

**Table S1.** Effect of CaCl<sub>2</sub> and sodium citrate on the fermentation process of gentamicin biosynthesis in *M. echinospora*.

Time (h)	Fermentation Potency (mg/L) *10 <sup>2</sup>		C1 Ratio (100%)		C1a Ratio (100%)		C2a Ratio (100%)		C2 Ratio (100%)	
	CK	CS	CK	CS	CK	CS	CK	CS	CK	CS
0	2.50±0.12	2.70±0.13	19.11±0.87	15.32±0.76	55.00±1.33	57.23±0.33	9.88±0.38	10.23±0.09	16.01±0.08	17.22±0.52
12	3.90±0.13	4.20±0.16	25.00±0.26	20.33±0.42	32.03±1.21	39.96±0.21	13.88±1.99	12.98±0.27	29.09±1.04	26.73±0.48
24	6.30±0.16	7.00±0.13	18.22±1.68	17.24±0.95	52.12±1.10	57.98±0.10	11.43±0.2	12.4±0.31	18.23±0.78	12.38±1.16
36	9.10±0.17	10.10±0.14	21.45±1.87	17.36±0.69	50.98±1.50	53.23±0.50	11.25±1.43	12.54±0.83	16.32±1.8	16.87±1.02
48	11.50±0.13	12.90±0.15	22.11±0.22	18.23±0.31	49.13±1.80	52.00±0.80	11.87±0.85	13.21±1.51	16.89±0.73	16.56±1.02
60	13.90±0.15	15.20±0.16	23.44±1.66	19.34±1.22	47.22±2.10	49.43±0.18	12.77±1.18	13.45±0.52	16.57±0.74	17.78±1.56
72	15.80±0.17	17.58±0.15	24.10±0.76	21.45±0.56	46.11±2.33	48.13±0.53	13.22±1.69	14.12±0.91	16.57±0.12	16.3±0.94
84	18.20±0.09	19.00±0.10	24.31±0.69	20.21±1.11	42.96±1.50	45.15±0.50	13.87±1.74	15.3±1.05	18.86±0.93	19.34±1.66
96	19.60±0.11	20.90±0.12	24.11±0.64	23.66±1.07	41.32±1.70	45.27±0.70	14.12±1.66	16.33±1.5	20.45±0.60	14.74±1.87
108	20.70±0.13	23.10±0.14	25.23±0.49	26.57±0.09	39.40±1.22	43.34±0.72	14.24±0.77	16.57±0.31	21.13±0.04	13.52±0.324
120	21.50±0.50	23.98±0.34	25.34±1.67	25.77±1.17	38.33±1.80	42.43±0.80	15.33±0.83	16.96±1.44	21±0.70	14.84±1.81

CK denotes fed batch without calcium chloride and sodium citrate; CS denotes fed batch with calcium chloride and sodium citrate; Three biological replicates were performed, and the data were presented as mean ± SD.

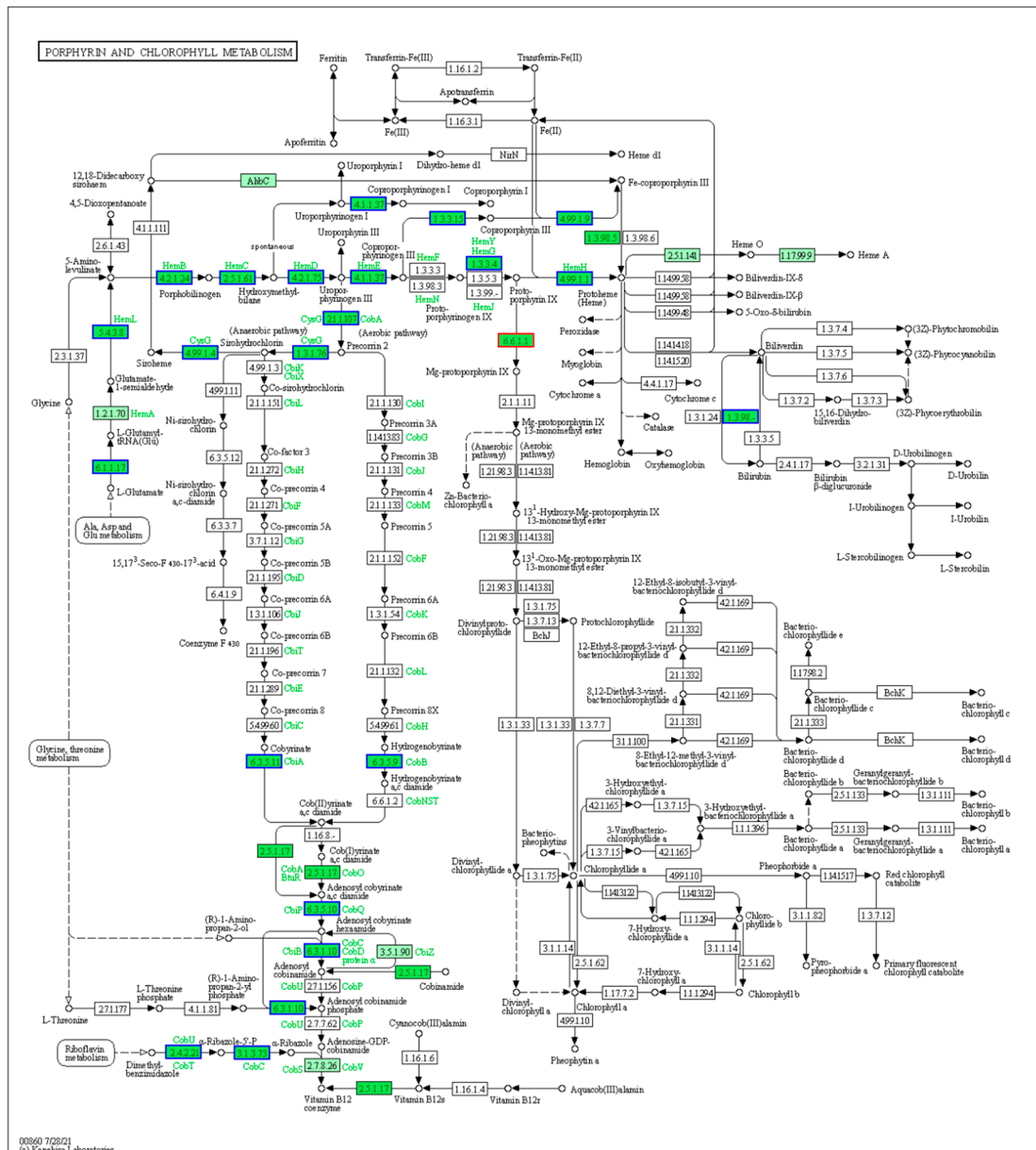
**Table S2.** Differentially expressed proteins potentially related to gentamicin biosynthesis.

Pathway	Uniprot AC	Gene name	Protein name	Fold changes*		<i>p</i> -value*	
				CaCl <sub>2</sub>	Sodium Citrate	CaCl <sub>2</sub>	Sodium Citrate
Gentamicin biosynthesis	A0A0K0K513	<i>genD1</i>	Putative gentamicin oxidoreductase/methyltransferase	(-)2.17	-	0.0034	-
	A0A0K0K515	<i>genB4</i>	Putative gentamicin aminotransferase IV	ND	(-)3.66	ND	0.0279
	A0A0K0K516	<i>genD2</i>	Putative gentamicin oxidoreductase	ND	ND	ND	ND
	A0A0K0K518	<i>genB3</i>	Putative gentamicin aminotransferase III	-	(-)8.47	-	0.001
	A0A0K0K520	<i>genX</i>	Putative gentamicin production protein	-	-	-	-
	A0A0K0K522	<i>genV</i>	Putative gentamicin exporter	-	-	-	-
	A0A0K0K523	<i>genS2</i>	Putative gentamicin aminotransferase II	-	(-)5.31	-	0.0316
	A0A0K0K525	<i>genU</i>	Putative gentamicin production protein	-	ND	-	ND
	A0A0K0K526	<i>genK</i>	Putative gentamicin C-methyltransferase	(-)12.36	-	0.045	-
	A0A0K0K527	<i>genM2</i>	Putative gentamicin	ND	ND	ND	ND

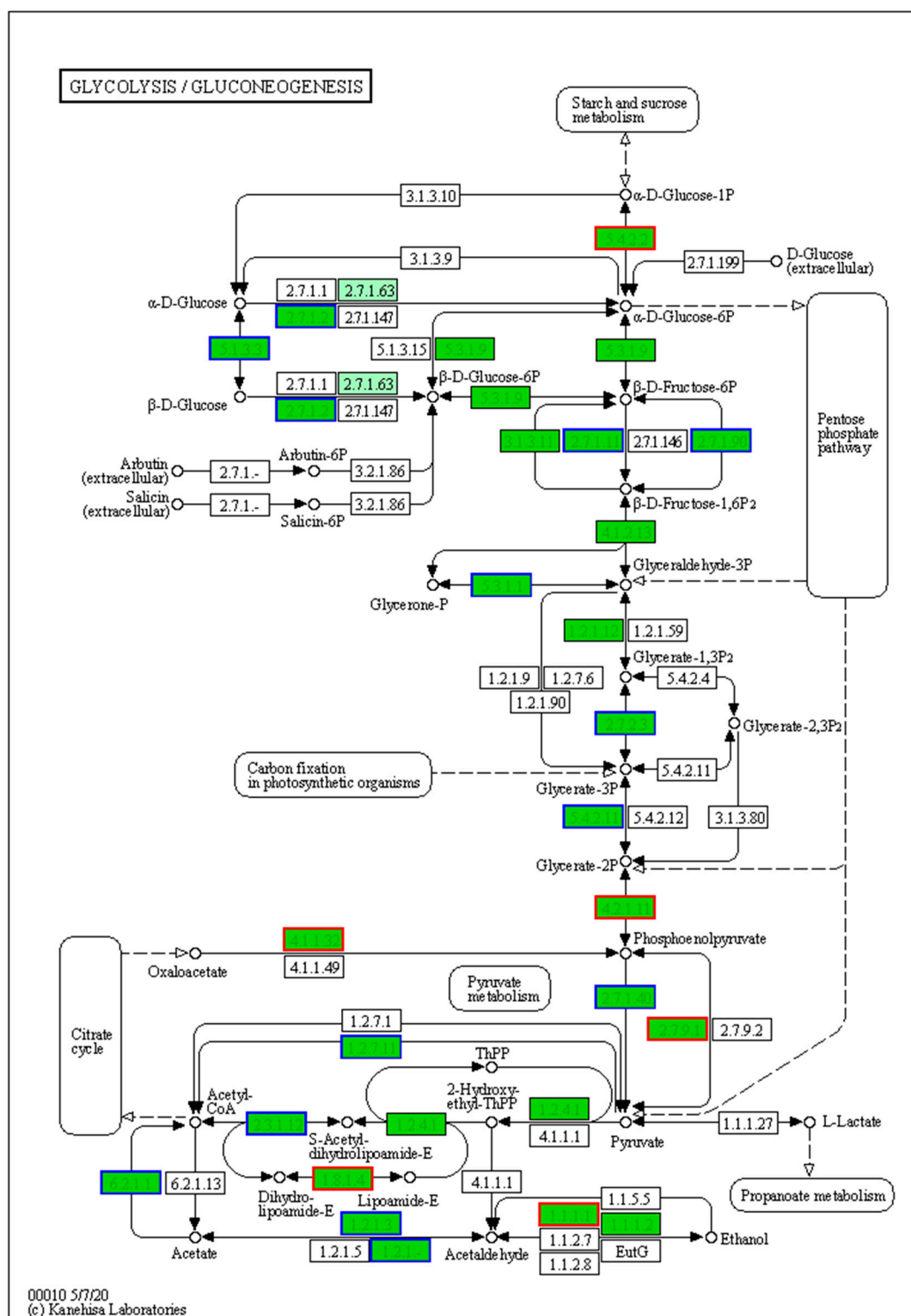
			glycosyltransferase II Gentamicin (Hexosaminyl-6-) aminotransferase I	-	-	-	-
	A0A0K0K528	<i>genB1</i>					
	A0A0K0K532	<i>genI</i>	<b>Putative gentamicin exporter</b>	(+)4.04	-	0.0007	-
	A0A0K0K535	<i>genQ</i>	Gentamicin hexosaminyl-6'- dehydrogenase	-	-	-	-
	A0A0K0K6A4	<i>genB2</i>	Putative gentamicin aminotransferase II	(-)3.20	-	0.0427	-
	A0A0K0K6B9	<i>genH</i>	<b>Putative gentamicin exporter</b>	-	(+)2.74	-	0.0005
Glycolytic pathway <sup>1</sup>	A0A1C4YCI0	<i>pfk</i>	Phosphofructokinase	(-)4.31	(-)5.14	0.0166	0.0024
	A0A1C4YHB7	<i>pk</i>	Pyruvate kinase	-	(-)4.45	-	0.0014
TCA pathway <sup>1</sup>	A0A1C5A2F4	<i>cs</i>	Citrate synthase	-	(-)2.46	-	0.0405

\* The fold changes with (+) stand for up-regulated proteins and those with (-) stand for down-regulated proteins. The symbol of “-” stands for non-differentially expressed protein and “ND” stands for non-detected protein.

<sup>1</sup> Only key rate-limiting enzymes in the glycolytic and TCA pathways are shown here. Other differential expressed proteins are shown in Supplementary Figures S2, S3.



**Figure S1.** The effect of adding  $\text{CaCl}_2$  on vitamin B12 biosynthesis pathway. Green background indicates proteins detected in proteomics, blue boxes indicate proteins that were significantly down-regulated, and red boxes color-code significantly up-regulated proteins.



**Figure S2.** The effect of adding sodium citrate on glycolytic pathway. Green background indicates proteins detected in proteomics, blue boxes indicate proteins that were significantly down-regulated, and red boxes color-code significantly up-regulated proteins. Phosphofructokinase EC 2.7.1.11, Pyruvate kinase EC 2.7.1.40.

