RESEARCH NOTE

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The transcriptome response of the ruminal ^{Const} methanogen *Methanobrevibacter ruminantium* strain M1 to the inhibitor lauric acid

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Abstract

Objective: Lauric acid (C_{12}) is a medium-chain fatty acid that inhibits growth and production of the greenhouse gas methane by rumen methanogens such as *Methanobrevibacter ruminantium*. To understand the inhibitory mechanism of C_{12} , a transcriptome analysis was performed in *M. ruminantium* strain M1 (DSM 1093) using RNA-Seq.

Results: Pure cell cultures in the exponential growth phase were treated with 0.4 mg/ml C_{12} , dissolved in dimethyl sulfoxide (DMSO), for 1 h and transcriptomic changes were compared to DMSO-only treated cells (final DMSO concentration 0.2%). Exposure to C_{12} resulted in differential expression of 163 of the 2280 genes in the M1 genome (maximum log₂-fold change 6.6). Remarkably, C_{12} hardly affected the expression of genes involved in methanogenesis. Instead, most affected genes encode cell-surface associated proteins (adhesion-like proteins, membrane-associated transporters and hydrogenases), and proteins involved in detoxification or DNA-repair processes. Enrichment analysis on the genes regulated in the C_{12} -treated group showed a significant enrichment for categories 'cell surface' and 'mobile elements' (activated by C_{12}), and for the categories 'regulation' and 'protein fate' (repressed). These results are useful to generate and test specific hypotheses on the mechanism how C_{12} affects rumen methanogens.

Keywords: Methanobrevibacter ruminantium, Methanogenesis, Fatty acid, Rumen, Gene expression, Lauric acid

Introduction

Ruminal methane-producing archaea acquire attention because ruminant livestock is estimated as the most important source of anthropogenic emission of the greenhouse gas methane [1]. Among the most-promising anti-methanogenic compounds are two medium chain fatty acids (MCFA), lauric acid (C_{12}) and myristic acid (C_{14}), which were shown to inhibit methanogenesis in vivo when supplemented to the diet of ruminants [2–4], in vitro in rumen fluid [5] and in methanogenic cultures [6]. MCFA cause leakage of K⁺ ions and decrease survival of *Methanobrevibacter ruminantium*, a

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dominant methanogen species in the rumen [6, 7]. Further, MCFA killed some, but not all methanogen cells, which implies that the cells may be capable to react to fatty acid-caused stress. In search of the mode of action, we investigated the transcriptional response of *M. ruminantium* to exposure of C_{12} in culture.

Main text

Methods

Experimental design

Methanobrevibacter ruminantium (strain M1, DSM 1093; 'Deutsche Sammlung von Mikroorganismen und Zellkulturen' (DSMZ), Braunschweig, Germany) was cultivated anaerobically in 50 ml of modified *Methanobacterium* medium (DSMZ No. 1523) in 116 ml bottles under a CO_2/H_2 (0.2:0.8) atmosphere at 150 kPa and at 39 °C in an incubation shaker as described previously [6]. Growth of the cultures was monitored by recording

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optical density at 600 nm and by methane (CH₄) formation after 24, 48, 60 and 61 h. The culture was inoculated with 5 ml of an exponentially growing pre-culture $(OD_{600} \sim 0.64)$ to 45 ml of medium. Cell survival was detected with the LIVE/DEAD BacLight Bacterial Viability Kit for microscopy and quantitative assays (Kit L7012; Invitrogen GmbH, Darmstadt, Germany) [6]. Lauric acid (> 97% purity) was obtained from Sigma-Aldrich (Buchs, Switzerland), and a stock solution with 200 mg/ml was prepared by dissolving the C₁₂ in sterile dimethyl sulfoxide (DMSO) (Sigma-Aldrich), a commonly used solvent for water-insoluble substances [8]. After 60 h of incubation, when cells reached the exponential phase, three bottles were supplemented with 0.1 ml of the C_{12} stock solution to reach a final concentration of 0.4 mg $C_{12}/$ ml (treatment group), three bottles were supplemented with 0.1 ml of DMSO (final concentration: 0.2%) (control group), and three bottles received no supplement (blank group). The concentration of C_{12} and the exposure time of 1 h chosen were in a range where most cells remained alive and where CH₄ formation was clearly but not completely inhibited. It was verified that, at 61 h of incubation, CH₄ formation rates and proportion of living cells did not differ between DMSO-exposed control cultures (measured: 0.71 \pm 0.03 $\mu mol/ml$ \times h and 97 \pm 0.3%, respectively) and untreated blank cultures (0.74 \pm 0.04 $\mu mol/ml$ \times h and 99 \pm 1.2%). At 61 h, i.e. after 1 h of exposure to C_{12} , CH_4 formation rates in the hour after exposure were suppressed by $40 \pm 6\%$ compared to the control cultures (P < 0.05), and cell viability was reduced down to 71 \pm 1.8% when compared to the control cultures (P < 0.05). At this time point, three samples per group (each 50 ml of culture) were anaerobically collected at 4 °C after centrifugation at $5000 \times g$ for 6 min. Cell pellets were immediately frozen in liquid nitrogen and stored at - 80 °C until RNA extraction.

RNA isolation

Total RNA was isolated from the frozen cell pellets by using TRIzol[®] Reagent (ThermoFisher, Waltham, MS, USA), according to the manufacturer's protocol. In order to remove genomic DNA from total RNA samples, a DNA digestion was performed with the RNase-Free DNase Set (Qiagen, Hilden, Germany) following manufacturer's instructions. Quantity and quality of extracted RNA were determined by a Qubit[®] 1.0 fluorometer with a Qubit RNA BR (Broad Range) assay kit (Invitrogen, Carlsbad, CA, USA) and by an Agilent 2200 TapeStation with the Agilent RNA ScreenTape assay (Agilent Technologies, Santa Clara, CA, USA), respectively. Nine purified total RNA samples with a yield of at least 5 µg and RNA integrity numbers (RIN) in a range of 5.6–7.6 were used for sequencing. These included three replicates per group: three DMSO-dissolved C_{12} -treated samples (T1, T2 and T3), three samples with DMSO supplementation alone (control samples C1, C2, C3) and three samples without supplement (blank samples B1, B2, B3).

Ribosomal RNA depletion

The Ribo-ZeroTM rRNA removal kit (Bacteria) (http:// www.illumina.com/products/ribo-zero-rrna-removalbacteria.html, Epicentre, San Diego, USA) was applied to deplete rRNA from the *M. ruminantium* total RNA samples (5 μ g) by following the Illumina user guide for the Ribo-Zero Magnetic kits (Part#15065382 Rev. A, November 2014). The rRNA-depleted samples were purified with AMPure RNAClean XP Beads (Beckman-Coulter Genomics, Nyon, Switzerland) as recommended in the Illumina protocol mentioned above.

Next generation sequencing

Enriched RNA samples were used to produce library constructs by following the Illumina TruSeq® Stranded total RNA protocol (Part#15031048 Rev. C, September 2012) with the Illumina TruSeg Stranded total RNA Sample Preparation Kit. Libraries were quantified and quality checked using qPCR with Illumina adapter specific primers (Roche LightCycler® system, Roche Diagnostics, Basel, Switzerland) and by the Agilent Technologies 2100 Bioanalyzer with DNA-specific chips, respectively. Diluted indexed libraries (10 nM) were pooled, used for cluster generation (Illumina TruSeq SR Cluster Kit v4-cBot-HS reagents) and further sequenced (Illumina TruSeq SBS Kit v4-HS reagents) on the Illumina HiSeq 2500 instrument in the high output mode according to the manufacturer's recommendations. Illumina single read approach (1 \times 125 bp) was used to generate raw sequencing reads with a depth of approximately 20-30 million reads per sample.

RNA-sequencing data analysis

Data analyses were performed as described by Tanner et al. [9]. Shortly, reads (125 bp) were mapped against the genome of *M. ruminantium* M1 using the CLC Genomics Workbench 6.5.1 (CLC, Aarhus, Denmark). Statistical analysis was performed using Bioconductor EdgeR software package in R. A false discovery rate (FDR) value < 0.05 was used as cutoff for significance of differentially expressed genes and log₂ fold change > 1 and < -1 was used as cutoff for differential transcription of genes higher (positive log₂-fold change values) or lower (negative log₂-fold change values) expressed in cultures [10]. To test for significant enrichment in each category listed in Table 1, a two-tailed Fisher test was performed at http://www.langsrud.com/fisher.htm.

Category	Gene count	Treatment vs. control		Control vs. blank		Treatment vs. blank	
		Up	Down	Up	Down	Up	Down
Amino acid metabolism	94	2 ^b	3	0	4	1	2
Cell cycle	29	1	0	0	0	0	0
Cell envelope	189	28 ^a	0 ^b	2	4	2	3
Cellular processes	14	3	1	1	0	2 ^a	0
Central carbon metabolism	61	2	1	0	1	2	0
Energy metabolism	141	9	9 ^a	6	3	6	0
Lipid metabolism	21	0	0	1	0	0	3 ^a
Mobile elements	87	37 ^a	0	0	37 ^a	0	0
Nitrogen metabolism	14	0	1	1	0	1	0
Nucleic acid metabolism	60	2	1	0	0	0	0
Protein fate	51	0 ^b	2	1	0	1	0
Protein synthesis	169	7	1	0 ^b	9	Ob	0
Purines and pyrimidines	47	2	0	0	0	0	0
Regulation	68	0 ^b	5 ^a	5 ^a	0	2	0
Secondary metabolites	12	4	0	0	0	0	0
Transcription	26	1	0	0	0	0	0
Transporters	97	11	1	7 ^a	3	7 ^a	1
Unknown function	183	10	8	4	2 ^b	3	0
Vitamins and cofactors	142	8	3	2	4	5	1
Total ^c	1505	127	36	30	67	32	10

Table 1 Number of genes significantly differential expressed within functional categories

^a Significant functional enrichment in a Fisher exact test (p < 0.05)

 $^{\rm b}$ Significant functional underrepresentation in a Fisher exact test (p < 0.05)

^c Non-conserved hypothetical genes and RNAs are omitted in the classification [11]. Treatment: with DMSO-dissolved C₁₂, control: with DMSO alone, blank: without C₁₂ and DMSO

Results and discussion

The Ribo-ZeroTM rRNA Removal Kit can be used to efficiently remove the rRNA fraction from total RNA samples isolated from the archaeon *M. ruminantium* M1. The Epicentre probes (directed to bind rRNA from a broad spectrum of bacteria species) reduced the rRNAs in all samples tested, which resulted in 40–85% of non-rRNA sequencing reads in the samples (Fig. 1). More than 10 million mRNA sequencing reads per sample were mapped to the genome of *M. ruminantium* M1 (Fig. 1), which is a sufficient coverage for transcriptome analyses [11].

First, we compared the untreated cultures to the control cultures treated with DMSO. DMSO affected the expression of 97 out of 2280 genes in the M1 genome (Additional file 1). DMSO induced changes in gene expression of cell surface-related proteins, cell membrane-associated transporters and intracellular proteins; the latter maybe related to the observation that DMSO penetrates cell membranes [8]. DMSO-regulated genes included genes encoding proteins related to the cell envelope, mainly adhesion-like proteins (six genes; four



down-regulated, two up-regulated). Others were classified as mobile genetic elements (38 genes including hypothetical genes; all down-regulated), and genes involved in energy metabolism, mainly hydrogen metabolism [nine genes, six up-regulated (frhA/B1/D/G, mtrA2, DsbD), three down-regulated (hypA/B, adh3)]. Genes involved in metabolism of vitamins and cofactors (six genes; four down-regulated, two up-regulated) as well as of amino acids (four genes, all down-regulated) were regulated. Moreover, cation transporters (five genes; four of five up-regulated), amino acid transporters (two genes; down-regulated), and other transporters (three genes, up-regulated) showed differential expression when untreated cultures were compared to DMSO-supplemented cultures. Overall, the set of genes regulated in the DMSO control group compared to the blank group was enriched for genes assigned to categories: 'Mobile elements', 'Transporters', and 'Regulation', whereas genes assigned to 'protein synthesis' and genes of unknown function were significantly underrepresented (Table 1).

The comparison between the C_{12} + DMSO-treated and the untreated cultures revealed 42 genes differentially regulated (Additional file 2), 26 of these also found in the DMSO-treated versus untreated comparison (Additional file 3).

Thereafter the transcriptome of the C_{12} + DMSOtreated and DMSO-treated cultures were compared to identify the mechanisms how MCFA affect methanogenesis. A total of 147 genes, 6.4% of all 2280 genes, were differentially regulated (Table 2).

The subcellular localization of the encoded protein could be identified for 75% of the regulated genes. Predominantly, genes associated with the cell envelope were affected, namely trans-membrane proteins or membraneassociated proteins. Enrichment analysis showed that, with C12 exposure, mainly adhesion-like proteins (category 'cell surface') and phage-related proteins ('mobile elements') were significantly enriched in the regulated genes data set (Table 1). This supports earlier suggestions that MCFA primarily target the cell envelope and processes that occur at the cell membrane [12]. For example, upon exposure to C₁₂ in the present study, the mRNA abundance of 26 adhesion-like proteins (ALPs) (part of the cell envelope [13]), i.e. of 25% of all ALPs of *M. rumi*nantium, and of two proteins involved in biosynthesis of teichoic acid and pseudomurein which are cell-wall related [14], were up-regulated compared to the DMSO control group (Table 2).

Two subunits of the membrane-bound energy-converting hydrogenase (Eha), which is involved in hydrogenotrophic methanogenesis [13, 15], were down-regulated by $\log_2 1.6$ - and 1.7-fold in cultures exposed to C_{12} , whereas two cytoplasmic hydrogenases (Frh, Mvh) were not. A gene encoding ferredoxin, a trans-membrane iron-sulfur protein involved in electron transfer from hydrogen, was up-regulated (log 2.6-fold upon C12 exposure). Expression of 3 genes encoding trans-membrane 4Fe-4S binding domain-containing proteins was affected by C₁₂ exposure. Two subunits of the methyl-H4MPT:coenzyme M methyltransferase (Mtr), which is membrane-bound and plays a crucial role in the methanogenesis pathway [15, 16], were down-regulated by log₂ 2.1- and 1.2-fold upon C12 exposure. In total 13 genes encoding mainly transporters of amino acids and cations displayed differences in transcript abundance after C_{12} exposure (Table 2). For example, several genes encoding subunits of cations transporters, like the nickel ABC transporter permease proteins or nickel ABC transporter ATP-binding proteins, NikA2, NikB1, NikB2, NikC2 and NikD2, were differentially regulated. These cation transporters belong to a large family of ABC transporters (peptide/nickel transporter family) in ABC-type nickel transporter system, which is composed of a periplasmic binding protein (NikA), two integral membrane proteins (NikB and NikC) and two ABC proteins (NikD and NikE) [17]. One P-type ATPase, which are membrane-bound efflux pumps involved in metal homeostasis of microorganisms [18], was down-regulated. In prokaryotes, ABC transporters and P-type ATPases have important functions in maintaining appropriate concentrations of transition metals such as Ni, Co, Fe, Cu, and Zn, which are essential components of many prokaryotic enzymes [18]. Two transmembrane cobalt transport proteins (mru 0540; mru_0539), and two membrane-associated proteins involved in molybdate transport (mru_0200, mru_0201) [19], were up-regulated.

In addition, genes encoding intracellular proteins were affected by C₁₂ exposure. These data support earlier observations that exposure to C_{12} causes leakage of intracellular K^+ ions in *M. ruminantium* [6, 7], thus damages the cell envelope. Amongst the regulated genes, mostly genes encoding proteins involved in DNA repair, and genes controlling transcription/translation and redox homeostasis were affected. For example, thioredoxins and rubrerythrins showed an altered expression; they are considered to form a system protecting Archaea against oxidative stress [20, 21]. Thioredoxin-like proteins exhibit biochemical activities similar to thioredoxin and help methanogens maintain redox homeostasis [7]. Genes which were up-regulated by C₁₂ included genes encoding proteins that are involved in nucleic acid metabolism and repair and in translation include a helicase (mru 0981), an exonuclease (mru 2097, recJ1), an anaerobic ribonucleosid-triphosphate reductase nrdD (mru_0241), a nucleotidase (mru_2104; SurE1), and a RNA-metabolizing metallo-beta-lactamase (mru 1978). Several genes

Anino acid metabolism True_0153 dysk Diaminopimelate decarboxykase bysk - 1.02 7.66 Halidine mm_0153 dysk Diaminopimelate decarboxykase bysk - 1.02 7.61 Halidine mm_0153 dysk Diaminopimelate decarboxykase bysk - 1.02 6.27 Serine mm_0158 scrin Phosphoglycerate dehydrogenase SerA 1.03 9.59 Tryptophan mm_1739 tryptopha synthase beta subunit Trypk2 1.00 1.31 Cell division mm_2160 mm_0160 Adhesin-like protein 1.02 6.70 Cell surface proteins mm_0160 Mmu_160 Adhesin-like protein 1.15 9.15 mm_0233 mm_1233 Adhesin-like protein 1.15 9.15 9.15 mm_0233 mm_0233 mm_0233 Adhesin-like protein 1.27 1.29 mm_0233 mm_0245 Adhesin-like protein 1.26 1.04 1.02 mm_0337 mm_0245 Adhesin-like protein 1.27 1.29 1.29 1.27 1.29 <th>Category and subcategory</th> <th>ORF</th> <th>Gene name</th> <th>Annotated function</th> <th>log2-fold change</th> <th>log2 counts per 106 reads</th>	Category and subcategory	ORF	Gene name	Annotated function	log2-fold change	log2 counts per 106 reads
Lysine mru_0152 lysA Diaminopimetate decatoxylase LysA - 1.02 7.66 Histoline mru_0153 dapf Diaminopimetate epimerase Dapf - 1.07 7.01 Serine mru_028 hsH Imidacole giverol phosphate synthase glutamine - 1.07 6.27 Serine mru_0273 tryR Thyptophan synthase beta subunit TryB2 1.03 9.99 Cell division mru_1200 mru_0160 Arbesin-like protein 1.08 5.46 Cell division mru_0160 mru_0160 Arbesin-like protein 1.00 6.37 Gell division mru_0160 mru_0160 Arbesin-like protein 1.08 5.46 Cell division mru_0131 Machai-like protein 1.01 4.54 mru_0133 mru_0133 Arbesin-like protein 1.17 8.53 mru_0147 mru_0147 Arbesin-like protein 1.27 1.23 mru_0134 mru_0245 Arbesin-like protein 1.27 1.28 mru_0147 mru_0147 Arbesin-like protein <td< td=""><td>Amino acid metabolism</td><td></td><td></td><td></td><td></td><td></td></td<>	Amino acid metabolism					
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Histidine mm_0182 hist Imdiacole glycend phosphate symbase glutamine -137 0.27 Serine mm_026/8 serA Phosphoglycerate delydrogenase SerA 1.03 9.39 Tryptophan mm_02159 trypt Tryptophan symbase beta subunit TryB2 1.03 1.131 Cell division mmu_2150 trypt Tryptophan symbase beta subunit TryB2 1.00 5.46 Cell division Mu_0160 mu_01500 mu_01500 Adhesin-like protein 1.02 6.70 Gell division ATPase MinD 1.08 Adhesin-like protein 1.02 6.70 mu_0263 mu_0233 Adhesin-like protein 1.15 1.04 mu_0233 mu_0233 Adhesin-like protein 1.23 1.04 mu_0245 mu_0245 Adhesin-like protein 1.23 1.046		mru_0153	dapF	Diaminopimelate epimerase DapF	- 1.00	7.01
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Typtophan mu_2159 trpi2 Typtophan synthase beta subunit Trpi2 1.00 11.31 Cell cycle	Serine	mru_0678	serA	Phosphoglycerate dehydrogenase SerA	1.03	9.59
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mru_0963 mru_0963 Makesin-like protein 1.08 12.13 mru_0331 mru_0231 Makesin-like protein 1.15 9.15 mru_0331 mru_0338 Adhesin-like protein 1.17 8.55 mru_0331 mru_0344 Adhesin-like protein 1.20 12.55 mru_0357 mru_0687 Adhesin-like protein 1.21 11.29 mru_0467 mru_0245 Adhesin-like protein 1.32 8.78 mru_1477 mru_1504 Adhesin-like protein 1.33 9.69 mru_1455 mru_1505 Adhesin-like protein 1.43 9.49 mru_1450 mru_1506 Adhesin-like protein 1.61 6.82 mru_1455 mru_0237 Adhesin-like protein 1.61 6.82 mru_0317 mru_0327 Adhesin-like protein 2.61 7.42 mru_0328 mru_0327 Adhesin-like protein 2.61 7.42 mru_0331 mru_0334 Adhesin-like protein 2.61 7.83 mru_0329 mru_0		mru_0160	mru_0160	Adhesin-like protein	1.02	6.70
mu_1263 mu_1263 mu_238 Adhesin-like protein 1.15 0.03 mu_038 mu_038 Adhesin-like protein 1.15 0.03 mu_038 mu_038 Adhesin-like protein 1.20 1255 mu_038 mu_0287 Adhesin-like protein 1.20 1255 mu_0687 mu_0285 Adhesin-like protein 1.20 1257 mu_0455 mu_0245 Adhesin-like protein 1.28 10.46 mu_1457 mu_1456 Adhesin-like protein 1.43 949 mu_1656 mu_1656 Adhesin-like protein 1.61 6.22 mu_1756 mu_1566 Adhesin-like protein 1.61 7.76 mu_0377 mu_0327 Adhesin-like protein 1.70 5.86 mu_038 mu_0249 mu_0374 Adhesin-like protein 2.07 6.71 mu_0384 mu_0374 Adhesin-like protein 2.27 8.58 1.23 mu_0375 mu_0374 Adhesin-like protein 2.32 8.37		mru_0963	mru_0963	Adhesin-like protein	1.08	12.13
mru_0331 mru_0333 Adhesin-like protein 1.15 10.34 mru_0383 mru_0383 Adhesin-like protein 1.17 8.55 mru_031 mru_031 Adhesin-like protein 1.20 12.55 mru_0431 mru_0647 Adhesin-like protein 1.22 11.29 mru_0647 mru_0647 Adhesin-like protein 1.22 8.78 mru_1650 mru_0147 Adhesin-like protein 1.43 9.49 mru_1650 mru_1650 Adhesin-like protein 1.61 6.82 mru_1566 mru_1475 Adhesin-like protein 1.61 7.76 mru_1566 mru_0327 Adhesin-like protein 1.61 7.76 mru_0321 mru_0324 Adhesin-like protein 2.07 6.71 mru_0323 mru_0324 Adhesin-like protein 2.07 6.71 mru_0324 mru_0343 Adhesin-like protein 2.07 6.71 mru_0324 mru_0444 Adhesin-like protein 2.51 13.38 mru_0403 mru_04		mru_1263	mru_1263	Adhesin-like protein	1.15	9.15
mru_0338 mru_0338 Adhesin-like protein 1.17 8.55 mru_124 mru_1124 Adhesin-like protein 1.20 1.25 mru_0331 mru_0031 Adhesin-like protein 1.20 1.23 mru_045 mru_0687 Adhesin-like protein 1.32 8.78 mru_147 mru_147 Adhesin-like protein 1.43 9.49 mru_1650 mru_1650 Adhesin-like protein 1.43 9.49 mru_1650 mru_1650 Adhesin-like protein 1.61 6.82 mru_1506 mru_1506 Adhesin-like protein 1.61 6.82 mru_0128 mru_0327 Adhesin-like protein 1.61 7.66 mru_0129 mru_0327 Adhesin-like protein 2.04 7.42 mru_0203 mru_0324 Adhesin-like protein 2.07 6.71 mru_0204 mru_034 Adhesin-like protein 2.07 6.71 mru_0204 mru_034 Adhesin-like protein 2.07 6.71 mru_2043 mru_044		mru_0331	mru_0331	Adhesin-like protein	1.15	10.34
mru_1124 mru_0031 Adhesin-like protein 1.20 11.29 mru_0033 mru_0036 Adhesin-like protein 1.22 11.29 mru_0245 mru_0245 Adhesin-like protein 1.28 0.46 mru_1126 mru_0245 Adhesin-like protein 1.32 8.78 mru_1126 mru_0245 Adhesin-like protein 1.43 9.49 mru_1150 mru_1147 Adhesin-like protein 1.61 6.82 mru_1505 mru_147 Adhesin-like protein 1.61 6.82 mru_0147 mru_0147 Adhesin-like protein 1.70 5.86 mru_0217 mru_0148 Adhesin-like protein 2.04 7.42 mru_0018 Adhesin-like protein 2.04 7.42 mru_0028 mru_0119 Adhesin-like protein 2.02 7.42 mru_0028 mru_0203 Adhesin-like protein 2.25 11.23 mru_0028 mru_1726 Mru_172 3.31 3.31 mru_1726 mru_1726 Adhesin-like protei		mru_0338	mru_0338	Adhesin-like protein	1.17	8.55
mru_0031 mru_0031 Adhesin-like protein 1.27 11.29 mru_0687 mru_02245 Adhesin-like protein 1.28 10.46 mru_1417 mru_1177 Adhesin-like protein 1.33 9.49 mru_1455 mru_1456 Adhesin-like protein 1.44 4.24 mru_1455 mru_1650 Adhesin-like protein 1.61 6.82 mru_1456 mru_1566 Adhesin-like protein 1.61 6.82 mru_0327 mru_0176 Adhesin-like protein 1.61 6.82 mru_0327 mru_0327 Adhesin-like protein 2.04 7.42 mru_0328 mru_0327 Adhesin-like protein 2.07 6.71 mru_0417 mru_2049 Adhesin-like protein 2.25 11.33 mru_2048 mru_2049 Adhesin-like protein 2.25 11.33 mru_2049 mru_2049 Adhesin-like protein 2.25 11.33 mru_2049 mru_2049 Adhesin-like protein 2.33 13.13 mru_2040 A		mru_1124	mru_1124	Adhesin-like protein	1.20	12.55
mm_0687 mm_0287 Adhesin-like protein 1.28 10.46 mm_0245 mm_0245 Adhesin-like protein 1.32 8.78 mm_1417 mm_1145 Adhesin-like protein 1.43 9.49 mmu_1650 mu_1650 Adhesin-like protein 1.61 6.82 mm_1506 mm_1465 Adhesin-like protein 1.61 6.82 mm_1009 mu_1037 Adhesin-like protein 1.61 6.82 mm_0197 mu_0137 Adhesin-like protein 1.73 0.86 mm_0019 mu_0019 Adhesin-like protein 2.07 6.71 mm_0048 mu_0039 Adhesin-like protein 2.25 11.33 mm_0049 mu_12049 Adhesin-like protein 2.32 8.37 mm_2049 mu_1226 Adhesin-like protein 2.51 1.388 mm_2047 mu_1276 Adhesin-like protein 2.51 1.338 mm_2049 mu_1276 Adhesin-like protein with cysteine protease domain 1.49 9.07 mu_2050 <td< td=""><td></td><td></td><td></td><td>Adhesin-like protein</td><td>1.27</td><td>11.29</td></td<>				Adhesin-like protein	1.27	11.29
mm_0245 mm_0245 Adhesin-like protein 1.32 8.78 mm_1417 mu_1417 Adhesin-like protein 1.43 9.49 mm_1650 mm_1650 Adhesin-like protein 1.61 6.82 mm_1650 mm_1750 Adhesin-like protein 1.61 6.82 mm_017 mru_0417 Adhesin-like protein 1.61 7.76 mru_017 mru_0327 Adhesin-like protein 1.73 10.86 mru_0020 mru_0327 Adhesin-like protein 2.04 7.42 mru_0044 mru_0034 Adhesin-like protein 2.07 6.71 mru_0049 mru_0049 Adhesin-like protein 2.25 11.23 mru_02049 mru_2043 Adhesin-like protein 2.32 8.37 mru_1720 mru_2043 Adhesin-like protein 2.51 13.88 mru_0205 mru_2049 Adhesin-like protein 2.51 13.88 mru_0204 mru_2026 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_0105 </td <td></td> <td></td> <td></td> <td>Adhesin-like protein</td> <td>1.28</td> <td>10.46</td>				Adhesin-like protein	1.28	10.46
mru_1417 mru_1417 mru_165 mru_1650 Adhesin-like protein 1.61 6.62 mru_1506 mru_1650 Adhesin-like protein 1.61 7.66 6.66 mru_0327 rru_0327 Adhesin-like protein 2.04 7.42 mru_0019 mru_0019 Adhesin-like protein 2.07 6.71 mru_0024 mru_2049 Adhesin-like protein 2.07 6.71 mru_2049 mru_2049 Adhesin-like protein 2.32 8.37 mru_2049 mru_2049 Adhesin-like protein 2.32 8.37 mru_2040 mru_2040 Adhesin-like protein 2.31 13.88 mru_1726 mru_1726 Adhesin-like protein 2.31 13.88 mru_2147 mru_1670 mru_1670 1.37 6.32 mru_0020 mru_0020		mru 0245	mru 0245	Adhesin-like protein	1.32	8.78
mru_1650 mru_1650 Adhesin-like protein 1.44 4.24 mru_1465 mru_1455 Adhesin-like protein 1.61 6.82 mru_1506 mru_1506 Adhesin-like protein 1.61 7.66 mru_0417 mru_0417 Adhesin-like protein 1.73 10.86 mru_0327 mru_0327 Adhesin-like protein 2.04 7.42 mru_0034 mru_0084 Adhesin-like protein 2.04 7.42 mru_0084 mru_0084 Adhesin-like protein 2.07 6.71 mru_2049 mru_2049 Adhesin-like protein 2.25 11.23 mru_2043 mru_2043 Adhesin-like protein 2.32 8.37 mru_1726 mru_2040 mru_2147 Adhesin-like protein 2.51 13.88 mru_1726 mru_2040 mru_2047 Adhesin-like protein 2.51 13.81 mru_0200 mru_2015 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_1020 mru_0015 Adhesin-like protein with cysteine protease domai		mru 1417	mru 1417	Adhesin-like protein	1.43	9.49
nmu_1465 mu_1465 Adhesin-like protein 1.61 6.82 mru_1506 mru_1506 Adhesin-like protein 1.61 7.76 mru_0417 mru_0327 Adhesin-like protein 1.73 10.86 mru_0019 mru_0327 Adhesin-like protein 1.73 10.86 mru_0019 mru_0327 Adhesin-like protein 2.07 6.71 mru_0044 mru_0044 Adhesin-like protein 2.07 6.71 mru_2043 mru_2049 Adhesin-like protein 2.25 11.23 mru_2043 mru_2043 Adhesin-like protein 2.32 8.37 mru_2040 mru_2040 Adhesin-like protein 2.32 8.37 mru_2045 mru_2040 Adhesin-like protein 2.51 13.88 mru_1266 mru_1726 Adhesin-like protein 2.73 13.13 mru_0105 mru_0200 Adhesin-like protein with cysteine protease domain 2.49 9.07 mru_0202 mru_0102 Adhesin-like protein with cysteine protease domain 2.78 7.86		mru 1650	mru 1650	Adhesin-like protein	1.44	4.24
mm_1_506 mmu_1506 Adhesin-like protein 1.61 7.76 mru_0417 mru_0417 Adhesin-like protein 1.70 5.86 mru_0327 mru_0327 Adhesin-like protein 1.73 10.86 mru_0019 mru_0019 Adhesin-like protein 2.04 7.42 mru_0248 mru_0084 Adhesin-like protein 2.07 6.71 mru_0243 mru_2049 Adhesin-like protein 2.25 11.23 mru_2043 mru_2043 Adhesin-like protein 2.32 8.37 mru_1726 mru_1726 Adhesin-like protein 2.32 8.37 mru_0209 mru_2090 Adhesin-like protein 2.51 13.88 mru_11726 mru_015 Adhesin-like protein 5.04 12.58 mru_015 mru_0015 Adhesin-like protein with cysteine protease domain 2.78 7.86 Teichoic acid biosynthesis mru_1079 mru_1079 CDP-glycerolpoly(glycerophosphate) glycerophospho- 1.27 6.32 Pseudomurein biosynthesis mru_1118 Cell wall b		mru 1465	mru 1465	Adhesin-like protein	1.61	6.82
mru_017 mru_0217 Adhesin-like protein 1.70 5.86 mru_0327 mru_0327 Adhesin-like protein 1.73 10.86 mru_0019 mru_0019 Adhesin-like protein 2.04 7.42 mru_0044 mru_0084 mru_0084 Adhesin-like protein 2.07 6.71 mru_2049 mru_2049 Adhesin-like protein 2.25 11.23 mru_2043 mru_2043 Adhesin-like protein 2.25 8.58 mru_2040 mru_2043 Adhesin-like protein 2.32 8.37 mru_2090 mru_2046 Adhesin-like protein 2.51 13.88 mru_2047 mru_2147 Adhesin-like protein 2.51 13.88 mru_0326 mru_0326 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_0015 mru_0020 mru_0020 Adhesin-like protein with cysteine protease domain 1.49 9.07 relichoic acid biosynthesis mru_11079 mru_0202 Adhesin-like protein with cysteine protease domain 1.49 9.07		mru 1506	mru 1506	Adhesin-like protein	1.61	7.76
mru_0327 mru_0327 mu_0327 Adhesin-like protein 1.7.3 10.86 mru_0019 mru_0019 Adhesin-like protein 2.04 7.42 mru_0084 mru_0019 Adhesin-like protein 2.07 6.71 mru_0084 mru_0084 Adhesin-like protein 2.25 11.23 mru_0243 mru_2043 Adhesin-like protein 2.32 8.37 mru_1726 mru_1726 Adhesin-like protein 2.32 8.37 mru_0090 mru_0204 Adhesin-like protein 2.51 13.88 mru_0105 mru_015 Adhesin-like protein 5.04 12.58 mru_0105 mru_015 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_01020 mru_0020 Adhesin-like protein with cysteine protease domain 2.78 7.86 Teichoic acid biosynthesis mru_1079 mru_1079 CDP-glycerol:poly(glycerophosphate) glycerophosphoto 1.27 6.32 Cellular processe mru_1015 fprA1 F420H2 oxidase FprA1 1.37 10.47		mru 0417	mru 0417	Adhesin-like protein	1 70	5.86
mru_0019 mru_0019 Adhesin-like protein 2.04 7.42 mru_0084 mru_0084 Adhesin-like protein 2.07 6.71 mru_0049 mru_2049 Adhesin-like protein 2.25 11.23 mru_2043 mru_2043 Adhesin-like protein 2.27 8.58 mru_1726 mru_1726 Adhesin-like protein 2.32 8.37 mru_2090 mru_2090 Adhesin-like protein 2.51 13.88 mru_2147 mru_2147 Adhesin-like protein 2.73 13.13 mru_0326 mru_0326 Adhesin-like protein 5.04 12.58 mru_015 mru_015 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_0020 mru_0020 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_0020 mru_0105 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_01015 mru_01020 Adhesin-like protein with cysteine protease domain 2.78 7.86 Teichoic acid biosynthesis mru_179 CDP-glycerol:ploly(glycerophosphate) glycerophospho- transferase 1.27 6.32		mru 0327	mru 0327	Adhesin-like protein	1 73	10.86
mru_0084 mru_0084 Adhesin-like protein 2.07 6.71 mru_0084 mru_0084 Adhesin-like protein 2.25 11.23 mru_2043 mru_2043 Adhesin-like protein 2.27 8.58 mru_2090 mru_2090 Adhesin-like protein 2.32 8.37 mru_2090 mru_2090 Adhesin-like protein 2.51 13.88 mru_2147 mru_2147 Adhesin-like protein 2.73 13.13 mru_0326 mru_0326 Adhesin-like protein 5.04 12.58 mru_0015 mru_0020 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_0020 mru_0020 Adhesin-like protein with cysteine protease domain 2.78 7.86 Teichoic acid biosynthesis mru_1079 mru_019 CDP-glycorephosphate) glycerophospho- transferase 1.27 6.32 Pseudomurein biosynthesis mru_1118 Cell wall biosynthesis protein Mur ligase family 1.07 9.37 Cellular processes mru_1137 fprA1 F420H2 oxidase FprA2 3.58 12.42 mru_1367 fbr2 Ruberythrin Rbr2 1.27		mru 0019	mru 0019	Adhesin-like protein	2.04	7.42
Inite_outer		mru 0084	mru 0084	Adhesin-like protein	2.07	671
Initial 2003 Initial protein 2.25 11.25 mru_2043 mru_2043 Adhesin-like protein 2.27 8.58 mru_1726 mru_1726 Adhesin-like protein 2.32 8.37 mru_2090 mru_2090 Adhesin-like protein 2.32 8.37 mru_2090 mru_2090 Adhesin-like protein 2.51 13.88 mru_2015 mru_2147 Mru_326 Adhesin-like protein 5.04 12.58 mru_0015 mru_0020 Adhesin-like protein with cysteine protease domain 1.49 9.07 mru_0020 mru_0020 Adhesin-like protein with cysteine protease domain 2.78 7.86 Teichoic acid biosynthesis mru_1079 mru_1079 CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase 1.27 6.32 Pseudomurein biosynthesis mru_1118 mru_1118 Cell wall biosynthesis protein Mur ligase family 1.07 9.37 Cellular processes mru_1136 fprA1 F420H2 oxidase FprA1 1.37 10.47 mru_0131 fprA2 F420H2 oxidase FprA2 3.58 12.42 mru_1367 rbr2 Rubrerythr		mru 2049	mru 2049	Adhesin-like protein	2.07	11.23
Initial constraint integrateInitial constraint integrateInitial constraint integrateInitial constraint integratemru_1726mru_1726Mahesin-like protein2.328.37mru_2000mru_2000Adhesin-like protein2.5113.88mru_2147mru_2147Adhesin-like protein2.7313.13mru_0326mru_0326Adhesin-like protein5.0412.58mru_0015mru_0015Adhesin-like protein with cysteine protease domain1.499.07mru_0020mru_0020Adhesin-like protein with cysteine protease domain2.787.86Teichoic acid biosynthesismru_1079mru_1079CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase1.276.32Pseudomurein biosynthesismru_1118mru_1118Cell wall biosynthesis protein Mur ligase family1.079.37Cellular processesmru_1131fprA1F420H2 oxidase FprA11.3710.47Mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein-1.197.79Central carbon metabolismmru_0628pgk2A2-Phosphoglycerate kinase Pgk2A1.857.69Othermru_1786mru_1786Transporter SS5 family-1.189.66		mru 20/13	mru 2043	Adhesin-like protein	2.23	8.58
Initia_1720Initia_1720Adhesin-like protein2.326.37mru_2090mru_2090Adhesin-like protein2.5113.88mru_2147mru_2147Adhesin-like protein2.7313.13mru_0326mru_0326Adhesin-like protein5.0412.58mru_0015mru_0015Adhesin-like protein with cysteine protease domain1.499.07mru_0020mru_0020Adhesin-like protein with cysteine protease domain2.787.86Teichoic acid biosynthesismru_1079mru_1079CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase1.276.32Pseudomurein biosynthesismru_1118cell wall biosynthesis protein Mur ligase family1.079.37Cellular processesmru_11307fprA1F420H2 oxidase FprA11.3710.47Mru_1367fbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183Protein disulfide-isomerase thioredoxin-related protein-1.197.79Central carbon metabolismmru_1265gde22-Phosphoglycerate kinase Pgk2A1.857.69Othermru_1265deoCDeoxyribose-phosphate aldolase DeoC5.1211.11Acretaremru_1786mru_1786Transporter SSS family-1189.66		mru 1726	mru 1726	Adhesin-like protein	2.27	8.30
Initial 2000Initial 2000Addressificitie protein2.5113.88mru_2147mru_2147Adhesin-like protein2.7313.13mru_0326mru_0326Adhesin-like protein5.0412.58mru_0015mru_0015Adhesin-like protein with cysteine protease domain1.499.07mru_0020mru_0020Adhesin-like protein with cysteine protease domain2.787.86Teichoic acid biosynthesismru_1079mru_1079CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase1.276.32Pseudomurein biosynthesismru_1118mru_1118Cell wall biosynthesis protein Mur ligase family1.079.37Cellular processesmru_1131fprA1F420H2 oxidase FprA11.3710.47mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein-1.197.79Central carbon metabolismmru_1685deoCDeoxyribose-phosphate aldolase DeoC5.1211.11Acetatemru_1786mru_1786Transporter SSS family-1.189.66		mru 2000	mru 2000		2.52	12.00
Initial and the protein2.7313.13mru_0326mru_0326Adhesin-like protein5.0412.58mru_0015mru_0015Adhesin-like protein with cysteine protease domain1.499.07mru_0020mru_0020Adhesin-like protein with cysteine protease domain2.787.86Teichoic acid biosynthesismru_1079mru_1079CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase1.276.32Pseudomurein biosynthesismru_1118mru_1118Cell wall biosynthesis protein Mur ligase family1.079.37Cellular processesmru_1118F420H2 oxidase FprA11.3710.47Mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubreythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein- 1.197.79Central carbon metabolismmru_1685deoCDeoxyribose-phosphate aldolase DeoC5.1211.11Acetatemru_1786mru_1786Transporter SS5 family- 1.188.66		mru 2147	mru 2147		2.21	13.00
Initial of the protein5.0412.56mru_0015mru_0015Adhesin-like protein with cysteine protease domain1.499.07mru_0020mru_0020Adhesin-like protein with cysteine protease domain2.787.86Teichoic acid biosynthesismru_1079mru_1079CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase1.276.32Pseudomurein biosynthesismru_1118mru_1118Cell wall biosynthesis protein Mur ligase family1.079.37Cellular processesmru_0131fprA2F420H2 oxidase FprA11.3710.47Mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein- 1.197.79Central carbon metabolismmru_0628pgk2A2-Phosphoglycerate kinase Pgk2A1.857.69Othermru_1786mru_1786Transporter SSS family- 1.188.66		mru 0226	mru 0226	Adhesin like protein	2.75	13.13
Initiation of the protein with cysterine protease domain1.499.07mru_0020mru_0020Adhesin-like protein with cysterine protease domain1.499.07Teichoic acid biosynthesismru_1079mru_1079CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase1.276.32Pseudomurein biosynthesismru_1118mru_1118Cell wall biosynthesis protein Mur ligase family1.079.37Cellular processesmru_0131fprA1F420H2 oxidase FprA11.3710.47mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein- 1.197.79Central carbon metabolismGluconeogenesismru_0628pgk2A2-Phosphoglycerate kinase Pgk2A1.857.69Othermru_1786mru 1786Transporter SSS family- 1.189.66		mmu_0015	mru_0320	Adhesia like protein	1.40	12.58
Teichoic acid biosynthesismru_1079mru_1079CDP-glycerol:poly(glycerophosphate) glycerophospho- transferase1.276.32Pseudomurein biosynthesismru_1118mru_1118Cell wall biosynthesis protein Mur ligase family1.079.37Cellular processes0xidative stress responsemru_1507fprA1F420H2 oxidase FprA11.3710.47mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein-1.197.79Central carbon metabolismGluconeogenesismru_1685deoCDeoxyribose-phosphate aldolase DeoC5.1211.11Acretatemru 1786mru 1786Trapsporter SSS family-1.189.66		mru 0020	mru 0020	Adhesin-like protein with cysteine protease domain	1.49	9.07
Pseudomurein biosynthesismru_1118mru_1118Cell wall biosynthesis protein Mur ligase family1.079.37Cellular processes0xidative stress responsemru_1507fprA1F420H2 oxidase FprA11.3710.47mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein-1.197.79Central carbon metabolismGluconeogenesismru_0628pgk2A2-Phosphoglycerate kinase Pgk2A1.857.69Othermru_1786deoCDeoxyribose-phosphate aldolase DeoC5.1211.11Acretatemru 1786mru 1786Trapsporter SSS family1189.66	Teichoic acid biosynthesis	mru_1079	mru_1079	CDP-glycerol:poly(glycerophosphate) glycerophospho- transferace	1.27	6.32
Oxidative stress responsemru_1507fprA1F420H2 oxidase FprA11.3710.47mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein- 1.197.79Central carbon metabolismGluconeogenesismru_0628pgk2A2-Phosphoglycerate kinase Pgk2A1.857.69Othermru_1685deoCDeoxyribose-phosphate aldolase DeoC5.1211.11Acetatemru_1786mru_1786Trapsporter SSS family- 1189.66	Pseudomurein biosynthesis	mru_1118	mru_1118	Cell wall biosynthesis protein Mur ligase family	1.07	9.37
Oxidative sites (sports)Intra_1307Intra_142012 Oxidase FprA21.5710.47mru_0131fprA2F420H2 oxidase FprA23.5812.42mru_1367rbr2Rubrerythrin Rbr21.2713.19Stress responsemru_0183mru_0183Protein disulfide-isomerase thioredoxin-related protein- 1.197.79Central carbon metabolismGluconeogenesismru_0628pgk2A2-Phosphoglycerate kinase Pgk2A1.857.69Othermru_1685deoCDeoxyribose-phosphate aldolase DeoC5.1211.11Acetatemru_1786mru_1786Trapsporter SSS family- 1189.66	Ovidative stress response	mru 1507	for A 1	E420H2 ovidase EprA1	1 37	10.47
mru_0131 hpr/2 142012 Oxidas (1pr/2 5.50 12.42 mru_1367 rbr2 Rubrerythrin Rbr2 1.27 13.19 Stress response mru_0183 mru_0183 Protein disulfide-isomerase thioredoxin-related protein - 1.19 7.79 Central carbon metabolism Gluconeogenesis mru_0628 pgk2A 2-Phosphoglycerate kinase Pgk2A 1.85 7.69 Other mru_1786 deoC Deoxyribose-phosphate aldolase DeoC 5.12 11.11 Acetate mru_1786 Trapsporter SSS family - 1.18 9.66	Oxidative stress response	mru 0131	for 42		3.58	10.47
Stress response mru_0183 mru_0183 Protein disulfide-isomerase thioredoxin-related protein - 1.19 7.79 Central carbon metabolism Gluconeogenesis mru_0628 pgk2A 2-Phosphoglycerate kinase Pgk2A 1.85 7.69 Other mru_1685 deoC Deoxyribose-phosphate aldolase DeoC 5.12 11.11 Acetate mru_1786 mru_1786 Transporter SSS family - 118 2.66		mru 1267	rbr2	Rubrerythrin Rhr2	1.27	13.10
Sites response Initigeness response Initigeness response 7.79 Central carbon metabolism Gluconeogenesis mru_0628 pgk2A 2-Phosphoglycerate kinase Pgk2A 1.85 7.69 Other mru_1685 deoC Deoxyribose-phosphate aldolase DeoC 5.12 11.11 Acetate mru_1786 mru_1786 Transporter SSS family – 118 8.66	Strass rasponso	mru 0102	mru 0102	Protoin disulfida-isomorasa thioradovia rolatad aratain	1.27	7 70
Gluconeogenesis mru_0628 pgk2A 2-Phosphoglycerate kinase Pgk2A 1.85 7.69 Other mru_1685 deoC Deoxyribose-phosphate aldolase DeoC 5.12 11.11 Acetate mru_1786 mru_1786 Transporter SSS family 118 8.66	Control corbon motobolism	1111u_0163	1111u_0165	notem disultue-isotherase thioredoxin-related protein	- 1.19	1.19
Other mru_1685 deoC Deoxyribose-phosphate aldolase DeoC 5.12 11.11 Acetate mru_1786 Transporter SSS family – 118 9.66	Glucopogoposis	mru 0620	nak2A	2-Phosphoglycorate kinase Pak2A	1.85	7.60
Acetate mru 1786 Transporter SSS family — 118 9.66	Other	mru 1605	HUKZA deoC	2 + 103p10919Cetate Nillase F9K2A	5.10	11 11
	Acetate	mru 1796	mru 1786	Transporter SSS family	- 1.18	8.66

Table 2 Significant changes of gene expression in *M. ruminantium* M1 cultures exposed to C₁₂

Table 2 (continued)

Category and subcategory	ORF	Gene name	Annotated function	log2-fold change	log2 counts per 106 reads
Energy metabolism					
Electron transfer	mru 0915	mru 0915	4Fe-4S binding domain-containing protein	- 1.06	764
	mru 2036	mru 2036	4Fe=4S binding domain-containing protein	1.00	5.60
	mru 1345	mru 1345	4Fe=4S binding domain containing protein	1.20	7.63
Methanogenesis pathway	mru 0569	mer	5 10-methylenetetrahydro-methanopterin reductase	- 136	12 71
methanogenesis patima)	a_00000	inci	Mer		
	mru_0526	hmd	Coenzyme F420-dependent N(5), N(10)-methenyltet- rahydromethanopterin reductase Hmd	1.41	10.96
	mru_1850	atwA2	Methyl-coenzyme M reductase component A2 AtwA2	1.05	10.86
	mru_1927	mcrD	Methyl-coenzyme M reductase D subunit McrD	— 1.43	11.33
	mru_0441	mtrA2	Tetrahydromethanopterin S-methyltransferase subunit A MtrA2	- 2.14	11.99
	mru_1918	mtrF	Tetrahydromethanopterin S-methyltransferase subunit F MtrF	- 1.24	9.71
Electron transfer	mru_0184	dsbD	Cytochrome C-type biogenesis protein DsbD	- 1.16	6.17
	mru_0830	mru_0830	Ferredoxin	2.56	9.31
H2 metabolism	mru_1410	ehaC	Energy-converting hydrogenase A subunit C EhaC	- 1.63	6.30
	mru_1408	ehaE	Energy-converting hydrogenase A subunit E EhaE	— 1.74	7.34
	mru_1632	hypB	Hydrogenase accessory protein HypB	2.25	7.90
	mru_1633	hypA	Hydrogenase nickel insertion protein HypA	2.19	7.47
Formate metabolism	mru_0332	fdhC	Formate/nitrite transporter FdhC	— 1.11	11.98
Alcohol metabolism	mru_1445	adh3	NADP-dependent alcohol dehydrogenase Adh3	6.42	7.81
	mru_1444	npdG2	NADPH-dependent F420 reductase NpdG2	3.84	5.32
Mobile elements					
Prophage	mru_0269	mru_0269	ATPase involved in DNA replication control MCM family	2.51	4.60
	mru_0323	mru_0323	dnd system-associated protein 2	1.11	6.63
	mru_0280	mru_0280	ParB-like nuclease domain-containing protein	2.52	1.87
	mru_0256	mru_0256	Phage integrase	1.69	6.95
	mru_0287	mru_0287	Phage portal protein	2.73	1.86
	mru_0315	mru_0315	Phage tail tape measure protein	2.47	3.39
	mru_0270	mru_0270	Phage-related protein	1.91	4.54
	mru_0288	mru_0288	Phage-related protein	2.21	2.32
	mru_0058	mru_0058	Phage-related protein	2.53	- 0.04
	mru_0282	mru_0282	Phage-related protein	2.64	1.93
	mru_0316	mru_0316	Phage-related protein	2.66	3.40
	mru_0317	mru_0317	Phage-related protein	2.89	3.42
	mru_0311	mru_0311	Phage-related protein	3.14	2.55
	mru_0310	mru_0310	Phage-related protein	3.18	1.56
	mru_0284	mru_0284	Phage-related protein	3.35	1.93
	mru_0307	mru_0307	Phage-related protein	3.38	2.86
	mru_0313	mru_0313	Phage-related protein	3.40	2.83
	mru_0308	mru_0308	Phage-related protein	3.48	3.46
	mru_0324	mru_0324	Type II restriction enzyme, methylase subunit	1.88	5.99
CRISPR-associated genes	- mru_0798	mru_0798	CRISPR-associated protein Cas1-1	1.93	4.09
2	- mru_1181	mru_1181	CRISPR-associated RAMP protein Csm3 family	1.03	7.23
Nitrogen metabolism					
Other	mru_2121	hcp	Hydroxylamine reductase Hcp	— 1.46	12.26
Nucleic acid metabolism	-				
Helicase	mru_0981	mru_0981	Rad3-related DNA helicase	1.09	7.97

Table 2 (continued)

Category and subcategory	ORF	Gene name	Annotated function	log2-fold change	log2 counts per 106 reads
Recombination and repair	mru_2097	recJ1	ssDNA exonuclease RecJ1	1.39	11.06
	mru_1383	mru_1383	Staphylococcal nuclease domain-containing protein	— 1.30	7.06
Protein fate					
Protein folding	mru_1511	mru_1511	Nascent polypeptide-associated complex protein	— 1.00	6.61
Protein secretion	mru_1581	mru_1581	Signal peptidase I	— 1.21	7.34
Protein synthesis					
RNA processing	mru_0589	mru_0589	NMD3 family protein	1.50	7.52
Translation factors	mru_0728	mru_0728	Peptide chain release factor aRF1	1.46	7.74
Ribosomal proteins	mru_0865	rpl5p	Ribosomal protein L5P Rpl5p	1.03	8.24
	mru_0868	rpl6p	Ribosomal protein L6P Rpl6p	1.05	7.92
	mru_2098	mru_2098	Ribosomal protein S15P Rps15p	1.19	9.21
Other	mru_0519	mru_0519	RNA-binding protein	— 1.68	8.08
	mru_1978	mru_1978	RNA-metabolising metallo-beta-lactamase	1.58	8.74
RNA processing	mru_1846	dusA2	tRNA-dihydrouridine synthase DusA2	1.06	6.58
Purines and pyrimidines					
Interconversion	mru_2104	surE1	5'-Nucleotidase SurE1	1.02	7.02
	mru_0241	nrdD	Anaerobic ribonucleoside-triphosphate reductase NrdD	1.47	11.08
Regulation					
Protein interaction	mru 1186	mru 1186	TPR repeat-containing protein	— 1.05	8.81
Transcriptional regulator			Transcriptional regulator	- 1.62	8.68
			Transcriptional regulator	— 1.55	8.56
			Transcriptional regulator ArsR family	- 1.21	7.78
	_ mru 0442	– mru 0442	Transcriptional regulator MarR family	- 1.68	4.74
Secondary metabolites	-	-			
Other	mru 0514	mru 0514	4'-Phosphopantetheinyl transferase family protein	1.26	6.32
	mru 0069	mru 0069	MatE efflux family protein	1.20	7.17
	mru 0352	mru 0352	MatE efflux family protein	1.64	6.73
NRPS	mru 0351	mru 0351	Non-ribosomal peptide synthetase	1.06	10.17
Transcription					
RNA polymerase	mru 0161	Foqr	DNA-directed RNA polymerase subunit F RpoF	1.05	9.66
Transporters		L.			
Amino acids	mru 1775	mru 1775	Amino acid ABC transporter ATP-binding protein	1.03	5.46
	mru 1776	mru 1776	Amino acid ABC transporter permease protein	1.25	4.94
Cations	mru 1861	mru 1861	Heavy metal translocating P-type ATPase	- 6.61	10.24
	mru 1706	nikD2	Nickel ABC transporter ATP-binding protein NikD2	1.15	6.54
	mru 1617	nikB1	Nickel ABC transporter permease protein NikB1	1.10	7.35
	mru 1709	nikB2	Nickel ABC transporter permease protein NikB2	1.43	7.34
	mru 1708	nikC2	Nickel ABC transporter permease protein NikC2	1.31	7.03
	mru 1710	nikA2	Nickel ABC transporter substrate-binding protein NikA2	1.14	11.86
Other	mru 0253	mru 0253	ABC transporter ATP-binding protein	1 97	7.23
outer	mru 0252	mru 0252	ABC transporter permease protein	1 71	7 40
	mru 0251	mru 0251	ABC transporter substrate-binding protein	2.06	913
	mru 0329	mru 0329	MotA/TolO/ExbB proton channel family protein	1.56	6.00
Vitamins and cofactors	11110_0525	11110_0329	Not viole, Exel proton channen anny protein	1.50	0.00
Riotin	mru 0527	bioB2	Biotin synthase BioB2	1 24	7 09
Cobalamin	mru 0520	chiM1	Cobalamin biosynthesis protein ChiM1	1.21	9.82
Coodaniin	mru 05/0	cbiN1	Cobalt transport protein ChiN1	1.18	9.02 8.30
	mru 0260	chiA1	Cobvrinic acid a c-diamide synthese ChiA1	- 1.60	8.09
	mru 1857	CVSG	Siroheme synthase CvsG	1.00	7.47
		-,	Shortenic Synthuse Cyse	1.20	1.17

Table 2 (continued)

Category and subcategory	ORF	Gene name	Annotated function	log2-fold change	log2 counts per 106 reads
Coenzyme B	mru_0385	aksA	Homocitrate synthase AksA	- 1.15	10.22
Metal-binding pterin	mru_0200	modB	Molybdate ABC transporter permease protein ModB	2.04	9.37
	mru_0201	modA	Molybdate ABC transporter substrate-binding protein ModA	2.83	10.54
Thiamine	mru_0247	thiC1	Thiamine biosynthesis protein ThiC1	— 1.18	9.24
	mru_0532	mru_0532	ThiF family protein	1.38	4.67
Others	mru_1769	nifB	Nitrogenase cofactor biosynthesis protein NifB	2.58	8.89
Unknown function					
Enzyme	mru_0455	mru_0455	Acetyltransferase	— 1.16	9.80
	mru_1758	mru_1758	Acetyltransferase	— 1.10	6.05
	mru_2170	mru_2170	Acetyltransferase	1.32	6.12
	mru_0574	mru_0574	Acetyltransferase GNAT family	— 1.92	1.81
	mru_1707	mru_1707	Acetyltransferase GNAT family	1.48	5.54
	mru_0560	mru_0560	ATPase	1.11	8.14
	mru_1613	mru_1613	SAM-dependent methyltransferase	1.58	4.18
Other	mru_0231	mru_0231	CAAX amino terminal protease family protein	— 1.09	8.53
	mru_1993	mru_1993	CBS domain-containing protein	— 1.65	10.72
	mru_1994	mru_1994	CBS domain-containing protein	— 1.31	11.57
	mru_0474	mru_0474	HD domain-containing protein	1.33	7.47
	mru_1034	mru_1034	HEAT repeat-containing protein	2.35	8.75
	mru_2109	mru_2109	Methanogenesis marker protein 12	— 1.01	7.90
	mru_0562	mru_0562	PP-loop family protein	1.59	7.50
	mru_1678	mru_1678	Redox-active disulfide protein	1.51	7.12
	mru_0561	mru_0561	Von Willebrand factor type A domain-containing protein	1.33	8.52
	mru_1510	mru_1510	YhgE/Pip-like protein	- 1.31	8.45
	mru_0627	mru_0627	ZPR1 zinc-finger domain-containing protein	2.04	6.70

 C_{12} -treated cultures were compared to DSMO-exposed control cultures (significant change with \log_2 fold changes < 1 and > 1 and a false discovery rate < 0.05). The list does not include the 71 regulated hypothetical proteins. The *M. ruminantium* (mru) open reading frame (ORF) codes are adopted from the Kyoto Encyclopedia of Genes and Genomes

involved in translation or post-translational modification were down-regulated, e.g. a staphylococcal nuclease domain-containing protein (mru_1383), a nascent polypeptide-associated complex protein (mru_1511), an RNA-binding protein (mru_0519) and a signal peptidase (mru_1581).

Conclusion

The transcriptional response of *M. ruminantium* to the fatty acid C_{12} does not involve repression of specific pathway such as the methanogenesis pathway. Instead, it implies that C_{12} provokes broad transcriptional changes, and targets primarily cell surface associated adhesion-like

proteins, phage-related proteins, and transmembrane proteins. How this response affects methanogens remains unclear. Future studies may investigate how different dosages of and prolonged exposure to C_{12} affect gene and protein expression and survival of *M. ruminantium*.

Limitations

One limitation of our study is the low number of replicates per group. In addition, only one dosage of C_{12} was tested and samples for RNA sequencing were collected only at one time point; this precludes generalization to situations where C_{12} affects $M\!\!\!\!$. ruminantium stronger or weaker.

Additional files

Additional file 1: Table S1. *M. ruminantium* M1 genes with significantly changed expression of genes in the DMSO control as compared to the blank group (\log_2 -fold change < 1 and > 1, false discovery rate < 0.05). The list does not include the 59 regulated hypothetical proteins. The *M. ruminantium* (mru) open reading frame (ORF) codes are adopted from the Kyoto Encyclopedia of Genes and Genomes.

Additional file 2: Table S2. *M. ruminantium* M1 genes with significantly changed expression of genes in the cultures exposed to C_{12} + DMSO as compared to the blank group (log₂-fold change < 1 and > 1, false discovery rate < 0.05). The list does not include the 15 regulated hypothetical proteins. The *M. ruminantium* (mru) open reading frame (ORF) codes are adopted from the Kyoto Encyclopedia of Genes and Genomes.

Additional file 3: Figure S1. Venn diagram indicates the number of differentially expressed genes between the experimental groups and the common overlapping differentially expressed genes. TC: treatment (C₁₂ + DMSO) vs. control (DMSO); TB: treatment (C₁₂ + DMSO) vs. untreated blank; CB: control (DMSO) vs. untreated blank. It should be kept in mind that it is not possible to distinguish between the DMSO and the C12 effect in the dataset comparing the treatment and the blank samples, and that the C_{12} effect is much better studied in the TC comparison (C12 + DMSO vs DMSO). The DMSO effect can be partial quenched by the C12 effect, so genes regulated in CB and TC are not necessarily regulated in the TB. The 26 common genes differentially expressed in M. ruminantium exposed to DMSO or DMSO + C_{12} compared to the untreated blank control are outlined in the tables on the right side. The 35 overlapping differentially expressed genes of the TC and CB comparisons are outlined in the table on the left side. The diagram was generated using the online tool at bioinformatics.psb.ugent.be/webtools/Venn/.

Abbreviations

MCFA: medium-chain fatty acids; C_{12} : lauric acid; DMSO: dimethyl sulfoxide; CH_{a} : methane.

Authors' contributions

XZ participated in designing the study, performed the data collection, and drafted the manuscript. MJAS performed the data analysis and contributed to data interpretation. SN participated in designing the study, data collection and data interpretation and revised the manuscript. AS participated in data collection and critically revised the manuscript. MK participated in designing the study and critically revised the manuscript. AB participated in designing the study, performed the sequencing experiment, wrote the methods section of the manuscript, contributed to interpretation of the data and revised the manuscript. JOZ designed the study and wrote introduction, results and discussion of the manuscript. All authors read and approved the final manuscript.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The raw data can be accessed in the NCBI Sequence Read Archive (SRA) under the series record GSE81199 at https://www.ncbi.nlm.nih.gov/geo/query/acc. cqi?acc=GSE81199.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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