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Survey and Detection of *Pectobacterium atrosepticum* in Major Potato-Growing Areas in Central Java Province, Indonesia

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ABSTRACT

Potato (*Solanum tuberosum*) is a seasonal shrub-tuber crop originated from sub-tropical area. Soft-rot is one of the most important diseases of potato. It can be caused by *Pectobactorium atrosepticum*, a pathogen within a status of quarantine plant pest A1 type I in Indonesia. The objective of this study was to know the incidence of potato soft rot disease and to detect *P. atrosepticum* in major potato-growing areas in Central Java Province by applying the serology method using DAS-ELISA technique. Survey of soft rot disease was carried out in some regencies in Central Java Province, i.e. Magelang, Banjarnegara, Wonosobo and Karanganyar. The field survey of potato plant in all the regencies indicated symptoms of stem rot which was black in color (blackleg) and foul-smelling, with disease incidence of about 10–90%. The laboratory testing showed that by applying DAS-ELISA method, *P. atrosepticum* was detected in samples collected from Pandean and Bagongan villages, district of Ngablak,Regency of Magelang, Central Java Province.

Keywords: Detection, ELISA, *Pectobacterium atrosepticum*, Potato

INTRODUCTION

As one of the main sources of carbohydrate, potato is an important commodity. Compared to other types of horticultural commodity, it has the most opportunity for agribusiness and agriculture development (Anonym, 2011). Potato production in Indonesia is lower (only 16 ton/ha) than Europe, where the average achieved 10% of the national demand; 4.9% of it is supplied domestically and the rest is imported (Baharuddin *et al.*, 2012). One of the constraints causing the low production in Indonesia is the soft rot and blackleg diseases. Soft rot is an important disease because it infects and causes severe disease to many types of vegetables, food, and ornamental crops (Joko *et al*., 2007a; 2007b). Soft rot disease causes major loss in worldwide agriculture. The cause of the disease is also reported to be spread from temperate, subtropics to tropical area (Perombelon and Kelman, 1980). It was reported in Orchidaceae that the soft rot disease in nursery with poor management can reach more than 50% (Joko *et al*., 2011a; 2011b). Various species of pathogenic bacteria within families of Enterobacteriaceae and Pseudomonadaceae can be isolated from orchid plant with soft rot symptoms (Joko *et al*., 2014).

The main cause of rot to the stem base of the potato and the tubers that requires attention is *Pectobacterium atrosepticum*. Other than, *P. atrosepticum*, which is dominant in cold area, Dickeya spp.and *P. carotovorum* are well developed in warmer area (above 25°C). They are also reported to cause rot in the trunk of potato plant (aerial rot) (Perombelon, 1992; Toth *et al*., 2003). *P. atrosepticum* is transmitted by the seed (seed-borne). This indicates that imported seed highly risky being entrance of pathogen into Indonesia. Efforts to trace the probability of the entrance through the imported tuber seed of *P. atrosepticum* can be done by detecting the existence of the pathogen in suspected area distribution of the imported seed.

It is important to take preliminary detection upon the existence of *P. atrosepticum* especially in imported seed being the entrance doors. This is to avoid the dispersion of the bacteria through the seed as the

carrier media. Also, it requires continual monitoring of potato production centers in Indonesia to confirm the existence of P. atrosepticum in the suspected area. Thus, a quick and accurate detection method is required. One of the mostly used is immunoassay. Various immunoassay methods are available; one of them is the Enzyme-Linked Immunosorbent Assay (ELISA). Immunoassay is a method to measure the unrefined substance using specific antibody. The enzyme used for the immunology indicator is the main characteristic of this technique (Burgess, 1995). This method is carried out by reacting the antiserum with the antigen of the microorganism. Antigen is used for identification due to its long lasting stability (Bowden, 1993). The objective of this study was to identify the soft rot disease incidence in potato plant centers in Central Java as well as to detect *P. atrosepticum* as the quarantine plant pest A1 type I by using the DAS-ELISA method.

MATERIALS AND METHODS

Survey and Sampling

The samples of the plant or part of the potato plant with symptoms of soft rot and blackleg diseases were collected from 9 locations within the 4 regencies of potato plant centers in Central Java. They were 1. Patakbanteng village, district of Kejajar, regency of Wonosobo at 2,565 m above sea level (asl) (7' 12' 33'. 18' LS and 109' 55' 25.44' BT); 2. Tieng village, district of Kejajar, regency of Wonosobo at 1,789 m asl (7º 13'. 37.109'.41' LS and 109' 52' 110' 64 BT); 3. Kejajar village, district of Kejajar, regency of Wonosobo at 2,302 m asl (7º 15' 20' LS and 109' 47' 35' BT); 4) Buntu village, district of Batur, regency of Banjarnegara at 1,010 m asl (7' 16' 24' LS and 109' 57' 17' E); 5. Batur village, district of Sumberejo, regency of Wonosobo at 1,663 m asl (7' 12' 31' LS and 109' 29' 45' BT); 6. Gembol village, district of Pejawaran, regency of Banjarnegara at 1,150 m asl (7° 14' 45' S 109°47' 47' E); 7. Bagongan village, district of Ngablak, regency of Magelang at 1,500 m asl (7° 22' 17° 110° 23' 52' E); 8. Pandean village, district of Ngablak, regency of Magelang at 1,500 m asl (7º 19' 24' 9' LS and 110' 36' 36'. 0; BT); and 9. Segorogunung village, district of Argoyoso, regency of Karanganyar at 1,500 m asl (7° 36' 37' S 111° 7' 58' E). The locations were selected based on the data where the potato plant commodity being the main host-plant. Observations were taken only on fields indicated with symptoms of soft rot and blackleg diseases under the purposive random sampling method. Survey and sampling were carried out two times in September 2012 and early January 2013. Samples included stem, trunk and tuber indicated with the symptoms of the diseases.

Samples were put on a paper and into an envelope before processed in the laboratory for serology assays (Joko *et al*., 2012).

Calculating the Disease Incidence

For each observed fields, 20 plants were selected with diagonal scouting and disease incidence was calculated (Wardhika *et al*., 2014). The age of the plant indicated disease symptom was recorded. The disease incidence can be calculated by using the following formula (Cooke, 2006):

$$\text{Disease Incidence} = \frac{n}{N} \times 100\ \%$$

Disease Incidence is the percentage of plant infected by pathogen (n) of the total observed plant (N).

Detecting the Pathogen using ELISA

Samples of stems, trunks and tuber of the potato plant indicating unique symptom of soft rot or blackleg diseases were detected using the double anti sandwich enzyme-linked Immunosorbent assay (DAS-ELISA) with antiserum specific to *P. atrosepticum*. Of each location, three repetitions were made and the DAS-ELISA testing were based on the protocols made by the antiserum producer (Agdia, Inc.). Coating humid box was given with wet towel paper under it and ELISA plate was stored to maintain the evaporation. Before used, capture antibody was diluted by using the coating buffer. A 100 µl antibody solvent was prepared and added into each plate well, incubated for 4 hours in room temperature and washed using the PBST. Samples with symptoms were extracted using the general buffer (GEB) within 1:10 ratio. A 100 µl sample diluted for each testing well was divided into wells of 100 µl positive control, 100 µl negative control and 100 GEB of buffer well. Samples were incubated for 2 hours in room temperature, then the conjugate was diluted using ECI buffer and washed using the PBST solvent by adding 100 µl conjugate to each plate well. The plate was incubated for 2 hours at room temperature and added with PNP solvent within concentration of 1 mg/ml of one tablet for 5 ml of PNP solvent. It was put into plate well and incubated for 60 minutes.

Color intensity in the testing results was measured by using the ELISA reader at wave length of 405 nm. Absorbance reading upon the sample using the ELISA reader was stated positive when the absorbency value of ELISA of the specimen sample

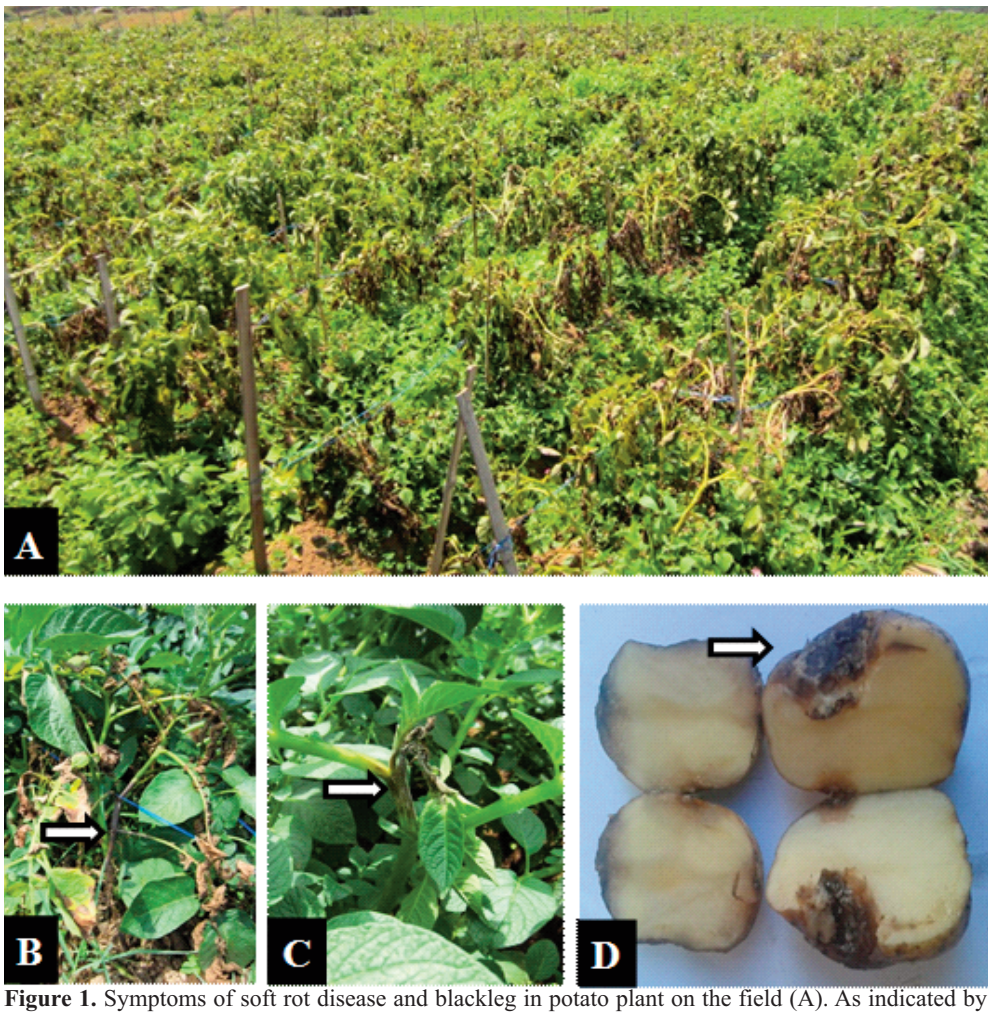

Figure 1. Symptoms of soft rot disease and blackleg in potato plant on the field (A). As indicated by arrow, the rot symptoms are visible in the stem (B) or can also experience rot in the trunk or tip of the infected plants (C), infected tuber shows soft rot symptom in blackish-brown color and produces foul-smelling aroma (D).

was larger or the same as 2 times of the average negative control (healthy plant) (Windari *et al*., 2015).

RESULT AND DISCUSSION

Disease Symptoms on the Field

The results of field survey upon the potato plant in all districts showed unique symptoms of soft rot, indicated from green turning yellow leaves with black color base-stem continued to tip of the plant. In most severe condition, it smelled strongly from the rotten wound. Plants infected by the disease would be withered and then died. Fields with severe infection showed mass wither with dried leaves and brownish color (Figure 1A). Infected plants showed early symptoms of lesion in the stem base of the plant. When the infection continued, it would cause lengthwise blackleg (Figure 1B), especially when the plant entered two months of age. Lesion could also occur on the top area of the trunk (aerial rot) (Figure 1C). Likewise, tuber in the plant with disease indicated unique symptom of soft rot and foul-smelling (Figure 1D). The development of soft rot disease was also worsening by the moist soil on the cultivation field in which the pathogen infection is spreading to the entire stem and led to dryness of the stem. Plant infected by pathogen would experience stunted growth that decreased the potato tuber product.

People in the cultivation location called the symptom of the disease as “liyer”, referring the permanent withered condition. This disease is commonly found in regencies of Wonosobo and Banjarnegara as they are the production centers of potato plant where the people continually plant potato with no planting rotation. The soft rot bacterial pathogen can survive long time in the soil (soil-borne pathogen) where it promptly infects the plants being continually planted in the same field. When the host-plant is unavailable, bacteria can last by the plant debris. The social-culture of the surrounding people is leaving the plant debris on the field. Such behavior plays important role in extending the disease cycle.

Table 1. Locations, age of the plant and disease incidence in potato plant

Location	Age of the Plant (months)		Disease incidence (%)	
	Observation I	Observation II	Observation I	Observation II
Patakbanteng village, district of Kejajar, reg. of Wonosobo	3	3.5	60	80
Kejajar village, district of Kejajar, reg. of Wonosobo	2	2.5	30	40
Tieng village, district of Tieng reg. of Wonosobo	2.5	3	70	90
Buntu village, district of Batur, reg. of Banjarnegara	3	3.5	10	45
Batur village, district of Sumberejo, reg. of Banjarnegara	2	2.5	40	65
Gembol village, district of Pejawaran, reg. of Banjarnegara	2	2.5	60	70
Bagongan village, district of Ngablak, reg. of Magelang	2	3	50	65
Pandean village, district of Ngablak, reg. of Magelang	2	3	60	65
Segorogunung village, district of Argoyoso, reg. of Karanganyar	2	3.5	50	75

Table 2. DAS-ELISA results on infected samples collected from locations of potato plant production centers in Central Java

Location	ELISA I testing result	ELISA II testing result
Patakbanteng village, district of kejajar, reg. of Wonosobo	(-)	(-)
Kejajar village, district of Kejajar, reg. of Wonosobo	(-)	(-)
Tieng village, district of Tieng, reg. of Wonosobo	(-)	(-)
Buntu village, district of Batur, reg. of Banjarnegara	(-)	(-)
Batur village, district of Sumberejo, reg. of Banjarnegara	(-)	(-)
Gembol village, district of Pejawaran, reg. of Banjarnegara	(-)	(-)
Bagongan village, district of Ngablak, reg. of Magelang	(+)	(+)
Pandean village, district of Ngablak, reg. of Magelang	(+)	(+)
Segorogunung village, district of Argoyoso, reg. of Karanganyar	(-)	(-)

Remarks: (+): infected; (-): not infected

Table 3. Average absorbency value of ELISA at wavelength of 405 nm

DAS-ELISA	Buffer	Average absorbency value of ELISA (405 nm)			
		Positive Control	Negative Control	Positive Sample	Negative Sample
I	0.201	0.633	0.248	2.561–2.762	0.111–0.270
II	0.154	0.669	0.226	2.797–3.173	0.108–0.380

Disease Incidence

Observed from various sampling locations, the disease incidence varied between 10–90% as well as in various age of the plant. Location with the highest incidence was Tieng village, Tieng district, Wonosobo, and the lowest was Buntu village, Batur district, Banjarnegara (Table 1).

Based on the table 1, the average age of the plants indicating symptom of disease was between 2–3.5 months and found in every district. Averagely, the potato plant was infected by soft rot pathogen during the vegetative period when the plant required water for the growth of the root, stem, and leaves. As soon as the plant showed the symptom during this phase,

it would experience stunted growth. If infection occured during the generative phase, when the plant experienced growth for the flowering period and tuber development, it would experience stunted flowering with small tuber. Such condition commonly happens in the regency of Wonosobo and Banjarnegara.

Detecting *P. atrosepticum* using the DAS-ELISA method

Serology method of DAS-ELISA was carried out upon samples collected from the field. The assay was taken twice at different times based on the time of survey. Samples with symptom were cut and crushed finely to be used as the antigen because *P. atrosepticum* exists in the tissue of the infected plant. Based on the DAS-ELISA assays (Table 2), two samples originated from two different locations (Pandean and Bagongan villages, district of Ngablak, Magelang) were positively infected by *P. atrosepticum* indicated by the changing of color of the samples compared to that of uncolored buffer. To determine the absorbency value, the color changing was read three times. The positive control was a solvent with identified antigen concentration. Absorbency resulted from the positive control was used as the indicator.

A sample is stated as positive based on DAS-ELISA when the absorbency value at wavelength of 405 nm is close to the absorbency value of the positive control, or twice or more than the absorbency value of the control buffer. DAS-ELISA assay was carried out twice (Table 3). Samples of infected plant collected from Pandean and Bagongan villages, district of Ngablak, Magelang were indicated positively with infection of *P. atrosepticum* at average ELISA absorbent value of 2,561–2,762 in assay I and 2,797–3,173 in assay II.

In this study, it was showed that in potato production centers in Central Java, i.e. the regencies of Wonosobo, Banjarnegara, Magelang, and Karanganyar, soft rot and blackleg diseases were found. However, based on the serological analysis using the DAS-ELISA method, it was identified that only samples from the regency of Magelang was detected with *P. atrosepticum* infection, and samples collected from the regencies of Wonosobo, Banjarnegara, and Karanganyar were not. This indicated that the soft rot and blackleg diseases in potato were able to be infected by the bacteria other than *P. atrosepticum*. Czajkowski *et al*. (2011), reported that *Dickeya* spp. and *Pectotobacterium carotovorum* are members of soft-rot enterobacteriaceae that can cause soft rot disease on many host-plants including potato. Meanwhile, *P. atrosepticum* is a bacterial pathogen with very specific host, i.e. in potato plant. *P. atrosepticum* can not survive more than a year in the soil except in the potato tuber or in infected plants (Diallo *et al*., 2009).

Indication of the existence of *P. atrosepticum* in Magelang regency should be further studied by confirmation using the PCR technique. This is in line to the study by Haerani *et al*. (2015) that the production centers of potato in Pangalengan, Garut, Dieng and Malang had been infected by *Dickeya* sp. based on the ELISA analysis. But when conformed by using the PCR using specific primer, the results were negative. Serology assay by using polyclonal antiserum was reported to evenly cross reaction to bacteria with close genetic relationship (Emantoko, 2001). It is valuable only for the early sreening of the target bacteria. Thus, the *P. atrosepticum* status as quarantine plant pathogen A1 type I for potato plant should be further considered and its existence requires awareness. Results of this study also support the efforts taken by the Indonesian Agency for Agricultural Quarantine which still gives quarantine plant pathogenas A1 status to *P. atrosepticum*.

CONCLUSION

ELISA-based survey of *P. atrosepticum* in Central Java revealed that the pathogen was detected in Magelang regency; however the result of this study indicated that the occurrence was found only in limited potato-growing areas.

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