Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Information

The transcriptome of *Euglena gracilis* reveals unexpected metabolic capabilities for carbohydrate and natural product biochemistry

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Figure S1: Aromatic amino acid biosynthesis

The shikimate pathway is present in *Euglena* as the pentafunctional fungal type.[1] There are four isoforms of the DHAP synthase, one of which is only apparent in the dark transcriptome. The pathway branches at chorismate: towards folate biosynthesis, via 4-aminobenzoate formed by a bifunctional enzyme; towards tryptophan biosynthesis, via a tetrafunctional enzyme from 2-aminobenzoate; or to prephenate via chorismate mutase, which is present either as a single domain protein or as part of a trifunctional protein, with dehydratase and dehydrogenase domains. Aromatic amino acid transaminases, for the final step in tyrosine and phenylalanine synthesis, are present in the transcriptome.

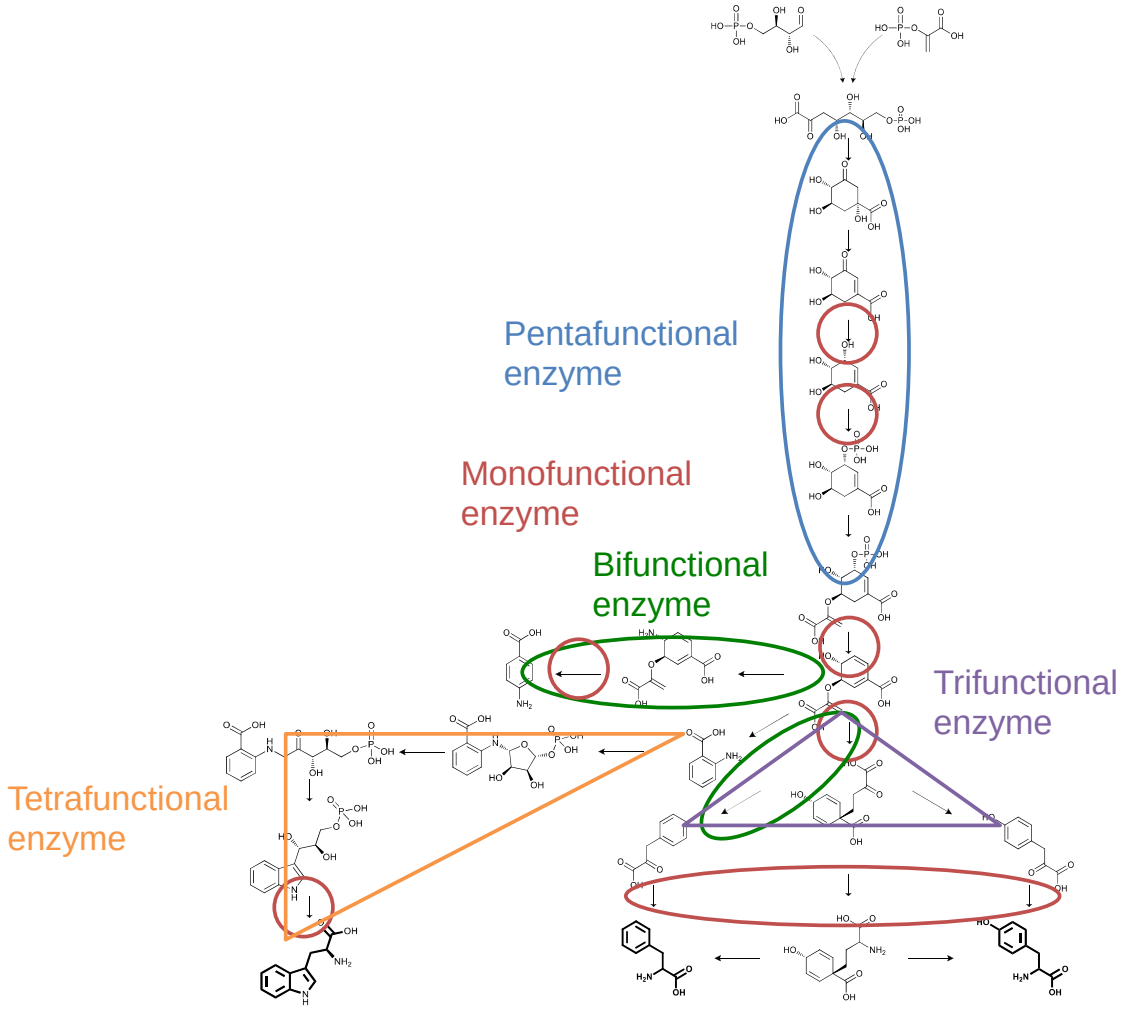


Table S1: The enzymes of N-glycan biosynthesis

Transcripts were identified which encode enzymes involved in N-glycan biosynthesis by homology with known enzymes.[2] Highlighted in yellow are transcripts only present in the dark grown cells and highlighted in green are present only in the light grown cells. FPKM values for each cognate transcript are given in parentheses.

Activity	EC no.	ORFs			
dolichyldiphosphatase	3.6.1.43	lm.53772 (10.03)	dm.71782 (2.44)		
dolichol kinase	2.7.1.108	lm.96623 (1.20)			
UDP-GlcNAc-dolichol phosphate GlcNAc-1-P-transferase	2.7.8.15	lm.96341 (2.12)			
beta-1,4-N-acetylglucosaminyltransferase	2.4.1.141	lm.87840 (1.67)			
beta-1,4-mannosyltransferase	2.4.1.142	lm.92144 (1.35)			
alpha-1,3/alpha-1,6-mannosyltransferase	2.4.1.132	lm.79157 (7.10)			
alpha-1,2-mannosyltransferase	2.4.1.131	lm.67740 (8.51)			
Flippase		lm.83408 (4.92)			
alpha-1,3-mannosyltransferase	2.4.1.258	lm.68532 (7.44)			
alpha-1,2-mannosyltransferase	2.4.1.259/ 261	dm.60522 (1.56)	lm.71029 (6.67)	dm.85690 (0.80)	dm.60521 (2.61)
alpha-1,6-mannosyltransferase	2.4.1.260	lm.71029 (6.67)			
dolichol-phosphate mannosyltransferase	2.4.1.83	lm.31276 (16,28)	lm.48352 (14.30)		
dolichyl-phosphate beta-glucosyltransferase	2.4.1.117	lm.48352 (14.30)			
alpha-1,3-glucosyltransferase	2.4.1.267	lm.99883 (0.54)	dm.96080 (1.2)		
alpha-1,3-glucosyltransferase	2.4.1.265	lm.99883 (0.54)	dm.96080 (1.2)		
alpha-1,2-glucosyltransferase	2.4.1.256	lm.100691 (0.72)			
dolichyl-diphosphooligosaccharide--protein glycosyltransferase	2.4.99.18	dm.41728 (5.62)	lm.68366 (11.63)	dm.17756 (44.10)	

Table S2: Annotation of enzymes involved in the biosynthesis of GPI anchors

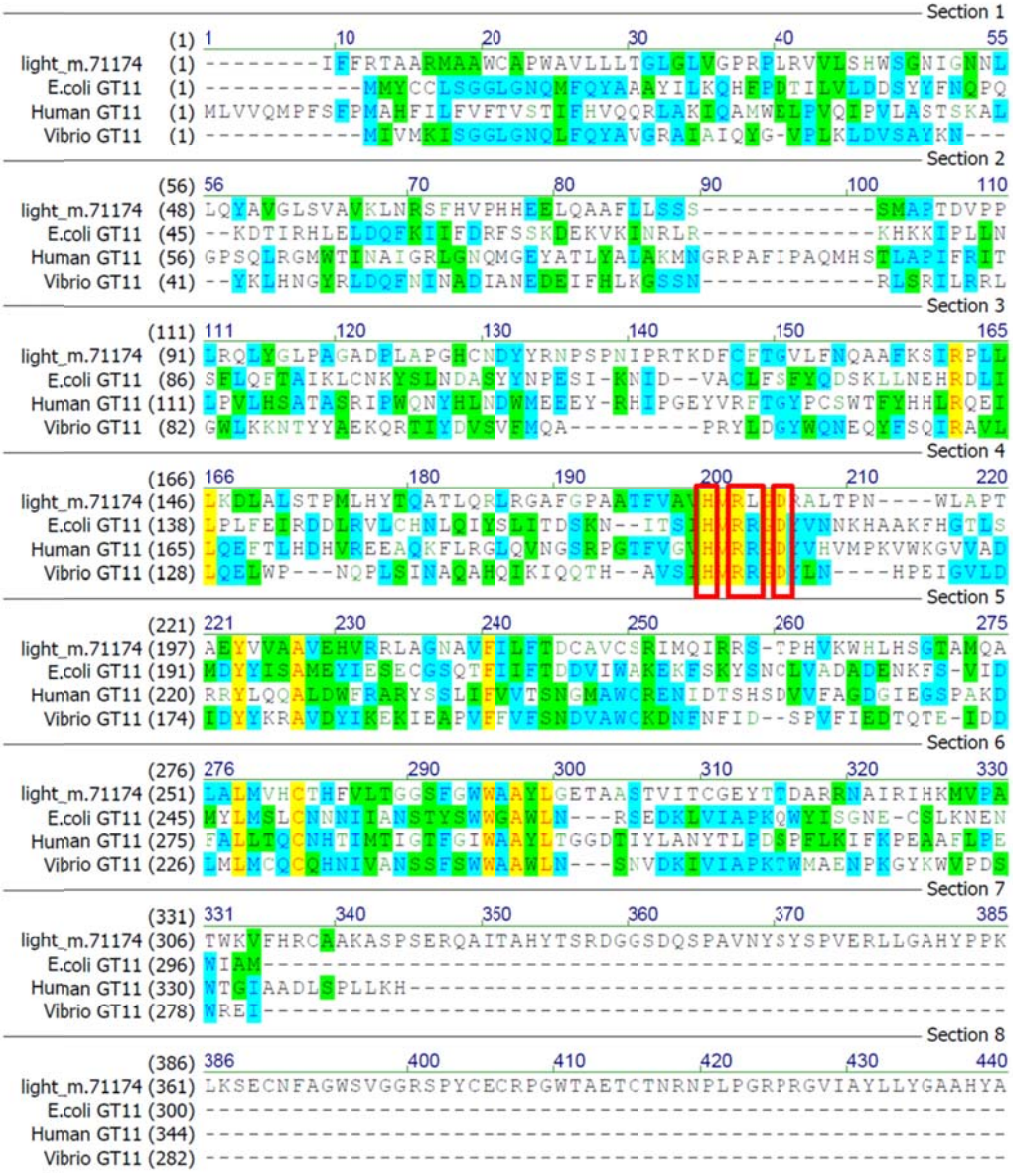
Transcripts were identified which encode enzymes involved in GPI anchor biosynthesis by homology with known enzymes.[3] Highlighted in yellow are transcripts only present in the dark grown cells and highlighted in green are present only in the light grown cells. FPKM values for each cognate transcript are given in parentheses.

EC number	Reaction	Mammalian protein	Euglena model number	Closest homologue	E-value
5.5.1.4	Inositol-3-phosphate synthase 1	INO1	lm.32631 (22.56)	Predicted inositol-3-phosphate synthase 1-like [Saccoglossus kowalevskii]	0.00E+00
3.1.3.25	Myo-inositol-1-phosphatase	IMPA1	lm.49313 (5.60)	Hypothetical protein TRIADDRAFT_52515 [Trichoplax adhaerens]	1.00E-60
2.7.8.11	CDP-diacylglycerol-inositol 3-phosphatidyl transferase	PIS1	lm.37320 (12.27)	Phosphatidylinositol synthase [Spathaspora passalidarum NRRL Y-27907]	3.00E-50
			lm.79366 (4.02)	Predicted protein [Phaeodactylum tricornutum CCAP 1055/1]	1.00E-47
2.4.1.198	GPI-GlcNAc transferase	PIG-A	dm.79942 (4.57)	UDP-GlcNAc:PI a1-6 GlcNAc-transferase [Trypanosoma cruzi marinkellei]	0.00E+00
		PIG-C	None		
		PIG-H	dm.82217 (4.59)	Predicted glycosyltransferase [Ectocarpus siliculosus]	1.00E-16
		PIG-P	dm.82867 (3.40)	Predicted protein [Populus trichocarpa]	4.00E-24
		PIG-Q	dm.48080 (1.57)	Hypothetical protein BATDEDRAFT_85480 [Batrachochytrium dendrobatidis JAM81]	5.00E-36
		PIG-Y	None		
		DPM2	lm.108666 (0.26)	Unknown [Picea sitchensis]	6.00E-09
3.5.1.89	GlcNAc-PI de-*N*-acetylase	PIG-L	dm.85635 (2.13)	N-Acetyl-D-acetylglucosaminylphosphatidyl inositoldeacetylase [Leishmania major strain Friedlin]	2.00E-52
			dm.27659* (7.68)	Hypothetical protein [Paramecium tetraurelia strain d4-2] - *Also contains N-ter ManT domain	3.00E-51
2.3.-.-	Inositol acyltransferase	PIG-W	lm.93529 (2.32)	Predicted protein At4g17910-like [Brachypodium distachyon]	2.00E-64
2.4.1.-	*α*-(1-4)-Mannosyltransferase	PIG-M	lm.94033 (1.57)	GPI mannosyltransferase 1 [Dicentrarchus labrax]	2.00E-100
		PIG-X	None		
2.7.-.-	EtNP transferase	PIG-N	lm.94615 (2.25)	Predicted GPI ethanolamine phosphate transferase 1-like [Brachypodium distachyon]	3.00E-177
2.4.1.-	*α*-(1-6)-Mannosyltransferase II	PIG-V	lm.98056 (1.15)	Dolichol-P-mannose mannosyltransferase [Selaginella moellendorffii]	4.00E-65
2.4.1.-	*α*-(1-2)-Mannosyltransferase III	PIG-B	dm.85690 (0.79)	Predicted protein [Physcomitrella patens subsp. patens]	3.00E-86
	GPI transamidase	PIG-K	dm.51731 (10.94)	Predicted protein [Physcomitrella patens subsp. patens]	3.00E-122
		GAA1	lm.88011 (2.90)	Predicted protein [Naegleria gruberi]	4.00E-25
		PIG-S	dm.90214 (1.83)	Unnamed protein product [Vitis vinifera]	8.00E-21
		PIG-T	dm.67348 (3.66)	Hypothetical protein BATDEDRAFT_35820 [Batrachochytrium dendrobatidis JAM81]	8.00E-52
		PIG-U	lm.71943 (4.97)	Gpi transamidase component pig-u [Colletotrichum gloeosporioides Nara gc5]	3.00E-31
	Glycosylphosphatidylinositol deacylase	PGAP1	lm.73955 (5.40)	GPI inositol-deacylase [Metarhizium anisopliae ARSEF 23]	2.00E-36

Figure S2. Alignments of didomain CAZys (lm.71174 and dm.47703) with well characterised single domain proteins.

Alignments were performed using AlignX (Invitrogen) and confirmed by modelling proteins using SWISS-MODEL in Automated mode.[4] The key catalytic residues are highlighted in red boxes. **A**. Alignment of lm.71174 with GT11s[5] (1) and GT15s[6] (2). **B**. Alignment of dm.47703 with GT1s[7] (1) and GH78s[8] (2). FPKM values are 0.90 and 3.61 respectively.

A1



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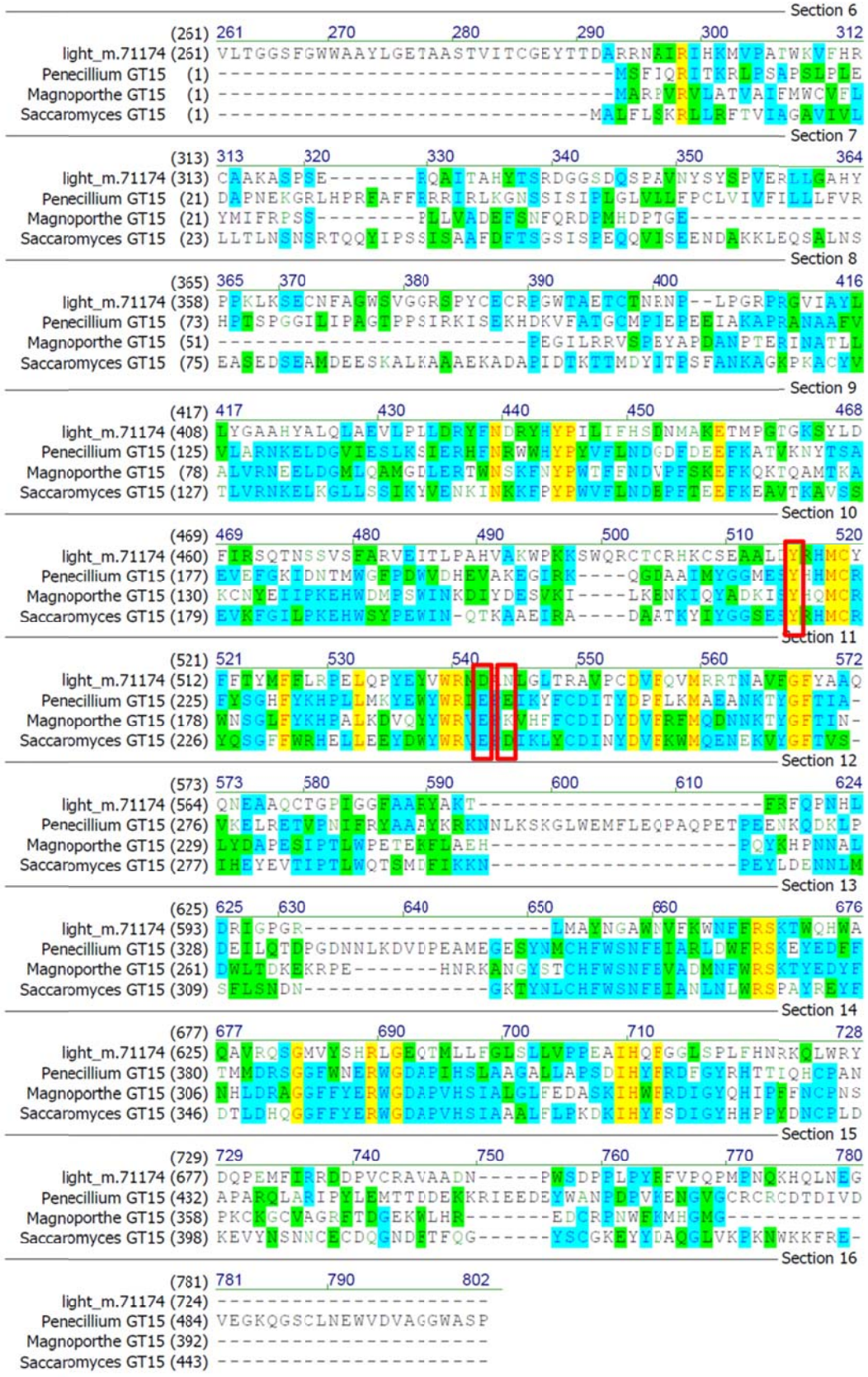
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Magnoporthe GT15 (1) ----------MARPVRVLATVAIFMWCVFL
Saccaromyces GT15 (1) ----------MALFLSKRLLRFTVIAGAVIVL
Section 7
(313) 313 320 330 340 350 364
light_m.71174 (313) CAAKASPSE-------EQAITAHYTSRDGGSDQSPAVNYSYSPVERLLGAHY
Penecillium GT15 (21) DAPNEKGRLHPRFAFFKRRIRLKGNSSISIPLGLVLLFPCLVIVFILLLFVR
Magnoporthe GT15 (21) YMIFRPSS--------PLLVADEFSNFQRDPMHDPTGE----------
Saccaromyces GT15 (23) LLTLNSNSRTQQYIPSSISAAFDFTSGSISPEQQVISEENDAKKLEQSALNS
Section 8
(355) 365 370 380 390 400 416
light_m.71174 (358) PPKLKSECNFAGWSVGGRSPYCECRPGWTAETCTNRNP--LPGRPRGVIAYL
Penecillium GT15 (73) HPTSPGGILIPAGTPPSIRKISEKHDKVFATGCMPIEPEEIAKAPRANAAFV
Magnoporthe GT15 (51) ----------PEGILRRVSPEYAPDANPTERINATLL
Saccaromyces GT15 (75) EASEDSEAMDEESKALKAAAEKADAPIDIKTTMDYITPSFANKAGKPKACYV
Section 9
(417) 417 430 440 450 468
light_m.71174 (408) LYGAAHYALQLAEVLPLLDRYFNDRYHYPILIFHSDNMAKETMPGTGKSYLD
Penecillium GT15 (125) VLARNKELDGVIESLKSIERHFNRWWHYPYVFLNDGDFDEEFKATVKNYTSA
Magnoporthe GT15 (78) ALVRNEELDGMLQAMGDLERTWNSKFNYPWTFFNDVPFSKEFKQKTQAMTKA
Saccaromyces GT15 (127) TLVRNKELKGLLSSIKYVENKINKKFPYPWVFLNDEPFTEEFKEAVTKAVSS
Section 10
(469) 469 480 490 500 510 520
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Magnoporthe GT15 (130) KCNYEIIPKEHWDMPSWINKDIYDESVKI----LKENKIQYADKIHYHQMCR
Saccaromyces GT15 (179) EVKFGILPKEHWSYPEWIN-QTKAAEIRA----DAATKYIYGGSESYRHMCR
Section 11
(521) 521 530 540 550 560 572
light_m.71174 (512) FFTYMFFLRPELQPYEYVWRNDRNLGLTRAVPCDVFQVMRRTNAVFGFYAAQ
Penecillium GT15 (225) FYSGHFYKHPLLMKYEWYWRDEHEIKYFCDITYDPFLKMAEANKTYGFTIA-
Magnoporthe GT15 (178) WNSGLFYKHPALKDVQWYWRVEHKMHFFCDIDYDVFRFMQDNNKTYGFTIN-
Saccaromyces GT15 (226) YQSGFFWRHELLEEYDWYWRVEHDIKLYCDINYDVFKWMQENEKVYGFTVS-
Section 12
(573) 573 580 590 600 610 624
light_m.71174 (564) QNEAAQCTGPIGGFAAEYAKT----------FRFQPNHL
Penecillium GT15 (276) VKELRETVPNIFRYAAAYKRKNNLKSKGLWEMFLEQPAQPETPEENKQDKLP
Magnoporthe GT15 (229) LYDAPESIPTLWPETEKFLAEH----------PQYKHPNNAL
Saccaromyces GT15 (277) IHEYEVTIPTLWQTSMLFIKKN----------PEYLDENNLM
Section 13
(625) 625 630 640 650 660 676
light_m.71174 (593) DRIGPGR----------LMAYNGAWNVFKWNFFRSKTWQHWA
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Magnoporthe GT15 (261) DWLTDKEKRPE-------HNRKANGYSTCHFWSNFEVADMNFWRSKTYEDYF
Saccaromyces GT15 (309) SFLSNDN----------GKTYNLCHFWSNFEIANLNLWRSPAYREYF
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(677) 677 690 700 710 728
light_m.71174 (625) QAVRQSGMVYSHRLGEQTMLLFGLSLLVPPEAIHQFGGLSPLFHNRKQLWRY
Penecillium GT15 (380) TMMDRSGGFWNERWGDAPIHSLAAGALLAPSDIHYFRDFGYRHTTIQHCPAN
Magnoporthe GT15 (306) NHLDRAGGFFYERWGDAPVHSIALGLFEDASKIHWFRDIGYQHIPFFNCPNS
Saccaromyces GT15 (346) DTLDHQGGFFYERWGDAPVHSIAAALFLPKDKIHYFSDIGYHHPPYDNCPLD
Section 15
(729) 729 740 750 760 770 780
light_m.71174 (677) DQPEMFIRRDDPVCRAVAADN-----PWSDPFLPYFFVPQPMPNQKHQLNEG
Penecillium GT15 (432) APARQLARIPYLEMTTDDEKKRIEEDEYWANPDPVKENGVGCRCRCDTDIVD
Magnoporthe GT15 (358) PKCKGCVAGRFTDGEKWLHR-------EDCRPNWFKMHGMG----------
Saccaromyces GT15 (398) KEVYNSNNCECDQGNDFTFQG------YSCGKEYYDAQGLVKPKNWKKFRE-
Section 16
(781) 781 790 802
light_m.71174 (724) ----------
Penecillium GT15 (484) VEGKQGSCLNEWVDVAGGWASP
Magnoporthe GT15 (392) ----------
Saccaromyces GT15 (443) ----------

B1


Section 1
dark_m.47703
Arabidopsis GT1
Amycolatopsis GT1
Human GT1
Section 2
Section 3
Section 4
Section 5
Section 6
Section 7
Section 8
Section 9
Section 10
Section 11

B2

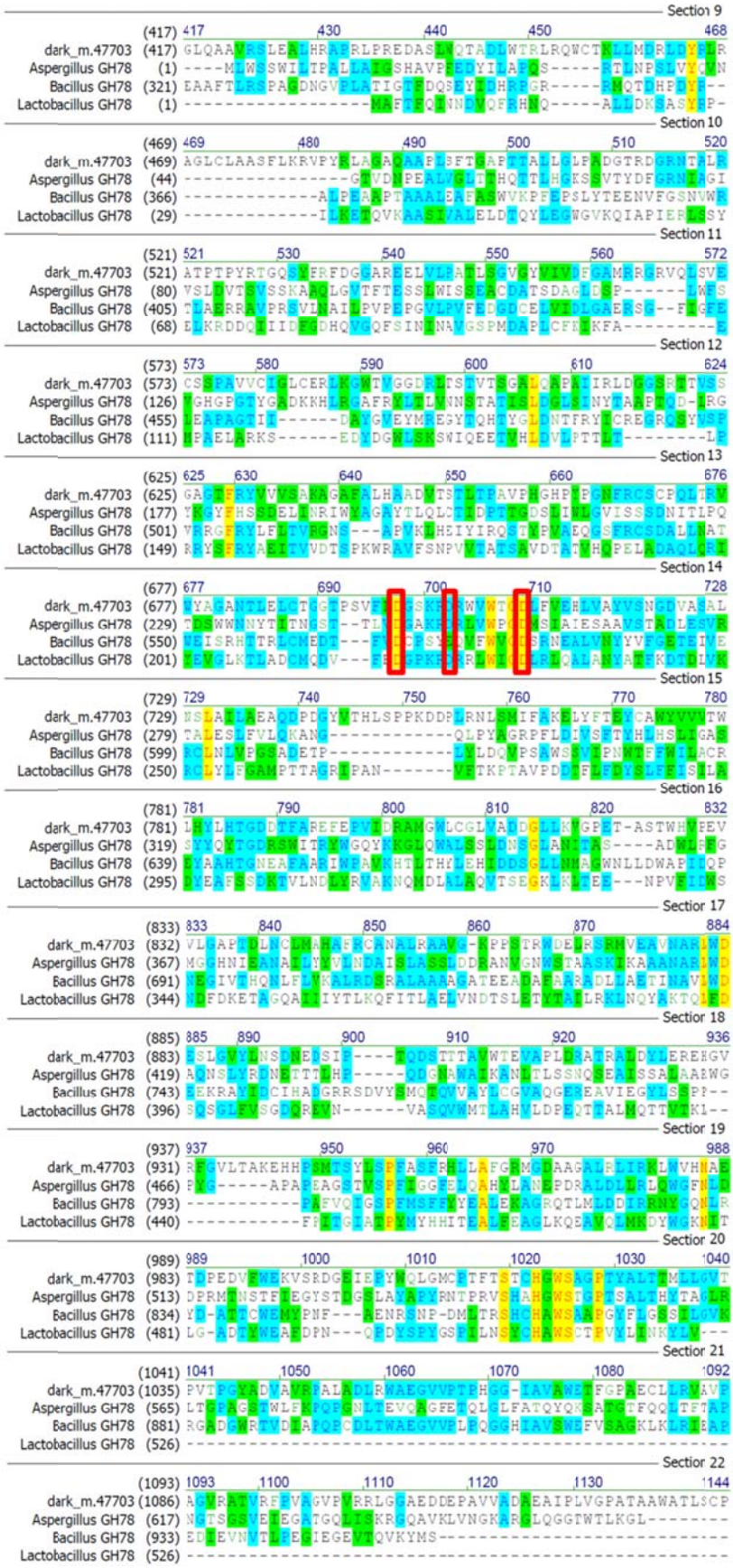

Section 9
Section 10
Section 11
Section 12
Section 13
Section 14
Section 15
Section 16
Section 17
Section 18
Section 19
Section 20
Section 21
Section 22
dark_m.47703
Aspergillus GH78
Bacillus GH78
Lactobacillus GH78

Figure S3: Tocopherol biosynthesis

Strong candidates could be found for each gene. There are many amino transferases in the transcriptome which may act on tyrosine and there are many methyltransferases whose substrates are difficult to conclusively assign. The genus of the closest homologue of each Euglena isoform is shown in blue.

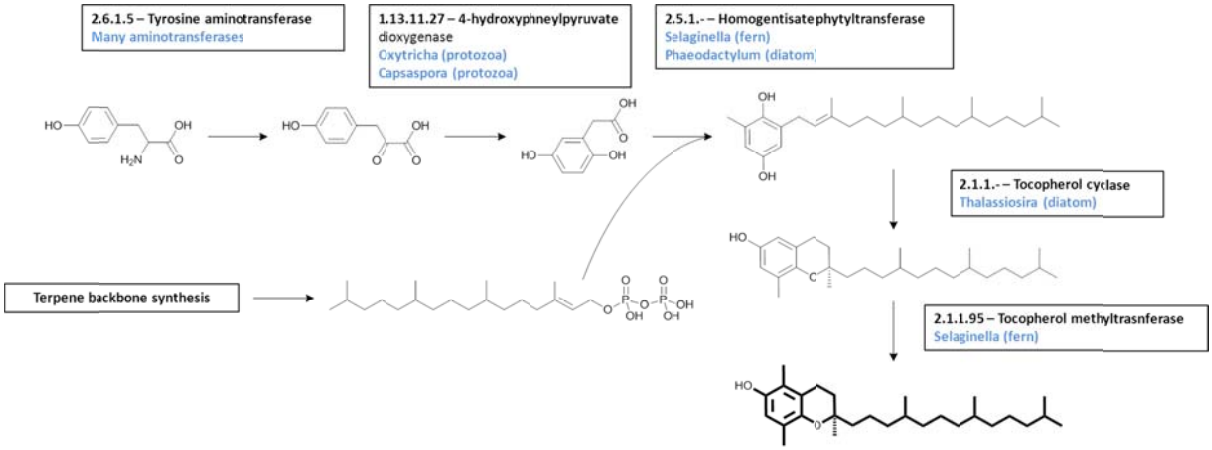


Figure S4: Thiol biosynthetic pathways

Trypanothione is synthesised by joining one molecule of glutathione to each end of spermidine. The proposed biosynthetic pathway for the novel *nor*-trypanothione is shown, whereby aminopropane is transferred to 1,3-diaminopropane, derived from aspartate semialdehyde, to form *nor*-spermidine. Glutathione is then attached to this, either sequentially by two trypanothione synthases or by the separate isoforms adding one molecule each. Ovothiol biosynthesis is shown in the inset. Only the first two steps are known. The genus of the closest homologue is shown in blue.

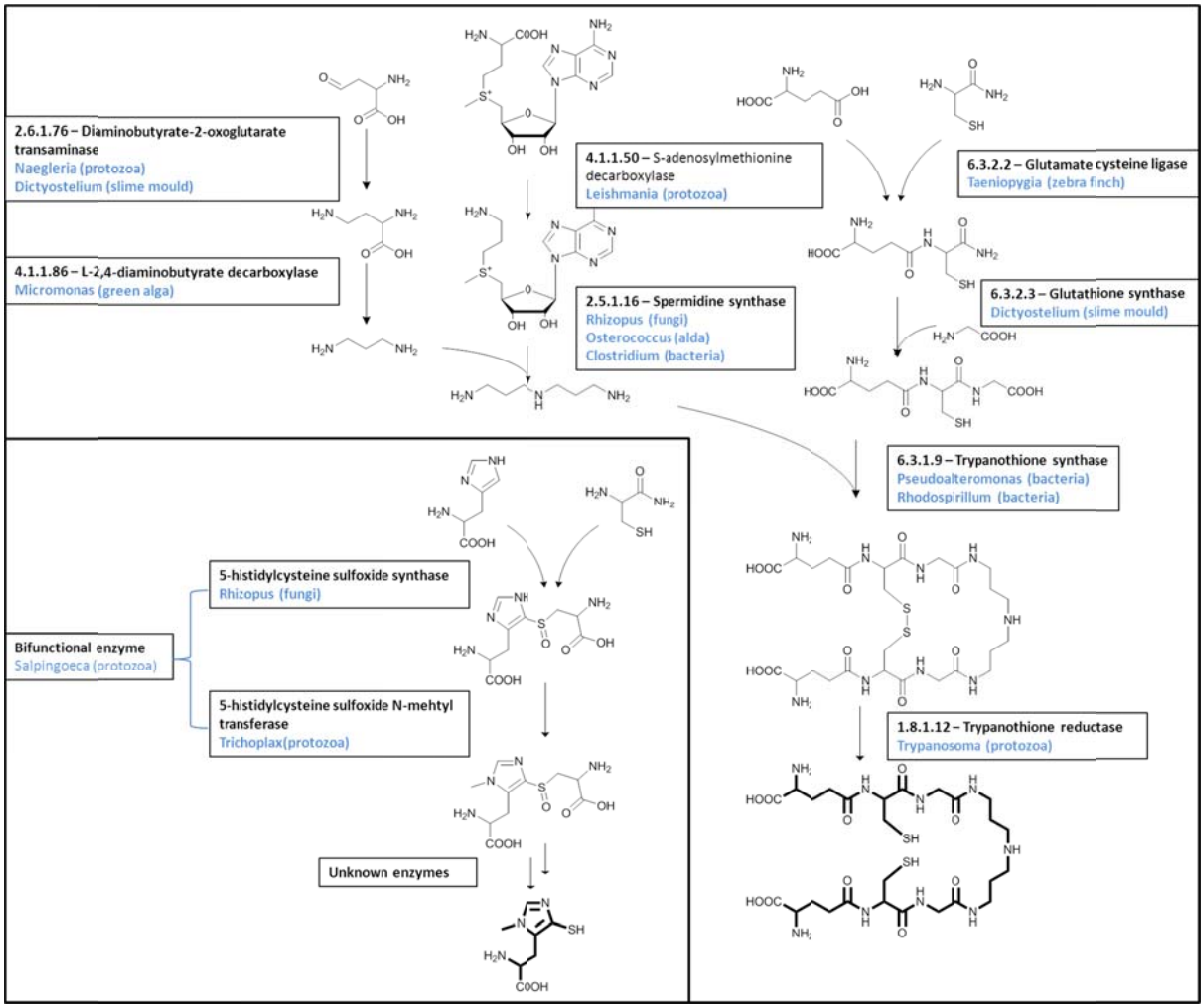
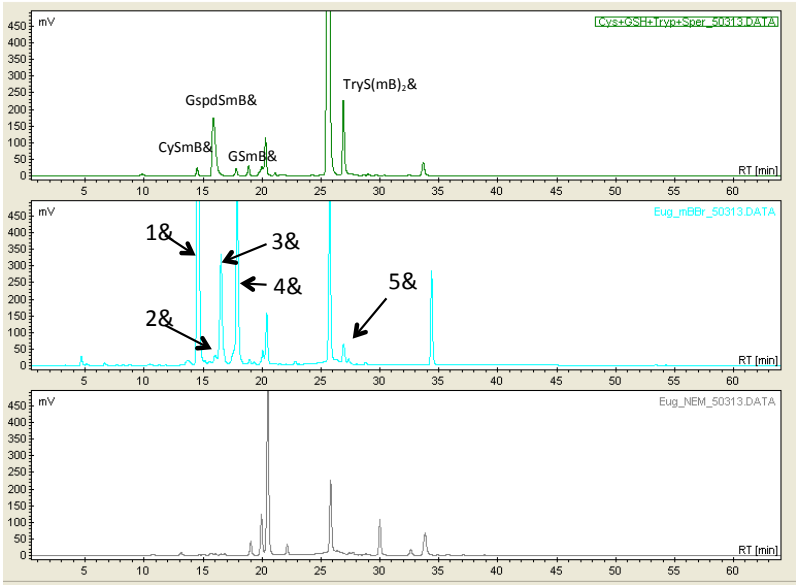


Figure S5: Analysis of the thiol content of *E. gracilis*

Thiols were labelled with monobromobimane and analysed by HPLC. Collected fractions were analysed by LC-MS (Figure S6). There are peaks that have retention time and masses matching cysteine (Peak 1) and glutathione (Peak 4). There is a small peak (5) that coelutes with trypanothione and contains masses, which match the diprotonated mass, as well as a 14 Da smaller analogue, namely *nor*-trypanothione. Additionally there is a small peak (2) matching glutathionyl spermidine, which also contains a 14 Da smaller analogue. Together these data, along with the MS2 fragmentation (Figure S6) indicate the presence of a novel analogue of trypanothione that has one fewer carbons in the spermidine chain, namely *nor*-trypanothione. Peak 3 has a retention time that matches the reported relative retention times of ovothiol,[9] though we were unable to obtain a standard. This peak has an exact mass matching mono-protonated ovothiol and its dimer, together with sodiated adducts, which fragment to give the expected monomers (see figure S6C). mB = monobromobimane. CyS = cysteine. GspdS = glutathionyl spermidine. GS = glutathione. TryS = trypanothione.



Peak	Retention time (min)	Compounds (bimane derivative)	Formula	Calculated m/z (Da)	Observed m/z (Da)
1	14.517	Cysteine	$[C_{13}H_{17}N_3O_4S+H]^+$	312.10124	312.10077
2	16.017	Glutathionyl spermidine/ Glutathionyl *nor*-spermidine	$[C_{27}H_{44}N_8O_7S+H]^+$/ $[C_{26}H_{42}N_8O_7S+H]^+$	625.31262/ 611.29697	625.31239/ 611.29658
3	16.517	Ovothiol	$[C_{17}H_{21}N_5O_4S+H]^+$	392.13869	392.13870
4	17.875	Glutathione	$[C_{20}H_{26}N_5O_8S+H]^+$	498.16529	498.16536
5	26.908	Trypanothione/ *nor*-Trypanthione	$[C_{46}H_{67}N_{13}O_{14}S_2+2H]^{2+}$/ $[C_{46}H_{67}N_{13}O_{14}S_2+2H]^{2+}$	552.73364/ 545.72585	552.73344/ 545.72543

!

Figure S6: Mass spectrometric analysis of bimane derivatives of thiol-containing fractions from HPLC

A. Peak 1. B. Peak 2. The glutathionyl spermidine containing masses were not selected from fragmentation. C. Peak 3. Ovothiol did not successfully fragment but the cluster ions fragmented to the monomers. D. Peak 4. E. Trypanothione in Peak 5. F. *nor*-Trypanothione in Peak 5. Note the homogeneity in the MS2 signals and deviation compared to the trypanothione masses indicates the 14 Da deviation cannot be in the glutamate or cysteine residues.

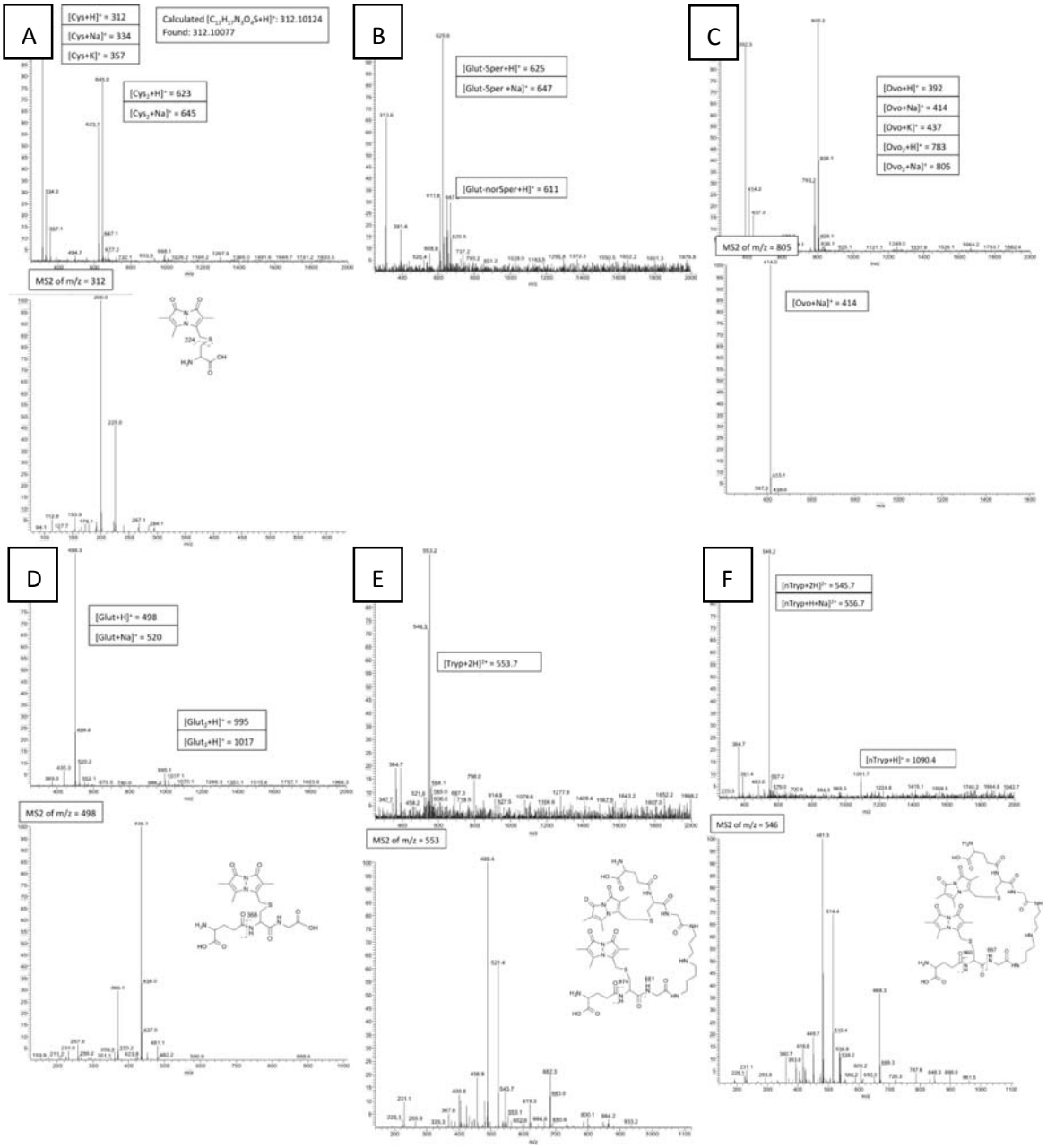


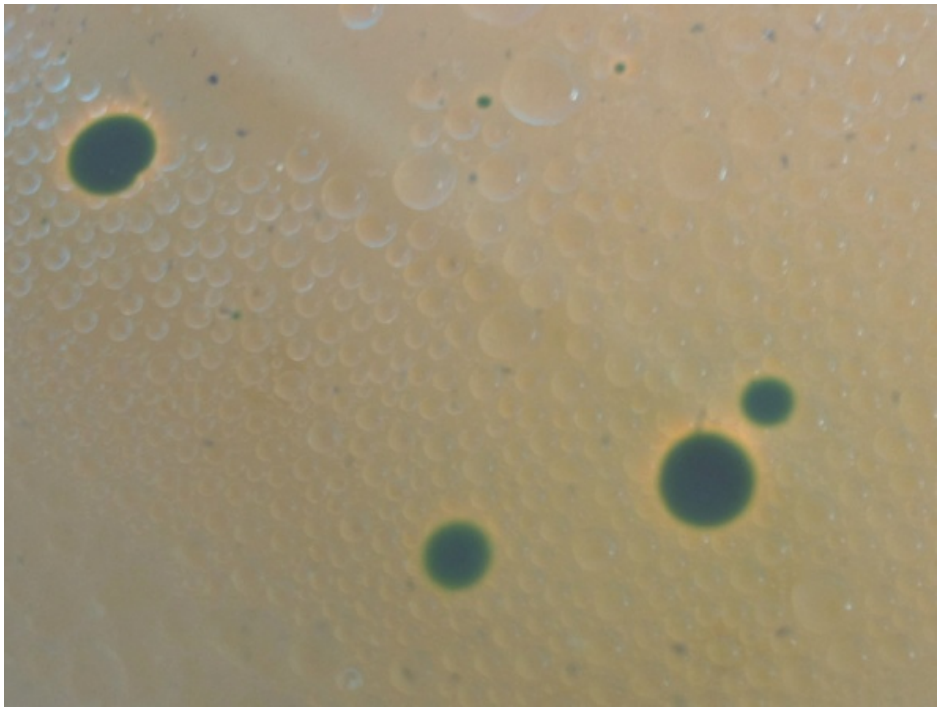
Table S3: Transcripts for polyketide and non-ribosomal peptide synthases.
Predicted proteins were identified using BLASTP to search for keto synthase (for PKSs), condensation, and adenylation (for NRPSs) domains. There is a high degree of uncertainty using this technique. The Kozak sequence for the predicted start codon is listed and the presence of any upstream stop codons is described, indicating the probability that this is the complete cognate transcript. FPKM values for each cognate transcript are given in parentheses.
A – Amino acid adenylation. AA-syn – Amino acid synthesis. ANK – Ankyrin domain. AT – Acyl transfer. C – Condensation. CoAL – Acyl-CoA ligase. DH – Dehydratase. AmT – Aspartate amino transferase. EH – Enoyl CoA hydratase ER – Enoyl reductase. HCS – HMGCoA synthase. KR – keto reductase. KS – Polyketide synthase. TE – thioesterase.

PKS		**Role**	**Domains**	Kozak sequence (Flagellate Kozak consensus is AnnATGnC[10])
1	lm.8157 (34.0)	PKS	KS-AT-DH-ER-KR-ACP-EH-EH-TE-HCS	ACGATGAT, also stop 4 codons upstream
2	lm.60697 (2.79)	PKS	DH-KS-ACP-AmT	CAGATGGC
3	lm.53854 (2.62)	PKS	KS-AT-KR-ACP-KS	CCCATGCC
4	lm.82030 (3.47)	PKS	KR-ACP-KS	GGCATGGC
5	lm.42557 (1.77)	PKS	KR-ACP-KS-DH-KR	GGGATGGC
6	lm.91532 (2.16)	PKS	A-ACP-KS	GTGATGCA
7	lm.95952 (1.04)	PKS	ACP-KS	CCAATGTT
8	lm.88225 (2.10)	PKS	KS-AT	GGCATGGC
9	lm.23151 (2.17)	PKS	KS	CACATGCT
10	lm.94376 (1.70)	PKS	C-ACP	
11	lm.88941 (1.50)	PKS	KS	GTCATGCT
12	lm.110121 (0.15)	PKS	KS	GGCATGGC
13	lm.102218 (0.45)	PKS	KS	GGGATGGC
14	lm.97081 (1.83)	PKS	ACP-KS	
NRPS		**Activity**		
1	lm.66007 (6.73)	NRPS	C-A-ACP	ACCATGGA
2	lm.9669 (8.76)	NRPS	C-C-A-ACP-TE	AACATGGC
3	lm.32232 (3.16)	NRPS	C-C-A-ACP-C-A	AGGATGCT
4	lm.96272 (1.73)	NRPS	C-A-A	AACATGAC
5	lm.21957 (19.80)	AA-Syn	C-A-ACP-TE	ACGATGGC
6	lm.23118 (33.25)	AA-syn	A-ACP-TE	GCCATGGC
7	lm.78138 (3.06)	A	A-ANK	TGTATGTG
8	lm.54590 (2.55)	A	A-ANK	AGCATGGC
9	lm.87820 (2.50)	A	A-ACP-TE	GACATGGC
10	lm.77877 (2.39)	A	A-ACP-ANK	CCGATGGA, also stop 11 codons upstream
11	lm.98982 (1.30)	A	A	CCGATGCT
12	lm.89785 (1.29)	A	A-ACP	GAAATGCA
13	lm.47668 (4.62)	A	A	TACATGAT
14	lm.94698 (1.87)	A	A	GTGATGCG
15	lm.44795 (2.15)	A	A	CGCATGGG, also stop 6 codons upstream
16	lm.44327 (5.36)	A	CoAL	CCTATGGT
17	lm.11010(127.33)	A	CoAL	ATCATGAC
18	lm.81072 (6.55)	A	CoAL	TTGATGGC, also stop 7 codons upstream
19	lm.97175 (0.69)	C	CoAL	
20	lm.26470 (20.15)	C	CoAL	CGGATGCC
21	lm.22119 (3.22)	C	CoAL	GCCATGAC
22	lm.37810 (6.84)	C	CoAL	CCCATGGC
23	lm.3119 (549.70)	C	CoAL	
24	lm.28443 (11.01)	C	CoAL	CAGATGAC
25	lm.9400 (16.40)	C	CoAL	ATCATGAT
26	lm.26875 (17.61)	C	CoAL	CGCATGGC
27	lm.12408 (12.41)	C	CoAL	

28	lm.3346 (17.23)	C	CoAL	
29	lm.44112 (13.17)	C	CoAL	CCAATGCG
30	lm.17087 (13.39)	C	CoAL	AACATGGC

Figure S7: Potential siderophore production by *E. gracilis*

The chrome azurol S (CAS) assay was used to show the production of siderophores.[11] High nutrient media agar was prepared containing CAS (60 mg/l), hexadecyltrimethylammonium bromide (73 mg/l) and additional $FeCl_3$ (2.7 mg/l); cells were diluted such that approximately 10 cells were plated per petri dish. After two weeks a colourless halo around these colonies, particularly vivid when viewed with an orange filter, indicates the uptake of iron, potentially via a siderophore-based mechanism.



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