Supplemental Materials

SUPPLEMENTAL METHODS

Primer design and PCR for Cys clade *fdhF* alleles.

Degenerate primers (Cys499F1b, 1045R) for a major clade of selenium independent (Cys) *fdhF* alleles present in lower termites and the wood roach *C. punctulatus* were designed manually using all sequences recovered from these insects in ref. (1). Forward primer Cys499F1b (5'– ATG TCS CTK TCS ATI CCG GAA A –3') specificity is as follows: 38.9% of the sequences are perfectly matched, 22.2% have 1 mismatch, 27.8% have 2 mismatches, and 8.3% have 3 mismatches. No mismatches are in located in the terminal 3' position. The reverse primer 1045R (5'– CIC CCA TRT CGC AGG YIC CCT G –3') was designed based on 154 sequences from higher termites, lower termites and *C. punctulatus*. The 1045R primer targets both Sec and Cys *fdhF* variants; 60.3% of the sequences have 0 primer mismatches, 32.4% have 1, 5.8% have 2, and 1.3% have 3 mismatches. All sequences are perfectly matched at the terminal 3' position. PCR reactions contained 0.4 ng · μl^{-1} of DNA template, 200 nM of Cys4991F1b, 200 nM 1045R, 1X FAILSAFE Premix D (EPICENTRE), and 0.05 U · μl^{-1} Taq polymerase (New England Biolabs). Thermocycling conditions were 2 min at 95°C, 30 cycles of (95°C for 30 sec, 60°C for 30 sec, 72°C for 45 sec), followed by 10 min at 72°C.

Primer design and PCR for AGR group *fdhF* alleles.

AGR clade *fdhF* sequences were amplified using a nested PCR approach in which the amplicon from the first PCR reaction, generating with universal *fdhF* primers (TgfdhF-unvF1, EntfdhF-unvF1, and fdhF-unvR1) (1), was used as the template for the second PCR reaction, containing the clade-specific primer set AGR193F and 1045R. Clade specificity was imparted by the forward primer, AGR193F (5'– AGG CTT ACC AAG CCG CCT ATC AGA – 3'), which targets 55.6% of the sequences in the AGR clade with 4 or fewer mismatches, none of them at the terminal 3' end. PCR amplification of all *fdhF* types was achieved using the PCR reaction compositions and thermocycling conditions (51°C annealing temperature) previously specified for inventories. Clade specific PCR reactions contained 1 μl of diluted product from the first reaction (1:1000 in water), 250 nM AGR193F, 250 nM 1045R, 1X FAILSAFE Premix D (EPICENTRE), and 0.07 U · μl^{-1} of EXPAND High Fidelity polymerase (Roche). Thermocycling conditions were 2 min at 95°C, 25 cycles of (95°C for 15 sec, 60°C for 30 sec, 72°C for 1 min), followed by 10 min at 72°C.

1. **Zhang X, Matson EG, Leadbetter JR.** 2011. Genes for selenium dependent and independent formate dehydrogenase in the gut microbial communities of three lower, wood-feeding termites and a wood-feeding roach. Environ. Microbiol. **13:**307-323.