (Figure 2). DNA Amplicon DNA from symptomized samples which were not able to visualize in agarose gel (PSimGrt, PKP1, PNTsk, and PKKP) were suspected due to too little or too much the amount of DNA. This could be due to fresh tissue plants or tissues that were still in shape and color used in the extraction process, using stored extracted DNA, adding high concentrations of primer or DNA template DNA, all of these resulting mispriming, that is, detecting non-specific target band or not forming target DNA bands. The presence of inhibitors that appear in plant tissues during the process of storing samples was included in the extraction process and interfered amplification process. The inhibitor could be a high salt concentration that in the PCR amplification process is able to inhibit Taq polymerase activity rate in synthesizing DNA (Handiyanti *et al.*, 2018).

According to Prasetyo *et al.* (2017) inhibitors in plant tissues can interfere with the amplification process. Nested-PCR was designed to increase sensitivity and specificity, especially for samples with less phytoplasma amplification so inhibitors can interfere with the success of PCR. In addition, the DNA amplicon from the first PCR which was diluted and subsequently became a template on the

Table 1. Samples of infected banana plants suspected of being caused by phytoplasma in the field

| No. | Sample code | Location | PCR result |
|---|---|---|---|
| 1 | PRTsk | Sirnajaya, Tasikmalaya | Negative |
| 2 | PNTsk | Sirnajaya, Tasikmalaya | Negative |
| 3 | PMuTsk | Sirnajaya, Tasikmalaya | Negative |
| 4 | PSiTsk | Sirnajaya, Tasikmalaya | Negative |
| 5 | PSeTsk | Sirnajaya, Tasikmalaya | Negative |
| 6 | PKTsk | Sirnajaya, Tasikmalaya | Negative |
| 7 | PBTsk | Sirnajaya, Tasikmalaya | Negative |
| 8 | PMTsk | Manonjaya, Tasikmaya | Negative |
| 9 | PCSTsk | Manonjaya, Tasikmaya | Positive |
| 10 | PNB | Banjar | Positive |
| 11 | PSiB | Banjar | Negative |
| 12 | PSiPC | Ciamis | Negative |
| 13 | PSiC | Ciamis | Negative |
| 14 | PNGrt | Lewok, Garut | Negative |
| 15 | PTGrt | Lewok, Garut | Negative |
| 16 | PSimGrt | Lewok, Garut | Negative |
| 17 | PNBgr | Bogor | Negative |
| 18 | PKeBgr | Bogor | Negative |
| 19 | PRBtl | Bantul | Negative |
| 20 | PKePuBtl | Bantul | Negative |
| 21 | PSuBtl | Bantul | Negative |
| 22 | PAmKBtl | Bantul | Negative |
| 23 | PNSlmn | Sleman | Negative |
| 24 | PRSlmn | Sleman | Negative |
| 25 | PPIAT | Sleman | Negative |
| 26 | PA | Sleman | Negative |
| 27 | PB | Sleman | Negative |
| 28 | PeP | Sleman | Negative |
| 29 | PGK1 | Semin, Gunung Kidul | Negative |
| 30 | PGK2 | Semin, Gunung Kidul | Negative |
| 31 | PGK3 | Semin, Gunung Kidul | Negative |
| 32 | PKP1 | Sentolo, Kulon Progo | Negative |
| 33 | PKP2 | Sentolo, Kulon Progo | Negative |
| 34 | PKP3 | Sentolo, Kulon Progo | Negative |
| 35 | PKP4 | Sentolo, Kulon Progo | Negative |
| 36 | PKKP | Sentolo, Kulon Progo | Negative |

second PCR was able to reduce the inhibitor concentration in the second PCR reaction so that the results of the phytoplasma DNA amplification were more optimal.

### *DNA Sequencing and Phylogenetic Analysis*

Nucleotide sequences were PCR product result amplified using a pair of primers (R16F2n/m23SR) measuring ± 1600 bp which have been sequenced. Sequencing results showed that these nucleotides had similarities > 90% with several phytoplasma strains in GenBank. The results of phylogenetic tree analysis showed that phytoplasma nucleotide sequences from manggala banana from Tasikmalaya had a genetic relationship of 93% and the raja nangka banana from Banjar had a genetic relationship of 94% with lethal wilt oil palm Phytoplasma (*Candidatus* Phytoplasma asteris) and the lowest genetic relationship from the manggala banana from Tasikmalaya and the raja nangka banana were 87% with *Candidatus* Phytoplasma oryzae (Figure 3). Description of GenBank from NCBI showed that lethal wilt of oil palm Phytoplasma (*Candidate Candidatus* Phytoplasma asterasteris) is grouped in the 116SrI-B (aster yellows).

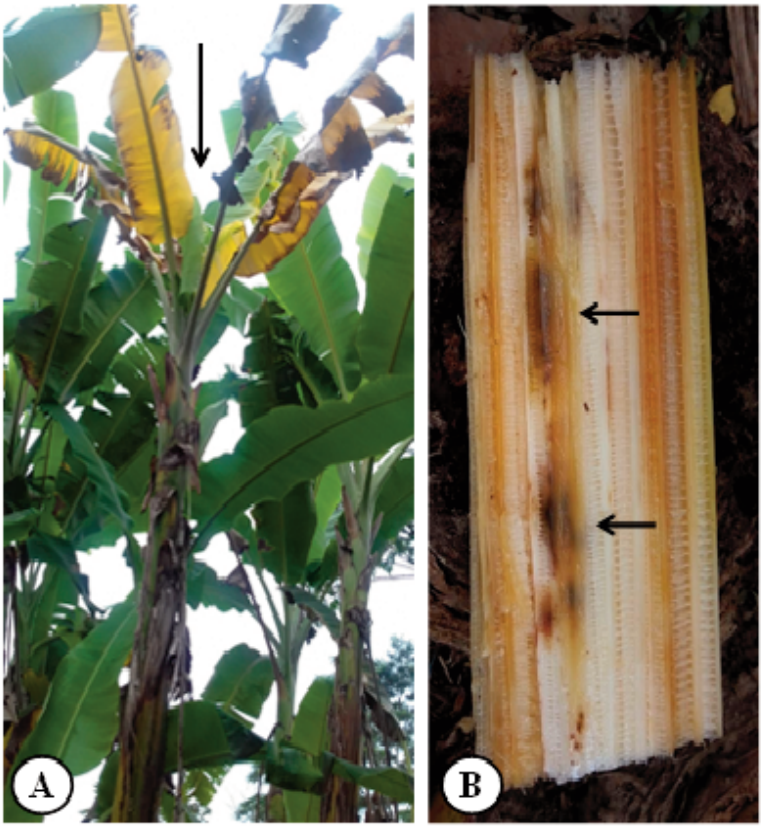


Figure 1. Symptoms of disease in banana plants associated with phytoplasma; (A) a symptom of infected plant that was suspected of being associated with phytoplasma; (B) brownish or black red pseudostem parts and necrotic sacs as shown by arrows

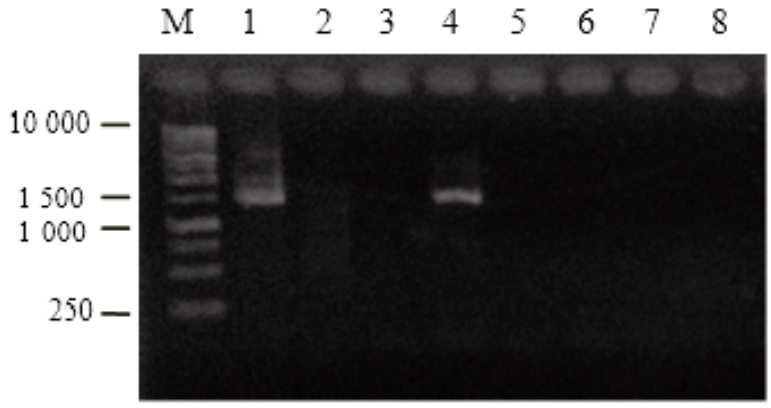


Figure 2. Visualization of nested-PCR phytoplasma DNA bands using primers P1/P7 R16F2n/m23SR from samples in various regions, (M) 1 Kb DNA marker, (1) Banjar nangka raja banana, (2) Tasikmalaya manggala banana, (3) Garut tanduk banana, (4) Tasikmalaya manggala banana, (5) Banjar siam banana, (6) Ciamis siam banana, (7) Garut siam banana, (8) Banjar white siam banana

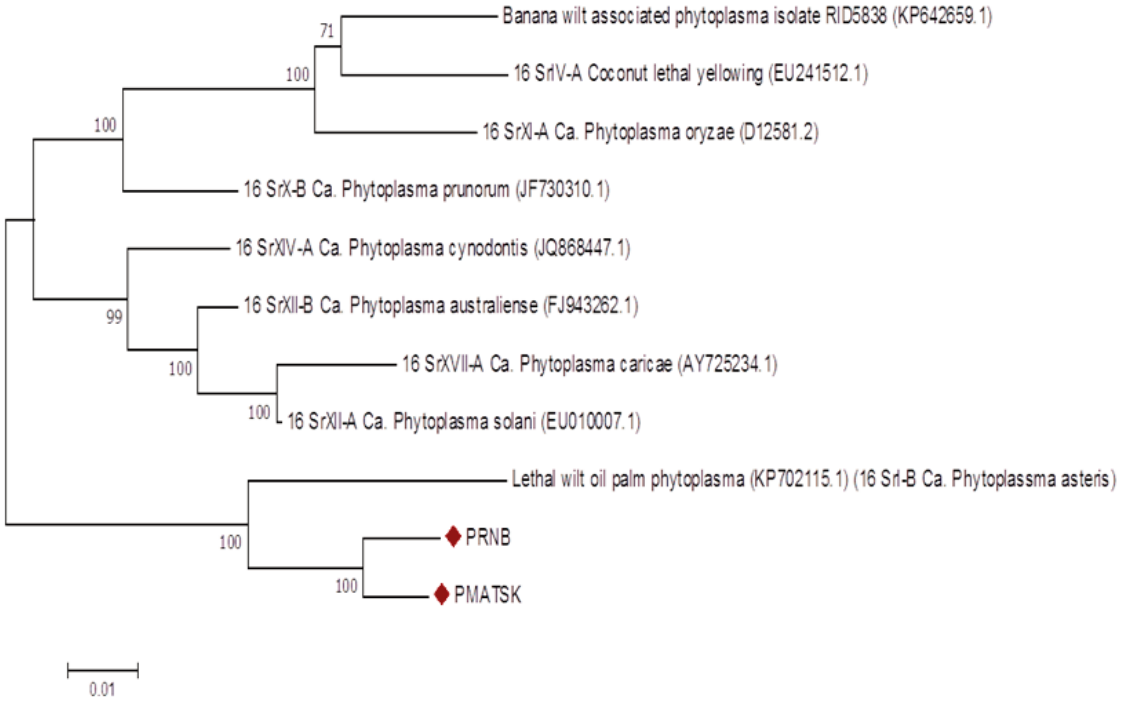


Figure 3. Phylogenetic tree describing the relationship between PCSTsk from Tasikmalaya and PNB from Banjar with several phytoplasma strains in GenBank with bootstrap neighbor-joining program MEGA 7.0 analysis

The possibility of Phytoplasma nucleotide sequences from of banana from Tasikmalaya and Banjar has might have similarities with lethal wilt oil palm Phytoplasma (*Candidatus* Phytoplasma asteris) found in oil palm (*Elaeis guineensis* Jacq.) from Ecuador because in the banana infected sample field there were oil palm trees that showed suspected phytoplasma infected symptoms. In Indonesia, there was a report that Kalimantan wilt disease that attacks coconut plants in the Sampit region caused by *Candidatus* Phytoplasma oryzae (Warokka *et al.*, 2006). Prasetyo *et al.* (2017) also reported on wilt that attacks coconut plants in Derawan Island, East Kalimantan caused by two different phytoplasma groups, which were witches broom Phytoplasma (16SrII group) and *Candidatus* Phytoplasma oryzae (16SrXI group). In Indonesia, it has never been reported about phytoplasma associated with banana plants. To prevent and suppress the spread of this disease, the centers of banana plants should not be adjacent to areas where there are many coconut plants. In addition, the role of the Agricultural Quarantine Agency in preventing the spread of this disease needs to be increased to avoid spreading to other regions.

## CONCLUSION

Phytoplasma nucleotide sequences from banana cv. manggala from Tasikmalaya and banana cv. the nangka king of Banjar has a relationship with lethal wilt oil palm Phytoplasma (*Candidatus* Phytoplasma asteris), 93% and 94% respectively, and was in the 16SrI-B group (aster yellows).

## ACKNOWLEDGMENTS

Author would like to thank to the Indonesian Agricultural Quarantine Agency, the Ministry of Agriculture for funding the research, and the Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada which facilitated this research. This manuscript is part of a thesis on behalf of Saurma Mona Astrid Sibarani.

## LITERATURE CITED

Agrios, G.N. 2005. *Plant Pathology*. Elsevier Academic Press, USA. 922 p.

Alhudaib, K. & A.A. Rezk. 2014. Molecular Characterization of Phytoplasm-Associated Disease in Tomato (*Lycopersicum esculentum* L.) in Saudi Arabia. *International Journal of Virology* 10: 10−191.

Al-Saleh, M. A. & M.A. Amer. 2014. Molecular Characterization of the 16SR II Group of Phytoplasm Associated with Faba Bean (*Vicia faba* L.) in Saudi Arabia. *The Journal of Animal & Plant Sciences* 24: 221−228.

Bertaccini, A. 2007. Phytoplasmas: Diversity, Taxonomy, and Epidemiology. *Frontiers in Bioscience* 12: 673–689.

Bertacini, A. & B. Duduk. 2009. Phytoplasm and Phytoplasm Diseases: A Review of Recent Research. *Phytopathologia Mediterranea* 48: 355−378.

Constable, F.E., K.S. Gibb, & R.H. Symons. 2003. Seasonal Distribution of Phytoplasm in Australian Grapevines. *Plant Pathology* 52: 267−276.

Davis, R.I., P. Kokoa, L.M. Jones, J. Mackie, J., F.E. Constable, B.C. Rodoni, T.G. Gunua, & J.B. Rossel. 2012. A New Wilt Diseases of Banana Plants Associated with Phytoplasms in Papua New Guinea (PNG). *Australian Plant Disease Notes* 7: 91−97.

Davis, R.I., J. Henderson, L.M. Jones, A.R. McTaggart, C. O'Dwyer, F. Tsatsia, C. Fanai, & Rossel, J.B. 2015. First Record of a Wilt Diseases of Banana Plants Associated with Phytoplasms in Solomon Islands. *Australian Plant Disease Notes* 10: 14.

Dellaporta, S.L., J. Wood, & J.B. Hicks. 1983. A Plant DNA Mini Preparation: Version II. *Plant Molecular Biology Reporter* 1: 19−21.

Deng, S. & C. Hiruki. 1991. Amplication of 16S rRNA genes from Culturable and Nonculturable Mollicutes. Journal of Microbiological Methods 14: 53−61.

Dickinson, M., M. Tuffen, & J. Hodgetts. 2013. The Phytoplasms: An Introduction, p. 1−14. *In* M. Dickinson & J. Hodgetts (eds.), *Phytoplasm Methods and Protocols*. Humana Press, New York (USA).

Duduk, B., S. Paltrinieri, I.M. Lee, & A. Bertaccini. 2013. Nested-PCR and RFLP Analysis Based on The 16S rRNA Gene, p. 159−172. *In* M. Dickinson & J. Hodgetts (eds.), *Phytoplasm Methods and Protocols*. Humana Press, New York (USA).

Dwimartina, F., T. Arwiyanto, & T. Joko. 2017. Potential of Endophytic and Rhizobacteria as an Effective Biocontrol for *Ralstonia syzygii* subsp. *syzygii*. *Asian Journal of Plant Pathology* 11: 191−198.

Gibb, K. & A. Padovan. 1994. A DNA Method that Allows Reliable PCR Amplification of MLO DNA from Difficult Plant Host Species. *PCR Method & Application* 4: 56−58.

Handiyanti, M., S. Subandiyah, & T. Joko. 2018. Deteksi Molekuler *Burkholderia glumae*, Penyebab Penyakit Hawar Malai Padi. *Jurnal Perlindungan Tanaman Indonesia* 22: 98−107.

Ismiyatuningsih, T. Joko, & S. Hartono. 2016. Survey and Detection of *Pectobacterium atrosepticum* in Major Potato-Growing Areas in Central Java Province, Indonesia. *Ilmu Pertanian* 1: 1−6.

Joko, T., S. Subandiyah, & S. Somowiyarjo. 2000. The Role of Extracellular Protein on the Pathogenicity of *Xanthomonas campestris* pv. *citri*. *Jurnal Perlindungan Tanaman Indonesia* 6: 32−38.

Joko, T., M. Umehara, T. Murata, H. Etoh, K. Izumori, & S. Tsuyumu. 2018. Hyperinduction of Pectate Lyase in *Dickeya chrysanthemi* EC16 by Plant-Derived Sugars. *Journal of Plant Interactions* 13: 141−150.

Kirkpatrick, B.C., C.D. Smart, S.L. Gardner, U. Ahrens, R. Ma'urer, B. Schneider, K.H.M. Lorenz, E. Seemuller, N.A. Harrison, S. Namba, & X. Daire. 1994. Phylogenetic Relationships of Plant Pathogenetic MLOs Established by 16/23S rDNA Spacer Sequences. International Organitation for Migration *Letter* 3: 228−229.

Kumar, S., G. Stecher, & K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Data Sets. *Molecular Biology and Evolution* 33: 1870−1874.

Lee, I.M., R.E. Davis, D.F. Gundersen, & Rindal. 2000. Phytoplasm, Phytopathogenic Mollicutes. *Annual Review of Microbiology* 54: 221−225.

Lou, B., X. Bai, Y. Bai, C. Deng, M.R. Chowdhury, C. Chen, & Y. Song. 2013. Phytoplasm in Grapefruit (*Citrus paradisi*) with Huanglongbing-Like Symptoms in China. *Journal of Phytopathology* 16: 387−395.

Maust, B.E., C.F. Espadas, M. Talavera, J.M. Aguilar, Santamaría, & C. Oropeza. 2003. Changes in Carbohydrate Metabolism in Coconut Palms Infected with the Lethal Yellowing Phytoplasm. *Phytopathology* 93: 976−981.

Mahfut, T. Joko, & B.S. Daryono. 2016. Molecular Characterization of Odontoglossum Ring Spot Virus (ORSV) in Java and Bali, Indonesia. *Asian Journal of Plant Pathology* 10: 9−14.

Musetti, R. 2010. Biochemical Changes in Plant Infected by Phytoplasm, p. 132–146. *In* P.G. Weintraub & P. Jones (eds.), *Phytoplasms: Genomes, Plant Hostsand Vectors*. CAB International, Wallingford (UK).

Nurjanah, N., T. Joko, & S. Subandiyah. 2017. Characterization of *Pantoea ananatis* Isolated from Garlic and Shallot. *Jurnal Perlindungan Tanaman Indonesia* 21: 120−126.

Prasetyo, A.E., K.H. Mutaqin, & Giyanto. 2017. Deteksi dan Identifikasi Phytoplasm yang Berasosiasi dengan Penyakit Layu Kelapa di Pulau Derawan Kalimantan Timur. *Jurnal Fitopatologi Indonesia* 13: 89−97.

Suyanti & A. Supriyadi. 2008. *Pisang, Budidaya, Pengolahan, dan Prospek Pasar*. Penebar Swadaya, Depok. 132 p.

Semangun, H. 2007. *Penyakit-Penyakit Tanaman Hortikultura di Indonesia*. Gadjah Mada University Press. Yogyakarta. 845 p.

Warokka, J.S., P. Jones, & M.J. Dickson. 2006. Detection of Phytoplasm Associated with Kalimantan Wilt Disease of Coconut by the Polymerase Chain Reaction. *Jurnal Penelitian Tanaman Industri* 12: 154–160.

Wibowo A., T. Joko, S. Subandiyah, I. Mariska, Y. Supriyati, Y. Suryadi, & I. Roostika. 2010. Peningkatan Ketahanan Tanaman Pisang Kepok Kuning terhadap Penyakit Darah melalui Variasi Somaklonal dan Simbiosis Endofitik. *Jurnal Perlindungan Tanaman Indonesia* 16: 15–21.

Windari, U., T. Joko, & S. Subandiyah. 2015. Deteksi Penyakit *Bacterial Fruit Blotch* pada Melon Menggunakan ELISA. *Jurnal Perlindungan Tanaman Indonesia* 19: 1−5.

Wei, W., R.E. Davis, I.M. Lee, & Y. Zhao. 2007. Computer-simulated RFLP Analysis of 16S rRNA Genes: Identification of Ten New Phytoplasm Groups. *International Journal of Systematic and Evolutionary Microbiology* 57: 1855–1867.