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Engineering Bacteria to Form a Biofilm and Induce Clumping in *Caenorhabditis elegans*

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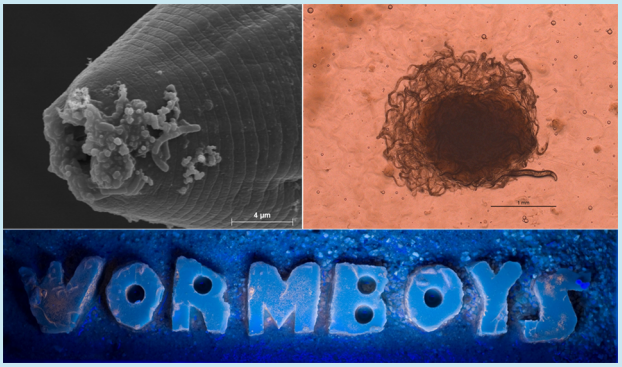
Supporting Information

ABSTRACT: Bacteria are needed for a vast range of biotechnological processes, which they carry out either as pure cultures or in association with other bacteria and/or fungi. The potential of bacteria as biofactories is hampered, though, by their limited mobility in solid or semisolid media such as agricultural or domestic waste. This work represents an attempt toward overcoming this limitation by associating bacterial biotechnological properties with the transport ability of the nematode *Caenorhabditis elegans*. We report here biofilm formation on *C. elegans* by engineered *Escherichia coli* expressing a *Xhenorhabdus nematophila* adhesion operon and induction of nematode social feeding behavior (clumping) through an *E. coli*-mediated iRNA blocking on the expression of FLP-21, a neuropeptide involved in worm solitary behavior.



From food processing to waste treatment, and from biofuels production to drug synthesis, the range of biotechnological processes mediated by bacteria is huge and diverse. Biotechnological processes might rely on the metabolic activity of a single strain/species, but the final product is often the result of the combined activity of microbial consortia. Engineering such consortia has been recently pointed out as one of the last frontiers of synthetic biology.[1] Here, we show a similar but yet parallel approach: the establishment of the first synthetic symbiosis between engineered bacteria and the nematode *Caenorhabditis elegans*, aiming at combining the biotechnological potency of the former with the transport ability of the latter.

In order to do so, we performed two independent genetic modifications in *Escherichia coli*. The ultimate goal was to use the nematode as a transporter for bacteria to reach nutrient-rich hotspots. We transformed *E. coli* XL1-Blue strain with the hmsHFRS operon of *Xhenorhabdus nematophila* cloned in a pUC57 plasmid. This gene network allows *X. nematophila* to form a symbiotic association with the nematode *Steinernema carpocapsae*, which accounts for the entomopathogenic properties of this bacteria-nematode complex against several insect species such as *Manduca sexta*,[2] *Galleria mellonella*,[3] and *Plutella xylostella*.[4] Although the particular function of each gene of the operon in not well understood, it has been demonstrated that the association is based on a lectin-dependent attachment of bacteria to the extracellular matrix of the worms, resulting in the formation of a biofilm on the nematode's head.[2] Engineered as well as control *E. coli* strains were induced with low-nitrogen minimal medium (glucose 10 g/L, K_2HPO_4 7 g/L, KH_2PO_4 2 g/L, $(NH_4)_2SO_4$ 0.6 g/L, $CaCl_2$ 0.02 g/L, bacteriological agar 15 g/L), incubated with a suspension of nematodes for 60 h at 20 °C, and samples prepared for scanning electron microscopy. As shown in Figure 1, engineered bacteria formed discrete and stable biofilm spots on the nematode surface, mainly in the vicinity of the mouth (Figure 1B), while nonengineered bacteria did not form any biofilm (Figure 1A).

The second modification was performed in another strain in order to modify the behavior of the nematode in such a way that worms—and thus the bacteria forming a biofilm on them—would concentrate in nutrient-rich hotspots. *E. coli* was engineered to transcribe an interference RNA matching *C. elegans flp-21* RNA coding for a neuropeptide. The interference of the *flp-21* mRNA alters *C. elegans* N2 strain normal solitary behavior and triggers clumping.[5] When *C. elegans* was fed with this engineered *E. coli* strain, the nematodes shifted from a solitary feeding behavior (Figure 1C) to the formation of clumps made of dozens to hundreds of individuals in less than a

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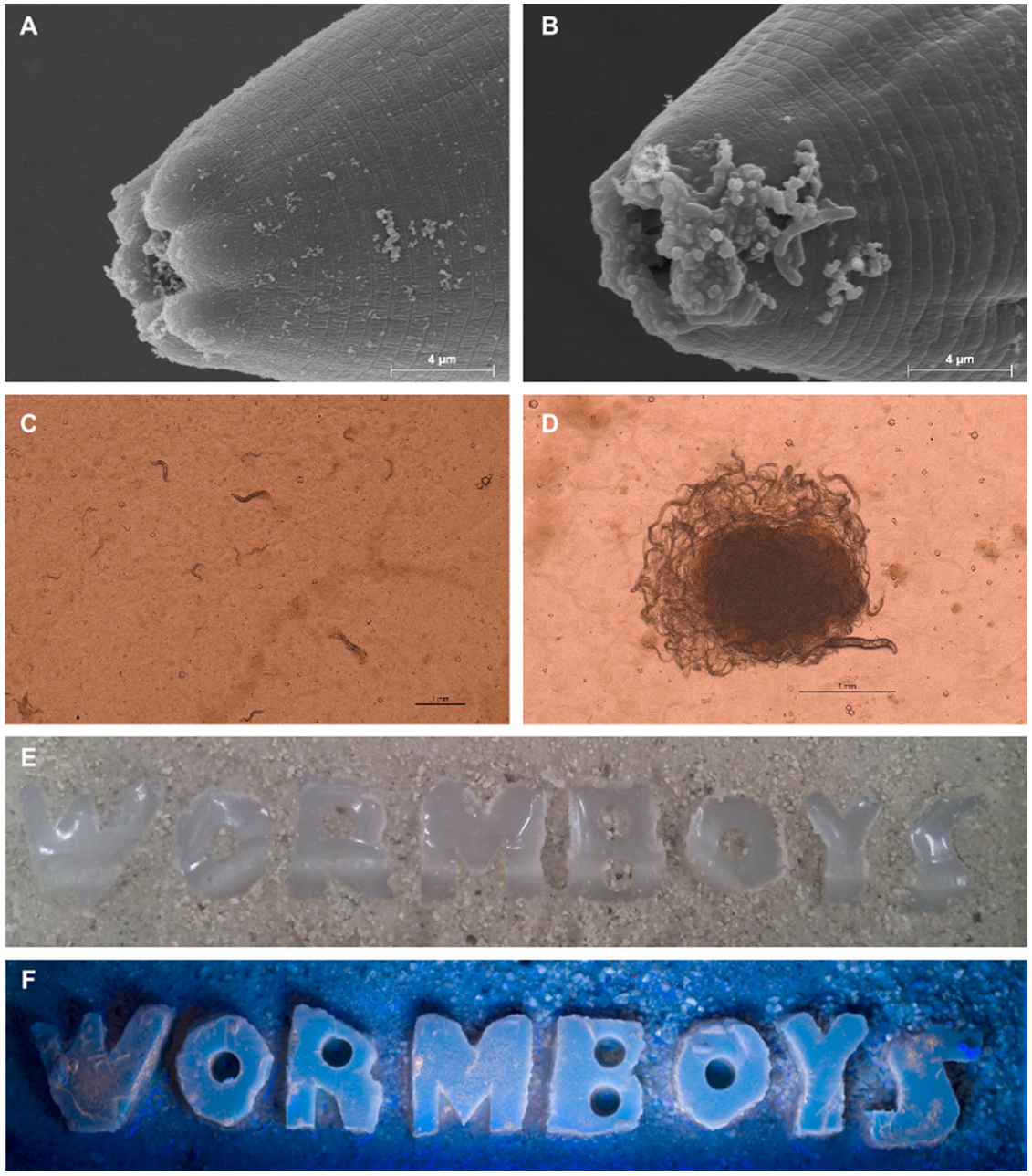

Figure 1. Nematode−bacteria artificial symbioses as described in the main text. Scanning electron microscopy showing the mouth and corpus area of *Caenorhabditis elegans* incubated with control (A) and engineered *Escherichia coli* suspensions expressing the hmsHFRS operon of *Xhenorhabdus nematophila* triggering biofilm formation (B). Optical microscopy photograph of a group of *C. elegans* fed with an untransformed *E. coli* (individual behavior, C) and engineered to transcribe an interference RNA matching *C. elegans flp-21* RNA coding for a neuropeptide (clumping formation, D). (E and F) Soil inoculated with clumping-inducing engineered *E. coli*, *Pseudomonas putida* KT2440 strain (naturally able to synthesize PHA), and a suspension of *C. elegans*. An imbibed agar block ("wormboys") containing the suitable medium for Bioplastic production and Nile Red for the detection of PHA was used (E). After two days of incubation, exposure under UV light reveals reddish Bioplastic nodules only in the agar medium (F).

minute (Figure 1D). Clumping behavior was observed during at least 12 h. Scanning electron microscopy revealed that clumps were in fact aggregates of both nematodes and bacteria, harvested from the surface of the agar plate by the clumping behavior of *C. elegans* (Figure S1 in Supporting Information).

Taken together, our results demonstrate that it is possible to force an artificial symbiosis between biotechnologically active bacteria and nematodes in order to combine their capacities for a bioprocess to occur. The association of bacteria through biofilm formation on fast moving nematodes might allow targeting nutrient-rich hotspots that would attract nematodes, dragging bacteria to specific locations. Once there, an iRNA mechanism would, as we have demonstrated here, allow mixed clumping of bacteria and worms to set in the desired place. As a proof of concept, we combined clumping-inducing engineered *E. coli* transcribing the interference RNA targeting *flp-21*, a *Pseudomonas putida* KT2440 strain naturally able to synthesize PHA (but not engineered to produce a biofilm), and a suspension of *C. elegans*, and used the mixture to inoculate a soil sample with an imbibed agar block containing the suitable

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medium for Bioplastic production and Nile Red for the detection of PHA. After two days of incubation, worms proved able to drag *P. putida* to the agar block where they produced Bioplastic nodules, as revealed after exposure under UV light (Figure 1E and F) and scanning electron microscopy, which revealed isolated *P. putida*-like cells in the vicinity of plastic nodules (Figure S2 in Supporting Information). The mechanism by which non-biofilm-producing *P. putida* were dragged to the agar blocks is unkown.

Further work should be performed in order to improve both biofilm and clumping induction processes. Biotechnologically active strains, such as *Pseudomonas putida* producing Bioplastic, would need to be engineered in order to both form a biofilm and mediate the bioprocess once targeted to the desired location. Theoretically, an "on and off" mechanism would be possible, since the biofilm operon was placed under the control of a nitrogen-sensitive promoter, which is only active under low nitrogen concentration and should thus allow bacteria to "get off" the nematode once a nutrient-rich location was reached.

This work is the first synthetic symbiosis specifically designed to use a nematode as a bacterial shuttle. Beyond its fundamental nature, it might be the first step toward developing a range of artificial associations between microorganisms and eukaryotic species with promising biotechnological applications.

■ ASSOCIATED CONTENT

Supporting Information

Growth and induction of engineered *E. coli*; biofilm formation and clumping induction experiments; SEM imaging. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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