

Supplementary material for :

Development of an RNA interference (RNAi) gene knockdown protocol in the anaerobic gut fungus *Pecoramyces ruminantium* strain C1A

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Supplementary Tables.

Table S1. Transcripts with a significant (False Discovery Rate (FDR) < 0.1) fold change in the *ldhD*-siRNA-treated cultures.

Transcript ID	IMG Gene accession number	Predicted function	Log ₂ fold change [TPM in siRNA-treated cultures/TPM in untreated cultures]	-log ₁₀ FDR
Significantly down-regulated transcripts				
TRINITY_DN25404_c0_g1_i1	2511056874	Actin and related proteins; ACTR8, ARP8, INO80N actin-related protein 8	-9.89	3.03
TRINITY_DN27647_c0_g2_i1	2511052130	Ankyrin repeat	-8.89	1.22
TRINITY_DN26914_c3_g1_i10	2511052359	Aspartyl aminopeptidase	-11.31	1.04
TRINITY_DN27318_c0_g1_i15	2511054689	Beta-1,4-xylanase	-10.27	3.79
TRINITY_DN27318_c0_g1_i3	2511054689	Beta-1,4-xylanase	-9.47	2.21
TRINITY_DN26767_c0_g1_i2	2518731831	chloride channel 3/4/5	-8.94	1.37
TRINITY_DN27688_c5_g4_i3	2511053193	DNA methylase	-9.97	3.24
TRINITY_DN26958_c2_g1_i9	2511057393	DnaJ family protein C member 3	-8.83	1.22
TRINITY_DN27149_c6_g1_i13	2511052023	Endoglucanase	-11.50	1.13
TRINITY_DN26597_c3_g6_i4	2511059612	Endoglucanase	-9.65	2.58
TRINITY_DN24006_c1_g2_i3	2511053004	Fumarase	-9.13	1.34
TRINITY_DN27371_c7_g1_i12	2511061203	Glycosyl hydrolase family 9	-9.31	1.47
TRINITY_DN23827_c0_g1_i1	2511061779	Hypothetical protein	-11.23	1.03
TRINITY_DN22650_c0_g1_i1	2511057661	Hypothetical protein	-11.21	1.08
TRINITY_DN26878_c4_g5_i1	2518722908	Hypothetical protein	-10.19	3.71
TRINITY_DN72241_c0_g1_i1	2511055911	Hypothetical protein	-9.79	2.88
TRINITY_DN25954_c3_g3_i7	2511052693	Hypothetical protein	-9.34	1.39
TRINITY_DN27282_c5_g1_i4	2511054952	Hypothetical protein	-9.32	1.70
TRINITY_DN20666_c0_g1_i2	2511055315	Hypothetical protein	-9.24	1.71
TRINITY_DN24836_c0_g1_i3	2511055352	Hypothetical protein	-9.10	1.57
TRINITY_DN27173_c0_g2_i1	2511052832	Hypothetical protein	-8.95	1.37
TRINITY_DN26709_c2_g3_i8	2511053925	Hypothetical protein	-8.83	1.18
TRINITY_DN22602_c0_g1_i7	2511058339	Hypothetical protein	-8.23	1.10
TRINITY_DN61803_c0_g1_i2	2511052980	Permeases of the drug/metabolite transporter (DMT)	-10.17	3.71
TRINITY_DN25216_c1_g1_i1	2511061481	Serine/threonine protein kinase	-8.74	1.10
TRINITY_DN26990_c2_g2_i2	2511049217	Uncharacterized conserved protein	-8.76	1.08

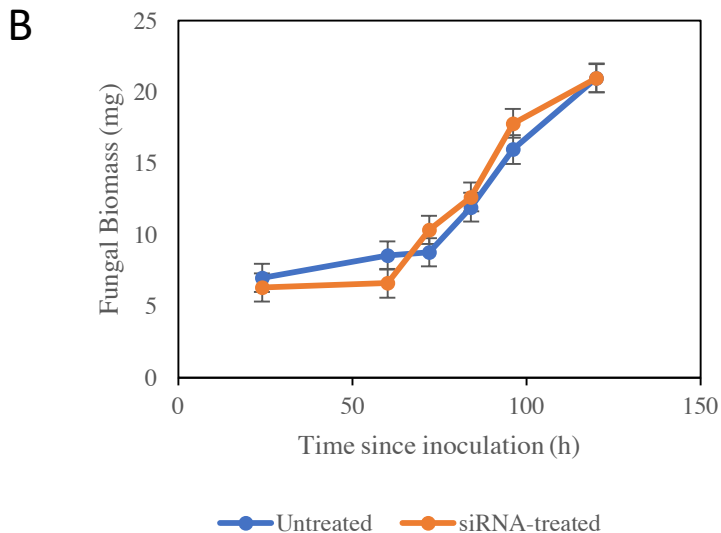
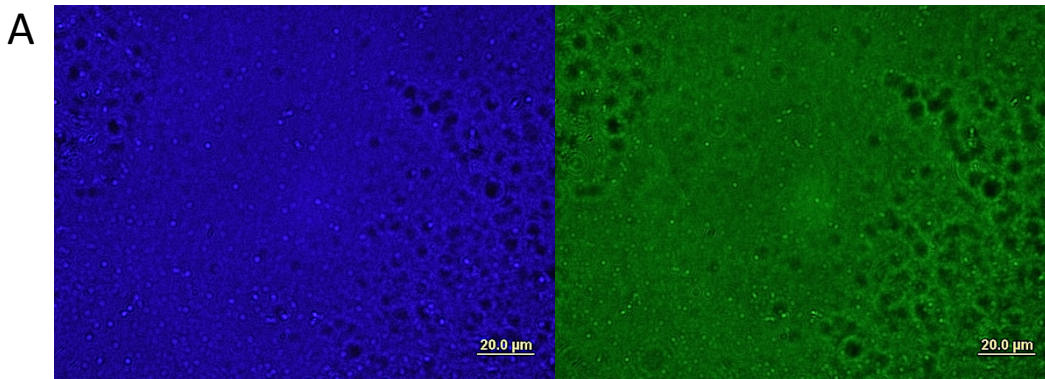
TRINITY_DN23344_c0_g2_i1	2511048410	V8-like Glu-specific endopeptidase	-9.42	1.67
TRINITY_DN26795_c1_g3_i15	2511062537	Vacuolar sorting protein 9	-9.72	2.64
TRINITY_DN27051_c5_g9_i6	2511059070	WD40-repeat-containing domain	-9.17	1.55
TRINITY_DN27737_c3_g9_i1	2511055262	d-Lactate dehydrogenase	-1.31	1.02
Significantly up-regulated transcripts				
TRINITY_DN23455_c1_g3_i1	2511053442	NAD-dependent 2-hydroxyacid dehydrogenase (Pfam00389)	10.59	1.67
TRINITY_DN26116_c0_g1_i2	2518732204	ABC-type multidrug transport system, ATPase component	9.03	1.08
TRINITY_DN27116_c8_g3_i1	2518720443	ABC-type multidrug transport system, ATPase component	9.04	1.22
TRINITY_DN25404_c0_g1_i6	2518716333	Actin and related proteins; ACTR8, ARP8, INO80N actin-related protein 8	9.23	1.67
TRINITY_DN25945_c5_g1_i1	2518724113	Ankyrin repeat	11.62	1.55
TRINITY_DN27367_c4_g7_i1	2518717840	Beta-ketoacyl synthase, N-terminal domain/AMP-binding	9.08	1.53
TRINITY_DN26659_c2_g1_i6	2518724693	Calponin	10.43	2.98
TRINITY_DN27260_c3_g1_i2	2518717204	Cell division protein	9.28	1.85
TRINITY_DN25040_c0_g1_i2	2518723847	Aminopeptidase	10.37	3.88
TRINITY_DN84844_c0_g1_i1	2518724165	Cyclin	9.38	2.05
TRINITY_DN27688_c5_g4_i4	2518731843	Zinc finger, PHD-type	8.13	3.79
TRINITY_DN27658_c7_g1_i9	2518727414	Enterochelin esterase and related enzymes	9.41	1.38
TRINITY_DN27456_c0_g1_i1	2518732213	Transposase	8.89	1.30
TRINITY_DN27284_c8_g3_i8	2518722845	Galactose binding lectin domain	9.56	1.53
TRINITY_DN27371_c7_g1_i8	2511050919	Glycosyl hydrolase family 9	8.94	1.26
TRINITY_DN27514_c12_g7_i3	2511058314	Glycosyl hydrolase family 9	9.10	1.50
TRINITY_DN26559_c2_g2_i3	2511061591	Hypothetical protein	4.35	1.04
TRINITY_DN26871_c0_g1_i6	2511048726	Hypothetical protein	5.66	1.08
TRINITY_DN26577_c7_g3_i6	2511060029	Hypothetical protein	7.06	3.71
TRINITY_DN27338_c2_g6_i9	2511050279	Hypothetical protein	7.91	3.71
TRINITY_DN20198_c0_g1_i1	2511054063	Hypothetical protein	8.64	1.04
TRINITY_DN59842_c0_g2_i1	2511053000	Hypothetical protein	8.67	1.08
TRINITY_DN26705_c9_g11_i5	2511061429	Hypothetical protein	8.79	1.10
TRINITY_DN24643_c0_g3_i3	2511061911	Hypothetical protein	9.24	1.78

TRINITY_DN26861_c6_g1_i1	2518722966	Hypothetical protein	9.36	1.68
TRINITY_DN26789_c7_g2_i2	2511060353	Hypothetical protein	9.63	2.59
TRINITY_DN25732_c3_g1_i2	2518716704	CAP-Gly domain	9.74	2.81
TRINITY_DN20193_c0_g1_i1	2511049097	Hypothetical protein	9.74	2.27
TRINITY_DN27291_c5_g1_i27	2511062706	Hypothetical protein	9.92	1.34
TRINITY_DN26268_c0_g6_i2	2511056562	Hypothetical protein	9.94	3.10
TRINITY_DN72241_c0_g4_i1	2511055911	Hypothetical protein	9.99	3.10
TRINITY_DN23108_c0_g1_i1	2511055897	Leucine-rich repeat (LRR) protein	8.67	1.04
TRINITY_DN26891_c1_g2_i3	2518723924	Long-chain acyl-CoA synthetases (AMP-forming)	9.22	1.59
TRINITY_DN27245_c11_g4_i1	2511055785	Metal-dependent hydrolase	11.86	1.14
TRINITY_DN26212_c0_g1_i6	2511051817	Mismatch repair ATPase (MutS family)	9.29	1.68
TRINITY_DN26388_c1_g1_i3	2511051700	Nucleosome-binding factor SPN, POB3 subunit	8.96	1.26
TRINITY_DN25459_c0_g1_i3	2518723044	Nucleotide-sugar transporter.	8.72	1.08
TRINITY_DN26193_c4_g1_i3	2518729432	Phosphatidylinositol-4-phosphate 5-Kinase	9.92	3.10
TRINITY_DN18878_c0_g1_i3	2511061142	Predicted Rossmann fold nucleotide-binding protein	12.17	9.52
TRINITY_DN27738_c2_g1_i2	2518732026	Reverse transcriptase (RNA-dependent DNA polymerase)/Integrase	9.48	1.71
TRINITY_DN26725_c0_g1_i2	2511052637	RhoGEF domain.	9.24	1.78
TRINITY_DN25303_c1_g1_i1	2511056187	Ribosomal protein L11 methylase	9.49	1.90
TRINITY_DN26960_c5_g2_i8	2518725849	Superfamily II DNA/RNA helicases, SNF2 family	9.01	1.35
TRINITY_DN11227_c0_g2_i1	2511055978	Trehalose-6-phosphate synthase	12.34	1.26
TRINITY_DN27057_c7_g3_i8	2511061879	WD40-repeat-containing domain	9.13	1.62
TRINITY_DN24706_c0_g1_i2	2518725042	WD40-repeat-containing domain	9.31	1.55
TRINITY_DN22853_c0_g1_i4	2518720070	WD40-repeat-containing domain	9.32	1.56
TRINITY_DN27087_c6_g1_i1	2511051329	WD40-repeat-containing domain	9.36	1.84
TRINITY_DN17665_c0_g1_i1	2518731672	Zinc metalloprotease (elastase)	9.56	2.22
TRINITY_DN26802_c4_g9_i1	NA ^a	1,4-alpha-glucan branching enzyme	9.21	1.55
TRINITY_DN27699_c2_g2_i5	NA	Hypothetical protein	9.65	2.62
TRINITY_DN27163_c4_g2_i1	NA	Hypothetical protein	11.75	1.13
TRINITY_DN19604_c0_g3_i1	NA	SH3 domain	8.89	1.26

a: NA Blastx comparison of the transcript sequence against C1A proteins showed no hits.

Supplementary Figures

Figure S1. Uptake of fluorescently (Cy3) tagged siRNA by C1A spores. (A) The *ldhD*-specific siRNA was added to the flooding solution 75 minutes after the onset of flooding followed by incubation for 15 more minutes at 39°C. Samples (a few microliters) were taken at regular intervals for visualization. The same field is shown for DAPI-, and Cy3-labeled germinating spores (Note that the spores were concurrently stained with DAPI and fluorescing green indicating the uptake of the Cy3-labeled siRNA) (bar=20 μm). (B) Effect of the siRNA treatment on fungal growth rate. siRNA-treated spores were collected and used to inoculate fresh RFC medium. Control cultures were started at the same time using siRNA-untreated spores. Headspace pressure was measured daily and used to calculate fungal biomass as described previously (1). Error bars are standard deviations from at least three replicate cultures for each condition.



References:

1. **Ranganathan A, Smith OP, Youssef NH, Struchtemeyer CG, Atiyeh HK, Elshahed MS.** 2017. Utilizing anaerobic fungi for two-stage sugar extraction and biofuel production from lignocellulosic biomass. *Front Microbiol* **8**:635.