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Interdomain Conjugal Transfer of DNA from Bacteria to Archaea▽†

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***Escherichia coli* transforms the methanogenic archaeon *Methanococcus maripaludis* at frequencies ranging from 0.2×10^{-6} to 2×10^{-6} per recipient cell. Transformation requires cell-to-cell contact, *oriT*, and *tra* functions, is insensitive to DNase I, and otherwise displays hallmarks of conjugation.**

Conjugal transfer of DNA involves a specific set of transfer (*tra*) functions that mediate the mobilization of DNA containing an origin of transfer (*oriT*) from a donor to a recipient in a process requiring cell-to-cell contact (9). While conjugation is often very efficient between members of a given species or genus, it can also occur at a lower efficiency between phylogenetically distant microorganisms with structurally distinct cell surfaces. *Escherichia coli*, for example, mediates conjugal transfer of DNA to such diverse bacterial recipients as cyanobacteria (23), spirochetes (14), and a variety of Gram-positive bacteria (17, 22); *E. coli* even mediates conjugal DNA transfer to members of the domain *Eukarya*, such as to *Saccharomyces cerevisiae* (6) and mammalian (20) cells. Because of its broad range of potential recipients, conjugation has proven to be a valuable genetic tool (11) and may be an important mechanism of horizontal gene transfer and a driver of genome evolution (7). Conjugation-like DNA transfer has also been demonstrated in members of the domain *Archaea* (5, 15). However, conjugation between *Bacteria* and *Archaea* has not been demonstrated, despite the observation that many whole-genome sequences of *Archaea* harbor DNA that appears to be of bacterial origin (7).

To investigate whether conjugation can occur between *Bacteria* and *Archaea*, the RP4 (IncPα group) conjugal-transfer system was used to attempt to mobilize DNA from *E. coli* to the anaerobic, methanogenic archaeon *Methanococcus maripaludis* strain S2 (21). The RP4 system was selected because previous work demonstrated that this plasmid supports the transfer of DNA from *E. coli* to phylogenetically distant recipients, including yeast (3) and mammalian (20) cells. Additionally, *E. coli* has been shown to successfully conjugate with strictly anaerobic bacterial strains (22). *M. maripaludis* was chosen as a recipient because it has growth parameters similar to those of *E. coli* and has readily available selectable markers (1). For all the experiments described, *M. maripaludis* was grown in liquid or solid (excluding cysteine) McCas medium (12), supplemented with 2.5 μg/ml puromycin (Pur) where appropriate, using standard anaerobic techniques (2). All plating for conjugation experiments, except for determination of viable-*E. coli* cell counts, was performed in an anaerobic chamber (Coy, Grass Lake, MI) with an atmosphere of 5:5:90 H_2-CO_2-N_2. *E. coli* was grown in Difco LB medium (Becton-Dickinson, Sparks, MD) supplemented where appropriate with 50 μg/ml kanamycin sulfate (Kan) and ampicillin (Amp).

To interrogate conjugal DNA transfer between *E. coli* and *M. maripaludis*, a set of vectors that either contained or lacked *cis*-acting sites required for mobilization by RP4 transfer functions were constructed (Table 1). Each of these vectors contained a Pur resistance (Pur^r) gene cassette (*pac*) (4) flanked by ~0.5 kb DNA homologous to regions 5′ and 3′ of the *M. maripaludis nrpR* gene (*nrpR*::*pac*), which allows for selection by Pur in *M. maripaludis* and provides sites for homologous recombination into the *nrpR* locus of the *M. maripaludis* chromosome. This construct was selected because it has previously been used to transform *M. maripaludis* to Pur resistance by recombination into the *nrpR* locus using a polyethylene glycol (PEG)-mediated transformation protocol (10, 18). After it was demonstrated that plasmids of the appropriate genotypes support conjugation from donor strain *E. coli* S17-1, which contains the RP4 *trans*-acting transfer (*tra*) functions on the chromosome via an integrated RP4-2-Tc::Mu-Km::Tn*7* cassette (16), to *E. coli* recipient cells (Table 1; see also the supplemental material), we investigated whether these same donor strains could support DNA transfer to *M. maripaludis.*

For initial conjugation experiments, 20-ml cultures of *E. coli* donor cells were pelleted by centrifugation, resuspended in 5 ml of the recipient culture, and transferred to 28-ml serum tubes under anaerobic conditions (see the supplemental material). Sealed tubes were removed from the chamber, centrifuged for 10 min at 750 × *g*, and returned to the anaerobic chamber, and cell pellets were resuspended in 1 ml of McCas medium without sulfide. Aliquots (10 to 50 μl) of the concentrated donor-recipient mixture were spread on Pur-containing McCas medium plates, and dilutions were plated on nonselective LB and McCas medium plates to determine total counts of viable cells of the donor and recipient, respectively. Preliminary experiments indicated that, although *E. coli* remained fully viable during at least the first 4 h of coincubation with *M. maripaludis* on

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TABLE 1. Transformation of *M. maripaludis* by *E. coli*

Plasmid[f]	Locus(i) from mobilizable plasmid[a]	Predicted mobilization phenotype	Mediates conjugation to *E. coli* recipient?[b]	No. of Pur^r colonies per 10^8 *M. maripaludis* cells[c]
pTAP1	*mob-oriT-rep*	Mob$^+$	Yes	24
pTAP2	*rep*	Mob$^-$	No	<1[d]
pTAP3	*oriT-rep*	Mob$^-$	No	<1[d]
pTAP4	*mob-oriT*	Mob$^+$	Yes	51
pTAP5	None	Mob$^-$	No	0[e]
pTAP6	*oriT* region	Mob$^+$	Yes	175

[a] From pBBR1MCS-2 (8) for pTAP1 to -4 or RP4 (13) for pTAP6 (see the supplemental material).
[b] Indicates whether recipient growth was observed (yes) or not (no) under appropriate selection conditions for transconjugants (see the supplemental material).
[c] Average of results from 3 experiments.
[d] Only one colony was observed in three experiments.
[e] No colonies observed.
[f] All vectors were based on pCR2.1 (Amp^r Kan^r) and contained *nrpR*::*pac*.

McCas medium plates (data not shown), significant growth was not observed; thus, no selection against the donor strain was necessary. Plates were incubated at 37°C for 1 day (LB medium) or 4 days (McCas medium), and colonies were counted. In a series of three experiments, only two Pur-resistant *M. maripaludis* colonies were observed when the *mob*-negative vectors pTAP2, -3, and -5 were used (Table 1). When these were restreaked onto selective McCas medium plates, either no or very poor growth occurred, suggesting that these were not true transformants. In contrast, many *M. maripaludis* colonies were observed when vectors that were capable of being mobilized to an *E. coli* recipient were used (pTAP1, -4, and -6) (Table 1). For these vectors, frequencies of transformation ranged from 0.2×10^{-6} to 2×10^{-6} per recipient cell, suggesting that the Pur-resistant colonies arose due to conjugation. These are similar to frequencies of RP4-mediated conjugation from *E. coli* to diverse recipients, such as yeast (6) and *Clostridium* spp. (22).

To confirm that the Pur-resistant colonies obtained in these experiments were indeed transformed with the *nrpR*::*pac*-containing vector, randomly selected colonies (5 each from matings using pTAP1 and pTAP4 or 19 from pTAP6) were screened by PCR and Southern hybridization (see the supplemental material). PCR using primers complementary to the 3′ or 5′ end of the *pac* cassette and to the *M. maripaludis* genome 3′ or 5′ of *nrpR* (outside the regions of homology in *nrpR*::*pac*) as well as Southern blots using a region of the *pac* gene as a probe indicated that all tested strains contained *nrpR*::*pac* recombined at the *nrpR* locus (Fig. 1). Approximately half of the

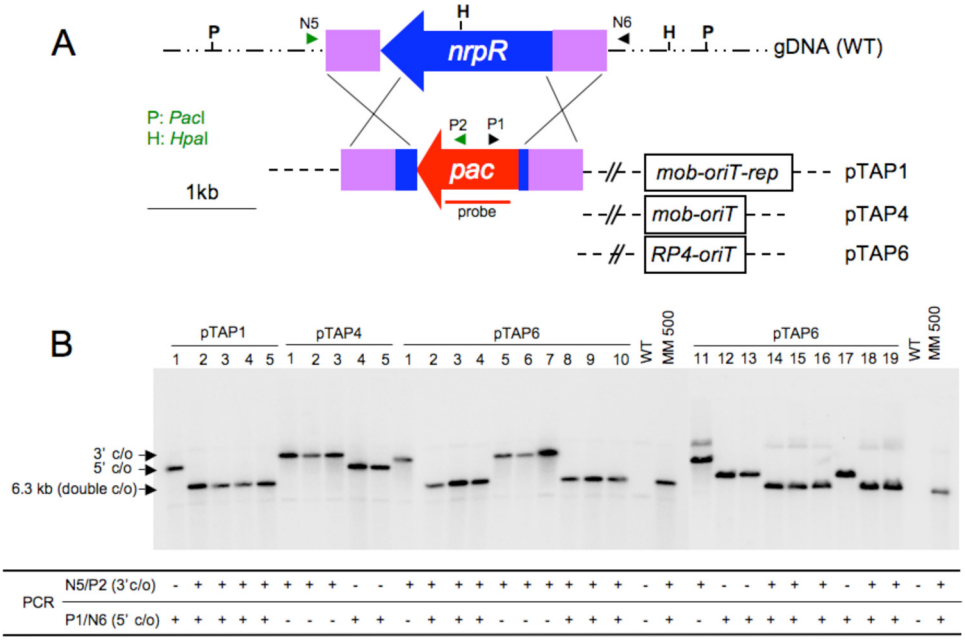

FIG. 1. Genetic analysis of *M. maripaludis* transformants. (A) A schematic diagram of the *nrpR* gene and flanking region in the *M. maripaludis* genome and the *nrpR*::*pac* region of the gene replacement constructs pTAP1, pTAP4, and pTAP6, harboring *mob-oriT-rep*, *mob-oriT*, and RP4-*oriT*, respectively (open boxes). Primers for PCR analyses are shown with arrowheads, and the probe for Southern analysis is indicated. gDNA, genomic DNA. (B) Southern blot and PCR analyses of DNA extracted from putative pTAP1, pTAP4, and pTAP6 transformants of *M. maripaludis*. Arrows indicate the signature band (6.3 kb) for double crossover (c/o), 5′ crossover, and 3′ crossover. Positive and negative PCR amplifications are shown as "+" and "−," respectively. WT, wild-type *M. maripaludis* S2; MM500, *nrpR* deletion mutant generated by PEG-mediated transformation with an *nrpR*::*pac*-containing construct (10).

TABLE 2. Requirements for transformation of *M. maripaludis* by *E. coli*[a]

E. coli donor	Plasmid in donor	Treatment	No. of Pur^r colonies observed[b]	Efficiency per recipient ($n = 4$)[c]
S17-1	pTAP6	None	28, 27, 26, 32	$(3.8 \pm 0.8) \times 10^{-7}$
S17-1	pTAP6	250 U DNase I spread on plates	24, 26, 16, 52	$(3.9 \pm 1.3) \times 10^{-7}$
S17-1	pTAP6	Both donor and recipient plated on a 0.45-μm filter	129, 170, 180, 167	$(2.1 \pm 0.5) \times 10^{-6}$
S17-1	pTAP6	Donor and recipient separated by a 0.45-μm filter	0, 0, 0, 0	$< (3.3 \pm 0.7) \times 10^{-9}$
S17-1	pTAP6	Heat-killed donor (80°C for 20 min)	0, 0, 0, 0	$< (3.3 \pm 0.7) \times 10^{-9}$
S17-1	pTAP5	None	0, 0, 0, 0	$< (3.3 \pm 0.7) \times 10^{-9}$
DH5α (Tra$^-$)	pTAP6	None	0, 0, 0, 0	$< (3.3 \pm 0.7) \times 10^{-9}$
S17-1	None	Purified pTAP6 (4 μg) plated with donor	0, 0, 0, 0	$< (3.3 \pm 0.7) \times 10^{-9}$
None	NA	No donor	0, 0, 0, 0	$< (3.3 \pm 0.7) \times 10^{-9}$
S17-1	pTAP6	No recipient	0, 0, 0, 0	NA

[a] All data are from a single experiment, where each treatment was performed in quadruplicate. Approximately 8×10^7 recipient cells were used, with donor/recipient ratios ranging from 7:1 to 13:1. NA, not applicable.
[b] The number of Pur^r *M. maripaludis* colonies observed on each plate.
[c] Efficiency represents the mean number of Pur^r colonies per viable recipient cell (±standard error of the mean). When no Pur^r colonies were observed, the efficiency is shown as being less than the calculated efficiency observed from one Pur^r colony ± the error in determining the total number of viable recipients.

strains were the result of double-crossover events, i.e., replacement of genomic *nrpR* with *nrpR*::*pac*.

Using the pTAP6 vector (GenBank accession no. HM536627), a series of controls were performed to determine whether transformation was a result of conjugation. Matings were performed as described above, except that donor and recipient cells were pelleted and resuspended separately, coming into contact only when plated on McCas medium plus Pur agar. This is essentially the "combined spread plate" method described by Walter et al. (19) and was used to simplify interpretation of results. To determine whether the mobilization functions present in S17-1 were required, *E. coli* strain DH5α (*tra* mutant) transformed with pTAP6 was used as a donor. To determine whether donor cells must be viable, concentrated S17-1(pTAP6) was heated to 80°C for 20 min under anaerobic conditions prior to being plated, which decreased donor viable counts >10,000-fold ($<10^5$/ml). To test if transformation could be achieved with naked DNA (via natural competence of *M. maripaludis*) and if the transferred plasmid must be inside the donor cell, 4 μg purified pTAP6 was plated along with S17-1 containing no intracellular plasmid. To test for inhibition by DNase, 250 U (0.2 ml of 1,250 Kunitz units/ml in McCas medium) of DNase I (Sigma, St. Louis, MO) was spread on plates immediately prior to plating; the efficacy of DNase under assay conditions was confirmed (see the supplemental material). To determine if cell-to-cell contact was required, 20-μl aliquots of the donor and recipient were spread either on the same or opposite sides of a 0.45-μm nylon filter laid on the plate surface. In all other cases, 20-μl aliquots of donor and recipient cells were spread on a section of the plate ~50 mm in diameter, consistent with the size of the nylon filters. Transformants were observed only with live S17-1(pTAP6) as a donor, with or without DNase on plates and only when the donor and recipient were not separated by the nylon filter, at frequencies ranging from 0.4×10^{-6} to 2×10^{-6} per recipient cell or 0.5×10^{-6} to 3×10^{-7} per donor cell (Table 2).

In summary, this work demonstrated that the transformation of *M. maripaludis* by *E. coli* displayed all of the hallmarks of conjugation: *oriT* was required in *cis* on the plasmid to be transferred, mobilization functions were required in the donor cell, the plasmid had to be inside the donor cells, donor cells had to be viable, cell-to-cell contact was required, and DNase I had no effect on the transformation. This shows that conjugation between *Bacteria* and *Archaea* can occur, thereby expanding the phylogenetic range of recipients that can be transformed using the RP4 conjugal-transfer system. Although the process described here is less efficient than standard PEG-mediated transformation of *M. maripaludis* (18), it is less laborious and may be useful for routine transformation of this methanogen. This approach may also prove fruitful for establishing genetic systems in other methanogens and *Archaea*.

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