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# Etiology of Bacterial Soft Rot of Orchids

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## ABSTRAK

*Gejala penyakit reput lembut bakteria telah diperhatikan pada pokok-pokok orkid jenis* Phalaenopsis *dan* Dendrobium. *Penyakit ini menyebabkan kematian banyak pokok-pokok orkid terutama sekali jenis* Phalaenopsis *pada peringkat benih dan pokok muda. Bakteria telah berulangkali diasingkan daripada pokok-pokok yang berpenyakit. Ujian menunjukkan asingan-asingan bakteria adalah patogenik pada orkid. Langkah-langkah mengikut dalil-dalil Koch telah dijalankan. Berdasarkan kepada ujian-ujian kultur, morfologi, fisiologi dan biokimia, asingan-asingan bakteria telah dikenalpasti sebagai* Erwinia chrysanthemi *Burk., Mc Fadden and Dimock, 1953.*

## ABSTRACT

*Symptoms of bacterial soft rot were observed on the* Phalaenopsis *sp. and* Dendrobium *sp. orchids. The disease caused death in many plants, especially those of the* Phalaenopsis *sp. at the seedling stage and of young plants. Bacteria were consistently isolated on diseased plants. Tests proved the pathogenicity of the isolates on orchids. Steps were carried out to complete Koch's postulate. Based on the cultural, physiological and biochemical properties the pathogen was identified as* Erwinia chrysanthemi *Burk., McFadden and Dimock 1953.*

**Keywords: Bacterial soft rot, *Phalaenopsis*, *Dendrobium*, *Erwinia chrysanthami***

## INTRODUCTION

Orchids have been known to be infected by bacteria from the genus *Erwinia.* Strider (1985) described soft rot caused by *Erwinia carotovora* (Jones) Holland, which affected a wide range of vegetable and ornamental plants, as being not too common on orchids, but can be the most destructive disease. In Malaysia, Singh (1973) listed soft rot of *Phalaenopsis* sp. caused by *E. carotovora* (Jones) Holland and indicated that the disease was not serious and of rare occurrence. However, since early 1989, rotting of *Dendrobium* sp. and *Phalaenopsis* sp. was commonly observed in the campus of Universiti Pertanian Malaysia on all stages of plant growth. The disease was observed to be more severe during the wet periods and on *Phalaenopsis* hybrids. The objective of this study was to determine the etiology of the disease on these orchids.

## MATERIALS AND METHODS

*Isolation of bacterial strains*

Leaves of plants showing soft rot symptoms were brought to the laboratory and washed under running tap water. The epidermis of the leaves between the rotted and healthy tissue were aseptically removed. A small portion of the tissue was then removed and squashed in a drop of sterile distilled water and allowed to stand for 15 min. A loopful of this was streaked on Difco nutrient agar (NA) plates and incubated at 30 $\pm$ 1°C for 24hr. Isolated colonies were purified by serial dilutions and spread on NA plates and incubated in the same manner. Isolated colonies were selected and streaked on NA and modified yeast extract-dextrose-calcium carbonate (YDC) agar (Dye, 1968) slants for stock preparation. Stocks were kept at 4 and 15°C for further studies. *Bacterial cultures:* In addition to the five bacterial isolates from orchids, an isolate of *Erwinia carotovora* pv. *carotovora,* that caused soft rot of cabbage was also included in the morphological, cultural, physiological and biochemical tests. All cultures were maintained at the Department of Plant Protection, Universiti Pertanian Malaysia.

*Morphological and cultural properties*

All bacterial strains were tested for Gram's stain and examined for shape. Gram's stain reaction was further confirmed with the KOH solubility test. Colour of growth on modified YDC and on glucose yeast extract calcium carbonate (GYCA) agar (Dye, 1968) was observed daily up to 1 week.

*Physiological and biochemical properties*

All tests were made using a 24-48hr culture from NA and incubated at 30 $\pm$ 1°C unless indicated otherwise. Cultures were tested for their ability to cause rotting of potato slices, phosphatase production and sensitivity to erythromycin (15 ul). These were carried out as described by Kelman and Dickey (1980). The methods described by Dye (1968) were used to test for : acetoin production, oxidation fermentation, gas from glucose, catalase, oxidase, growth in 5% NaCl, reducing substance from sucrose, glatin hydrolysis (Cowen's method), growth at 40°C, production of nitrite from nitrate and production of acid from glucose, sucrose, lactose, maltose, trehalose, cellobiose, rhamnose, arabinose, sorbitol, dulcitol, mannitol, melibiose and alpha-methyl-d-glucoside using medium C. In addition, acid production from glucose, sucrose, lactose, maltose, trehalose, cellobiose, sorbitol, dulcitol and mannitol were also tested using Bacto OF medium (Difco). A 10% (w/v) aqueous solution of the above carbon sources was filter sterilized were aseptically added to the basal medium to give a final concentration of 1.0% (w/v). A change in the color of the medium from green to yellow was scored as a positive reaction. Readings were done at 3, 7, 14 and 21 days. To test for the production of indole, bacterial strains were cultured in 3 media for indole production as given in i) Lelliott (1957), ii) Bradshaw (1963) and iii) Dye (1968). Cultures were tested after 2 and 5 days by addition of 0.5 ml xylene which was mixed with the culture before addition of Kovacs' reagent. Hydrogen sulphide production was tested from cystein hydrocloride by the method described in Dye (1968) and from sodium thiosulphate by using Kligler Iron agar (Oxoid). Bacto Malonate broth (Difco) and Bacto-Koser Citrate Medium (Difco) were used to test for the utilization of malonate and citrate respectively. Production of lecithinase was determined as described in Fahy and Hayward (1983).

*Pathogenicity test*

Bacterial suspensions were made from a 24 - 48hr culture in sterile distilled water. These were adjusted to approximately 6 x $10^9$ cfu/ml using a spectrophotometer. Fifteen ul of the bacterial suspension was then placed on the surface of the leaves of *Phalaenopsis* hybrids and the leaves were lightly pricked twice through the bacterial suspension. Control plants were similarly inoculated but with sterile distilled water.

All bacterial strains from the pathogenicity test that produced soft rot after 24 - 48hr were reisolated. Morphological, cultural, physiological and biochemical test as indicated above were repeated with these isolates.

## RESULTS AND DISCUSSION

*Morphological and cultural properties*

Five bacterial isolates examined were all rod shaped with peritrichous flagella. All grew readily on modified YDC and GYCA. Orchid isolates consistently produced non-diffussible blue pigment on GYCA media. On modified YDC medium, pigment production was variable and was observed only on the third or fourth day while on GYCA pigment production was observed on the first day. *E. carotovora* pv. *carotovora* did not produced any pigment on both YDC and GYCA. On NA, all isolates produce small translucent colonies, that could not be differentiated.

*Physiological and biochemical properties*

Distinct differences could be seen in the physiological and biochemical properties of bacterial isolates from orchids and cabbage (Table 1). The distinctive properties of *E. chrysanthemi* according to Dickey (1979); Dye (1969); Cother and Sivasithamparam (1983), such as: gas production from glucose, production of phosphatase and lecithinase, sensitive to erythromycin (15 ug), produced blue non-diffusible pigment on modified YDC and GYCA media; utilization of sodium malonate was apparent for the orchid isolates (Table 1). Based on their cultural, physi-

TABLE 1
Physiological and biochemical properties of isolates of *Erwinia* spp. from orchid and cabbage

| Property | Origin of *Erwinia* species: orchids (5 isolates) | cabbage (1 isolate) |
|---|---|---|
| Acid production from: | | |
| Glucose (aerobic & anaerobic) | + | +** |
| Sucrose | + | + |
| Maltose | – | – |
| Cellobiose | + | + |
| Lactose* ) (in 1 week) | – | + |
| Trehalose* ) | – | + |
| Rhamnose | + | + |
| Arabinose | + | + |
| Sorbitol | – | – |
| Dulcitol | – | – |
| Mannitol | + | + |
| Alpha–methyl–d–glucoside | – | – |
| Melibiose | + | + |
| Gas from glucose* | + | – |
| Potato soft rot | + | + |
| Gelatin liquefaction | + | + |
| Sensitivity to erythromycin (15 g)* | + | – |
| Phosphatase* | + | – |
| Lecithinase* | + | – |
| Blue non–diffusible pigment on GYCA media* | + | – |
| Catalase | + | + |
| Oxidase | – | – |
| Indole* | + | – |
| Methyl Red | – | + |
| $H_2S$ production | – | – |
| Nitrite from nitrate | + | + |
| Reducing sub. from sucrose (48 hr.) | – | – |
| Beta–galactosidase | + | + |
| Arginine dihydrolase | + | + |
| Utilization of: | | |
| Sodium citrate | + | + |
| Sodium malonate* | + | – |
| Gram stain | – | – |
| KOH test | + | + |

* Determinative properties according to Dye (1969)
** + = Positive reaction; – = Negative reaction
GYCA = Glucose yeast extract calcium carbonate agar (Dye, 1969).

ological and biochemical properties isolates from *Dendrobium* sp. and *Phalaenopsis* sp. were thus identified as *E. chrysanthemi* Burk., Mc Fadden & Dimock, 1953. This findings corroborate the work of Lim and Khaw (1984) who indicated that the causal organism of bacterial soft rot of orchids, previously attributed to *Erwinia carotovora* (Jones) Holl. in Singapore and Peninsular Malaysia, to be *Erwinia chrysanthemi* Burk., Mc Fadden & Dimock, 1953.

In Malaysia, *E. chrysanthemi* had so far been isolated from two other hosts, namely, *Ananas comosus* (L.) Merr. (Lim, 1974) and *Zea mays* (Abdullah, 1982).

### *Symptoms and pathogenicity*

Soft rot symptoms on orchids were observed on *Phalaenopsis* sp. and *Dendrobium* sp. at all stages of plant growth (Plates 1a & 1b). However, the disease was most severe on seedlings and young plants of the *Phalaenopsis* sp. during wet periods. On seedlings, soft rot commonly occurs at the base of the leaves, thus resulting in the death of the plants soon after infection (Plate 2). On inoculated plants, initial symptom was a watersoaked rot which enlarged rapidly with no apparent yellowing of the margin after 1-2 days (Plate 3). On mature plants, the margin of the

*A*

*B*

*Plate 1. Natural infection of soft rot on A)* Phalaenopsis *sp. and B)* Dendrobium *sp.*

*Plate 2. Infection of* Phalaenopsis *seedlings at the base of the leaves resulted in the death of the plants.*

*Plate 3. Symptoms of soft rot on* Phalaenopsis *seedlings 2 days after inoculation.*


*Plate 4. Symptoms of soft rot on mature* Phalaenopsis *plants 5 days after inoculation.*

rotted area usually produce yellowing, 4-6 days after infection (Plate 4). *E. chrysanthemi* isolates were found to be highly pathogenic to *Phaelaenopsis* hybrids while *E. carotovora* pv. *carotovora* from cabbage was not.

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