

Supplementary Materials

Enhanced Pentostatin Production in *Actinomadura* sp. by Combining ARTP Mutagenesis, Ribosome Engineering and Subsequent Fermentation Optimization

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Table S1. RT-qPCR analysis of the transcription levels of different genes in *Actinomadura* sp. S-15 compared with those in *Actinomadura* sp. ATCC 39365 on the 5th day.

Gene	$\Delta\Delta\text{Ct}_1$	$\Delta\Delta\text{Ct}_2$	$\Delta\Delta\text{Ct}_3$	$\bar{\Delta\Delta\text{Ct}}$	$2^{-\bar{\Delta\Delta\text{Ct}}} \pm \text{SD}$
<i>AdeA</i>	0.6305	0.6197	0.6386	0.6296	0.6464 \pm 0.0043
<i>AdeB</i>	0.0299	0.0401	0.0443	0.0381	0.9740 \pm 0.0050
<i>AdeC</i>	-0.1699	-0.1803	-0.1862	-0.1788	1.1320 \pm 0.0065
<i>AdeK</i>	0.0093	0.0203	0.0049	0.0115	0.9921 \pm 0.0055
<i>AdeM</i>	-0.4698	-0.4815	-0.4701	-0.4738	1.3888 \pm 0.0064

Table S2. RT-qPCR analysis of the transcription levels of different genes in *Actinomadura* sp. S-15 compared with those in *Actinomadura* sp. ATCC 39365 on the 8th day.

Gene	$\Delta\Delta\text{Ct}_1$	$\Delta\Delta\text{Ct}_2$	$\Delta\Delta\text{Ct}_3$	$\bar{\Delta\Delta\text{Ct}}$	$2^{-\bar{\Delta\Delta\text{Ct}}} \pm \text{SD}$
<i>AdeA</i>	-1.5693	-1.5802	-1.5647	-1.5714	2.9719 \pm 0.0164
<i>AdeB</i>	-0.6803	-0.6698	-0.6854	-0.6785	1.6005 \pm 0.008
<i>AdeC</i>	-0.5310	-0.5192	-0.5158	-0.5220	1.4360 \pm 0.0079

<i>AdeK</i>	-0.1804	-0.1699	-0.1864	-0.1789	1.1320±0.0065
<i>AdeM</i>	-1.9289	-1.9405	-1.9350	-1.9348	3.8233±0.0154

Table S3. Effect of different carbon sources on pentostatin production and biomass in *Actinomadura* sp. S-15.

Carbon sources (4 g/L)	Pentostatin Production (g/L)					Biomass (DCW) (mg/mL)				
	X ₁	X ₂	X ₃	\bar{X}	SD	X ₁	X ₂	X ₃	\bar{X}	SD
Xylitol	86.9760	79.7280	87.3630	84.6890	4.3007	8.1700	7.3333	7.3133	7.6056	0.4889
Maltitol	94.2080	104.5280	98.1670	98.9677	5.2064	7.7833	7.4333	7.4867	7.5678	0.1886
Soluble starch	89.0990	94.6910	90.7840	91.5247	2.8686	7.9333	7.54	7.4733	7.6489	0.2486
Rice meal	80.6330	79.6170	79.5210	79.9237	0.6162	7.4667	7.5667	7.2733	7.4356	0.1491
Dextrin	89.3580	73.3810	86.6210	83.1200	8.5445	7.0800	7.3033	7.9867	7.4567	0.4724

Table S4. Effect of different maltitol concentrations on pentostatin production and biomass in *Actinomadura* sp. S-15.

Maltitol (g/L)	Pentostatin Production (g/L)					Biomass (DCW) (mg/mL)				
	X₁	X₂	X₃	\bar{X}	SD	X₁	X₂	X₃	\bar{X}	SD
2	72.1460	75.8580	75.4240	74.4760	2.0295	7.8233	6.7967	6.9733	7.1978	0.5489
4	97.2120	94.6480	96.7610	96.2070	1.3688	7.1167	8.1000	8.1533	7.7900	0.5837
6	78.0020	80.2180	80.6030	79.6077	1.4038	7.6333	7.7367	8.0067	7.7922	0.1928
8	75.2660	82.1285	77.8735	78.4227	3.4641	8.0233	7.9967	7.9667	7.9956	0.0284
10	69.7110	76.4290	72.5000	72.8800	3.3751	8.5400	9.5800	8.5167	8.8789	0.6073

Table S5 Effect of the initial pH of the fermentation medium on pentostatin production and biomass of *Actinomadura* sp. S-15

pH	Pentostatin Production (g/L)					Biomass (DCW) (mg/mL)				
	X ₁	X ₂	X ₃	\bar{X}	SD	X ₁	X ₂	X ₃	\bar{X}	SD
6.9	79.5770	76.2030	75.7240	77.1680	2.1000	7.4200	6.6900	7.3500	7.1533	0.4028
7.2	89.2860	86.9110	90.5880	88.9283	1.8644	7.2933	7.4400	7.4300	7.3878	0.0819
7.5	92.5470	89.7570	92.6930	91.6657	1.6546	7.6367	7.2967	7.7200	7.5511	0.2243
7.8	95.1810	93.5860	92.9830	93.9167	1.1357	7.4367	8.3533	7.8133	7.8678	0.4608
8.1	79.4940	74.8470	80.0760	78.1390	2.8658	6.6600	6.8033	7.5000	6.9878	0.4494

Table S6 Effect of the seed age on pentostatin production and biomass of *Actinomadura* sp. S-15.

Seed	Pentostatin Production (g/L)					Biomass (DCW) (mg/mL)				
age (h)	X ₁	X ₂	X ₃	\bar{X}	SD	X ₁	X ₂	X ₃	\bar{X}	SD
40	80.2280	83.5210	83.4570	82.4020	1.8830	7.2367	6.8667	6.6967	6.9333	0.2761
48	86.2260	88.7210	83.5090	86.1520	2.6068	7.2633	7.5000	7.2867	7.3500	0.1304
56	86.2350	89.8450	88.3170	88.1323	1.8121	7.6033	7.8700	8.0433	7.8389	0.2216
64	74.6980	75.3120	78.5160	76.1753	2.0502	7.1633	7.0800	7.6133	7.2856	0.2869
72	78.0860	73.5560	72.3390	74.6603	3.0285	6.8733	6.9233	6.9733	6.9233	0.0500

Table S7 Effect of the culture time on pentostatin production and biomass of *Actinomadura* sp. S-15.

Culture	Pentostatin Production (g/L)					Biomass (DCW) (mg/mL)				
time (d)	X₁	X₂	X₃	\bar{X}	SD	X₁	X₂	X₃	\bar{X}	SD
5	81.1920	88.4690	87.1340	85.5983	3.8739	6.7967	6.7333	6.3600	6.6300	0.2360
6	98.2050	105.3960	108.0190	103.8733	5.0811	6.9233	6.9900	6.7100	6.8744	0.1463
7	92.5470	89.7570	92.6930	91.6657	1.6546	6.4100	6.0900	6.8933	6.4644	0.4044
8	86.2270	95.3350	94.2490	91.9370	4.9747	6.7200	6.6700	7.0833	6.8244	0.2256
9	93.3860	93.8380	95.9790	94.4010	1.3852	7.2700	6.8867	6.2867	6.8144	0.4956