S1. Construction of strains

S1.1. Construction of *E. coli* Strain MG1655 *cat*-P_L-*cycA*

To construct *E. coli* strain MG1655 *cat*-P_L-*cycA*, the native regulatory region of the *cycA* gene was replaced with the phage lambda P_L promoter via λRed-mediated integration. For that purpose, we amplified the *cat*-P_L fragment using the oligonucleotide P1 and P2 with 36 nt homology to the region upstream of the *cycA* gene. The chromosomal DNA of strain BW25113 *cat*-P_L-*yddG* was used as a template for PCR. The method for obtaining BW25113 *cat*-P_L-*yddG* has been previously described in detail [39].

S1.2. Construction of *E. coli* Strain MG1655 *cat*-P_{cycA}-*lacZ*

To construct the *cat*-P_{cycA}-*lacZ* expression unit, the *cat* gene was first introduced upstream of the *cycA* gene on the chromosome of *E. coli* strain MG1655 using λRed-mediated integration. A DNA fragment bearing the λattL-*cat*-λattR cassette was amplified by PCR using the oligonucleotide primers P3 and P4, and the plasmid pMW118-λattL-*cat*-λattR [44] was used as the template. Secondly, the fragment *cat*-P_{cycA} including λattL-*cat*-λattR and the P_{cycA} regulatory region containing the first 33 nt of the coding sequence (−223; +117 relative to the transcription start site, TSS) was PCR-amplified using the oligonucleotide primers P5 and P6 with 36 nt homology to the region upstream of the *lacZ* gene, and DNA of the *E. coli* MG1655 *cat*-*cycA* strain was used as a template. The obtained PCR fragment was inserted into the *E. coli* MG1655/pKD46 genome in a region upstream of the *lacZ* gene via λRed-mediated integration. As a result, the strain *E. coli* MG1655 *cat*-P_{cycA}-*lacZ* was obtained.

S1.3. Construction of *E. coli* K12 2Δ P_L-*ilvBN*^{fbr} *cat*-P_{cycA}-*lacZ*

The strain MG1655 *cat*-P_{cycA}-*lacZ* was used as a donor for the transfer of the cassette *cat*-P_{cycA}-*lacZ* into the Val-resistant strain K12 2Δ P_L-*ilvBN*^{fbr} (kindly provided by Dr. Sycheva, [40]) using the P1 transduction method [46].

S1.4. Construction of the Set of *E. coli* Strains with Deleted Transcription Factor Genes and Possessing *cat*-P_{cycA}-*lacZ* Cassette

The strain MG1655 *cat*–P_{cycA}–*lacZ* was used as a donor for the transfer of the cassette *cat*-P_{cycA}-*lacZ* into the strains K12 BW25113 from the KEIO collection with different individual knockouts of genes encoding transcription factors (*gcvB*, *hns*, *ihfA*, *crp*, *farR*, *rpoS*, and *lrp*) [45] using the P1 transduction method [46].

S1.5. Construction of *E. coli* Strain MG1655 *cat*-P_{cycA}-5′-UTR$_{lacZ}$-*lacZ* and Its *Δcrp* Derivative

The strain MG1655 *cat*–P_{cycA}–5′-UTR$_{lacZ}$–*lacZ* was constructed as described for MG1655 *cat*-P_{cycA}-*lacZ* above, except for the position of the P_{cycA} regulatory region (−223; −1 relative to TSS). The strain BW25113 Δ*crp* from the KEIO collection [45] was used as a donor for the transfer of the chromosome knockout into the MG1655 *cat*-P_{cycA}-5′-UTR$_{lacZ}$-*lacZ* strain by means of the P1 transduction method [46].

S1.6. Construction of *E. coli* Strain 44-3-15 Scr *kan*-P$_L$-*cycA*

To obtain the Ile-producing strain with overexpressed *cycA* gene, in the strain 44-3-15 Scr (B7 *ilvG*ilvA** mini-Mu-P$_R$-*ilvG*MEDA*YC* mini-Mu-P$_R$-*thrA*BC-cat Scr*, [42]), the native regulatory region of the *cycA* gene was replaced with the "strong" promoter P$_L$. In this case, we used kanamycin resistance (*kan)* as a marker since the strain 44-3-15 Scr already had chloramphenicol resistance. The construct *kan*-P$_L$-*cycA* was obtained using the method of *λ*-Red integration in the chromosome of the MG1655 strain (as described for *cat*-P$_L$-*cycA* above, but the source of the marker gene was pMW118-*λ*attL-*kan*-*λ*attR). The strain MG1655 *kan*-P$_L$-*cycA* was used as a donor for the transfer of the cassette *kan*-P$_L$-*cycA* into the Ile-producing strain 44-3-15 Scr via the P1 transduction method [46].

Table S1. Sequences of the PCR primers used in this study.

No.	Sequence 5′–3′	Description
P1	CCCGTAAGCGTGTATTTTTGTGAGCTGTTTCGCGTTCGCTCAAGTTAGTATAAAAAAGCTGAAC	Replacement of the native regulatory region of the *cycA* gene with phage lambda P$_L$ promoter
P2	GATCATCGGCAACGACTTTTACCTGATCTACCATGTTTAGTTCTCCTTCCGGCCAATGCTTCGTT	Replacement of the native regulatory region of the *cycA* gene with phage lambda P$_L$ promoter
P3	TGTTCCTCAATCATAGCCTATGAATAAGCTAACGCTTGAAGCCTGCTTTTTTATACTAAGTTGG	Integration of the *cat* gene upstream of the *cycA* gene
P4	GTAATGAACACGAACGCGGTCGGTACGTGCCGGAATCGCTCAAGTTAGTATAAAAAAGCTGAAC	Integration of the *cat* gene upstream of the *cycA* gene
P5	GTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGATCATCGGCAACGACTTTTACC	Integration of the *cat*-P$_{cycA}$ expression unit upstream of the *lacZ*
P6	GCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGATGAAGCCTGCTTTTTTATACTAAGTTGG	Integration of the *cat*-P$_{cycA}$ or *cat*-*P$_{cycA}$-5′*-UTR$_{lacZ}$ expression unit upstream of the *lacZ*
P7	CTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTTCATCGATATTAGGTAACAATACGCGGG	Integration of the *cat*-*P$_{cycA}$-5′*-UTR$_{lacZ}$- expression unit upstream of the *lacZ*
P8	TAMRA-CCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAG	Amplification of the fragment of the *lacZ* gene promoter region (−238; −20 relative to the TSS)
P9	TAMRA-AAGCATAAAGTGTAAAGC	Amplification of the fragment of the *lacZ* gene promoter region (−238; −20 relative to the TSS)
P10	TAMRA-CGTTCAGCACCCTGGTGTTT	Amplification of the fragments of the *cycA* gene promoter region #1 (−261; −63 relative to the TSS) and #3 (−261; −123 relative to the TSS)
P11	TAMRA-TGTTTAAAGTGAGTGTCATATCACGGTGA	Amplification of the fragment of the *cycA* gene promoter region #1 (−261; −63 relative to the TSS) and #2 (−136; −63 relative to the TSS)
P12	CAAAGACCCCGTAAGCGTGTATTTTTGTGAGCTGTTTCGCGTTATCACCGTGATATGACACTCACTTTAAACA	Amplification of the fragment of the *cycA* gene promoter region #2 (−136; −63 relative to the TSS)
P13	TACGGGGTCTTTGCAACACA	Amplification of the fragment of the *cycA* gene promoter region #3 (−261; −123 relative to the TSS)