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Genetically engineered termite gut bacteria (*Enterobacter cloacae*) deliver and spread foreign genes in termite colonies

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Abstract Indigenous gut bacteria of the Formosan subterranean termite (*Coptotermes formosanus* Shiraki, Isoptera: Rhinotermitidae) were used as shuttle systems to deliver, express and spread foreign genes in termite colonies. The gut bacterium *Enterobacter cloacae* was transformed with a recombinant plasmid (pEGFP) containing genes encoding ampicillin resistance and green fluorescent protein (GFP). In laboratory experiments, termite workers and soldiers from three colonies were fed with filter paper inoculated with transformed bacteria. Transformed bacteria were detected in termite guts by growing the entire gut flora under selective conditions and checking the cultures visually for fluorescence. We demonstrated that (1) transformed bacteria were ingested within a few hours and the GFP gene was expressed in the termite gut; (2) transformed bacteria established a persistent population in the termite gut for up to 11 weeks; (3) transformed bacteria were efficiently transferred throughout a laboratory colony, even when the donor (termites initially fed with transformed bacteria) to recipient (not fed) ratio was low; (4) transformed *E. cloacae* were transferred into soil; however, they did not accumulate over time and the GFP plasmid was not transferred to other soil bacteria. In the future, transgenic bacteria may be used to shuttle detrimental genes into termite colonies for improved pest control.

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Introduction

Termites harbor a diverse community of microbes in their gut and are dependent upon these microbes for survival in many ways. The main roles of the gut protozoa (Eucarya) and bacteria (Archaea, Eubacteria) are to supplement the nitrogen, carbon and energy requirements of the termite host (Breznak 2000). In addition, the gut flora possibly protects the termite host from invasion by foreign bacteria by providing "colonization resistance" (Dillon and Dillon 2004; Veivers et al. 1982). Microbes are naturally exchanged between colony members through grooming, food-exchange (trophallaxis) and coprophagy (La Fage and Nutting 1978).

In the future, it may be possible to use gut bacteria and protozoa of termites as tools and targets for termite control. The key is paratransgenesis, i.e., the use of genetically engineered microorganisms that reside within the host for use as "shuttles" or "Trojan horses" to express foreign genes in a host. Previous studies have been aimed at solitary insects (e.g., Bextine et al. 2004; Peloquin et al. 2002; Watanabe et al. 2000). In this study we present the first use of recombinant bacteria as shuttles to deliver, express and spread foreign genes in a social insect colony.

We have recently developed such a shuttle system using a genetically engineered laboratory strain of *Escherichia coli* to express green fluorescent protein (GFP) in the Formosan subterranean termite *Coptotermes formosanus* Shiraki. Although genetically modified bacteria were rapidly ingested by the termites and passed into the hindgut where the natural microbial flora of termites occurs, the *E. coli* strain survived less than a week in the termite gut (Husseneder et al. 2005). The number of termites retaining a stable population of genetically modified bacteria with continuous expression of marker genes could most likely be increased by engineering bacteria that occur naturally in the insect gut (Thimm et al. 1998; Chapco and Kelln 1994; Dillon and Dillon 2004). Bacteria derived from the termites' own indigenous gut flora should not trigger defensive or immune responses and should be well adapted to the living conditions and selective pressures in the termite gut. About a dozen indigenous bacterial strains have been cultured from the diverse

gut flora of *C. formosanus*, ranging from novel species to common Enterobacteriaceae (Higashiguchi et al. 2002).

The purpose of this study was to assess the utility of *Enterobacter cloacae*, which was isolated from the termite gut, as a shuttle bacterium to deliver, express and spread genes in termite colonies. We genetically engineered *E. cloacae* to express GFP. The bacterial shuttle was then evaluated according to some of the criteria and methods for successful application of paratransgenesis as defined by Durvasula et al. (2003): we (1) monitored ingestion of bacteria into the termite gut (method of delivery), (2) assessed the long term stability of the bacterial population in the gut (fitness of the transgenic bacteria), (3) measured the transfer of transgenic bacteria among workers and soldiers (method of dispersal), and (4) measured persistence and accumulation of transformed bacteria in soil, and determined if the plasmid was transferred to soil bacteria (method of assessing environmental spread).

Materials and methods

Isolation of gut bacteria

Termite workers and soldiers were collected from three colonies (M: Miller, G: Gilmore and P: Publication) on the Manoa campus of the University of Hawaii. Freshly collected termites were chilled, mouth and anus were sealed with paraffin, and then the surface of the termites was sterilized with 70% ethanol. Guts were extracted and homogenized under sterile conditions. The total gut extract was subjected to serial dilution and subsequently streaked on brain heart infusion (BHI) medium (Difco/Becton Dickinson, Sparks, Md.). Plates were incubated aerobically for 3 days at 30°C. Single colony forming units (cfu) were purified and described using standard morphological and biochemical techniques, including API 20E kits (bioMerieux, Hazelwood, Mo.) to identify Enterobacteriacea species.

Transformation

Plasmid-mediated transformation was used to introduce marker genes into isolated Enterobacteriaceae according to standard protocols (e.g., Peloquin et al. 2000). The vector plasmid (pEGFP; Clontech, Palo Alto, Calif.) contained genes for ampicillin resistance and GFP under the control of a lacZ promoter. The plasmid has a narrow host range (including Enterobacteriaceae) and no mobilizing (mob−, nic−, bom−) or conjugation functions (tra−). Transformed bacteria were identified by expressing GFP, a visual marker that causes the bacteria to glow bright green when exposed to UV light. Successfully transformed Enterobacteriacea species from the termite gut included *Citrobacter amalonaticus*, *Klebsiella pneumoniae* and *Enterobacter cloacae*. Plasmid stability and gene expression in serial culture under nonselective conditions was comparable to other studies (e.g., Peloquin et al. 2000). *Enterobacter cloacae* was selected as shuttle bacterium for introducing GFP plasmid into termite colonies.

Overnight cultures for feeding experiments

Genetically modified *E. cloacae* (GFP bacteria) were kept on selective BHI source plates with 10 μg/ml ampicillin (Sigma-Aldrich, Milwaukee, Wis.), and were restreaked each month. To grow liquid cultures for feeding termites, BHI broth was inoculated with GFP bacteria from the source plate and grown overnight at 30°C. Overnight cultures for feeding purposes did not contain ampicillin to avoid toxic effects on termites. An aliquot of each overnight culture was tested for contamination, and the number of GFP bacteria was determined by plating dilution series on selective BHI plates. If the culture was contaminated or did not contain at least 10^8 cfu/ml, the feeding experiment was repeated. Overnight cultures were diluted (1:2) with distilled water, and 400 μl was applied to a 9 cm filter paper circle placed in a 100×15 mm Petri dish (Fisher Scientific, Pittsburgh, Pa.). The 400 μl solution contained between 0.93 and 2.44×10^9 cfu [mean 1.79×10^9, standard deviation (SD) 0.46×10^9, n=12].

Feeding experiments

Termites were placed in Petri dishes at room temperature and were allowed to feed on filter paper inoculated with GFP bacteria solution. Controls were fed filter paper treated with 400 μl distilled water. Every day, dead individuals were removed; Petri dishes and filter papers were replaced to avoid reinoculation through feces. Termites were checked for presence of GFP bacteria by sterilizing their surface with 70% ethanol, removing and crushing their whole guts aseptically in sterile BHI broth containing 10 μg/ml ampicillin, and growing the cultures overnight. The presence or absence of GFP bacteria was confirmed by streaking the overnight culture on selective BHI agar plates and observing fluorescence under UV light (254–365 nm). The controls were processed simultaneously in the same way to exclude contamination. Unless noted otherwise, each experiment was conducted with three termite colonies (M, G, P). Means and SD were calculated over three replicates per colony.

Ingestion of GFP bacteria

One hundred workers from three colonies (M, G, P) were fed with 400 μl recombinant *E. cloacae* inoculated on filter paper in separate Petri dishes. Five individuals from each colony were then tested for GFP bacteria in their guts 1 h after initial exposure, then every 2 h for the next 12 h, and then again after 24, 28, 32, 36, and 48 h. Several guts were examined with a fluorescence microscope (LEICA DM LB; Meyers, Houston, Tex.) after 2 days to determine if bacteria were expressing GFP in the termite gut.

Persistence of the bacterial population in the termite gut

From each of the three colonies, 270 workers and 30 soldiers were placed in separate large Petri dishes. The 10:1 ratio of workers to soldiers mimics the natural ratio in a termite colony (Haverty 1977). Termites were fed with GFP bacteria on filter paper for 2 days, transferred to new Petri dishes, and fed daily with filter paper moistened with 400 μl water. For 3 months, ten guts were extirpated weekly from worker termites and screened for GFP bacteria. The mortality rate of workers and soldiers was assessed weekly.

Transfer of GFP bacteria

To distinguish between donor termites and recipients we dyed either donors or recipients by feeding them with filter paper containing 1% (w/w, 6.0 mg stain per paper) Sudan Red 7B (Aldrich) for a week. The dye had no influence on ingestion, spread, or stability of GFP bacteria in the termites. Donor termites of the three colonies were prepared by feeding with GFP bacteria for 2 days, and subsets of these were tested to ensure that donors contained GFP bacteria before beginning the transfer experiment. Recipients were maintained under the same conditions as the donors, but were fed with water-treated filter paper.

First, transfer of genetically modified bacteria was observed between workers every 2 h for 12 h. For this experiment 150 donors of colony P were combined with 150 recipients in a Petri dish, and, for the first 12 h, guts were extirpated every 2 h from five recipients and screened for the presence of recombinant bacteria. The experiment was repeated three times for one colony (P). Second, bacterial transfer between workers was observed daily for 6 days with different donor/recipient ratios. One thousand workers from each of the three colonies with four different donor/recipient ratios (1:1, 1:5, 1:10, 1:25) were placed in large Petri dishes on moist filter paper. Guts of five donors and five recipients were extirpated daily to determine the percentage of termites containing GFP bacteria. Third, transfer between workers and soldiers and vice versa was measured. Worker donors and soldier donors were kept separately on filter paper inoculated with GFP bacteria for 2 days. Recipient workers and soldiers were fed with water-treated filter paper. One hundred worker donors were combined with 100 recipient soldiers and the same number of soldier donors was combined with recipient workers. Each day for 7 days guts were extirpated from ten donors and ten recipients to screen for GFP bacteria. This experiment was repeated three times for one colony (P).

Persistence, accumulation, and gene transfer of GFP bacteria in soil

One hundred workers per colony were fed on filter paper inoculated with GFP bacteria for 2 days. Termites were then placed into a Petri dish containing 20 g soil from the termite collection site. After 24 h, termites were removed from the first dish and placed into a second dish with 20 g soil. Once per week for 18 weeks, 1 g soil from each dish was subjected to serial dilution to determine the number of GFP bacteria. Fluorescent bacteria recovered from the soil were tested to determine if they were still the originally transformed *E. cloacae*, using standard biochemical tests for identification of Enterobacteriaceae species (API 20E, bioMerieux).

Results

Ingestion of GFP bacteria

Termite workers from the three colonies (M, G, P) ingested the recombinant bacteria rapidly. After only 1 h of feeding on inoculated filter paper, an average of 10% (M) to 40% (G) of the workers contained GFP bacteria in their guts (Fig. 1). The percentage of termites containing recombinant bacteria increased within the first 12 h to a maximum of 90% (G, P) to 100% (M). Screening between 24 and 48 h of continuous feeding showed no additional increase; the percentage of termites with GFP bacteria present in their guts varied between 60% and 100%. The results demonstrated rapid ingestion of recombinant bacteria by a high percentage of termite workers. Using fluorescence microscopy we found fluorescent bacteria in freshly dissected hindguts, which confirmed that transgenic bacteria were not only ingested, but also expressed GFP in the gut.

Persistence of recombinant bacteria in the termite gut

In the first 6 weeks after termites were fed with GFP bacteria, an average of 60% to 100% of the termites from each colony contained GFP bacteria (Fig. 2). In our study, recombinant bacteria continued to persist as long as the termites were healthy. For the first 6 weeks, the mortality rate of the termites was low (under 5%). After 8 weeks the mortality rate increased and the termite populations in the Petri dishes declined rapidly, which contributed to the loss of the GFP bacteria. The experiment ended after 11 weeks, because all termites had died. However, bacteria were not entirely lost during that period; 55% of the last surviving termites (P) still harbored GFP bacteria in their guts. The results showed that the GFP bacteria established a stable, replicating and self-perpetuating population in the termite gut without augmentation.

Transfer of GFP bacteria

First, we measured the transfer rate of GFP bacteria between workers for the first 12 h after initial combination of donors and recipients (50:50). As early as 2 h after combining donors and recipients, an average of 20% of the

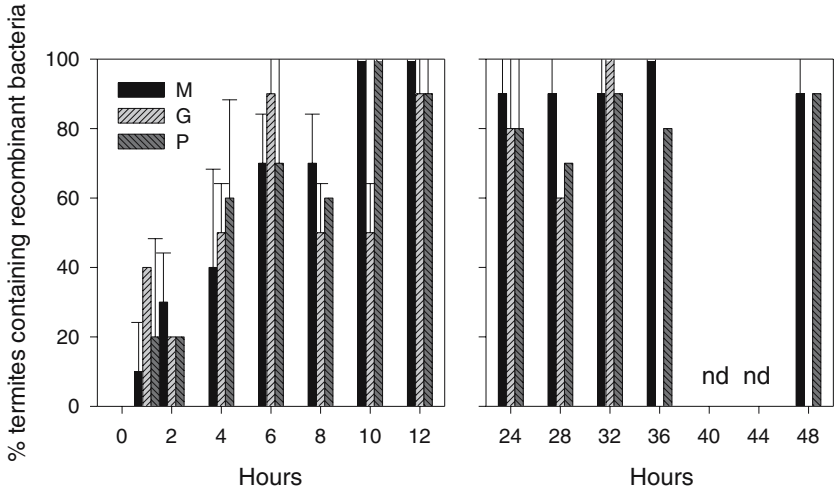

Fig. 1 Ingestion of genetically modified *Enterobacter cloacae* expressing green fluorescent protein (GFP bacteria) by workers of three termite colonies (*M*, *G*, *P*) from filter paper. *Left graph* Percentages of termites containing GFP bacteria for the first 12 h, *right graph* additional screenings for up to 48 h. Percentages at 40 and 44 h were not determined (*nd*). Results were averaged over three replicates per colony. *Error bars* Standard deviation (SD)

recipients had GFP bacteria in their guts. The percentage of recipients containing recombinant bacteria increased to 75% after 12 h (Fig. 3). Second, we measured bacterial transfer between workers with donor/recipient ratios ranging from 1:1 to 1:25. During the experiment at least 85%, and up to 100%, of the donors contained GFP bacteria on any one day (Fig. 4). Donors passed GFP bacteria to at least 25% (G) and up to 95% (P) of the recipients on the first day. The proportions of recipients containing recombinant bacteria increased over time and reached 80–100% after 6 days in all colonies. Overlapping SDs indicated that there was no significant difference in transfer rate among the four donor/recipient ratios. Even a ratio of 1 donor per 25 recipients was sufficient to spread recombinant bacteria throughout the laboratory colony in less than 6 days. Third, transfer of recombinant bacteria was observed between workers and soldiers of colony P and vice versa. All of the donor workers had ingested GFP bacteria within 2 days of feeding on inoculated filter paper. Surprisingly, a high percentage of donor soldiers (57%) were also able to ingest GFP bacteria without workers present to feed them (Fig. 5). Recombinant bacteria were transferred from workers to soldiers. Two days after combining donor workers with recipient soldiers, a small proportion of soldiers, on average 3%, harbored GFP bacteria in their guts. This proportion increased steeply to 95% after 1 week. Soldiers transferred GFP bacteria to workers even faster. After 24 h of exposure to donor soldiers, 73% of the recipient workers showed the presence of GFP bacteria, increasing to 97% after 3 days.

Persistence, accumulation, and gene transfer of GFP bacteria in soil

After keeping termites (fed recombinant bacteria prior to the experiment) on soil for 24 h, 50,000 (SD 7,100; colony G) to 151,000 GFP bacteria (SD 35,000; colony M) were recovered per gram soil (Fig. 6). This number decreased within 1 month, but a few recombinant bacteria persisted

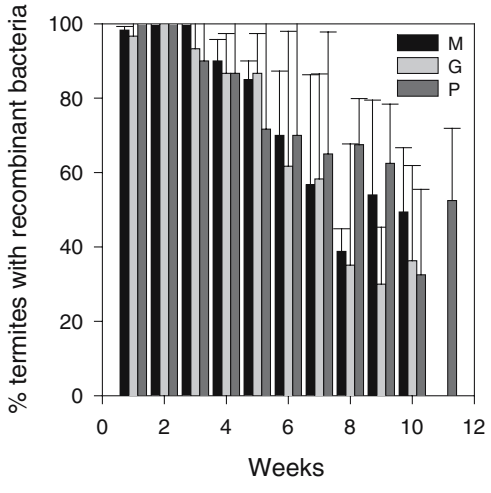

Fig. 2 Persistence of recombinant bacteria in three different colonies (*M*, *G*, *P*). Results were averaged over three replicates per colony. *Error bars* SD

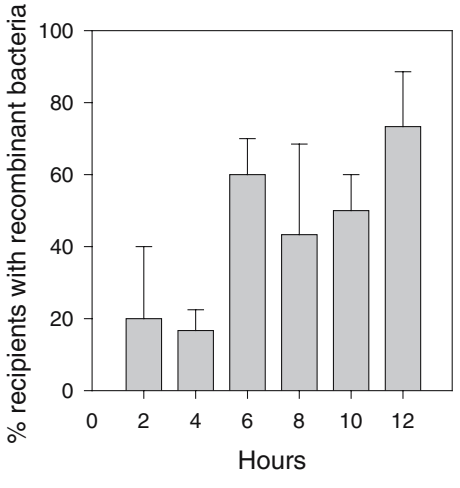

Fig. 3 Transfer of GFP bacteria between workers in the first 12 h. *Columns* Percentage of recipients containing GFP bacteria after combining them with donors. Results for one colony (P) were averaged over three replicates. *Error bars* SD

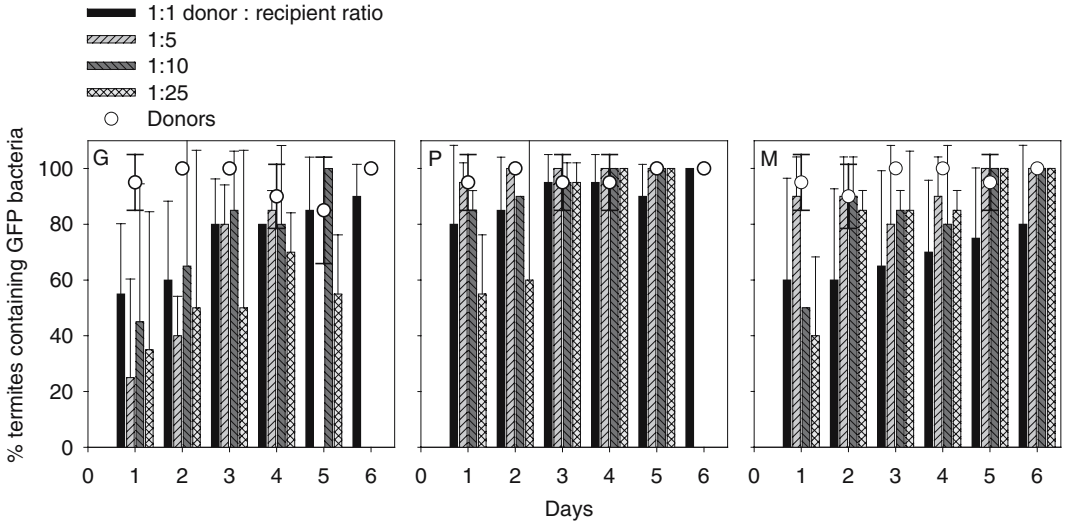

Fig. 4 Transfer of recombinant bacteria among workers from three colonies (*G*, *P*, *M*) with different donor/recipient ratios (1:1, 1:5, 1:10, 1:25). *Circles* Percentages of donors containing GFP bacteria, *columns* percentages of recipients with GFP bacteria. *Error bars* SD (three replicates per colony)

in the soil for more than 4 months without new inoculation. However, GFP bacteria did not accumulate in soil, even when donor termites were continuously present. Numbers of GFP bacteria in the soil of colony M, initially high at about 108,000 cfu/g soil (SD 2,830), declined rapidly in the first 4 weeks; recombinant bacteria in the soil of colony G stayed at a low level between 23,850 (SD 7,140) and 30,000 (SD 3,960) cfu/g soil during the first 5 weeks, then the numbers also declined towards zero. Although the number of GFP bacteria increased to a maximum of 108,000 (SD 9,900) cfu/g soil of colony P within the first 4 weeks, the number of GFP bacteria in soil decreased rapidly after 1 month. After 9 weeks the termites started to die and the experiment was ended. Weekly samples of fluorescent cfu recovered from soil showed the same biochemical characteristics as the originally transformed *E. cloacae*.

Discussion

Research into gene delivery and expression by transgenic bacteria has previously focused on solitary insects (e.g., Bextine et al. 2004; Peloquin et al. 2002; Watanabe et al. 2000). Our study is the first to provide data not only on ingestion and persistence, but also on transfer of recombinant bacteria among colony members of a social insect species. In this study, we describe a novel use of genetically modified indigenous gut bacteria of termites as shuttles for spreading and expressing foreign genes within

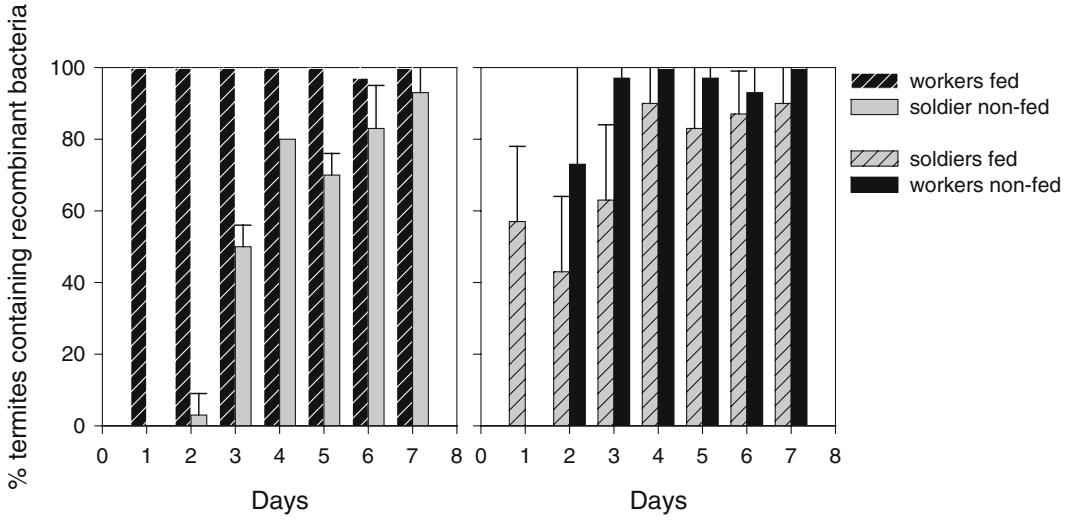

Fig. 5 Ingestion of recombinant bacteria in workers and soldiers and transfer from workers to soldiers and vice versa. Results averaged over three replicates for one colony (P). *Error bars* SD

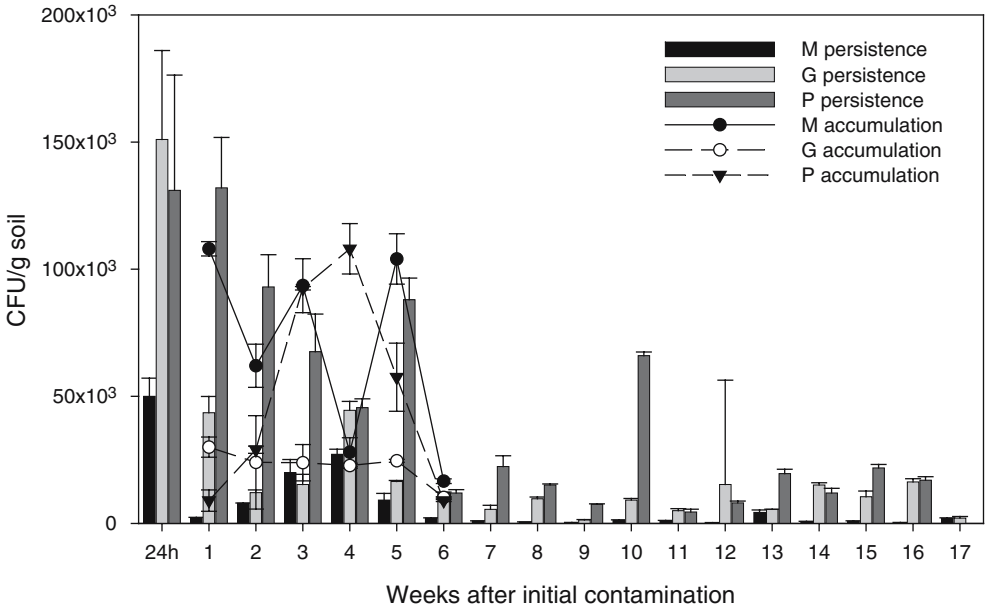

Fig. 6 Persistence and accumulation of GFP bacteria in soil. The number of colony forming units (CFU) of GFP bacteria per gram of soil was measured weekly to determine persistence (termites removed from the soil after 24 h, *columns*) and possible accumulation of bacteria (termites kept on soil, *symbols*). Results averaged over three replicates per colony. *Error bars* SD

termite colonies. The goal of this study was to design a functional bacterial shuttle system that establishes a stable population in the termite gut to spread GFP marker genes efficiently among termites. Such a shuttle system will make it possible to investigate the role of certain bacteria in termite biology; for example, the specific location of bacteria groups, interaction between trophic levels, and the vertical and horizontal transfer of gut symbionts. In addition, such a shuttle system would be useful in delivering and expressing genes in a termite colony that produce detrimental substances against the gut flora or the termites themselves.

As the shuttle bacterium we chose *E. cloacae*, a strain found in ample amounts in the gut of *C. formosanus* (Higashiguchi et al. 2002). Transgenic strains of the genus *Enterobacter* have previously been used for microbial insect control. Kuzina et al. (2002) transformed *Enterobacter gergoviae*, which was isolated from the gut of pink bollworm (Lepidoptera: Gelechiidae), to express *Bacillus thuringiensis* toxin. Also, *E. cloacae* was transformed with an ice nucleation gene to successfully reduce the cold hardiness of the mulberry pyralid moth, *Glyphodes pyloalis* (Watanabe et al. 2000).

In our experiments, transgenic *E. cloacae* were rapidly ingested from inoculated filter paper by termite workers and soldiers. It was not surprising that workers, who are responsible for feeding the colony, ingested bacteria quickly. Soldiers, however, are morphologically adapted to defend the colony. Their mandibles are enlarged, which supposedly makes it impossible for them to feed and groom themselves. As such, they are dependent upon the workers for these activities (Grassé 1949, p 498). Nevertheless, we found GFP bacteria in the gut of soldiers after only 1 day on inoculated filter paper without workers. Future studies on the route of infection in soldiers are warranted. Rapid ingestion of transgenic bacteria through feeding was also demonstrated in solitary insect species (Peloquin et al. 2002; Chapco and Kelln 1994; Dillon and Dillon 2004). Rapid ingestion alone, however, does not guarantee stable expression of the foreign gene over time if the transgenic bacteria do not persist in the gut. For example, our previous studies, using a laboratory strain of *E. coli*, failed to produce a persistent population in the termite gut. Due to this instability, the percentage of termites containing modified bacteria never exceeded 70%, and bacterial populations declined rapidly without continuous inoculation (Husseneder et al. 2005). Possible explanations for the low success of the *E. coli* strain, which is not indigenous to the termite gut, include colonization resistance by the indigenous gut flora (Dillon and Dillon 2004; Veivers et al. 1982), and the fact that a non-indigenous strain might not be competitive among the natural community (Leff and Leff 1996; Chao and Feng 1990).

In other insects, researchers have found that indigenous bacteria originally derived from the host insect could be easily reintroduced, and establish a stable population despite genetic modifications. Whereas genetically tagged non-indigenous *E. coli* vanished in 1 day from the gut of collembola, the indigenous *Alcaligenes faecalis* survived for almost 2 months (Thimm et al. 1998). Chapco and Kelln (1994) fed grasshoppers with genetically modified indigenous *Enterobacter agglomerans*, and the bacteria persisted for at least 3 weeks in the insects' digestive system. In our study, transgenic *E. cloacae* established a self-sustaining and replicating population in the termite guts for almost 3 months, which suggests the superiority of this indigenous strain over the previously used *E. coli* laboratory strain.

Our previous studies using *E. coli* as a shuttle bacterium have shown that transformed bacteria were transferred among termites in a day, but the maximum proportion of recipients receiving GFP bacteria was only 33% (Husseneder et al. 2005). However, indigenous gut bacteria such as *E. cloacae* are well adapted to the living conditions and selective pressures in the termite gut and should not elicit

colonization resistance or immune defenses (Dillon and Dillon 2004). We found that transfer of *E. cloacae* was more likely to be promoted by social interactions of termites than was *E. coli*. Transgenic *E. cloacae* were transferred quickly and efficiently. Fluorescent bacteria appeared in recipient termites within the first few hours after combination with donors. Even with 1 donor termite per 25 recipients, spread of GFP bacteria to at least 80% recipients of the laboratory colony occurred in less than a week. The key to the fast spread of bacteria to a large number of individuals was probably the multiplicative effect of recipients becoming secondary donors. In addition, both workers and soldiers were able to ingest bacteria and pass them on to other colony members. Although ingestion and transfer of bacteria by soldiers was not as efficient as by workers, it still added to the overall efficiency of the shuttle system. The efficient transfer and the stable persistence of transgenic *E. cloacae* should make it possible to spread a foreign gene throughout a termite field colony for termite control, even though mature colonies of *C. formosanus* can contain millions of termites (Su et al. 1984).

Before field experiments involving genetically modified organisms can be conducted it is imperative to gather detailed knowledge about the potential environmental impact, including the persistence of the transgenic bacteria in soil and possible gene transfer between bacterial strains. When termites that contained GFP bacteria were kept on soil, they transferred the recombinant bacteria to the soil. There, GFP bacteria persisted for several weeks even after termites were removed. However, no accumulation of GFP bacteria in soil was observed. Even when donor termites were kept continuously on soil, the bacterial population declined within 2 months suggesting limited survival capability among the community of soil bacteria. Although bacteria have a high generation turnover and mutate frequently, the fluorescent *E. cloacae* recovered from the soil each week had the same morphological and biochemical characteristics as the original strain. No microevolution in adaptation to the new habitat could be detected. Dillon and Dillon (2004) proposed that the guts of soil-inhabiting insects provide ideal conditions for transconjugation between bacterial strains. For example, plasmid transfer was recorded in the guts of the collembolan *Folsomia candida* (Hoffmann et al. 1998), and larvae of the silkworm *Bombyx mori* (Watanabe and Sato, 1998). However, within the time and scope of our experiments we did not detect GFP expression in any bacterial strain other than *E. cloacae*. Because the expression vector used in this study (pEGFP) has a narrow host range, and its mobilizing and conjugation functions were disabled, plasmid transmission to soil bacteria was considered unlikely. In contrast to our study, Armstrong et al. (1990) used highly mobile plasmids of *E. cloacae* to investigate transconjugation in the gut of cutworms. He came to a similar conclusion 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." Nevertheless, because the insect gut is an alleged "hot spot" for gene transfer in the soil (Dillon and Dillon 2004), more detailed research is required, especially prior to any future releases of genetically modified bacteria for field applications (Hoffmann et al. 1998).

Durvasula et al. (2003) listed prerequisites necessary for a successful strategy using paratransgenesis. The transgenic *E. cloacae* shuttle system fulfills most of these requirements. For example, Enterobacteriaceae belong to the natural, stable flora of the target pest (termites), and they are amenable to isolation, culture and transformation with foreign genes. A method of delivery and dispersal in the population of the target exists: as shown in this study, transgenic bacteria can be introduced into termite colonies by feeding, and bacteria are subsequently spread throughout the colony by social interactions. Furthermore, it is possible to monitor environmental spread and non-target effects. The use of GFP, which does not affect the fitness of the bacteria, as a marker gene makes transfer of bacteria and the plasmid traceable (Valdivia et al. 1996). Thus, we have established a proof-of-concept for the use of *E. cloacae*, an indigenous termite gut bacterium, as a shuttle system to deliver and express foreign genes in termite colonies. A crucial requirement for application of paratransgenesis in termite control is that future detrimental expression products must target the pest species itself or their obligate symbionts. To that end, we are currently screening gene products for their efficacy against termites and their obligate protozoan symbionts.

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