Supplemental Material for:

Cellulose as an architectural element in spatially structured *Escherichia coli* biofilms

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Contents:

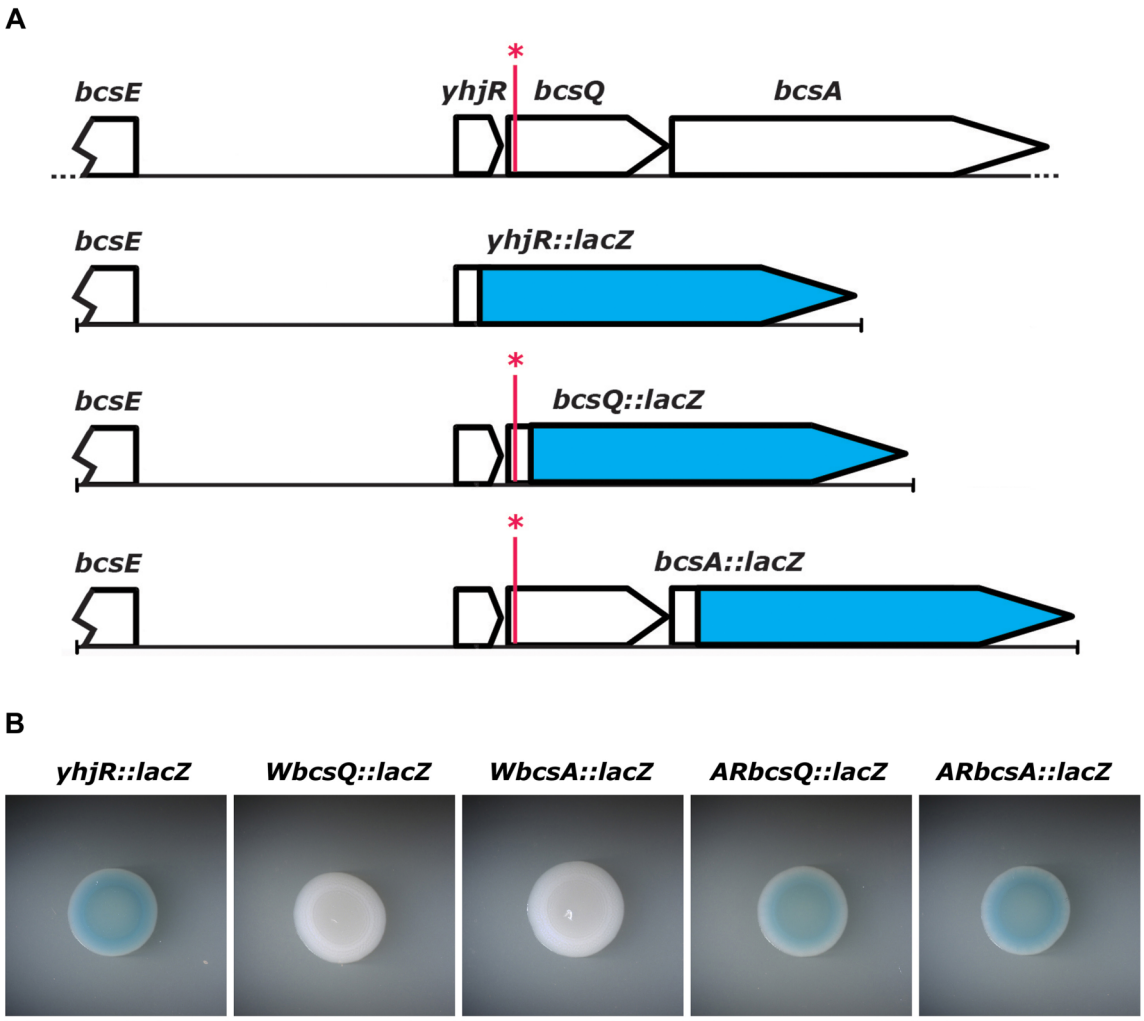

Figure S1. Construction of single copy *lacZ* reporter fusions to *yhiR*, *bcsQ* and *bcsA* and β-galactosidase activities in colonies of fusion-carrying strains. A: The latter two fusions were constructed with TAG ('W' fusions) or TTC ('AR' fusions) in codon 6 of *bcsQ* (position indicated by a red asterisk). Lengths of genes and intergenic regions are not drawn to scale. **B:** Different β-galactosidase activities conferred by these *lacZ* fusions integrated into the chromosome of strain W3110 are shown qualitatively in colonies grown for 2 days on salt-free LB containing the indicator XG.

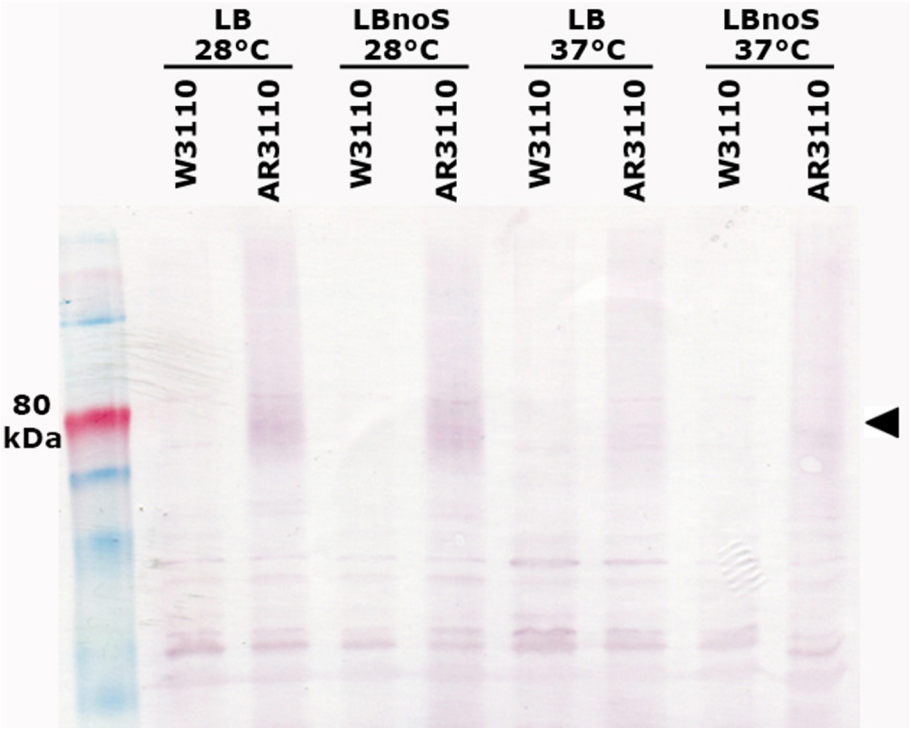

Figure S2. FLAG-tagging reveals the expression and temperature regulation of cellulose synthase (BcsA) in strain AR3110 but not in W3110. W3110 and AR3110 carrying FLAG-tagged *bcsA* in the chromosome were grown in LB or salt-free LB at 28ºC or 37ºC as indicated and FLAG-tagged proteins were detected in colonies growing for four days at 28 °C (40 μg total protein per lane) by immunoblot analysis using an anti-FLAG serum. Note that as an overall hydrophobic membrane protein, BcsA runs as a diffuse band and more rapidly than expected for its actually molecular mass (99.8 kDa).

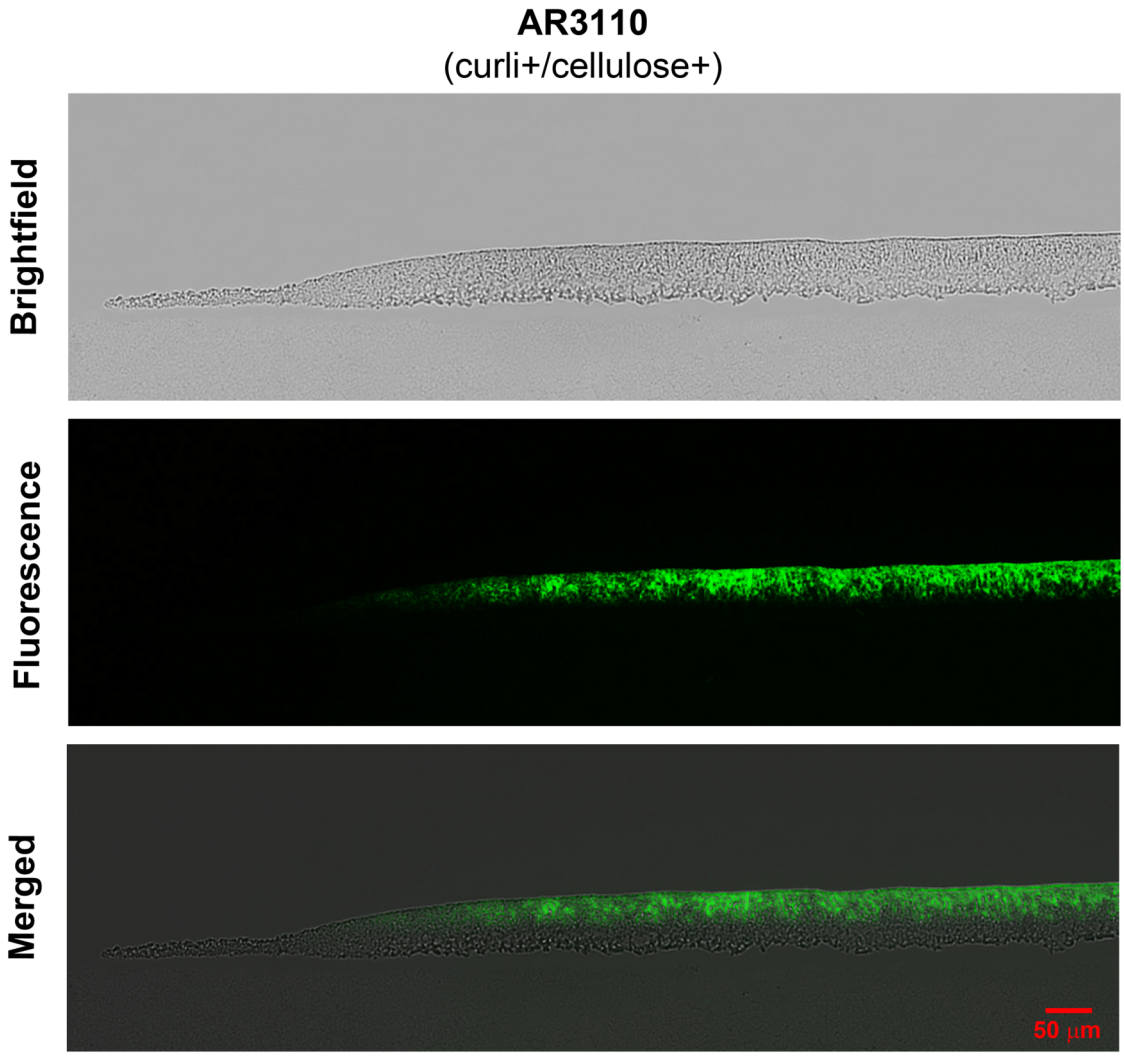

Figure S3. Cryosection through the outer growth zone of a 5-day-old macrocolony of strain AR3110 grown in the presence of thioflavine S. A thin section (5 μm) of a representative AR3110 macrocolony was visualized at low magnification by brightfield and fluorescence microscopy, the latter was false-colored green for TS and the two images were merged. Brightfield in the merged image appears on a dark grey background to better visualize the location of the fluorescence.

Table supplement 1. Oligonucleotides used in this study.

I. Primers for 2-step mutagenesis of *bcsQ* SNP[1;2]

Primer	Sequence
BcsQ HP1 pKD54	5'-CTGCCTGATCCCGCGATAGGCTATATCTTCCAGAATGATATT GTGGCGTT**TCAGA AGAACTCGTCAAGAAG** -3'
bcsQ HP2 pKD45	5'-TAGCGCAACCCAGCGTCACGCCAGTCCTGGCCATCCAGCA TCGCTCTGGCC**GGA TATTATCGTGAGGATG** -3'
bcsQ mut1 rev	5'-CTGGGATTGCAGGGGG -3'
bcsQ mut2 fw	5'-CCCTGCAATCCCAGTACG -3'

[1]Cat/Kan cassette-specific sequences are shown in **boldface.**
[2]mutation introduced at the natural SNP position is underlined

II. Primers for generating knockout mutations by one-step inactivation[1]

ΔbcsQ::cat	5'- TGGCCGTACTGGGATTGCAGGGGGTGCGGGGAGGCGTGG GGACAACAACC**GTGTAGGCTGGAGCTGCTTC** -3' 5'- TGGTGGGTTATCTGCGAGGCATCACGCGGTAAGTCGATTA AAATCCACTG**CATATGAATATCCTCCTTAG** -3'
ΔbcsEFG::kan	5'- GATAAGTTTTAATTTCAATGGTAGGTTTATTTCTTAGCTTTC GCTAG**GTGTAGGCTGGAGCTGCTTC**-3' 5'-TTACTGCGGGTAAGGCACCCAGTCGCCGCCGTTCAGGCGA ACGTACGG**ATTCCGGGGATCCGTCGACC** -3'
ΔyhjR::cat	5'- ATGAATAACAATGAACCAGATACTCTGCCTGATCCCGCGATAG GCTATAT**GTGTAGGCTGGAGCTGCTTC** -3' 5'- CTACTTTTGTTGCGCAAACTCTGCCAGCAACGGCCAGCGTTTT AATGCCG**CATATGAATATCCTCCTTAG** -3'

[1]Cat/Kan cassette-specific sequences are shown in **boldface.**

III. Primers for generating *lacZ* reporter fusions[3]

yhjR EcoRI	5'-GCTCAG**GAATTC**TGATTCGCCAGACTGATAGC -3'
yhjR HindIII	5'-GC**AAGCTT**GCAGAGTATCTGGTTCATT -3'
bcsQ HindIII	5'-GC**AAGCTT**GATGGTTGTTGTCCCCACGCC -3'
bcsA HindIII	5'-GC**AAGCTT**GGATAAGCAACCACCGGGTCAG -3'

[3]Restrictions sites are shown in **boldface.**

IV. Primers used for C-terminally 3xFLAG-tagging of BcsA

bcsA H1Flag	5'-CGGCACAACCATCGGATCAGGCTTTGGCTCAACAA -3'
bcsA H2Flag	5'-AAATCCAGAATAGTTTTCTTTTCATCGCGTTATCA -3'