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A bottom-up approach towards a bacterial consortium for the biotechnological conversion of chitin to L-lysine

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| **Table S1a** Strains used in this study |
|------------------------------------------|

**Escherichia coli**

| Abbreviation | Relevant characteristics | Reference |
|--------------|--------------------------|-----------|
| EcWT         | *E. coli* W3110, F- lambda- IN(rrnD-rrnE)1 rph-1 | (Bachmann 1972) |
| EcNagE*      | EcWT with ΔnagE ΔmanXYZ | This work |
| EcCHB        | *E. coli* W3110 with ΔnagE ΔmanXYZΔchbBCA::CM, CmR | This work |
| EcCHB*       | EcCHB; chloramphenicol resistance gene removed | This work |
| EcLPP        | EcCHB with Δlpp::CM; CmR | This work |
| EcLPP*       | EcLPP; chloramphenicol resistance gene removed | This work |
| EcLPPLYSA    | EcLPP* with ΔlysA::CM; CmR | This work |
| EcLPPLYSA*   | EcLPPLYSA; chloramphenicol resistance gene removed | This work |
| EcLPP* [empty] | EcLPP* with [pPRII+ (empty-vector)], CmR | This work |
| EcLPP* [TkCDA] | EcLPP* with [pPRII+::C.vio-TkCDA-StrepII], CmR | This work |
| EcLPPLYSA* [TkCDA] | EcLPPLYSA* with [pPRII+::C.vio-TkCDA-StrepII], CmR | This work |
| EcLPP* [TK]   | EcLPP* with [pPRII+::pelB-TK-StrepII], CmR | This work |
| EcLPP* [ChiB] | EcLPP* with [pPRII+::pelB-ChiB-StrepII], CmR | This work |
| EcNagE* [TkCDA] | EcNagE* [pPRII+::C.vio-TkCDA-StrepII], CmR | This work |
| EcLPP* [ChiB_TK_TkCDA] | EcLPP* with [pPRII+::pelB-ChiB-StrepII_pelB-TK-StrepII_pelB-TkCDA-StrepII], CmR | This work |

**Corynebacterium glutamicum**

| Abbreviation | Relevant characteristics | Reference |
|--------------|--------------------------|-----------|
| DM1729       | *C. glutamicum* ATCC 13032 carrying chromosomal mutations *pycP458S, homV59A, lysCT311I* | (Georgi et al. 2005) |
| CgLYS4       | *C. glutamicum* DM1729 Δpta-ackA Δcat ΔldhA ΔaceAB ΔnanR | (Sgobba et al. 2018) |

CmR: chloramphenicol resistance
| Name                                      | Relevant characteristics                                                                 | Reference   |
|-------------------------------------------|------------------------------------------------------------------------------------------|-------------|
| pPRII+::pelB-TK-StrepII                   | pPRII+ with pelB signal peptide, TK (glucosaminidase) from *Thermococcus kodakarensis* KOD1 and StrepII-Tag | This work  |
| pPRII+::pelB-ChiB-StrepII                | pPRII+ with pelB signal peptide, ChiB (chitinase) from *Serratia marcescens* and StrepII-Tag | This work  |
| pPRII+::C.vio-TkCDA-StrepII              | pPRII+ with sequence for *C. violaceum* signal peptide, TkCDA (chitin deacetylase) from *Thermococcus kodakarensis* KOD1 and StrepII-Tag | This work  |
| pPRII+::pelB-ChiB-StrepII_pelB-TK-StrepII_TkCDATstreplpelB-StrepII | pPRII+ ChiB, TK and TkCDA; all enzymes include a pelB signal peptide and a StrepII-tag | This work  |
| pPRII+ (empty vector)                    | pPRII+ empty-vector control                                                               | EP2848691A1 (patent) |
**Table S2 Oligonucleotides used in this study**

| No. | Name            | Oligonucleotide sequence (5'-3')                                                                 | Description                                                                 |
|-----|-----------------|---------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| 1   | nagE_fow        | AAAAATACGGCTTTAAACGAGCCAA ATAGGTTCTCTGAGGGGAATAAG GTGTAGGCTGGAGCTGCTTC                         | Amplification of Cm\(^R\) from pKD3 for disruption of nagE                  |
| 2   | nagE_rev        | TTGTCAATTGTTGGATGAGGAGCTCA AGCCTGCATCAAGGAGGATAAAGAGGAGA CATATGAATATCCTCTCTCTAG               | Amplification of Cm\(^R\) from pKD3 for disruption of nagE                  |
| C1  | nagE_contr_FW  | TATATCTGAGGACCTGTTGAGGAGC AATTAAAAGGAGGATGCTGGAGGAGA CATATGAATATCCTCTCTCTAG                 | Control of nagE deletion                                                     |
| C2  | nagE_contr_rev | TATATCTGAGGACCTGTTGAGGAGC AATTAAAAGGAGGATGCTGGAGGAGA CATATGAATATCCTCTCTCTAG                 | Control of nagE deletion                                                     |
| 3   | manXYZ_fow      | AAAAAAATACCTCTGGCAGTGAGGAGGAGGAGA CATATGAATATCCTCTCTCTAG                                      | Amplification of Cm\(^R\) from pKD3 for disruption of manXYZ                 |
| 4   | manXYZ_rev      | CACTGAGAGGAGGAGGAGGAGGAGA CATATGAATATCCTCTCTCTAG                                               | Amplification of Cm\(^R\) from pKD3 for disruption of manXYZ                 |
| C3  | manXYZ_contr_FW| CGATTCGATTTGTGGAGGAGC     | Control of manXYZ deletion                                                     |
| C4  | manXYZ_contr_rev| ACCAGTCCGGTGATTGTCAT                             | Control of manXYZ deletion                                                     |
| 5   | chbBCA_fow      | AGGCTTGCGGAGGAGGAGGAGGAGA CATATGAATATCCTCTCTCTAG                                               | Amplification of Cm\(^R\) from pKD3 for disruption of chbBCA                 |
| 6   | chbBCA_rev      | GGGCAGTTGCGGAGGAGGAGGAGA CATATGAATATCCTCTCTCTAG                                               | Amplification of Cm\(^R\) from pKD3 for disruption of chbBCA                 |
| C5  | chbBCA_contr_FW| ATCTTTCGCAATTATTTGTGGC | Control of chbBCA deletion                                                      |
| C6  | chbBCA_contr_rev| ATTTCCGCGCGCTTAATCAG                             | Control of chbBCA deletion                                                      |
| 7   | lysA_fow        | CTTTTATGAA TGTTGCGTTA AAATCAAGAGGAGGAGGAGGAGA CATATGAATATCCTCTCTCTAG                           | Amplification of Cm\(^R\) from pKD3 for disruption of lysA                   |
| 8   | lysA_rev        | CAAATCTGGCTGAGGAGGAGGAGGAGA CATATGAATATCCTCTCTCTAG                                           | Amplification of Cm\(^R\) from pKD3 for disruption of lysA                   |
| C7  | lysA_contr_FW  | CAAACAGAGGCGAGTTGCTTTGC | Control of lysA deletion                                                      |
| C8  | lysA_contr_rev | AGTGGTATTGCGCGCCTATGAGA | Control of lysA deletion                                                      |
| 9   | lpp_fow         | AAATTTCTGCAACGCTACAGGGAGAT TAATCTCATCTGGAGGAGGAGA CATATGAATATCCTCTCTCTAG | Amplification of Cm\(^R\) from pKD3 for disruption of lpp                 |
|   | Primer Name   | Sequence                      | Description                        |
|---|---------------|-------------------------------|------------------------------------|
| 10| lpp_rev       | ACAAAAAAAATGGCGCACAATGTGC GCCATTTTTCACTTCACAGGTACTAC ATATGAATATCCTCCTTAG | Amplification of Cm\(^R\) from pKD3 for disruption of lpp |
| C9| lpp_contr_fw  | GTAACGCTACATGGAGATTAAC        | Control of lpp deletion            |
| C10| Lpp_contr_rev | GACGCAGTAGCGGTAAACGGCAG       | Control of lpp deletion            |

Underlined letters restriction site
| Name                     | Oligonucleotide sequence (5'-3') | Description                                                                 |
|--------------------------|----------------------------------|-----------------------------------------------------------------------------|
| pPRII+::chiB_for         | ATGACTCGAGCTGAGAGAC             | Excision of TK and TkCDA from pPRII+::chiB_Tk_TkCDA to construct pPRII+::pelB-chiB-StrepII |
| pPRII+::chiB_for         | CTAAGATTATTTTCAAATTGCGGGTGCC    | Excision of TK and TkCDA from pPRII+::chiB_Tk_TkCDA to construct pPRII+::pelB-chiB-StrepII |
| pPRII+::TkCDA_for        | ATGAAATACCTGCTGCCG              | Excision of chiB and TK from pPRII+::chiB_Tk_TkCDA to construct pPRII+::pelB-TkCDA-StrepII |
| pPRII+::TkCDA_rev        | TTGGAATTCTGTTCCTGTGG            | Excision of chiB and TK from pPRII+::chiB_Tk_TkCDA to construct pPRII+::pelB-TkCDA-StrepII |
| C. vio-TkCDA_for         | ATGTTGTTAGCATTTGGGACAA CATGCTTTGCGCTGCTGCAATTG GTGTTCGAAGAATTTAACAA CTTTG | Exchange of pelB-leader for C. violaceum-Tag |
| C. vio-TkCDA_rev         | GCCCATTTGCGATAGCACGACC TGTAGTGCCGCGCATTGTGA ATACTTCTTCTGTGTAAGAA TTG | Exchange of pelB-leader for C. violaceum-Tag |
| TK_GA_for                | CACAGGAAACAGAATCC AAATGAAATACCTGCTGCGC | Amplification of TK from pPRII+::chiB_Tk_CDA |
| TK_GA_rev                | GCTTCTGCAGCTCGAGTGT CATTTTCAAATGTGCGGTGAG | Amplification of TK from pPRII+::chiB_Tk_CDA |

Italic letters: *C. violaceum*-Tag; Bold letters: Overlapping region
| Plasmid                          | Template                          | Primers                          | Cloning method                                   |
|--------------------------------|-----------------------------------|----------------------------------|--------------------------------------------------|
| pPRII+::pelB-chiB-StrepII      | pPRII::Syn_OP                      | -                                | Digestion with EcoRV, religation of vector       |
| pPRII+::pelB-chiB-TkCDA-StrepII| pPRII::pelB-chiB-StrepII::pelB-Tk-| pPRII+::chiB_for, pPRII+::chiB_rev| Back-to-back PCR, religation                      |
| pPRII+::pelB-TkCDA-StrepII     | pPRII::pelB-chiB-StrepII::pelB-Tk-| pPRII+::TkCDA_for, pPRII+::TkCDA_rev| Back-to-back PCR, religation                      |
| pPRII+::pelB-Tk-                 | pPRII::pelB-TkCDA-StrepII (vector | pPRII+::chiB_for and pPRII+::TkCDA_rev for vector backbone | Gibson assembly using Gibson Assembly® Master Mix (NEB, Ipswich, MA, USA) |
|                                 | backbone)                          | TK_GA_for and TK_GA_rev for insert|                                                  |
| pPRII+::C.vio-TkCDA-StrepII     | pPRII::pelB-TkCDA-StrepII         | C.vio-TkCDA_for, C.vio-TkCDA_rev | Back-to-back PCR, religation                      |
Fig. S1 Secretion of chitin deacetylase TkCDA. Amount of chitin deacetylase TkCDA in the extracellular medium (filled bars) and in the cellular fraction (open bars) of EcLPP* [TkCDA] and E. coli W3110 ΔnagE ΔmanXYZ [pPRII::C.vio-TkCDA-StrepII] (EcNagE* [TkCDA]). Error bars indicate standard deviation (n = 3). Statistically significant difference at *** P < 0.001.
**Fig. S2** Growth of *E. coli* strains on GlcNAc and quantification of metabolites. (a) Optical density (OD$_{600}$) of the strain EcLPP* [empty] (red squares and dashed line) and EcLPP* [TkCDA] (blue squares and solid line) cultivated with 20 mM *N*-acetylglucosamine (GlcNAc) as sole carbon and energy source. *E. coli* cells were induced with 0.2 mM IPTG at $t_0$. Error bars indicate standard deviation ($n = 3$). (b) Concentration of GlcNAc (filled bars) and glucosamine (open bars) in the supernatant of EcLPP* [TkCDA] measured using UHPLC-ELSD-ESI-MS$^+$ as described in Stumpf et al. 2019.
Fig. S3 Extracted ion chromatograms (EICs) of UHPLC-ESI-MS analysis of culture supernatants of substrate converters expressing ChiB, TK, or TkCDA cultured in the presence of their respective substrates (TkCDA: GlcNAc, TK: GlcN₂, ChiB: colloidal chitin) at time point t₃ (72 h). Inserts show the mass to charge ratio of the respective peaks. A) EIC of mass 425.18 (GlcNAc₂, H⁺ adduct): EcLPP* [ChiB] cultured in M₉ minimal medium with 20 mM glucose as carbon source and 0.1% (wt/vol) colloidal chitin. B) EIC of mass 359.17 (two GlcN units, H⁺ adduct): EcLPP* [TK] cultured in M₉ minimal medium with 20 mM glucose as carbon source and 12 mM GlcN₂. C) EIC of mass 359.17 (two GlcN units, H⁺ adduct): EcLPP* [TkCDA] cultured in M₉ minimal medium with 40 mM GlcNAc, no additional carbon source.
**Fig. S4** Cultivation of synthetic microbial consortia on colloidal chitin. Cultivation of the co-culture of EcLPP* [ChiB], EcLPP* [TK], and ECLPP* [TkCDA] together with CgLYS4 and co-culture of EcLPP* [empty-vector] and CgLYS4 on 0.5% colloidal chitin supplemented with 5 mM acetate as sole carbon and energy sources. *E. coli* cells harbouring a plasmid were induced with 0.2 mM IPTG at t₀. (a) CFUs of strains EcLPP* [ChiB], EcLPP* [TK], and ECLPP [TkCDA] (blue squares and solid line) and EcLPP* [empty-vector] (red squares and dashed line). (b) CFUs of strain CgLYS4 in co-culture with EcLPP* [ChiB], EcLPP* [TK], and ECLPP* [TkCDA] (blue dots and solid line) and in co-culture with EcLPP* [empty-vector] (red dots and dashed line). Error bars indicate standard error of the mean (n = 3).

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