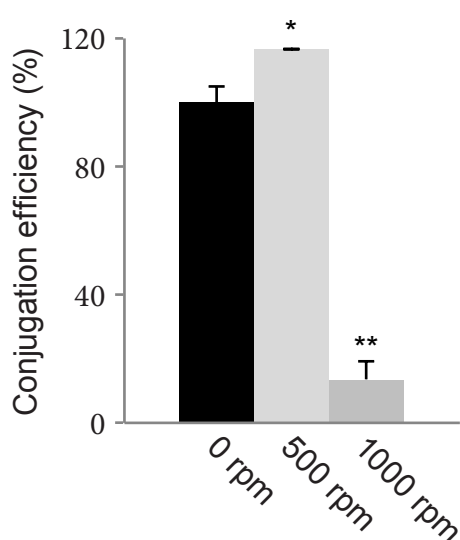


Supplementary Figure S1: Relationship of growth and the conjugation efficiency of pLS20. (A) Growth curve of cells carrying pLS20 (triangles) or lacking pLS20 (squares). Arrows indicate time points used for sampling of the cells for transcriptional profiling. Note that 30 min marks the onset of conjugation, 90 min represents the time of highest conjugation efficiency and 480 min represents the time of no conjugation. (B) Conjugation efficiency of pLS20 during growth of *Bacillus subtilis* cells. Data from independent mating experiments (n=9) were averaged and the transfer rate in transconjugants/ml/donor was plotted against the optical density of the corresponding cultures. Note that under our growth conditions conjugation activity reaches its maximum at OD₆₀₀ = 0.4.



Supplementary Figure S2: Strong shaking conditions repress conjugation. Conjugation efficiency of pLS20 carrying cells during mating under different conditions of culture shaking (rpm = revolutions per minute), using an Eppendorf Thermomixer. Conjugation experiments were performed in parallel and in duplicates. *P* values were calculated by using one-way analysis of variance (ANOVA) with tukey's post-test. **P* < 0.05, ***P* < 0.001 compared to the non-shaking condition.

Supplementary Table S1: List of differentially expressed genes in pLS20-carrying cells.

| Functional category | gene | gene function | fold change (<i>P</i> -value) | | |
|---------------------|-------------|---|--------------------------------|--------|---------|
| | | | 30 min | 90 min | 480 min |
| Cell wall | <i>gcaD</i> | bifunctional N-acetylglucosamine-1-phosphate uridylyltransferase/glucosamine-1-phosphate acetyltransferase | | 2.0*** | |
| Cell wall | <i>lytB</i> | modifier protein of major autolysin LytC | | -3.1** | |
| Cell wall | <i>lytC</i> | N-acetylmuramoyl-L-alanine amidase, required for flagellar function | | -3.2** | |
| Cell wall | <i>pbpA</i> | penicillin-binding protein PBP 2A | -2.0** | | |
| Cell wall | <i>pbpE</i> | penicillin-binding protein PBP 4* (spore cortex) | | 2.0** | |
| Cell wall | <i>tagE</i> | UDP-glucose:polyglycerol phosphate glucosyltransferase | | 1.9*** | |
| Cell wall | <i>tuaB</i> | biosynthesis of teichuronic acid | | 4.7* | |
| Cell wall | <i>tuaC</i> | sugar transferase | | 1.9* | |
| Cell wall | <i>tuaD</i> | biosynthesis of teichuronic acid (UDP-glucose 6-dehydrogenase) | | 2.5* | |
| Cell wall | <i>tuaE</i> | biosynthesis of teichuronic acid | | 3.4** | |
| Cell wall | <i>tuaF</i> | biosynthesis of teichuronic acid | | 2.0** | |
| Cell wall | <i>tuaG</i> | sugar transferase | | 1.8** | |
| Cell wall | <i>yojL</i> | D,L-endopeptidase, peptidoglycan hydrolase | | | 2.6* |
| Cell wall | <i>ypjH</i> | L-malic acid glycosyltransferase, involved in bacillithiol synthesis | | | 2.0* |
| Cell wall | <i>ywhE</i> | penicillin-binding protein 2d | | | -1.9* |
| Cell wall | <i>ywtD</i> | gamma-DL-glutamyl hydrolase | -2.0** | | |

| | | | | | |
|----------------------------|-------------|--|-------|---------|-------|
| Transport/binding proteins | <i>aapA</i> | amino acid permease | | -2.2* | |
| Transport/binding proteins | <i>amyC</i> | melibiose transport protein | | 2.6* | |
| Transport/binding proteins | <i>amyD</i> | probably ABC transporter for melibiose (permease) | | | -2.5* |
| Transport/binding proteins | <i>araN</i> | L-arabinose ABC transporter | | 1.9* | |
| Transport/binding proteins | <i>cydD</i> | ABC transporter required for expression of cytochrome bd (ATP-binding protein) | | -5.2** | |
| Transport/binding proteins | <i>cysC</i> | adenylyl-sulfate kinase | | -2.5** | |
| Transport/binding proteins | <i>cysP</i> | sulfate transport in via proton symport | | -6.6*** | |
| Transport/binding proteins | <i>czcD</i> | cation diffusion facilitator | | | -2.1* |
| Transport/binding proteins | <i>dctP</i> | C4-dicarboxylate transport protein | | 2.8** | |
| Transport/binding proteins | <i>dppD</i> | dipeptide ABC transporter (ATP-binding protein) | -2.8* | | |
| Transport/binding proteins | <i>gamP</i> | glucosamine-specific phosphotransferase system | | 2.0** | |
| Transport/binding proteins | <i>glcP</i> | probable glucose/mannose:H ⁺ symporter | | 5.6* | |
| Transport/binding proteins | <i>gntP</i> | gluconate permease | 2.3** | 3.3*** | |
| Transport/binding proteins | <i>hutM</i> | histidine permease | | 1.8* | |
| Transport/binding proteins | <i>iolF</i> | D-chiro-inositol transport protein | | 2.0** | |
| Transport/binding proteins | <i>lctP</i> | lactate permease, excretion | | -16.1** | |
| Transport/binding proteins | <i>levD</i> | fructose-specific phosphotransferase system, EIIA PTS component | | -3.6* | |
| Transport/binding proteins | <i>licA</i> | lichenan-specific phosphotransferase system, EIIA PTS component | | 2.4* | |
| Transport/binding proteins | <i>licC</i> | lichenan-specific phosphotransferase system, EIIC PTS component | | 2.0** | |
| Transport/binding proteins | <i>lplC</i> | transmembrane lipoprotein, putative ABC transporter | | 2.1** | |

| | | | | | |
|----------------------------|--------------|---|-------|--------|-------|
| Transport/binding proteins | <i>maeN</i> | Na ⁺ /malate symporter | | 2.1** | |
| Transport/binding proteins | <i>malP</i> | maltose-specific phosphotransferase system, EIICB of the PTS | | 3.0*** | |
| Transport/binding proteins | <i>manP</i> | mannose-specific phosphotransferase system, EIIBCA of the PTS | | 1.9* | |
| Transport/binding proteins | <i>mleN</i> | malate-H ⁺ /Na ⁺ -lactate antiporter | | 2.3*** | |
| Transport/binding proteins | <i>mrpA</i> | Na ⁺ transporter subunit of the Na ⁺ /H ⁺ antiporter, multiple resistance and pH homeostasis | | | 1.8* |
| Transport/binding proteins | <i>msmE</i> | multiple sugar-binding protein | | 5.0*** | |
| Transport/binding proteins | <i>mtlA</i> | mannitol-specific phosphotransferase system, EIICB of the PTS | | -3.0** | |
| Transport/binding proteins | <i>nagP</i> | N-acetylglucosamine-specific phosphotransferase system, EIICB of the PTS | | | 2.4** |
| Transport/binding proteins | <i>natB</i> | Na ⁺ ABC transporter (export) (ATP-binding protein) | -1.8* | | |
| Transport/binding proteins | <i>nupC</i> | pyrimidine nucleoside transport protein | | 2.1*** | |
| Transport/binding proteins | <i>pstBB</i> | phosphate ABC transporter (ATP-binding protein) | | 3.1* | |
| Transport/binding proteins | <i>pstC</i> | phosphate ABC transporter (permease) | | 2.0* | |
| Transport/binding proteins | <i>pyrP</i> | uracil permease | 1.8** | -1.9** | |
| Transport/binding proteins | <i>rbsA</i> | ribose ABC transporter (ATP-binding protein) | | 4.8*** | |
| Transport/binding proteins | <i>rbsB</i> | ribose ABC transporter (ribose-binding protein) | | 4.7*** | |
| Transport/binding proteins | <i>rbsC</i> | ribose ABC transporter (permease) | | 2.9* | |
| Transport/binding proteins | <i>rbsD</i> | ribose ABC transporter (membrane protein) | | 6.3*** | |
| Transport/binding proteins | <i>rocE</i> | amino acid permease | | 2.2*** | |
| Transport/binding proteins | <i>sacP</i> | PTS sucrose-specific enzyme IIBC component | | 3.2*** | |

| | | | | |
|----------------------------|-------------|---|-------|---------|
| Transport/binding proteins | <i>slp</i> | small peptidoglycan-associated lipoprotein | | 2.1* |
| Transport/binding proteins | <i>treP</i> | PTS trehalose-specific enzyme IIBC component | | 2.5** |
| Transport/binding proteins | <i>trkA</i> | cation export | -2.0* | |
| Transport/binding proteins | <i>xynP</i> | beta-xyloside permease | | 2.9** |
| Transport/binding proteins | <i>ybaE</i> | similar to ABC transporter (ATP-binding protein) | | -2.7*** |
| Transport/binding proteins | <i>ybxA</i> | similar to ABC transporter (ATP-binding protein) | | -2.1** |
| Transport/binding proteins | <i>yceA</i> | ABC transporter for zinc (permease) | | 1.9* |
| Transport/binding proteins | <i>yclF</i> | similar to transporter | | 2.0* |
| Transport/binding proteins | <i>ydeR</i> | similar to antibiotic resistance protein | | 2.0** |
| Transport/binding proteins | <i>ydfA</i> | similar to arsenical pump membrane protein | | 1.8* |
| Transport/binding proteins | <i>ydhM</i> | glucomannan-specific phosphotransferase system, EIIB of PTS | | 2.3** |
| Transport/binding proteins | <i>ydhN</i> | glucomannan-specific phosphotransferase system, EIIA of PTS | | 2.6** |
| Transport/binding proteins | <i>ydhO</i> | glucomannan-specific phosphotransferase system, EIIC of PTS | | 1.9** |
| Transport/binding proteins | <i>yhaQ</i> | similar to ABC transporter (ATP-binding protein) | | 1.9* |
| Transport/binding proteins | <i>yhcL</i> | cystine transporter, membrane protein | | -4.3** |
| Transport/binding proteins | <i>yodF</i> | similar to proline permease | | -1.8** |
| Transport/binding proteins | <i>ytcQ</i> | similar to ABC transporter (solute-binding lipoprotein) | | 1.8* |
| Transport/binding proteins | <i>yvfM</i> | arabinogalactan oligomer permease | | 3.5** |
| Transport/binding proteins | <i>yvgW</i> | cadmium transporting ATPase, resistance to cadmium | | 2.1* |
| Transport/binding proteins | <i>yvmA</i> | similar to multidrug transporter | | -1.8* |
| Transport/binding proteins | <i>ywcJ</i> | similar to nitrite transporter | | -3.5* |

| | | | | |
|----------------------------|-------------|--|---------|------|
| Transport/binding proteins | <i>ywoA</i> | undecaprenyl pyrophosphate phosphatase, bacitracin resistance | -2.2* | |
| Transport/binding proteins | <i>yxeM</i> | similar to amino acid ABC transporter (binding protein) | -4.9* | |
| Transport/binding proteins | <i>yxeN</i> | similar to amino acid ABC transporter (permease) | -9.2*** | |
| Transport/binding proteins | <i>yxeO</i> | similar to amino acid ABC transporter (ATP-binding protein) | -7.4** | |
| Transport/binding proteins | <i>yxeR</i> | ethanolamine transporter in/out via proton symport | -2.1* | |
| Signal transduction | <i>yesM</i> | two-component sensor kinase | 2.1* | |
| Signal transduction | <i>yvqE</i> | two-component sensor kinase, response to bacitracin | 1.8** | |
| Membrane bioenergetics | <i>atpB</i> | ATP synthase (subunit a) | | 2.0* |
| Membrane bioenergetics | <i>ctaA</i> | heme A synthase | -2.1* | |
| Membrane bioenergetics | <i>cydA</i> | cytochrome bd ubiquinol oxidase (subunit I), terminal oxidase | -3.8** | |
| Membrane bioenergetics | <i>cydB</i> | cytochrome bd ubiquinol oxidase (subunit II), terminal oxidase | -3.6* | |
| Membrane bioenergetics | <i>etfA</i> | electron transfer flavoprotein (alpha subunit) | 1.8* | |
| Membrane bioenergetics | <i>ldh</i> | L-lactate dehydrogenase | -8.7** | |
| Membrane bioenergetics | <i>narG</i> | nitrate reductase (alpha subunit) | -3.5* | |
| Membrane bioenergetics | <i>narH</i> | nitrate reductase (beta subunit) | -2.5** | |
| Membrane bioenergetics | <i>qcrA</i> | menaquinol:cytochrome c oxidoreductase (iron-sulfur subunit), component of the cytochrome bc complex | 1.8*** | |
| Membrane bioenergetics | <i>qcrC</i> | menaquinol:cytochrome c oxidoreductase (cytochrome b/c subunit), component of the cytochrome bc complex | 2.5* | |
| Membrane bioenergetics | <i>yfkO</i> | similar to NAD(P)H-flavin oxidoreductase | 2.3*** | |
| Membrane bioenergetics | <i>ykuP</i> | flavodoxin, replaces ferredoxin under conditions of iron limitation | -2.0* | |

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|-------------------------|-------------|--|--------|---------|
| Membrane bioenergetics | <i>yneN</i> | similar to thiol:disulfide interchange protein | -3.2** | |
| Membrane bioenergetics | <i>yrhE</i> | unknown, similar to formate dehydrogenase | 2.1*** | -1.8* |
| mobility and chemotaxis | <i>cheV</i> | modulation of CheA activity in response to attractants | -1.9* | -2.0* |
| mobility and chemotaxis | <i>flgK</i> | flagellar hook-associated protein 1 (HAP1) | -2.4* | |
| mobility and chemotaxis | <i>fliJ</i> | flagellar protein required for formation of basal body | -2.1* | |
| mobility and chemotaxis | <i>fliS</i> | chaperone for the export of flagellin | -1.9** | |
| mobility and chemotaxis | <i>mcpA</i> | membrane-bound methyl-accepting chemotaxis receptor | | -2.1** |
| mobility and chemotaxis | <i>mcpB</i> | membrane-bound chemotaxis receptor, methyl-accepting chemotaxis protein, receptor for asparagine | | -2.0** |
| mobility and chemotaxis | <i>motA</i> | H ⁺ -coupled MotAB flagellar stator | | -2.5*** |
| mobility and chemotaxis | <i>motB</i> | H ⁺ -coupled MotAB flagellar stator | | -2.5*** |
| mobility and chemotaxis | <i>yfmS</i> | soluble chemotaxis receptor | -1.8* | |
| mobility and chemotaxis | <i>yvyC</i> | similar to flagellar protein | -2.0* | |
| mobility and chemotaxis | <i>yvyG</i> | similar to flagellar protein | -1.9* | |
| protein secretion | <i>ffh</i> | signal recognition particle (SRP) component | | -2.2** |
| protein secretion | <i>lytA</i> | secretion of major autolysin LytC | | -2.9*** |
| protein secretion | <i>yobE</i> | similar to general secretion pathway | | -2.2* |
| Sporulation | <i>soj</i> | ATPase, centromere-like function involved in forespore chromosome partitioning / negative regulation of sporulation initiation | | 1.9** |
| sporulation | <i>sspH</i> | small acid-soluble spore protein (minor) | | 2.0*** |

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|-------------------|--------------|--|--------|---------|-------|
| sporulation | <i>tasA</i> | major component of biofilm matrix, forms amyloid fibers | | 2.0** | |
| Germination | <i>gerBB</i> | nutrient receptor, germination response to the combination of glucose, fructose, and KCl | -1.9* | | |
| Specific pathways | <i>acsA</i> | acetyl-CoA synthetase | | 2.1*** | |
| Specific pathways | <i>amyE</i> | alpha-amylase | | 2.0*** | |
| Specific pathways | <i>araA</i> | L-arabinose isomerase | | 2.2*** | |
| Specific pathways | <i>araB</i> | L-ribulokinase | | 2.7* | |
| Specific pathways | <i>araD</i> | L-ribulose-phosphate 4-epimerase | | 2.4** | |
| Specific pathways | <i>araM</i> | glycerol-1-phosphate dehydrogenase | | 3.3** | |
| Specific pathways | <i>bglH</i> | phospho-beta-glucosidase | | -2.4** | |
| Specific pathways | <i>gamA</i> | glucosamine-6-phosphate deaminase | | 2.4*** | |
| Specific pathways | <i>glpD</i> | glycerol-3-phosphate dehydrogenase (menaquinone 7) | -1.9** | -3.6*** | |
| Specific pathways | <i>glpK</i> | glycerol kinase | 1.9* | | |
| Specific pathways | <i>gntK</i> | gluconate kinase | 2.9** | 2.7** | |
| Specific pathways | <i>idh</i> | myo-inositol 2-dehydrogenase | | 3.3** | |
| Specific pathways | <i>iolB</i> | myo-inositol catabolism | | 3.6*** | |
| Specific pathways | <i>iolC</i> | myo-inositol catabolism | | 3.6*** | |
| Specific pathways | <i>iolD</i> | myo-inositol catabolism | | 3.4** | |
| Specific pathways | <i>iolE</i> | myo-inositol catabolism | | 10.5* | -2.0* |
| Specific pathways | <i>iolH</i> | myo-inositol catabolism | | 2.8** | |
| Specific pathways | <i>lacA</i> | beta-galactosidase | | 2.0** | |

| | | | | |
|-------------------|-------------|---|--------|---------|
| Specific pathways | <i>licH</i> | 6-phospho-beta-glucosidase | | 1.8*** |
| Specific pathways | <i>manA</i> | mannose-6-phosphate isomerase, required for cell wall synthesis | | 2.2* |
| Specific pathways | <i>mela</i> | alpha-galactosidase | | 2.9** |
| Specific pathways | <i>mtlD</i> | mannitol-1-phosphate 5-dehydrogenase | | -2.1*** |
| Specific pathways | <i>nagB</i> | glucosamine-6-phosphate deaminase | | 2.0* |
| Specific pathways | <i>pps</i> | similar to phosphoenolpyruvate synthase | | 2.1** |
| Specific pathways | <i>rbsK</i> | Ribokinase | | 4.1*** |
| Specific pathways | <i>sacA</i> | sucrase-6-phosphate hydrolase | | 3.6*** |
| Specific pathways | <i>treA</i> | trehalose-6-phosphate hydrolase | 2.0* | 2.5** |
| Specific pathways | <i>uxaC</i> | glucuronate isomerase (D-glucuronate, D-galacturonate) | | 1.9** |
| Specific pathways | <i>uxuA</i> | D-mannonate hydrolase | | 1.8* |
| Specific pathways | <i>xsa</i> | alpha-L-arabinofuranosidase | | 2.1* |
| Specific pathways | <i>ydhP</i> | similar to beta-glucosidase | | 2.5** |
| Specific pathways | <i>ydhR</i> | carbohydrate kinase | | 2.2* |
| Specific pathways | <i>ydhS</i> | similar to mannose-6-phosphate isomerase | | 2.4* |
| Specific pathways | <i>ydhT</i> | similar to mannan endo-1,4-beta-mannosidase | | 2.3** |
| Specific pathways | <i>ykfC</i> | D-glutamyl-L-amino acid peptidase | | -1.8* |
| Specific pathways | <i>ylxY</i> | similar to deacetylase | | -2.0** |
| Specific pathways | <i>ysfC</i> | unknown | -2.3** | |
| Specific pathways | <i>ysfD</i> | similar to glycolate oxidase subunit | -2.5** | |
| Specific pathways | <i>ytiB</i> | similar to carbonic anhydrase | | -23.4** |

| | | | | |
|---------------------------|-------------|--|------|---------|
| Specific pathways | <i>yvaM</i> | similar to hydrolase | 1.9* | |
| Specific pathways | <i>yvfO</i> | arabinogalactan endo-1,4-beta-galactosidase | | 4.2* |
| Specific pathways | <i>yvfV</i> | lactate catabolic enzyme | | -7.1*** |
| Specific pathways | <i>yvkC</i> | similar to pyruvate water dikinase | | -8.1*** |
| Specific pathways | <i>ywdH</i> | similar to aldehyde dehydrogenase | | 2.2* |
| Specific pathways | <i>ywjI</i> | class II fructose-1,6-bisphosphatase, gluconeogenesis | | 1.8** |
| Specific pathways | <i>yxiA</i> | endo-1,5-alpha-L-arabinosidase | | 3.4** |
| main glycolytic pathways | <i>acuA</i> | protein acetylase for the control of AcsA activity | | 1.9* |
| main glycolytic pathways | <i>acuB</i> | unknown | | 2.6* |
| main glycolytic pathways | <i>acuC</i> | protein deacetylase for the control of AcsA activity | | 2.1** |
| TCA cycle | <i>mleA</i> | malic enzyme | | 1.8*** |
| TCA cycle | <i>odhA</i> | 2-oxoglutarate dehydrogenase (E1 subunit) | | 1.9*** |
| TCA cycle | <i>odhB</i> | 2-oxoglutarate dehydrogenase complex (E2 subunit) | | 1.9*** |
| TCA cycle | <i>sdhA</i> | succinate dehydrogenase (flavoprotein subunit) | | 2.1** |
| TCA cycle | <i>sdhB</i> | succinate dehydrogenase (iron-sulfur protein) | | 1.9* |
| TCA cycle | <i>sdhC</i> | succinate dehydrogenase (cytochrome b558 subunit) | | 2.2** |
| Metabolism of amino acids | <i>ansA</i> | L-asparaginase | | 1.9* |
| Metabolism of amino acids | <i>ansB</i> | L-aspartase | | 2.1** |
| Metabolism of amino acids | <i>cysH</i> | phosphoadenosine phosphosulfate sulfotransferase | | -2.4* |
| Metabolism of amino acids | <i>gabD</i> | succinate-semialdehyde dehydrogenase, general stress protein | | -2.9** |
| Metabolism of amino acids | <i>gcvH</i> | glycine cleavage system protein H, 2-oxo acid dehydrogenase, | 1.9* | |

| | | | | |
|---------------------------|--------------|--|-------|---------|
| | | lipoyl-binding site | | |
| Metabolism of amino acids | <i>glmS</i> | glutamine-fructose-6-phosphate transaminase | | -1.9* |
| Metabolism of amino acids | <i>nasE</i> | assimilatory nitrite reductase (subunit) | 2.0* | |
| Metabolism of amino acids | <i>proB</i> | glutamate 5-kinase | 2.0* | |
| Metabolism of amino acids | <i>rocG</i> | glutamate dehydrogenase (major) | | 2.5* |
| Metabolism of amino acids | <i>speE</i> | spermidine synthase | | 1.9**** |
| Metabolism of amino acids | <i>ypwA</i> | carboxypeptidase, metalloprotease | | 2.1* |
| Metabolism of nucleotides | <i>dra</i> | deoxyribose-phosphate aldolase | | 2.0** |
| Metabolism of nucleotides | <i>nrdE</i> | ribonucleoside-diphosphate reductase (major subunit), control of DNA/cell mass ratio | | 1.9* |
| Metabolism of nucleotides | <i>purN</i> | phosphoribosylglycinamide formyltransferase, irreversible | | -2.1*** |
| Metabolism of nucleotides | <i>pyrAA</i> | carbamoyl-phosphate synthetase (glutaminase subunit) | 2.7** | -4.0** |
| Metabolism of nucleotides | <i>pyrAB</i> | carbamoyl-phosphate synthetase (catalytic subunit) | | -2.4* |
| Metabolism of nucleotides | <i>pyrB</i> | aspartate carbamoyltransferase | 2.9* | -3.7** |
| Metabolism of nucleotides | <i>pyrC</i> | dihydroorotase | | -2.4* |
| Metabolism of nucleotides | <i>pyrD</i> | dihydroorotic acid dehydrogenase (catalytic subunit) | 1.8* | -1.9* |
| Metabolism of nucleotides | <i>pyrE</i> | orotate phosphoribosyltransferase | | -4.6* |
| Metabolism of nucleotides | <i>pyrF</i> | orotidine 5'-phosphate decarboxylase | | -4.2** |
| Metabolism of nucleotides | <i>pyrK</i> | ihydroorotic acid dehydrogenase (electron transfer subunit) | | -2.0* |
| Metabolism of nucleotides | <i>yabR</i> | similar to polyribonucleotide nucleotidyltransferase | | -2.8* |
| Metabolism of lipids | <i>fabHB</i> | beta-ketoacyl-acyl carrier protein synthase III | | -1.9* |

| | | | | |
|--------------------------|-------------|---|---------|-------|
| Metabolism of lipids | <i>yngF</i> | methylglutaconyl-CoA hydratase | 2.1*** | |
| Metabolism of lipids | <i>ytkK</i> | similar to 3-oxoacyl- acyl-carrier protein reductase | | 1.9* |
| Metabolism of lipids | <i>yvrD</i> | similar to ketoacyl-carrier protein reductase | | 2.9* |
| Metabolism of coenzymes | <i>ylbQ</i> | 2-dehydropantoate 2-reductase | 2.1* | |
| Metabolism of coenzymes | <i>ylnD</i> | probable uroporphyrin-III C-methyltransferase | -8.2*** | |
| Metabolism of sulfur | <i>sat</i> | sulfate adenylyltransferase | -9.9** | |
| DNA replication | <i>dnaA</i> | replication initiation protein | 2.0** | |
| RNA synthesis-Initiation | <i>sigD</i> | RNA polymerase sigma factor SigD | -2.0*** | |
| Regulation RNA synthesis | <i>acoR</i> | transcriptional activator of the acetoin dehydrogenase operon | 3.9** | |
| Regulation RNA synthesis | <i>ccpA</i> | Carbon catabolite control protein A | 2.0* | |
| Regulation RNA synthesis | <i>ccpC</i> | transcriptional repressor of citZ and citB | 2.1** | |
| Regulation RNA synthesis | <i>fnr</i> | transcriptional regulator of anaerobic genes | -3.2** | |
| Regulation RNA synthesis | <i>gntR</i> | transcriptional repressor of the gluconate operon | 6.0* | 2.0** |
| Regulation RNA synthesis | <i>hpr</i> | transcriptional repressor of genes expressed in the transition phase (ScoC) | -2.3* | |
| Regulation RNA synthesis | <i>msmR</i> | transcriptional regulator | 3.3** | |
| Regulation RNA synthesis | <i>phoP</i> | two-component response regulator, regulation of phosphate metabolism | 1.9*** | |
| Regulation RNA synthesis | <i>rbsR</i> | transcriptional repressor of the ribose operon | 2.9** | |
| Regulation RNA synthesis | <i>sacT</i> | transcriptional antiterminator for the sacP-sacA-ywdA operon | -2.2* | |
| Regulation RNA synthesis | <i>treR</i> | transcriptional repressor of the trehalose operon | 2.5** | |

| | | | | |
|---------------------------------|-------------|---|-------|---------|
| Regulation RNA synthesis | <i>ydeB</i> | similar to transcriptional regulator | -2.1* | |
| Regulation RNA synthesis | <i>ydhQ</i> | transcriptional repressor of the <i>gmuB</i> operon | | 1.9* |
| RNA modification | <i>truA</i> | pseudouridylate synthase I, universally conserved protein | | -1.8* |
| Synthesis of ribosomal proteins | <i>rpmD</i> | ribosomal protein | -2.0* | |
| Detoxification | <i>ydjP</i> | similar to chloroperoxydase | 1.9** | |
| Detoxification | <i>ygaF</i> | similar to thioredoxin-dependent hydroperoxide peroxidase | | 1.9* |
| Detoxification | <i>yxeK</i> | similar to monooxygenase | | -8.4** |
| Antibiotic production | <i>alba</i> | antilisterial bacteriocin (subtilosin) production | | -1.9** |
| Antibiotic production | <i>pksE</i> | involved in polyketide synthesis | | 1.9* |
| Antibiotic production | <i>pksG</i> | involved in polyketide synthesis | | -1.9* |
| Antibiotic production | <i>sboA</i> | subtilosin A | | -4.4** |
| Antibiotic production | <i>sboX</i> | bacteriocin-like product | | -5.5** |
| Phage-related functions | <i>xlyA</i> | N-acetylmuramoyl-L-alanine amidase | | -2.5* |
| Membrane organization | <i>yuaF</i> | NfedD2 membrane protein, associated with FloT | | 2.7** |
| Membrane organization | <i>yuaG</i> | FloT, similar to flotillin 1 | | 2.7*** |
| Similar to unknown proteins | <i>ybaF</i> | similar to ABC transporter (membrane protein) | | -2.7** |
| Similar to unknown proteins | <i>ybbA</i> | putative bacillibactin esterase | -1.9* | -1.8*** |
| Similar to unknown proteins | <i>ybyB</i> | general stress protein, survival of ethanol stress | | -2.2* |
| Similar to unknown proteins | <i>yczJ</i> | unknown | | 1.9* |
| Similar to unknown proteins | <i>yeeF</i> | unknown | | 2.0* |
| Similar to unknown proteins | <i>yjfD</i> | unknown | | 2.2* |

| | | | | | |
|-----------------------------|-------------|---|--------|---------|--------|
| Similar to unknown proteins | <i>yhjN</i> | unknown | | -1.8** | |
| Similar to unknown proteins | <i>ykuL</i> | unknown | | 2.3** | |
| Similar to unknown proteins | <i>ylnE</i> | probable siroheme ferrochelatase | | -2.2*** | |
| Similar to unknown proteins | <i>ylqD</i> | unknown | | -1.8*** | |
| Similar to unknown proteins | <i>ylxM</i> | unknown | | -2.3** | |
| Similar to unknown proteins | <i>ymxH</i> | unknown | | -2.1* | |
| Similar to unknown proteins | <i>yneK</i> | unknown | | | 1.9* |
| Similar to unknown proteins | <i>yrkH</i> | unknown | 2.6* | | |
| Similar to unknown proteins | <i>yrkR</i> | unknown | -2.1** | | |
| Similar to unknown proteins | <i>yrzE</i> | unknown | | | -1.9* |
| Similar to unknown proteins | <i>ytpB</i> | salt stress protein | 1.9* | | |
| Similar to unknown proteins | <i>yueB</i> | receptor for phage SPPI | 2.0** | | |
| Similar to unknown proteins | <i>yukB</i> | unknown | | 2.0*** | |
| Similar to unknown proteins | <i>yukC</i> | membrane-associated enzyme involved in bacteriocin production | | 2.4* | |
| Similar to unknown proteins | <i>yukD</i> | unknown | | 2.1** | |
| Similar to unknown proteins | <i>yurX</i> | synthesis of Fe-S-clusters | 2.5** | | |
| Similar to unknown proteins | <i>yusA</i> | methionine ABC transporter (binding lipoprotein) | 1.9* | | |
| Similar to unknown proteins | <i>yvfW</i> | lactate catabolic enzyme | | -2.2* | |
| Similar to unknown proteins | <i>yvlB</i> | unknown | 1.8* | | |
| Similar to unknown proteins | <i>yvqG</i> | maybe involved in resistance to bacitracin | | 2.1** | -2.3** |
| Similar to unknown proteins | <i>yvqH</i> | phage shock protein A; LiaH | | 4.3* | -2.9* |

| | | | | | |
|-----------------------------|-------------|---|-------|---------|-------|
| Similar to unknown proteins | <i>ywfE</i> | alanine-anticapsin ligase | | | 1.9* |
| Similar to unknown proteins | <i>ywpE</i> | unknown | 1.8* | | |
| Similar to unknown proteins | <i>ywsB</i> | Bacterial SH3 domain homologues; teichoic acids export protein | | 2.6** | |
| Similar to unknown proteins | <i>yxaA</i> | putative glycerate kinase | | 1.8** | |
| Similar to unknown proteins | <i>yxbB</i> | putative glycerate kinase | | 2.0* | |
| Similar to unknown proteins | <i>yxeL</i> | unknown | | -8.4* | |
| Similar to unknown proteins | <i>yxeQ</i> | unknown | | -5.2** | |
| Similar to unknown proteins | <i>yxiC</i> | unknown | | 1.8** | |
| Similar to unknown proteins | <i>yxiD</i> | unknown | | 2.0** | |
| Similar to unknown proteins | <i>yxiE</i> | unknown | | -2.1*** | |
| Similar to unknown proteins | <i>yxkO</i> | general stress protein, survival of ethanol stress | 2.6* | | |
| No similarity | <i>ycdA</i> | unknown | | 2.5*** | |
| No similarity | <i>yczI</i> | unknown | | 2.3** | |
| No similarity | <i>ydaK</i> | unknown | -1.9* | | |
| No similarity | <i>ydaN</i> | unknown | | -2.1** | |
| No similarity | <i>ydbN</i> | Fur-regulated basic protein, acts as RNA chaperone for <i>fsrA</i> , response to iron limitation | | -1.8* | |
| No similarity | <i>ydhK</i> | general stress protein, survival of ethanol stress | | 2.9* | |
| No similarity | <i>ydjA</i> | component of the BsuM DNA restriction system | | | 1.9* |
| No similarity | <i>ydjN</i> | unknown | | 2.1* | |
| No similarity | <i>yeaA</i> | unknown | | | -2.2* |

| | | | | | |
|---------------|-------------|---|-------|---------|-------|
| No similarity | <i>yeeB</i> | unknown | | -2.2* | |
| No similarity | <i>yfhL</i> | general stress protein, important for survival at low temperature (4°C) and during ethanol stress | 1.8* | | |
| No similarity | <i>yjfA</i> | unknown | | 2.2** | |
| No similarity | <i>yjfB</i> | unknown | | 2.7** | |
| No similarity | <i>yfmQ</i> | unknown | | -2.2* | |
| No similarity | <i>yhdS</i> | unknown | | 2.8*** | |
| No similarity | <i>yjfB</i> | unknown | | -1.8* | |
| No similarity | <i>yjhA</i> | unknown | | 2.3*** | |
| No similarity | <i>ykoF</i> | thiamine ABC transporter (binding protein) | | 1.9* | |
| No similarity | <i>ykoL</i> | unknown | 1.8* | | |
| No similarity | <i>yoeB</i> | inhibits in vitro activity of cell wall endopeptidases LytE and LytF | | -2.7* | |
| No similarity | <i>yokK</i> | unknown | | | 2.1* |
| No similarity | <i>yolA</i> | unknown | | -2.1*** | |
| No similarity | <i>yomN</i> | unknown | | -2.0* | |
| No similarity | <i>yonO</i> | unknown | | -2.6** | |
| No similarity | <i>yoqO</i> | unknown | -2.0* | | |
| No similarity | <i>yoqX</i> | unknown | | 1.9* | |
| No similarity | <i>yorH</i> | unknown | | -1.8** | |
| No similarity | <i>yqxI</i> | unknown | | | 2.1* |
| No similarity | <i>yqxJ</i> | unknown | | | 2.3** |

| | | | | | |
|---------------|-------------|---|-------|---------|-------|
| No similarity | <i>yrkG</i> | unknown | | 2.9* | |
| No similarity | <i>yrkN</i> | unknown | | -2.0* | |
| No similarity | <i>yrrT</i> | putative AdoMet-dependent methyltransferase | | -3.4*** | |
| No similarity | <i>ysbA</i> | murein hydrolase regulator LrgA | | 2.9** | |
| No similarity | <i>ysfE</i> | unknown | | 2.3** | |
| No similarity | <i>yukE</i> | unknown | | 2.1** | |
| No similarity | <i>yvcB</i> | unknown | | 2.8*** | |
| No similarity | <i>yvdJ</i> | unknown | | 1.9* | |
| No similarity | <i>yvqI</i> | permease | | 5.3*** | -2.1* |
| No similarity | <i>yvrI</i> | putative sigma factor (sigma70) | | 3.2* | |
| No similarity | <i>ywqB</i> | unknown | -2.0* | | |
| No similarity | <i>yxcD</i> | unknown | | -2.0** | |
| No similarity | <i>yxiF</i> | unknown | -1.9* | | |
| No similarity | <i>yxnB</i> | unknown | | 2.3* | |

**P*-value <0.2

***P*-value <0.05

****P*-value <0.01

Supplementary Table S2: Primers and plasmids used in this study

| Primer | Sequence (5' to 3') |
|---------------|---|
| 1 | TAAGGGCCCATGACAGAACAACGAAAAAGC |
| 2 | TAACTCGAGCGCTTCATACGTTTCATCG |
| 3 | TAAGCTAGCAAGGAGATTCCTAGGATGGATGAAAAAGCTATTTTAAGT GATGA |
| 4 | TAAGCATGCCTACATTAGAAGCAGAACACTGC |
| 5 | TAAGCTAGCAAGGAGATTCCTAGGATGGATTTGAATAAAGGTATTGAA |
| 6 | TAAGCATGCCTATACCTTTAATTCACCATTCTTCT |
| 7 | TAAGCTAGCATGTTGTCCAAAGTAAAAAAGTA |
| 8 | TAAGCATGCTCATCCTAACGCCTCCGTTATTC |
| tuaB_fw | CTGCTGCAGTCCAGTAAAGG |
| tuaB_rv | CCTGTTTACAAGACGGGATG |
| yueB_fw | CACGGAGAATACAGGAACCTC |
| yueB_rv | CCCTGTAGCCCATCACTTTG |
| phoP_fw | GTGCACATCAGCCATCTTCG |
| phoP_rv | GGCTCCTCCAGTTTATACCC |
| ccpA_fw | GAAGAGCAGGAGGAAACACC |
| ccpA_rv | GATCGGTTCTGCCATTGGTC |
| virB11_fw | TGAAGATACGCGGGAAGGAC |
| virB11_rv | TACCCCAGGAGAAGTAAGCC |
| mleN_fw | CGGTGGAATTGTTCGGTATGC |
| mleN_rv | CCGAGTTTCTCAAGCAGACC |
| ldh_fw | CCAAGGGATCACAGATGAGC |
| ldh_rv | CCTTTCCGTGGGGTAAATCC |
| ydhK_fw | CGTTACAACCCGGAGATCAG |
| ydhK_rv | GGAATCGATCTCGGCTGTTG |
| ybyB_fw | TCAGGTCTCGCTGTTTCAAC |
| ybyB_rv | GCTGTGAATGAATTCTCTTGCC |
| pbpE_fw | ATGGGACGGTAACTCTGTC |
| pbpE_rv | ACGCAGATTCCTTAGAAGCC |
| cheV_fw | CGTGGGTGAAAATGCTTTTCG |
| cheV_rv | TACATGCTGATGGGAGTGAG |
| sigD_fw | GAAGATTGGCTGCCCAGAAC |

| sigD_rv | CATCCTGTACCGTCATTCCG | |
|---------|---|-------------------------|
| motA_fw | GAAAAACGGGCTCAGCATGG | |
| motA_rv | CCTCCATTGCTTCGACTTCC | |
| rpsJ_fw | GCGGTGCACAAATACAAAG | |
| rpsJ_rv | TCGCATAAGAGCATCAACAG | |
| sigA_fw | GCAACTTCACCTTCTGACCAC | |
| sigA_rv | CCGAATCGAAGACGCAATAC | |
| Plasmid | genotype | reference |
| pSG1193 | <i>amp spec yfp 5`amyE 3`amyE</i> | Feucht and Lewis, 2001 |
| pDP150 | <i>amp spec mls</i> | Kearns and Losick, 2005 |
| pTCR06 | <i>amyE::P_{xyt}-yueB-yfp amp spec</i> | this study |
| pLH1 | <i>thrC::P_{hyspank}::cds7 amp spec mls</i> | this study |
| pLH2 | <i>thrC::P_{hyspank}::cds8 amp spec mls</i> | this study |
| pWG8 | <i>thrC::P_{hyspank}::rap_{LS20} amp spec mls</i> | this study |

References

Feucht, A., & Lewis, P. J. (2001). Improved plasmid vectors for the production of multiple fluorescent protein fusions in *Bacillus subtilis*. *Gene*, 264(2), 289–297.

Kearns, D. B. (2005). Cell population heterogeneity during growth of *Bacillus subtilis*. *Genes & Development*, 19(24), 3083–3094. doi:10.1101/gad.1373905