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IDENTIFICATION OF *Ralstonia solanacearum* ISOLATED FROM A NEW HOST: *Cosmos caudatus* IN INDONESIA

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ABSTRACT

A new host of bacterial wilt was observed on the wild *Cosmos (Cosmos caudatus)* crop *in* Pacet-Cianjur, Indonesia. A novel strain of *Ralstonia solanacearum* was isolated and characterized by morphological, physiological and biochemical tests and host range. The results confirmed that the wild *Cosmos* was infected with *R. solanacearum*. Strains were characterized based on the symptoms expressed, morphological, physiological, biochemical and host pathogenicity. The new strains were classified into group 7, race 1 and biovar 3. This was the first report of *R. solanacearum* infecting wild *cosmos* in Indonesia. Information from this report will serve as a basis for control methods development.

Keywords: *Cosmos* (*Cosmos caudatus),* new host, bacterial wilt *Ralstonia solanacearum*, physiological and biochemical identifications

INTRODUCTION

Plant host-pathogen relationship is a complex interaction involving numerous internal and external factors. The invasion of host tissue by pathogens initiates a complex host and pathogen battle which continues throughout the course of their coexistence. Aside from physical environment, the difference in the timing and the level of infection relative to the current physiological and morphological states of the host organism genotypes also affect their susceptibility or resistance to invasion. As consequences, similar variations in pathogens influence their growth rates and virulence (Loomis & Adams 1983).

Pathogens are categorized into two broad groups, which are necrotrophs and biotrophs. These two groups are distinguished by their different substrate requirements. Necrotrophs, usually have a wider host range, act by killing host plant cell before parasitizing them. Naturally, host and pathogen cells cannot coexist harmoniously, thus pathogen releases toxins that act on metabolic targets common to

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many plants. Biotrophs on the other hand are generally obligate parasites which obtain nutrients from living cells. They typically infect through natural opening or by directly penetrating their host's surface. Some interactions between individual pathogen and plant cell, may lead to successful pathogen establishment (Alfano & Collmer 1996).

The successful host infection by pathogenic bacteria largely depends on the coordinated expression of a multitude of virulence factors and genes involved in the infection process. The complexities of bacterial gene expression are regulated at many different levels, including transcriptional control at the DNA level (Caldelari *et al.* 2013). There are a number of genetic changes that can alter the host range such as point mutations, duplications and horizontally acquired elements. These processes contribute to the evolutionary adaptation of the pathogen to new hosts (Kiliny & Almedia 2011). There are a number of examples of plant pathogens that have adapted and changed colonizing new hosts and environments, i.e. *Xylella fastidiosa* (Doddapaneni *et al.* 2006), *Xanthomonas* (Newman *et al.* 2008) and *Ralstonia* (Hanudin 1997; He *et al.* 1983; Hong *et al.* 1993; Wicker *et al.* 2002; Abdullah 1980).

Ralstonia solanacearum is known to be a major plant pathogen primarily in the tropical and warm temperate zones that causes bacterial wilt disease of over 200 species, including potato, tomato, eggplant, heliconia, and pepper (Hanudin 1997), mulberry, olive, and casuarina (He *et al.* 1983), *Perilla crispatan* (Hong *et al.* 1993), *Cucumis melo, Cucumis sativus, Cucurbita moscata*, and *Anthurium* spp (Wicker *et al.* 2002), winged been, ginger, and clove (Abdullah 1980). Strains of this generally soil borne betaproteobacterium form a heterogeneous species complex. Distribution patterns and phylogenetic analyses suggest that it has associated with plants for over 160 million years (Hayward 1991).

In the last decade, at the district of Pacet, Cianjur, West Java, Indonesia, *Cosmos caudatus* (Asteraceae; Compositae) has been infected with an unknown bacterial disease. *Cosmos caudatus* is also known as Kenikir (Indonesia), Ulam Raja (Malaysia) or Daoruang-phama (Thailand). *Cosmos* plant is an annual tropical herb that is used as medicine, vegetables and in food preparation (Baedeker 2009).

When a new disease outbreak occurs it is imperative that it be identified and characterized. Initial information can then serve as a basis for the development of new control recommendations. Many new bacterial identification systems have been developed that have been useful in distinguishing many new pathogen groups with unique new described host range (Suryadi & Machmud 1996; Joko *et al.* 2007; Zinniel *et al.* 2002). The most commonly used method of identifying pathogens is through the use of physiological and biochemical tests. This method needs less complicated equipment, skills and is cost-effective compared to unconventional method. In these scheme bacteria were classified on the basis of many characteristics, like cell shape, nature of multicelled-aggregates, motility, multiplication pattern and reaction to the gram stain (Suryadi & Machmud 1996). The information gained from the assessment may serve as a primary report of the existence of new bacterial strains and/or new hosted plants of a targeted pathogen.

Laboratory tests were conducted to identify the bacteria species associated with *C. caudatus*. The objectives of this research project were to (1) identify the pathogen

causing wilt disease on the wild *Cosmos* in Indonesia using physiological and biochemical tests, (2) describe the characteristic symptoms on *Cosmos*, 3) describe colony morphology on various media, and (4) conduct a limited host for, race determination.

MATERIALS AND METHODS

Disease Survey

The survey was conducted in the farmer's field about 1 km^2 around Indonesian Ornamental Crops Research Institute (IOCRI), Pacet, Cianjur West Java, Indonesia (latitude 6^o 45.5393'S, longitude 107^o 3.2193' E, 1100 m asl). Plants showing symptoms of wilt were collected and location of collection was recorded.

Glasshouse and Laboratory Assessments

The study was conducted at the IOCRI from April 2010 to May 2011. The objective was to determine the extent of outbreak and to make a positive identification of the pathogen using physiological and biochemical methods. Individual isolates were tested for hypersensitivity on tobacco and Koch's postulates were performed.

Isolation of the bacterial isolates

Bacteria were isolated by immersing roots and stem sections of symptomatic plants in tap water to release the bacteria. The plant suspension was streaked on tetrazolium chloride (TZC) medium containing 5% glucose, 10% peptone, 1% casamino acid, 15% bactoagar, and 1 ml of 1% (w/v) aqueous solution of 2,3,5 triphenyl tetrazolium chloride (Kelman 1954). Plates were then, incubated at 30 ^{0}C for 48 h (Patrice 2008).

The predominate colonies were mucoid, convex with red centers and whitish periphery on TZC. The strains were then compared biochemically, hypersensitive response, and Koch's postulates were performed. Previously identified *R. solanacearium* strains from IOCRI collection were used as controls.

Hypersensitivity test

The hypersensitive reaction (HR) was done on tobacco leaves (*Nicotiana tabacum* cv. Glutinosa) following the procedures of Lelliot and Stead (1987). Each strain was streaked on nutrient agar medium and incubated for 24 h. The growth from each strain was then suspended to sterile water and adjusted to a cell density of 10^8 cfu/ml using spectrophotometer (equivalent to 28% light transmission on 580 nm wavelength optical density). This cell suspension was infiltrated into fully expanded tobacco leaves using a hypodermic syringe (0.6 mm external diameter). The reactions were recorded 24 and 48 h after inoculation. A positive reaction was given by a rapid collapse with water soaked symptoms which later became dry light-brown necrotic tissue.

Koch's postulates test

Koch's postulates were done to verify that bacterial isolates were the causal agent of the suspected disease. Each strain was cultured on nutrient agar for 24 h, suspended in sterile water to a cell density of 10^8 cfu/ml using spectrophotometer (equivalent to 28% light transmission on 580 nm wavelength optical density). The cell suspension was injected into the stem of a healthy plant. Symptoms were recorded 2 weeks after inoculation.

Host range

A limited host range test was performed to help identify the bacterial race of *Ralstonia*. Six different hosts were used: tobacco, potato (cv. Granola), tomato (cv. Permata), eggplant (cv. Reza long purple), sweet pepper (cv. California wonder), peanut (cv. Local Cianjur) and wild *Cosmos*. The seeds of respected plants were sown in pots containing sterilized media (mixture of soil and horse manure at 1:1 v/v). The pots were then placed and maintained for seed germination under glasshouse conditions for 20 days. After 20 days, the plants had reached a height of 10-15 cm. Each strain was suspended and inoculated following the procedures of Alfarez *et al.* (1993). Ten individual plants per hosts were inoculated with each strain of the bacterium. A syringe containing 50 µl of inoculum (density of 10^8 cfu/ml) was inserted into the stem at the axil of the third fully expanded leaf (Lum & Kelman 1981) to facilitate the inoculum infiltrating entirely. The inoculated plants were then, maintained under glasshouse conditions for 20 days with wilt symptoms being rated every 3 days. Severity of wilting was rated using the following scale i.e. 1 = no symptoms, 2 = inoculated leaf wilted, 3 = two or more leaves wilted, and 5 = dead plant (He *et al.* 1983).

Culture for physiological and biochemical tests

Two additional *R. solanacearum* isolates from tomato (Tom 048), and eggplant (Egg 036) were used for controls as well as six isolates (Pot 006, Tom 008, Pin 016, Hel 019, Tom 022, Lec 023) from the Laboratory of Bacteriology IOCRI and two isolates (ACH 114 and 319) from Department of Microbiology, University of Queensland, Australia were also included in these tests and served as control (Table 1).

Isolate characterization based on physiological and biochemical tests

The observation on the physiological and biochemical tests included morphology, gram stain, fermentative-oxidative reaction, and the ability to use various carbon sources (Price *et al.* 1999). Colony morphology (color) was described for the bacteria while grown on the TZC medium. The gram test was carried out using potassium hydroxide (KOH). The KOH test consisted of placing 10 µl of 3% KOH solution onto glass slide. Bacterial isolate droplet was then, transferred into glass slide containing KOH and gently stirred in. If the suspension became sticky within 50-60 seconds, then the bacterium was categorized as gram negative. In contrast, if there was no such mucus, the bacterium was categorized as gram positive (Suslow *et al.* 1982).

For biovar determination, a basal medium containing 1% $NH_4H_2PO_4$, 0.2% KCl, 0.2% $MgSO_4.7H_2O$, 1% peptone, 0.3% aqueous bromothymol blue 1%, 1.5% oxoid agar no. 3 (Hayward 1964) was used for oxidation of carbohydrates. Six different kinds

Table 1. Isolates of *R. solanacearum* used in physiological and pathogenicity tests

No. Isolates	Host plants	Locations	Latitude (m asl)*)	Isolated date	Collectors
Pot 006	Potato	Lembang-Bandung (West Java, Indonesia)	1250	16-05-1994	Hanudin *et al.*
Tom 008	Tomato	Landbouw- Cianjur (West Java, Indonesia)	1,100	09-06-1994	Hanudin *et al.*
Pin 016	Peanut	Limbangan-Brebes (Central Java, Indonesia)	8	08-08-1994	Hanudin *et al.*
Hel 019	Heliconia	Ampelgading-Semarang (Central Java, Indonesia)	1,100	08-08-1994	Hanudin *et al.*
Tom 022	Tomato	Werasari-Subang (West Java, Indonesia)	100	16-05-1994	Hanudin *et al.*
Lec 023	*Solanum nigrum*	Panyekaran-Majalengka (West Java, Indonesia)	250	22-12-1994	Hanudin *et al.*
Egg 036	Eggplant	Pacet-Cianjur (West Java, Indonesia)	1100	08-04-2010	Ridwan *et al.*
Cos 037	*Cosmos caudatus*	Pacet-Cianjur (West Java, Indonesia)	1100	14-04-2010	Hanudin *et al.*
Tom 048	Tomato	Pacet-Cianjur (West Java, Indonesia)	1100	14-04-2010	Hanudin *et al.*
ACH 114	Potato	Wee Woa, NSW - Australia	NA	22-04-1966	Hayward, AC.
ACH 319	*Solanum nigrum*	Perwillaven-Australia	NA	24-01-1968	Hayward, AC.

Remarks = '*' NA = not available data

of carbohydrate sources i.e. lactose, maltose, cellobiose, mannitol, sorbitol and dulcitol were separately filter-sterilized and then, supplemented into the basal media.

The bacterial suspensions were inoculated into these prepared media and the cultures were then, incubated under room temperature for 21 days. The observations on the acid production were conducted every day with gradual color change for acid production from green to yellow.

RESULTS AND DISCUSSIONS

Disease Symptoms of Collected Plants

The surveyed location was a highland area characterized by high relative humidity throughout the year. The survey activities were conducted during the months of May to August, since the bacterial wilt incidences were higher in dry season. Wild *Cosmos* was commonly used by farmer for bordering their primary vegetables and ornamentals sites, since the *Cosmos* plants have been known as insect repellent to protect their plants and indigenous vegetable. According to local farmer, the first appearance of bacterial wilt on *Cosmos* was observed 11 years ago and based on the surveys conducted on the farmer's field, the disease incidence reached 35% of all visited sites. Similar findings was also reported by Sequeira and Kelman (1976) that *R. solanacearum* isolated United States was found highest during hot seasons.

Samples of suspecious infected *Cosmos* plants were collected and recorded. Typical symptoms of wilt were observed on infected plants (Fig. 1b) with vascular

Figure 1. (a) Healthy and (b) wilting plants as suspected to be infected by bacterial wilt at farmer's field.

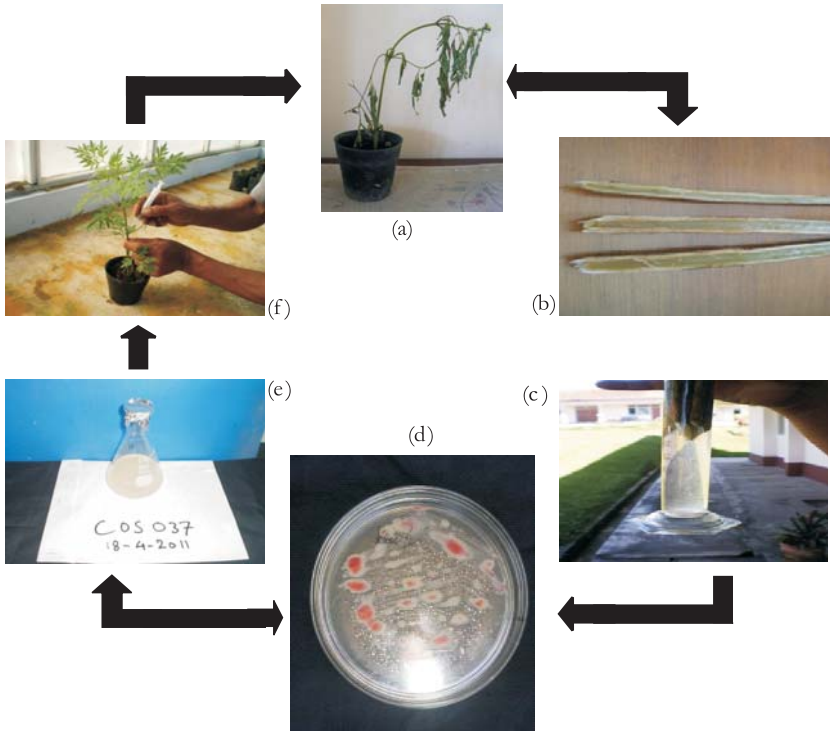

Figure 2. Steps in pathogenicity assays of *R. solanacearum* conducted in this experiment; (a) collection of the infected plant as indicated by wilted shoot and (b) discoloration of stem vascular tissue, (c) whitish bacterial fluid secretion after soaking the stem in sterile water (as indicated by blue arrow), (d) mucous, convex bacterial colony with red at center and whitish at peripheral after the exudates were inoculated in TZC medium, (e) inoculation of isolate in nutrient agar medium and, (f) reinoculation of isolated bacterial culture into healthy *Cosmos* plant. The above steps were carried out according to Koch's postulates.

discoloration as presented on Figure 2a and b. Similar symptoms was observed in tomato plant when infected by *R. solanacearum* as reported by Loreti *et al.* (2008). Bacterial wilt infection occurred mainly through wounded tissues, though some reports suggested that infection could occur without injury, thus injuries might further accelerate root tissue infections (Caitilyn *et al.* 2006). At the early infection, the plant showed collapse of the growing apex. Stunted lateral emerged but then wilted and died, while adventitious root appeared on the stems. Soaking the infected stem and root into sterile water in few minutes would secrete cloudy white bacterial fluid like condensed milk from the plant tissue (Fig. 2c).

Bacteria Isolation and Koch's Postulate Test

Isolation of suspected bacterial pathogen on TZC medium was succesfully conducted. The exudates inoculated in medium grew into bacterial colonies after 24 h incubation. The colonies were mucous, opaque, pleomorphic, convex, with red center and whitish periphery as presented in Figure 2d. These findings were compatible with that found in tomato as reported by Romano *et al.* (2012).

After inoculated into nutrient agar medium (Fig. 2e), the bacterial isolate was suspended in sterile water in certain density then reinoculated into healthy plants (Fig. 2f). After 2 weeks, the injected plants were wilting with brown discoloration on xylem stem tissue. These symptoms were similar to those observed on infected *Cosmos* plants within farmer's fields. These results confirm Koch's postulates. These relationships may be referred to a congruent taxonomical life forms and features (Kwon & Hong 2005).

Isolate Culture Characteristics

Culture characteristic was an important feature in identifying the unknown bacteria. These included colonial shape and appearance, such as pigmentation and optical characteristics. The physical characteristics were often specific and distinctive for the type or species of bacteria making the colony and could be used as a mean of recognition. Cultural characteristics or morphology were determined by the respective microorganism, physical condition during culture and the nutrient medium being used. Thus, the colonial morphology of the same bacteria might vary on different media or under different conditions. It is suggested then, the identification of targeted bacteria be cultured under the same circumstances together with the well-identified bacteria as a control (Dithal *et al.* 2001).

The observation on the cultured isolates on TZC medium after 24 h revealed that the isolates of Cos 037 was similar to control (ACH 114, and ACH 319). They produced rough fluidal and butyrous colonies with pink or red at the centers (Fig. 2d). Similar patterns were also observed on Egg 036, Tom 022, Tom 048 and Hel 019 isolates, which were known as virulent *R. solanacearum* strains isolated from eggplant, tomato and heliconia. These similar features indicated that targeted bacterial isolates from Cosmos (Cos 037) was presumably also a virulent bacterial strains.

All strains tested had rod shaped cells and were gram negative. Gram-staining response of bacteria is an empirical criterion (Patrice 2008). The basis laid in the

marked differences in the ultrastructure and chemical compositions. These two kinds of cells (negative and positive grams) were distinguished upon the presence or absence of an outer lipid membrane which was a fundamental characteristic of a bacterial cell (Chaudhry & Rashid 2011). Unlike gram positive bacteria, gram negative bacteria has thinner (single) peptidoglycan layer and low murein (10-20%), with many outer membranes with high lipopolysaccaride (LPS) and lipid/lipoprotein contents. Mostly, they were characterized by high resistances to physical disruption, drying and antibiotic (Zhu *et al.* 2010).

Hypersensitivity and Pathogenicity Test

Hypersensitivity (HR) tests was carried out on tobacco leaves, only isolate Pot 006 (isolated from Potato) produced yellowing on the infiltrated leaf, while the rest tested bacteria strains showed typical positive HR. Lelliot and Stead (1987) reported that yellowing or browning without collapse on infiltrated area of tobacco leaf was not a positive reaction. A positive HR was given by a rapid collapse and water soaking of inoculated tissue in 24 h and at most 48 h, the tobacco leaf showed dry, light-brown necrosis of water soaked tissue.

For host range testing all bacterial strains were inoculated on six potential hosts (tobacco, potato, tomato, eggplant, pepper and peanut), while only three strains i.e. Egg 036, Cos 037 and Tom 048 were inoculated into wild *Cosmos* plants. Most of the

Table 2. Hypersensitivity evaluation on tobacco leaf, pathogenicity reaction on tester plants on 15 days after inoculation and classification of tested bacterial strains

Isolates	Hypersensitivity*) (HR)	Tester Plants**)							Group	Race
		Potato	Tomato	Eggplant	Tobacco	Peanut	Pepper	*Cosmos*		
Pot 006	-	M	H	0	0	0	0	Nt	5	3
Tom 008	+	H	H	M	0	L	H	Nt	2	1
Pin 016	+	L	M	M	0	L	M	Nt	2	1
Hel 019	+	L	L	H	L	0	M	Nt	6	1
Tom 022	+	M	H	H	H	0	H	Nt	3	1
Lec 023	+	L	M	M	0	0	L	Nt	3	1
Egg 036	+	H	L	H	H	0	H	0	8	1
Cos 037	+	H	H	H	L	0	H	H	7	1
Tom 048	+	H	H	H	H	0	H	0	8	1
ACH 114	+	L	L	L	0	0	L	Nt	3	3
ACH 319	+	L	M	L	0	0	M	Nt	3	1

Remarks: *) Hypersensitivity '+' = infiltrated tobacco leaf became necrotic after 24 h inoculation
'-' = infiltrated tobacco leaf showed yellowing even after 48 h inoculation

**) Pathogenicity H = high (wilted tester plants ≥ 60%)
M = medium (wilted tester plants 30-59%)
L = low (wilted tester plants ≤ 29%)
0 = no reaction
Nt = not inoculated

(a) Tobacco (b) Potato (c) Tomato (d) Eggplant

(e) Pepper (f) Peanut (g) Wild *Cosmos*

Figure 4. Performance of tester plants; (a) tobacco, (b) potato, (c) tomato, (d) eggplant, (e) pepper and (f) peanut inoculated by all tested bacterial strains and (g) wild *Cosmos* inoculated by strains of Egg 036, Cos 037 and Tom 048, 15 days after inoculation.

strains caused wilting on tomato, potato, and tobacco, eggplant and pepper, while the least was found in peanut (less than 29% wilted tester plants caused by strains Tom 008 and Pin 016). From the three strains (Egg 036, Cos 037 and Tom 048) inoculated into wild *Cosmos* plant, only Cos 037 triggered symptom (Table 2 and Fig. 4). Wilting symptoms began to appear in different times in each tester plants and tested strains. However, the symptom generally appeared at 2-5 days after inoculation consisted of wilting of the inoculated leaf and stunted growth.

On the basis of these host reactions, the strains of *R. solanacearum* could then be grouped into eight pathogenicity groups. He *et al.* (1983) classified *R. solanacearum* strains into 6 groups, since *Cosmos* has not yet been observed and confirmed to be a host of *R. solanacearum*. According to them, group 1 was categorized to be virulent on all tester plants (hosts), excluding wild *Cosmos*. Group 2 represented virulent strain in all tester plants except tobacco and wild *Cosmos*, while those avirulent to tobacco, peanut and wild *Cosmos* but virulent to potato, tomato, eggplant, and pepper were included in *R. solanacearum* group 3. Group 4 represented virulent strain on tobacco and pepper. Group 5 included a single strain from potato which was known to be highly virulent only on potato and tomato. Group 6 included strains from tobacco, tomato, pepper, heliconia, and paprika.

Based on the pathogenicity testing on *Cosmos* plant then, two groups were purposely confirmed as group 7, which was a strain isolated from *C. caudatus* infected all seven hosts, except peanut. The next was a strain which was able to infect all hosts, except peanut and *C. caudatus* (group 8). This is apparently the first report of *R. solanacearum* as a natural host of *C. caudatus* or even a species of Asteraceae in Indonesia. This information is important to define strategies for bacterial wilt control in Indonesia, as related to crop area selection and rotation. Additionally, since "wild *Cosmos*" is propagated by seeds, it is necessary to avoid spreading of the disease through infected soil and planting material.

Biochemical Tests

The biochemical evaluation of all bacterial isolates was carried out to categorize the isolates into biovar based on their ability to oxidize different kinds of carbohydrate sources (Ahmed *et al.* 2013). The result of the biovar evaluation showed that all bacterial isolates oxidized sugar alcohols (mannitol, dulcitol and sorbitol), except isolates Pot 006, Tom 008 and ACH 114. On the other hand, only isolate ACH 319 from all isolates that did not produce acid from lactose, maltose and cellobiose (Table 3). The oxidation reaction was indicated by color changes of the medium from green into yellow, thus in the absent of these reaction, the color of medium would remain unchanged (green). The color changes reflected the production of acid as a result of carbohydrate oxidation by bacterial colony (Huang *et al.* 2012). The appearance of slight yellow color was firstly observed surrounding the colony at 3-5 days after inoculation. The medium color then, changed completely into yellow after 21 days under $28 \pm 3\ ^{0}C$.

The typical oxidation reaction of bacterial strains on these different carbon sources served as basis on the classification of bacterial biovars (Hayward 1964; He *et al.* 1983; Kumar *et al.* 1993). From 11 tested bacterial isolates, 3 isolates belonged to biovar 2 which oxidized only sugar alcohols. Biovar 3 represented the strains that consumed both polysaccharides and sugar alcohols (7 isolates), while ACH 319 included in biovar 4, which showed positive oxidation reaction only on sugar alcohols.

This study also confirmed a negative correlation between the biovar types and distribution of isolates where they were commonly found. All biovar 2 strains from tomato and potato were collected from higher altitudes (Pot 006 was collected from Lembang, West Java at 1250 m asl and Tom 008 was collected from Landbouw-Cipanas, Cianjur, West Java at 1100 m asl). Some isolates that were collected from high altitudes like Egg 036, Cos 037, Tom 048 (collected from eggplant, cosmos and tomato respectively at Pacet, Cianjur, West Java, 1100 m asl) and Hel 019 (collected from heliconia at Ampelgading, Semarang, Central Java, 1100 m asl), however, were belonged to biovar 3 (not biovar 2). On the other hand, most bacterial strains collected from lowland areas were belonged to biovar 3. These findings were in accordance with Bandara (1983) and He *et al.*(1983) that the patterns of *R. solanacearum* biovars and races were not always affected by altitudes.

Another study by Hayward (1976) and Buddenhagen *et al.* (1962) indicated that biovar classification of *R. solanacearum* might indicate their adaptability on certain ranges of temperature. Biovar 2 was observed to have more rapid growth at $27\ ^{0}C$ than $37\ ^{0}C$. Biovar 3 and 4, however, showed an opposite characteristic, which grew better at higher temperature ($37\ ^{0}C$). These features might reflect their natural incidences in relation to the availability of their hosts. Hayward (1964) suggested that geographical isolation by latitudes approached these conditions. He observed that biovar 2 were commonly found as a sole biovar and they hosted only on specific plants (potato and/or tomato) in higher latitudes. Certain most Northern and Southern latitudes might be the limits of the geographical distribution of *R. solanacearum*.

Table 3. Biovar determination of tested bacterial isolates based on biochemical reaction on different carbohydrate sources

Bacterial isolates	Carbohydrate sources *)						Biovar
	Mannitol	Dulcitol	Sorbitol	Lactose	Maltose	Cellobiose	
Pot 006	-	-	-	+	+	+	2
Tom 008	-	-	-	+	+	+	2
Pin 016	+	+	+	+	+	+	3
Hel 019	+	+	+	+	+	+	3
Tom 022	+	+	+	+	+	+	3
Lec 023	+	+	+	+	+	+	3
Egg 036	+	+	+	+	+	+	3
Cos 037	+	+	+	+	+	+	3
Tom 048	+	+	+	+	+	+	3
ACH 114	-	-	-	+	+	+	2
ACH 319	+	+	+	-	-	-	4

Remarks : *) '+' = color changes on medium from green into yellow
'-' = no color changes on the medium

CONCLUSIONS

The incidence of bacterial wilt on wild *Cosmos* at Pacet, Cianjur, and West Java, Indonesia was shown to be caused by the pathogen *R. solanacearum*. The infected plants were stunted and wilted. The xylem of stems had red/brown discoloration on xylem tissue and produced ooze when dipped in water. The bacterial cultures on TZC medium showed mucous, pleomorphic and convex colony with red center and whitish periphery. The colony was rod in shape and classified as gram negative on their gram-stain retention. Tobacco leaf showed positive hypersensitivity reaction to these bacterial isolate and based on biochemical evaluation, it was included on the strain that could utilize polysaccharides and sugar alcohols for carbon sources.

Based on the symptoms, morphological, physiological, biochemical and pathogenicity evaluations, this *R. solanacearum* belonged to race 1, biovar 3 and group 7. This was the first report of the *R. solanacearum* hosted in wild *Cosmos* in Indonesia. The findings of the present study will be useful for designing the study of the population structures of *R. solanacearum* using the molecular approaches with special emphasis on its integrated management. These new findings may also serve as a basis on the protection management and quarantine policy for the incoming plant/plant material on certain area.

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