Synthetic Biology
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# Transcriptomic Insights into Selenite Response and Biotransformation in a Novel Selenium-Enriching Lactic Acid Bacterium

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**Table S1.** Composition of MRS basal medium.

| Component | Concentration (g·L$^{-1}$) |
|---|---|
| Glucose | 20.0 |
| Peptone | 10.0 |
| Beef extract | 10.0 |
| Yeast extract | 5.0 |
| Sodium acetate | 5.0 |
| Triammonium citrate | 2.0 |
| $K_2HPO_4$ | 2.0 |
| $MgSO_4·7H_2O$ | 0.41 |
| $MnSO_4·H_2O$ | 0.05 |
| Tween 80* | 1.0 mL·L$^{-1}$ |

Note: The initial pH of the MRS basal medium was adjusted to 6.2 ± 0.2 with 1 M HCl or 1 M NaOH prior to sterilization at 121 °C for 15 min. After cooling to ≤ 50 °C, filter-sterilized sodium selenite stock solution was aseptically added to obtain the indicated final $Na_2SeO_3$ concentrations. The composition listed corresponds to the MRS basal medium used for the isolation and cultivation of lactic acid bacteria in this study. *For Tween 80, the final concentration is expressed as volume per liter ofculture medium.

**Table S2.** Primers for 16S rRNA gene amplification.

| Primer | Sequence (5′→3′) | Notes |
|---|---|---|
| 27F | AGAGTTTGATCMTGGCTCAG | Universal bacterial forward primer |
| 1492R | TACGGYTACCTTGTTACGACTT | Universal bacterial reverse primer |

Note: Primers used for amplification of the nearly full-length 16S rRNA gene from lactic acid bacteria isolates.

**Table S3.** PCR reaction mixture and thermocycling program for 16S rRNA gene amplification.

| Step | Temperature (°C) | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 5 min | 1 |
| Denaturation | 95 | 30 s | 30 |
| Annealing | 55 | 30 s | 30 |
| Extension | 72 | 90 s | 30 |
| Final extension | 72 | 10 min | 1 |

Note: PCR reaction mixture (50 µL total volume): 25 µL 2× Taq Master Mix, 1 µL of each primer (10 µM), 2 µL template DNA (10–50 ng), and nuclease-free water to a final volume of 50 µL.

**Table S4.** RNA extraction, library preparation, and sequencing.

| Sample ID | Time (h) | Clean reads | GC content (%) | Q20 (%) | Q30 (%) | Uniquely mapping rate (%) | Total mapping rate (%) |
|---|---|---|---|---|---|---|---|
| Control_1 | 4 | 7532358 | 43.97 | 99.45 | 97.58 | 95.78 | 97.24 |
| Control_2 | 4 | 7767406 | 43.97 | 99.4 | 97.24 | 95.27 | 96.67 |
| Control_3 | 4 | 7433992 | 44.05 | 99.38 | 97.27 | 95.78 | 97.39 |
| Treatment_1 | 12 | 7708484 | 44.04 | 99.41 | 97.43 | 94.59 | 97.71 |
| Treatment_2 | 12 | 7830278 | 44.06 | 99.42 | 97.46 | 94.59 | 97.72 |
| Treatment_3 | 12 | 7582428 | 44.02 | 99.44 | 97.5 | 94.83 | 97.85 |

Note: Total RNA was extracted using TRIzol$^{\text{TM}}$ reagent (Invitrogen, Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Ribosomal RNA was removed using the Ribo-Zero$^{\text{TM}}$ rRNA Removal Kit (Bacteria) (Epicentre, USA). Strand-specific libraries were prepared using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (New England Biolabs, USA) and sequenced by Novogene (Tianjin, China) on an Illumina platform as described in the main text. Quality control summary (as reported in main text): RIN > 9.5; Q30 > 97%; mapping efficiency 96.7–97.9%.

**Table S5.** Key selenium-related genes highlighted by RNA-seq (treatment vs. control).

| Gene ID | Direction | Log2 FC | Padj |
|---|---|---|---|
| HN015_RS09075 | Down | -0.204 | $7.67 \times 10^{-3}$ |
| HN015_RS05170 | Up | 0.866 | $6.51 \times 10^{-3}$ |
| HN015_RS07050 | Down | -1.804 | $8.04 \times 10^{-17}$ |
| HN015_RS07215 | Up | 2.250 | $1.32 \times 10^{-59}$ |
| HN015_RS06610 | Up | 1.668 | $3.40 \times 10^{-79}$ |
| HN015_RS02045 | Up | 3.123 | $<1.0 \times 10^{-300}$ |
| HN015_RS06785 | Up | 0.942 | $9.10 \times 10^{-52}$ |
| HN015_RS03615 | Up | 5.767 | $5.40 \times 10^{-110}$ |
| HN015_RS03620 | Up | 5.569 | $<1.0 \times 10^{-300}$ |
| HN015_RS03625 | Up | 4.943 | $1.54 \times 10^{-224}$ |
| HN015_RS04060 | Down | -0.132 | $4.46 \times 10^{-1}$ |
| HN015_RS02370 | Down | -1.085 | $3.12 \times 10^{-41}$ |
| HN015_RS06795 | Up | 2.197 | $2.46 \times 10^{-128}$ |
| HN015_RS06190 | Up | 0.989 | $9.83 \times 10^{-80}$ |
| HN015_RS00075 | Down | -1.090 | $1.12 \times 10^{-3}$ |
| HN015_RS02375 | Down | -0.915 | $9.72 \times 10^{-13}$ |
| HN015_RS01990 | Down | -3.475 | $2.41 \times 10^{-159}$ |
| HN015_RS01995 | Down | -3.063 | $9.70 \times 10^{-129}$ |
| HN015_RS02000 | Down | -2.789 | $6.03 \times 10^{-82}$ |

Note: Padj values are adjusted P values from DESeq2 (Benjamini–Hochberg correction). Entries with padj reported as 0 in the DESeq2 output are shown as "$< 1.0 \times 10^{-300}$" due to numerical underflow. Full DEG lists were provided as machine-readable spreadsheets (Supplementary Data S1).

**Table S6.** GO enrichment analysis of selenium-responsive genes (top terms).

| Category (BP/MF/CC) | GO Term | Gene Count | Adjusted p-value |
|---|---|---|---|
| BP | cell redox homeostasis | 9 | 0.993 |
| BP | cellular response to stress | 16 | 0.993 |
| BP | cofactor biosynthetic process | 6 | 0.993 |
| BP | drug metabolic process | 14 | 0.993 |
| BP | ion transport | 19 | 0.993 |
| BP | response to stress | 17 | 0.993 |
| BP | tRNA aminoacylation for protein translation | 18 | 0.993 |

| | | | |
|---|---|---|---|
| CC | membrane | 149 | 0.916 |
| CC | ribosome | 33 | 0.916 |
| MF | ATP binding | 119 | 0.794 |
| MF | inorganic molecular entity transmembrane transporter activity | 17 | 0.794 |
| MF | ion binding | 178 | 0.794 |
| MF | metal ion binding | 26 | 0.794 |
| MF | methyltransferase activity | 17 | 0.794 |
| MF | oxidoreductase activity | 56 | 0.090 |

Note: Full GO enrichment results generated by clusterProfiler are provided in Supplementary Data S2.

**Table S7.** KEGG pathway enrichment (top 15).

| **Pathway** | **KEGG ID** | **Gene Count** | **Adjusted p-value** |
|---|---|---|---|
| Bacterial secretion system | paci03070 | 8 | 0.971238 |
| Protein export | paci03060 | 10 | 0.971238 |
| Phosphotransferase system (PTS) | paci02060 | 29 | 0.971238 |
| ABC transporters | paci02010 | 35 | 0.924681 |
| Carbon metabolism | paci01200 | 44 | 0.807333 |
| Microbial metabolism in diverse environments | paci01120 | 71 | 0.194182 |
| One carbon pool by folate | paci00670 | 5 | 0.994052 |
| Pyruvate metabolism | paci00620 | 26 | 0.807333 |
| Glycerophospholipid metabolism | paci00564 | 8 | 0.994052 |
| Teichoic acid biosynthesis | paci00552 | 17 | 0.924681 |
| Peptidoglycan biosynthesis | paci00550 | 13 | 0.971238 |
| Galactose metabolism | paci00052 | 20 | 0.924681 |
| Fructose and mannose metabolism | paci00051 | 16 | 0.924681 |
| Pentose phosphate pathway | paci00030 | 19 | 0.924681 |
| Glycolysis / Gluconeogenesis | paci00010 | 30 | 0.807333 |

Note: Full KEGG pathway enrichment results generated by clusterProfiler are provided in Supplementary Data S3.

**Supplementary Data** Machine-readable spreadsheets associated with the RNA-seq analyses.

**Supplementary Data S1** (all_compare.xlsx) contains the full list of differentially expressed genes, including gene ID, gene name, baseMean, log2(fold change), standard error, test statistic, P value, and Benjamini–Hochberg adjusted P value (padj) for the selenium treatment versus control comparison.

**Supplementary Data S2** (ALL_GOenrich.xlsx) provides the complete Gene Ontology (GO) enrichment results generated by clusterProfiler, including GO ID, GO term description, category (BP/MF/CC), gene counts, gene ratios, and adjusted P values for enriched GO terms.

**Supplementary Data S3** (ALL_KEGGenrich.xlsx) provides the complete KEGG pathway enrichment results generated by clusterProfiler, including pathway name, species-specific KEGG pathway ID, gene counts, gene ratios, and adjusted P values for enriched pathways.