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Nitrogenase Activity in the Queensland Fruit Fly, *Dacus tryoni*

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Abstract

When 5-day-old laboratory-raised Queensland fruit flies (*Dacus tryoni*) were fed a dinitrogen-fixing bacterial strain of *Klebsiella oxytoca* isolated from the crop of a wild fly, acetylene reduction (nitrogenase) activity associated with the flies was detected after 2 to 3 days and persisted for at least 22 days. Flies not fed the dinitrogen-fixing strain were negative for acetylene reduction until 21 days after emergence. Presumably such dinitrogen-fixing bacteria are able to supply some Queensland fruit flies with a small part of their nitrogen requirements, but its importance is unknown.

Extra key words: dinitrogen-fixing bacteria, *Klebsiella oxytoca*, alimentary tract.

Introduction

The Queensland fruit fly (*Dacus tryoni*, Diptera : Tephritidae) is the most important pest of cultivated fruits in eastern Australia. Fecundity of this species depends on an adequate supply of nutrients and laboratory-reared adult females fed a diet of sucrose and water produce virtually no eggs (Drew *et al.* 1983). It has been proposed that the source of nutrients, presumably amino acids (Hagen 1958), in nature is phyllosphere and fructosphere bacteria, which are consumed and digested by the flies to yield the necessary nutrients (Drew *et al.* 1983; Drew and Lloyd 1987). Laboratory-raised *D. tryoni*, *D. jarvisi*, *D. neohumeralis* and *D. cacuminatus* produced many eggs when fed cultures of bacteria isolated from the alimentary tracts of *Dacus* spp. (Drew *et al.* 1983; Fitt and O'Brien 1985).

When *Dacus* spp. and other tephritid flies ingest bacteria, some bacteria persist in, and apparently colonize, the gut (Fitt and O'Brien 1985; Howard *et al.* 1985; Lloyd *et al.* 1986). The digestible biomass produced by the growth of these bacteria may be an important additional factor in the nutrition of the fly. Colonizers are primarily members of the Enterobacteriaceae. They include dinitrogen-fixing species that occur in the rhizosphere and phyllosphere of tropical plants (Sengupta *et al.* 1981; Murphy and Mac Rae 1985).

Using the acetylene reduction (AR) assay (Hardy *et al.* 1968), we found that out of a total of 18 bacterial isolates obtained from wild fruit flies (*Dacus* spp.), one isolate of *Enterobacter cloacae* and two isolates of *Klebsiella oxytoca* had nitrogenase activity. Populations of such diazotrophs have been found in several other classes of invertebrates including termites (Breznak 1982), soil animals (Citernesi *et al.* 1977), marine shipworms (Carpenter and Culliney 1975) and sea urchins (Guerinot *et al.* 1977), with possible benefits to the nitrogen nutrition of these host animals arising from dinitrogen fixation. However, results of AR assays using live larvae and adult flies belonging to the tephritid genus *Rhagoletis* (Howard *et al.* 1985) were negative and thus did not support the assignment of a significant nitrogen input from dinitrogen fixation by bacteria such as *K. oxytoca*, which

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are the dominant gut colonizers of these temperate-zone fruit flies. Nevertheless, we felt that the association between dinitrogen-fixing bacteria and the subtropical *Dacus* species was worthy of investigation.

Since laboratory-raised groups of *D. tryoni* will feed on pastes of live bacterial cells (Drew *et al.* 1983), the possibility that detectable rates of nitrogenase activity would be found in flies fed a dinitrogen-fixing strain was examined.

Materials and Methods

Feeding of Flies

In the first experiment approximately 1500 pupae of *D. tryoni* were obtained from the laboratory colony maintained by the Queensland Department of Primary Industries, Brisbane. These were divided equally among six gauze cages (20 × 20 × 30 cm) and held at room temperature (20–25°C); 5 days later most flies had emerged. Five days after emergence flies in four cages were offered an agar-supported bacterial lawn grown for 16 h at 28°C on peptone yeast extract agar (10 g peptone, 5 g yeast extract, 5 g NaCl, 15 g agar in 1000 ml distilled water). The strain used, *Klebsiella oxytoca* F15C, was isolated from the crop of a wild *D. tryoni* by A. C. Lloyd, Queensland Department of Primary Industries, Brisbane. The dinitrogen-fixing capability of this strain was initially determined, and frequently confirmed, in our laboratory by methods described elsewhere (Murphy and Mac Rae 1985). Flies fed freely on the bacteria for 24 h. All cages of flies were supplied with distilled water and sucrose cubes for the duration of the experiment.

The second experiment was similar to the first except that only three cages of flies were used, and flies in two of these were allowed to feed on a lawn of *K. oxytoca* F15C 3 days after emergence from pupae.

Acetylene Reduction Assays

In the first experiment, groups of 13–19 flies with total weights of 0·19–0·34 g, were sealed into a 14·75 ml glass serum bottle containing a gauze-topped tube with Ascarite II (Arthur H. Thomas Co., Philadelphia) to absorb carbon dioxide, and a few grains of silica gel to absorb moisture. Air in the vessels was replaced with acetylene to give a 20% content. A control without flies was included in each assay series. After 3 h at room temperature (24°C), 0·5 ml of gas was analysed for ethylene using a Shimadzu GC4A(PTF) gas chromatograph equipped with flame ionization detectors and a 2 m × 3 mm stainless steel column packed with Porapak N 80–100 mesh at 66°C. Retention times and peak heights were compared with known concentrations of ethylene.

On the seventh day after feeding, some of the flies were also tested after 24 h incubation, a period longer than the normal 3 h. Three groups of flies from all cages were incubated for 24 h in serum bottles with Ascarite II and silica gel in the 20% acetylene atmosphere. Controls without flies and of flies without acetylene were included in the assay. Flies were provided with oxygen by connecting the vessels to a gas feed system with a slight (40 mm of water) positive pressure. All flies, except those indicated in Table 2, were alive at the end of the incubation.

Groups of flies in the second experiment were assayed for acetylene reduction activity as for the first, except that tests were run 8 days after feeding *K. oxytoca* F15C and flies were tested for AR after 2, 6·75 or 24 h.

Results

In the first experiment acetylene reduction was first detected from 2 to 3 days after feeding the flies with the bacterium, reached a peak in 6 to 12 days and was still present at the termination of the experiment 22 days after feeding. In the unfed control groups of flies, acetylene reduction was not detected until 21 days after the start of the experiment (Table 1).

When the exposure of flies to acetylene was only 3 h, rates of ethylene production were sometimes over 100 nmol C_2H_4 g^{-1} fresh wt h^{-1} (Table 1). When exposures to acetylene were extended to 24 h, rates of ethylene production were always less than 30 nmol C_2H_4 g^{-1} fresh wt h^{-1} (Table 2).

Table 1. Nitrogenase activity associated with *Dacus tryoni*
All groups of flies were fed sucrose cubes and water; 5 days after emergence, flies in four of the six cages were fed from a lawn of the dinitrogen fixing bacterium, *Klebsiella oxytoca* F15C. All flies were still alive after exposure to acetylene for 3 h and were released back into their respective cages

Days since feeding bacterium	Nitrogenase activity (nmol C_2H_4 produced g^{-1} fresh wt h^{-1})					
	Flies fed *K. oxytoca* F15C				No supplement	
	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6
0	0	0	0	0	0	0
2	0	0	0	22	0	0
3	38	47	54	58	0	0
4	78	98	61	56	0	0
7	25	66	32	39	0	0
9	45	120	108	65	0	0
10	47	100	61	40	0	0
11	49	86	75	59	0	0
14	90	95	54	72	0	0
21	21	82	20	21	11	31
22	22	42	42	15	13	12

Table 2. Nitrogenase activity associated with *Dacus tryoni*
Replicate groups of flies were incubated with acetylene for 24 h starting 7 days after 5-day-old flies were fed *Klebsiella oxytoca* F15C

Group	Nitrogenase activity (nmol C_2H_4 produced g^{-1} fresh wt h^{-1})					
	Flies fed *K. oxytoca* F15C				No supplement	
	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6
1	7	27[A]	6	4	0	0
2	7[A]	15	5[A]	8	0[A]	0[A]
3	12	19	4	10[A]	0	0

[A] Due to a fault in the O_2 feed, flies in these vessels were dead after 6 h exposure to C_2H_2.

In the second experiment AR rates in the flies exposed to acetylene for 2 or 6·75 h were similar, but the rate declined to about half when the flies were incubated with acetylene for 24 h (Table 3).

Table 3. Nitrogenase activity associated with *Dacus tryoni*
Flies in two of the three cages had been fed *K. oxytoca* F15C 3 days after emergence. Nitrogenase activity was measured three times in 24 hours starting 8 days after feeding

Hours of incubation with C_2H_2	Nitrogenase activity (nmol C_2H_4 produced g^{-1} fresh wt h^{-1})		
	Flies fed *K. oxytoca* F15C		No supplement
	Cage 1	Cage 2	Cage 3
2	44	48	0
6·75	48	40	0
24	28	24	0

In control vessels with acetylene but without flies, with flies fed the dinitrogen-fixing bacterium but without acetylene, with flies not fed the bacterium but without acetylene, or without both flies and acetylene, no ethylene was detected. The retention times of all peaks designated as ethylene were identical and corresponded exactly to the retention time of an authentic standard of ethylene.

Discussion

The AR activity associated with Queensland fruit flies fed *K. oxytoca* F15C (Table 1, Cages 1–4) can be explained in terms of colonization of the alimentary tract by the dinitrogen-fixing strain. The delayed advent of AR activity in control flies not fed *K. oxytoca* F15C (Table 1, Cages 5–6) may have been caused by the delayed build up of gut populations of AR-active dinitrogen fixers, possibly including contaminating *K. oxytoca* F15. Spread of such bacteria between cages in our laboratory has been demonstrated on two occasions (unpublished data).

There was an apparent inhibition of the insect-associated nitrogenase system after continued exposure to acetylene. The figures in Table 2 for AR activity over 24 h are very low compared with those in Table 1 determined after only 3 h incubation in acetylene. Similarly in the second experiment the AR rates for 24 h are low compared with rates for 2 and 6·75 h exposure to acetylene (Table 3). This disparity implies that the rate of reduction of acetylene by the fly-associated dinitrogen-fixing system decreased with time. Breznak *et al.* (1973) reported that AR in termites exhibited this tendency, whereas Guerinot *et al.* (1977) found that nitrogenase systems associated with sea urchins had relatively constant rates of ethylene production over periods of up to 30 h. Toxic effects might be responsible for the inhibitory effect of acetylene in insects.

The 'normal' AR activities recorded in Table 2 for fly groups that did not survive the 24 h incubation in acetylene indicated that most of the ethylene was produced within the first 6 h of exposure. No additional ethylene production has been detected in this laboratory in vessels incubated a further 18 h after death of the contained flies (unpublished data) and experience with termites (Breznak *et al.* 1973; Benemann 1973), marine shipworms (Carpenter and Culliney 1975) and sea urchins (Guerinot *et al.* 1977) showed that AR activity was lost or considerably impaired when the invertebrates were dead or damaged.

The exact extent of any advantage conferred by a dinitrogen-fixing bacteria/Queensland fruit fly association remains to be determined. Times required by the experimental flies to double cellular nitrogen (TDN) were calculated according to the formula TDN = (ln 2) [(N_2 fixed per day)/(N content)], where nitrogen concentrations are per milligram fresh weight (Carpenter and Culliney 1975). Values ranged from 632 to 997 days. These compare favourably with the lowest TDN estimates attributed to termites by Breznak (1982) but would not obviate the need for substantial nitrogen input through the diet.

Fixed nitrogen may supplement nutrition either directly, e.g. by giving the fly access to additional amino acids from digested gut diazotrophs, or indirectly, e.g. by providing suitable substrates for the growth of bacteria possibly more important to the insect as sources of vitamins and growth factors.

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