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NOTES

Transconjugation between Bacteria in the Digestive Tract of the Cutworm *Peridroma saucia*

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Transconjugants arising from transfer of plasmid R388::Tn*1721* between donor and recipient strains of *Enterobacter cloacae* were detected in samples from the digestive tracts and fecal pellets of variegated cutworms (*Peridroma saucia*).

Previous reports (1, 2) have described the ingestion, transport, and regrowth of plasmid-bearing bacteria in the digestive tract of the variegated cutworm, *Peridroma saucia* (order Lepidoptera, family Noctuidae). Since *Enterobacter cloacae* effectively colonizes cutworm guts and insect frass (feces) (2), we wondered if these bacteria could be transported by a host insect from one site to another and continue to be active in the conjugal transfer of DNA between donor and recipient strains. To maximize the probability of detecting transfer events between bacteria associated with insects, we used a highly transmissible plasmid and sprayed microcosm contents with a heavy suspension of bacteria.

To produce the donor strain, plasmid-free *E. cloacae* (2) was transformed (3) with plasmid R388::Tn*1721* (4), which encodes resistance to trimethoprim and tetracycline. A spontaneous nalidixic acid-resistant (Nal^r) mutant was used as a recipient in the matings. Bacteria were grown for 18 h at 30°C with shaking in LB broth (3) amended with antibiotics (donors received trimethoprim [50 μg/ml] and tetracycline [15 μg/ml]; recipients received nalidixic acid [500 μg/ml]), washed twice by centrifugation in water (10 min, 5,000 × *g*, 4°C), and suspended at 10^9 CFU/ml in water. Samples were diluted in 10 mM Tris buffer (pH 7.5), spread on selective LB media, and incubated for 18 h at 30°C. For donors and recipients, colonies from pairs of plates were averaged to calculate numbers of CFU. For transconjugants (plated on LB containing trimethoprim, tetracycline, and nalidixic acid in concentrations mentioned above), up to eight plates were each spread with 0.1 ml of an undiluted sample. Nalidixic acid was used to select against donors and to prevent plasmid transfer after samples were spread on agar surfaces (M. Walter, personal communication). Cycloheximide (25 μg/ml) was used in all media to inhibit fungi (all antibiotics were purchased from Sigma Chemical Co., St. Louis, Mo.).

Microcosms consisted of Blue Lake bush bean plants grown in plastic trays containing potting mix (1) and were kept in plastic chambers (1) for the duration of the experiment. Two microcosms were sprayed with a suspension of either *E. cloacae*(R388:Tn*1721*) (trial 1) or plasmid-free *E. cloacae* Nal^r (trial 2) at 10^9 CFU/ml (2). About 2 h later, approximately 50 fourth- or fifth-instar cutworms, reared as previously described (1), were placed on the plants. After feeding on the plants for 3 days, larvae were transferred to a second set of microcosms that had been spray inoculated 2 h earlier with bacterial suspensions at 10^9 CFU/ml. Cutworms feeding on plants sprayed with *E. cloacae*(R388::Tn*1721*) were transferred to plants sprayed with plasmid-free *E. cloacae* Nal^r (trial 1). Those feeding at first on plants sprayed with plasmid-free *E. cloacae* Nal^r were transferred to plants inoculated with *E. cloacae*(R388::Tn*1721*) (trial 2). The insects

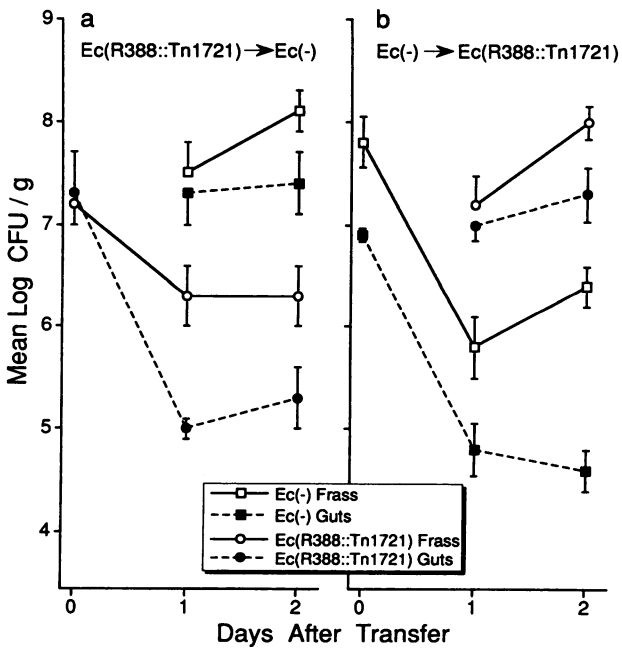

FIG. 1. Numbers of plasmid-free *E. cloacae* Nal^r [Ec(−)] and *E. cloacae*(R388::Tn*1721*) in digestive tracts and frass of cutworms during experiments demonstrating plasmid transfer. (a) Cutworms were fed for 3 days on bean plants sprayed with *E. cloacae*(R388::Tn*1721*) and then transferred to plants sprayed with plasmid-free *E. cloacae*. (b) Cutworms were fed for 3 days on bean plants sprayed with plasmid-free *E. cloacae* and then transferred to plants sprayed with *E. cloacae*(R388::Tn*1721*). The plotted values are based on bacterial numbers in three leaf samples, three soil samples, six gut samples, and six frass samples. Bars show standard errors of the means.

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TABLE 1. Occurrence of verified transconjugants from leaf, soil, cutworm guts, and cutworm frass samples[a]

Trial no.	Order of spraying bacteria into microcosms	Time of count[b]	CFU of transconjugants/g of sample (mean of detection threshold[c])			
			Leaf	Soil	Gut	Frass
1	Donor, recipient	1	ND[d] (43)	ND (30)	ND (35)	ND (49)
		2	ND (38)	ND (36)	93	75
2	Recipient, donor	1	ND (26)	ND (20)	78	ND (62)
		2	30	151	ND (14)	80

[a] See text for full description of experiment.
[b] Days after transfer of insects to second microcosm.
[c] Calculated for all replicates of a particular sample type collected on the same day.
[d] ND, None detected.

foraged for 2 days in the second set of microcosms. All leaf, soil, gut, and frass samples were collected and prepared for analysis of bacterial numbers as previously reported (2).

By spraying the first set of microcosms with 10^9 CFU/ml, plants were initially inoculated with 6×10^7 to 4×10^8 CFU/g. After 3 days on these plants, insects had about 1×10^7 to 6×10^7 CFU of donor or recipient bacteria per g of guts or frass. Figure 1 shows the means of the logarithms of bacterial numbers after the larvae had been transferred to the second set of microcosms. The 10- to 100-fold decrease of bacteria used in the first spray probably reflected replacement of gut contents with fresh plant material. While these populations decreased, the same bacteria were deposited from insect surfaces and frass onto soil and leaves. These numbered 10^4 to 10^5 CFU/g of foliage or soil. At the same time, bacteria used in the second spray persisted at about 10^7 to 10^8 CFU/g of guts or frass (Fig. 1) and about 1×10^7 to 5×10^8 CFU/g of foliage or soil by the second day after the transfer.

Table 1 summarizes the numbers of transconjugants recovered during the experiments depicted in Fig. 1. Transconjugants were verified as *E. cloacae*(R388::Tn*1721*) Nal^r by (i) growth on LB plus trimethoprim, tetracycline, and nalidixic acid; (ii) identification with API 20E strips (Analytab Products, Plainview, N.Y.); and (iii) the presence of plasmid R388::Tn*1721*, detected by the rapid boiling method for DNA extraction (3) and analysis by electrophoresis (3). In all cases, transconjugants were present in only one of the several replicate samples analyzed. In trial 1, transconjugants were observed at numbers that were equivalent to 25 CFU per gut and 3 CFU per frass pellet. In trial 2, the observed numbers were equivalent to 6.3 CFU per gut and 2 CFU per frass pellet. To calculate the frequency of transconjugants in these trials, the number of transconjugants in a specific sample was compared with the number of bacteria of the type applied to the second microcosm which were present in the sample [i.e., trial 1, plasmid-free *E. cloacae* Nal^r; trial 2, *E. cloacae*(R388::Tn*1721*)]. The number of transconjugants per 10^6 recipient bacteria that were recovered during trial 1 were 0.33 for gut and 2.7 for frass. In trial 2, transconjugants per 10^6 donors were 0.057 for leaves, 3.2 for soil, 3.9 for gut, and 1.7 for frass. It is not known whether multiple isolates from a single sample resulted from multiple transfer events or from one event followed by reproduction of the transconjugant. This causes uncertainty in the accuracy of the frequency of transconjugation. However, this is an inherent consideration relevant to any reporting of transconjugation frequencies. We also compared the frequencies observed for transfer in the microcosms with in vitro matings; donors and recipients were incubated at 5×10^8 CFU/ml in LB broth for 18 h at 30°C without shaking and plated on LB agar amended with tetracycline, trimethoprim, and nalidixic acid to select for transconjugants. For the two experiments performed in LB broth, the numbers of transconjugants per 10^6 recipient bacteria were 0.38 and 1.6. These values are similar to those observed for transfer in the microcosms.

Table 1 also presents the detection thresholds on days when all samples of a given type lacked observable transconjugants. It is not known if the samples contained transconjugants below these values. Transconjugants were not detected in trial 3, when a lower titer (3.5×10^7 CFU/ml) of donor and recipient was used in the respective sprays (data not shown). This absence is attributed to the lower probability of donor-recipient cell contact. The possibility that transconjugants occurred at numbers lower than our detection limits could not be ruled out.

Plasmid transfer was more likely to occur when donor and recipient numbers were relatively high (based on transfer in LB broth, at levels about 10^6 CFU/ml or greater). Thus, we consider two scenarios more probable for transconjugation, since sites that harbor higher numbers of *E. cloacae* are involved: (i) in the digestive tract at the interface of spray-inoculated leaves that were ingested in the first microcosm and spray-inoculated leaves that were ingested after the insects were transferred to the second microcosm and (ii) in or near frass pellets that were deposited on spray-inoculated leaves or soil after transfer of insects to the second microcosm. Other possible explanations could involve transfer on leaves or in soil. However, these possibilities are relatively unlikely, since bacteria carried by insects from the first microcosm were present in relatively low numbers (between 10^4 and 10^5 CFU/g) in the soil and on the plants of the second microcosm. The results reported in this paper indicate that even when mobility of recombinant DNA is encouraged by using a transmissible plasmid under optimized conditions, transfer is relatively rare. We suggest that transfer of recombinant DNA from field-released, genetically altered bacteria to indigenous organisms in the environment may occur, but that the events will be very rare.

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