# Biotin-independent Strains of Escherichia coli for Enhanced Streptavidin Production



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# Supplementary Information for

## Biotin-independent Strains of *Escherichia coli* for Enhanced Streptavidin Production

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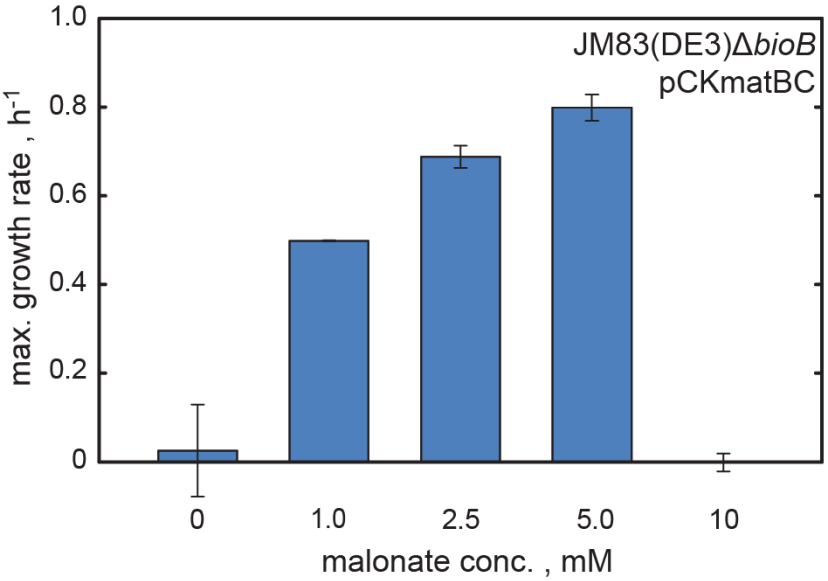
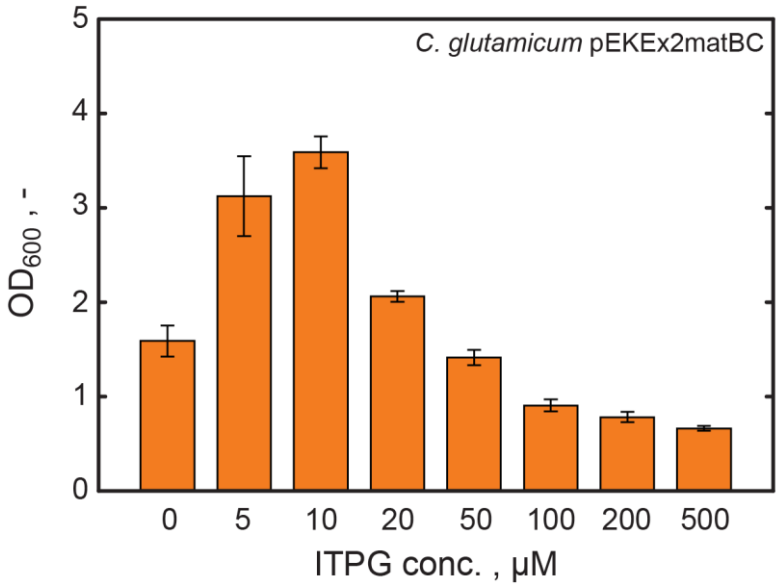
**This PDF file includes:**

Supplementary Figures 1 to 4
Supplementary Tables 1 to 3
Supplementary References 65 to 68

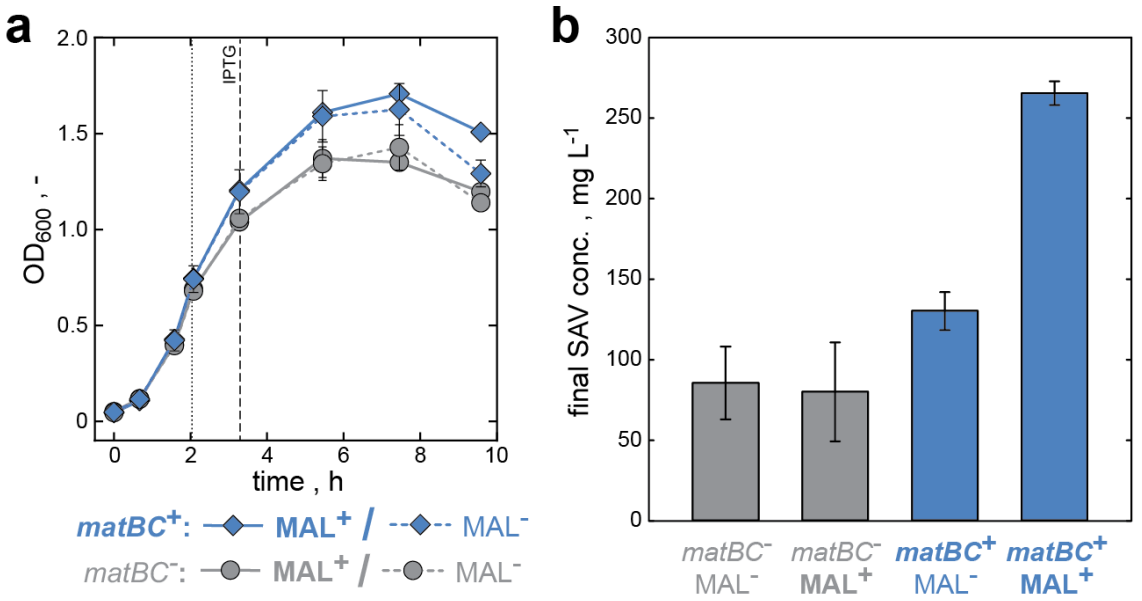
## Supplementary Results



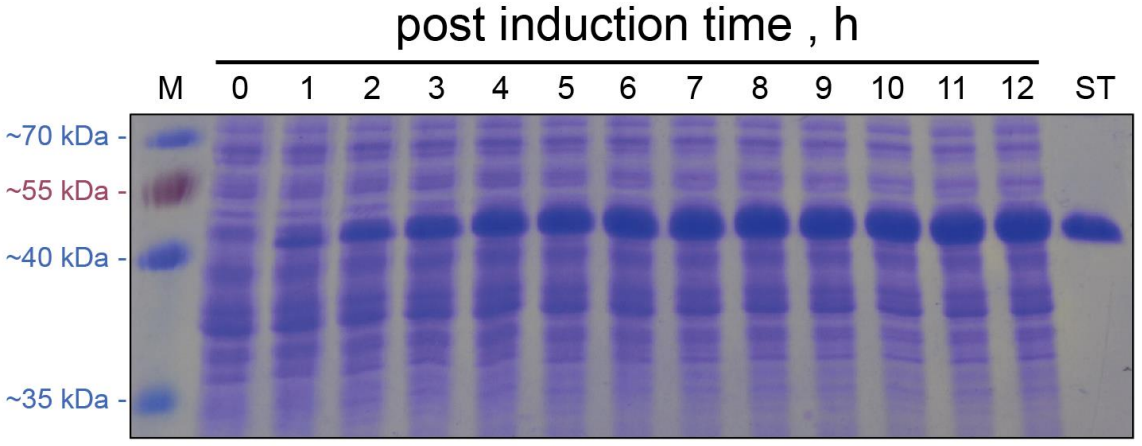

**Supplementary Fig. 1 | Complementation of biotin auxotrophy in shake flask cultures of *E. coli*.** The MatBC bypass allows to restore growth of the biotin auxotrophic mutant JM83(DE3)Δ*bioB* depending on the amount of malonate supplemented to the biotin-free medium (M9). Bars represent average growth rates of two independent shake flask cultures with s.d. (n=2).




**Supplementary Fig. 2 | Complementation of biotin auxotrophy in *C. glutamicum* depends on the expression level of *matBC* genes.** Cultivations were performed in biotin-free CGXII medium supplemented with 10 mM malonate and varying IPTG concentrations to alter the expression of *matBC* genes. Bar heights are mean $OD_{600}$ values after 48 h of cultivation in 96-deepwell plates with s.d. (n=4).




**Supplementary Fig. 3 | Shake flask cultivation of biotin-independent strains for SAV production.** (a) Cultivations were performed with *E. coli* JM83(DE3) both in the presence (***matBC***$^{+}$) and absence (*matBC*$^{-}$) of pCKmatBC as well as with (**MAL**$^{+}$) and without (MAL$^{-}$) supplementation of 5 mM malonate to the biotin-free medium (M9). To improve SAV expression the cultivation temperature was lowered from 37°C to 20°C (dotted vertical line) prior to induction of SAV expression with IPTG (dashed vertical line). (b) Analysis of the final SAV titers revealed a significantly improved product formation for the biotin-independent strain (***matBC***$^{+}$ **MAL**$^{+}$). Data points/bars represent average $OD_{600}$ (a) values and final SAV titers (b) of two independent shake flask cultivations with s.d. (n=2).




**Supplementary Figure 4 | SDS-PAGE analysis of cell lysates from biotin-independent SAV production.** Samples from 0 to 12 hours after induction from the best replicate process with the biotin-independent strain were run together with a purified, lyophilized SAV standard (ST) corresponding to 4 g $L^{-1}$ as well as a protein ladder (M). Please note that due to its extraordinary stability SAV does not denature under standard SDS-PAGE conditions if the samples are not heat-denatured prior to loading of the gel and consequently the prominent band appearing over time corresponds to native, tetrameric SAV.

**Supplementary Tab. 1 | Strains and plasmids used in this study.**

| Strain or plasmid | Genotype/Description | Source/Reference |
|---|---|---|
| *E. coli* strains | | |
| TOP10 | F$^-$ *mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL*(Str$^R$) *endA1* λ$^-$; general cloning strain | Thermo Fisher Scientific, Reinach, Switzerland |
| JM83 | *ara* Δ(*lac-proAB*) *rspL*(+*strA*) *ϕ*80 *lacZΔM15* | Yanisch-Perron *et al.* (1985)[65] |
| JW0758 | BW25113 carrying an insertional knockout of the biotin synthase gene, *bioB*:*kan* | Keio collection[66] |
| BL21(DE3) | Strain used for SAV expression experiments | Studier & Moffatt (1986)[67] |
| JM83(DE3)*ΔbioB* | Product of JM83(DE3) transduction with P1 phage lysate from JW0758 and removal of *kan$^R$* by FRT recombination; *bioB* deficient, biotin auxotroph | This study |
| JM83(DE3) | λDE3 lysogen of JM83 | This study |
| *C. glutamicum* strains | | |
| DSM 20300 | Wild type strain (ATCC 13032), biotin auxotroph | DSMZ, Braunschweig, Germany |
| Plasmids | | |
| pCK01 | $P_{lac}$ promoter, pSC101 ori, *cat* (Cam$^R$); used for cloning of pCKmatBC and as empty vector control. | Fernandez *et al.* (1995)[29] |
| pET-30b(+) | $P_{T7}$ promoter, pBR322 ori, Kan$^R$ | Merck Millipore, Darmstadt, Germany |
| pEKEx2 | *E. coli*/*C. glutamicum* shuttle vector; $P_{tac}$ promoter, *kan$^R$* | Eikmanns *et al.* (1991)[30] |
| pCP20 | Yeast Flp recombinase, temperature sensitive replicon, *amp*, *cat*; used for removal on kanamycin resistance gene from insertional knockout *bioB*:*kan* | Datsenko & Wanner (2000)[28] |
| pET30T7SAV | Derivative of pET-30b(+) containing the gene coding for T7-tagged SAV[68] under transcriptional control of $P_{T7}$ | Jeschek *et al.* (2016)[48] |
| pCKmatBC | Derivative of pCK01 containing the *matBC* cassette from *R. trifolii* under the control of a $P_{lac}$ promoter | This study |
| pET30matBC | Derivative of pET-30b(+) containing the *matBC* cassette from *R. trifolii* under transcriptional control of $P_{T7}$ and the natural GTG start codon in *matB* | This study |
| pET30matBC* | Derivative of pET-30b(+) containing the *matBC* cassette from *R. trifolii* under transcriptional control of $P_{T7}$ and the an ATG start codon in *matB* | This study |
| pEKEx2matBC | Derivative of pEKEx2 containing the *matBC* cassette from *R. trifolii* under transcriptional control of $P_{tac}$ | This study |

**Supplementary Tab. 2 | Oligonucleotides used in this study.**

| No. | Sequence (5'-3') | Description |
|---|---|---|
| 1 | CCAGATATGGCGTTGGTCAAAGG | Forward primer flanking *bioB* |
| 2 | CGCTATAACCGCTGACGTGACC | Reverse primer flanking *bioB* |
| 3 | TATAGGATCCAGGAGGGCGAAAGTGAGCAACC | Amplification of *matBC*, *Bam*HI site |
| 4 | TATAGATATCGGCGCGCCTCAAACCAGC | Amplification of *matBC*, *Eco*RV site |
| 5 | TAGGATCCCCGGGTACCGAGCTC | Amplification of pCK01, *Bam*HI site |
| 6 | TAGATATCCTGCAGGCATGCAAGCTTGC | Amplification of pCK01, *Eco*RV site |
| 7 | GCCGCATCATATGTAATAAGGAGG | Amplification of *matBC*, *Nde*I site |
| 8 | TATACATATGAGCAACCATCTTTTCGACGC | Amplification of *matBC*, *Nde*I site, ATG start codon |
| 9 | TATAGGATCCGGCGCGCCTCAAACCAGC | Amplification of *matBC*, *Bam*HI site |
| 10 | TAGGATCCAGGAGGGCGAAAG | Amplification of *matBC*, *Bam*HI *site* |
| 11 | ATGGTACCTCAAACCAGCCCGGGCAC | Amplification of *matBC*, *Kpn*I *site* |

**Supplementary Tab. 3 | Synthetic *matBC* DNA construct used in this study.** Genes (*matB* and *matC*) are underlined.

| Name/Description | Sequence (5'-3') |
|---|---|
| *matBC* cassette from *R. trifolii* | catatgtaataaggagggcgaaagtgagcaaccatcttttcgacgccatgcgggccgccgcg<br>cccggtaacgcaccattcatccggatcgataacacgcgcacatggacctatgacgacgccttt<br>cgctctttccggccgcattgccagcgcgatggacgcgctcggcattcgccccggcgaccgcg<br>ttgcggtgcaggtcgagaaaagtgccgaggcattgatcctctatctcgcctgtcttcgaagc<br>ggcgccgtctacctgccgctcaacaccgcctatacgctggctgagctcgattattttatcgg<br>cgatgcggagccgcgtttggtggttgtcgcatcgtcggctcgagcgggcgtggagacaatcg<br>ccaagccccgcggtgcgatcgtcgaaactctcgacgctgctggcagcggctcgttgctggat<br>ctcgcccgcgacgagccggccgactttgtcgatgcctcgcgctccgccgatgatctggcggc<br>gatcctctacacgtccggaacgacgggacgctccaaggggggcgatgctcacgcatgggaacc<br>tgctctcgaacgccctgaccttgcgagatttttggcgcgtcaccgccggcgatcgactgatc<br>catgccttgccgatcttccacacgcatggactgttcgtcgccacgaacgtcacactgctcgc<br>cggcgcctcgatgttcctgctgtcgaagttcgacccggaggagatcctgtcgctgatgccgc<br>aggcaacgatgctgatgggcgtgccgaccttctacgtgcgcctcctgcagagcccgcgcctc<br>gacaagcaagcggtcgccaacatccgcctcttcatttccggttcggctccactgcttgcaga<br>aacacataccgagttccaggcacgtaccggtcacgccattctcgagcgctacggcatgacgg<br>aaaccaatatgaacacgtccaacccttatgaggggaaacggattgccggaacggtcggcttc<br>ccgctgcctgatgtgacggtgcgcgtcaccgatcccgccaccgggctcgcgctgccgcccga<br>acaaaccggcatgatcgagatcaaggggccgaacgttttcaagggctattggcgcatgcccg<br>aaaaaaccgcggccgaattcaccgccgacggtttcttcatcagcggcgatctcggcaagatc<br>gaccgcgacggttatgtccacatcgtcggccgcggcaaggatctggtgatttcgggtggata<br>caacatctatccgaaagaggttgagggcgagatcgaccagatcgagggtgtggttgagagcg<br>ctgtgatcggcgtgccgcatcccgatttcggagaaggcgtaacggccgtcgtcgtgcgcaag<br>cccggcgctgccctcgatgaaaaggccatcgtcagcgccctccaggaccggctcgcgcgcta<br>caaacaacccaagcgcatcatctttgcagaggacttgccgcgcaacacgatgggtaaggttc<br>agaaaaacatcctgcggcagcaatacgccgatctttataccaggacgtaaggcgaccgcgct<br>ctctgggaggagagtgcgtcgacatcccgcatcaatcttgaaaacagcaactgcgacgcgga<br>ggcgtcggagggaggggaatcatgggtattgaattactgtccataggcctgctgatcgccat<br>gttcatcattgcgacgatccagccaatcaacatgggtgcgctcgcctttgccggcgccttcg<br>tgctcggctcgatgatcatcgggatgaaaaccaacgaaatatttgccggctttccgagtgat<br>ctgttcctgacgctcgtcgccgtcacctacctcttcgccatagcgcagatcaacggcacgat<br>cgactggctcgtcgaatgtgccgtccgcctggtacgcgggcggatcggcttgattccctggg<br>tgatgttccttgtcgccgccatcattactggcttcggtgcacttgggcctgctgcggtcgcc<br>attctcgcacccgtcgcgttgagctttgccgtgcagtaccgcattcatccggtgatgatggg<br>tctgatggtgatccacggcgcgcaggcaggcggcttctcgccgatcagcatctatggcggaa<br>tcaccaaccagatcgttgcgaaggccggcctgcctttcgctccgacctcgctgtttctttcc<br>agcttcttctttaacctggcgatcgcggtgctggtgttcttcgtgttcggcggcgcgagggt<br>gatgaagcacgatcccgcatcacttggccccttgcccgaactccatcccgagggcgtatcgg<br>cgtcgatcagaggccacggcggcacgccggcaaaaccgatcagagagcatgcctatggtacg<br>gcggccgataccgcgacgacgttgcgtctgaacaatgagagaattaccaccttgatcggcct<br>gacggcgctcggcatcggcgccctggttttcaagttcaatgttggcctcgtcgccatgaccg<br>tcgccgtcgtcctcgcgctgctgtcaccgaagacccagaaggccgcaatcgacaaggtcagt<br>tggtcgaccgtgctgctgattgccggcatcatcacctatgtcggcgtcatggagaaggccgg<br>tacggtcgactacgtggcgaatggcatatccagtctcggcatgccgctactggtagcgctcc<br>tgctttgctttacgggcgccatcgtctcggcctttgcttcctcgaccgcgctgctcggcgcg<br>atcatcccgcttgccgttccattcctcctgcaagggcacatcagcgccatcggtgtggtcgc<br>ggcgatcgccatctcgacgacgatcgtcgacaccagcccattctccaccaacggcgcccttg<br>tcgtcgccaatgcgccggacgacagccgtgagcaggtgttgcgacagctactgatctacagc<br>gccttgatcgctatcatcggtccgatcgttgcctggttggtgttcgtcgtgcccgggctggt<br>ttgaggcgcgcc |

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