Supplementary Material

Killing two birds with one stone – strain engineering facilitates the development of a unique rhamnolipid production process

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# Supplementary Figures and Tables

**Supplementary Table 1:** List of oligonucleotides used in this work.

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Direction | Used for | Sequence |
| IB-5IB-6IB-278IB-279IB-280IB-281IB-282IB-283IB-311IB-312IB-313IB-314IB-315IB-316rpoB frpoB r | fwdrevfwdrevfwdrevfwdrevfwdrevfwdrevfwdrevfwdrev | pEMG-*fleQ*pEMG-*fleQ**P. putida* gDNA*P. putida* gDNA*P. putida* gDNA*P. putida* gDNA*P. putida* gDNA*P. putida* gDNA*P. putida* cDNA*P. putida* cDNA*P. putida* cDNA*P. putida* cDNA*P. putida* cDNA*P. putida* cDNA*P. putida* cDNA*P. putida* cDNA | CAAGGCGATTAAGTTGGGTCCGGCTCGTATGTTGTGtaacagggtaatctgaattcCACCCCGCCAGGCAGCAAttgctattgcTGTTTGGGCTGGGTGTTCGCagcccaaacaGCAATAGCAACTTCCCTAGTCATATCttgcatgcctgcaggtcgacGCCTCCGCCGAGCAATAATACCAATTGGCCGATCAGAGGGAAGTCGCGCAGTTGATGGGACATCTGGAACGAAGGCAAGCACGCCGATGTAGTCTTCGTTCATGGCCGAGGTGATGATGTCGTACTGTTGCTCGGACGGTCCTCGAGCTGCTGGACAGTACGTCCTCTTCGAGCCATACGGCGCGGCATACACGTTCACGTCGTCCGACTT |

Supplementary Table 2: Identified mutations after genome re-sequencing of *P. putida* KT2440 E1 and *P. putida* KT2440 E1.1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Strain** | **Region** | **Position** | **Mutation** | **Outcome** |
| *P. putida* KT2440 E1*P. putida* KT2440 E1.1 | PP\_0441 (*secE*) | 534281 | G**A**C (T) 🡪 G**G**C (A) | Missense mutation |
| *P. putida* KT2440 E1.1 | PP\_2682 (*yiaY*) | 3071601 | **C**AG (Q)🡪 **T**AG (Stop) | Nonsense mutation |
| *P. putida* KT2440 E1*P. putida* KT2440 E1.1 | PP\_2682 (*yiaY*) | 3072054 | **C**GT (A) 🡪 **T**GT (C) | Missense mutation |
| *P. putida* KT2440 E1.1 | PP\_4373 (*fleQ*) | 4964766 | **A** 🡪 **ACGGCGCTTCG** | Frameshift |
| *P. putida* KT2440 E1*P. putida* KT2440 E1.1 | PP\_4373 (*fleQ*) | 4964949 | **C**AG (Q) 🡪 **T**AG (Stop) | Nonsense mutation |
| *P. putida* KT2440 E1.1 | intergenic region upstream of PP\_3839 (*adhP*) | 4362872 | **C** 🡪 **T** | Predicted -10 element affected |



**Supplementary Figure 1.** Predicted promoter region upstream of *adhP* using the Softberry BPROM tool (Solovyev and Salamov, 2011). Shown is the alteration of the -10 element based on the introduced mutation (bold, red) in the evolved strain *P. putida* KT2440 E1.1 compared to the reference (*P. putida* KT2440).

**Reference**

Solovyev, V., and Salamov, A. (2011). “Automatic annotation of microbial genomes and metagenomic sequences,” in *Metagenomics and its Applications in Agriculture, Biomedicine and Environmental Studies*, ed. R. W. Li (Hauppauge, NY: Nova Science Publishers), 61–78.