



Full Length Article

First Report on Bacterial Soft Rot Disease on Dragon Fruit (*Hylocereus* spp.) Caused by *Enterobacter cloacae* in Peninsular Malaysia

MASANTO MASYAHIT¹, KAMARUZAMAN SIJAM, YAHYA AWANG[†] AND MOHD GHAZALI MOHD SATAR[‡]

Department of Plant Protection, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

[†]*Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

[‡]*Department of Land Management, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

¹Corresponding author's e-mail: masantoug@yahoo.com

ABSTRACT

This study was specifically carried out to isolate and identify the pathogenic bacteria causing disease on dragon fruit in Peninsular Malaysia as well as to study the correlation of disease occurrence with environmental factors. Among 43 surveyed areas, field observations found that disease occurred on 11 sampling areas with the maximum disease occurrence in Ayer Hitam, Kedah (disease incidence, 36% & disease severity, 10.6%); while statistical analysis significantly revealed that the maximum mean disease occurrence was found in Johor (disease incidence, 17.33% & disease severity, 4.53%) and the minimum in Kedah (disease occurrence, 1.30%). The *in vitro* pathogenicity test and Biolog analysis resulted in *Enterobacter cloacae* as the main pathogenic agents of yellowish to brownish soft and watery symptoms on infected stem and fruit. Pearson coefficient correlation highlighted that disease intensity was significantly correlated with temperature (r value -0.478 & -0.485) and altitude of surveyed areas (r value 0.508 & 0.540). Disease prevalence was more influenced by plant condition and environmental factors. This could be considered as the first scientific report of bacterial soft rot disease on dragon fruit in Peninsular Malaysia.

Key Words: Dragon fruit; Soft rot; *Enterobacter cloacae*; Disease occurrence; Temperature; Altitude

INTRODUCTION

Pitaya (*Hylocereus* spp.) originated principally from the tropical and subtropical forest regions of Latin Americas, including North, Central and South America (Crane & Balerdi, 2005; Luders & McMahon, 2006), is known as dragon fruit in Asia as its skin is covered with bracts (scales) like dragon (Mizrahi *et al.*, 2002). Since pre-Columbian times, it has been very common in its native countries and consumed by the general population (Crane & Balerdi, 2005). From the original areas, about 100 years ago, the French brought it into Vietnam, where it was exclusively grown for the king as ornamental crop (Luders & McMahon, 2006).

In Malaysia, dragon fruit was firstly introduced in large scale at the end of 1990s by Golden Hope Company locating at Sungai Wangi Estate (Perak). Furthermore, at the early of 1999, the commercial cultivations were then developed in Kluang (Johor), Kuala Pilah (Negeri Sembilan) and Sitiawan (Perak). Since then, the farmers

have been cultivating dragon fruit in various lands, such as low and high land, rice-planted land, mined land and even housing yard (Halimi & Satar, 2007).

The acreage of dragon fruit-cultivating lands in Malaysia increased from 47.3 ha in 2002 to 962.3 ha in 2006 with 363.2 ha production areas and 2,534.2 tons (production value around RM12, 670,755 equal to USD3.5 million). Recently, this crop has been nationwide planted in Peninsular and East Malaysia (Cheah & Zulkarnain, 2008).

Many investigations on dragon fruit in Malaysia are particularly aimed to enhance its production technologies and to improve its potential product in downstream industries. Those researches are more focused on its great health benefits (Ching *et al.*, 2005), farming technologies (Martini *et al.*, 2006), physico-chemical characteristics (Novita *et al.*, 2006; Novita *et al.*, 2008), post-harvest handling (Lau *et al.*, 2008), downstream potential products (Chuah *et al.*, 2008; Harivaindaran *et al.*, 2008; Norziah *et al.*, 2008; Rebecca *et al.*, 2008) and nutritional contents (Ariffin *et al.*, 2008).

Although there is no scientific report of bacteria infecting dragon fruit, some plant pathogenic bacteria, such as *Erwinia* sp. and *Xanthomonas campestris*, have been observed on soft watery stem rot of dragon fruit in Vietnam (Hoa, 2008), Central America and Australia (Le Bellec *et al.*, 2006). Considering that there is no scientific report documenting bacterial diseases on dragon fruit, this study was then specifically carried out to isolate and identify the pathogenic bacteria causing disease on dragon fruit in Peninsular Malaysia as well as to study the correlation of disease occurrence with environmental factors.

MATERIALS AND METHODS

Assessment of disease occurrence. At least two dragon fruit orchards/plantations, as representative district sampling area of each state in Peninsular Malaysia, were surveyed since December 2007 until August 2008. Fifty posts of dragon fruit plants were randomly sampled from each surveyed plantation, with at least 0.4 ha in acreage. Disease incidence (DI) was calculated by the following equation (Cooke, 2006):

$$DI = \frac{\text{No. of infected plant units}}{\text{Total no. of plant units assessed}} \times 100$$

Meanwhile, the disease severity (DS) was determined according to alternative rating scale proposed by Bowen (2004) in which scale 0 = no symptom, scale 1 = 0–20% of severity level on infected plants, scale 2 = 20–40%, scale 3 = 40–60%, scale 4 = 60–80% and scale 5 = 80–100%, respectively and then measured using the equation proposed by Kranz (1988) as follows:

$$DS = \frac{\sum(a \times b)}{N \cdot Z} \times 100\%$$

$\Sigma(a \times b)$ = Sum of the symptomatic plant and their corresponding score scale.

N = Total number of sampled plant.

Z = Highest score scale.

Cultural data such as acreage of farms and age of plants were also noted. Longitude and latitude data of the surveyed areas were recorded using GPS device (SILVA Multinav-Version 2.01) and then mapped using Mapinfo Software (Troy, New York; USA). Meanwhile, weather data including rainfall, relative humidity (RH), temperature and wind velocity were obtained from Malaysian Meteorological Department (period of 1998–2008).

Isolation and identification of pathogen. The symptomatic parts of plants were collected, brought to laboratory and then isolations were made following the Janse (2005) with a slight modification. Pieces of plant samples taken from the margin of healthy and diseased tissues were briefly disinfected with 70% alcohol and suspended in a test tube

containing 20 mL of sterile distilled water (SDW). These sliced tissues were then agitated at 150 rpm of rotary shaker for 5 min at room temperature to allow bacteria diffuse out of the tissue into the water. A loopful of bacterial suspension was directly streaked onto nutrient agar (NA) medium (Oxoid Ltd., Basingtoke, Hampshire; England) and incubated for 24–48 h at room temperature. The single colonies of bacteria were then transferred onto fresh petri dishes to obtain pure culture.

The isolated bacteria were specifically identified and characterized according to their gram reaction (Ishimaru, 2001) for determining their proper testing protocol in further identification following manufacturer's instruction using Biolog Bacterial Identification System (Microbiolog™ System, Release 4.0). Bacterial suspension was prepared at specified cell density, inoculated into Microplate (BiOLOG, Hayward CA 94545; USA) and incubated in incubator at suitable temperature. The bacterial suspension within Microplate was then read and its ID was determined.

Pathogenicity test. This *in vitro* assay was conducted with a slight modification of the methodology by Lacy and Lukezic (2004). Bacterial colony was cultured in nutrient broth (NB) medium (Oxoid Ltd., Basingtoke, Hampshire; England) and incubated at 150 rpm of rotary shaker overnight at room temperature. Bacterial suspension was harvested by centrifugation at 8,000 rpm for 15 min and the pellet was resuspended in SDW. The bacterial suspension was adjusted spectrophotometrically to approximately 10^7 – 10^8 colony forming units per mL (CFU mL⁻¹) with SDW (an optical density (OD) of 1.0–1.3 at 550–600 nm using UV Spectrophotometer VARIAN Cary Series 50 Bio). The suspension was then immediately inoculated to fresh and healthy surface-disinfected organs (stem & fruit). Artificial wounds approximately 2 mm deep were aseptically made on tested stem using sterile needle. Twenty microliters of bacterial suspension was inoculated onto the wounded site. The control organs were inoculated with 20 µL of SDW. The inoculated organs were put into moisturized filter paper-layered plastic trays, wrapped using transparent wrapping plastic and then incubated for 48–96 h. The symptom development was daily observed.

Statistical data analysis. Prior to analysis, test of normality for disease occurrence data (DI & DS) was employed to determine whether those data should be transformed either to log, *ln* arcsine or square root transformations in order to achieve the best linear severity–incidence (Cardoso *et al.*, 2004). Linear regression analysis using Microsoft Excel 2003 program (Microsoft Corporation, Washington; USA) was used to examine severity as a function of incidence.

Disease occurrence data from all surveyed states was analyzed under General Linear Modeling (GLM) procedure with Duncan Multiple Range Test (DMRT) using SAS® System for Windows V8 software (SAS Institute Cary, North California; USA) to compare different states with respect to disease incidence and severity. Using SAS® System for Windows V8 software (SAS Institute Cary,

North California; USA), Pearson correlation analysis was performed to describe the relationship of disease occurrence with weather factors and cultural data.

RESULTS

Assessment of disease occurrence. A total of 43 dragon fruit orchards in Peninsular Malaysia had been successfully surveyed since December 2007 until August 2008. The disease occurred in 11 surveyed farms (25.58%). The maximum DI and DS (36% & 10.4%, respectively) were recorded from Ayer Hitam Kedah. The disease occurred not only on red-fleshed dragon fruit (*Hylocereus polyrhizus* the major cultivated species), but also appeared on white-fleshed (*H. undatus*) and yellow species (*Selenicereus megalanthus*) (Table I).

Isolation and identification of pathogen. The suspected pathogenic bacteria were subsequently isolated from diseased stems and fruits. The infected plants showed yellowish to brownish soft and watery symptoms on stem as well as fruit. On the stem, the smelly lesion tissues subsequently softened and rotted leaving the main vein intact; while the rotten fruits would be totally destroyed after three days initial symptoms appeared (Fig. 1).

At least five members of enterobacteriaceae, namely *Enterobacter cloacae*, *En. aerogenes* (*Klebsiella mobilis*), *Kleb. oxytoca*, *Pantoea dispersa* and *Rahnella aquatilis*, had been successfully isolated from the infected plant organs with various colony color (cream to yellowish) and size ($1.9\text{--}6.3 \times 0.5\text{--}2.8 \mu\text{m}$ in range). The Biolog system identified these bacteria with range of probability and similarity around 97–100% and 51–67.3%, respectively (Table II).

Pathogenicity test. The results of *in vitro* pathogenicity test showed that only *En. cloacae* could show soft rot symptoms, which appeared 24 h and 48 h after inoculation on fruit and stem, respectively; while artificial inoculation with other bacteria and SDW did not result in any symptom. Both symptomatic stem and fruit completely deteriorated in less than 5 days after inoculation (Fig. 2).

Statistical data analysis. As test of normality resulted in normal distribution data, DI and DS data were directly analyzed without any transformation. Linear correlation between DI and DS revealed positive *r* value around 0.995 (Fig. 3).

Meanwhile, GLM procedure with Duncan's test highlighted that the maximum occurrence of disease was recorded from Johor state, with mean of DI $17.33\% \pm 1.33$ and mean of DS $4.53\% \pm 0.53$; whilst the minimum disease prevalence was found in Kedah state with mean of DI and DS, $4.50\% \pm 4.50$ and $1.30\% \pm 1.30$, respectively (Table III).

The significant negative correlations of disease occurrence was recorded with temperature namely -0.478 for DI and -0.485 for DS; whereas disease prevalence had nearly zero correlations with other weather factors such as rainfall and wind velocity, with range of *r* value from -0.066 to -0.090. Although the *r* values were highly positive i.e., 0.428

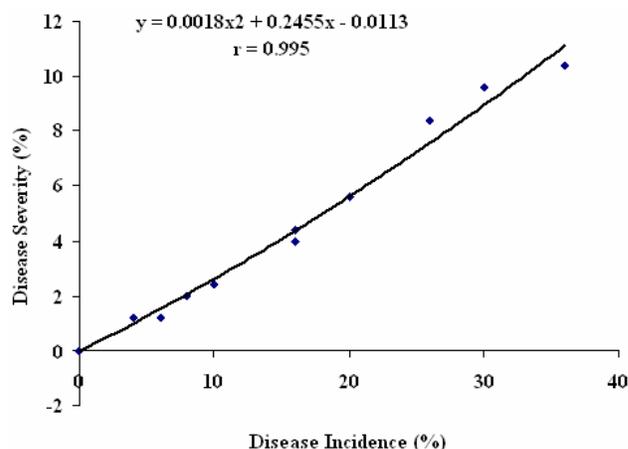
Fig. 1. The symptoms of soft rot disease on infected stem and fruit



Fig. 2. The symptoms appeared on the inoculated stem and fruit during *in vitro* pathogenicity test



Fig. 3. The relationship between incidence and severity of disease



for DI and 0.416 for DS, non-significant correlation was found between the occurrence of disease and RH (Table IV).

On the other hand, disease prevalence had a significant positive correlation with altitude of surveyed areas (*r* value 0.508 for DI & 0.540 for DS). No significant correlations were revealed between disease intensity and other cultural factors, such as acreage of farms and age of plants, though their *r* value was quite positive, with 0.255–0.331 in range (Table IV).

DISCUSSION

This work was concurrently conducted in conjunction

Table I. Occurrence of soft rot disease sampled from 43 of surveyed dragon fruit-growing areas in Peninsular Malaysia

| Location ^a | Dragon fruit species | Altitude (m asl) | Age (years) ^b | Acreage (ha) ^c | Incidence (%) | Severity (%) |
|------------------------|---------------------------|------------------|--------------------------|---------------------------|---------------|--------------|
| Johor | | | | | | |
| Batu Pahat | Rf (Hp) | 12.50 | 4.5 | 1.42 | 16 | 4.0 |
| Kluang | Rf (Hp) | 81.38 | 4 | 1.42 | 20 | 5.6 |
| Mersing | Rf (Hp), Wf (Hu), Yf (Sm) | 47.55 | 6 | 10.11 | 16 | 4.0 |
| Malacca | | | | | | |
| Durian Tunggal | Rf (Hp) | 10.36 | 6 | 3.24 | 0 | 0.0 |
| Machap Umboo | Rf (Hp) | 61.26 | 5 | 2.43 | 0 | 0.0 |
| Naning | Rf (Hp) | 42.36 | 1.5 | 1.21 | 0 | 0.0 |
| Negeri Sembilan | | | | | | |
| Rembau | Rf (Hp) | 54.56 | 1.5 | 0.48 | 6 | 1.2 |
| Kuala Pilah | Rf (Hp) | 80.77 | 1.5 | 2.83 | 0 | 0.0 |
| Pajam | Rf (Hp) | 60.96 | 2.5 | 2.43 | 16 | 4.4 |
| Selangor | | | | | | |
| Sepang | Rf (Hp) | 51.20 | 3 | 0.65 | 8 | 2.0 |
| Hulu Langat | Rf (Hp) | 75.90 | 0.67 | 2.43 | 4 | 1.2 |
| Sabak Bernam | Rf (Hp) | 13.41 | 3 | 1.21 | 10 | 2.4 |
| Perak | | | | | | |
| Slim River | Rf (Hp) | 44.50 | 4 | 0.81 | 0 | 0.0 |
| Teluk Intan | Rf (Hp) | 5.80 | 4 | 0.40 | 0 | 0.0 |
| Tapah | Rf (Hp) | 40.54 | 3.5 | 0.81 | 0 | 0.0 |
| Batu Gajah | Rf (Hp) | 42.36 | 2.5 | 0.61 | 0 | 0.0 |
| Pantai Remis | Rf (Hp) | 6.40 | 1.5 | 2.43 | 0 | 0.0 |
| Taiping | Rf (Hp) | 20.42 | 1 | 0.81 | 0 | 0.0 |
| Pahang | | | | | | |
| Pekan | Rf (Hp) | 11.88 | 3 | 4.45 | 0 | 0.0 |
| Kuantan | Rf (Hp) | 17.37 | 4 | 2.02 | 0 | 0.0 |
| Raub | Rf (Hp) | 146.91 | 4 | 3.64 | 0 | 0.0 |
| Jerantut | Rf (Hp) | 121.31 | 0.5 | 1.62 | 30 | 9.6 |
| Terengganu | | | | | | |
| Paka | Rf (Hp) | 8.53 | 0.6 | 0.48 | 0 | 0.0 |
| Merchang | Rf (Hp) | 6.09 | 3 | 1.01 | 26 | 8.4 |
| Marang | Rf (Hp) | 4.57 | 2.5 | 2.02 | 0 | 0.0 |
| Setiu | Rf (Hp) | 5.79 | 1.5 | 0.40 | 0 | 0.0 |
| Kerteh | Rf (Hp) | 11.27 | 2.5 | 0.81 | 0 | 0.0 |
| Kelantan | | | | | | |
| Batang Merbau | Rf (Hp) and Wf (Hu) | 51.81 | 3 | 0.40 | 0 | 0.0 |
| Kota Bharu | Rf (Hp) | 10.97 | 2 | 0.40 | 0 | 0.0 |
| Gua Musang | Rf (Hp) | 31.67 | 4 | 1.21 | 0 | 0.0 |
| Kuala Krai | Rf (Hp) | 34.74 | 2 | 2.02 | 0 | 0.0 |
| Pulau Pinang | | | | | | |
| Bukit Mertajam | Rf (Hp) | 30.78 | 3 | 2.02 | 0 | 0.0 |
| Seberang Perai Tengah | Rf (Hp) | 22.55 | 1.5 | 0.81 | 0 | 0.0 |
| Seberang Perai Utara | Rf (Hp) | 9.44 | 5 | 5.95 | 0 | 0.0 |
| Kedah | | | | | | |
| Merbau Pulas | Rf (Hp) | 14.63 | 0.58 | 0.40 | 0 | 0.0 |
| Pokok Sena | Rf (Hp) and Yf (Sm) | 19.81 | 0.4 | 0.40 | 0 | 0.0 |
| Gurun | Rf (Hp) | 25.60 | 2 | 2.43 | 0 | 0.0 |
| Yan | Rf (Hp) | 3.96 | 2 | 0.40 | 0 | 0.0 |
| Ayer Hitam | Rf (Hp) | 3.35 | 2 | 0.81 | 36 | 10.4 |
| Mata Ayer | Rf (Hp) | 28.95 | 3.5 | 2.02 | 0 | 0.0 |
| Pantai Kok | Rf (Hp) | 27.43 | 1.5 | 0.40 | 0 | 0.0 |
| Ayer Hangat | Rf (Hp) and Wf (Hu) | 3.96 | 4 | 0.81 | 0 | 0.0 |
| Perlis | | | | | | |
| Beseri | Rf (Hp) | 25.60 | 2 | 0.61 | 0 | 0.0 |

^a: Locations were arranged successively from the northern (Johor, Malacca and Negeri Sembilan), western (Selangor and Perak), eastern (Pahang, Terengganu and Kelantan) and southern (Pulau Pinang, Kedah and Perlis). ^b: Age of crops which were recorded until surveyed date; ^c: Generally, growers cultivated in acre acreage. 1 acre consists of approximately 449 posts in which 1 post has 4 plants. 1 acre = 0.4 ha; asl = above sea level; Rf = Red-fleshed species (Hp = *Hylocereus polyrhizus*); Wf = White-fleshed species (Hu = *Hylocereus undatus*); Ys = Yellow species (Sm = *Selenicereus megalanthus*)

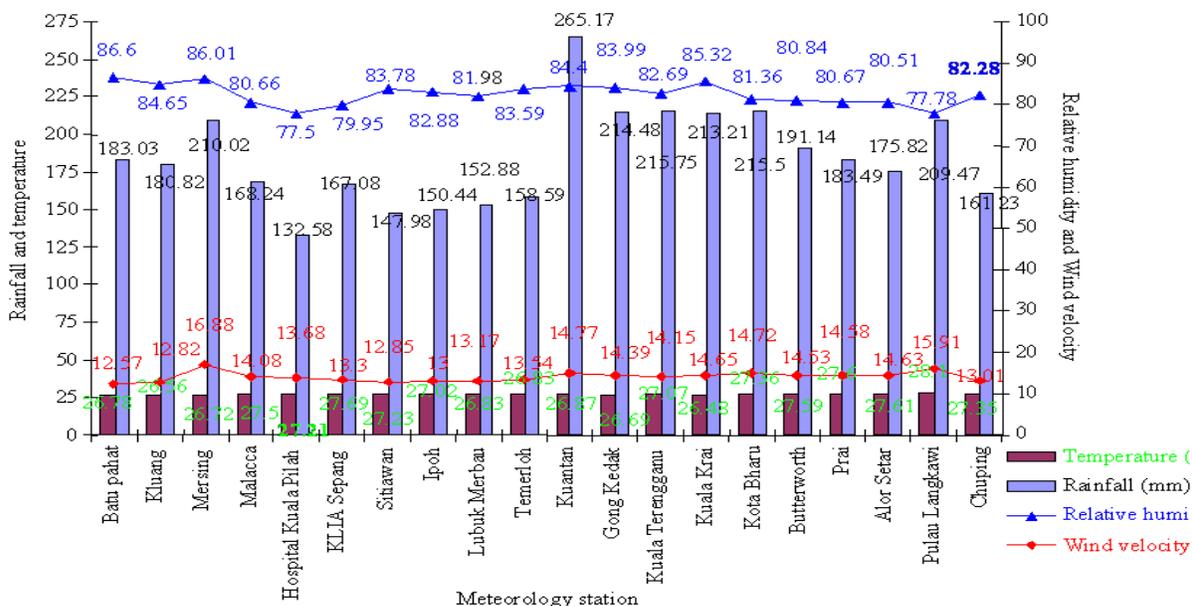
to study important diseases of dragon fruit in Peninsular Malaysia. Our previous study has reported the occurrence of anthracnose disease caused by *Colletotrichum gloeosporioides* as one of those important diseases on this crop in Peninsular Malaysia (Masyahit *et al.*, 2009). On the

previous report, we noted that 90.7% of surveyed farms were planted with red-fleshed species (*H. polyrhizus*). So, it was not wondering if the occurrence of soft rot disease reported in this current study was mostly found on that species. Since there is no any scientific documentation of

Table II. Characteristics of isolated bacteria from infected plants

| Isolated bacteria | Characteristics | | | Pathogenicity test | Biolog analysis | |
|---|-----------------|------------------------|--------------|--------------------|-----------------|----------------|
| | Gram reaction | Shape and size (µm) | Colony color | | Probability (%) | Similarity (%) |
| <i>Enterobacter cloacae</i> | Negative | Rod, 5.3–5.6 × 2.1–2.4 | Cream | + | 99 | 61 |
| <i>Enterobacter aerogenes</i> (<i>Klebsiella mobilis</i>) | Negative | Rod, 5.3–5.8 × 2.1–2.6 | Cream | - | 98 | 57 |
| <i>Klebsiella oxytoca</i> | Negative | Rod, 5.5–6.3 × 2.4–2.8 | Cream | - | 100 | 58 |
| <i>Pantoea dispersa</i> | Negative | Rod, 5.3–5.8 × 2.3–2.5 | Yellowish | - | 99 | 67.3 |
| <i>Rahnella aquatilis</i> | Negative | Rod, 1.9–2.3 × 0.5–1.0 | Yellowish | - | 97 | 51 |

Fig. 4. The 11-years (1998–2008) of weather data in Peninsular Malaysia obtained from Department of Meteorological Malaysia



bacterial soft rot disease on dragon fruit, this study might be likely considered as the first report of the status of that disease in Peninsular Malaysia and even among other dragon fruit-producing countries the world. On other cacti species, the bacterial soft rot disease has been reported on graft-cactus (*Chamaecereus silvestrii*) in Korea caused by *Pectobacterium carotovorum* subsp. *carotovorum* syn. *Erwinia carotovora* subsp. *carotovora* (Kim *et al.*, 2007).

Biolog analysis identified that all isolated bacteria were members of enteric bacteria among which only *En. cloacae* could artificially produce symptoms similar to those observed in the field after *in vitro* pathogenicity test. Despite of the fact that genus *Erwinia* has been frequently reported as the pathogenic agent of bacterial soft rot diseases on many crops (Charkowski, 2006), we did not obtain any bacteria species belonging to this genus during this study. One of *Erwinia* species found to be pathogenic in cacti plant was *Erwinia carotovora* subsp. *carotovora* which has been documented causing watery brown spot symptom on prickly pear cactus (*Opuntia ficus-indica*) in Italy (Valvaro *et al.*, 1992).

Other four bacterial species, which did not show any symptoms after *in vitro* pathogenicity test were actually known as environmental bacteria. They have been more

frequently isolated from water, sewage, soil, dairy products and human blood as well as in the feces of humans and animals (Caroff *et al.*, 1998; Podschun *et al.*, 2001; Grimont & Grimont, 2006; Brady *et al.*, 2008). In the plants, these species have been more often found as endophytic bacteria, which have been utilized as bio-control agents of plant disease (Bacon & Hinton, 2006).

Although *En. cloacae* has a role as commensal in water, sewage, soil, meat, hospital environments, the skin as well as in the intestinal tracts of humans and animals (Grimont & Grimont, 2006), this species has also been found to be pathogenic on several crops in many places such as internal yellowing disease on papaya fruits (*Carica papaya* L.) in Hawaii (Nishijima *et al.*, 1987), internal bulb decay on onions (*Allium cepa* L.) in California (Bishop & Davis, 1990) and in Colorado (Schwartz & Otto, 2000), leaf rot disease on odontioda orchids (*Odontioda* sp.) in Tocighi Prefecture, Japan (Takahashi *et al.*, 1997), rhizome rot of edible ginger (*Zingiber officinale* Roscoe) in Hawaii (Nishijima *et al.*, 2004) and gray kernel disease on macadamia (*Macadamia integrifolia*) in Hawaii (Nishijima *et al.*, 2007). Reisolation of pathogenicity test on wetwood of elm (*Ulmus americana*) in Maine (Orono, USA) performed by Murdoch and Campana (1983) and on internal

breakdown of onion bulbs in Riverina (New South Wales, Australia) conducted by Cother and Dowling (1986) found that *En. cloacae* and *Klebsiella oxytoca* were concurrently pathogenic on those two plants.

The absence of disease from majority of farms (74.42% of total surveyed farms) may be assumed due to favorable environmental conditions, especially RH and temperature, for crop growth. Another possible explanation for this phenomenon may be the natural role of *En. cloacae* as endophytic microorganism in the plant (Bacon and Hinton, 2006).

On the contrary, disease occurring on other several farms might be attributed by both disadvantageous plant conditions, especially wounds caused mechanically by agricultural tools and insects' bites, and environmental factors. Zimmerman and Granata (2002) explained that the bacterial agents on prickly pear cactus only could penetrate into the plant tissue through wounds as cacti species had waxy epidermal skin. Those lesions were potential for the entry point of bacteria infecting the plant; while the moist and nutrient-rich sites within the plant were ideal condition for fostering bacterial growth (Beattie, 2006).

The linear regression between DI and DS with positive significant *r* value (0.995) described that DI progressively affected DS. It meant that the disease could severely develop once the infection occurred. Zimmerman and Granata (2002) studied that the evolution of diseases within the tissues of cacti plant was very rapid as the biochemical characteristics of their cell juices, which were appropriate for the growth conditions of various biotic agents.

Our results on the negative significant correlation of disease occurrence and temperature were supported by Zimmerman and Granata (2002) who reviewed that bacterial infection on cacti plants necessitated specific weather conditions characterized by low temperatures and elevated atmospheric humidity favored by the presence of juicy tissues in the plant. Other pathogenic bacterium on cacti species required optimal temperatures around 10 to 15°C though it could grow at temperature up to 36 °C (Valvaro *et al.*, 1992). For encouraging the infection and development of *Xanthomonas compestris* pv. *citri* causing canker on citrus (*Citrus* spp.) in nursery, a temperature range of 20 to 30°C with wet weather was appropriate (Burhan *et al.*, 2007).

Present findings, however, were not in line with other previous works on both same and different bacteria. Cother and Dowling (1986) postulated that the change of *En. cloacae* as endophytic bacteria to opportunistic pathogens on onion bulbs was triggered by the alteration of host physiological conditions, particularly high temperature at bulb maturity. Meanwhile, Nishijima *et al.* (2004) concluded that the presence of *En. cloacae* as pathogenic agent of rhizome rot of edible ginger was enhanced by the high level of RH and temperature combined with low atmospheric oxygen during post harvest handling and storage, which affected the development of decay.

Table III. Mean and standard error of the occurrence of soft rot disease on dragon fruit in Peninsular Malaysia

| State | Disease occurrence | |
|-----------------|-----------------------|----------------------|
| | Disease incidence (%) | Disease severity (%) |
| Johor | 17.33 ± 1.33a* | 4.53 ± 0.53a |
| Malacca | 0.00 ± 0.00b | 0.00 ± 0.00a |
| Negeri Sembilan | 7.33 ± 4.67ab | 1.87 ± 1.31a |
| Selangor | 7.33 ± 1.76ab | 1.87 ± 0.35a |
| Perak | 0.00 ± 0.00b | 0.00 ± 0.00a |
| Pahang | 7.50 ± 7.50ab | 2.40 ± 2.40a |
| Terengganu | 5.20 ± 5.20ab | 1.68 ± 1.68a |
| Kelantan | 0.00 ± 0.00b | 0.00 ± 0.00a |
| Pulau Pinang | 0.00 ± 0.00b | 0.00 ± 0.00a |
| Kedah | 4.50 ± 4.50ab | 1.30 ± 1.30a |
| Perlis | 0.00 ± 0.00b | 0.00 ± 0.00a |

*Means followed with same letter are not significantly different at 95% confidence interval ($\alpha = 0.05$) analyzed using GLM procedure with DMRT test

Table IV. Pearson correlation coefficient (r) between soft rot disease occurrence and weather and cultural data

| | Weather factors | | | Acreage of farm | Age of plants | Altitude | |
|----|-----------------|-------------------|-------------|-----------------|---------------|----------|--------|
| | Rainfall | Relative humidity | Temperature | | | | |
| DI | -0.068 | 0.428 | -0.478* | -0.078 | 0.331 | 0.324 | 0.508* |
| DS | -0.066 | 0.416 | -0.485* | -0.090 | 0.280 | 0.255 | 0.540* |

*Correlation is significant at the 0.05 level

The study on status of citrus canker caused by *Xanthomonas axonopodis* pv. *citri* in Peninsular Malaysia found that temperature increasingly influenced the incidence of disease (Derso *et al.*, 2007). Our findings resulted in positive correlation of disease occurrence and RH despite of the Pearson coefficient correlation value was not significantly different, either at the 0.01 level or at the 0.05 level (Table IV). From the field investigations, we observed that disease occurred in the surveyed states with range of RH level around 77.5 to 86.6% (Fig. 4).

Although our field surveys did not constantly detect the presence of disease on each surveyed farm with high elevation, statistical analysis revealed that disease intensity had the significant positive correlation with altitude. The field study of other bacterial disease on citrus in Peninsular Malaysia conducted by Derso *et al.* (2007) discovered that disease incidence negatively correlated with the elevation of surveyed areas and they exposed the negative significant correlation of temperature and elevation as well. This current investigation did not correlate those two factors each other as our results on the negative significant correlation of disease occurrence with temperature and positive significant with altitude could indirectly explain the reverse correlation of those two environmental factors.

CONCLUSION

Bacterial soft rot disease on dragon fruit occurred in several surveyed areas in Peninsular Malaysia with various

disease intensities. Disease prevalence was more influenced by plant condition as well as environmental factors, especially temperature and altitude. This report could be considered as the first scientific documentation of bacterial infection causing soft rot disease on dragon fruit in Peninsular Malaysia.

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