

## Supplementary Material

The Catenulida flatworm can express genes from its microbiome or from the DNA it ingests

**Supplementary movie1** – A comparison between *Stenostomum leucops* SL0-sm02 that consumed bacteria harbouring plasmids with *gfp* gene and worms that have ingested bacteria without the plasmids, under bright-field and fluorescence microscopy.

**Supplementary Table 1 - Summary statistics of plasmids assembly**

Plasmid	Assemblage	Length bp	Reads used	Mismatch	Max. coverage	Average coverage
pBac [3xp3-EGFPafm]	1 contig (circularized)	8,876	1969	4.1	152	57.961
pBASac	1 contig (circularized)	5,906	1368	3.8	147	48.781

The *S. leucops* genome was sequenced using the Illumina MiSeq platform and generated 13,643,983 reads with size among 35-301 bp. After trimming, it was filtered 10,583,723 reads with size among 50-300 bp, these data were used for the plasmids assemblage. Both plasmids (pBac [3xp3-EGFPafm] and pBASac) were assembled complete and circularized.

**Supplementary Table 2 - Summary of microbiome associated with the *Stenostomum* strains used in this study:** *Stenostomum leucops* SL0-sm01 and SL0-sm02; *Stenostomum sp.* Ssp-sm09. The results are expressed in % of reads. In blue are highlighted the bacterial species shared among the three strains; in red between SL0-sm02 and Ssp-sm09; in yellow between SL0-sm01 and Ssp-sm09.

Bacteria species	SL0-sm01 % of reads	SL0-sm02 % of reads	Ssp-sm09 % of reads
<i>Acidovorax delafieldii</i>	0	0.82	0.06
<i>Acidovorax soli</i>	0	0	0.28
<i>Acinetobacter johsonii</i>	0	0	0.38
<i>Acinetobacter lwoffii</i>	0	0	0.03
<i>Acinetobacter ursingii</i>	0	0	0.50
<i>Actinomyces oris</i>	0	0	0.06
<i>Actinomyces viscosus</i>	0	0	0.06
<i>Aeromonas hydrophila</i>	0	0	0.03
<i>Agrobacterium tumefaciens</i>	0	0.49	1.28
<i>Altererythrobacter epoxidivorans</i>	0	2.70	3.54
<i>Arthrobacter ureafaciens</i>	0	0	0.03
<i>Aureimonas jatrophae</i>	0	0	0.03
<i>Bacillus cereus sp. group</i>	85.02	1.23	0.22
<i>Bacillus oshimensis</i>	0	0	0.06
<i>Bacillus subtilis</i>	0.06	0	0
<i>Blastomonas natatoria</i>	0	1.96	0
<i>Bosea massiliensis</i>	0	2.13	0
<i>Bosea thiooxidans</i>	0	0	0.09
<i>Brachybacterium faecium</i>	0	0	0.06
<i>Brachybacterium sacelli</i>	0	0	0.13
<i>Bradyrhizobium elkanii</i>	0	0	0.22
<i>Bradyrhizobium genosp. SA-1</i>	0	0	0.19
<i>Brassicogethes aeneus</i>	0.05	0	0
<i>Brevibacterium aureum</i>	0	0	0.16
<i>Brevibacterium epidermidis</i>	0	0	0.03
<i>Brevibacterium iodinum</i>	0	0	0.03
<i>Brevibacterium pityocampae</i>	0	0	0.03

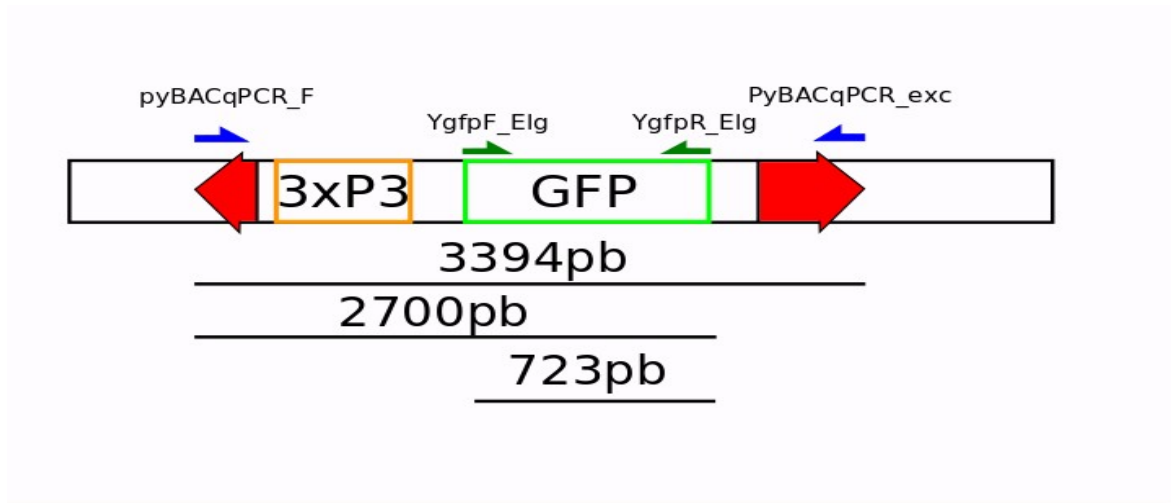
<i>Brevibacterium senegalense</i>	0	0	0,03
<i>Brevundimonas alba</i>	0	9.82	0
<i>Brevundimonas subvibrioides</i>	0	0	0,13
<i>Brevundimonas vesicularis</i>	0	0	0,03
<i>Candidatus Prevotella conceptionensis</i>	0	0	0,03
<i>Caulobacter sp.</i>	0	0	1,44
<i>Caulobacter vibrioides</i>	0	0	0,03
<i>Chryseobacterium viscerum</i>	0	0	0,03
<i>Corynebacterium accolens</i>	0	0	0,78
<i>Corynebacterium afermentans</i>	0	0	0,06
<i>Corynebacterium coyleae</i>	0	0	0,06
<i>Corynebacterium durum</i>	0	0	0,03
<i>Corynebacterium fastidiosum</i>	0	0	0,03
<i>Corynebacterium imitans</i>	0	0	0,03
<i>Corynebacterium jeikeium</i>	0	0	0,03
<i>Corynebacterium mucifaciens</i>	0	0	0,06
<i>Corynebacterium singulare</i>	0	0	0,38
<i>Corynebacterium thomssenii</i>	0	0	0,13
<i>Corynebacterium tuberculostearicum</i>	0	0	0,41
<i>Cryobacterium arcticum</i>	0	0	0,03
<i>Cryobacterium psychrotolerans</i>	0	0	1,69
<i>Cupriavidus metallidurans</i>	0	0	0,09
<i>Curvibacter delicatus</i>	0	0	0,16
<i>Dermabacter hominis</i>	0	0	0,06
<i>Devosia insulae</i>	0	0	2,82
<i>Dietzia psychrhalcaliphila</i>	0	0	0,06
<i>Dietzia timorensis</i>	0	0	0,22
<i>Dolosigranulum pigrum</i>	0	0	0,03
<i>Dyadobacter fermentans</i>	0	0	0,03
<i>Dyadobacter soli</i>	0	0	2,74
<i>Empedobacter falsenii</i>	0	0	0,03
<i>Ensifer adhaerens</i>	0	19.48	0
<i>Enterobacter falsenii</i>	0	0	0,03
<i>Enterobacter aerogenes</i>	0	0	0,03
<i>Escherichia coli</i>	14.73	0	0,03
<i>Finegoldia magna</i>	0	0	0,03
<i>Gemmobacter aquaticus</i>	0	0.49	0
<i>Gemmobacter lanyensis</i>	0	0	0,03
<i>Globicatella sanguinis</i>	0	0	0,03
<i>Haemophilus parainfluenzae</i>	0	0	0,06
<i>Hyphomicrobium vulgare</i>	0	12.93	0,28
<i>Hyphomicrobium zavarzinii</i>	0	3.85	0
<i>Janibacter limosus</i>	0	0	0,19
<i>Kingella oralis</i>	0	0	0,03
<i>Kocuria kristinae</i>	0	0	0,03
<i>Kocurria palustris</i>	0	0	0,03
<i>Lactobacillus acidophilus</i>	0	0	0,03
<i>Lactococcus lactis</i>	0	0	0,06
<i>Lautropia mirabilis</i>	0	0	0,03
<i>Leifsonia xyli</i>	0	0	0,03
<i>Massilia haematophila</i>	0	0	0,03

<i>Massilia suwonensis</i>	0	0.41	0
<i>Mesorhizobium amorphae</i>	0	0	0,13
<i>Mesorhizobium loti</i>	0	0	0,06
<i>Methylobacterium hispanicum</i>	0	0	0,03
<i>Methylobacterium komagatae</i>	0	0	0,5
<i>Methylobacterium radiotolerans</i>	0	0	0,34
<i>Methyloversatilis universalis</i>	0	0	0,03
<i>Moraxella sp.</i>	0	0	0,03
<i>Mycobacterium mucogenicum</i>	0	0.49	0
<i>Nevskia ramosa</i>	0	9.74	40,68
<i>Nitrospira sp.</i>	0	1.39	0
<i>Nocardioides fonticola</i>	0	0.82	0
<i>Novosphingobium aromaticivorans</i>	0	0	0,03
<i>Novosphingobium nitrogenifigens</i>	0	0	0,03
<i>Novosphingobium panipatense</i>	0	3.27	0
<i>Novosphingobium soli</i>	0	0	0,03
<i>Novosphingobium subterraneum</i>	0	0	0,13
<i>Ochrobactrum anthropi</i>	0	0	0,06
<i>Ochrobactrum pseudogrignonense</i>	0	0	0,03
<i>Panacagrimonas perspica</i>	0	7.77	0
<i>Pantoea ananatis</i>	0	0	0,03
<i>Pedobacter agri</i>	0	0	0,03
<i>Pedomicrobium americanum</i>	0	0	0,06
<i>Pelomonas puraquae</i>	0	0	0,28
<i>Pelomonas saccharophila</i>	0	0.41	0,22
<i>Peptoniphilus gorbachii</i>	0	0	0,03
<i>Phenylobacterium murchangponense</i>	0	0	0,06
<i>Polynucleobacter acidiphobus</i>	0	0	4,58
<i>Porphyrobacter donghaensis</i>	0	0	0,41
<i>Porphyrobacter neustonensis</i>	0	0	0,5
<i>Pseudarciella hirudinis</i>	0	0	0,09
<i>Pseudomonas alcaligenes</i>	0	0.49	0
<i>Pseudomonas fluorescens</i>	0	0.65	0,06
<i>Pseudomonas pseudoalcaligenes</i>	0	0	0,56
<i>Pseudomonas putida</i>	0	4.17	0,38
<i>Pseudonocardia alni</i>	0	0.41	0
<i>Ralstonia pickettii</i>	0	0	0,5
<i>Rhizobium nepotum</i>	0	0	0,06
<i>Rothia aerea</i>	0	0	0,06
<i>Rothia amarae</i>	0	0	0,13
<i>Rubrobacter xylanophilus</i>	0	0	0,03
<i>Sandarakinorhabdus limnophila</i>	0	0	0,06
<i>Sediminibacterium salmoneum</i>	0	3.03	18,27
<i>Shinella yambaruensis</i>	0	1.15	0
<i>Smaragdicoccus niigatensis</i>	0	2.95	0
<i>Serratia marcenscens</i>	0	0	0,28
<i>Sphingobium yanoikuyae</i>	0	0.74	0,06
<i>Sphingomonas canadensis</i>	0	0	0,13
<i>Sphingomonas changbaiensis</i>	0	0.65	0
<i>Sphingomonas cynarae</i>	0	0	0,03
<i>Sphingomonas echinoides</i>	0	0	0,38
<i>Sphingomonas leidyi</i>	0	0	1,19

<i>Sphingomonas melonis</i>	0	0	0,69
<i>Sphingomonas sanxanigenens</i>	0	0	0,03
<i>Sphingomonas sp.</i>	0.06	0	0
<i>Staphylococcus aureus</i>	0	0	0,03
<i>Staphylococcus capitis</i>	0	0	0,13
<i>Staphylococcus cohnii</i>	0	0	0,06
<i>Staphylococcus epidermidis</i>	0	0.57	0,56
<i>Staphylococcus haemolyticus</i>	0	0	0,03
<i>Staphylococcus hominis</i>	0	0	0,06
<i>Staphylococcus pasteurii</i>	0	0	0,16
<i>Staphylococcus pseudintermedius</i>	0	0	0,06
<i>Staphylococcus saprophyticus</i>	0	0	1,35
<i>Staphylococcus warneri</i>	0	0	0,47
<i>Stella vacuolata</i>	0	0.74	0
<i>Stenotrophomonas maltophilia</i>	0	0	0,06
<i>Stenotrophomonas rhizophila</i>	0	0	0,06
<i>Streptococcus australis</i>	0	0	0,03
<i>Streptococcus macedonicus</i>	0	0	0,03
<i>Streptococcus oralis</i>	0	0.49	0,13
<i>Streptococcus salivarius</i>	0	0	0,09
<i>Streptococcus sanguinis</i>	0	0	0,09
<i>Uncultured Oscillatoria sp.</i>	0	0	0,03
<i>Variovorax paradoxus</i>	0	0.41	0
<i>Veillonella parvula</i>	0	0	0,03
<i>Woodsholea maritima</i>	0	1.47	0,03
<i>Zavarzinia compransoris</i>	0	1.88	0

**Supplementary Table 3 – Primers used, PCR parameters**

Amplification	Primers	Parameters	Amplicon size
plasmid pBac [3xp3-EGFPafm] / <i>gfp</i> gene and flanking sequences  (see Supplementary Figure 1)	pyBACqPCR_F: CAATCTCACAGACAG CGTTGACAATCTCAC  pyBACqPCR_exc CGACTTGGTTTGCCA TTCTT  ygfR_Elg TTACTTGTACAGCTCT CGTCCATGC  ygfF_Elg ATGGTGAGCAAGGGC GAG	95°C-5 min [95°C-1min 58°C-30sec 72°C-2min: 30 sec]X35 72°C-7 min 4°C-∞	3394 bp 2700bp 723bp  (see Supplementary Figure 1)
Bacterial 16S V3-V4 ribosomal gene (rRNA)	341F CCTACGGGRSG CAGCAG  806R GGACTACHVGGGTW TCTAAT	95°C for 5 min [95°C for 45s, 55°C for 30s and 72°C for 45s] x35 72°C for 2 min	305bp

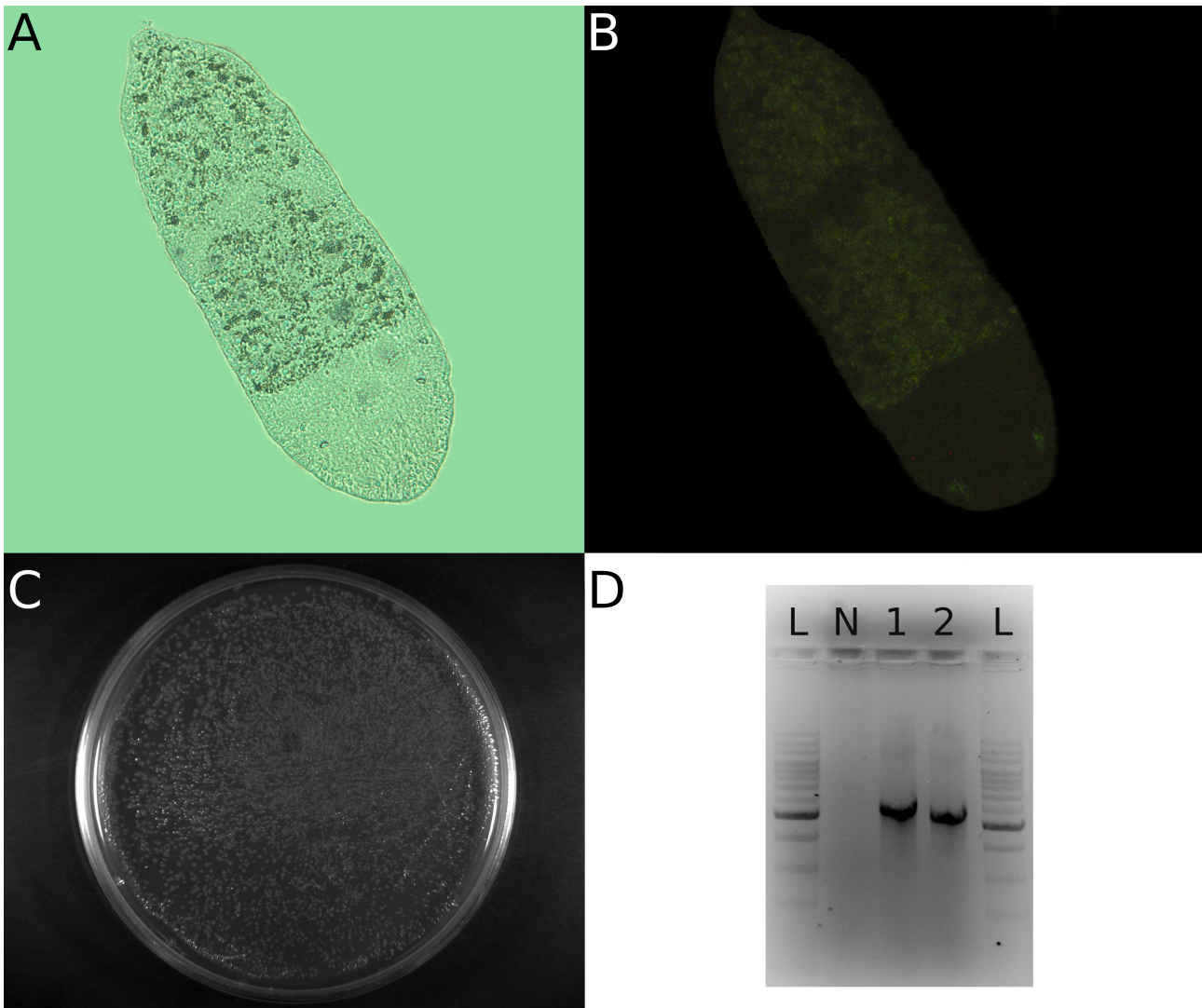


**Supplementary Figure 1: Schematic representation of primers used and amplicons produced in PCR assays, testing the presence of pBac [3xp3-EGFPafm] plasmid, in worms fed with bacteria harbouring this plasmid.**

**Supplementary Table 4: Results of PCR assays, using different set of primers, for SL0-sm01 and SL0-sm02 worms fed with *E. coli* harbouring pBac [3xp3-EGFPafm] plasmid, 3 and 15 days after of the bacterial ingestion**

PCR primers/amplicon size	SL0-sm01		SL0-sm02	
	3 days after	15 days after	3 days after	15 days after
pyBACqPCR_F - PyBACqPCR_exc 3394 pb	+	+	+	-
pyBACqPCR_F - ygfpR_Elg 2700 pb	+	+	+	-
YgfpF_Elg - ygfpR_Elg 723 pb	+	+	+	-

**Supplementary Figure 2- Long term maintenance of wild *E. coli* bacteria in *Stenostomum leucops* SL0-sm02 strain**



**Supplementary Figure 2- maintenance of native *E. coli* bacteria in the *S. leucops*.**

*Stenostomum leucops* SL0-sm02 strain three months after receive wild *E. coli* bacteria harbouring the pBac [3xp3-EGFPafm] plasmid. These worms were fed only once with this bacterium, which is thereafter kept in the worm microbiome. A) Bright-field microscopy of SL0-sm02 worm; B) under fluorescence microscopy; C) worms were mechanically disintegrated and plated in a LB medium with ampicillin antibiotic. As can be seen, *E. coli* bacteria were maintained (confirmed by Sanger sequencing of rRNA 16 gene; five bacterial colonies had de DNA sequenced); D) PCR assay showing amplification of a region of pBac [3xp3-EGFPafm] plasmid (L = Kb ladder; N = control-none DNA template; 1= amplicom -2.7kb- generated using DNA extracted from a colony of bacteria obtained three months after feeding the worms with bacteria harbouring the plasmid; 2 = positive control, DNA of pBac [3xp3-EGFPafm] plasmid used as template). Fifteen bacterial colonies were tested by PCR and showed amplicons similar to the one showed in lane 1.