**Supplementary Material**

**Enhanced ε-poly-L-lysine production by the synergistic effect of ε-poly-L-lysine synthetase overexpression and citrate in *Streptomyces albulus***

**Aixia Wang1, Wenzhe Tian1, Lei Cheng2, Youqiang Xu2, Xiuwen Wang1\*, Jiayang Qin1\*, Bo Yu3**

1College of Pharmacy, Binzhou Medical University, Yantai 264003, PR China

2Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology & Business University (BTBU), Beijing 100048, PR China

3CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

\*Corresponding author

Email addresses:

AW: 406076940@qq.com; WT: 1158807207@qq.com;

LC: chenglei@btbu.edu.cn; YX: xuyouqiang@btbu.edu.cn;

XW: wangxiuwen0822@163.com;

JQ: qinjysdu@163.com; BY: yub@im.ac.cn

**Table S1.** Oligonucleotides used to construct overexpression plasmids.

|  |  |
| --- | --- |
| Oligonucleotides | Sequence |
| pro1 | gccaagcttgggctgcaggtcgactctagatgttcacattcgaacggtctctgctttg |
| pro2 | tgttcacattcgaacggtctctgctttgacaacatgctgtgcggtgttgtaaagtcgtggccaggagaata |
| pro4 | atggacactccttacttagatctagtattctcctggccacgactttacaacaccgcacagcatgtt |
| pls-F2 | tactagatctaagtaaggagtgtccatatgtcgtcgccccttctcgaatcgtccttc2 |
| pls-R | caggaaacagctatgacatgattacgaattctcacgcggccgcacctccctccgcgcg |

1The underlined sequence is RBS2.

**Table S2.** Primers for qRT-PCR analysis with target gene information

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene ID** | **Description** | **Primers (5′→3′)** | **Length** |
| SAZ\_36495 | ε-Poly-L-lysine synthase | GCGAGATGTGGAACACCTACGGGCGAGCTGCCAGCCCTTCA | 115 bp |
| SAZ\_12395 | RNA polymerase sigma factor (hrdB) | CTGACCAGATTCCGCCAACCCGCCTCTGCGGCACTGACCAT | 100 bp |

**Fig. S1.** Diagram of the integrative plasmids containing *pls* gene, strong promoter, and RBS2.



**Fig. S2.** The description and assembled sequences used in this study.

Sequence of *pro-rbs2-pls*

(5′ flank-XbaI-*kasO*p\* promoter-RBS2-*pls* gene-EcoRI-3′ flank)

gccaagcttgggctgcaggtcgactctagatgttcacattcgaacggtctctgctttgacaacatgctgtgcggtgttgtaaagtcgtggccaggagaatactagatctaagtaaggagtgtccatatgtcgtcgccccttctcgaatcgtccttcgagccgtccgagccagcgccccaacaggccctgtaccgcaccgccggcaacccggccccgcggaccctgctcgacgtgctcgatgccaccgccgccgcacatccccaggcgatcgccctggacacgggctccgaggcgctcacctaccgcgacctgtgtatcgagatcgaacgccgcgcacggcagctcagggaccgcggcatcggtcccggcgaccgggtcggagtccgcgtcccctccgggaccgccgagctgtacctgtccatcctcgccgtcctgcgcagcggagcggcctacgtgccggtcgacgccgacgaccccgacgagcgggccgccaccgtcttccgcgaggccgccgtctgcgccgtcctcggccccgacggcccgctgcccggcccggcccggcccctcggcgacccgcgttccgcgggcccccaggacgacgcctggatcatcttcacctcgggttcgaccggcgcgcccaagggcgtggcggtcagccaccgctccgccgccgccttcgtcgacgccgaggccgacctgttctgccaggaccagccgttgggccccggcgaccgggtgctggccgggctgtccgtcgccttcgacgcctcctgcgaggagatgtggctcgcctggcggtacggcgcctgcctggtgcccgcaccccgcgcgctggtccgggccggccacgaactcggcccctggctcgtcgagcgcggcatcaccgtcgtctccaccgtgcccaccctcgccgcgctctggccggacgaggcgatgcgccgggtccgcctgctgatcgtcggcggcgaatcctgcccggccgggctcgtcgaccgcttcgccggacccggccgcgagatgtggaacacctacggcccgaccgagaccaccgtcgtcgcctgcgccgcccgcctgctgccgggcgagccggtccgcatcggcctgcccctgaagggctggcagctcgccgtcgtcgaccgcaccgggcagccggtgcccttcggcgccgagggcgaactgctgatcagcggcgtcggcacggcccgctacctcgaccccgccaaggacgccgaacggttccggcccgacgacgccctgggggccgcccgcgtctaccgcaccggcgacctggtccgggccgaacccgagggcctgctcttcgtcggccgcgccgacgaccagatcaaactcggcggccgccgcatcgagctgggcgagatcgacgccgccctggccgccctgcccggcgtccgcggggccgccgcggccgtccagacgacgccggccggcacccaggtgctggtcggctacgtcgttcccgagcagcgcaccgccgacggttccagcttccagcaggacaaggcccgcgcactgctccaggaacgcctgcccgcgcagttggtcccggtcctcgcggaggtcgagtccctgcccacccggacctccggcaaggtcgaccgcaaggcgctgccctggccgctgccgtccgccccggtcgactccgccaccggcgatccggccacggcgctggacggcaccgccgcccggctcgccgggatctgggaggaactcctcggcgtccggcccggcccggacagcgacttcgtctccctcggcggcaccagcctggtcgccgcccgcatggcgtcccagctccgcatccaccaccccggcgtctcggtcgccgacctctaccgccacccggtgctgcgcgacatggccgagcacctcgactcgctgggcggcccggtggacgaggtccgcccggtccgccccgtcccgcgccgcaccggattcgtccaactcctcgtccagaccggcctgtacggcatcgccggcctgcgcggactggtcgggctcgcgctcgcggacaacgtcctcggcctgctcgccccgcaggtctgggccccgcacaccgcgtggtggctgatcatcgtcggctgggtggtgctctacagcgccccgatgcgttgcgccctcggcgcactggccgcccgcgcgctcgccggcaccatcaagcccggcgcctacccgcgcggcggcgccacccacctgcgcctgtggaccgccgaacgcgtcgtcgccgccttcggcgtcccctccctgctcggcaccccctgggcgcggctctacgcccggagcctgggctgcgccacagggcggaacgtggcgctgcacaccatgccgccggtcaccggcctcgccgaactcggcgacggctgcagcgtcgaacccgaggccgacatctccggctggtggctcgacggcgacaccctgcacatcggcgcggtccggatcggcgccggcgcccgggtcgcccaccgcagcatgctgatgcccggcgccgtcgtcggccagggcgccgaactcgcctccggcgcctgcctggacggagagatccccgacggcgcctcgtggtccggctccccggcccgcccggccggcgccgccgagcggatggccggcgccgcctggcccgcccccgcctggcagcgctcgcgccgctggagcgccgcctacggactgaccctgctgggcctgccgctgctggccctgctgtccaccgcgcccgccctggtcggcgcgtacttcctgctccgcgacagcggcaccctcgccacagccgggcttcgcctgctgctggccgtcccggtcttcacgctcctgaccactggctgctccctcctcgtcaccgccgccgtggtgcgcctcctcggccgcggcatcacgccgggactgcaccccgcgagcggtggcgtcgcctggcgcgcctggctggtcacccgcctcctggacggcgcccgcggcagcctcttcccgctctacgccagcctcggcaccccgcactggctgcggctgctcggcgccaaggtcggccggcacgcggagatctccaccgtgctgccgctgccctccctgctgcacgtcgaggacggcgcgttcctcgccgacgacaccctggtggcgcccttcgaactccgcggcggctggctgcggttggggaccgtccggatcggtcgccgggccttcgtcggcaactccggcatcgtcgaccccggccacgacgtgcccgatcacagcctggtcggcgtgctctccaacgcccccgccgacggcgagcccggctcgtcctggctgggccggcccgccatgccgctgccccgggtggcgacccaggccgacccggcgcgcaccttcgcaccgccgcgcaggctggtccgggcccgcgccgccgtcgagctgtgccgggtgctgccgctgatgtgcggcctggcgctcgccgagggcgtgttcctcaccgagcaggacgccttcgcccagggcggcctcggtctcgccgcactggtcggcgccccgctgctgctggcctcgggcctcgtggcgctgctcgtcaccaccctcgcgaagtggctgctggtcggccgcttcacggtgagcgagcaccccctgtggtcgtcgttcgtgtggcgcaacgagctctacgacaccttcgtcgaatcgctcgccgtgccgtcgatggccggcgcgttcaccggcaccccggtcctgaactggtggctgcgcaccctcggcgccaagatcgggcgcggggtctggttggagagctactggctgccggagaccgacctgatcaccgtcgccgacggcgtcagcgtcaaccgcggctgcgtcctgcagacccacctcttccacgaccggatcatgcggctggacaccgtccgcctcgccgaaggctcctcgctcggcccgcacggcatcgtgctccccggcaccgaggtcggggcgcgcgcctcgatcgcgccgtcgtccctggtcatgcgcggcgagagcgtcccggcccacacccggtgggccggcaacccgatcgccggcgaacgccccgcccgccccgtcccggcacgcgcggagggaggtgcggccgcgtgagaattcgtaatcatgtcatagctgtttcctg