

## *Supplementary material*

### **Food-like growth conditions support production of active vitamin B12 by *Propionibacterium freudenreichii* without DMBI or cobalt supplementation.**

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#### **16S rDNA sequence analysis**

The *P. freudenreichii* strain 2067kk (Valio Ltd) isolated from dairy environment was subjected to genotypic analysis by the partial sequencing of a 500 bp internal fragment (nucleotides 27–518) of the 16S rDNA gene. The strain was cultured in 10 mL of PPA medium at 30°C for four days under microaerophilic conditions. Cells were harvested by centrifugation (3220 g, 10 min, 4°C) and washed once with ice-cold 50mM TRIS pH 8.0. Cells were disrupted with glass beads ( $\leq 106 \mu\text{m}$  in diameter; Sigma) in a Fast Prep-24 homogeniser (MP Biomedicals, France) in three 30-seconds 6,5 m/s cycles with one-minute cooling on ice between the cycles. PCR was performed from cell lysates using the universal 16S rDNA primers p8F (5'AGAGTTTGATCCTGGCTCAG3') and pfRev (5'ACGAGCTGACGACAGCCATG3') (Edwards et al., 1989), and the PCR products were sequenced using a BigDye terminator cycle sequencing kit with the primer pdRev (5'GTATTACCGCGGCTGCTG3'), in the DNA sequencing laboratory of the Institute of Biotechnology, University of Helsinki, Helsinki, Finland. Sequencing reactions were run on an ABI 3700 automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The sequence data was compared to all 16S-rRNA sequences of bacteria in the gene bank, using the BlastN tool (<http://www.ncbi.nlm.nih.gov/BLAST/>). The Blast search using the obtained sequence as query revealed 100% identity with the annotated *Propionibacterium freudenreichii* subsp. *shermanii* CIRM-BIA (Figure S1) 16S rDNA gene sequence (Falentin et al. 2010), while 99% identity to the sequence from the type strain of *Propionibacterium freudenreichii* subsp. *freudenreichii* DSM 20271 (Koskinen et al. 2015) was found and therefore we concluded that the identity of the selected strain is *P. freudenreichii* subsp. *shermanii*.

Score	Expect	Identities	Gaps	Strand
1421 bits(769)	0.0	769/769(100%)	0/769(0%)	Plus/Plus
Query 1		CCCCGTGGAAGGGACCCACACCTAGTACCCACCGTTTACGGCGTGGACTACCAGGGTATC		60
Sbjct 1539504		CCCCGTGGAAGGGACCCACACCTAGTACCCACCGTTTACGGCGTGGACTACCAGGGTATC		1539563
Query 61		TAAGCCTGTTTGCTCCCCACGCTTTCGCTTCTCAGCGTCAGGAAATGTCCAGAGAACCGC		120
Sbjct 1539564		TAAGCCTGTTTGCTCCCCACGCTTTCGCTTCTCAGCGTCAGGAAATGTCCAGAGAACCGC		1539623
Query 121		CTTCGCCACTGGTGTCTTCCCGATATCTGCGCATTCACCGCTCCACCGAGAGTTCAT		180
Sbjct 1539624		CTTCGCCACTGGTGTCTTCCCGATATCTGCGCATTCACCGCTCCACCGAGAGTTCAT		1539683
Query 181		TCTCCCCTACATTCTCAAGTCAACCCGATCGAAAGCAAGCCCAGAGTTAAGCCCTGGA		240
Sbjct 1539684		TCTCCCCTACATTCTCAAGTCAACCCGATCGAAAGCAAGCCCAGAGTTAAGCCCTGGA		1539743
Query 241		ATTTCACTTCCGACGTGATCAACCGCCTACAAGCTCTTTACGCCAATAATTCCGGACAA		300
Sbjct 1539744		ATTTCACTTCCGACGTGATCAACCGCCTACAAGCTCTTTACGCCAATAATTCCGGACAA		1539803
Query 301		CGCTCGCACCCCTACGTATCACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTCTTCTCC		360
Sbjct 1539804		CGCTCGCACCCCTACGTATCACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTCTTCTCC		1539863
Query 361		CACTACCGTCACTTGCCTTCGTATGGATGAAAGCGGTTTACAACCCGAAGCCGTCAT		420
Sbjct 1539864		CACTACCGTCACTTGCCTTCGTATGGATGAAAGCGGTTTACAACCCGAAGCCGTCAT		1539923
Query 421		CCCGCACGCGGCGTTGCTGCATCAGGCTTGCGCCATTGTGCAATATCCCCACTGCTGC		480
Sbjct 1539924		CCCGCACGCGGCGTTGCTGCATCAGGCTTGCGCCATTGTGCAATATCCCCACTGCTGC		1539983
Query 481		CTCCCGTAGGAGTCTGGGCGTATCTCAGTCCCAATGTGGCCGGTCACCCTCTCAGGCCG		540
Sbjct 1539984		CTCCCGTAGGAGTCTGGGCGTATCTCAGTCCCAATGTGGCCGGTCACCCTCTCAGGCCG		1540043
Query 541		GCTACCCGTCGCTGCCTTGGTAGGCCATTACCCACCAACAAGCTGATAGGCCGCGAGAC		600
Sbjct 1540044		GCTACCCGTCGCTGCCTTGGTAGGCCATTACCCACCAACAAGCTGATAGGCCGCGAGAC		1540103
Query 601		GATCCCTGACCGCATAAAGCTTTCCAACCCAACCCATGCAGGCCAGGCTCATATTCGGGA		660
Sbjct 1540104		GATCCCTGACCGCATAAAGCTTTCCAACCCAACCCATGCAGGCCAGGCTCATATTCGGGA		1540163
Query 661		TTACCACCAGTTTCTGGAGCTATACCGAAGTCAAGGGCACGTTCTCAGTGTACTCA		720
Sbjct 1540164		TTACCACCAGTTTCTGGAGCTATACCGAAGTCAAGGGCACGTTCTCAGTGTACTCA		1540223
Query 721		CCCGTTCGCCACTCGTGTACCCCCGAAAGGGGCCTTACCGTCCGACTT	769	
Sbjct 1540224		CCCGTTCGCCACTCGTGTACCCCCGAAAGGGGCCTTACCGTCCGACTT	1540272	

**Figure S1** The 16S rDNA sequence alignment. Query: *P. freudenreichii* 2067, Subject: *P. freudenreichii* CIRM-BIA1<sup>T</sup>

**Table S1** List of proteins detected by nano-LC-MS/MS from spots with relative abundance fold change  $\geq 1.4$  (ANOVA,  $p \leq 0.05$ ) from PPA and WBM media during exponential growth phase. Spots with single identification are in bold. From spots with multiple protein identification those with the highest Mascot identification score are indicated and marked with an asterisk. Proteins identified from the spots with fold change 1.4 are marked gray.

Spot no.	Fold change (PPA/WBM)	Mw	ID Score < 0.05	(p	No of pept.	Seq. cov. (%)	pI	Locus in reference organism	Name	Annotation	Category
1	1.5	77306	571		6	15%	5.3	PFREUD_22890	NadE	<i>Amino acid metabolism</i> glutamine-dependent NAD(+) synthetase	Metabolism of amino acids and related molecules
3	1.4	65568	592		7	20%	4.86	PFREUD_08920	LeuA1	2-isopropylmalate synthase	Metabolism of amino acids and related molecules
13	1.6	40364	213		2	11%	4.97	PFREUD_18160	SerC	phosphoserine aminotransferase	Metabolism of coenzymes and prosthetic groups
20*	-1.7	36744	387		4	20%	5.16	PFREUD_20100	ASADH	aspartate-semialdehyde dehydrogenase	Metabolism of amino acids and related molecules
12	2.1	39475	304		8	24%	4.77	PFREUD_19880	Ino1	inositol-1-phosphate synthase	<i>Carbohydrate metabolism</i> <i>Inositol metabolism</i> Specific carbohydrate metabolic pathway
21*	-1.7	38657	323		6	19%	4.84	PFREUD_19010	IoIE	2-keto-myo-inositol dehydratase	Specific carbohydrate metabolic pathway
4	2.3	63781	147		4	12%	5.71	PFREUD_16710	D-LDH	<i>Pyruvate metabolism</i> d-lactate dehydrogenase	Membrane bioenergetics
7*	1.6	54126	1831		36	78%	5.17	PFREUD_15390	Pyk1	pyruvate kinase 1	Main glycolytic pathways
1*	1.5	80242	1411		28	53%	5.29	PFREUD_07650	MutB	<i>Wood Werkman -TCA</i> Methylmalonyl-CoA mutase large subunit	Metabolism of carbohydrates and related molecules
21	-1.7	35059	141		2	7%	4.95	PFREUD_06860	MDH	malate dehydrogenase	Metabolism of carbohydrates and related molecules
7	1.6	59283	343		4	14%	5.34	PFREUD_18870	PycB	methylmalonyl-CoA carboxyltransferase 5S subunit monomer	Metabolism of carbohydrates and related molecules
22	-2.8	35214	180		2	11%	5.37	PFREUD_02620	GalE	<i>Lactose metabolism</i> UDP-glucose 4-epimerase	Specific carbohydrate metabolic pathway
23*	-1.5	37022	584		8	32%	5.21	PFREUD_19150	Fba1	Fructose-bisphosphate aldolase II	Main glycolytic pathways
4*	2.3	59534	1731		41	73%	5.58	PFREUD_12990	GlpA	<i>Lipid metabolism</i> anaerobic glycerol-3-phosphate dehydrogenase subunit A	Specific carbohydrate metabolic pathway
9*	1.5	45884	1629		29	66%	5.27	PFREUD_12980	GlpB	anaerobic glycerol-3-phosphate dehydrogenase subunit B	Specific carbohydrate metabolic pathway

10	1.5	45884	999	11	40%	5.27	PFREUD_12980	GlpB	anaerobic glycerol-3-phosphate dehydrogenase subunit B	Specific carbohydrate metabolic pathway
10	1.5	43861	989	15	73%	5.07	PFREUD_09410	FabF	3-oxoacyl-ACP synthase	2.4 Metabolism of lipids
11*	1.5	45661	1249	16	46%	4.79	PFREUD_02430	CaiB	crotonobetainyl-CoA:carnitine CoA-transferase	2.4 Metabolism of lipid
13*	1.6	43073	1150	22	58%	4.89	PFREUD_02420	CaiA	putative acyl-CoA dehydrogenase	2.4 Metabolism of lipids
<b>14</b>	<b>1.5</b>	<b>31894</b>	<b>487</b>	<b>9</b>	<b>15%</b>	<b>4.8</b>	<b>PFREUD_09430</b>	<b>FabH</b>	<b>3-oxoacyl-ACP synthase</b>	<b>2.4 Metabolism of lipids</b>
<b>15</b>	<b>1.4</b>	<b>32914</b>	<b>1038</b>	<b>27</b>	<b>65%</b>	<b>5.4</b>	<b>PFREUD_02450</b>	<b>MaoC</b>	<b>MaoC acyl dehydratase</b>	<b>2.4 Metabolism of lipids</b>
17*	2.2	28116	396	4	34%	4.59	PFREUD_19890	MmsB	beta-hydroxyacid dehydrogenase	2.4 Metabolism of lipids
17	2.2	28117	326	8	18%	4.6	PFREUD_23160	UgpQ2	glycerophosphoryl diester phosphodiesterase	2.4 Metabolism of lipids
<i>Membrane bioenergetics</i>										
<b>5</b>	<b>1.4</b>	<b>60757</b>	<b>1691</b>	<b>33</b>	<b>65%</b>	<b>5.45</b>	<b>PFREUD_01830</b>	<b>GLS</b>	<b>Putative glutamate synthase</b>	<b>1.4 membrane bioenergetics (electron transport subunit and ATP synthase)</b>
<b>8</b>	<b>2.2</b>	<b>52579</b>	<b>1706</b>	<b>30</b>	<b>69%</b>	<b>4.67</b>	<b>PFREUD_10490</b>	<b>AtpD</b>	<b>ATP synthase subunit beta</b>	<b>1.4 Membrane bioenergetics</b>
<b>16</b>	<b>2.3</b>	<b>29633</b>	<b>676</b>	<b>12</b>	<b>51%</b>	<b>4.54</b>	<b>PFREUD_02490</b>	<b>FixB</b>	<b>electron transfer flavoprotein FixB</b>	<b>1.4 Membrane bioenergetics</b>
<i>Transport proteins</i>										
3	1.4	62791	157	2	6%	4.9	PFREUD_07970	<u>ChvD</u>	ABC transporter ATP-binding protein	1.2 Transport/binding proteins and lipoproteins
22*	-2.8	30956	577	17	43%	5.88	PFREUD_01620	SalX	ABC transporter ATP-binding protein	1.2 Transport/binding proteins and lipoproteins
<i>Protein synthesis</i>										
<b>2</b>	<b>1.5</b>	<b>64546</b>	<b>709</b>	<b>9</b>	<b>22%</b>	<b>5.16</b>	<b>PFREUD_11940</b>	<b>GlnRS</b>	<b>glutaminyl-tRNA synthetase</b>	<b>Aminoacyl-tRNA synthetases</b>
3*	1.4	64150	1792	24	47%	4.83	PFREUD_14690	ProRS	prolyl-tRNA synthetase	Aminoacyl-tRNA synthetases
3	1.4	76982	445	6	14%	4.83	PFREUD_05640	EF-G	elongation factor G	Translation elongation
<b>6</b>	<b>1.5</b>	<b>56027</b>	<b>274</b>	<b>2</b>	<b>20%</b>	<b>5.79</b>	<b>PFREUD_13090</b>	<b>MiaB1</b>	<b>2-methylthioadenine synthetase</b>	<b>Translation elongation</b>
10*	1.5	35694	1472	18	71%	5.12	PFREUD_14780	RpsB	30S ribosomal protein S2	Ribosomal proteins
11	1.5	46219	819	7	38%	4.82	PFREUD_14130	CysRS1	cysteinyl-tRNA synthetase	Aminoacyl-tRNA synthetases
11	1.5	39306	233	2	14%	4.78	PFREUD_10360	PrfA	peptide chain release factor 1	Nonribosomal protein synthesis
17	2.2	20647	318	4	20%	4.66	PFREUD_11510	EF-P	elongation factor P	Translation elongation
<i>Peptidases</i>										
10	1.5	44572	590	6	22%	5.23	PFREUD_18560	AbgB	peptidase, M20/M25/M40 family	Metabolism of amino acids and related molecules
10	1.5	45372	112	2	5%	5.14	PFREUD_16940	ApeB	aminopeptidase	Protein degradation
<i>Stress proteins</i>										
1	1.5	94317	1003	15	16%	5.33	PFREUD_17920	ClpB2	ATP-dependent Clp protease B2	Protein folding
4	2.3	56504	203	5	13%	4.72	PFREUD_18470	GroL2	molecular chaperone GroEL	Protein folding
19	-1.5	93458	3811	61	71%	5.07	PFREUD_19250	ClpB1	ATP-dependent Clp protease B1	Protein folding
23	-1.5	33620	362	4	22%	5.14	PFREUD_16420	Cys2	cysteine synthase	Metabolism of amino acids and related molecules
<b>24</b>	<b>-1.5</b>	<b>33620</b>	<b>903</b>	<b>16</b>	<b>58%</b>	<b>5.14</b>	<b>PFREUD_16420</b>	<b>Cys2</b>	<b>cysteine synthase</b>	<b>Metabolism of amino acids and related molecules</b>
<b>25</b>	<b>-1.9</b>	<b>33620</b>	<b>1557</b>	<b>34</b>	<b>82%</b>	<b>5.14</b>	<b>PFREUD_16420</b>	<b>Cys2</b>	<b>cysteine synthase</b>	<b>Metabolism of amino acids and related molecules</b>

<b>26</b>	<b>-1.5</b>	<b>22619</b>	<b>392</b>	<b>4</b>	<b>26%</b>	<b>5.28</b>	<b>PFREUD_06110</b>	<b>SodA</b>	<b>Superoxide dismutase</b>	<b>Miscellaneous</b>
<b>27</b>	<b>-1.5</b>	<b>22619</b>	<b>611</b>	<b>16</b>	<b>47%</b>	<b>5.28</b>	<b>PFREUD_06110</b>	<b>SodA</b>	<b>Superoxide dismutase</b>	<b>Miscellaneous</b>
28*	-2.4	22828	719	13	68%	5.28	PFREUD_06110	SodA	superoxide dismutase	Miscellaneous
									<b><i>B12 biosynthesis</i></b>	
13	1.6	35968	740	12	49%	4.89	PFREUD_18910	HemB	delta-aminolevulinic acid dehydratase	Metabolism of coenzymes and prosthetic groups
									<b><i>Miscellaneous</i></b>	
<b>18</b>	<b>1.6</b>	<b>23335</b>	<b>530</b>	<b>7</b>	<b>44%</b>	<b>5.02</b>	<b>PFREUD_23970</b>	<b><u>SDR</u></b>	<b>Oxidoreductase</b>	<b>Miscellaneous</b>
20	-1.7	40589	147	2	8%	5.34	PFREUD_19130	AdhC	zinc-containing alcohol dehydrogenase	Miscellaneous
									<b><i>Other</i></b>	
9	1.5	46935	174	2	8%	5.3	PFREUD_19280	PurA	adenylosuccinate synthetase	Metabolism of nucleotides and nucleic acids
17	2.2	29040	179	2	15%	4.81	PFREUD_03380	ThiM	Hydroxyethylthiazole kinase	Metabolism of coenzymes and prosthetic groups
20	-1.7	39350	256	3	22%	5.19	PFREUD_21640	<u>CysD</u>	sulfate adenylyltransferase	Metabolism of sulfur
28	-2.4	19697	174	2	15%	5.24	PFREUD_23460	SSB	ssDNA-binding protein	DNA replication
12*	2.1	40517	1261	30	80%	4.75	PFREUD_16140	PF47	protein mrp (ATP-binding protein involved in chromosome partitioning )	Adaptation to atypical conditions

**Table S2** List of proteins detected by nano-LC-MS/MS from spots with relative abundance fold-change  $\geq 1.5$  (ANOVA,  $p \leq 0.05$ ) from PPA and SWP media during stationary growth phase. Spots with single identification are in bold. From spots with multiple protein identification those with the highest Mascot identification score are indicated and marked with an asterisk

Spot no.	Fold-change (PPA/SWP)	Mw (Da)	pI	ID score	No. match. pept.	Seq. cov. (%)	Locus in reference organism	Name	Annotation	Category
<b><i>Amino acid metabolism</i></b>										
44*	2.3	52727	5.17	620	7	20	PFREUD_08280	ThrC	threonine synthase	Metabolism of amino acids and related molecules
44	2.3	52087	5.28	274	3	9	PFREUD_18740	GlyA	glycine hydroxymethyltransferase	Metabolism of amino acids and related molecules
48	1.5	48354	5.28	147	2	5	PFREUD_20440	MAO	flavin-containing amine oxidase	Metabolism of amino acids and related molecules
50*	3	39604	4.81	829	34	26	PFREUD_00370	AlaDH	alanine dehydrogenase	Cell wall
51*	2	39604	4.81	804	20	26	PFREUD_00370	AlaDH	alanine dehydrogenase	Cell wall
64*	-1.6	55513	4.95	848	11	27	PFREUD_11910	TrpE	anthranilate synthase component I	Metabolism of amino acids and related molecules
65	-1.5	52727	5.17	411	6	12	PFREUD_08280	ThrC	threonine synthase	Metabolism of amino acids and related molecules
65	-1.5	48354	5.28	201	2	5	PFREUD_20440	MAO	flavin-containing amine oxidase	Metabolism of amino acids and related molecules
71	-	43566	5.53	193	2	7	PFREUD_13960	ArgD	acetylornithine aminotransferase	Metabolism of amino acids and related molecules
73	-2.1	44997	5.13	168	3	7	PFREUD_14000	AlaT	aspartate aminotransferase	Metabolism of amino acids and related molecules
77	-1.8	36744	5.16	196	2	11	PFREUD_20100	ASADH	aspartate-semialdehyde dehydrogenase	Metabolism of amino acids and related molecules
88	-1.6	30334	5.02	659	11	38	PFREUD_11900	TrpC	indole-3-glycerol phosphate synthase	Metabolism of amino acids and related molecules
88	-1.6	30601	4.85	128	2	4	PFREUD_16230	LDC	lysine decarboxylase	Metabolism of amino acids and related molecules
<b><i>Aspartate metabolism</i></b>										
43*	3.6	53385	5.14	825	62	25	PFREUD_16320	AspA1	aspartate ammonia-lyase	Metabolism of amino acids and related molecules
43	3.6	53526	5.12	748	48	26	PFREUD_16330	AspA2	aspartate ammonia-lyase	Metabolism of amino acids and related molecules
45*	2.4	53526	5.12	805	27	28	PFREUD_16330	AspA2	aspartate ammonia-lyase	Metabolism of amino acids and related molecules

45	2.4	53385	5.14	776	28	25	PFREUD_16320	AspA1	aspartate ammonia-lyase	Metabolism of amino acids and related molecules
45	2.4	52812	4.99	145	2	6	PFREUD_01460	ArgG	argininosuccinate synthase	Metabolism of amino acids and related molecules
65	-1.5	48951	5.17	777	9	23	PFREUD_13940	ArgH	argininosuccinate lyase	Metabolism of amino acids and related molecules
<b>Valine leucine and isoleucine biosynthesis</b>										
76*	-2.1	37335	5.01	819	15	46	PFREUD_13390	IlvC	ketol-acid reductoisomerase	Metabolism of amino acids and related molecules
76	-2.1	40036	5.02	259	3	15	PFREUD_13390	IlvE	branched-subunit amino acid aminotransferase	Metabolism of amino acids and related molecules
<b>Carbohydrate metabolism</b>										
<b>Pentose phosphate</b>										
32	2	97159	5.61	239	2	8	PFREUD_12930	Phk	phosphoketolase pyrophosphate	Main glycolytic pathways
33	3	97159	5.61	430	7	10	PFREUD_12930	Phk	phosphoketolase pyrophosphate	Main glycolytic pathways
34	4.2	97159	5.61	650	7	14	PFREUD_12930	Phk	phosphoketolase pyrophosphate	Main glycolytic pathways
44	2.3	55556	5.21	538	9	20	PFREUD_00760	XylB	xylulokinase	Metabolism of carbohydrates and related molecules
65	-1.5	52863	5.25	915	14	20	PFREUD_08090	Gnd2	6-phosphogluconate dehydrogenase	Main glycolytic pathways
66*	-1.5	52863	5.25	1117	14	25	PFREUD_08090	Gnd2	6-phosphogluconate dehydrogenase	Main glycolytic pathways
74*	-1.8	38056	4.84	651	10	36	PFREUD_04970	Tal1	transaldolase	Main glycolytic pathways
87	-3.6	32249	5.4	477	4	31	PFREUD_15070	OpcA	glucose 6-phosphate dehydrogenase effector OpcA	Main glycolytic pathways
69	-1.7	42603	5.44	200	2	10	PFREUD_00690	AckA	acetate kinase	Metabolism of carbohydrates and related molecules
71	-	42603	5.44	196	3	10	PFREUD_00690	AckA	acetate kinase	Metabolism of carbohydrates and related molecules
<b>Glycolysis</b>										
35	1.9	96275	4.81	1272	12	28	PFREUD_03230	PPDK	pyruvate phosphate dikinase	Metabolism of carbohydrates and related molecules
50	3	43333	4.94	798	11	35	PFREUD_12040	PFP	pyrophosphate phosphofructokinase	Carbohydrate metabolism
51	2	43333	4.94	531	8	27	PFREUD_12040	PFP	pyrophosphate phosphofructokinase	Carbohydrate metabolism
62	-1.9	63416	4.88	417	6	16	PFREUD_23740	AgIA	alpha-1,4-glucosidase	Specific carbohydrate metabolic pathway (galactose)
63	-	58031	5.05	265	2	9	PFREUD_19470	PtsA	PTS enzyme I	Protein of unknown function, without similarity to other proteins (carbohydrate transport and metabolism)
67*	-1.8	42524	5.21	1217	23	48	PFREUD_15120	PGK	phosphoglycerate kinase	Main glycolytic pathways

68	-1.5	42524	5.21	251	3	14	PFREUD_15120	PGK	phosphoglycerate kinase	Main glycolytic pathways
74	-1.8	34225	4.91	413	5	19	PFREUD_12840	L-LDH	L-lactate dehydrogenase	Main glycolytic pathways
<b>75</b>	<b>-3</b>	<b>34225</b>	<b>4.91</b>	<b>661</b>	<b>15</b>	<b>31</b>	<b>PFREUD_12840</b>	<b>L-LDH</b>	<b>L-lactate dehydrogenase</b>	<b>Main glycolytic pathways</b>
77*	-1.8	36261	5.26	1401	48	65	PFREUD_15130	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Metabolism of coenzymes and prosthetic groups
<b>78</b>	<b>-1.6</b>	<b>35532</b>	<b>5.17</b>	<b>609</b>	<b>8</b>	<b>27</b>	<b>PFREUD_15130</b>	<b>GAPDH</b>	<b>glyceraldehyde-3-phosphate dehydrogenase</b>	<b>Metabolism of coenzymes and prosthetic groups</b>
79*	-1.8	35532	5.17	667	7	34	PFREUD_15130	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Metabolism of coenzymes and prosthetic groups
79	-1.8	35795	XX	XX	11	33	PFREUD_02200	BkdA2	2-oxoisovalerate dehydrogenase subunit beta	Metabolism of amino acids and related
80	-2.1	32398	5.51	415	5	25	PFREUD_22330	<u>YeaD</u>	aldose 1-epimerase	Metabolism of carbohydrates and related molecules
<b>81</b>	<b>-2</b>	<b>32368</b>	<b>5.02</b>	<b>582</b>	<b>8</b>	<b>34</b>	<b>PFREUD_23890</b>	<b>FBA2</b>	<b>Fructose-bisphosphate aldolase class I</b>	<b>Main glycolytic pathways</b>
82*	-2.4	37022	5.21	525	10	27	PFREUD_19150	FBA1	Fructose-bisphosphate aldolase class II	Main glycolytic pathways
83	-3.1	37022	5.21	180	2	8	PFREUD_19150	FBA1	Fructose-bisphosphate aldolase class II	Main glycolytic pathways
84*	-1.6	37022	5.14	861	24	16	PFREUD_19150	FBA1	Fructose-bisphosphate aldolase class II	Main glycolytic pathways
84	-1.6	35795	5.21	174	2	38	PFREUD_02200	BkdA2	2-oxoisovalerate dehydrogenase subunit beta	Metabolism of amino acids and related
87*	-3.6	35532	5.17	1032	10	54	PFREUD_15130	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Metabolism of coenzymes and prosthetic groups
88	-1.6	32368	5.02	521	6	29	PFREUD_23890	FBA2	Fructose-bisphosphate aldolase class I	Main glycolytic pathways
89*	-1.5	27830	5.05	424	5	26	PFREUD_15110	TIM1	triosephosphate isomerase 1	Main glycolytic pathways
<b>Wood Werkman-TCA</b>										
<b>29</b>	<b>1.6</b>	<b>137778</b>	<b>5.22</b>	<b>2689</b>	<b>40</b>	<b>33</b>	<b>PFREUD_01840</b>	<b>NifJ1</b>	<b>pyruvate synthase/pyruvate-flavodoxin oxidoreductase</b>	<b>Membrane bioenergetics</b>
<b>30</b>	<b>1.9</b>	<b>137778</b>	<b>5.22</b>	<b>2525</b>	<b>59</b>	<b>34</b>	<b>PFREUD_01840</b>	<b>NifJ1</b>	<b>pyruvate synthase/pyruvate-flavodoxin oxidoreductase</b>	<b>Membrane bioenergetics</b>
<b>31</b>	<b>1.7</b>	<b>137778</b>	<b>5.22</b>	<b>3124</b>	<b>71</b>	<b>42</b>	<b>PFREUD_01840</b>	<b>NifJ1</b>	<b>pyruvate synthase/pyruvate-flavodoxin oxidoreductase</b>	<b>Membrane bioenergetics</b>
<b>36</b>	<b>1.7</b>	<b>96275</b>	<b>4.81</b>	<b>2351</b>	<b>50</b>	<b>39</b>	<b>PFREUD_03230</b>	<b>PPDK</b>	<b>pyruvate phosphate dikinase</b>	<b>Metabolism of carbohydrates and related molecules</b>
<b>39</b>	<b>2.4</b>	<b>75276</b>	<b>6</b>	<b>1488</b>	<b>36</b>	<b>36</b>	<b>PFREUD_14311</b>	<b>SdhA3</b>	<b>succinate dehydrogenase flavoprotein subunit</b>	<b>Membrane bioenergetics</b>
40*	1.9	75276	6	1227	21	31	PFREUD_14310	SdhA3	succinate dehydrogenase flavoprotein subunit	Membrane bioenergetics
41	1.7	66057	5.22	373	4	10	PFREUD_18860	MmdA	methylmalonyl-CoA carboxyltransferase subunit 12S monomer	Specific carbohydrate metabolic pathway



45	2.4	53022	5	200	1	6	PFREUD_19100	lolA	myo-inositol catabolism protein lolA	Specific carbohydrate metabolic pathway
45	2.4	57987	5.97	184	2	6	PFREUD_18870	<u>PycB</u>	methylmalonyl-CoA carboxyltransferase 5S subunit monomer	Metabolism of carbohydrates and related molecules
46*	2	49814	5.08	483	5	22	PFREUD_10890	LPD	dihydrolipoamide dehydrogenase	Metabolism of carbohydrates and related molecules
48*	1.5	51435	5.34	897	56	39	PFREUD_16300	FumC	fumarate hydratase class II	TCA cycle
49	1.6	48580	5.67	276	3	14	PFREUD_01090	GitA2	citrate synthase	TCA cycle
53	1.9	27939	5.42	117	0	9	PFREUD_09260	SdhB	succinate dehydrogenase subunit B	Membrane bioenergetics
55*	2.8	27939	5.42	269	3	18	PFREUD_09260	SdhB	succinate dehydrogenase subunit B	Membrane bioenergetics
64	-1.6	49814	5.08	466	7	19	PFREUD_10890	LPD	dihydrolipoamide dehydrogenase	Metabolism of carbohydrates and related molecules
65	-1.5	51435	5.34	310	2	17	PFREUD_16300	FumC	fumarate hydratase class II	TCA cycle
66	-1.5	51435	5.34	420	5	18	PFREUD_16300	FumC	fumarate hydratase class II	TCA cycle
67	-1.8	47181	5.29	628	6	24	PFREUD_09540	GabT	<b>4-aminobutyrate aminotransferase</b>	Metabolism of amino acids and related molecules
68	-1.5	47181	5.29	719	9	29	PFREUD_09540	GabT	<b>4-aminobutyrate aminotransferase</b>	Metabolism of amino acids and related molecules
80	-2.1	31447	5.22	504	5	28	PFREUD_16110	CitE	citrate lyase subunit beta	TCA cycle
87	-3.6	31447	5.22	220	2	9	PFREUD_16110	CitE	citrate lyase subunit beta	TCA cycle
<b>Lipid metabolism</b>										
51	2	45661	4.79	123	2	4	PFREUD_02430	CaiB	crotonobetainyl-CoA:carnitine CoA-transferase	Metabolism of lipids
56	2	25770	4.72	132	2	8	PFREUD_02480	FixA	electron transfer flavoprotein FixA	Membrane bioenergetics (electron transport subunit and ATP synthase)
61*	-2.3	61241	5.08	806	9	24	PFREUD_23780	CaiC	Fatty-acyl-CoA synthase	Metabolism of lipids
<b>Metabolism of coenzymes and prosthetic groups</b>										
52	1.5	39443	5.22	176	2	8	PFREUD_06270	PanK	pantothenate kinase	Metabolism of coenzymes and prosthetic groups
52	1.5	41862	5.19	175	2	7	PFREUD_10010	BioB	biotin synthase	Metabolism of coenzymes and prosthetic groups
73	-2.1	41862	5.19	161	2	7	PFREUD_10010	BioB	biotin synthase	Metabolism of coenzymes and prosthetic groups
77	-1.8	36833	5.26	473	7	25	PFREUD_07540	MenB	naphthoate synthase	Metabolism of coenzymes and prosthetic groups
82	-2.4	31387	5.14	181	2	11	PFREUD_15040	PdxS	pyridoxal biosynthesis lyase PdxS	Metabolism of coenzymes and prosthetic groups
88*	-1.6	31387	5.14	696	12	34	PFREUD_15040	PdxS	pyridoxal biosynthesis lyase PdxS	Metabolism of coenzymes and prosthetic groups

88	-1.6	32235	5.05	291	3	19	PFREUD_12810	KPR	2-dehydropantoate 2-reductase	Metabolism of coenzymes and prosthetic groups
83	-3.1	30812	5.23	184	2	11	PFREUD_06840	FoID	methylenetetrahydrofolate dehydrogenase (bifunctional protein FoID)	Metabolism of coenzymes and prosthetic groups
84	-1.6	30812	5.29	177	2	14	PFREUD_06840	FoID	methylenetetrahydrofolate dehydrogenase (bifunctional protein FoID)	Metabolism of coenzymes and prosthetic groups
<b>Membrane bioenergetics</b>										
32*	2	10170 7	5.68	1185	22	22	PFREUD_04320	<u>GlcD/</u> <u>GlpC</u>	FAD-linked oxidase multidomain	Membrane bioenergetics (electron transport subunit and ATP synthase)
33*	3	10170 7	5.68	1780	36	28	PFREUD_04320	<u>GlcD/</u> <u>GlpC</u>	FAD-linked oxidase multidomain	Membrane bioenergetics (electron transport subunit and ATP synthase)
34*	4.2	10170 7	5.68	1727	38	29	PFREUD_04320	<u>GlcD/</u> <u>GlpC</u>	FAD-linked oxidase multidomain	Membrane bioenergetics (electron transport subunit and ATP synthase)
41*	1.7	60757	5.45	1613	41	47	PFREUD_01830	<u>GLS</u>	Putative glutamate synthase	Membrane bioenergetics
42	1.9	60757	5.45	1966	67	62	PFREUD_01830	GLS	<b>Putative glutamate synthase</b>	<b>Membrane bioenergetics</b>
47	1.5	47953	5.7	678	11	27	PFREUD_05140	GGR	<b>Electron transfer oxidoreductase (Geranylgeranyl reductase superfamily)</b>	<b>Membrane bioenergetics</b>
49*	1.6	49297	5.72	1432	36	48	PFREUD_05210	NuoF	NADH dehydrogenase I subunit F	Membrane bioenergetics (electron transport subunit and ATP synthase)
58	-1.8	85720	5.1	185	1	5	PFREUD_05220	NuoG	NADH dehydrogenase I subunit G	Membrane bioenergetics (electron transport subunit and ATP synthase)
63*	-	61453	5.06	1182	20	36	PFREUD_10470	AtpA	ATP synthase subunit alpha	Membrane bioenergetics (electron transport subunit and ATP synthase)
<b>Transport proteins</b>										
53	1.9	28958	5.4	281	6	18	PFREUD_14840	FepC	ABC transporter ATP-binding protein ( ABC-type cobalamin/Fe3+-siderophores transport systems)	Transport/binding proteins and lipoproteins
62	-1.9	61661	5.06	932	13	27	PFREUD_05930	DdpA	ABC transporter substrate-binding protein	Transport/binding of proteins/peptides
87	-3.6	29828	5.26	513	5	35	PFREUD_19980	PstB	phosphate ABC transporter substrate-binding protein	Transport/binding of inorganic ions
87	-3.6	31039	5.45	291	3	19	PFREUD_10060	DppF	ABC transporter ATP-binding protein ( ABC-type dipeptide/oligopeptide/nickel transport system)	Transport/binding of inorganic ions
80	-2.1	36077	5.35	172	3	8	PFREUD_07640	ArgK	kinase	Transport/binding of amino-acids

<b>Cell wall biogenesis</b>										
38	2.6	78318	4.94	180	2	5	PFREUD_19400	PBP-1A	penicillin-binding protein	Cell wall
68*	-1.5	45318	5.29	886	14	41	PFREUD_00570	UGM	UDP-galactopyranose mutase	Cell wall
74	-1.8	40727	4.85	472	5	21	PFREUD_13250	DdIA	D-alanine--D-alanine ligase	Cell wall
<b>DNA recombination and repair</b>										
52	1.5	39046	5.11	365	5	18	PFREUD_20160	DisA	DNA integrity scanning protein DisA	DNA recombination, and repair
73	-2.1	39046	5.11	398	6	18	PFREUD_20160	DisA	DNA integrity scanning protein DisA	DNA recombination, and repair
76	-2.1	36678	4.96	183	2	12	PFREUD_11700	RuvB	Holliday junction ATP-dependent DNA helicase	DNA recombination, and repair
<b>Transcription regulation</b>										
53	1.9	26294	5.33	103	1	9	PFREUD_01400	OmpR	transcriptional regulator	Transcription regulation
79	-1.8	28725	5.26	309	4	18	PFREUD_15890	PspA	phage shock protein A	Transcription regulation
88	-1.6	29867	5	570	7	37	PFREUD_23070	RpiR	RpiR family transcriptional regulator	Transcription regulation
<b>Protein synthesis</b>										
35*	1.9	90689	4.74	1610	20	33	PFREUD_14010	PheRS	phenylalanyl-tRNA synthetase subunit beta	Aminoacyl-tRNA synthetases
38*	2.6	75867	4.88	1555	35	33	PFREUD_06230	EF-G	GTP phosphohydrolase, elongation factor G	Translation elongation
<b>46</b>	<b>2</b>	<b>47518</b>	<b>4.99</b>	<b>270</b>	<b>4</b>	<b>11</b>	<b>PFREUD_01350</b>	<b>SerRS1</b>	<b>seryl-tRNA synthetase</b>	<b>Aminoacyl-RNA synthetases</b>
<b>Peptidases</b>										
44	2.3	53283	5.2	363	3	14	PFREUD_16090	PepP	Xaa-Pro aminopeptidase	Protein degradation
65*	-1.5	53283	5.2	1080	15	34	PFREUD_16090	PepP	Xaa-Pro aminopeptidase	Protein degradation
66	-1.5	53283	5.2	975	13	33	PFREUD_16090	PepP	Xaa-Pro aminopeptidase	Protein degradation
72*	-2.4	42925	5	946	22	33	PFREUD_00340	AbgB	amidohydrolase	Protein degradation
73	-2.1	39184	5.31	327	3	19	PFREUD_08810	PIP2	proline iminopeptidase	Protein degradation
74	-1.8	33462	4.8	132	1	5	PFREUD_16060	MAP	methionine aminopeptidase	Protein degradation
<b>Selenocompound metabolism</b>										
51	2	45129	4.93	593	7	20	PFREUD_22880	CBL	cystathionine beta-lyase	Metabolism of amino acids and related molecules
52	1.5	41688	5.17	283	3	13	PFREUD_18590	CGS	cystathionine gamma-synthase	Metabolism of amino acids and related molecules
62*	-1.9	62897	4.68	1023	11	33	PFREUD_06960	MetRS	methionyl-tRNA synthetase	Aminoacyl-tRNA synthetases

69*	-1.7	45374	5.44	884	11	36	PFREUD_14940	SufS	cysteine desulfurase	Transport/binding proteins and lipoproteins
71*	-	45374	5.44	946	9	36	PFREUD_14940	SufS	cysteine desulfurase	Transport/binding proteins and lipoproteins
73*	-2.1	41688	5.17	411	8	18	PFREUD_18590	CGS	cystathionine gamma-synthase	Metabolism of amino acids and related molecules
73	-2.1	39350	5.19	255	2	17	PFREUD_21640	<u>CysD</u>	sulfate adenylyltransferase	Metabolism of sulfur
<b>Pirimidine metabolism</b>										
72	-2.4	42817	5.08	396	4	14	PFREUD_11480	CarA	carbamoyl-phosphate synthase small subunit	Transport/binding of nucleosides, nucleotides, purines and pyrimidines
77	-1.8	37599	5.18	316	4	12	PFREUD_17440	PyrB	aspartate carbamoyltransferase	Metabolism of nucleotides and nucleic acids
<b>Stress proteins</b>										
<b>37</b>	<b>2.3</b>	<b>94317</b>	<b>5.33</b>	<b>2756</b>	<b>48</b>	<b>44</b>	<b>PFREUD_17920</b>	<b>ClpB2</b>	<b>ATP-dependent Clp protease B2</b>	<b>Protein folding</b>
58*	-1.8	93458	5.07	2390	38	42	PFREUD_19250	ClpB1	ATP-dependent Clp protease B1	Protein folding
<b>59</b>	<b>-2</b>	<b>93458</b>	<b>5.07</b>	<b>2766</b>	<b>46</b>	<b>50</b>	<b>PFREUD_19250</b>	<b>ClpB1</b>	<b>ATP-dependent Clp protease B1</b>	<b>Protein folding</b>
<b>60</b>	<b>-1.5</b>	<b>93458</b>	<b>5.07</b>	<b>2527</b>	<b>49</b>	<b>46</b>	<b>PFREUD_19250</b>	<b>ClpB1</b>	<b>ATP-dependent Clp protease B1</b>	<b>Protein folding</b>
68	-1.5	43958	5.3	480	7	23	PFREUD_22290	MshA	group 1 glycosyl transferase (D-inositol-3-phosphate glycosyltransferase)	Cell wall (mycothiol biosynthesis)
<b>70</b>	<b>-2</b>	<b>49226</b>	<b>5.5</b>	<b>1290</b>	<b>20</b>	<b>51</b>	<b>PFREUD_17610</b>	<b>GDH</b>	<b>glutamate dehydrogenase</b>	<b>Metabolism of amino acids and related molecules</b>
71	-	49226	5.5	120	2	4	PFREUD_17610	GDH	glutamate dehydrogenase	Metabolism of amino acids and related molecules
79	-1.8	33620	5.14	231	2	16	PFREUD_16420	Cys2	cysteine synthase A	Metabolism of amino acids and related molecules
80*	-2.1	33620	5.14	650	17	33	PFREUD_16420	Cys2	cysteine synthase A	Metabolism of amino acids and related molecules
82	-2.4	33620	5.14	418	5	21	PFREUD_16420	Cys2	cysteine synthase A	Metabolism of amino acids and related molecules
83*	-3.1	33620	5.14	522	5	35	PFREUD_16420	Cys2	cysteine synthase A	Metabolism of amino acids and related molecules
84	-1.6	33620	5.14	299	3	13	PFREUD_16420	Cys2	cysteine synthase A	Metabolism of amino acids and related molecules
<b>85</b>	<b>-3.7</b>	<b>33620</b>	<b>5.14</b>	<b>431</b>	<b>6</b>	<b>28</b>	<b>PFREUD_16420</b>	<b>Cys2</b>	<b>cysteine synthase A</b>	<b>Metabolism of amino acids and related molecules</b>
<b>86</b>	<b>-</b>	<b>33620</b>	<b>5.14</b>	<b>826</b>	<b>47</b>	<b>54</b>	<b>PFREUD_16420</b>	<b>Cys2</b>	<b>cysteine synthase A</b>	<b>Metabolism of amino acids and related molecules</b>
87	-3.6	33620	5.14	842	11	35	PFREUD_16420	Cys2	cysteine synthase A	Metabolism of amino acids and related molecules
90*	-1.7	26907	5.18	849	17	58	PFREUD_14950	SufC	ABC transporter ATPase	Transport/binding proteins and lipoproteins

<b>92</b>	<b>-1.5</b>	<b>22619</b>	<b>5.28</b>	<b>314</b>	<b>6</b>	<b>18</b>	<b>PFREUD_06110</b>	<b>SodA</b>	<b>Superoxide dismutase</b>	<b>Miscellaneous</b>
<b>93</b>	<b>-5.2</b>	<b>16830</b>	<b>4.93</b>	<b>575</b>	<b>8</b>	<b>58</b>	<b>PFREUD_09500</b>	<b>IbpA</b>	<b>Molecular chaperone (small heat shock protein)</b>	<b>Protein folding</b>
									<b><i>B</i><sub>12</sub> biosynthesis</b>	
65	-1.5	49597	5.19	764	11	31	PFREUD_19260	HemL2	glutamate-1-semialdehyde 2,1-aminomutase	<b>Metabolism of coenzymes and prosthetic groups</b>
66	-1.5	49597	5.19	949	15	37	PFREUD_19260	HemL2	glutamate-1-semialdehyde 2,1-aminomutase	<b>Metabolism of coenzymes and prosthetic groups</b>
72	-2.4	46227	5.1	216	2	11	PFREUD_18880	HemL1	glutamate-1-semialdehyde 2,1-aminomutase	<b>Metabolism of coenzymes and prosthetic groups</b>
89	-1.5	26867	4.91	404	7	34	PFREUD_07680	CbiL	CbiL. Precorrin-2 C20-methyltransferase	<b>Metabolism of coenzymes and prosthetic groups</b>
90	-1.7	25015	5.22	260	3	25	PFREUD_05110	CbiO	ABC transporter ATP-binding protein	Transport/binding proteins and lipoproteins
									<b>Miscellaneous</b>	
53*	1.9	29476	5.56	311	8	17	PFREUD_18650	GlpC	Oxidoreductase	Miscellaneous
54*	2	29476	5.56	507	14	34	PFREUD_18650	GlpC	Oxidoreductase	Miscellaneous
55	2.8	29476	5.56	156	3	9	PFREUD_18650	GlpC	Oxidoreductase	Miscellaneous
<b>57</b>	<b>1.6</b>	<b>24018</b>	<b>5.18</b>	<b>352</b>	<b>21</b>	<b>33</b>	<b>PFREUD_22440</b>	<b><u>SDR_c</u></b>	<b>Short subunit dehydrogenase (classical)</b>	<b>Miscellaneous</b>
78*	-1.6	37025	5.36	755	12	37	PFREUD_13440	<u>Kv</u>	aldo/keto reductase (voltage-dependent potassium channel beta subunit)	Miscellaneous
87	-3.6	31191	5.49	685	9	39	PFREUD_03160	<u>DkgA</u>	aldo/keto reductase (2,5-diketo-D-gluconate reductase A)	Miscellaneous
<b>91</b>	<b>-1.8</b>	<b>23335</b>	<b>5.02</b>	<b>476</b>	<b>6</b>	<b>46</b>	<b>PFREUD_23970</b>	<b><u>SDR</u></b>	<b>Oxidoreductase</b>	<b>Miscellaneous</b>
									<b>Other</b>	
40	1.9	68281	5.95	404	6	15	PFREUD_21620	CysI	Sulfite reductase [ferredoxin]	2.7 Metabolism of sulfur
50	3	42499	5.17	212	2	10	PFREUD_03730	<u>Csb1-like</u>	CRISPR-associated protein with similarity to Csb1	6. Protein of unknown function, without similarity to other proteins
51	2	43375	4.87	306	3	18	PFREUD_03120	<u>DUF1015</u>	Protein of unknown function (DUF1015)	Protein of unknown function similar to proteins from other organisms
53	1.9	27851	5.4	190	2	12	PFREUD_16080	<u>COG1579</u>	Zn-ribbon protein, possibly nucleic acid-binding [General function prediction only]	Protein of unknown function similar to proteins from other organisms
54	2	27851	5.4	439	6	36	PFREUD_16080	<u>COG1579</u>	Zn-ribbon protein, possibly nucleic acid-binding [General function prediction only]	Protein of unknown function similar to proteins from other organisms
56*	2	23227	4.7	804	34	52	PFREUD_18630	<u>COG1556</u>	Uncharacterized conserved protein [Function unknown]	Protein of unknown function similar to proteins from other organisms
61	-2.3	62746	5.05	575	7	17	PFREUD_20030	ArsA	arsenic ABC transporter ATPase	Detoxification

## Supplementary Material

74	-1.8	36506	4.78	251	2	13	PFREUD_15430	-	Protein of unknown function	Protein of unknown function similar to proteins from other organisms
76	-2.1	39568	5.13	139	2	6	PFREUD_11600	-	Protein of unknown function, highly conserved in Propionibacteria	Protein of unknown function similar to proteins from other organisms