Additional file 1

**Engineering marine fungi for conversion of d-galacturonic acid to galactaric acid**

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**Table S1.** List of primers used.

**Figure S2.** The *gar2* sequences obtained for Trichoderma sp. LF328 and Coniochaeta sp. MF729 *gar2* gene

**Table S1.** List of primers used.

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| --- | --- | --- | --- |
| **Description** | **Name** | **Sequence** | **Target gene** |
| Primers for cassette construction | udh\_FRW | ACCTCTACAACTCCAATACACTTAATTAAAATGGCCATGAAGCGCCTG | *udh* in GUDH\_A-tum plasmid |
| udh\_REV | ATAATAAAAATCATAAATCATAAGAAATTCG GATC TCAGGACTGCTTGAAGATGG | *udh* in GUDH\_A-tum plasmid |
| 5’\_flank\_FRW\_328 | CAGGAAACAGCTATGACCATGATTACGCCAAGCTAGATCTGCCTCACCCTCTTCGACCTG | LF328 *gar2*  |
| 5’\_flank\_REV\_328 | TTGTTCCCTTTAGTGAGGGTTAATTCGGCGCGGGCGTCGGCATCGACCTT | LF328 *gar2*  |
| 5’\_flank\_FRW\_729 | CAGGAAACAGCTATGACCATGATTACGCCAAGCTAGATCTGGCGAGGCAAGGTCGGGC | MF729 *gar2* |
| 5’\_flank\_REV\_729 | TTGTTCCCTTTAGTGAGGGTTAATTCGGCGCGGGGAACGAGTCGAACGAGGT | MF729 *gar2* |
| 3’\_flank\_FRW | TGTCTATGCCCTGCCCCTAATAGATGCATGGCCATCACCATCTTCACCCC | LF328 and MF729 *gar2*  |
| 3’\_flank\_REV\_328 | GATCCTCTAGAGTCGACCTGCAGGCATGCAAGATCTGCTTGTGGTGCTCGATGAAGA | LF328 *gar2*  |
| 3’\_flank\_REV\_729 | GATCCTCTAGAGTCGACCTGCAGGCATGCAAGATCTGCTTGTGGTGCTCGATGTAGA | MF729 *gar2*  |
| SES\_prom\_REV | TTTAATTAAG TGTATTGGAGTTGTAGAGG | SES promoter |
| udh\_REV | ATAATAAAAATCATAAATCATAAGAAATTCG GATC TCAGGACTGCTTGAAGATGG | *udh* |
| Primers for *gar1* and *gar2* amplification | gar1\_An\_FRW | GAGATCCCTGCTCTCGGACTCG | *A. niger putative gar1* |
| gar1\_An\_REV | GGGTGGTTCTCAATCTGGTTG | *A. niger putative gar1* |
| gar1\_Bc\_FRW | GAGATTCCGGCATTAGGACTCG | *B. cinerea gar1* |
| gar1\_Bc\_REV | GGGTGATTTTCAATTTGGTTGACG | *B. cinerea gar1* |
| gar1\_Tr\_FRW | GAGATTCCAGCTGTTGGTCTCGG | *T. reesei gar1* |
| gar1\_Tr\_REV | GGGTGGTTCTCAATCTGGTTGA | *T. reesei gar1* |
| gaaA\_An\_FRW | GGAACAGGCGAGTACACGACC | *A. niger gaaA* |
| gaaA\_An\_REV | GTAGGCCGGGTCGAAGCG | *A. niger gaaA* |
| gar2\_Bc\_FRW | GGAACCGGTGAATATACCACCG | *B. cinerea gar2* |
| gar2\_Bc\_REV | GTACGCGGGATCGAATCGTTT | *B. cinerea gar2* |
| gar2\_Nd\_FRW | GGTACCGGCGAGTACACCACC | *N. diffluens GAR2* |
| gar2\_Nd\_REV | GTAGGCCGGGTCGTACCG | *N. diffluens GAR2* |
| gar2\_Hj\_FRW | GGCACCGGCGAGTACACG | *T. reesei gar2* |
| gar2\_Hj\_REV | GTAGGCCGGGTCGAAGCG | *T. reesei gar2* |
| Protospacer sequences | crRNA LF328 | CTTCAATGAGCTTGTACGTACGG | LF328 *gar2* |
| crRNA MF729 | TCCCTGCTGACGTCGTTGGGCGG | MF729 *gar2* |
| Primers for transformant verification  | SES\_FRW | CGCGCCGAATTAACCCTCAC | SES promoter |
| udh\_screen\_FRW | ACCCCATCTTCAAGCAGTCCTG | udh |
| TEF\_REV | GAACATCAGGTGTTGATGATGGGT | TEF1t |
| 328\_screen\_FRW | GACAAAAAGGTGGGCGTTGT | LF328 gar2  |
| 328\_screen\_REV | CTTGTAGGCCGGGTCGAA | LF328 gar2  |
| 729\_screen\_FRW | ACACGACCGGCTTCGTCGG | MF729 gar2  |
| 729\_screen\_REV | GTAGGCCGGGTCGAAGC | MF729 gar2  |

**Figure S2**. The fungal pathway for d-galacturonic acid metabolism (reactions 1-4) and the reactions (5-6) necessary to produce mucic acid from d-galacturonic acid. The enzyme are: (1) d-galacturonate reductase EC 1.1.1.365, (2) l-galactonate dehydratase EC 4.2.1.146, (3) 2-keto-3-deoxy-galactonate aldolase EC 4.1.2.54 and (4) l-glyceraldehyde reductase EC 1.1.1.372, (5) d-galacturonate (uronate) dehydrogenase EC 1.1.1.203, (6) lactonase or spontaneous opening. (See Richard P, Hilditch S. d-Galacturonic acid catabolism in microorganisms and its biotechnological relevance. Appl Microbiol Biotechnol. 2009; 82:597-604 and Kuivanen J, Biz A, Richard P. Microbial hexuronate catabolism in biotechnology. AMB Expr 201; 9:16 for references and review of other pathways of d-galacturonic acid metabolism, including details on the enzymatic opening of the galactarolactone to the linear mucic acid, reaction 6.)



**Figure S2**. The *gar2* sequences obtained for *Trichoderma* sp. LF328 and *Coniochaeta* sp. MF729 *gar2* gene

> *Trichoderma* sp. LF328 *gar2*

TGGGCGTTGTCGGCCTCACCCTCTTCGACCTGCGACGACGAGGCAAAGTCGGCCAGCTGGGCATGGTCGGCGTCAATGGCACCAAGTTTCCCGCAATCCGTACGTACAAGCTCATTGAAGATGCTCCCGGGCCAAAGTTGAGACTGACTGTGAACTCGCCGCATTGTAGGAGAGCACCTCAACAAGAACATCACCCAAGTCTACAACAACCTGGACACCTCGTTCGACTCATTCCCAGCCAACGACAAGGTCGATGCCGACGCCTACAAGGCCGCCATTGACCAGCTCAAGCCCGGCGATGCCATCACCATCTTCACCCCCGATCCTACCCACTTCCCCATTGCGCTGTACGCCATCGAGCGCGGCATCCACGTCCTCATCACTAAGCCCGCCGTGAAGCTGCTGGAGCACCACCTGGAGCTGGCCCAAAAGGCCGCCGAAAAGGGCGTCTACGTCTTCATCGAGCACCACAAGCAAATTCGACCCGGCCTACAAGCG

> *Coniochaeta* sp. MF729 *gar2*

AGTCGGCGTCGTCGGCCTGTCCATGTTCGACCTGCGGCGGCGAGGCAAGGTCGGCAAGCTGGGCATGGTCGGCACAAACGGCACAAAGTTCCCGGCCATCCGCGAGCACCTGCGCAAGAACATCCAGCAGGTCTACAACAACCTCGACACCTCGTTCGACTCGTTCCCGCCCAACGACGTCAGCAGGGACCCGGACTCGTACAAGGCCGCCATCGACAGCCTCGGCAAGGGCGACGCCATCACCATCTTCACCCCGGACACCACCCACTTCCCCATCGCCCTCTACGCCATCGAGCGCGGCGTCCACGTCATGATCACCAAGCCCGCCGTCAAGCTGCTCGAGCACCACCAGGCCCTCATCGACGCCGCCCGCACCCACGGCGTCTACGTCTACATCGAGCACCACAAGCGCTTCG