

FIG S1 *L. plantarum* WCFS1 genes in functional groups that were up-regulated and downregulated in CDM compared to MRS grown cells, using total RNA (dark grey), enriched mRNA (light grey) and both (black). Dotted grey bar represent genes with opposite transcript direction between total RNA and enriched mRNA.





FIG S2 Distance of predicted terminators downstream of protein encoding genes employed
for the prediction for 3'-UTR length of *L. plantarum* WCFS1 (panel A) and increase of 3'UTR sequencing transcripts mapping percentage to the CDS based on 100bp, 200bp and
300bp extension (panel B)



FIG S3 Comparison of mean-normalized values between signal intensity of microarray (X axis) and number of read counts (Y-axis) of direct cDNA sequencing (panel A) and 3'-UTR sequencing (panel B) in CDM. A saturation range was observed for the highest expression values obtained by microarray analysis, while the read counts of direct cDNA and 3'-UTR sequencings still appeared to be within the dynamic range of the technology.

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TABLE S1 RNA-sequencing analysis summary of direct cDNA sequencing and 3'-UTR
 sequencing of *L. plantarum* WCFS1 grown in CDM or MRS

| | Direct cDNA sequencing | | | | 3'-UTR sequencing | | | | |
|-----------------------------------|------------------------|-------|------------|-------|-------------------|-------|------------|-------|--|
| | CDM | | MRS | | CDM | | MRS | | |
| | Reads | % | Reads | % | Reads | % | Reads | % | |
| Total no. of reads | 25,980,870 | 99.65 | 28,117,544 | 99.79 | 17,542,374 | 94.58 | 27,268,505 | 95.49 | |
| No. of reads mapped to the genome | 25,112,191 | 96.66 | 27,488,120 | 97.76 | 16,628,389 | 94.79 | 25,392,216 | 93.12 | |
| No. of reads mapped to CDS | 14,616,459 | 56.26 | 18,547,758 | 65.97 | 3,388,642 | 19.32 | 4,106,265 | 15.06 | |
| No of reads mapped to non-CDS | 10,495,732 | 40.40 | 8,940,362 | 31.80 | 13,239,747 | 75.47 | 21,285,951 | 78.06 | |

TABLE S2 An example of operon predictions which show increase in the frequency of mapped sequence reads at the last gene or the gene next to the

22 3'-UTR after mapping extension of 100bp (highlighted in grey).

| Gene | Gene | | | CDM | MRS | CDM + | MRS + |
|---------|---------|--|--------------------------------------|--------|--------|-------|-------|
| ID | name | Function | Category | normal | normal | 100bp | 100bp |
| lp_0643 | lp_0643 | prophage P1 protein 20 | | 14 | 14 | 16 | 13 |
| lp_0644 | lp_0644 | prophage P1 protein 21 | | | 0 | 1 | 1 |
| lp_0645 | lp_0645 | prophage P1 protein 23 | Phage and prophage related functions | 11 | 18 | 5 | 11 |
| lp_0646 | lp_0646 | prophage P1 protein 24 | | 18 | 13 | 19 | 15 |
| lp_0647 | lp_0647 | prophage P1 protein 25 | | 19 | 13 | 19 | 10 |
| lp_0648 | lp_0648 | prophage P1 protein 26 | | 7 | 3 | 7 | 4 |
| lp_0649 | lp_0649 | prophage P1 protein 27 | | 1 | 10 | 1 | 10 |
| lp_0650 | lp_0650 | prophage P1 protein 28 | | 10 | 3 | 12 | 3 |
| lp_0651 | lp_0651 | prophage P1 protein 29 | | 7 | 1 | 7 | 7 |
| lp_0652 | lp_0652 | prophage P1 protein 30 | | 3 | 9 | 5 | 17 |
| lp_0653 | lp_0653 | prophage P1 protein 31 | | 9 | 15 | 11 | 20 |
| lp_0654 | lp_0654 | prophage P1 protein 32 | | 19 | 32 | 22 | 35 |
| lp_0655 | lp_0655 | prophage P1 protein 33, phage transcriptional regulator, ArpU family | | 18 | 40 | 11760 | 13765 |
| lp_2719 | purD | phosphoribosylamineglycine ligase | | 1694 | 49 | 6436 | 14373 |
| | | bifunctional protein: phosphoribosylaminoimidazolecarboxamide | | | | | |
| lp_2720 | purH | formyltransferase; IMP cyclohydrolase | | 753 | 47 | 742 | 48 |
| lp_2721 | purN | phosphoribosylglycinamide formyltransferase | Purine ribonucleotide biosynthesis | 233 | 18 | 260 | 18 |
| lp_2722 | purM | phosphoribosylformylglycinamidine cyclo-ligase | | 517 | 38 | 517 | 38 |
| lp_2723 | purF | amidophosphoribosyltransferase precursor | | 886 | 100 | 885 | 96 |
| lp_2724 | purl | phosphoribosylformylglycinamidine synthase II | | 710 | 87 | 730 | 88 |
| lp_2725 | purQ | phosphoribosylformylglycinamidine synthase I | | 98 | 26 | 111 | 29 |
| lp_2726 | purS | phosphoribosylformylglycinamidine synthase, PurS component | | 48 | 7 | 51 | 3 |
| lp_2727 | purC | phosphoribosylaminoimidazole-succinocarboxamide synthase | uccinocarboxamide synthase | | 14 | 104 | 19 |
| lp_2728 | purK1 | phosphoribosylaminoimidazole carboxylase, ATPase subunit | | 159 | 40 | 153 | 41 |
| lp_2729 | pure | phosphoribosylaminoimidazole carboxylase, catalytic subunit | | 94 | 22 | 109 | 24 |
| lp_1672 | acpA2 | acyl carrier protein | | 433 | 1318 | 476 | 1401 |
| lp_1673 | fabD | (acyl-carrier protein) S-malonyltransferase | | 949 | 1698 | 949 | 1690 |
| lp_1674 | fabG1 | 3-oxoacyl-(acyl-carrier protein) reductase | | 500 | 1067 | 566 | 1209 |
| lp_1675 | fabF | 3-oxoacyl-(acyl-carrier protein) synthase II | | 1454 | 2194 | 1455 | 2211 |
| lp_1676 | accB2 | acetyl-CoA carboxylase, biotin carboxyl carrier protein | | 392 | 970 | 381 | 965 |
| lp_1677 | fabZ2 | (3R)-hydroxymyristoyl-(acyl carrier protein) dehydratase | Fatty acid biosynthesis | 374 | 746 | 431 | 839 |
| lp_1678 | accC2 | acetyl-CoA carboxylase, biotin carboxylase subunit | 1 | 1974 | 2518 | 2000 | 2558 |
| lp_1679 | accD2 | acetyl-CoA carboxylase, carboxyl transferase subunit beta | 724 | 1012 | 758 | 1074 | |
| lp_1680 | accA2 | acetyl-CoA carboxylase, carboxyl transferase subunit alpha | | 925 | 1523 | 976 | 1606 |
| lp_1681 | fabl | enoyl-(acyl-carrier protein) reductase (NADH) | | 572 | 806 | 528 | 760 |
| lp_1682 | lp_1682 | Phosphopantetheinyltransferase | | 3475 | 4049 | 5079 | 7654 |