Elaeis Vol. 7 No. 2, December, 1995, pp.118–121.

ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH *Elaeidobius kamerunicus* AND OIL PALM FLOWERS

Keywords: Isolation; bacteria; *Elaeidobius kamerunicus* and oil palm flowers

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Original manuscript received: 16 September 1994
Revised manuscript received: 14 December 1994

A study was carried out to isolate and identify Gram-negative bacteria associated with the larvae of Elaeidobius kamerunicus *and male oil palm spikelets. Nine species of floral bacteria and seven species of larval bacteria were identified. They belong to four genera, namely* Enterobacter, Serratia, Klebsiella *and* Escherichia. *Most of the bacteria isolated from larvae were also found in both the soil and male spikelets. Two predominant species of entomopathogenic bacteria isolated from the weevils and spikelets were* Enterobacter cloacae *and* Serratia marcescens. *Often, the same species of bacteria isolated from the weevil's haemocoel were also found in the intestine. The frequencies of isolation were 17%, 12% and 5% for samples collected from oil palm plantations at Banting, Labu and Serdang, respectively.*

INTRODUCTION

The introduction of the weevil, *Elaeidobius kamerunicus* Faust, into Malaysia in 1982 in order to improve pollination and fruit set in the oil palm became a landmark in the history of the industry (Syed *et al.*, 1982). The weevil population has established itself and adapted well to the local environment (Hussein *et al.*, 1991). The absence of major natural enemies (predators, parasitoids and pathogens) was identified as the principal environmental factor which determines the weevil's low mortality and high survival rate (Liau, 1985; Chiu *et al.*, 1986 and Hussein *et al.*, 1991). Rats are considered the only important predator: the effects of rat predation on the weevil life system were reported by Liau (1985) and Chiu *et al.*,

(1986). On the other hand, very little is known about the activities of microorganisms, especially entomopathogenic bacteria associated with the various life stages of *E. kamerunicus*. We now report the results of a field survey of the bacterial flora of the weevil's larvae and male spikelets of the oil palm.

MATERIALS AND METHODS

Field survey

Post-anthesis male spikelets of oil palm were collected from plantations at three localities, namely Serdang (Selangor), Banting (Selangor) and Labu (Negeri Sembilan). Five spikelets were taken from the proximal, median and distal parts of the male inflorescence making a total of 15 spikelets per sample. Each spikelet was then cut into three segments: apex, median and basal. Each segment was diced into 2–3 mm thick and one floret from each segment was plated onto nutrient agar medium. The agar plates were incubated at 37°C for 24 hours.

Larvae of *E. kamerunicus* were physically separated from the spikelets by carefully teasing up the florets. Healthy larvae were distinguished from diseased ones by their firm body, and agile and shiny integument. Diseased larvae were surface-sterilized with 10% sodium hypochlorite solution before making a tiny slit on the integument in order to obtain the haemolymph. Samples of the haemolymph were then streaked onto nutrient agar plates, which were incubated at 37°C for 24 hours. Similarly, samples of the intestinal fluid of the diseased larvae were obtained and streaked onto nutrient agar plates, which were incubated for 24 hours (Alexander and Marshall, 1982).

The identification of bacteria to the species level was done using a 24 well Microbact 24E System Kit (Diagnostic and Disposable Products, Australia) which was specially developed for identifying gram-negative Enterobacteriaceae. A bacterial suspension was prepared by picking a single colony (18–24 hour culture) from nutrient agar medium (GIBCO Ltd.) and emulsifying it in 3 ml of sterile physiological saline. The suspension was homogenized before placing it into the wells. Besides the gram-negative tests, the identification kit also gives confirmatory classification to biovar by evaluating the series of reagents that corresponds to the various biochemical reactions. Interpretation of the output of the system was based on the octal index of the reaction's code, which was linked to the Microbact computer, aided identification system.

RESULTS AND DISCUSSION

Table 1 shows the results of the survey on the incidence and rate of isolation of bacteria from samples of larvae collected at three localities. The variation in frequency of isolation between localities – from 5% (Serdang) to 17% (Banting) – was to be expected since the weevil density per spikelet also varies considerably between plantations (Basri *et al.*, 1984). The field rate of isolation at Serdang was much lower than the figures of 60% reported by Hussein and Rahman (1991) and 45% reported by Mohamed (1985). The larval isolation rates for Banting and Labu were not known prior to this study. The large differences in rates of infection reported in this study as compared with earlier surveys by Mohamed (1985) might be due to the prevailing environmental conditions and weevil populations during the survey.

Table 2 lists the bacteria which were isolated from larvae, spikelets and soil. They are gram-negative and belong to the family Enterobacteriaceae. Members of this family are well known as pathogens of insects and are more or less indigenous to the host while acting as opportunistic invaders (Steinhouse, 1963). A total of seven species of bacteria were isolated and identified from the intestinal fluid and

TABLE 1. FIELD RATES OF ISOLATION OF BACTERIA FROM LARVAE OF *E. kamerunicus* COLLECTED FROM THREE LOCALITIES

Locality	Number of larvae	% Isolation (± s.d.)
Serdang, Selangor	28923	5 ± 1%
Banting, Selangor	2988	17 ± 3%
Labu, Negeri Sembilan	6248	12 ± 1%

TABLE 2. SPECIES OF BACTERIA ISOLATED FROM LARVAE OF *Elaeidobius kamerunicus* AND MALE SPIKELETS OF OIL PALM

Bacteria		*E. kamerunicus* larvae		Spikelets of oil palm		
		Haemocoel	Intestine	Apex	Median	Base
1.	*E. cloacae*	+	+	+	+	+
2.	*E. agglomerans*	+	–	+	+	+
3.	*E. aerogenes*	+	+	+	–	–
4.	*S. marcescens*	–	+	+	–	–
5.	*S. dysenteriae*	+	+	–	+	+
6.	*S. rubidae*	–	–	–	+	–
7.	*C. fruendii*	–	–	–	+	–
8.	*C. diversus*	+	–	–	–	–
9.	*K. oxytoka*	–	+	–	–	+
10.	*K. ozanae*	–	–	–	–	+

\+ Indicates successful isolation
– indicates no isolation

haemolymph of *E. kamerunicus*.

The two predominant species of bacteria isolated were *Serratia marcescens* and *Enterobacter cloacae*. *E. cloacae* has been isolated from the intestine of the housefly, blister beetle and cabbage butterfly (Steinhouse, 1963), the American cockroach (Mischerlich and Marth, 1984) and the silkworm (Sedlak and Rische, 1968).

The red coloration produced by colonies of *S. marcescens* is due to the red pigment prodigiosin, which functions as a virulence factor. The colour makes the bacterium readily recognizable (James and Herman, 1974). However, one strain of *S. marcescens* isolated in this study was non-pigmented.

The occurrence of the above two entomopathogens is not limited to the spikelets and larvae of *E. kamerunicus*: they are also in the intestine of rats which feed on the larvae (Chiu *et al.*, 1986).

The results of pathogenicity tests carried out against the third instar larvae of *E. kamerunicus* showed no significant difference in the infection rates between the treated and untreated (control) larvae. The larval mortality rates in the controls were only slightly lower (45%–65%) than in the treated ones (60%–80%). The unexpected high mortality of larvae in the control might have been due to greater stress and dehydration as a result of the larvae being forced to live outside their natural habitat (Hussein and Rahman, 1991). Accordingly, it was not possible to confirm the pathogenicity of *S. marcescens* in terms of Koch's postulates.

ACKNOWLEDGEMENTS

The authors wish to acknowledge financial support from the Ministry of Science, Technology and Environment to carry out this research under IRPA Grant 1-07-05-019. Our special thanks go to the management of the oil palm plantations concerned for permission to collect insect and plant samples.

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