



RESEARCH PAPER

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Distribution and diversity *Ralstonia solanacearum* wilt disease bacterial causes of banana (Kepok: Local Indonesia) and intensity of attack in South Kalimantan, Indonesia

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Abstract

Ralstonia solanacearum is a bacterial wilt disease in South Kalimantan, Indonesia. These bacteria have been scattered in banana (kepok: local Indonesia) planting which is a superior commodity, with a loss rate of almost 70-80%. Since 2007 the losses due to this disease have increased until mid-2014, the high level loss of resulted 100%. The purpose of this research was to determine the extent of the strain diversity and distribution of these bacteria extensively both of which attacked bananas, as well as alternative host plants in South Kalimantan. Methods used (1) Field surveys to districts that planted many banana and bacterial wilt disease, dyeing test on sterile water to ascertain the cause, (2) The sampling of plants attacked by climber and plant samples around the bacterial wilt disease, identification on *nutrient agar* (NA) and *Triphenyl tetrazolium chloride* (TZC). Results that the bacteria were found in all districts in South Kalimantan, with no different diversity, the appearance of bacteria on NA and TZC, showed a round shape colony with serrated, middle red and clear surrounding edges. Distribution of bacteria ranging from the district of Banjar, Tanah Laut, Banjarbaru, Tapin and to the farthest district Tabalong and Kotabaru. The highest level of attacks in the districts of Banjar, Banjarbaru and Kotabaru (100%). Other host plants found as many as 8 species in Banjar and Banjarbaru. Conclusion of the distribution of this bacteria is evenly distributed in South Kalimantan, the level of strain diversity is not different and found many alternative host plants.

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Introduction

Ralstonia solanacearum infected various types of plants in the world and causes wilt disease. In Indonesia bacterium-causing wilt disease causes major loss for farmers, especially those growing plantains. Bacterial wilt disease caused by *R. solanacearum* has become one of the major obstacles in production for all types of plants grown areas with tropical and sub-tropical climate. *Ralstonia solanacearum* causes bacterial wilt disease in many plant species worldwide. In Indonesia, diseases caused by bacterial diseases are very detrimental to banana cultivation, especially banana kepok. These bacteria have distribution over the banana plantation which is a pre-eminent commodity in South Kalimantan, with a loss rate of almost 70-80%. Since 2007 the disease has been on the rise until mid-2014, the high level of attacks caused losses of up to 100%.

Some plants that become host of the bacteria are cabbage, peanut, red bean and several types of weeds (Hayward, 1994). There are approximately 50 other familial plants such as *Solanaceae*, *Musaceae*, *Asteraceae*, *Fabaceae*, including forest trees, bushes and weeds. *P. (Pseudomonas) solanacearum*, a bacterium that causes wilt disease, (Yabuuchi, *et. al.*, 1995) is notorious to have vast geographical spread and various strains and identification of the strains and hosts is needed in order to control the spread. *Ralstonia solanacearum*, another wilt-disease-causing bacterium, is categorized into biovar group (Hartman, *et. al.*, 1993) and strains where each of them has its distinctive phenotypes and hosts.

Bacterial wilt disease is one of the most frequently found disease and can spread fairly quickly in sub-tropical and tropical areas more particularly to plants with economic value grown for example potato, tomato, eggplant, pepper, ginger, chili, peanut, plantain and tobacco (Hayward, 1990; Hayward, 1994). Machmud (1986), Machmud (1989) state that species of weeds grown in peanut plantation is potential host for *P. solanacearum*. Weed as important source of inoculums plays an important role because bacteria infecting weeds may sometimes not give obvious symptoms.

The bacterium has various strains and different level of severity towards various types of plants; different strains are found in different locations with distinctive characteristics. Some researchers distinguish by biovar and host plants (Horita and Tsuchiya, 2001; Ji, *et. al.*, 2007).

The research focuses on level of severity and spread of the bacterium in South Kalimantan (Yusriadi, *et. al.*, 2013). It is expected that the findings give recommendations about types of plants that can successfully be grown in particular areas as well as preventif steps to overcome and, even, avoid wilt disease and its spread. In South Kalimantan, bacterial wilt disease caused by *R. solanacearum* is the major obstacle in production of plantains since 2007 up to the until 2014. In South Kalimantan bacterial wilt disease *R. solanacearum* is the main limiting factor in banana cultivation (2007 to present). At the present time, the attack of bacteria has spread to other crops that are excellent commodities. Some alternative controls have not been successful to suppress its development, therefore it is very necessary that this study to know the diversity of strains and its spread, so as to facilitate the control (Jaunet and Wang, 1999).

The purpose of this research was to determine the extent of strain diversity and the distribution of this bacterium extensively both attacking bananas, as well as alternative host plants and main host and intensity of attack in South Kalimantan, Indonesia.

Materials and methods

Location of research and observation

The research sites were conducted on banana cultivation (kepok) in South Kalimantan, in some of the most planted districts. The survey was conducted by visiting and seeing the plants directly affected by bacterial wilt disease. The diseased plants in the collection and identification of the cause of the disease and isolate isolates are stored in the laboratory at appropriate temperatures. This research was conducted in Laboratory of Biological Control and Greenhouse of Department of Pest and Disease of Faculty of Agriculture, University Lambung Mangkurat, Banjarbaru, (December 2014- December 2015).

Media and equipment

The media used are NA (Nutrient Agar), TZC (*Triphenyl Tetrazolium Chloride*) and the samples of infectious disease from *Banjar* and *Banjarbaru*. The equipments are cups, test tube, erlenmeyer tube, pipette, measuring glass, scale, microscope, autoclave, oven, isolated box, camera, double tape, stationaries and other tools used in the glass house like plastic bucket, shovel, and manure.

Research methods

Research methodology is survey and taking samples of plants infected by wilt disease. The study is conducted through some steps (Djaya, 1994; Fahy and Persley, 1983) namely: (1) field survey to some areas where plantains are grown and infected by the disease. The plantains are then soaked in sterilized water to identify the cause of the disease; (2) taking samples of infected plants, (3) isolation and identifying causes (Yusriadi, *et. al.*, 1998), (4) patogenic test for the tobacco.

Taking samples of infected plants is located in a municipality called "Banjar" and some areas where vegetables are the most frequent type of crop to grow. Samples of the infected plants are taken, cleaned, and cut on the roots. The plants are soaked in sterilized water and when there is ose in the water, the conclusion is that the disease is caused by bacterium (*Suryadi and Mahmud, 2004*). Identification is conducted based on the area where the host is grown and alternative hosts. After that, observation is carried out to identify biochemical, cerological and other natures of the media. Identification based on the host and alternative hosts is conducted by planting the infected disease to various testing plants. One isolate from one type of peanut is tested to 10 types of plantains and 10 types of ginger; one isolate from one type of plantain is tested to 10 types of peanuts and 10 types of ginger and vice-versa. The testing plants are healthy plants and have grown for at least 30 days for peanut and ginger and 75 days for plantain.

Pathogenic level becomes the result of the test. *R. solanacearum* isolates are obtained from ginger, peanuts, eggplant, red chili, celery and plantain with wilt disease symptoms. The stem and roots are washed, and the roots are cut on the stem. The plants are then soaked into 70% alcohol for a few minutes and washed with sterilized water for 3 times. The next steps are soaking them into diluents solution andresting them until the "bacterial ooze" comes out. After that, they are scratched into the TZC and being incubated (Álvarez, *et. al.*, 2008).

Results and discussion

The plants infected by the bacteria have various types of plants in South Kalimantan. The distinctive characteristics of *R. solanacearum* causing wilt disease is sudden wither to all parts of plants and adventives roots on the stems. On the severe level, the stem is rotten, watery and eventually the plants die. The wilt disease infects plants in highland and lowland with waterlogged soil, high soil temperature and high moisturizer. When plants are infected by wilt disease, they will die or have a few, small fruits or even not produce any fruit when the plants are infected with the disease when the buds are blossoming or before it happens.

Almost all type of vegetables grown in "Banjar, Banjarbaru" and "Kotabaru" can be the host of the bacterium. The finding is presented in Table 1.

Decreasing number of plantain results in the bacterium infecting other plants around the plantains. There are vegetables grown near the plantain being infected by the bacterium. Such condition may be the reason why other types of plants in South Kalimantan can easily be infected by the type of wilt disease.

Table 1. Level of severity in some types of plants and *R.solanacearum* isolates.

Sample Number	Types of Plants	Severity	Place where the Plant is Grown
1.	Banana	+++	Banjar
2.	Peanut	++	Banjar
3.	Ginger	++	Banjar
4.	Tomato	++	Banjar
5.	Eggplant	+	Banjar
6.	Chili	+	Banjar
7.	Celery	+++	Banjar
8.	Banana	+++	Banjarbaru
9.	Peanut	++	Banjarbaru
10.	Ginger	++	Banjarbaru
11.	Tomato	+++	Banjarbaru
12.	Eggplant	++	Banjarbaru
13.	Chili	++	Banjarbaru
14.	Celery	+++	Banjarbaru
15.	Banana	+++	Kotabaru
16.	Peanut	++	Kotabaru
17.	Ginger	++	Kotabaru
18.	Tomato	+++	Kotabaru
19.	Eggplant	++	Kotabaru
20.	Chili	++	Kotabaru
21.	Celery	+++	Kotabaru

Note: level of severity + low, ++ mild, +++ severe.

Considering there are two types of media used, namely Nutrient Agar (NA) and *Triphenyl Tetrazolium Chloride* (TTZ)

(Jaunet and Wang, 1999), there is difference in the formation of the sides of the colonies.

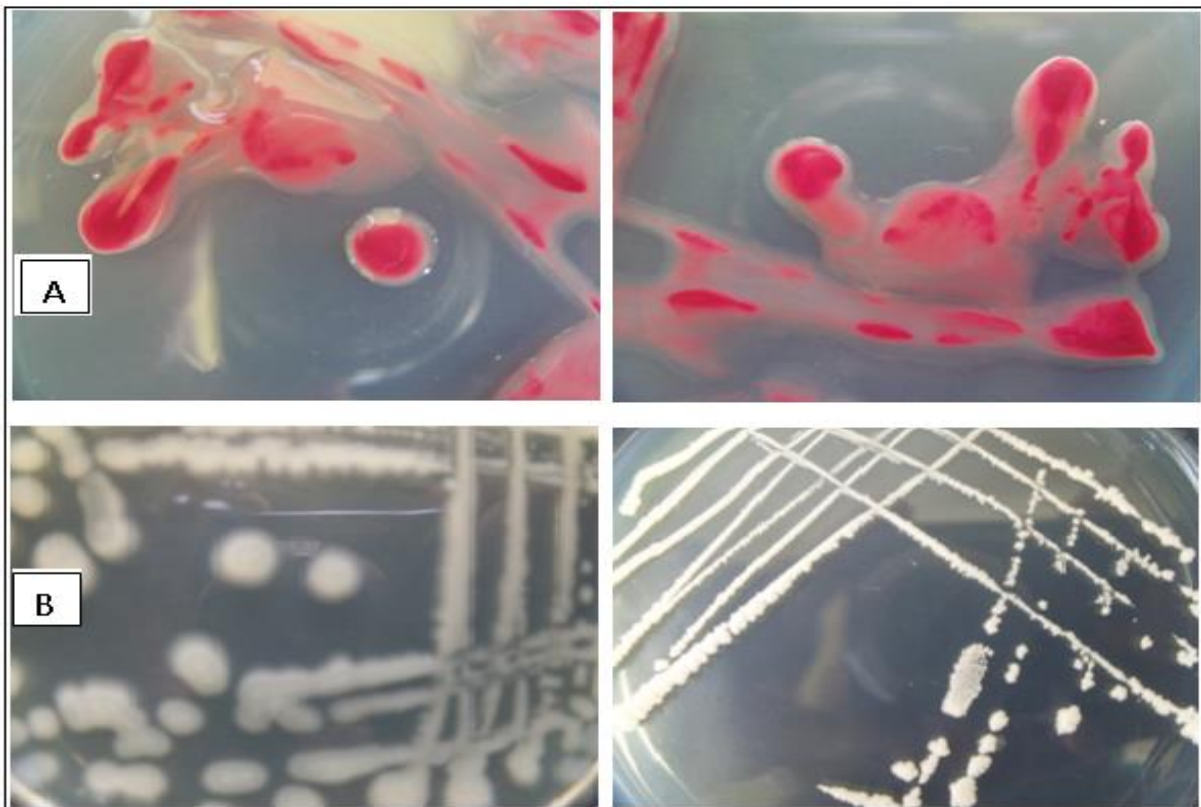


Fig. 1. (A). *R. solanacearum* Growth on medium TZC, (B) *R. solanacearum* Growth on medium NA.

The types of bacteria causing wilt disease are various and can be found in nearly all regions in South Kalimantan. The most frequent types of plants infected by the disease are plantain, celery and

tomato. Plantain is the most dominant type of plant infected by wilt disease; it is caused by the spread of plantain seed in infected areas.

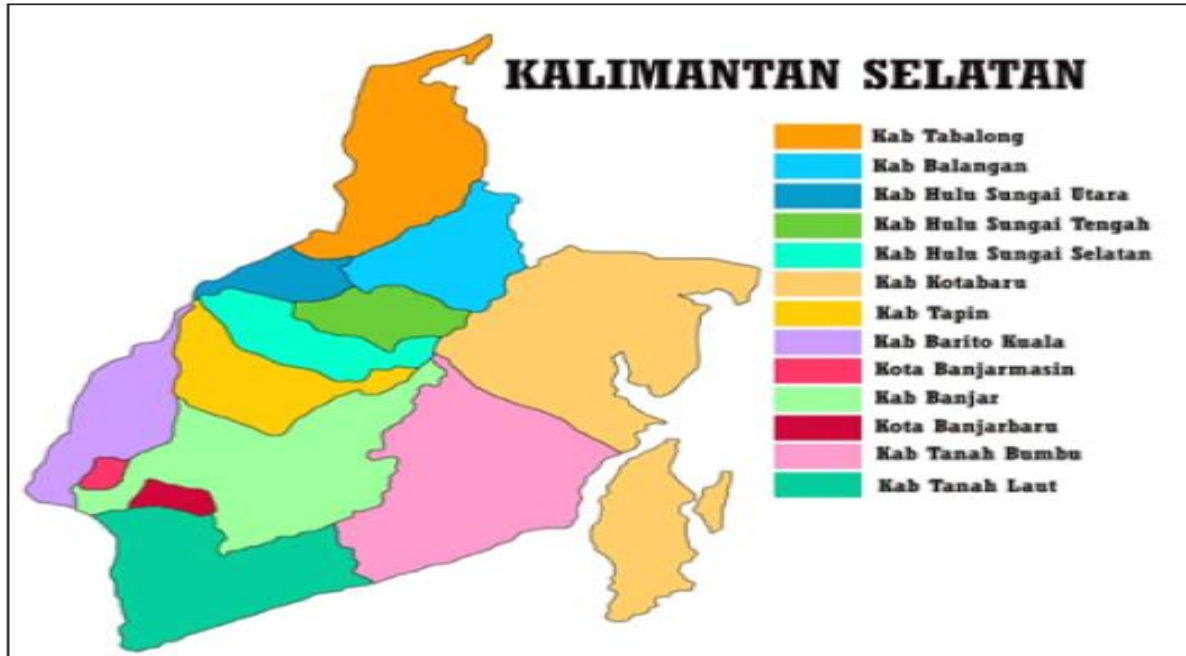


Fig. 2. Distribution of *R. solanacearum* causing bacterial wilt disease in South Kalimantan.

The distribution begins in Banjar, Banjarbaru, Tanah Laut, and Kotabaru, while on the southern area the spread begins in Tapin, Hulu Sungai Selatan, Hulu Sungai Tengah and Tabalong. The distribution of the disease in South Kalimantan, Indonesia can be seen in Fig. 2.

Conclusion

Distribution of *R. solanacearum* bacterial wilt disease in banana (kepok) has been prevalent in South Kalimantan, Indonesia with the intensity of attacks up to 100%. The diversity of strains found no significant difference between districts. The discovery of many host plants that become an alternative for this bacterial, so it can be a source of inoculum.

Suggestions

The need for continued research for the inventory of alternative host plants and harmful weeds in South Kalimantan. Control can be done with non-host plants for two seasons, so it can break the life cycle of bacteria.

References

- Álvarez B, Vasse J, Le-Courtois V, Trigalet-Démery D, López MM, Trigalet A.** 2008. Comparative behavior of *Ralstonia solanacearum* biovar 2 in diverse plant species. *Phytopathology* **98**, 59-68.
<http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO-98-1-0059>
- Djaya AA.** 1994. Efforts of Bacterial Late Control (*Pseudomonas solanacearum* E. F. Smith) On Ginger with Microorganisms Antagonists, Seed and Land Treatment. Thesis Prog. Postgraduate IPB, Bogor.
- Hayward AC.** 1990. Diagnosis, Distribution and Status of Groundnut Bacterial Wilt. In Middleton & Hayward (Eds.). Proceeding of an ACIAR/ICRISAT collaborative research planning meeting held at Genting Highlands, Malaysia 1990. ACIAR Proceedings **31**, 12-17.

- Hayward AC.** 1994. The host of *Pseudomonas solanacearum*. In Hayward, A.C. & G.L. Hartman (Eds.). Bacterial Wilt, the Disease and its Causative Agent *P. solanacearum*. CAB Int., U.K.:9-24.
- Hartman GL, Hong, Hanudin WF, Hayward AC.** 1993. Potensial of Biological and Chemical Control of Bacterial Wilt. In Hartman, G.L. & A.C Hayward (Eds). Bacterial Wilt. Proc. of an international conference held at Kaohsiung, Taiwan, October 1992. ACIAR Proceedings No.45:322-326.
- Horita M, Tsuchiya K.** 2001. Genetic Diversity of Japanese Strain of *Ralstonia solanacearum*. APS Press. **91(4)**, 399-407.
- Jaunet TX, Wang JF.** 1999. Variation in genotype and aggressiveness diversity of *Ralstonia solanacearum* race1 isolated from tomato in Taiwan. Phytopathology **89**, 320-327.
<http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO.1999.89.4.320>
- Ji Pingshen, Allen C, Sanchez-Perez A, Yao J, Elphinstone JG, Jones JB, Momol MT.** 2007. New Diversity of *Ralstonia solanacearum* Strains Associated with Vegetable and Ornamental Crops In Florida. Plant Disease **91**, 195-203.
<http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-91-2-0195>
- Fahy EM, Persley GJ.** 1983. Plant Bacterial Disease a Diagnostic Guide. Academic Press. Australia. page303.
- Machmud M.** 1986. Bacterial wilt in Indonesia. In Persley G.J. (Ed). Bacterial Wilt Disease in Asia and the South Pacific. Proc. Of an Int. Workshop held at PCARRD-ACIAR, Philippines. ACIAR Proceedings **13**, 32-34.
- Machmud M.** 1989. Resistance of Varieties and Plasma of Nutfah Peanut to Lute Disease (*Pseudomonas solanacearum*). In Sham, Mahyudin (ed) Seminar on Bogor Food Crops Research. 471-482.
- Suryadi Y, Machmud M.** 2004. Advances in Detection and Identification Techniques of *Pseudomonas solanacearum*. Journal of Scientific Review of Biological Research and Agricultural Biotechnology **1(1)**.
- Yabuuchi E, Osaka Y, Yano I, Hotta H, Nishiuchi Y.** 1995. Transfer of two Burkholderia and Alkali Genes Species to *Ralstonia* Gen; Proposal of *Ralstonia picketti* (ralston, palleroni and Doudroff, 1973) Comb., *Ralstonia solanacearum* (Smith, 1986) Comb. Nov. and *Ralstonia eutropha* (Davis, 1969) Com. Nov.
- Yusriadi B, Tjahjono M, Sinaga S, Machmud M.** 1998. The Influence of Giving Antagonist Mikroorganisme (*P. fluorescens* and *Trichoderma* spp.) To the development of Bacterial Lands Disease (*P. solanacearum* E.F. Smith) peanut plant. Bulletin of IPT IPT. **9(2)**.
- Yusriadi A, Abadi L, Halim H, Djauhari S.** 2013. Growth Pattern of *Ralstonia Solanacearum* Bacteria Causes of Lute Disease In South Kalimantan. Proceedings of the 22nd National Seminar of Indonesian Biological Society. Purwokerto, Indonesia