**Supplementary Information (****Online Resource 1)**

**Journal:** **Antonie van Leeuwenhoek**

**Expression of toxic genes in *Methylorubrum extorquens* with a tightly repressed, cumate-inducible promoter**

Laura Pöschel1,2, Elisabeth Gehr1, Paulina Jordan1, Frank Sonntag1, Markus Buchhaupt1\*

1 DECHEMA-Forschungsinstitut, Microbial Biotechnology, Theodor-Heuss-Allee 25, 60486 Frankfurt am Main, Germany

2 Faculty of Biological Sciences, Goethe University Frankfurt am Main, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany

\*Corresponding author,

e-mail address: markus.buchhaupt@dechema.de

**Description of content:**

**Table S1** Bacterial strains and plasmids used in this work

**Table S2** Oligonucleotides used in this study

**Figure S1** Tolerance of *M. extorquens* AM1 towards *cis*-abienol dissolved in aqueous phase

**Table S1** Bacterial strains and plasmids used in this work.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Relevant features/Cloning strategy** | **Application/Source** | **Reference** |
| **Bacterial strains** |
| *E. coli* DH5α | F– φ80*lac*ZΔM15, Δ(*lac*ZYA-*arg*F)U169, *rec*A1, *end*A1, *hsd*R17(rK–, mK+) *pho*A, *sup*E44, λ–, *thi*-1 *gyr*A96 *rel*A1 | Standard cloning applications | ATCC |
| *M. extorquens* AM1 | CmR, gram-negative, facultative methylotrophic, obligate aerobic, α‑proteobacterium  |  | Peel and Quayle 1961 |
| **Plasmids** |
| pFS62b | pQ2148F-*zssI-ERG20-hmgs*-MVA | Expression vector for *M. extorquens* AM1 for α‑humulene synthesis  | Sonntag et al. 2015 |
| pFS62b-s6 | pQ2148-s6-*zssI-ERG20-hmgs*-MVA | α‑humulene synthesis under Ps6-control | This work |
| pTE105\_mCherry | TetR | mCherry expression vector for *M. extorquens* AM1 | Schada von Borzyskowski et al. 2015 |
| ppjo16 | pQ2148F-*AbCAS‑ERG20F96C*‑MVA, *AbCAS* (Zerbe et al. 2012) and *ERG20F96C* (Ignea et al. 2015)were codon optimized and a new RBSa was inserted. For detailed sequence information see international patent WO 2016/142503 (Schrader et al.) | *cis*-abienol production plasmid | This work |
| ppjo16s1 | pQ2148F\_*AbCAS*(mut.)*\_*MVA(mut.) | Isolated from ppjo16-suppressor mutant | This work |
| ppjo16s3 | pQ2148F\_*AbCAS\_ERG20F96C*\_MVA | Isolated from ppjo16-suppressor mutant | This work |
| ppjo16s4 | pQ2148F\_*AbCAS*(mut.) | Isolated from ppjo16-suppressor mutant | This work |
| ppjo16s6 | pQ2148F\_*AbCAS\_ERG20F96C*\_MVA (mutated PQ2148) | Isolated from ppjo16-suppressor mutant | This work |
| ppjo16L1 | pQ2148F\_*AbCAS*(mut.)*\_ERG20F96C*(mut.)\_MVA | Isolated from ppjo16-suppressor mutant | This work |
| pQ2148 | PQ2148, TetR, oriT, pBR322ori | Expression vector for *M. extorquens* harboring cumate inducible promoter  | Kaczmarczyk et al. 2013 |
| pQ2148F | pQ2148 with adapted multiple cloning site, TetR, oriT, pBR322ori | Expression vector for *M. extorquens* harboring cumate inducible promoter  | Sonntag et al. 2015 |
| pQ2148\_mCherry | PQ2148, mCherry, TetR, oriT, pBR322ori | mCherry reporter plasmid for PQ2148  | This work |
| pQ2148-s6\_mCherry | Ps6, mCherry, TetR, oriT, pBR322ori | mCherry reporter plasmid for Ps6 | This work |
|  |  |  |  |
| pQ2148L\_mCherry | PQ2148, mCherry, TetR, oriT, pBR322ori, contains linker region of pQ2148-lux (Kaczmarczyk et al. 2013) | mCherry reporter plasmid for PQ2148 with same GOI-upstream sequence like pQ2148-lux (Kaczmarczyk et al. 2013) | This work |
| pQ2148L-s6\_mCherry | Ps6, mCherry, TetR, oriT, pBR322ori, contains linker region of pQ2148-lux (Kaczmarczyk et al. 2013) | mCherry reporter plasmid for Ps6 with same GOI-upstream sequence like pQ2148-lux (Kaczmarczyk et al. 2013) | This work |

a Optimization of RBS sequences was done with the RBS Calculator (Salis 2011)

**Table S2** Oligonucleotides used in this studya.

|  |  |  |
| --- | --- | --- |
| EGe119 | ACAATCTGGTCTGTTTGTAACTAGTATGGTGAGCAAGGGCGAG | Construction of pQ2148F\_mCherry and pQ2148F-s6\_mCherry |
| EGe121 | TTGTAAAACGACGGCCAGTGAATTCTTACTTGTACAGCTCGTCCATGCC |
| LPoe1 | AGCCTGAATTCGGATCCTGCAGGTACCGGGATCCGGCCCTCTAGTTACAAACAGACCAGATTGTCTGTTTGTTGTGGCGCGCTTCTAC | Construction of pQ2148\_mCherry and pQ2148-s6\_mCherry |
| LPoe2 | CATGGACGAGCTGTACAAGTAAGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGG |
| LPoe3 | CGGTACCTGCAGGATCCGAATTCAGGCTTGGAGGATACGTATGGTGAGCAAGGGCGAGG |
| LPoe4 | GTAAAACGACGGCCAGTGAATTCTTACTT |
| LPoe5 | GTATCATGAGCGGATACATACTGGTCTGTTTGTACAGCATTGACG |
| LPoe6 | TATGTATCCGCTCATGATACAATAACCCTGATGC |
| LPoe7 | GCCTCGCGCGGGATTTTCTT |
| LPoe8 | CTGTTCACCACGCGCAACAAG |
| PJo113 | TTCGGCGACATGATGAC | Sequencing of constructs |

a For oligonucleotides and PCR templates used for construction of ppjo16, see international patent WO 2016/142503 (Schrader et al.)



**Fig. S1** Tolerance of *M. extorquens* AM1 towards *cis*-abienol. Maximum growth rates (μmax) in medium without *cis*-abienol were compared to growth rates (μ) with different *cis*-abienol concentrations dissolved in aqueous phase. Three to four independent replicates were measured. Error bars represent standard deviations

**References**

Ignea C, Trikka FA, Nikolaidis AK, et al (2015) Efficient diterpene production in yeast by engineering Erg20p into a geranylgeranyl diphosphate synthase. Metab Eng 27:65–75. https://doi.org/10.1016/j.ymben.2014.10.008

Kaczmarczyk A, Vorholt JA, Francez-Charlot A (2013) Supplemental material Cumate-inducible gene expression system for sphingomonads and other Alphaproteobacteria. Appl Environ Microbiol 79:6795–6802. https://doi.org/10.1128/AEM.02296-13

Peel D, Quayle JR (1961) Microbial growth on C1 compounds. 1. Isolation and characterization of *Pseudomonas* AM 1. Biochem J 81:465–469. https://doi.org/10.1042/bj0810465

Salis HM (2011) The ribosome binding site calculator. Methods Enzymol 498:19–42. https://doi.org/10.1016/B978-0-12-385120-8.00002-4

Schada von Borzyskowski L, Remus-Emsermann M, Weishaupt R, et al (2015) A Set of Versatile Brick Vectors and Promoters for the Assembly, Expression, and Integration of Synthetic Operons in *Methylobacterium extorquens* AM1 and Other Alphaproteobacteria. ACS Synth Biol 4:430–443. https://doi.org/10.1021/sb500221v

Schrader J, Buchhaupt M, Sonntag F, et al PROCESS FOR DE NOVO MICROBIAL SYNTHESIS OF TERPENES. WO 2016/142503 A1, 2016

Sonntag F, Kroner C, Lubuta P, et al (2015) Engineering *Methylobacterium extorquens* for de novo synthesis of the sesquiterpenoid α-humulene from methanol. Metab Eng 32:82–94. https://doi.org/10.1016/j.ymben.2015.09.004

Zerbe P, Chiang A, Yuen M, et al (2012) Bifunctional *cis*-Abienol Synthase from *Abies balsamea* Discovered by Transcriptome Sequencing and Its Implications for Diterpenoid Fragrance Production. J Biol Chem 287:12121–12131. https://doi.org/10.1074/jbc.M111.317669