

Supplementary File to

High-efficiency production of the antimicrobial peptide pediocin PA-1 in metabolically engineered *Corynebacterium glutamicum* using a microaerobic process at acidic pH and elevated levels of bivalent calcium ions

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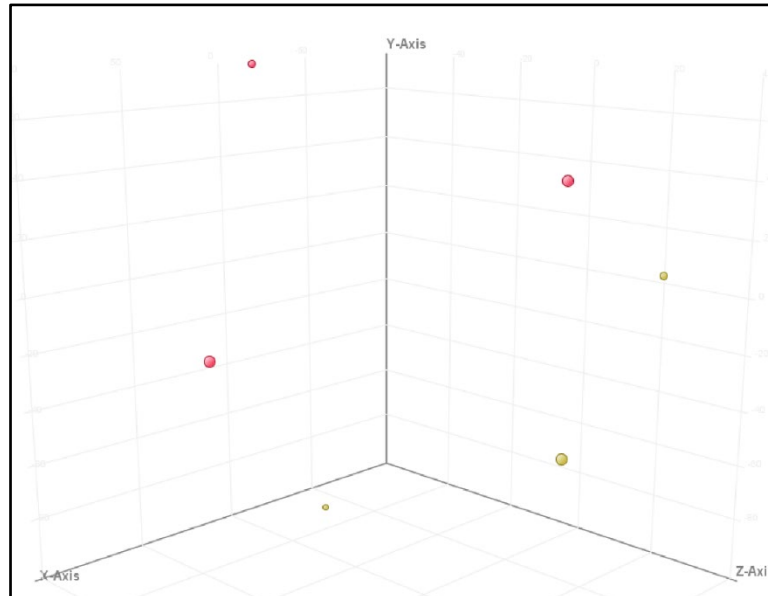


Figure S1: Principal component analysis of the transcriptome data set comprising the pediocin producer *C. glutamicum* CR099 pXMJ19 P_{tac} pedACD and its reference *C. glutamicum* CR09 pXMJ19, expressing the empty plasmid. n=3.

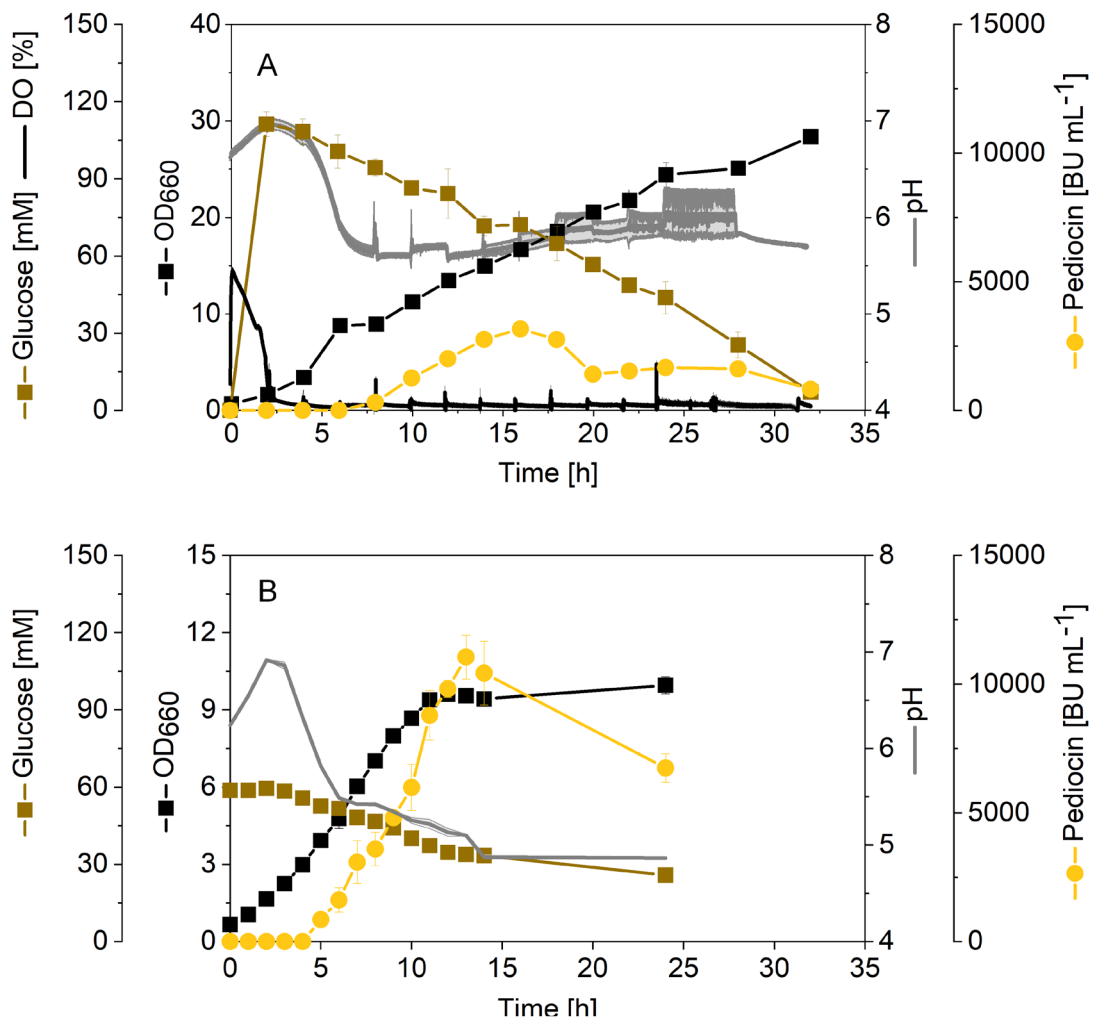


Figure S2: Pediocin production in recombinant *C. glutamicum* CR099 *pXMJ19 P_{tac} pedACD^{Cg}* using TY medium (A). Glucose (20 g L⁻¹) was added after 2 h. Pediocin production was induced, also after 2 h, by the addition of 0.2 mM IPTG. During the process, pH value and DO were monitored online. GY medium based pediocin production with additional pH monitoring (B). n=3.

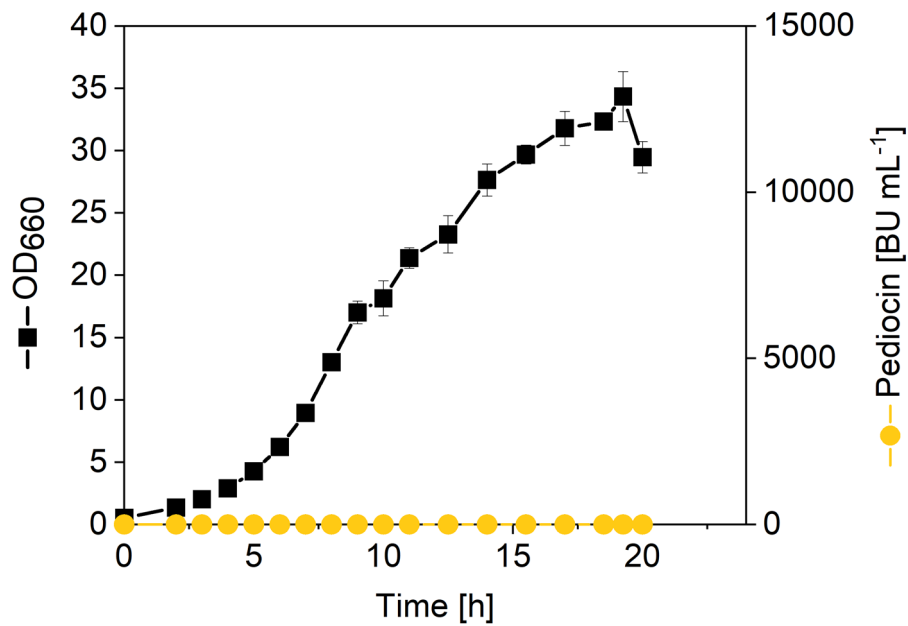


Figure S3: Pediocin production in recombinant *C. glutamicum* CR099 $pEKEx2 P_{tac} pedAM31LCD^{Cg}$ using TY medium in baffled shake flasks (aerobic conditions). Glucose (20 g L^{-1}) was added after 2 h. Pediocin production was induced, also after 2 h, by the addition of 0.2 mM IPTG. The used plasmid is a well-established vector for *C. glutamicum* [1]. n=3.

Table S1: Primers for strain construction.

Primer	Sequence	Annealing T [C°]
Primer 1	GAAGTCCAGGAGGACATACAATGAAGAAGATTGAG AAGCTGACCGA	59
Primer 2	CGCTTCCTTTAGCAGCCCGCTAGCGGATCATTTAAA TCCCTTATTCCTGGTTATGAATGAGGCGTGC	59
Primer 3	GCTCTTCTGCGTTAATTAACAATTGGGATCCTCTAG ACCCTGGCCGTTACCCTGCGAA	59
Primer 4	AGCTTCTCAATCTTCTTCATTGTATGTCCTCCTGGAC TTCGT	59

Literature

1. Bakkes PJ, Ramp P, Bida A, Dohmen-Olma D, Bott M, Freudl R: **Improved pEKEx2-derived expression vectors for tightly controlled production of recombinant proteins in *Corynebacterium glutamicum***. *Plasmid* 2020, **112**:102540.