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The 4th International Conference on Sustainable Future for Human Security SUSTAIN 2013

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4th International Conference on Sustainable Future for Human Security, SustaiN 2013

Editorial

The 4th International Conference on a Sustainable Future for Human Security (SUSTAIN 2013) was held at Kyoto University (Japan) on 19-21 October, 2013. The conference was organized by SustaiN Society and the Indonesian Students Associations of Kyoto, with the support of the Organization for the Promotion of International Relations (OPIR) Kyoto University, Research Institute for Sustainable Humanosphere (RISH), Global Center for Education and Research on Human Security Engineering (HSE), Global COE Program for Sustainability / Survivability Science for a Resilient Society Adaptable to Extreme Weather Conditions (GCOE-ARS), and Inter-Graduate School Program for Sustainable Development and Survivable Societies (GSS).

The conference originated from the need to provide an inter-disciplinary forum where the most serious problems affecting a sustainable future for human security could be discussed, in recognition of the fact that many future problems cannot be solved by a "siloed" approach. The emphasis on sustainable futures is in response to the general awareness of the need to solve numerous human-related problems resulting from the rapid growth of modern society. The topic of sustainable futures for human security needs to be discussed in an integrated way, in accordance with the principles of sustainability, considering energy and materials supply, economies and trade, technology, cities, agriculture, social and environmental aspects.

To continue providing adequate technology to cope with the demands of human quality of life requires intensive research and development with multidisciplinary perspectives. Research and development towards achieving future human security should embrace sustainability perspectives, to avoid negatively impacting the environment and necessitating or exacerbating inefficient use of natural reserves, increasing emissions and hazardous wastes and jeopardizing human health and society.

The conference covered a wide range of issues with the aim of highlighting potential issues and paths towards a sustainable future. It attracted a high level of attendance from countries of the global North and South, with a wide geographical coverage. Overall, 160 participants were involved, with 120 presentations over the course of the conference. The quality of papers received was a testament to the reputation that the conference has been building over the past 3 years.

Papers presented at SUSTAIN 2013 were divided into five thematic areas: (1) Energy and Environment (EnE); (2) Sustainable Forestry and Agriculture (FA); (3) Sustainable Built Environment in Tropical Hemisphere Countries (BE); (4) River Basin and Disaster Management (RnD); (5) Social Science and Economics (SE). Under these broad areas, a wide-ranging series of presentations was given, which elaborated on current research across Asia and the world. Being held in Kyoto, a city of great cultural heritage, the participants also took part in a tour of some of the main sights and experiences that link modern and ancient Japan.

Selection and peer-review under responsibility of the SustaiN conference committee and supported by Kyoto University; (RISH), (OPIR), (GCOE-ARS) and (GSS) as co-hosts
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The two programmed days of the conference each commenced with keynote presentations which, like the conference itself, were wide-ranging. In the first session on day one, Dr. Ir. Edi Effendi Tedjakusuma, delivered an address on issues of a sustainable future for human security in the context of Indonesia. Dr. Puppim de Oliveira, Assistant Director and Senior Research Fellow at the United Nations University Institute of Advanced Studies (UNU-IAS), then discussed the future sustainability of cities in Asian nations. In the last keynote, Professor Satoshi Fujii, a Japanese cabinet adviser on Disaster Prevention and Reduction, introduced Japanese policy towards a more resilient country.

More than 230 participants attended the conference from 23 countries in Asia, North America and Europe. Around 161 papers were presented in the two days of conference. Only selected papers will be published in the Procedia Environmental Science and a special issue of the International Journal for Sustainable Futures for Human Security (J-SUSTAIN).

The organizers appreciate the support and assistance of the co-operating organizations, the participants, presenters and staff. The next SUSTAIN conference is highly anticipated by all the attendees of SUSTAIN 2013 and the committee expect to further build on the success of this year's event.

Selection and peer-review under responsibility of the SustaiN conference committee and supported by Kyoto University; (RISH), (OPIR), (GCOE-ARS) and (GSS) as co-hosts

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Host Range for Bacteriophages that Infect Bacterial Blight Pathogen on Soybean

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Abstract

Bacterial blight disease caused by *Pseudomonas syringae* pv. *glycinea* becomes one among several important factors that affects soybean production. To control this pathogen, most farmers apply synthetic pesticides that have negative effect for human, animal, and environment. Therefore, an alternative control must be developed including the use of bacteriophages. Moreover, identification and detection of this pathogen should be accurate for optimal diseases management. On the other hand, bacteriophage, a virus that infects specific host-bacterium, is largely known to have the ability to kill their specific host-bacterium. In addition, bacteriophage is also used as diagnostic tool for bacterial detection and identification of purpose, known as phatovar classification. This research was aimed to isolate the bacterial blight pathogen and their bacteriophage from soybean field in Jember, determine the pathogen pathogenicity and virulence, and determine the host-range of bacteriophage that infects several isolates of bacterial blight pathogen on soybean. The results has shown that among 12 isolates of bacterial blight pathogens that had similar characteristic to *P. syringae* pv. *glycinea*, only 10 isolates showed positive hypersensitive response (HR) on tobacco leaf. However, all isolates were virulent when inoculated to soybean leaves by showing particular bacterial blight symptom. In addition, one bacteriophage was found to infect isolate H3, called ϕPSGH3, and was to have 6 hosts among 12 bacterial isolates including isolates H3, SK4-2, BT4-1, KR1-1, KR4-2 dan MG4-1. The results from plaque assay PSGH3 with ϕH3-1 found out three only particle bacteriophage which are ϕGH1, ϕGH2 and ϕGH3.

Keywords: Soybean; bacterial blight; bacteriophages

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Nomenclature

PSG : *Pseudomonas syringae* pv. *glycinea*
NA : Nutrient Agar
NB : Nutriet Broth
UV : Ultraviolet
CFU : Colony Forming Unit
HR : Hypersensitive Response
PFU : Plaque Forming Unit

1. Introduction

Bacterial blight disease is one among several important disease on soybean that causes the loss of soybean productivity in Indonesian for about 65,88% [1]. The pathogen is generally recognized belong to fluorescent-group of Pseudomonad, *Pseudomonas syringae* pv. *glycinea* [2]. The symptoms can be easily observed on leaves of diseased plants in form of small, water-soaked spots with yellow halo surrounding the spot [3]. Unfortunately, the use of synthetic pesticides to control this pathogen is not recommended due to the environmental impacts including to animal and human [4]. Alternatively, the use of biological control has been widely developed for many pathogens including bacterial blight pathogen on soybean [5] including the use of bacteriophages. Addy *et al*., [6] have shown that the use of ϕRSM phage may protect a tomato plant from bacterial wilt pathogen, caused by *Ralstonia solanacearum*. Moreover, the use of Lytic phages, φRSA1, φRSB1, and φRSL1, are successful in protecting plant from bacterial wilt on tomato [7].

Bacteriophage is a virus that infects bacteria. Studies with bacteriophage as biocontrol of phytopathogenic bacteria have increased in the recent years [8]; [6]; [7] mainly due to the emergence of bacterial resistance to a number of antimicrobial agents [9]. Some publications have also shown that the bacteriophage is useful for controlling animal pathogen [10]; [11] to human [12] and foodborne pathogen [13]. Besides as biocontrol, bacteriophages is also useful as a tool for detection and identification of bacteria [14].

During phage-cell interaction, several stages are done by phage to complete their life cycle such as adsorption on host cell surface, penetration into cell, synthesis of virus components and assembly of virions, lysis of bacterial cell and phage release [15]. Since the early stage of interaction is adsorption, the phage needs a receptor to attach and begin their penetration. Some bacteriophages, especially lytic phages, the LPS and its related component inclugin O-antigen are important for recognition [16]; [17]. However, a filamentous group phage, the type IV pilus is important to infect and complete their cycle in the host cell [18]; [19].

This research was aimed to isolate the bacterial blight pathogen of soybean and their bacteriophages following to test their bacteriophages host range among several isolates of bacterial blight pathogen.

2. Materials and methods

2.1 Isolation of bacteria and bacteriophages

Bacterial pathogens were isolated from soybean leaves and pods with halo blight symptom in the some soybean field in Jember. Briefly, samples were cut and disinfected with 70% ethanol for 5-10 minutes then rinsed with sterile water. Sample were then grown on King's medium B and incubated for 24 hours. The single colony has been chosen by examining a colony under ultraviolet (UV) irradiation for fluorescent colony. For routine use, bacteria were cultured in the Nutrient Broth (NB) medium. The reference strain of bacterial blight pathogen we used *P. syringae* pv. *glycinea* 1a/96 as previously described by Ullrich *et al.,* [20].

Bacteriophages were isolated from irrigation water and soybean rhizosphere from several soybean fields in Jember, Indonesia. For screening phages capable of lysing *P. syringae,* we followed the method described previously for isolating *E. coli* phages [21]. Briefly, a 100 µl of irrigation water was added in to PSG culture prepared previously and incubated overnight at 28°C. The phage mixture was centrifuged at 4,000 rpm for 10 min and the supernatant was filtered through membrane filter (0.45 µm, Whatman), then the filtrate was subjected to

plaque-forming assay, as described by Yamada *et al*., [8]. Phages were purified by repeatedly plating and picking individual plaques and stored in SM buffer at 4°C.

2.2 Pathogenicity and virulence assay

Pathogenicity assay for bacterial blight pathogen was done through hypersensitive assay on the leaves of tobacco plants. HR-inducing ability was tested on leaves of the tobacco leaf. Inocula were prepared in sterile distilled water as described for the pathogenicity test. About 0.5 ml of bacterial suspension with a concentration of 10^8 CFU/ml was infiltrated on the surface of the tobacco leaf using sterile syringe. The tobacco plants were kept in Green house for about 24-48 hours.

Virulence tests were done on two weeks soybean plants. Inocula were prepared by suspending PSG cells in sterile water to a final concentration of about 1 x 10^8 CFU/ml. Plants were grown in individual pots in a greenhouse at 27 to 30°C. Plants were inoculated by the prick technique as described by May *et al.,* [22]. Bacterial suspensions (approximately 10^8CFU/ml) were sprayed (5 µl) on wounded-leaves of pin-pricked leaves. Plants were monitored daily for symptom development. All greenhouse experiments were repeated at least three times, independently.

2.3 Phage susceptibility assay

Phage host ranges were examined using both the spot test [23] and plaque-forming assay [8]. Briefly, for spot test, a 4.5 ml of soft agar was added with 100 µl of an overnight bacterial culture (cell density of 10^8 CFU/ml), and was gently vortexed and poured on the surface of Nutrient Agar (NA) medium. Single drops of each phage suspension were spotted on the inoculated hard NA plates, and the plates were incubated overnight at 28°C. The clear or turbid plaque formed on the surface of agar indicated susceptible result.

In the other hand, Plaque assay was done by diluted a 100 µl of phage solution (10^6 PFU/ml) with a 100 µl of a bacterial overnight culture (10^8 CFU/ml), and was gently mixed with 4,5 ml of molten soft agar and poured on the surface of Nutrient Agar (NA) medium. The inoculated NA medium then incubated at 28^0C for 24-48 hours prior to plaque examination.

3. Results

About 12 isolates of bacterial blight pathogen (Table 1) were isolated from diseased soybean-plant samples from several areas in Jember. All isolates were characterized according to their phenotypical and physiological properties such as Gram, fluorescent pigment production, coronatine production, ice nucleation activity, DNA assay and were shown to have similar properties with references strain of *P. syringae* pv. *glycinea* 1a/96 [24].

Table 1. Hypersensitive response assay and virulence test

Bacterial isolates	Isolates Origin	Assay result*	
		Hypersensitive	Virulence
H3	Collection	+	+
KR1-1	Desa Keramat	+	+
KR4-2	Desa Keramat	+	+
BT4-1	Desa Botosari	+	+
BT3-2	Desa Botosari	+	+
MG4-1	Desa Manggisan	+	+
SK2	Desa Sukorambi	-	+
SK2-1	Desa Sukorambi	+	+
SK2-2	Desa Sukorambi	+	+
SK3-1	Desa Sukorambi	+	+
SK3-2	Desa Sukorambi	+	+
SK4-2	Desa Sukorambi	-	+

+ symbol means positive result while (-) symbol means negative result. All results were obtained from three independent replication.

The result showed that among 12 isolates, only 10 isolates showing hypersensitive reaction on tobacco leaf (Table 1). The hypersensitive reaction on tobacco leaf was earliest shown about 12 hours post infiltration and was

more clear about 24 hours post infiltration with dark-brown necrotic only in the infiltrated-area on tobacco leaf (Fig 2). Interestingly, all isolates were showing similar symptom as natural diseased-soybean plant (Fig 1c), that was yellow "halo" surrounding the blight (Fig. 1b). The symptom was also observed to appear about 2 days post inoculation with bacterial suspension at 10^8 (CFU/ml) following the methods as described above.

In addition, phage isolation was done on NA medium following the methods as described above. Three phages were collected and purified based on their plaque morphology and were named as ϕGH1, ϕGH2 and ϕGH3. All phages were vary in sizes but similar on plaque morphology (Table 2). The characteristic of plaques were also observed in detail. In low concentration, the plaques were clear within 24 hours (Fig 2a), while in high concentration, the plaque was also clear but followed with small turbid plaque surronding the edge of clear plaques in more extended incubation periods (Fig. 2b).

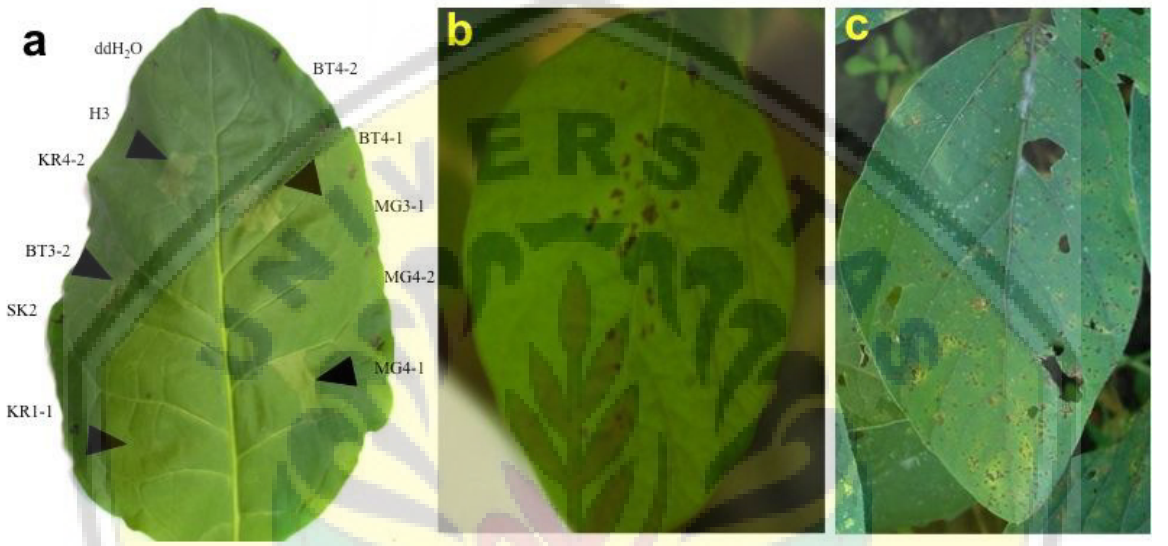

Fig. 1. Tobacco hypersensitive response (HR) after 24 hour post infiltration (a). Virulence assay on soybean leaf with symptom (b) with similar "halo" blight to natural diseased-leaf (c).

Table 2. Phages and plaques characteristics on *P.syringae* pv. *glycinea*

Phages	Plaque characteristics	
	Sizes (mm)	Morphology
GH1	< 2	Clear
GH2	2-4	Clear
GH3	> 4	Clear

The result was obtained from three independent replication using PSGH3 strain.

Furthermore, to know the host range of phages, we infected each phage in to all PSG isolates by following phage susceptibility assay. The result showed that all phages have similar host range, about 6 isolates among 12 PSG isolates. Interestingly, all phages were able to infect all representative PSG isolates that were isolated from Keramat (KR), Botosari (BT), Manggisan (MG) and Sukorambi (SK) (Table 3).

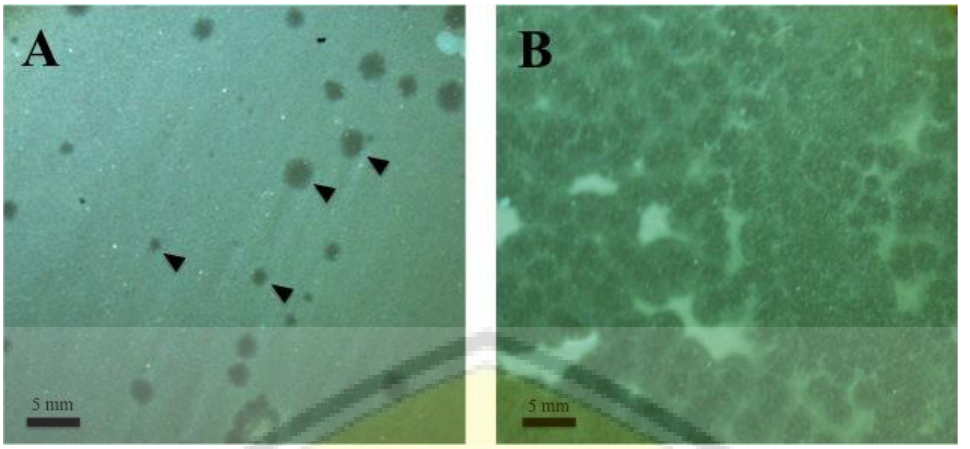

Fig. 2. Plaque forming on the lawn of PSGH3 by phage with low concentraiton of phage particles within 24 hours of incubation (A) and at high concentration of phages after 48 hours of incubation (B). Arrow-head indicated clear plaque.

Table 3. Host range assay of several bacteriophages against *P. syringae* pv. *glycinea* isolated from Jember area

Bacterial Host	Bacteriophages		
	ϕGH1	ϕGH2	ϕGH3
H3	+	+	+
KR1-1	+	+	+
KR4-2	+	+	+
BT4-1	+	+	+
BT3-2	-	-	-
MG4-1	+	+	+
SK2	-	-	-
SK2-1	-	-	-
SK2-2	-	-	-
SK3-1	-	-	-
SK3-2	-	-	-
SK4-2	+	+	+

*+ symbol meanpositive resultwhile(-) symbol meannegative result. All results were obtained from three independent replication both through plaque assay and spot test.

4. Discussion

All the isolates studied, originating from diseased soybean plants in Jember - Indonesia, belonged to *P. syringae* pv. *glycinea*. They were very homogeneous in morphological, physiological and biochemical characteristics and did not differ from the reference strain 1a/96 [5] obtained from Jacobs University, Germany.

Pathogenicity test on tobacco showed that only two isolates did not show hypersensitive reaction after 24 hours (Table 2), but after 48 hours after infiltration (data not shown) as the consequence of incompatible relationship between pathogen and host [2]. This normally occurs during hypersensitive test on tobacco leaves. Ignjatov *et al.,* [3] showed that HR appears on tobacco leaf at 24 hours after infiltration with *P. syringae* pv. *glycinea* suspension. In contrast, Kuarabachew *et al.,* [25] observed that HR on tobacco appears at 48 hours after infiltration with *R. solanacearum* suspension. Moreover, Schaad *et al*., [2] described that observation of HR could be observed until 48 hour after infiltration. Although the isolates are different in inducing HR, all isolates still show similar virulence on host plant (Table 1). All isolates were able to induce symptom on soybean leaf as it natural occurrences. The specific symptom, a yellow "halo" is also present in all tests (Fig 1b). Budde and Ullrich [26] found that the specific symptom on soybean leaf caused by the production of coronatine toxin by the pathogen. Moriwaki *et al.,*[27] studied with 25 isolates of *P. savastanoi* pv. *glycinea* and found that only 7 isolates formed spots accompanied with yellow "halo".

During interaction with the bacterial-host, bacteriophage may cause lyses the bacteria and inhibits the growth of

bacterial [15]. In the *in vitro* condition, inhibition of bacterial growth is indicated by plaque formation [28]. We have shown that three categories of plaque were determined by their size, small (<2 mm), medium (2-4 mm) and large (>4mm) (Table 2; Fig 3). The difference of the plaque size is normally on plaque assay and might be due to the difference of bacteriophage type or strain. Yamada *et al.,*[8] showed that about four types of bacteriophage were detected to infect *R. solanacearum* with different size of plaque. Moreover, Askora *et al.,*[2] and Addy *et al.,*[6] also showed that although different in phage strains, but ϕRSM phages showed indistinctive plaque.

Interestingly, all bacteriophages have similar host range, but not all bacterial isolates were infected by these bacteriophages (Table 3). Although the species of hosts are the same species, but their might have different host of bacteriophage as reported by Askora *et al.,*[18]. Moreover, Yamada *et al*., [8] showed that the same type bacteriophages, ϕRSS-type, have different host ranges among *R. solanacearum* strains. On the other hand, the host range of bacteriophage is also related on the species of the bacterial-host. Sundar *et al.,* [12] showed that bacteriophages isolated from *Salmonella typhi*, *P. aeruginosa and E. coli* were only able to infect bacterial species from their original bacterial-host. In Addition, the differences of host range of bacteriophage occurs probably due to the differences of phages receptor located on the bacterial-host cell surfaces. The specificity of the bacteriophages againts target bacteria due to their protein like type IV pili and /or lipopolysaccharide that is constitute in the bacterial membrane that serves as a receptor for adsorption of bacteriophage 15]; [18]. For example, ϕRSM1 and ϕRSM3 are the similar phages but their different on host range due to the difference of the component of minor pili of the host [18]. Moreover, phages ϕO4, ϕO5, ϕO7 and ϕO6 are different host range among *E.coli* strain due to variabilty O-antigen structure as a component of lipopolysacccharides [29].

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