*Supplementary Material*

**Transcriptome analysis revealed growth phase-associated changes of a centenarian-originated probiotic *Bifidobacterium animalis* subsp*. lactis* A6**

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**Table S1** The number of reads before and after the trimming step.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample name | Raw reads | Clean reads | clean bases | Q20(%) | Q30(%) | GC content (%) |
| A6log1 | 23311618 | 23261948 | 3.49G | 98.58 | 95.71 | 60.6 |
| A6log2 | 19689878 | 19652184 | 2.95G | 98.53 | 95.58 | 60.54 |
| A6log3 | 22553776 | 22508958 | 3.38G | 98.49 | 95.52 | 60.54 |
| A6log4 | 23882120 | 23526324 | 3.53G | 98.23 | 94.72 | 61.04 |
| A6WD2 | 23893390 | 23835722 | 3.58G | 98.29 | 94.88 | 59.87 |
| A6WD3 | 23501046 | 23447790 | 3.52G | 98.38 | 95.08 | 60.05 |
| A6WD4 | 21352442 | 21096818 | 3.16G | 98.15 | 94.5 | 60.75 |

**Table S2** The information of reads mapping to the reference genome.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sample name | A6log1 | A6log2 | A6log3 | A6log4 | A6WD2 | A6WD3 | A6WD4 |
| Total reads | 23261948 | 19652184 | 22508958 | 23526324 | 23835722 | 23447790 | 21096818 |
| Total mapped | 23115123 (99.37%) | 19531490 (99.39%) | 22330874 (99.21%) | 23392844 (99.43%) | 23605032 (99.03%) | 23247112 (99.14%) | 20996723 (99.53%) |
| Multiple mapped | 464994 (2%) | 338173 (1.72%) | 447809 (1.99%) | 305964 (1.3%) | 642864 (2.7%) | 585452 (2.5%) | 309065 (1.46%) |
| Uniquely mapped | 22650129 (97.37%) | 19193317 (97.67%) | 21883065 (97.22%) | 23086880 (98.13%) | 22962168 (96.34%) | 22661660 (96.65%) | 20687658 (98.06%) |

**Table S3** Genes differentially expressed at the transcriptional level in *B. lactis* A6 during the stationary phase compared to the exponential phase a.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene\_ID** | **Gene** | **Protein\_ID** | **Fold Change (FC)**b | **Description**c |
| **Carbohydrate transport and metabolism** |
| BAA6\_1092 |  | WP\_039700917.1 | -5.37 | putative glucose uptake permease |
| BAA6\_0481 |  | WP\_004268765.1 | 5.18 | galactoside symporter  |
| BAA6\_0050 |  | WP\_012619616.1 | 3.17 | MFS transporter  |
| BAA6\_1585 |  | WP\_042994610.1 | 3.11 | MalG-type ABC sugar transport system permease component |
| BAA6\_1587 |  | WP\_004218962.1 | 2.72 | sugar binding protein of ABC transporter system |
| BAA6\_0517 |  | WP\_004219354.1 | 2.45 | putative sugar transporter solute-binding protein |
| BAA6\_0518 |  | WP\_044059192.1 | 1.89 | putative binding protein-dependent transporter |
| BAA6\_1415 | *cebG* | WP\_004219192.1 | 3.37 | carbohydrate ABC transporter permease |
| BAA6\_1416 | *cbpA* | WP\_004219191.1 | 5.6 | cellobiose phosphorylase |
| BAA6\_0488 |  | WP\_004268778.1 | -2.27 | MalE-type ABC sugar transport system substrate-binding protein |
| BAA6\_0491 |  | WP\_004268783.1 | -2.10 | sugar ABC transporter permease |
| BAA6\_1555 |  | WP\_004219253.1 | -3.20 | MalG-type ABC sugar transport system permease component |
| BAA6\_1548 |  | WP\_004219261.1 | -2.01 | putative sugar ABC transport system membrane protein |
| BAA6\_1549 |  | WP\_004219260.1 | -2.46 | putative sugar ABC transport system membrane protein |
| BAA6\_0968 | *xfp* | WP\_004217768.1 | -1.97 | Xylulose-5-phosphate/fructose-6-phosphate phosphoketolase |
| BAA6\_0838 | *tkt* | WP\_004217933.1 | -2.07 | transketolase |
| BAA6\_0839 | *tal* | WP\_004217932.1 | -2.19 | transaldolase |
| BAA6\_1489 |  | WP\_004219087.1 | -2.49 | PfkB family sugar kinase[ribose kinase] |
| BAA6\_0794 | *rpe* | WP\_004217990.1 | -1.54 | ribulose-phosphate 3-epimerase |
| BAA6\_0298 | *gpi* | WP\_004268405.1 | -1.72 | glucose-6-phosphate isomerase |
| BAA6\_0431 | *pgM* | WP\_004268650.1 | -1.76 | phosphoglucomutase |
| BAA6\_0846 |  | WP\_004217924.1 | -2.38 | triosephosphate isomerase |
| BAA6\_1161 | *gap* | WP\_004218695.1 | -1.61 | Glyceraldehyde-3-phosphate dehydrogenase |
| BAA6\_0847 | *pgk* | WP\_004217923.1 | -1.72 | phosphoglycerate kinase |
| BAA6\_0756 | *pgm* | WP\_004218045.1 | -2.81 | phosphoglycerate mutase |
| BAA6\_0753 |  | WP\_004218048.1 | -2.77 | glucosyl-3-phosphoglycerate phosphatase |
| BAA6\_0939 |  | WP\_004217812.1 | -2.29 | phosphoglycerate mutase |
| BAA6\_1012 | *eno* | WP\_004269110.1 | -1.76 | enolase |
| BAA6\_0979 | *pyk* | WP\_004269104.1 | -1.73 | pyruvate kinase |
| BAA6\_0329 | *ldh2* | WP\_004268463.1 | -3.11 | lactate dehydrogenase |
| BAA6\_1078 | *pfl* | WP\_004217631.1 | -4.14 | formate acetyltransferase |
| BAA6\_0969 | *pat* | WP\_004217766.1 | -3.44 | phosphate acetyltransferase |
| BAA6\_0970 | *ackA* | WP\_004269103.1 | -1.67 | acetate kinase |
| BAA6\_0061 |  | WP\_004219336.1 | 1.91 | alpha-L-arabinofuranosidase |
| BAA6\_0062 | *araB* | WP\_004219337.1 | 2.93 | L-ribulokinase |
| BAA6\_0063 | *araD* | WP\_004219338.1 | 4.36 | L-ribulose-5-phosphate 4-epimerase |
| BAA6\_0064 | *araA* | WP\_004219339.1 | 3.38 | L-arabinose isomerase |
| BAA6\_0525 | *xylB* | WP\_004219345.1 | 2.00 | xylulose kinase |
| BAA6\_1351 | *galK* | WP\_004218283.1 | -3.10 | galactokinase |
| BAA6\_0274 | *lacZ* | WP\_004268361.1 | -1.69 | beta-galactosidase |
| BAA6\_0523 | *xyn* | WP\_004219346.1 | 2.64 | endo-1,4-β-xylanase |
| **Amino acids uptake and biosynthesis** |
| BAA6\_0566 | *oppA* | WP\_004218801.1 | 5.63 | oligopeptide transporter substrate-binding protein |
| BAA6\_0567 | *oppB* | WP\_004218802.1 | 2.10 | ABC transporter, oligopeptide transporter permease component |
| BAA6\_0568 | *oppC* | WP\_004218803.1 | 2.02 | ABC transporter, oligopeptide transporter permease component |
| BAA6\_0570 | *oppF* | WP\_004218805.1 | 2.93 | ABC transporter, oligopeptide transporter ATPase component |
| BAA6\_1193 | *dppD* | WP\_004218656.1 | 5.65 | ABC transporter, dipeptide transporter ATPase component |
| BAA6\_1194 | *dppC* | WP\_012619827.1 | 4.62 | ABC transporter, dipeptide transporter permase component |
| BAA6\_1269 |  | WP\_004218550.1 | -3.09 | peptide ABC transporter permease |
| BAA6\_1270 |  | WP\_004218548.1 | -2.15 | ATP binding protein of ABC transporter for Glu/Asp |
| BAA6\_0681 | *gluB* | WP\_004218145.1 | -1.99 | ABC-type amino acid transport system periplasmic component |
| BAA6\_0682 | *gluC* | WP\_004218144.1 | -2.58 | ABC-type amino acid transport system permease component |
| BAA6\_0683 | *gluD* | WP\_004218142.1 | -2.48 | permease protein of ABC transporter for glutamate |
| BAA6\_0680 | *gluA* | WP\_004218146.1 | -2.15 | ATP-binding protein of ABC transporter for glutamate |
| BAA6\_1082 | *MetI* | WP\_004217625.1 | -3.99 | ABC-type amino acid transport system permease component |
| BAA6\_1083 | *MetN* | WP\_004217623.1 | -1.83 | ATP binding protein of ABC transporter |
| BAA6\_0186 |  | WP\_004268977.1 | 1.6 | dipeptidase |
| BAA6\_0230 |  | WP\_004219304.1 | 2.0 | dipeptidase |
| BAA6\_0261 |  | WP\_004268336.1 | -1.51 | aspartate aminotransferase [aspartate biosynthesis] |
| BAA6\_0881 | *metE* | WP\_004217882.1 | -1.57 | homocysteine methyltransferase [methionine biosynthesis] |
| BAA6\_1150 | *pheA* | WP\_004218708.1 | -2.22 | bifunctional chorismate mutase/prephenate dehydratase [tyrosine and phenylalanine biosynthesis] |
| BAA6\_0731 | *argF* | WP\_004218077.1 | -4.87 | ornithine carbamoyltransferase [arginine biosynthesis] |
| BAA6\_0733 | *argG* | WP\_004218073.1 | -15.45 | argininosuccinate synthase [arginine biosynthesis] |
| BAA6\_0734 | *argH* | WP\_004218072.1 | -4.68 | argininosuccinate lyase [arginine biosynthesis] |
| BAA6\_1052 | *proC* | WP\_004217670.1 | -3.02 | pyrroline-5-carboxylate reductase [proline biosynthesis] |
| **Peptidoglycan biosynthesis** |
| BAA6\_0562 | *glmS* | WP\_004269062.1 | -2.03 | D-fructose-6-phosphate amidotransferase |
| BAA6\_1325 | *glmM* | WP\_004269221.1 | -1.75 | phosphoglucosamine mutase |
| BAA6\_0751 | *glmU* | WP\_004218050.1 | -3.00 | bifunctional protein glmU |
| BAA6\_1199 | *murC* | WP\_004218652.1 | -2.55 | UDP-N-acetylmuramate--L-alanine ligase |
| BAA6\_1202 | *murD* | WP\_004218648.1 | -1.94 | UDP-N-acetylmuramoyl-L-alanine-D-glutamate ligase |
| BAA6\_1204 | *murF* | WP\_004218644.1 | -1.53 | UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D- alanine ligase |
| BAA6\_1612 | *murJ* | WP\_004218997.1 | -1.62 | putative peptidoglycan lipid II flippase |
| BAA6\_0083 |  | WP\_004218373.1 | -1.71 | transpeptidase |
| BAA6\_0084 |  | WP\_004218375.1 | -2.01 | Bacterial cell division membrane protein |
| BAA6\_0550 | *ftsQ* | WP\_004218773.1 | -3.41 | cell division protein FtsQ |
| BAA6\_1198 | *ftsQ* | WP\_012619828.1 | -2.61 | cell division protein FtsQ |
| BAA6\_1201 | *ftsW* | WP\_004218649.1 | -2.71 | cell division protein FtsW |
| **Acid tolerance response** |
| BAA6\_0572 | *cysK* | WP\_004218807.1 | 6.30 | cystathionine beta-synthase |
| BAA6\_0947 | *metC* | WP\_077385089.1 | 2.72 | cystathionine beta-lyase |
| BAA6\_0010 | *gdhA* | WP\_004219034.1 | 2.67 | glutamate dehydrogenase |
| BAA6\_0643 | *serB* | WP\_004218899.1 | 1.8 | phosphoserine phosphatase SerB [serine biosynthesis] |
| BAA6\_0708 | *glnA1* | WP\_004218110.1 | -6.23 | glutamine synthetase 1 [glutamine biosynthesis] |
| BAA6\_1220 | *glnA2* | WP\_004218616.1 | -1.89 | glutamine synthetase 2 [glutamine biosynthesis] |
| BAA6\_0174 | *livK* | WP\_004218478.1 | 3.1 | BCAAs transport system substrate-binding protein |
| BAA6\_0283 | *ilvB* | WP\_014482615.1 | 2.31 | acetolactate synthase 1 catalytic subunit |
| BAA6\_0146 | *ilvC* | WP\_004218445.1 | 3.17 | ketol-acid reductoisomerase [isoleucine biosynthesis] |
| BAA6\_0370 | *groES* | WP\_004268536.1 | 1.20 | co-chaperone GroES |
| BAA6\_0665 | *groEL* | WP\_004218937.1 | 1.56 | chaperonin GroEL |
| BAA6\_1543 | *dnaK* | WP\_004219269.1 | 1.20 | molecular chaperone DnaK |
| BAA6\_0675 | *clpE* | WP\_004218154.1 | 1.65 | ATP-dependent Clp protease ATP-binding subunit |
| BAA6\_1072 | *clpP1* | WP\_004217639.1 | 1.55 | ATP-dependent Clp protease proteolytic subunit |
| BAA6\_1073 | *clpP2* | WP\_035136458.1 | 3.19 | ATP-dependent Clp protease proteolytic subunit 2 |
| **Adhesion**  |
| BAA6\_0205 | *tadZ* | WP\_004218511.1 | 9.37 | Flp pilus assembly protein tadZ |
| BAA6\_0206 | *tadA* | WP\_004268985.1 | 6.50 | Flp pilus assembly protein ATPase component |
| BAA6\_0207 | *tadB* | WP\_004218513.1 | 12.05 | Flp pilus assembly protein |
| BAA6\_0208 | *tadC* | WP\_004218514.1 | 9.48 | Flp pilus assembly protein |
| BAA6\_0211 | *tadE* | WP\_004218516.1 | 8.60 | pseudopilin TadE |
| BAA6\_0212 | *tadF* | WP\_004218517.1 | 8.14 | pseudopilin TadF |
| BAA6\_0224 | *tadV* | WP\_004219310.1 | 1.65 | prepilin peptidase |

a Selected genes which were discussed in the Results and discussion section.

b Fold change in gene or protein expression. The minus number means downregulated change.

c Functions were assigned from the KEGG pathways for *B.* *lactis* A6.

**Table S4** Alignment of amino acid sequences of tad pili in *B. lactis* A6 compared with *B. breve* UCC2003 by SnapGene software (version 5.3) in a local alignment (Smith-Waterman) module.

|  |  |  |
| --- | --- | --- |
| Gene number | Gene name | BLAST results |
| A6 | UCC2003 | length | Identity |  Similarity | Gap |
| BAA6\_0205 | Bbr\_0132 | *tadZ* | 280 | 38.93% | 56.43% | 11.07% |
| BAA6\_0206 | Bbr\_0133 | *tadA* | 327 | 59.33% | 70.03% | 0.00% |
| BAA6\_0207 | Bbr\_0134 | *tadB* | 206 | 39.32% | 48.06% | 16.02% |
| BAA6\_0208 | Bbr\_0135 | *tadC* | 168 | 41.07% | 57.14% | 17.86% |
| BAA6\_0210 | Bbr\_0136 | *flp* | 84 | 58.33% | 76.19% | 4.76% |
| BAA6\_0211 | Bbr\_0137 | *tadE* | 103 | 38.83% | 51.46% | 0.00% |
| BAA6\_0212 | Bbr\_0138 | *tadF* | 117 | 34.19% | 52.99% | 5.93% |
| BAA6\_0933 | Bbr\_0901 | *tadV* | 159 | 36.48% | 55.97% | 5.03% |
| BAA6\_0224 | Bbr\_0901 | *tadV* | 135 | 25.19% | 37.78% | 5.93% |



**Fig. S1.** Pearson correlation between samples. A6log: *B. lactis* A6 strain collected at the exponential phase with OD600 around 0.6~0.8 during growth (n=4). A6WD: *B. lactis* A6 strain collected at the stationary phase with OD600 around 4.0~4.2 during growth (n=3).



**Fig. S2.** Histogram of gene ontology (GO) and scatter plot of KEGG pathway enrichment of differentially expressed genes (DEGs). The most enriched 30 GO terms of regulated genes is shown in Column 1, while mainly metabolic pathway enrichment of regulated genes is shown in Column 2. (A) and (B) indicates the up-regulated biological processes or metabolic pathways, conversely, (c) and (d) indicates the down-regulated biological processes or metabolic pathways. Asterisk (\*) indicates a significantly enriched process during the stationary phase compared with the log phase.



**Fig. S3.** Graphical representation of DAP-type peptidoglycan biosynthesis of *B. lactis* A6 in the stationary phase compared to the log phase. Fru-6P, fructose-6-phosphate; GlcN-6P, glucosamine-6-phosphate; D-GlcN-1P, glucosamine-1-phosphate; GlcNAc-1P, N-acetylglucosamine-1-phosphate; UDP-GlcNAc, uridine diphosphate-N-acetylglucos-amine; UDP-MurNAc, UDP-N-acetylmuramate; Und-PP, undercaprenyl diphosphate; GlmU, N-acetylglucosamine-1-phosphate uridyltransferase; MurC, UDP-N-acetylmuramate L-alanine ligase; MurD, UDP-N-acetylmuramoylalanine D-glutamate ligase; MurF, UDP-N-acetylmuramoyl-tripeptide D-alanyl-D-alanine ligase. Arrows and characters in black and green color represent genes which were un-changed and down-regulated, respectively. Partial structural formulas are referenced from the KEGG pathway (https://www.kegg.jp/kegg/pathway.html).