

Supporting Information

Enhanced undecylprodigiosin production using collagen hydrolysate:

A cost-effective and high-efficiency synthesis strategy

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Section 1: Supplementary Notes 1-4

Supplementary Note 1: Effect of COH on pH value

The pH value was increased when 2% COH was added into GM medium, and the pH value in GM_2COH media at 79 h was slightly higher than that in GM media at 120 h, suggesting that COH can influence the metabolism of *Streptomyces* sp. SLL-523 (Fig. S1).

Supplementary Note 2: Morphological observation and taxonomic identification

Streptomyces sp. SLL-523 was cultured on the GYM solid medium at 30 °C to observe its phenotype characterization and cellular morphology. The strain was taxonomically identified using 16S rRNA gene sequence analysis, which was performed by PCR amplification with the primers PA (5'-CCGTCGACGAGCTCAGAGTTTGATCCTGGCTCAG-3') and PB (5'-CCCGGGTACCAAGCTTAAGGAGGTGATCCAGCCGCA-3')¹. Sequencing analysis was carried out by the Beijing Genomics Institute (Beijing, PR China). The 16S rRNA fragments from PCR were submitted to NCBI's BLAST search (www.blast.ncbi.nlm.nih.gov/Blast.cgi)^{2,3} and the EzBioCloud server (<https://www.ezbiocloud.net/>)^{4,5} to identify phylogenetic neighbours and calculate pairwise sequence similarities. A phylogenetic tree was generated with the neighbour-joining method from 1000 replicates for bootstrap analysis available in the program MEGA 11.0^{6,7}. Kimura's two-parameter model was used to calculate the evolutionary distances⁸.

The strain SLL-523 sporulated on the solid media, which had white to grey spores (Fig. S2a). Meanwhile, the large amounts of diffusible deep red to purple or blue pigments on plates were observed by naked eyes during the cultivation of the strain on the GYM solid medium. The result indicated the strain SLL-523 synthesizes abundant secondary metabolites. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SLL-523 belongs to genus *Streptomyces*, showing the highest homology to *Streptomyces anthocyanicus* NBRC 14892, with a 100% similarity (Fig. S2b). Besides, the result was further obtained recognition and support from the morphological feature examined by SEM (Fig. 2c). The air mycelium and spore filaments of the strain SLL-523 were obviously observed. Moreover, the spores were arranged in chains, and the length of spores was 1-2 μm , which showed the typical characteristics of mycelium morphology of *Streptomyces* genus.

Supplementary Note 3: Products characterization of *Streptomyces* sp. SLL-523

The red pigment was extracted from the strain SLL-523 by HCl methanol solution. Actually, undecylprodigiosin (UDP) is a liposoluble red pigment with a tri-pyrrole ring skeleton structure, showing a characteristic absorption peak around 530 nm^9 . When the red pigment extracted from the SLL-523 culture was analyzed by HPLC, the pigment mainly eluted at 14.8 min (Fig. 2d). The major peak pseudomolecular ion was observed at m/z 394.28 $[\text{M}+\text{H}]^+$, which is agreement with the $\text{C}_{25}\text{H}_{35}\text{N}_3\text{O}$ molecular formula of UDP (m/z 394.2859 $[\text{M}+\text{H}]^+$, calc.). Moreover, the LC-MS/MS analysis of the red pigment containing ions m/z 379.26, m/z 362.26,

m/z 336.21, m/z 308.18, m/z 266.13, m/z 252.11, m/z 238.10, m/z 222.10, m/z 175.09, m/z 161.07, m/z 134.10 (parent ion: m/z 394.28). All prodigiosin analogues have a common fragment ion at m/z 252, which derives from the loss of the alkyl chain¹⁰. The most intensive product ion m/z 238.1 is a result of cleavage of the aliphatic side chain. Besides, m/z 379.26 is caused by $-CH_3$ loss from UDP⁹. These results indicated that the fermentation products of *Streptomyces* sp. SLL-523 contain UDP.

Supplementary Note 4: COG analysis of *Streptomyces* sp. SLL-523

CDSs from the strain SLL-523 were analyzed and assigned to COG by EggNOG (Fig. 4b). The largest proportion of all genes belonged to the S category (Unknown function genes), which accounted for 24.53% of the entire CDSs, respectively. Genes related to transcription were the second largest COG, with 7.73% for the strain. 339 and 148 genes were assigned to category C (energy production and conversion) and H (coenzyme transport and metabolism). Especially, genes coupled with carbohydrate transport and metabolism (Category G), amino acid transport and metabolism (Category E), and secondary metabolites biosynthesis, transport and catabolism (Category Q), were accounted for 12.29% among the strain SLL-523. These results indicated that a considerable number of genes related to substances transport, substrates utilization and products synthesis were contained in the genomics of the strain SLL-523.

Section 2: Supplementary Figure S1-S7

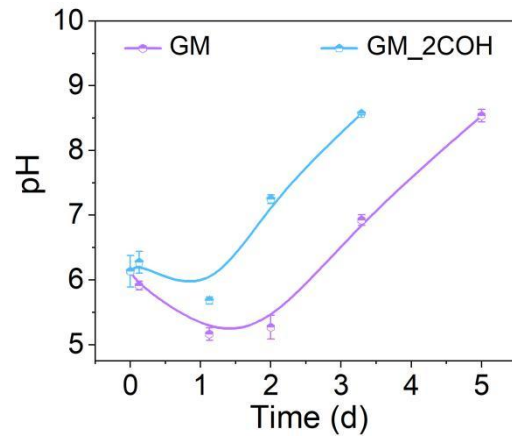


Fig. S1 pH value changes in GM and GM_2COH media over time.

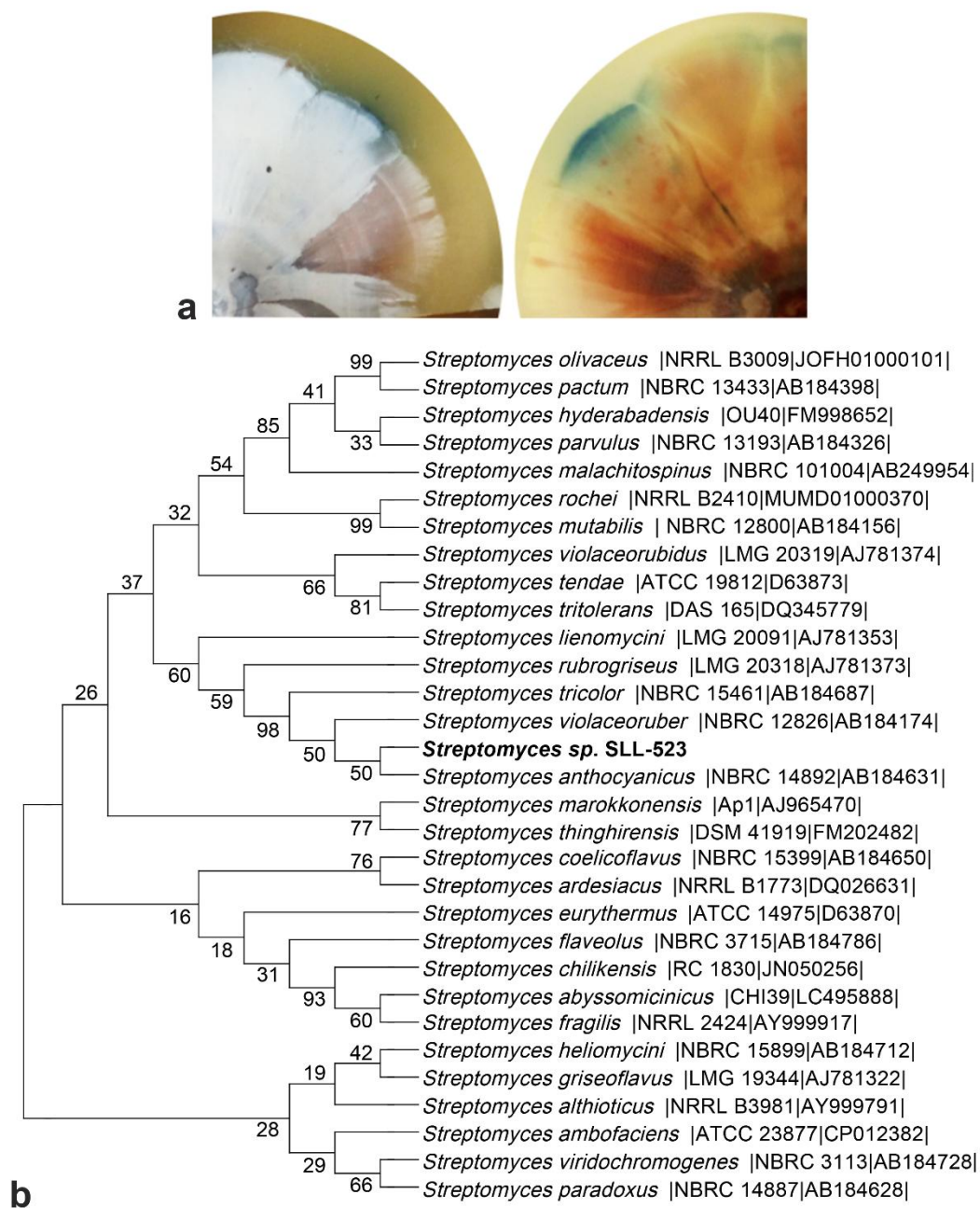


Fig. S2. Morphological observation and taxonomic identification of *Streptomyces* sp. SLL-523. (a) Phenotypes characterization of *Streptomyces* sp. SLL-523 on GYM agar medium. (b) Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between *Streptomyces* sp. SLL-523 and related taxa. Numbers at branch points are bootstrap values (percentages based on 1000 replications). Bar, 0.001 substitutions per nucleotide position.

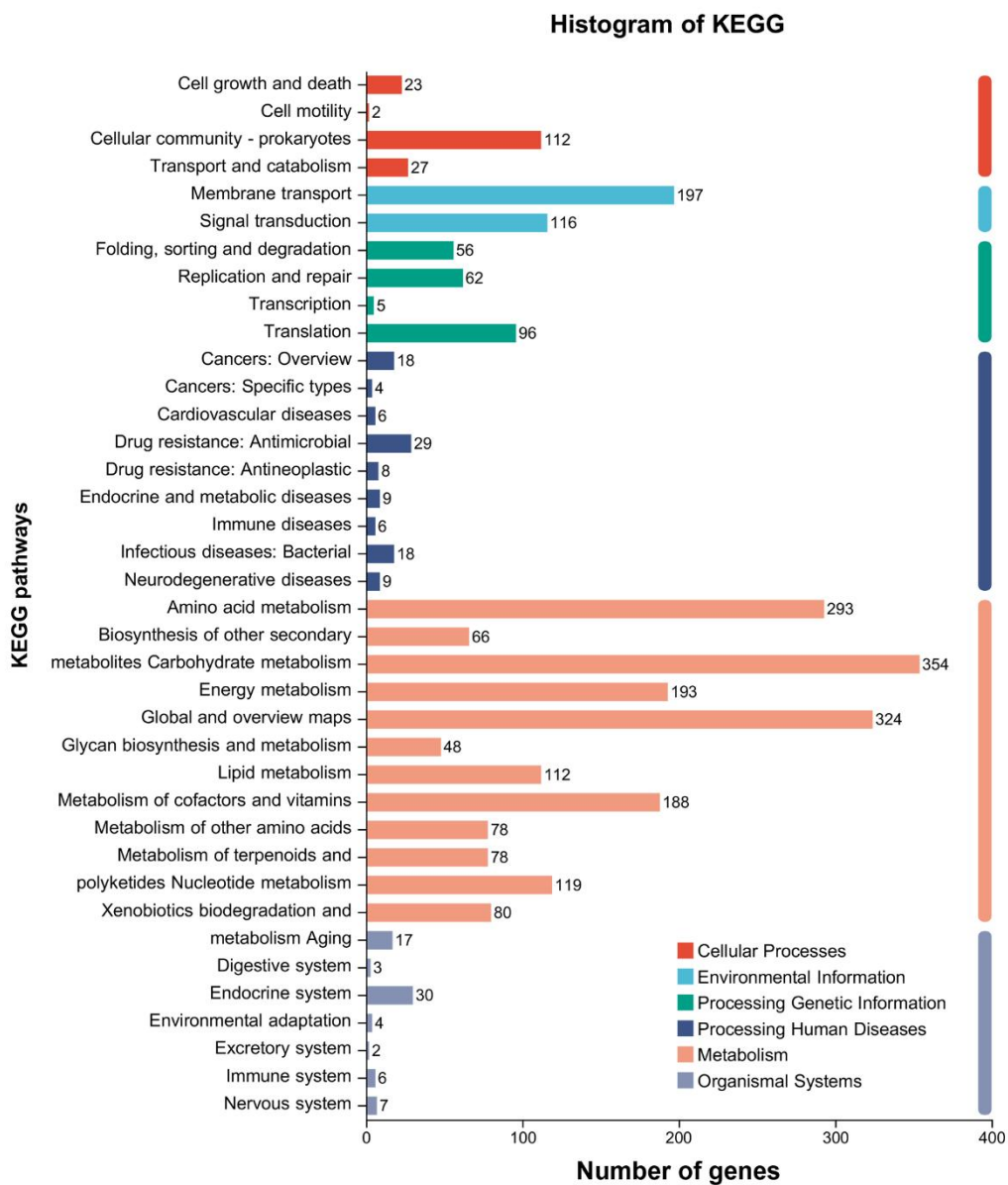


Fig. S3 KEGG clustering annotation of genes from the strain SLL-523. The ordinate represents the classification of second level KEGG pathway, and the abscissa represents the number of genes under the annotation of this classification. Different column colors represent the classification of first level KEGG pathway.

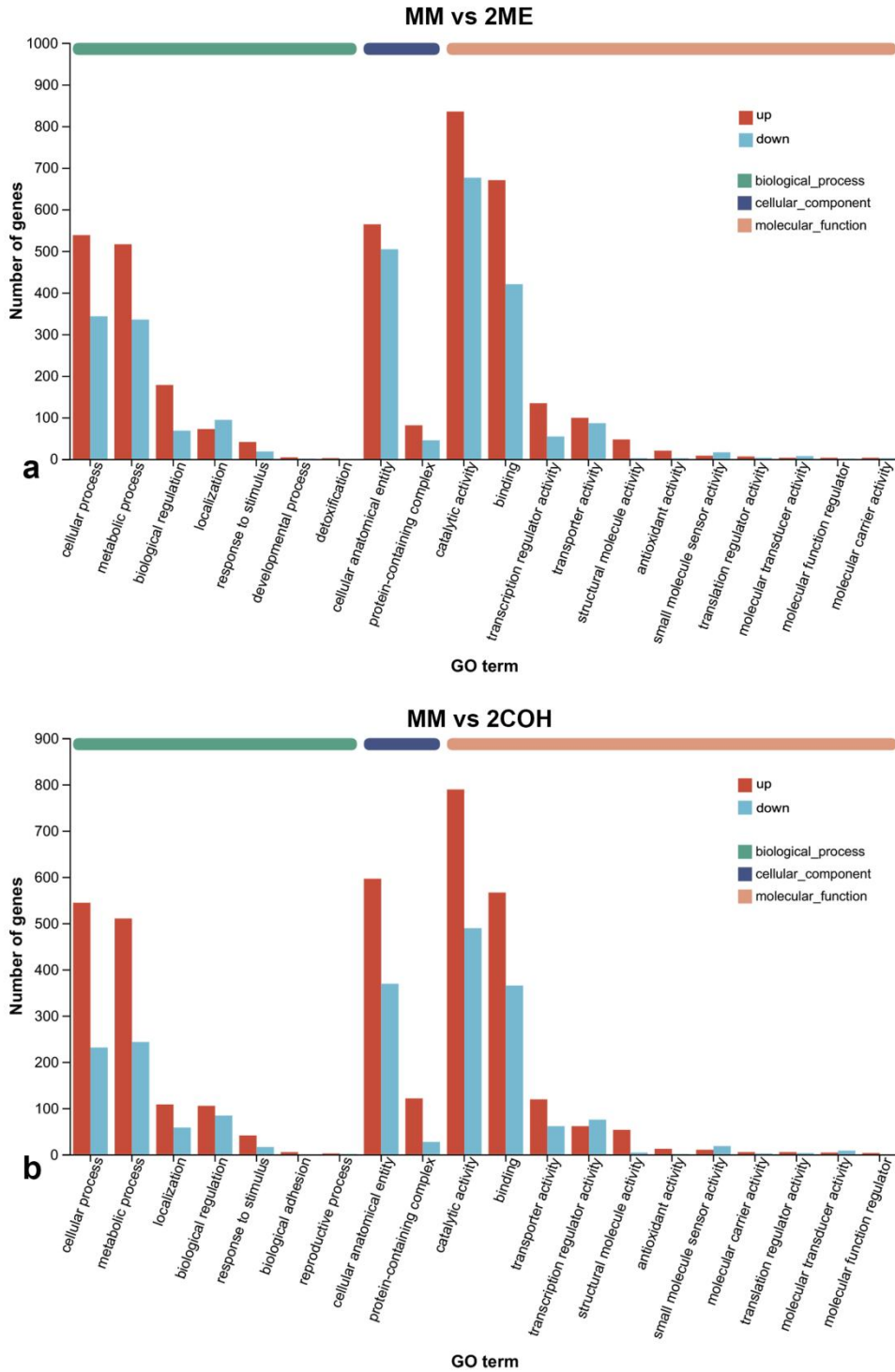


Fig. S4 Function annotation analysis of the intersection differentially expressed genes (DEGs) of *Streptomyces* sp. SLL-523. (a-b) GO annotation analysis of the DEGs in MM vs 2ME, and MM vs 2COH, respectively. The genes were assigned to three GO categories, biological process, cellular component, and molecular function. The horizontal axis indicated the top 20 ranked GO term of DEGs. The vertical axis represented the genes numbers of each sub-category of GO terms annotated successfully by GO assignment, respectively.

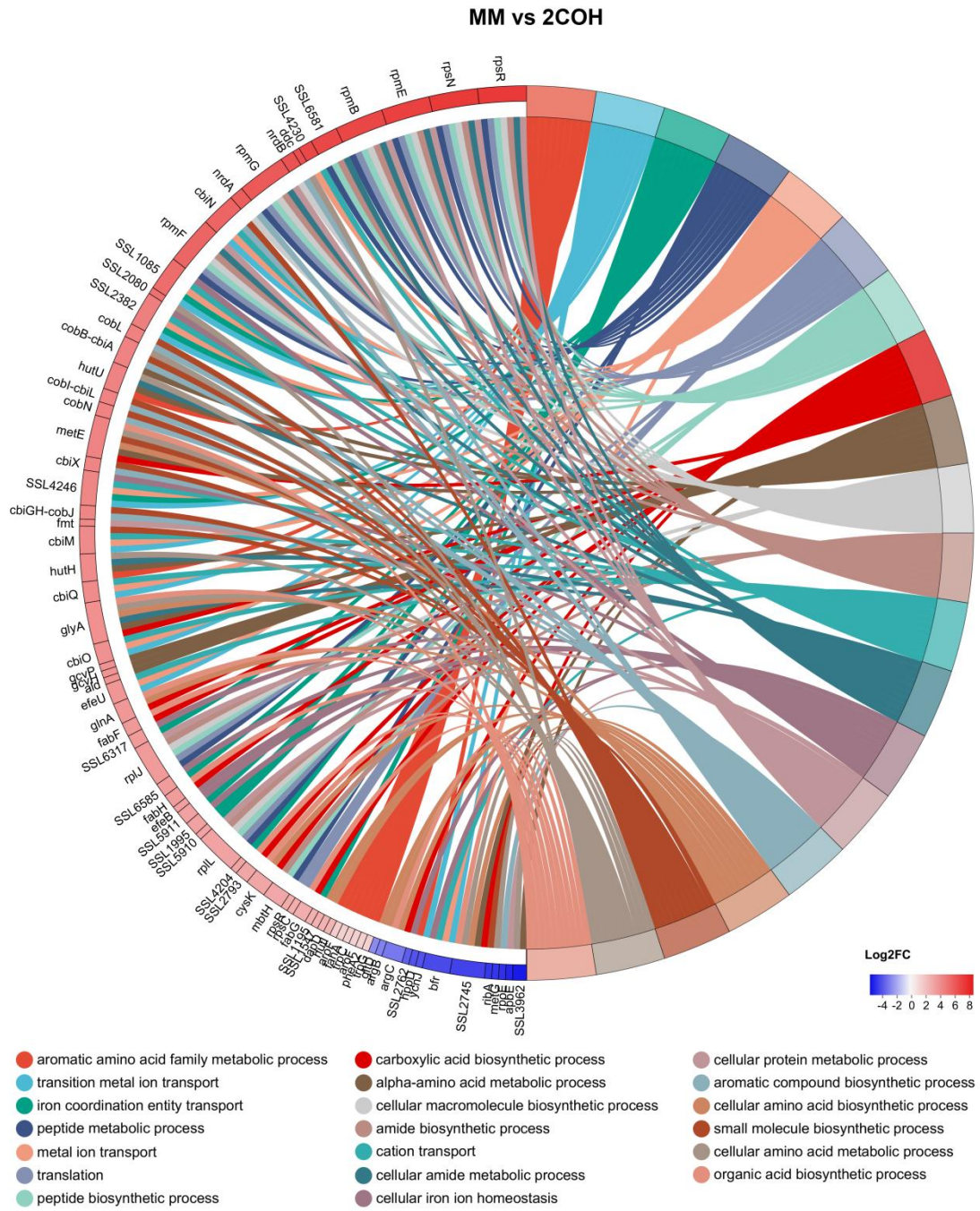


Fig. S5 GO Chord plot of top 20 ranked over-represented GO terms of DEGs for *Streptomyces* sp. SLL-523 in MM vs 2COH.

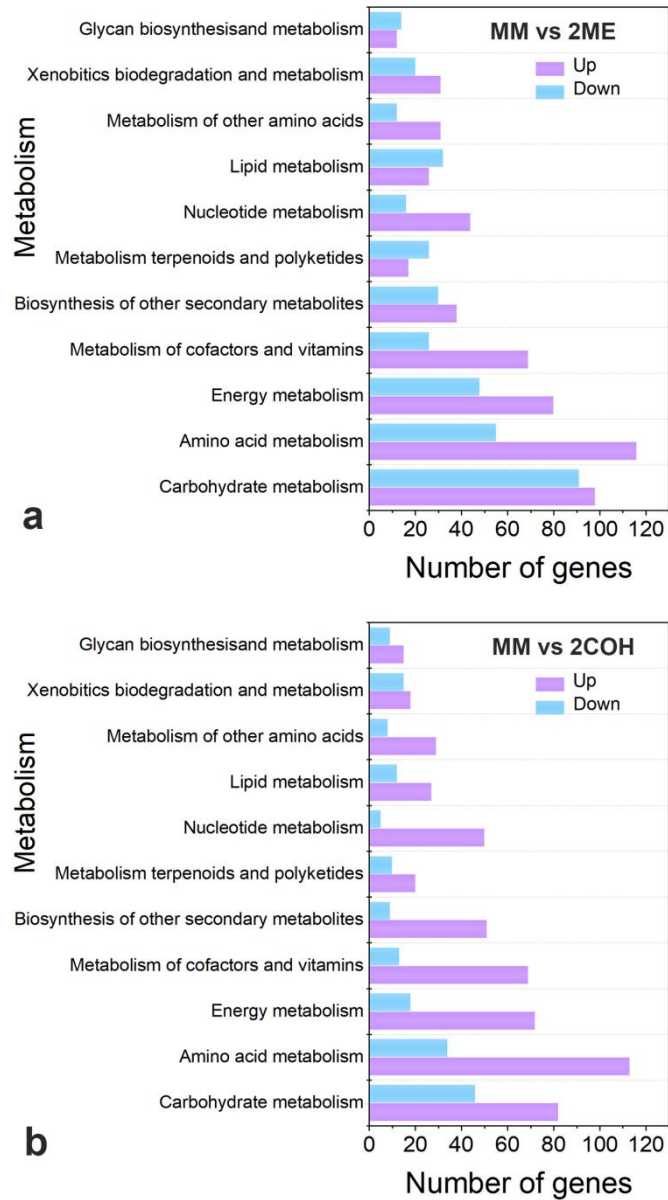


Fig. S6 Changes in gene expression of *Streptomyces* sp. SLL-523 related to metabolism. (a-b) The DEGs analysis related to metabolism in MM vs 2ME, and MM vs 2COH, respectively.

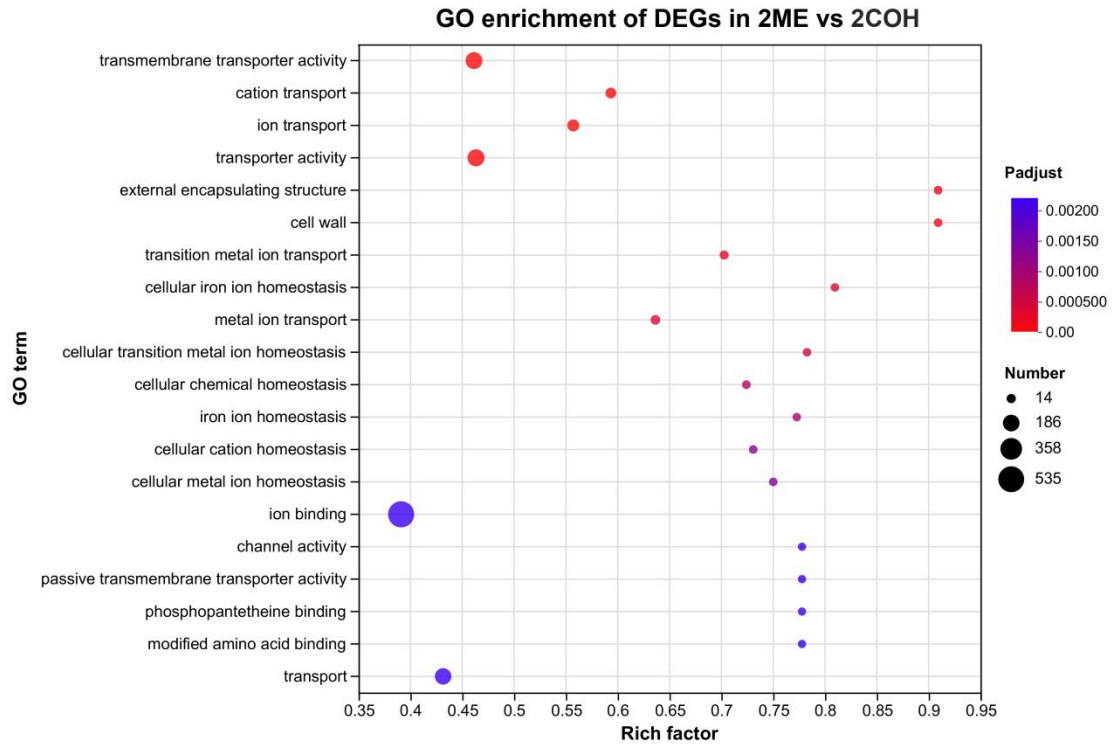


Fig S7 GO enrichment analysis of the intersection DEGs of the strain SLL-523 in 2ME vs 2COH by bubble diagram. Bubble diagram of the top 20 ranked GO terms of DEGs in 2ME vs 2COH. The horizontal axis indicated the Rich Factor and the vertical axis represented GO terms. The sizes of dots manifested the number of genes in the GO term. The enrichment degree was higher with a bigger Rich Factor.

Section 3: Supplementary Table S1-S8

Table S1. Primers used for mRNA expression analysis by qRT-PCR using SYBR Green.

Gene id	Forward primer (5' -3')	Reverse primer (5' -3')
<i>hrdB</i>	GTCATGGCGCTCATTG	ACCATCAGCGTCACAC
SSL0392	GCCACGTCTACCTCAACATCTC	GGGACTTCATCTGCGTCATCT
SSL0401	ATGAGCACCACCTACGACAAG	ATGACGAGCAGCAGTTCCAC
SSL4069	TCGGCTTCCTGCTCTCCT	GCCGTACCAGCGGATCA
SSL1211	CTTCGACATCACCGACTGGG	AGATCGACGAACCGAACTGC
SSL6492	CAAGGCGCGGATATCGG	TGCTGGCCGATCGTGTAGTA
SSL6490	AGTTCGAGTCCTACAACCCCG	ATGTCGTTGGCGTTGGTGA
SSL3085	GCCCTGGCGTTCATCTTCTA	TTGGTGATGCTGAGGGTGGT
SSL6930	CCAAGGGCGTCATCAACC	GTGCGGTACTTCTCGATGTTGT
SSL7396	TCGAAGTTGATGGCGAAGAC	ATGTTGCCGAGGACCTTCTT
SSL0396	GTGACCGAGGACCTCTACAC	GAGCCTGAGGTGGATGGAG
SSL8172	TCCTGATCAACTCCAAGGCC	TACTCGGTCTCCCAGTCCTC
SSL0398	AGGACTTCACCGAACAGCG	ACATGAGGAACTCGCGCTT
SSL0409	CGGCTCCAAGTGCTTCATC	TAGCAGTCGTCTGAAGTGGAC

Table S2. Genomic information of the strain SLL-523.

Features	Genome
Total Scaffolds Number	130
Total bases in Scaffolds (bp)	8,605,615
Scaffold N50 (bp)	245,692
Scaffold N90 (bp)	63,021
Largest Scaffold length (bp)	618,787
G+C (%)	72.20
Genes number	8,163
Gene total length (bp)	7,548,672
Gene average length (bp)	924.73
Gene density	0.95
tRNA	62
rRNA	4

Table S3. Summary of genes involved in the pathway of Gly and Ser.

Gene id	KO id	KO name	KO description
SSL0052	K15634	gpmB	probable phosphoglycerate mutase [EC:5.4.2.12]
SSL0497	K00836	ectB	diaminobutyrate-2-oxoglutarate transaminase [EC:2.6.1.76]
SSL0637	K00133	asd	aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]
SSL0638	K00928	lysC	aspartate kinase [EC:2.7.2.4]
SSL0958	K01758	-	cystathionine gamma-lyase [EC:4.4.1.1]
SSL1168	K15634	gpmB	probable phosphoglycerate mutase [EC:5.4.2.12]
SSL1289	K00382	lpd	dihydrolipoamide dehydrogenase [EC:1.8.1.4]
SSL1327	K00865	glxK	glycerate 2-kinase [EC:2.7.1.165]
SSL1442	K01696	trpB	tryptophan synthase beta chain [EC:4.2.1.20]
SSL1443	K01695	trpA	tryptophan synthase alpha chain [EC:4.2.1.20]
SSL1465	K13745	ddc	L-2,4-diaminobutyrate decarboxylase [EC:4.1.1.86]
SSL1810	K01754	ilvA	threonine dehydratase [EC:4.3.1.19]
SSL1874	K00382	lpd	dihydrolipoamide dehydrogenase [EC:1.8.1.4]
SSL2137	K15634	gpmB	probable phosphoglycerate mutase [EC:5.4.2.12]
SSL2530	K01620	ltaE	threonine aldolase [EC:4.1.2.48]
SSL2837	K00281	gcvP	glycine dehydrogenase [EC:1.4.4.2]
SSL3049	K17103	pssA	CDP-diacylglycerol---serine O-phosphatidyltransferase [EC:2.7.8.8]
SSL3050	K00865	glxK	glycerate 2-kinase [EC:2.7.1.165]
SSL4288	K00003	-	homoserine dehydrogenase [EC:1.1.1.3]
SSL4740	K01697	-	cystathionine beta-synthase [EC:4.2.1.22]
SSL5135	K01079	serB	phosphoserine phosphatase [EC:3.1.3.3]
SSL5196	K06718	ectA	L-2,4-diaminobutyric acid acetyltransferase [EC:2.3.1.178]
SSL5197	K00836	ectB	diaminobutyrate-2-oxoglutarate transaminase [EC:2.6.1.76]
SSL5198	K06720	ectC	L-ectoine synthase [EC:4.2.1.108]
SSL5199	K10674	ectD	ectoine hydroxylase [EC:1.14.11.-]

SSL5402	K00133	asd	aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]
SSL5456	K01754	ilvA	threonine dehydratase [EC:4.3.1.19]
SSL5531	K15634	gpmB	probable phosphoglycerate mutase [EC:5.4.2.12]
SSL5704	K00130	betB	betaine-aldehyde dehydrogenase [EC:1.2.1.8]
SSL5715	K00130	betB	betaine-aldehyde dehydrogenase [EC:1.2.1.8]
SSL5814	K00003	-	homoserine dehydrogenase [EC:1.1.1.3]
SSL5815	K01733	thrC	threonine synthase [EC:4.2.3.1]
SSL5816	K00872	thrB1	homoserine kinase [EC:2.7.1.39]
SSL5825	K00600	glyA	glycine hydroxymethyltransferase [EC:2.1.2.1]
SSL6154	K01754	ilvA	threonine dehydratase [EC:4.3.1.19]
SSL6482	K01752	sdaA	L-serine dehydratase [EC:4.3.1.17]
SSL6483	K00600	glyA	glycine hydroxymethyltransferase [EC:2.1.2.1]
SSL6484	K02437	gcvH	glycine cleavage system H protein
SSL6485	K00605	gcvT	aminomethyltransferase [EC:2.1.2.10]
SSL6556	K15634	gpmB	probable phosphoglycerate mutase [EC:5.4.2.12]
SSL6584	K13745	ddc	L-2,4-diaminobutyrate decarboxylase [EC:4.1.1.86]
SSL6722	K01733	thrC	threonine synthase [EC:4.2.3.1]
SSL6898	K01834	gpmA	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase [EC:5.4.2.11]
SSL6925	K00058	serA	D-3-phosphoglycerate dehydrogenase [EC:1.1.1.95]
SSL7114	K00600	glyA	glycine hydroxymethyltransferase [EC:2.1.2.1]
SSL7124	K00130	betB	betaine-aldehyde dehydrogenase [EC:1.2.1.8]
SSL7636	K00058	serA	D-3-phosphoglycerate dehydrogenase [EC:1.1.1.95]
SSL7675	K00273	aaO	D-amino-acid oxidase [EC:1.4.3.3]
SSL7718	K00382	lpd	dihydrolipoamide dehydrogenase [EC:1.8.1.4]
SSL7887	K00058	serA	D-3-phosphoglycerate dehydrogenase [EC:1.1.1.95]
SSL7931	K00639	kbl	glycine C-acetyltransferase [EC:2.3.1.29]
SSL7932	K00060	tdh	threonine 3-dehydrogenase [EC:1.1.1.103]
SSL8054	K15633	gpml	2,3-bisphosphoglycerate-independent

			phosphoglycerate mutase [EC:5.4.2.12]
SSL8120	K00831	serC	phosphoserine aminotransferase [EC:2.6.1.52]
SSL8163	K15634	gpmB	probable phosphoglycerate mutase [EC:5.4.2.12]

Table S4. Change of main genes expression related to oxidative phosphorylation in MM vs 2ME and 2ME vs 2COH.

Gene_id	Gene name	Gene description	FC(MM vs 2ME)	FC(2ME vs 2COH)
SSL4367	<i>nuoH</i>	NADH-quinone oxidoreductase subunit H	0.046	22.578
SSL4361	<i>nuoN</i>	NADH:ubiquinone oxidoreductase subunit N	0.048	18.272
SSL4363	<i>nuoL</i>	NADH-quinone oxidoreductase subunit L	0.056	15.855
SSL4366	<i>nuoI</i>	NADH-quinone oxidoreductase subunit I 1	0.086	15.373
SSL5832	<i>atpA</i>	ATP synthase subunit alpha	0.307	14.698
SSL4365	<i>nuoJ</i>	NADH:ubiquinone oxidoreductase subunit J	0.062	14.603
SSL4368	<i>nuoG</i>	NADH-quinone oxidoreductase subunit G	0.058	14.505
SSL5831	<i>atpH</i>	ATP synthase subunit delta	0.296	14.444
SSL5830	<i>atpF</i>	ATP synthase F ₀ 2C B subunit	0.314	13.805
SSL5834	<i>atpD</i>	ATP synthase subunit beta	0.562	13.197
SSL4362	<i>nuoM</i>	NADH-quinone oxidoreductase subunit M	0.054	11.656
SSL5833	<i>atpG</i>	ATP synthase subunit gamma	0.503	11.452
SSL4364	<i>nuoK</i>	NADH-quinone oxidoreductase subunit K 2	0.065	10.728
SSL5828	<i>atpB</i>	ATP synthase F ₀ subunit A	0.254	8.504
SSL5829	<i>atpE</i>	ATP synthase subunit C	0.305	8.126
SSL4370	<i>nuoE</i>	NADH-quinone oxidoreductase subunit E	0.111	7.456

SSL4371	<i>nuoD</i>	NADH-quinone oxidoreductase subunit D 2	0.161	6.648
SSL4373	<i>nuoB</i>	NADH-quinone oxidoreductase subunit B 1	0.279	4.013
SSL4369	<i>nuoF</i>	NADH-quinone oxidoreductase subunit F	0.171	3.703
SSL4372	<i>nuoC</i>	NADH-quinone oxidoreductase subunit C	0.274	2.751
SSL5835	<i>atpC</i>	ATP synthase epsilon chain	1.737	2.65
SSL4374	<i>nuoA</i>	NADH dehydrogenase	0.424	2.059
SSL0984	<i>cydB</i>	cytochrome c oxidase assembly protein	1.207	1.875
SSL7823	<i>ppk</i>	PPK_STRCO RecName	2.187	1.872
SSL3323	<i>sdhB</i>	fumarate reductase iron-sulfur subunit	0.063	1.623
SSL3322	<i>sdhA</i>	succinate dehydrogenase flavoprotein subunit	0.11	1.511
SSL3615	<i>ndh</i>	dehydrogenase	0.274	1.506
SSL5427	<i>ndh</i>	oxidoreductase	0.485	1.367
SSL1322	<i>qcrC</i>	cystathionine beta-lyase	4.048	1.344
SSL3635	<i>qcrB</i>	menaquinol-cytochrome c reductase cytochrome b subunit	0.315	1.162
SSL7095	<i>sdhA</i>	succinate dehydrogenase flavoprotein subunit	2.817	1.153
SSL3019	<i>ndh</i>	dehydrogenase	1.046	1.131
SSL8077	<i>nuoN</i>	NADH-quinone oxidoreductase subunit N	3.423	1.13
SSL0983	<i>cydA</i>	cytochrome ubiquinol oxidase subunit I	2.661	1.128

SSL1317	<i>coxA</i>	cytochrome ubiquinol oxidase subunit I		7.788	1.115
SSL1323	<i>qcrA</i>	ubiquinol-cytochrome reductase iron-sulfur subunit	c	4.182	1.076
SSL7094	<i>sdhD</i>	succinate dehydrogenase		2.102	1.057
SSL8075	<i>nuoL</i>	NADH dehydrogenase		2.287	1.04
SSL8076	<i>nuoM</i>	NADH-quinone oxidoreductase subunit M		2.122	1.026
SSL6839	<i>ndh</i>	NADH dehydrogenase		0.411	0.91
SSL7093	<i>sdhC</i>	Succinate dehydrogenase cytochrome b-556 subunit		2.041	0.898
SSL8071	<i>nuoH</i>	NADH dehydrogenase		2.419	0.878
SSL3743	<i>SSL3743</i>	polyphosphate kinase 2		0.647	0.852
SSL1324	<i>qcrB</i>	ubiquinol-cytochrome reductase cytochrome b subunit	c	6.762	0.851
SSL2949	<i>nuoF</i>	NAD-dependent dehydrogenase gamma subunit	formate	0.279	0.851
SSL5616	<i>ppa</i>	Inorganic pyrophosphatase		2.457	0.834
SSL5599	<i>nuoD</i>	NADH-ubiquinone oxidoreductase chain D		4.389	0.818
SSL5264	<i>SSL5264</i>	cytochrome b561		2.632	0.8
SSL7096	<i>sdhB</i>	succinate dehydrogenase iron-sulfur subunit		4.6	0.796
SSL8072	<i>nuoI</i>	NADH-quinone oxidoreductase subunit I 2		5.171	0.762
SSL8073	<i>nuoJ</i>	NADH dehydrogenase		2.063	0.762
SSL5513	<i>qcrB</i>	menaquinol-cytochrome reductase cytochrome b subunit	c	1.329	0.76
SSL1321	<i>coxC</i>	cytochrome c oxidase subunit 3		4.236	0.739

SSL1316	<i>coxB</i>	cytochrome c oxidase subunit 2	7.514	0.717
SSL1916	<i>sdhA</i>	succinate dehydrogenase flavoprotein subunit	39.846	0.685
SSL4723	<i>ndh</i>	NADH dehydrogenase	2.212	0.516
SSL8074	<i>nuoK</i>	NADH-quinone oxidoreductase subunit K 1	2.693	0.509
SSL8069	<i>nuoB</i>	NADH-quinone oxidoreductase subunit B 2	2.589	0.504
SSL8068	<i>nuoA</i>	NADH-quinone oxidoreductase subunit A	3.518	0.496
SSL1917	<i>sdhC</i>	cytochrome B subunit	45.972	0.483
SSL1915	<i>sdhB</i>	succinate dehydrogenase	49.002	0.451
SSL5268	<i>cyoE</i>	protoheme IX farnesyltransferase	6.939	0.444
SSL5515	<i>coxA</i>	cytochrome ubiquinol oxidase subunit I	1.068	0.431
SSL3623	<i>sdhA</i>	oxidoreductase	0.822	0.417

Table S5. Change of main genes expression related to ABC transporter in MM vs 2ME and 2ME vs 2COH.

Gene_id	Gene name	Gene description	FC(MM vs 2ME)	FC(2ME vs 2COH)
SSL1211	<i>oleC5</i>	ABC transporter	0.189	17.168
SSL4068	<i>znuC</i>	ABC transporter	0.599	15.831
SSL4069	<i>znuB</i>	ABC transporter	0.457	24.574
SSL4916	<i>tagG</i>	ABC transporter	0.386	6.656
SSL8050	<i>pstB</i>	ABC transporter	2.703	0.198
SSL8052	<i>pstS</i>	ABC transporter	2.417	0.282
SSL0272	<i>xylG</i>	ABC transporter protein ATP-binding	4.275	0.341
SSL1143	<i>ecfA1</i>	ABC transporter protein ATP-binding	4.42	2.992
SSL1472	<i>livF</i>	ABC transporter protein ATP-binding	0.06	131.572
SSL1473	<i>livG</i>	ABC transporter protein ATP-binding	0.069	115.585
SSL3976	<i>miaF</i>	ABC transporter protein ATP-binding	0.187	8.898
SSL0271	<i>xylH</i>	ABC transporter permease	6.241	0.299
SSL1239	<i>ganP</i>	ABC transporter permease	0.41	0.136
SSL1240	<i>ganQ</i>	ABC transporter permease	0.519	0.158
SSL3960	<i>gguB</i>	ABC transporter permease	0.398	2.861
SSL3974	<i>miaE</i>	ABC transporter permease	0.395	4.276
SSL3975	<i>miaE</i>	ABC transporter permease	0.292	4.837
SSL4900	<i>aglF</i>	ABC transporter permease	0.138	7.521
SSL8051	<i>pstC</i>	ABC transporter permease	3.162	0.364
SSL3971	<i>miaD</i>	ABC transporter substrate-binding	0.286	7.166

		protein			
SSL3972	<i>mldA</i>	ABC transporter substrate-binding	0.101	19.193	
		protein			
SSL4866	<i>dasA</i>	ABC transporter substrate-binding	13.27	0.056	
		protein			
SSL4067	<i>znuA</i>	ABC-transporter metal-binding	0.69	13.969	
		lipoprotein			
SSL4899	<i>aglE</i>	Alpha-glucosides-binding	0.11	10.5	
		periplasmic protein AglE precursor			
SSL7217	<i>bioY</i>	biotin synthase	1.726	6.162	
SSL1476	<i>livK</i>	branched chain amino acid ABC	0.037	173.827	
		transporter substrate-binding			
		protein			
SSL1474	<i>livM</i>	branched chain amino acid	0.036	240.38	
		transport permease			
SSL7366	<i>livK</i>	branched-chain amino acid ABC	1.276	0.362	
		transporter			
SSL1475	<i>livH</i>	branched-chain amino acid ABC	0.035	204.389	
		transporter permease			
SSL4888	<i>ftsX</i>	cell division protein FtsX	1.386	2.275	
SSL0324	<i>cbiM</i>	cobalamin biosynthesis protein	0.48	59.077	
		CbiM			
SSL0327	<i>cbiO</i>	cobalt ABC transporter	1.104	21.188	
SSL1144	<i>ecfT</i>	cobalt ABC transporter permease	1.44	3.662	
SSL0326	<i>cbiQ</i>	cobalt ECF transporter T	0.43	63.844	
		component CbiQ			
SSL4653	<i>cbiQ</i>	cobalt ECF transporter T	1.969	5.767	
		component CbiQ			
SSL0325	<i>cbiN</i>	cobalt transporter CbiN	0.434	213.518	

SSL1212	<i>oleC4</i>	daunorubicin resistance protein DrrA family ABC transporter ATP-binding protein	0.158	10.345
SSL5725	<i>thiQ</i>	Fe ²⁺ ions import ATP-binding protein FbpC	1.232	8.448
SSL0523	<i>gluD</i>	glutamate ABC transporter permease	0.346	2.1
SSL6315	<i>proV</i>	glycine/betaine ABC transporter ATP-binding protein	21.191	0.092
SSL6314	<i>proW</i>	glycine/betaine ABC transporter permease	26.895	0.068
SSL3968	<i>mIaD</i>	hypothetical protein	0.136	11.556
SSL3969	<i>mIaD</i>	hypothetical protein	0.356	3.21
SSL4654	<i>cbiM</i>	hypothetical protein	3.031	4.441
SSL5726	<i>thiP</i>	iron ABC transporter permease	1.418	6.766
SSL0277	<i>SSL0277</i>	lipoprotein	0.416	3.021
SSL1906	<i>dasA</i>	lipoprotein	1.959	0.375
SSL7292	<i>metQ</i>	lipoprotein	6.381	2.33
SSL8222	<i>bmpA</i>	lipoprotein	1.266	6.397
SSL5234	<i>smoG</i>	mannitol ABC transporter permease	0.269	3.532
SSL0731	<i>modB</i>	molybdate ABC transporter permease	6.572	0.438
SSL0730	<i>modA</i>	molybdate-binding protein	9.846	0.284
SSL0220	<i>opuA</i>	proline/glycine betaine ABC transporter ATP-binding protein	4.696	0.127
SSL0273	<i>xyIF</i>	solute-binding protein	3.913	0.436
SSL0691	<i>xyIF</i>	solute-binding protein	0.452	2.265
SSL0275	<i>SSL0275</i>	sugar ABC transporter permease	0.55	3.108
SSL0276	<i>SSL0276</i>	sugar ABC transporter permease	0.384	3.946

SSL3182	<i>dasB</i>	sugar ABC transporter permease	0.378	3.746
SSL4901	<i>aglG</i>	sugar ABC transporter permease	0.076	15.593
SSL1238	<i>cycB</i>	sugar ABC transporter substrate-binding protein	0.388	0.253
SSL3958	<i>chvE</i>	sugar ABC transporter substrate-binding protein	0.284	2.878
SSL5232	<i>smoE</i>	sugar ABC transporter substrate-binding protein	0.137	4.204
SSL2508	<i>lacE</i>	sugar transporter	0.702	0.39
SSL3183	<i>dasA</i>	sugar transporter	0.552	4.684
SSL4865	<i>dasB</i>	sugar transporter membrane protein	1.62	0.28
SSL3538	<i>bxIE</i>	sugar-binding lipoprotein	0.257	2.284
SSL7348	<i>msmF</i>	sugar-transporter integral membrane protein	0.066	18.352
SSL4917	<i>tagH</i>	teichoic acid ABC transporter ATP-binding protein	0.582	7.605
SSL5727	<i>thiB</i>	thiamine ABC transporter substrate-binding protein	1.421	4.528
SSL3959	<i>gguA</i>	xylose ABC transporter ATP-binding protein	0.285	2.032

Table S6. Change of main genes expression involved in peptide in MM vs 2ME and 2ME vs 2COH.

Gene_id	Gene name	Gene description	FC(MM vs 2ME)	FC(2ME vs 2COH)
SSL2080	<i>SSL2080</i>	non-ribosomal peptide synthetase	0.636	97.856
SSL2079	<i>SSL2079</i>	non-ribosomal peptide synthetase	1.145	77.301
SSL0378	<i>SSL0378</i>	peptide-binding protein, collagenase	0.397	32.242
SSL6433	<i>SSL6433</i>	lanthionine-containing SapB	0.169	25.38
SSL8180	<i>SSL8180</i>	non-ribosomal peptide synthetase	0.201	18.928
SSL6015	<i>SSL6015</i>	CDA peptide synthetase III	0.199	17.072
SSL8187	<i>SSL8187</i>	non-ribosomal peptide synthetase	0.31	10.762
SSL6492	<i>SSL6492</i>	dipeptide/oligopeptide/nickel ABC transporter ATP-binding protein	0.43	9.837
SSL6493	<i>SSL6493</i>	peptide ABC transporter ATP-binding protein	0.566	5.894
SSL6490	<i>SSL6490</i>	peptide ABC transporter substrate-binding protein	1.026	4.719
SSL4209	<i>SSL4209</i>	non-ribosomal peptide synthetase	6.75	3.249
SSL4203	<i>pvdA</i>	peptide monooxygenase	13.675	3.192
SSL3853	<i>SSL3853</i>	lantipeptide	1.397	2.991

SSL3027	<i>SSL3027</i>	putative acyl-peptide hydrolase	1.379	2.767
SSL3312	<i>SSL3312</i>	peptide ABC transporter ATP-binding protein	0.81	2.615
SSL1678	<i>SSL1678</i>	ATP-grasp ribosomal peptide maturase	0.273	2.214
SSL3311	<i>SSL3311</i>	dipeptide/oligopeptide/nickel ABC transporter ATP-binding protein	1.224	2.049
SSL0163	<i>SSL0163</i>	peptide ABC transporter substrate-binding protein	1.743	0.475
SSL5821	<i>prfA</i>	peptide chain release factor 1	3.145	0.468
SSL2660	<i>def</i>	peptide deformylase 2	2.857	0.381
SSL1873	<i>def</i>	peptide deformylase 1	4.565	0.16
SSL0221	<i>msrB</i>	peptide-methionine (R)-S-oxide reductase	7.915	0.12
SSL6148	<i>msrA</i>	peptide methionine sulfoxide reductase	16.282	0.061
SSL3084	<i>SSL3084</i>	non-ribosomal peptide synthetase	0.873	0.057
SSL3085	<i>SSL3085</i>	non-ribosomal peptide synthetase	0.882	0.049

Table S7. Change of genes expression related to Pro metabolism in MM vs 2ME, and 2ME vs 2COH.

Gene id	Gene name	KO id	KO name	Gene description	FC(MM vs 2ME)	FC(2ME vs 2COH)
SSL7396	<i>proS</i>	K01881	proS	proline--tRNA ligase	1.166	2.165
SSL8174	<i>proB</i>	K00931	proB	glutamate 5-kinase	2.444	0.488
SSL8172	<i>proA</i>	K00147	proA	gamma-glutamyl-phosphate reductase	2.355	0.735
SSL7918	<i>rocD</i>	K00819	rocD, OAT	aspartate aminotransferase family protein	4.867	0.117
SSL7712	SSL7712	K00128	ALDH	aldehyde dehydrogenase	16.951	0.579
SSL7675	<i>aao</i>	K00273	aao	amino acid oxidase	8.841	0.287
SSL7587	SSL7587	K01777	prdF	proline racemase	0.854	37.951
SSL6937	<i>aguA</i>	K10536	aguA	agmatine deiminase	7.104	0.114
SSL6930	SSL6930	K00294	-	1-pyrroline-5-carboxylate dehydrogenase	0.816	5.374
SSL6929	SSL6929	K00318	putB, PRODH	proline dehydrogenase	0.952	2.526
SSL6708	<i>speG</i>	K00657	speG, SAT	acetyltransferase	1.734	0.773
SSL6655	<i>amiE</i>	K01426	amiE	amidase	0.773	0.837
SSL6596	<i>speB</i>	K01480	speB	agmatinase	1.682	1.935
SSL5994	SSL5994	K00128	ALDH	aldehyde dehydrogenase	3.006	0.475
SSL5690	SSL5690	K00128	ALDH	aldehyde dehydrogenase	2.149	6.849
SSL5627	SSL5627	K00128	ALDH	aldehyde dehydrogenase	0.867	3.157
SSL5544	<i>proC</i>	K00286	proC	pyrroline-5-carboxylate reductase	0.97	1.074
SSL4943	<i>codA</i>	K01485	codA	hydrolase	0.427	1.5

SSL4011	<i>speE</i>	K00797	speE, SRM, SPE3	polyamine aminopropyltransferase 1	0.678	1.7
SSL3608	<i>pip</i>	K01259	pip	hydrolase	0.051	11.583
SSL2734	<i>patA</i>	K09251	patA	aminotransferase	0.07	4.027
SSL2672	<i>rocD</i>	K00819	rocD, OAT	ornithine--oxo-acid transaminase	0.123	97.762
SSL2205	<i>SSL2205</i>	K01470		creatinine amidohydrolase	0.751	1.212
SSL2169	<i>amiE</i>	K01426	amiE	amidase	2.719	0.305
SSL1497	<i>pip</i>	K01259	pip	proline iminopeptidase	1.384	0.613
SSL0681	<i>speE</i>	K00797	speE, SRM, SPE3	polyamine aminopropyltransferase 2	0.199	2.998
SSL0247	<i>speC</i>	K01581	ODC1, speC, speF	lysine/ornithine decarboxylase	1.123	0.093

Table S8. Change of main genes expression related to UDP biosynthesis in MM vs 2ME and 2ME vs 2COH.

Gene id	Gene name	KO id	KO name	Gene description	FC(MM vs 2ME)	FC(2ME vs 2COH)
SSL0396	SSL0396	K21792	<i>redL</i>	Polyketide synthase	0.096	327.907
SSL0397	SSL0397	K21780	<i>pigI</i> , <i>redM</i>	L-proline---[L-prolyl-carrier protein] ligase	0.156	263.326
SSL0392	<i>pps</i>	K21787	<i>pigC</i> , <i>redH</i>	Phosphoenolpyruvate-utilizing enzyme;Prodigiosin synthesizing transferase PigC.	0.096	222.932
SSL0395	SSL0395	K21793	<i>redK</i>	Oxidoreductase	0.096	188.901
SSL0393	SSL0393	K21786	<i>pigF</i> , <i>redI</i>	Methyltransferase	0.12	182.623
SSL0399	SSL0399	K21781	<i>pigG</i> , <i>redO</i>	Acyl carrier protein;peptidyl carrier protein.	0.338	137.161
SSL0398	SSL0398	K21784	<i>pigH</i> , <i>redN</i>	8-amino-7-oxononanoate synthase;4-hydroxy-2,2'-bipyrrole-5-methanol synthase.	0.196	124.899
SSL0406	SSL0406	K21785	<i>pigM</i> , <i>redV</i>	RedV protein;4-hydroxy-2,2'-bipyrrole-5-methanol dehydrogenase	0.188	107.814
SSL0402	<i>fabF</i>	K21789	<i>fabF</i> , <i>redR</i>	3-oxoacyl-[acyl-carrier-protein] synthase; dodecanoy-ACP synthase.	0.206	85.359
SSL0394	SSL0394	K21791	<i>redJ</i>	Thioesterase	0.161	74.471
SSL0400	<i>fabH</i>	K21788	<i>redP</i>	3-oxoacyl-ACP synthase III; dodecanoy-ACP synthase.	0.237	70.807
SSL0401	<i>acpP</i>	K21790	<i>acpP</i> , <i>redQ</i>	Acyl carrier protein	0.347	21.31
SSL0410	SSL0410	K21783	<i>pigJ</i> , <i>redX</i>	Polyketide synthase;beta-ketoacyl ACP synthase.	0.737	6.624
SSL4440	<i>fabG</i>	K00059	<i>fabG</i>	3-oxoacyl-ACP reductase	0.369	5.467
SSL0409	SSL0409	K21782	<i>pigA</i> , <i>redW</i>	Acyl-CoA dehydrogenase; L-prolyl-PCP dehydrogenase.	1.466	4.324

SSL5142	<i>fabG</i>	K00059	<i>fabG</i>	Beta-ketoacyl-ACP reductase	2.372	3.88
SSL5141	<i>fabI</i>	K00208	<i>fabI</i>	Enoyl-(Acyl carrier protein) reductase I	2.265	3.396
SSL3939	<i>fabD</i>	K00645	<i>fabD</i>	Malonyl coA-acyl carrier protein ;[acyl-carrier-protein] S-malonyltransferase	1.268	1.93
SSL7526	<i>fabG</i>	K00059	<i>fabG</i>	3-oxoacyl-ACP reductase	0.946	1.356
SSL5482	<i>fabG</i>	K00059	<i>fabG</i>	3-oxoacyl-ACP reductase	1.522	1.012
SSL0005	<i>SSL0005</i>	K00059	<i>fabG</i>	Short-chain dehydrogenase; 3-oxoacyl-ACP reductase.	1.329	1.008
SSL5160	<i>fabG</i>	K00059	<i>fabG</i>	Oxidoreductase; 3-oxoacyl-ACP reductase.	2.276	0.979
SSL2803	<i>fabG</i>	K00059	<i>fabG</i>	3-oxoacyl-ACP reductase	0.961	0.974
SSL7645	<i>fabG</i>	K00059	<i>fabG</i>	3-ketoacyl-ACP reductase	1.544	0.922
SSL2802	<i>fabG</i>	K00059	<i>fabG</i>	3-ketoacyl-ACP reductase	1.49	0.796
SSL4994	<i>fabG</i>	K00059	<i>fabG</i>	3-ketoacyl-ACP reductase	17.774	0.026

Section 4: Supplementary References

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