Supplementary Material

Improvement of *Lactobacillus plantarum* Aerobic Growth, Directed by Comprehensive Transcriptome Analysis

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**Detailed description of transcriptome analysis and results:**

**Sampling, RNA extraction, and quality control**
To avoid undesired alterations in the transcriptome due to a cellular responses after sampling, 1 volume of culture was quenched in 4 volumes of -40°C 60% methanol-HEPES buffer as described by Pieterse (2). After quenching, cells were harvested by centrifugation (13182 * g, 10 min, -20°C) using a Sorvall RC5B plus centrifuge (Sorvall, Newton, US). Immediately after harvesting, cell pellets were rapidly transferred with a pre-chilled spatula to a screw-crap tube containing 500 mg zirconium beads, 500µl phenol-chloroform-mix 1:1, 30 µl 3 M Na-Acetate (pH 5.2), 30 µl 10% SDS, and 400 µl MRS-medium (Merck, Darmstadt, Germany), carefully avoiding thawing of the cell pellet material. The tubes containing the cells were shaken, frozen in liquid nitrogen and stored at -80°C.

Cells were disrupted by bead-beating four times for 40 s at speed 4.0 using a Fastprep cell disrupter (QBiogene Inc., Cedex, France), interspaced with cooling intervals on ice. The tubes were centrifuged for 1 min at 22800 x g (4°C) and the aqueous-phase was transferred into an Eppendorf-tube. Residual phenol traces were removed by extraction with pre-chilled chloroform. The resulting aqueous phase was mixed with an equal volume of binding buffer (provided in the kit) and applied to a RNA purification column
from the High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany).
Further purification was performed following the protocol provided by the manufacturer, including on-column incubation with DnaseI for one hour and using a 50 µl elution volume.

The yield and purity of the RNA were determined by measurement of the absorption at 260 nm and 280 nm (Ultraspex 3000, Pharmacia Biotech, Roosendaal, The Netherlands). The RNA quality was assessed using the RNA 6000 Nano Assay in an Agilent 2100 Bioanalyzer (Agilent technologies, Palo Alto, Ca, USA) following the manufacturer’s instructions. Only RNA samples displaying 16S/23S-rRNA ratios of 1.6 or higher, were labeled and used for micro array experiments.

**cDNA-synthesis, labeling, and hybridization**

The Cyscribe Post-labeling kit was used to synthesize cDNA out of 25 µg of total RNA, which was subsequently labeled according to the manufacturer’s protocol (Amersham Biosciences, Amersham, UK). Subsequently, labeled cDNA solutions were concentrated in a Hetovac VR-1 (Heto Lab Equipment A/S, Birkerod, Denmark) to a final volume of 10 µl.

Two individual, differentially labeled cDNAs were incubated at 95° C for 3’, cooled down to 68° C, and mixed (final-volume 20 µl). To these mixed cDNAs 180 µl of pre-heated (68° C) Slidehyb#1 hybridization buffer (Ambion, Austin, USA) was added and the resulting solution was applied on a pre-heated slide (68° C). Slides were then hybridized at 44° C for 16 hours. Subsequently, slides were washed at 42°C, once in 1 x SSC/0.2% SDS and twice in 1 x SSC and dried by centrifugation (1 x SSC is 0.15 M NaCl and 0.15 M Sodium Citrate).

**Open reading frame-based micro array design and spotting**

DNA-microarrays were prepared using PCR- amplicons of 2917 genes in the genome of *Lactobacillus plantarum* WCFS1 (EMBL database, accession number AL935263) resulting in a coverage of 97% (GEO design GPL6368). Primers were designed to amplify unique regions of these genes using UniFrag and GenomePrimer (5). The optimal amplicon length was set at 750 bp unique regions within the genes. Genes smaller then 750 bp were amplified entirely. Gene-specific primers were extended with a universal 15 bp sequence at the 5’end (TGGCGCCCTAGATG for the 5’-primers and
CGCGATGCTGATTGC for the 3’-primers), to enable universal-primer based re-amplification, which was used to generate two-sided terminally aminated gene-specific amplicons. Aminated amplicons were mixed 1:1 with telechem spotting buffer (ArrayIT, Sunnyvale, USA) and spotted in duplicate on SMM superamine slides (ArrayIT, Sunnyvale, USA). After spotting the slides were washed and blocked with Na$_2$BH$_4$ as described previously (1). The slide-quality was checked with SpotCheck (Genetix, New Milton, UK) according to the manufacturer’s protocol.

**Scanning, Data extraction, and analyses**
The slides were scanned with a Scan Array Express 4000 scanner (Perkin Elmer, Wellesley, USA) and the images were analyzed with Imagene software 4.2 (BioDiscovery, El Segundo, USA).
Statistical analyses were performed with R [http://www.r-project.org/] using the linear models for microarray data library limma (3). Background corrected spot intensities in both channels (I1 and I2) were converted to M-A coordinates, where M=log2 (I1/I2) and A=log2 (I1/I2)/2 and subsequently normalized using a LOESS fit, assuming that, on average, M is independent of A and centered around 0 (3).
Biological replicates were treated as duplicate measurements and their averages were compared using linear modeling functions from the Limma package. The statistical significance of differences was calculated using the eBayes function in Limma (cross-probe variance estimation). The linear modelled, average ratios were projected on the metabolic map of *L. plantarum* WCFS1 (4) using the Sympheny software (Genomatica, Inc., San Diego, USA) and the cut off for differential expression was set at $^{2}\log 0.9$. 
Transcriptome results:

Table 1 shows all genes of *L. plantarum* displaying significant difference (p< 0.05) in expression level in cells harvested before (P1) compared to cells harvested after the growth stagnation (P2). The ratios are given as $^{2}\log$ ratio ($^{2}\log$ (P1/P2)). Gene products which are plotted on the metabolic map in figure 3A are depicted in bold.

<table>
<thead>
<tr>
<th>ORF</th>
<th>Name</th>
<th>Product</th>
<th>$^{2}\log$ (P1/P2)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>lp_0168</td>
<td>dak1B</td>
<td>glycerone kinase</td>
<td>-1.24</td>
<td>0.009</td>
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<td>lp_0169</td>
<td>dak2</td>
<td>dihydroxyacetone phosphotransferase, dihydroxyacetone binding subunit</td>
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<td>lp_0170</td>
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<td>dihydroxyacetone phosphotransferase, phosphoryl donor protein</td>
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<td>lp_0171</td>
<td>dhaP</td>
<td>dihydroxyacetone transport protein (putative)</td>
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<td>0.012</td>
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<td>lp_0202</td>
<td></td>
<td>acetyltransferase (putative)</td>
<td>0.96</td>
<td>0.013</td>
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<tr>
<td>lp_0203</td>
<td>serA1</td>
<td>phosphoglycerate dehydrogenase</td>
<td>1.30</td>
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<td>lp_0232</td>
<td>pts2A</td>
<td>mannitol PTS, EIIA</td>
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<td>unknown</td>
<td>-1.03</td>
<td>0.022</td>
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<td>pts3C</td>
<td>cellbiose PTS, EIIIC</td>
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<td>0.020</td>
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<td>lp_0259</td>
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<td>integral membrane protein</td>
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<td>0.023</td>
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<td>lp_0316</td>
<td>potC</td>
<td>spermidine/putrescine ABC transporter, permease protein</td>
<td>0.92</td>
<td>0.009</td>
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<td>lp_0318</td>
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<td>spermidine/putrescine ABC transporter, ATP-binding protein</td>
<td>0.98</td>
<td>0.087</td>
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<td>transcription regulator</td>
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<td>0.082</td>
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<td>acdH</td>
<td>acetaldehyde dehydrogenase</td>
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<td>lp_0350</td>
<td>hicD1</td>
<td>L-2-hydroxyisocaproate dehydrogenase</td>
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<td>lp_0482</td>
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<td>transcription regulator</td>
<td>1.38</td>
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<td>lp_0492</td>
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<td>transport protein</td>
<td>0.94</td>
<td>0.003</td>
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<td>lp_0558</td>
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<td>cation transport protein</td>
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<td>spx1</td>
<td>regulatory protein Spx</td>
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<tr>
<td>lp_0914</td>
<td>guaA</td>
<td>GMP synthase (glutamine-hydrolysis)</td>
<td>0.91</td>
<td>0.057</td>
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<tr>
<td>lp_1101</td>
<td>ldlh2</td>
<td>L-lactate dehydrogenase</td>
<td>-1.20</td>
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<tr>
<td>lp_1102</td>
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<td>cation transport protein</td>
<td>-1.53</td>
<td>0.002</td>
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<tr>
<td>lp_1105</td>
<td>mae</td>
<td>malic enzyme, NAD-dependent</td>
<td>-1.61</td>
<td>0.001</td>
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<tr>
<td>lp_1106</td>
<td>citC</td>
<td>[citrate (pro-3S)-lyase] ligase</td>
<td>-1.62</td>
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<tr>
<td>lp_1107</td>
<td>citD</td>
<td>citrate lyase, acyl carrier protein</td>
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<td>lp_1108</td>
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<tr>
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<td>citF</td>
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<td>0.044</td>
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<td>oppB</td>
<td>oligopeptide ABC transporter, permease protein</td>
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<td>lp_1477</td>
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<td>flavodoxin</td>
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<td>lp_1579</td>
<td>miaA</td>
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<td>Gene</td>
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<td>Log2FoldChange</td>
<td>P-Value</td>
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<td>lp_1593</td>
<td>ribosomal protein (putative)</td>
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<td>translation factor, GTPase</td>
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<td>0.041</td>
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<td>lp_1715</td>
<td>prenyltransferase</td>
<td>2.52</td>
<td>0.035</td>
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<tr>
<td>lp_1822</td>
<td><em>gshR3</em> glutathione reductase</td>
<td>0.97</td>
<td>0.008</td>
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<tr>
<td>lp_1856</td>
<td>unknown</td>
<td>0.95</td>
<td>0.028</td>
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<td>lp_2095</td>
<td><em>fruR</em> transcription regulator of fructose operon</td>
<td>-1.09</td>
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<tr>
<td>lp_2096</td>
<td><em>fruK</em> 1-phosphofructokinase</td>
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<td>0.004</td>
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<tr>
<td>lp_2101</td>
<td><em>cps4H</em> polysaccharide polymerase</td>
<td>-0.93</td>
<td>0.008</td>
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<td>lp_2110</td>
<td><em>ginQ3</em> glutamine ABC transporter, ATP-binding protein</td>
<td>-1.04</td>
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<td>lp_2111</td>
<td><em>ginPH2</em> glutamine ABC transporter, substrate binding and permease protein</td>
<td>-1.28</td>
<td>0.075</td>
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<td>lp_2153</td>
<td><em>pdhB</em> pyruvate dehydrogenase complex, E1 component, beta subunit</td>
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<td>aminotransferase with N-terminal regulator domain</td>
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<td><em>patB</em> aminotransferase</td>
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<td>0.011</td>
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<td>lp_3045</td>
<td>short-chain dehydrogenase/oxidoreductase</td>
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<td>oxidoreductase</td>
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<td>acetyltransferase (putative)</td>
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<td>0.039</td>
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References:


