## Supplementary material

**Table S1.** Summary results of fluorescence binding analysis obtained with twelve different lectins in *A. thiooxidans* wild type cells.

| Lectin | Specificity[a] | *A. thiooxidans*$^T$ |
|---|---|---|
| **AAL** | L-Fucose α(1,6) N-Acetyl-D-Glucosamine | + |
| **BPA** | N-Acetyl-D-Galactosamine | + |
| **ConA** | Internal D-Mannose and D-Glucose | + |
| **DBA** | Terminal α-N-Acetyl-D-Galactosamine | - |
| **GS-I** | α-D-Galactose | - |
| **GS-II** | N-Acetyl-D-Glucosamine | + |
| **LPA** | N-acetylneuraminic acid | - |
| **MPA** | α-D-Galactose | - |
| **PNA** | Galactoseβ(1,4)Glucose | - |
| **SBA** | Galactose | - |
| **UEA-I** | Linked α(1,2) L-Fucose | - |
| **WGA** | GlcNacβ(1,4) GlcNacβ(1,4) GlcNac | - |

[a], according to the manufacturer´s information (EY Laboratories®, San Mateo, CA, USA).

**Table S2.** Primers used in this work.

| Name | Sequence 5′–3′ [1] |
|---|---|
| *pelA1*_F | CCGATTGCCGCAGTTATTTATT |
| *pelA1*_R | GCTGTCTTGATGGCTTTGATG |
| *pelD*_F | CACAAGTTGGCATCCTGGTTCGTT |
| *pelD*_R | CATGCTGCCTGCGAAAGGTAACAA |
| *wgcA*_F | GAACTTGTCAATGCGCCATC |
| *wgcA*_R | GGCCAGCAATAAATCCTGAATAC |
| *flaA*_F | CTGGTCACGGCCATCAATAA |
| *flaA*_R | CAAAGTCCCGCCAGATGTAATA |
| *16S*-F3 | ATGGCCTTTATGTCCAGGGCTACA |
| *16S*-R3 | AATCCGAACTACGACGCGCTTTCT |
| *map*_F | GGACCGGATTTGTCACGATTA |
| *map*_R | GACGTGGTTGAGGGAAATACA |

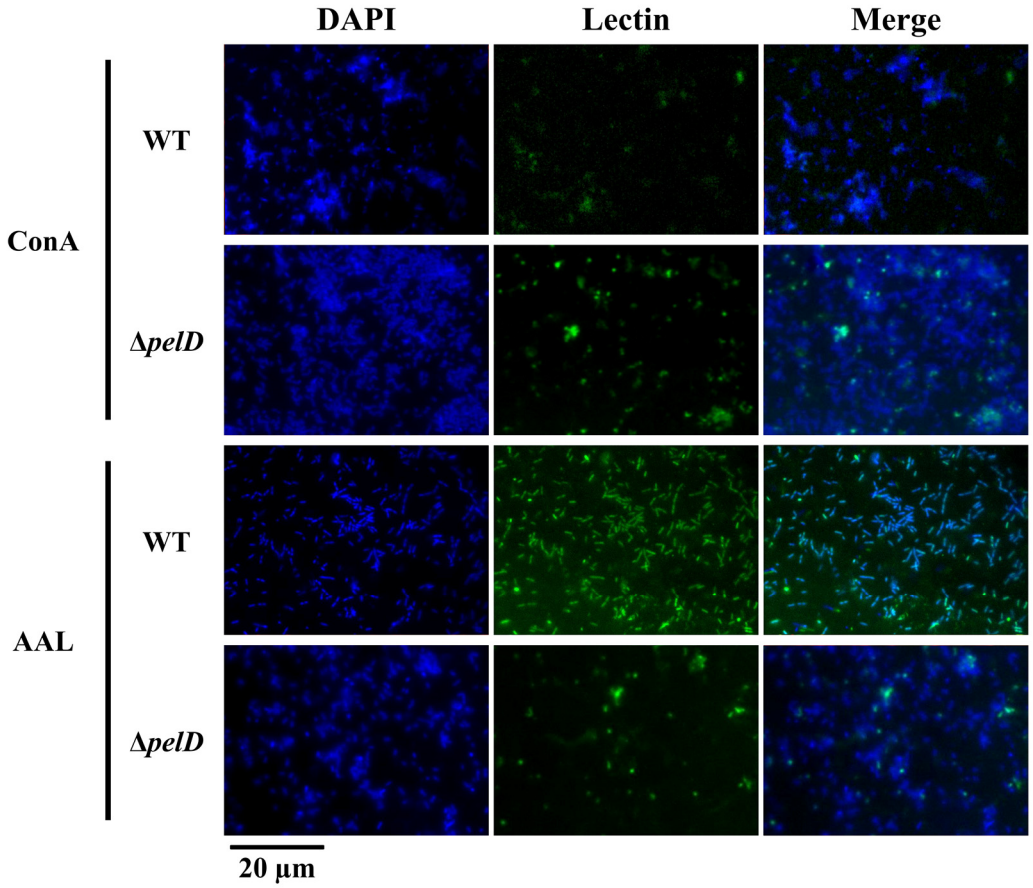


**Figure S1.** Analysis of PEL exopolysaccharide glycoconjugate composition by using epifluorescence microscopy coupled to FLBA. S⁰-coupons colonized by *A. thiooxidans*$^{T}$ (WT) or mutant derived (Δ*pelD*) cells were extracted from 5-days growth cultures and incubated with FITC-conjugated AAL or ConA lectins. Then, they were stained with DAPI before microscopy imaging. Size bars represent 20 µm.

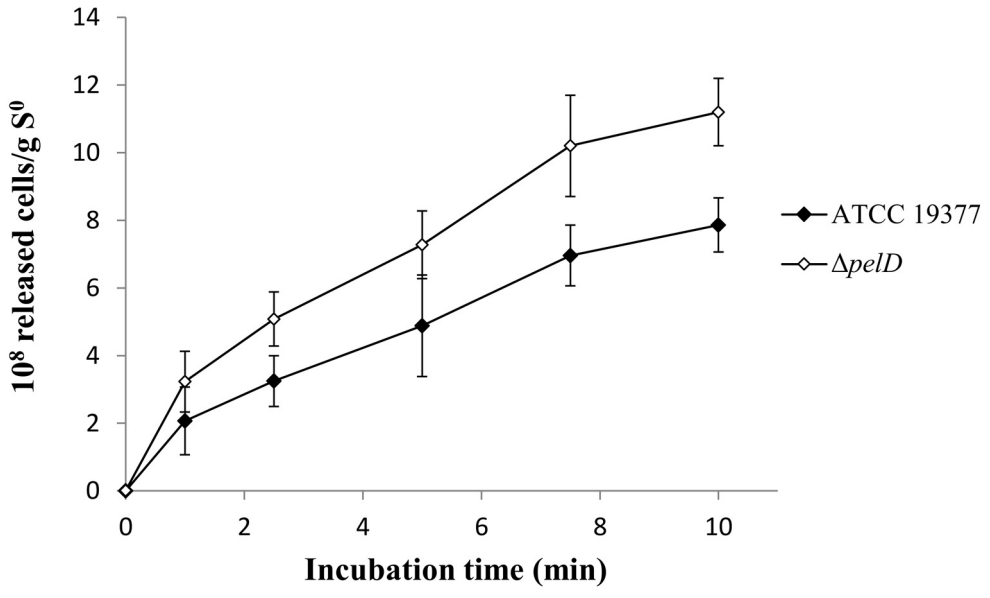


**Figure S2.** The loss of PEL exopolysaccharide contributes to the production of a fragile biofilm in *A.*

*thiooxidans*. Inoculated S$^0$-coupons were extracted from 5-days growth cultures and treated with 0.05 % Triton X-100. Then they were vortexed during 10 min. Number of cells released from the wild type and Δ*pelD* null-mutant biofilms subjected to the mechanical stress was determined with a Petroff-Hausser counting chamber and normalized against mass of sulfur. Standard deviations were calculated from three independent cultures.

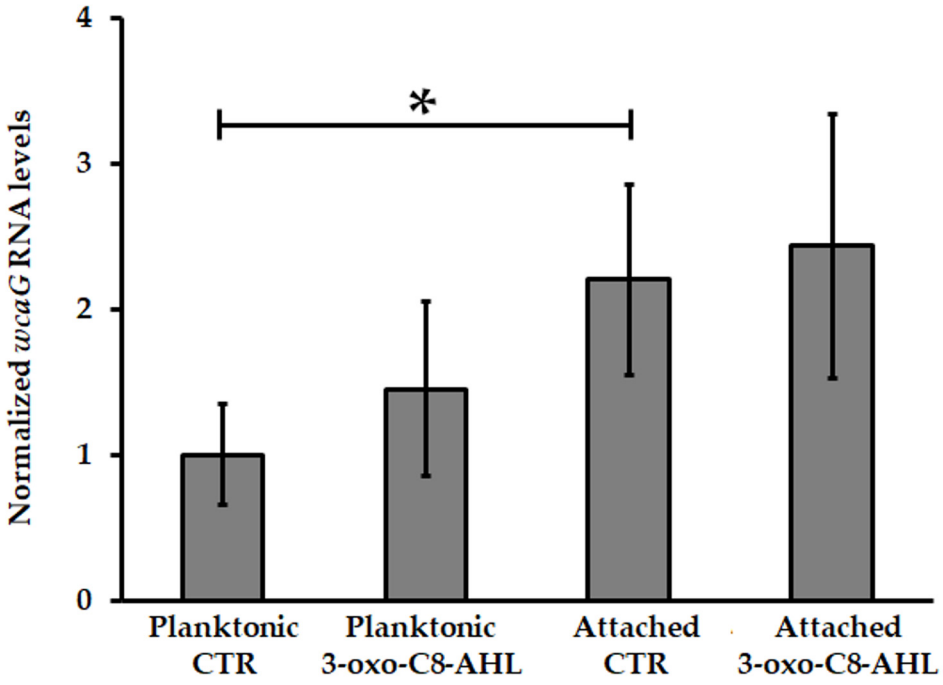


**Figure S3.** Addition of QS molecule 3-oxo-C8-AHL has no significant effect on transcription levels of *wcaG* from *A. thiooxidans*$^T$. Total RNAs were extracted from *A. thiooxidans* WT cells obtained from 5-days growth cultures. Transcript levels of *wcaG* were measured by qPCR and then normalized using DNA *16S* and *map* genes. Values represent the average of 4 independent experiments ± standard deviation. Significant differences calculated by a one-way ANOVA test ($p < 0.05$) are noted (*). CTR, DMSO 0.01 % without AHL.

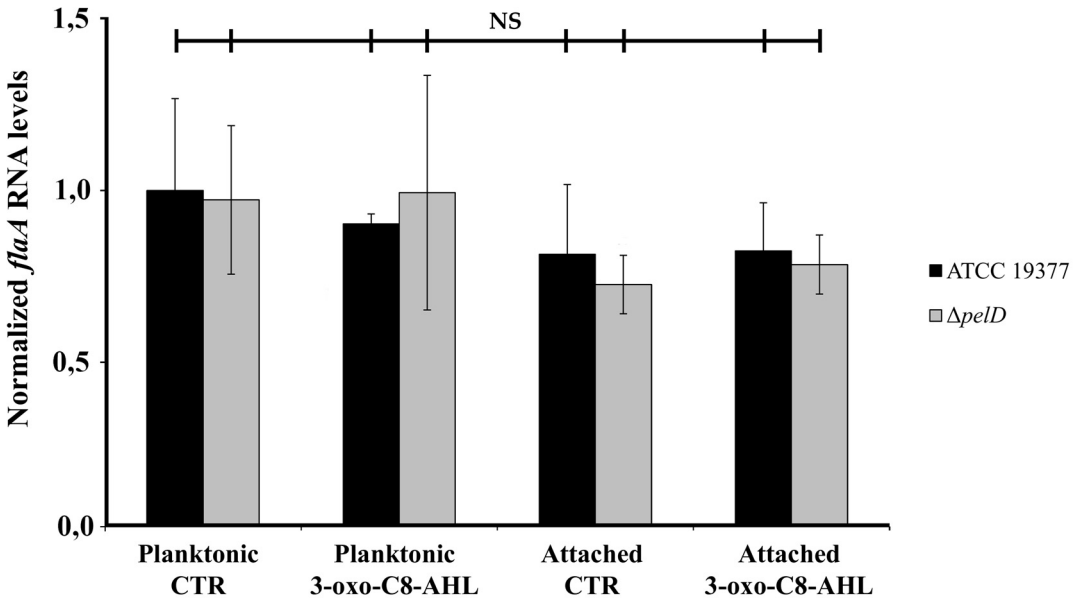


**Figure S4.** Transcriptional analysis of *flaA* gene from *A. thiooxidans*$^{T}$. Total RNAs were extracted from *A. thiooxidans* cells obtained from 5-days growth cultures. Transcript levels were measured by qPCR and then normalized using DNA *16S* and *map* genes. Values represent the average of 4 independent experiments ± standard deviation. **NS**, None significant differences were observed by a one-way ANOVA test ($p < 0.05$). CTR, DMSO 0.01 % without AHL.

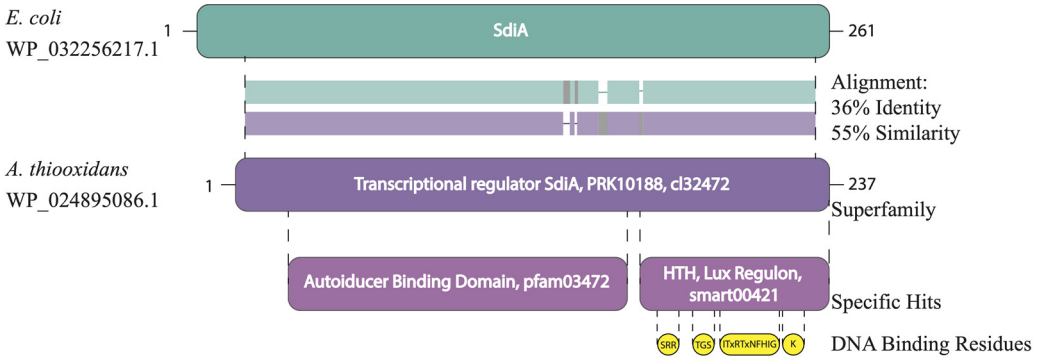


**Figure S5.** Bioinformatic characterization of WP_024895086.1, a SdiA-like protein present in the new available genome sequences of *A. thiooxidans*. (Top) Schematic representation of SdiA (green) and WP_024895086.1 (purple) amino acid sequence alignment. As noted, both proteins share high similarity and strong identity along over 88% of coverage. Few identified gaps are shown as lines without background color. (Bottom) Domain structure of WP_024895086.1 protein shows the distinctive two domains of canonical QS regulators: Autoinducer Binding Domain (pfam03472) and Helix-Turn-Helix Lux Domain (smart00421), whose combination is classified as PRK10188 Superfamily. Amino acids responsible for DNA binding are depicted in yellow.