

Engineering Probiotic *Escherichia coli* for Inflammation-Responsive Indoleacetic Acid Production Using RiboJ-Enhanced Genetic Circuits

Seung-Gyun Woo^{1,2}, Seong Keun Kim¹, Seung-Goo Lee^{1,3,4*} and Dae-Hee Lee^{1,3,4,5*}

¹Synthetic Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Republic of Korea

²Department of Molecular Biosciences, University of Texas at Austin, Austin, Texas 78712, USA

³Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, University of Science and Technology (UST), Daejeon 34113, Republic of Korea

⁴Graduate School of Engineering Biology, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea

⁵Department of Integrative Biotechnology, College of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon-si, Gyeonggi-do 16419, Republic of Korea

***Corresponding authors.**

Dae-Hee Lee, Ph.D., Tel: +82 42 879 8225; E-mail: dhlee@kribb.re.kr

Seung-Goo Lee, Ph.D., Tel: +82 42 860 4373; E-mail: sglee@kribb.re.kr

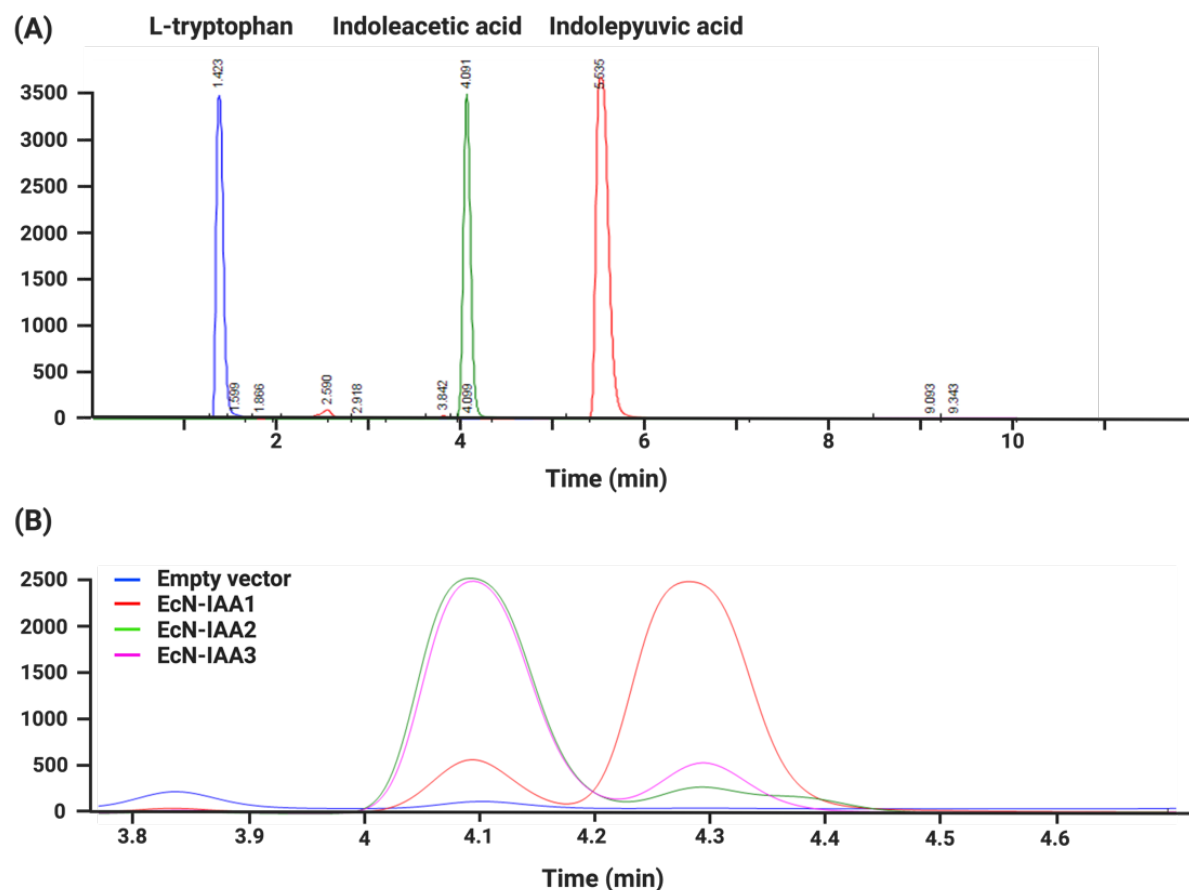


Figure S1. Representative HPLC chromatograms of both standard substances and analytes. All analytes are Identified, along with their retention times, as shown in panels A and B. Panel A represents the standard mixture, while Panel B illustrates the analyte samples.

Table S1. Bacterial strains and plasmids used in this study.

Name	Description	Reference
<i>E. coli</i> strains		
DH5α	<i>F</i> ⁻ , Φ80 <i>lacZ</i> ·Δ <i>M15</i> ·f(<i>lacZYA-argF</i>)U169 <i>deoR recA1 endA1 hsdR17</i> (<i>rk</i> , <i>mk</i> +) <i>phoA supE44 thi-1 gyrA96 relA1</i>	Enzynomics
Nissle 1917 (EcN)	Wild-type strain (serotype O6:K5:H1)	GmbH
Plasmids		
pTrc99A	P _{trc} promoter, <i>lacI</i> ^q , pBR322 ori, Amp ^R	GE Healthcare
pTSN-IAA1	pTrc99A derivatives containing <i>aspC</i> of <i>E. coli</i> , codon-optimized <i>ipdC</i> of <i>Enterobacter cloacae</i> , and codon-optimized <i>iad1</i> of <i>Ustilago maydis</i> , pBR322 ori, Amp ^R	This study
pTSN-IAA2	pTrc99A derivatives containing <i>iad1-aspC-ipdC</i> gene cluster, pBR322 ori, Amp ^R	This study
pTSN-IAA3	pTrc99A derivatives containing RiboJ- <i>iad1-aspC-ipdC</i> gene cluster, pBR322 ori, Amp ^R	This study
pSEVA131-J23114-TPG	Derivative of pSEVA131 harboring <i>thsS</i> (L547T) <i>R</i> genes, constitutive promoter (J23114), output promoter P _{<i>phsA</i>} , and <i>sfgfp</i> gene, pBBR1 ori, Amp ^R	(1)
pSEVA131-J23114-T(D57A)PG	Derivative of pSEVA131 harboring <i>thsS</i> (L547T) <i>R</i> (D57A) genes, constitutive promoter (J23114), output promoter P _{<i>phsA</i>} , and <i>sfgfp</i> gene, pBBR1 ori, Amp ^R	(1)
pPhsA-IAA4	Derivative of pSEVA131 harboring <i>thsS</i> (L547T) <i>R</i> genes, constitutive promoter (J23114), output promoter P _{<i>phsA</i>} , and <i>iad1-aspC-ipdC</i> gene cluster, pBBR1 ori, Amp ^R	This study
pPhsA-IAA5	Derivative of pSEVA131 harboring <i>thsS</i> (L547T) <i>R</i> (D57A) genes, constitutive promoter (J23114), output promoter P _{<i>phsA</i>} , and <i>iad1-aspC-ipdC</i> gene cluster, pBBR1 ori, Amp ^R	This study
pPhsA-IAA6	Derivative of pSEVA131 harboring <i>thsS</i> (L547T) <i>R</i> genes, constitutive promoter (J23114), output promoter P _{<i>phsA</i>} , and RiboJ- <i>iad1-aspC-ipdC</i> gene cluster, pBBR1 ori, Amp ^R	This study
pPhsA-IAA7	Derivative of pSEVA131 harboring <i>thsS</i> (L547T) <i>R</i> (D57A) genes, constitutive promoter (J23114), output promoter P _{<i>phsA</i>} , and RiboJ- <i>iad1-aspC-ipdC</i> gene cluster, pBBR1 ori, Amp ^R	This study
pSEVA131-J23113-NPG	Derivative of pSEVA131 harboring <i>narXL</i> genes, constitutive promoter (J23113), output promoter P _{<i>yeaR</i>} , and <i>sfgfp</i> gene, pBBR1 ori, Amp ^R	(1)
pSEVA131-J23113-N(H399A)PG	Derivative of pSEVA131 harboring <i>narX</i> (H399A) <i>L</i> genes, constitutive promoter (J23113), output promoter P _{<i>yeaR</i>} , and <i>sfgfp</i> gene, pBBR1 ori, Amp ^R	(1)
pYeaR-IAA1	Derivative of pSEVA131 harboring <i>narXL</i> genes, constitutive promoter (J23113), output promoter P _{<i>yeaR</i>} , and RiboJ- <i>iad1-aspC-ipdC</i> gene cluster, pBBR1 ori, Amp ^R	This study
pYeaR-IAA2	Derivative of pSEVA131 harboring <i>narX</i> (H399A) <i>L</i> genes, constitutive promoter (J23113), output promoter P _{<i>yeaR</i>} , and RiboJ- <i>iad1-aspC-ipdC</i> gene cluster, pBBR1 ori, Amp ^R	This study
placA-sfGFP	Derivative of pSEVA131 harboring codon-optimized <i>iacR</i> of <i>Pseudomonas putida</i> , constitutive promoter (J23114), output promoter P _{<i>iacA</i>} , and <i>sfgfp</i> gene, pBBR1 ori, Amp ^R	This study

Table S2. DNA oligonucleotides used in this study.

Name	Sequence (5' to 3')	Purpose
aspC-IF	ttcacacaggagggttaaaccatgtttgagaacattaccgc	For the construction of the pTSN-IAA1 plasmid
aspC-IR	tttctcctctttgcggccgcttacagcactgccacaatcg	
aspC-VF	gcggccgcaaagaggagaaatactagatgcgtaccccgtg	
iad1-IR	cttgcatgcctgcaggtcgatcacgctttcgggatccaca	
iad1-VF	tgtggatcccgaagcgtgatcgacctgcaggcatgcaag	
aspC-VR	gcggtaattgttctcaaacatggtttaacctcctgtgtgaa	
iad1-IF	ttcacacaggagggttaaaccatgaccgttggtgttcgctc	For the construction of the pTSN-IAA2 plasmid
iad1-IR	ctagtatttctcctctttgcggccgctcacgctttcgggatccaca	
aspC(B0034)-IF	gcggccgcaaagaggagaaatactagatgtttgagaacattaccg	
ipdC-IR	cttgcatgcctgcaggtcgatcacgctgtgttacgcgctt	
rrnBT-VF	tcgacctgcaggcatgcaagcttggctgttttggcggatg	
lacO-VR	ggtttaacctcctgtgtgaaattgtatccgctcacaatt	
RiboJ-IF	agcggataacaattagctgtcacccgatgtgctt	For the construction of the pTSN-IAA3 plasmid
RiboJ-IR	aacctcctgtgtgattaacaaaaattattgtag	
RiboJ(iad1)-VF	gtttaatcacacaggagggttaaaccatgaccgtt	
ipdC-IR	cttgcatgcctgcaggtcgatcacgctgtgttacgcgctt	
rrnBT-VF	tcgacctgcaggcatgcaagcttggctgttttggcggatg	
RiboJ(lacO)-VR	acagctaattgttatccgctcacaattccacaca	
iad1(B0034)-IF	aaagaggagaaatactagatgaccgttggtgttg	For the construction the of pPhsA-IAA1 and pPhsA-IAA2 plasmids
ipdC-IR1	aggatctcacgcgtgtgttacgcgttccagcgct	
ipdC-VF	gtaacaacgcgtgagatcctacaaggagggtacca	
ThsS(B0034)-VR	atctagtatttctcctctttgttaaatctccaatgacag	
RiboJ(PphsA)-IF	tttaacagctgtcacccgatgtgctttccggctt	For the construction the of pPhsA-IAA3 and pPhsA-IAA4 plasmids
ipdC-IR1	aggatctcacgcgtgtgttacgcgttccagcgct	
ipdC-VF	gtaacaacgcgtgagatcctacaaggagggtacca	
RiboJ(PphsA)-VR	atccgggtgacagctgttaaatctccaatgacag	
RiboJ(PyeeR)-IF	aaaaggagctgtcacccgatgtgctttccggctt	For the construction the of pYeeR-IAA1 and pYeeR-IAA2 plasmids
ipdC-IR1	aggatctcacgcgtgtgttacgcgttccagcgct	
ipdC-VF	gtaacaacgcgtgagatcctacaaggagggtacca	
RiboJ(PyeeR)-VR	atccgggtgacagctccttttccctccttcgggtga	
iacR-IF	acacaggaaaagcttttacagggtttccaggtgtg	For the construction of the placA-sfGFP plasmid
PiacA(B0034)-IR	tatttctcctctttggacattctccgtctcaggc	
PiacA(B0034)-VF	atgtccaaagaggagaaatactagatgagcaaaag	
iacR-VR	ctgtaaaagctttcctgtgtgaaattgttatccg	

Table S3. DNA sequences of *iad1*, *aspC*, *ipdC*, and *iacR* used in the study.

>*iad1*

atgaccgttggtgtgctctaaactgctggacgcgccgttctctacgttatcaacggtaaactctgttgacacccagggtttcaaaacccctggacg
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gtggatcccgaagcgtga

>*aspC*

atgtttgagaacattaccgccgctcctgccgacccgattctgggcctggccgatctgttctgcccgatgaacgtcccgcaaaattaacctcggg
attggtgtctataaagatgagacgggcaaaaccccggtactgaccagcgtgaaaaaggctgaacagtatctgctcgaaaaatgaaaccacaaaa
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>*ipdC*

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>iacR

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