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Supplementary Information for

Bacterial lipopolysaccharide induces settlement and metamorphosis in a marine larva.

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Supplementary Information Text

Small molecule analysis of cell pellets from *C. lytica:*

Cell pellets of *C. lytica* were extracted with MeOH twice followed by repeated extraction with MeOH: DCM (1:1). The MeOH: DCM extract was subjected to semi-preparative reverse chromatography HPLC (C18, ACN: 10-25%, 20 mins; 25-40%, 10 mins; 40-100%, 20 mins; 100% 10 mins). Fractions were collected every minute, dried and resuspended at 20 mg/ml and tested for metamorphic activity at a concentration of (10 µg/ml). Activity was detected in fraction 7 and fraction 29; fraction CLP-29 did not contain enough material or any indicative absorbance to pursue further.

Semi-preparative reverse chromatography of CLP7 (C18; MeOH 10%, 30 mins) yielded activity in the 2^{nd} and 4^{th} fractions (Fig S2). LC-Mass spectrometry and ^{1}H NMR spectroscopy were consistent with the presence of phenylalanine and adenosine (Fig S3). To confirm the activity and further elucidate the stereochemistry, both isolated and commercial versions of adenosine (Sigma-Aldritch) and phenylalanine (D and L; Sigma-Aldritch) were tested for metamorphic induction (Fig S4 and S5). Additional commercially available compounds (thymidine, leucine (D and L) and niacin) were also tested (Fig S5).

Small molecule analysis of cell-free supernatant from *C. lytica* containing OMVs*:*

Conditioned media of *C. lytica* cultures (3.7 L) were extracted with ethyl acetate (EtOAc) and dried (363 mg). The EtOAc extract was subjected to semi-preparative reverse chromatography HPLC (C18, ACN: 10-25%, 20 mins; 25-40%, 10 mins; 40-100%, 20 mins; 100% 10 mins). Fractions were collected every minute, dried and resuspended at 20 mg/ml ethanol and tested for metamorphic activity at a concentration of (10 µg/ml). Activity was identified in the 25^{th} and 26^{th} fractions which were combined. LC-MS revealed a compound consistent with the presence of a diketopiperazine of phenylalanine and proline. Isolated and commercial versions of this compound were subjected to metamorphosis assay as previously described (Fig S6).

Genome Mining for Secondary Metabolites

Analysis of the genome of *C. lytica* for the presence of secondary metabolite biosynthesis gene clusters revealed a single contig *(ctg1_392)* located between 455,027 - 455,863. A protein blast of this contig, identified it as a phytoene synthase (Table S1). The Minimum Information about a Biosynthetic Gene cluster (MIBiG) failed to match any enzymes with greater than 50% sequence identity (Table S2). Similar low matches were generated using NaPDos (Table S3).

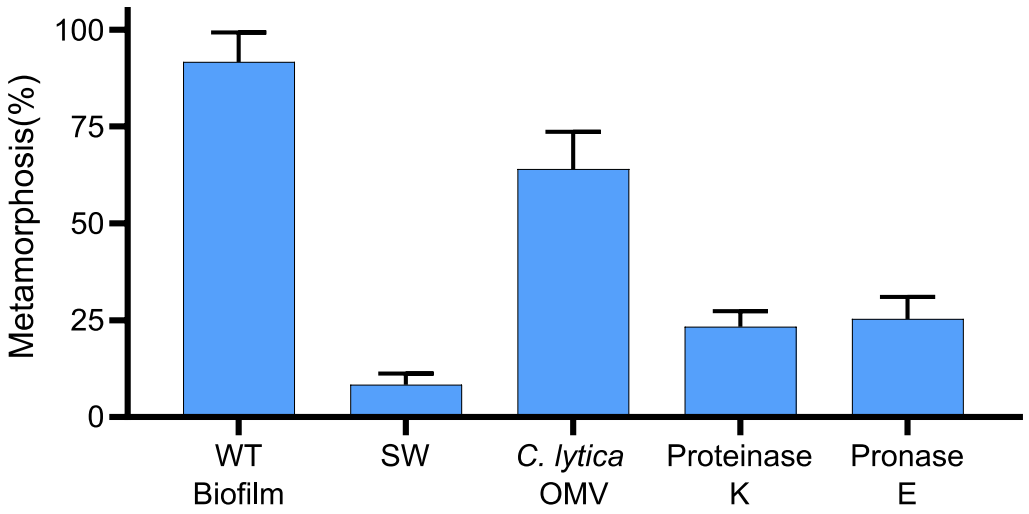

Fig. S1. Proteases induce metamorphosis in larvae of *C. lytica*. Larvae of *H. elegans* were exposed to either Proteinase K (5 U) or Pronase E (5 U). Metamorphosis was counted after 24 h exposure. Filtered seawater (SW) served as a negative control, and a multispecies biofilm and untreated OMVs were used as positive controls.

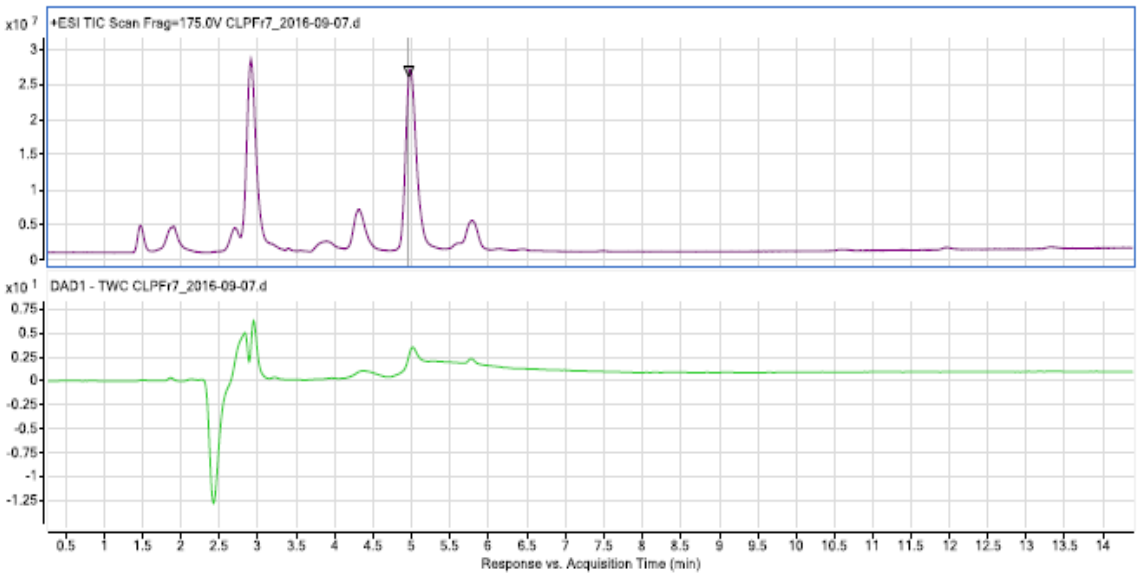

Fig. S2. Positive mode LCMS data from most active cell pellet fraction (CLP7) yielded m/z of 123.0459 and 268.1043. Ions were detected within a mass range m/z 100-1100.

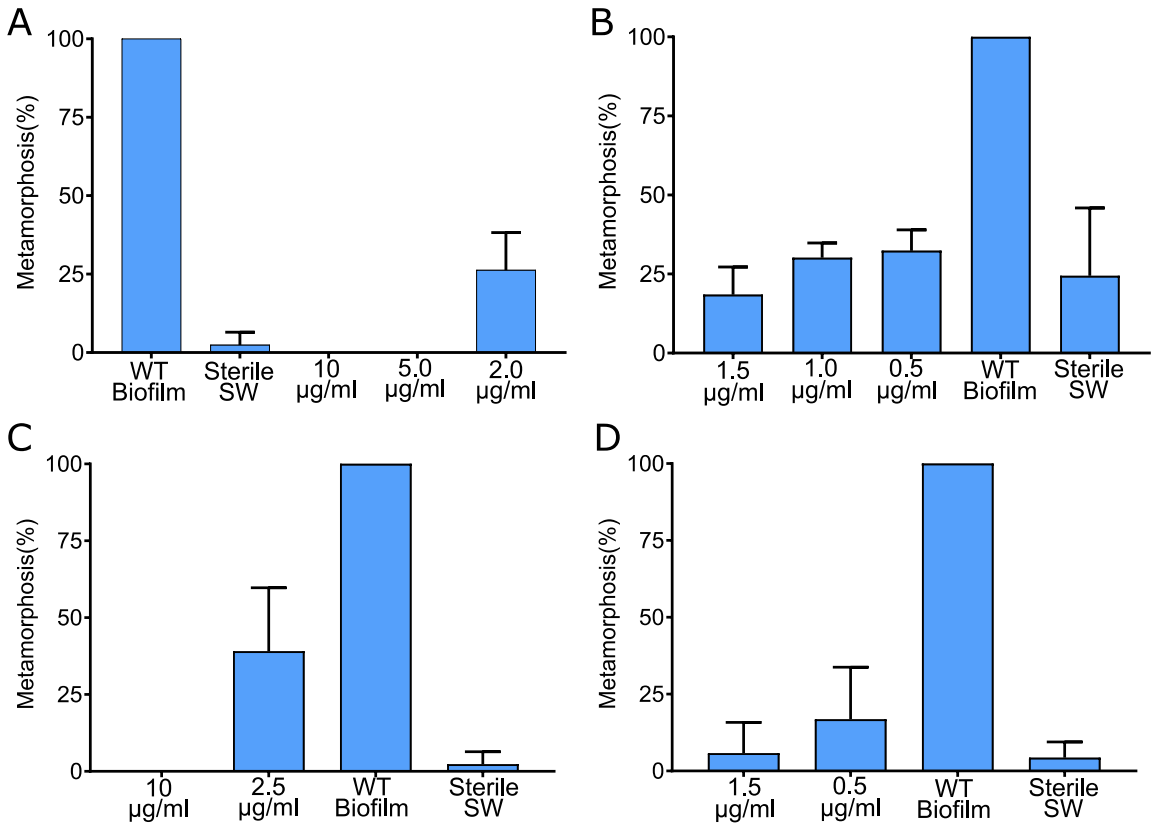

Fig. S3. Metamorphosis of larvae of *H. elegans* when exposed to isolated A and B) adenosine and C and D) phenylalanine from Fraction 7 of the cell pellet for 24 hrs. Positive Control: Wild Type (WT) Biofilm. Negative Control: Sterile Seawater (SW).

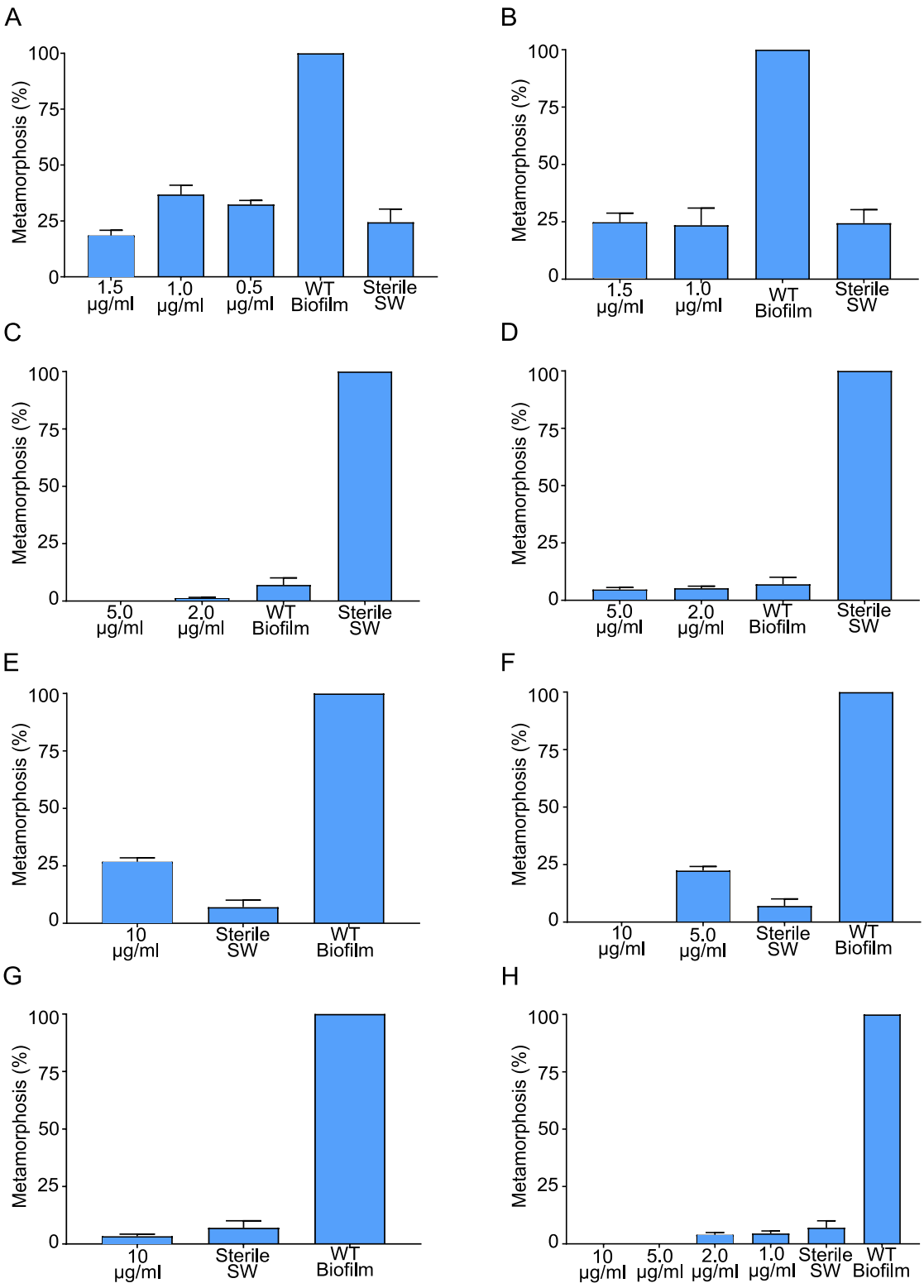

Fig S4. Metamorphosis of larvae of *H. elegans* when exposed to commercial versions of compounds detected in fraction 7 for 24 hrs. A) Adenosine; B) DL-Phenylalanine; C) L-Phenylalanine; D) D-Phenylalanine; E) Niacin; F) Thymidine; G) L=Leucine; H) D-Leucine. Positive Control: Wild Type (WT) Biofilm. Negative Control: Sterile Seawater (SW).

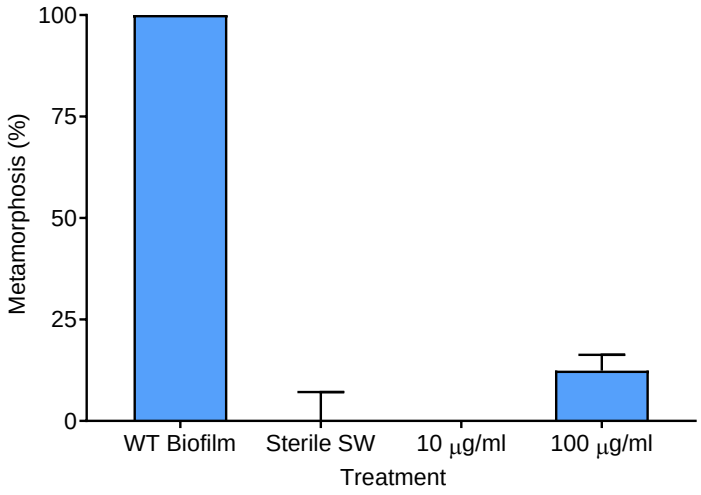

Fig S5. Metamorphosis of larvae of *H. elegans* when exposed to commercial version of the diketopiperizine of phenylalanine and proline detected in fraction 25 of the supernatant from a *C. lytica* culture for 24 hrs. Positive Control: Wild Type (WT) Biofilm. Negative Control: Sterile Seawater (SW).

Table S1. NCBI BlastP on ctg1_392 from genome of *C. lytica* generated by AntiSMASH (1)

Accession	Description	Max Score	Total Score	Query Cover	E value	% Ident
WP_013620042.1	phytoene synthase [*Cellulophaga lytica*]	563	563	100%	0.0	100.00%

Table S2. Minimum Information about a Biosynthetic Gene cluster (MIBiG) search results from genome of *C. lytica* generated by AntiSMASH (1)

MIBiG Protein	Description	MIBiG Cluster	MiBiG Product	% ID	% Coverage	BLAST Score	E-value
ABB88949.1	CrtB	BGC0000650	terpene	50.0	99.6	255.0	1.2e-67
AAK64298.1	Phytoene synthase	BGC0000637	terpene	37.0	100.4	168.0	1.5e-41
ABD24399.1	Phytoene synthase	BGC0000644	terpene	33.0	97.1	155.0	9.9e-38
AAF65581.1	Phytoene synthase	BGC0000636	terpene	34.0	102.5	134.0	2.4e-31
ABP56869.1	Phytoene synthase	BGC0001087	saccharide-terpene	25.0	89.9	80.0	5.3e-15

Table S3: Polyketide Synthase (KS) domains within the genome of *C. lytica* generated by NaPDos (2). Search was run with relaxed parameters.

	Query ID	Database match ID	% ident.	Align length	e-val	Pathway product
KS2	CP009239.1_3_157_15_425	FabF_Bacillus_FAS	55	409	$5e^{-112}$	fatty acid synthesis
KS1	CP009239.1_4_290_11_391	FabF_Bacillus_FAS	26	414	$8e^{-24}$	fatty acid synthesis
KS3	CP009239.1_6_112_8_221	Myca_YP880565_1KSB	30	131	$2e^{-06}$	mycocerosic acid synthase

SI References

1. M. H. Medema, *et al.*, AntiSMASH: Rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* **39**, W339–W346 (2011).
2. N. Ziemert, *et al.*, The natural product domain seeker NaPDoS: A phylogeny based bioinformatic tool to classify secondary metabolite gene diversity. *PLoS One* **7**, e34064 (2012).