Table S1 Primers used in this study.

Primer	Reference	Sequence 5'-3'	Tm (°C)	Target site
610IIF	a	GTG CCA GCA GCC GCG GT	57	569 on16S
609F	-	GGA TTA GAT ACC CBD GTA	50 - 54	840 on 16S
1390F	b	TTG TAC ACA CCG CCC GTC	53	1390-1407 on 16S
606F	-	ACC GCC CGT MRC C	48-52	1385 on 16S
606Fa	this study	ACC GCC CGT CAC A	44	1385 on 16S
630R	c	CAK AAA GGA GGT GAT CC	50 - 52	1529 on 16S
1035R	d	TTC GCT CGC CRC TAC	48 - 50	242 on 23S

[a] **Ustinova I, Krienitz L, Huss VAR.** 2001. *Closteriopsis acicularis* (G. M. Smith) Belcher *et S*wale is a fusiform alga closely related to *Chlorella kessleri* Fott *et* Nováková (Chlorophyta, Trebouxiophyceae). Eur. J.Phycol. **36:** 341-351.

[b] **Zheng DD, Alm EW, Stahl DA, Raskin L.** 1996. Characterization of universal small-subunit rRNA hybridization probes for quantitative molecular microbial ecology studies. Appl. Environ. Microbiol. **62:** 4504-4513.

[c] **Juretschko S, Timmermann G, Schmid M, Schleifer KH, Pommerening-Roser A, Koops HP & Wagner M.** 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. Appl. Environ. Microbiol. **64:** 3042-3051.

[d] **Ludwig W, Kirchhof G, Klugbauer N, Weizenegger M, Betzl D, Ehrmann M, Hertel C, Jilg S, Tatzel R, Zitzelsberger H, Liebl S, Hochberger M, Shah J, Lane D, Wallnofe, PR & Scheifer KH.** (1992). Complete 23S ribosomal-RNA sequences of Gram-positive bacteria with a low DNA G+C content. Syst. Appl. Microbiol. **15:** 487-501.

Description of the purpose-built CS_2 distribution system

A CS_2 distribution system was developed to continuously flush the headspaces of the bottles and jars. In this system N_2 was led through a jar filled one third with liquid CS_2 that was kept at 15°C. The resulting CS_2 saturated nitrogen gas was mixed with air in a mixing vessel (2.5 l) that was kept under constant pressure by a back pressure valve. To obtain the desired CS_2 concentration, the mixing ratio of air with CS_2 saturated nitrogen gas was controlled by mass flow controllers (Brooks instrument BV, Ede, The Netherlands). The CS_2 containing gas from the mixing vessel was split into 15 individual gas lines of which the flow could be individually controlled by Sho-Rate Purgemeters (Brooks instrument BV, Ede, The Netherlands).

Growth characteristics on plate and in liquid culture

Distinctly different colony morphologies were observed between the 16 isolated strains. Five strains (BBW1, BAW3, G8, S1p and BDW2) formed large dry, white colonies on Gelrite plates and white or yellow aggregates in stationary liquid culture. Strains G8, BAW3, BDW2 and BBW1 spread out over the Gelrite plates, ultimately covering the plates completely (Fig. S1A). Colonies of strain S1p remained compact. The white or yellow aggregates and whiteness of the colonies was not a result of more sulfur production by these strains. The other 11 strains formed smooth, shiny, compact colonies (Fig. S2A) and showed usually less aggregation in stationary liquid culture. The organisms that produced the large dry and white colonies are mainly present in cluster 1 and 4 of the phylogenetic tree constructed from the 16S-ISR sequences (Fig. 1).

There was a clear difference in the colony surface of the two types of strains when visualized by cryoSEM: those of the ‘white dry’ colonies were highly irregular, with valleys and peaks consisting of clumps of aligned cells. On central parts of colonies crystals were sometimes visible (Fig. S1B). The edges of the spread-out areas of the ‘white dry’ strains appeared to consist of a monolayer of cells, without crystals (Fig. S1C). The surfaces of ‘compact shiny’ colonies were smooth with defined holes and they were covered with a layer, preventing distinction of individual cells (Fig S2B-D). This is likely to be an extracellular polymeric substance (EPS), which usually consists of polysaccharides. Our results suggest that at least on solid media, the compactly growing strains produce more EPS than the spreading strains.

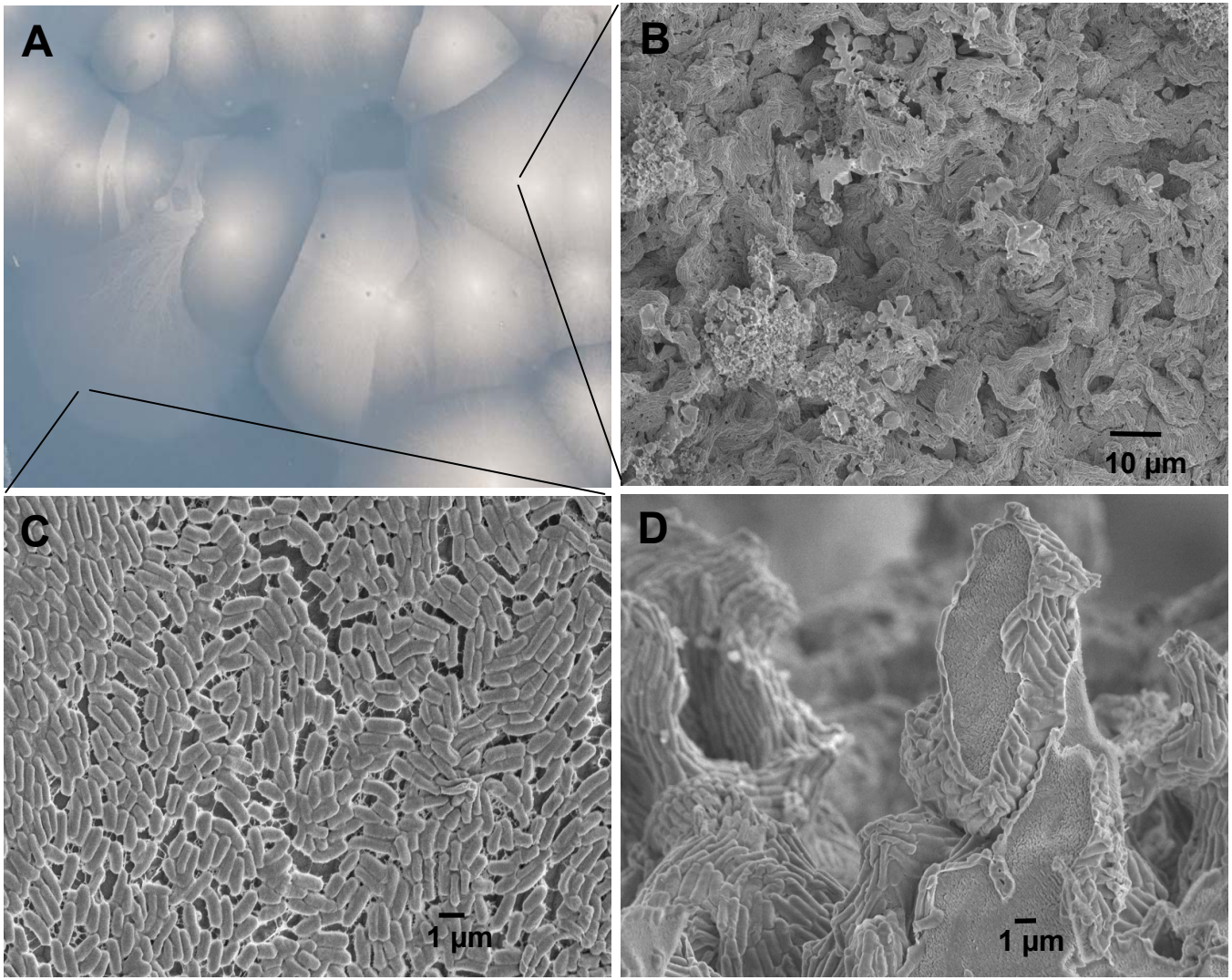

FIG S1 Colony morphology on a Gelrite plate of strain G8 by photography (A) and cryoSEM (B-D). A) Large, white spread-out colonies. B) Surface of the centre of one of the colonies. C) Surface of the spread-out edge of one of the colonies. D) Cross-section through a central part of the colony.

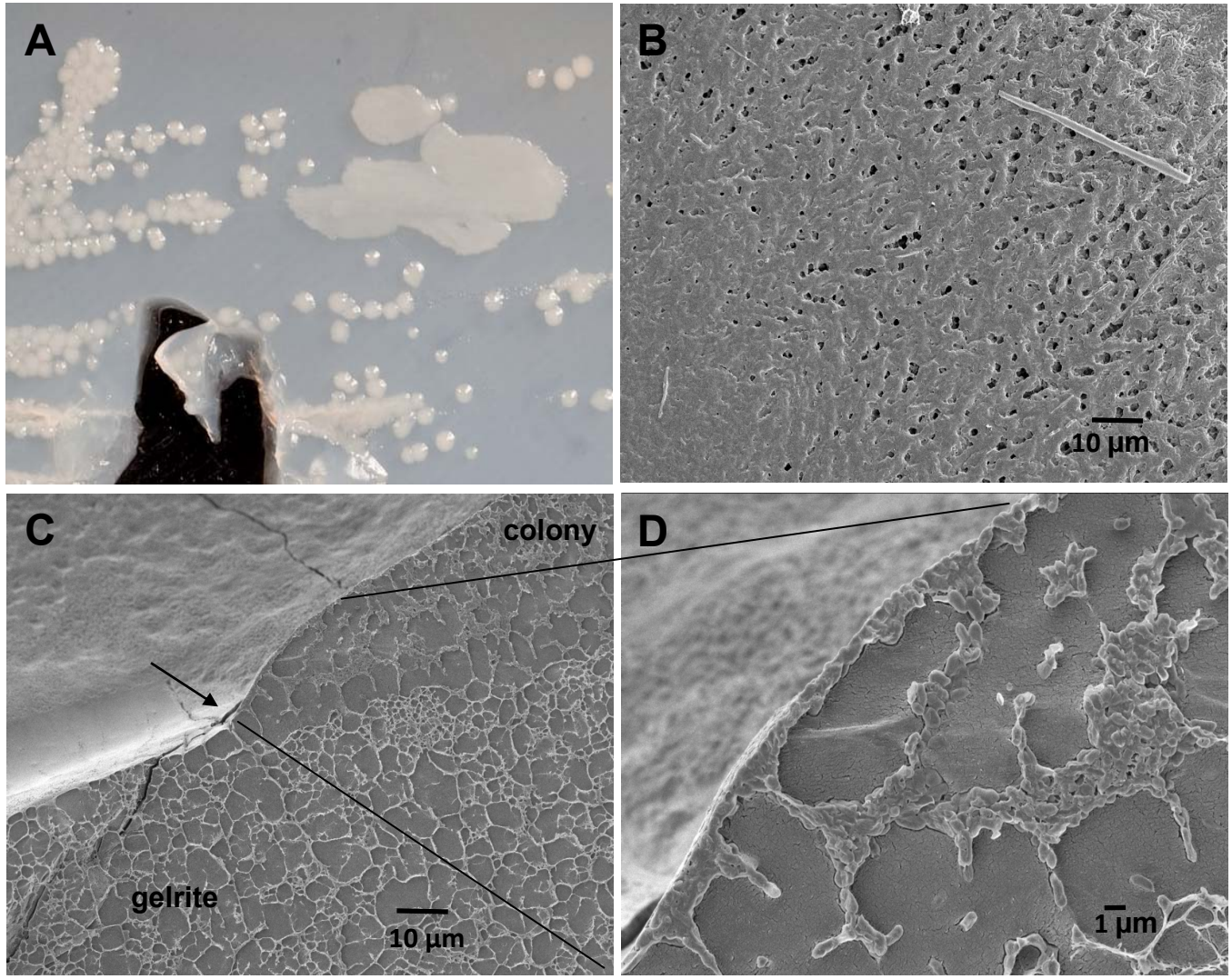

FIG S2 Colony morphology on a Gelrite plate of strain 2Bp by photography (A) and cryoSEM (B-D). A) Small, shiny, smooth colonies. The hole indicates a spot where colonies were excised for cryoSEM. B) Surface of one of the colonies showing a smooth surface with gaps. C) Cross-section through a colony and underlying Gelrite. The arrow indicates the start of the edge of the colony. D) Enlargement of cross-section showing the top of the colony and the clusters of cells in the colony.

Table S2 16S-23S ISR length of the isolated CS_2 converting *A.thiooxidans* strains and the number of base pairs (bp) that differ from strain G8, which is used as reference strain. Isolate BDW2 from the Oy Visko reactor had an exceptionally small ISR of 439 bp. This is characteristic of *A. ferrooxidans* ISRs as proposed by Ni *et al*. (2007, 2008) and it therefore forms an exception to their grouping method.

Strain	Total	ISR1	tRNA Ile	ISR 2	tRNA Ala	ISR 3	nr. different bp	GenBank Acc.
G8	458	46	77	3	77	255		KC902819
Sts 4-3	458	46	77	3	77	255	1	KC902820
1Bp	460	48	77	3	77	255	2 + 2 insertions	KC902818
2Ap	458	46	77	3	77	255	2	KC902816
2Bp	458	46	77	3	77	255	2	KC902817
BEF1	458	46	77	3	77	255	4	KC902823
BEF3	458	46	77	3	77	255	4	KC902829
BAD2	458	46	77	3	77	255	1	KC902821
BBF2	459	47	77	3	77	255	4 + 1 insertion	KC902827
BED2	458	46	77	3	77	255	2	KC902828
S1p	456	46	77	3	77	253	6 + 2 deletions	KC902824
BC6-1	456	46	77	3	77	253	7 + 2 deletions	KC902831
BDW2	439	43	77	3	76	240	18 + 19 deletions	KC902822
BAW3	458	45	77	3	77	256	57 + 3 deletions + 3 insertions	KC902825
BBW1	458	45	77	3	77	256	57 + 3 deletions + 3 insertions	KC902826

Ni YQ, Yang Y, Bao JT, He KY, Li HY. 2007. Inter- and intraspecific genomic variability of the 16S-23S intergenic spacer regions (ISR) in representatives of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*. FEMS Microbiol. Lett. **270:** 58-66.

Ni Y, Wan D, He K. 2008. 6S rDNA and 16S-23S internal transcribed spacer sequence analyses reveal inter- and intraspecific *Acidithibacillus* phylogeny. Microbiology-SGM. **154:** 2397-2407.

Table S3 Comparison of protein, carbon and sulfur yields of CS_2 converting *A. thiooxidans* strains growing in continuous culture with CS_2 as the sole energy source. Measurements were performed over a period of 40 days and values are average ± SEM for 6 to 10 replicates. For strain BBW1, only one optical density measurement was done and yield was not determined (n.d.).

	Yield (g·mole CS_2^{-1} ± SEM)			
Strain	**OD_{600}**	**Protein**	**Carbon**	**Sulfur**
2Bp	0.279 ± 0.015	13.1 ± 1.6	5.4 ± 0.3	1.4 ± 0.3
Sts4-3	0.205 ± 0.006	11.6 ± 1.2	4.6 ± 0.1	0.4 ± 0.2
S1p	0.233 ± 0.012	12.9 ± 1.4	4.7 ± 0.2	1.7 ± 0.4
G8	0.261 ± 0.011	11.7 ± 1.4	4.4 ± 0.2	2.2 ± 0.6
BBW1	0.238	n.d.	n.d.	n.d.

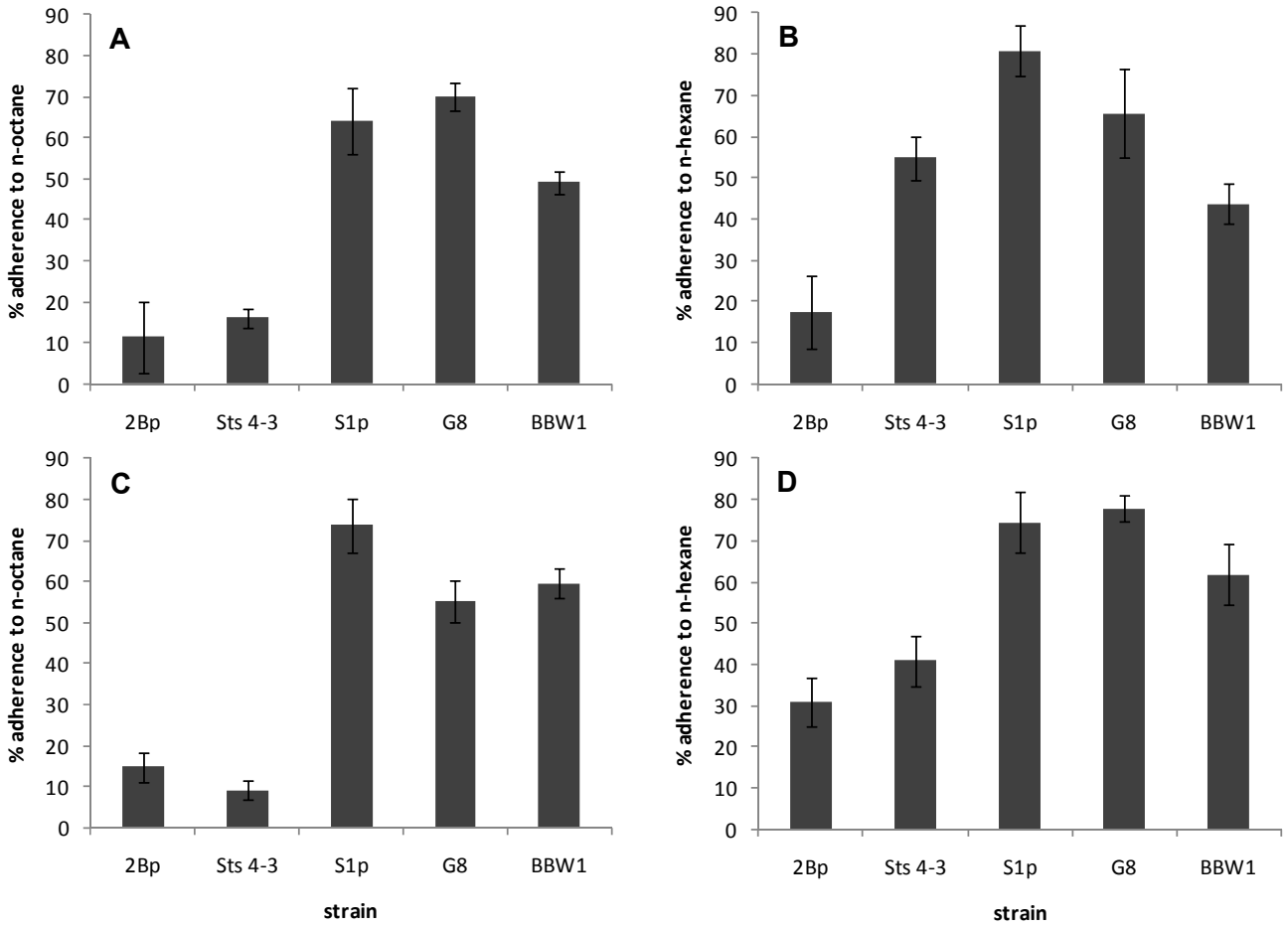

FIG S3 Cell surface hydrophobicity of five CS_2 converting *A. thiooxidans* strains at pH 7 (A, B) and pH 3 (C, D). Values are means ± SEM from three (pH 3) or four (pH 7) independent experiments. % adherence was calculated as (1-At/A0)×100, with A0: absorbance of the culture before mixing with solvent and At: absorbance of the aqueous phase after mixing and 20 min settling. Absorbance was measured at 400 nm.

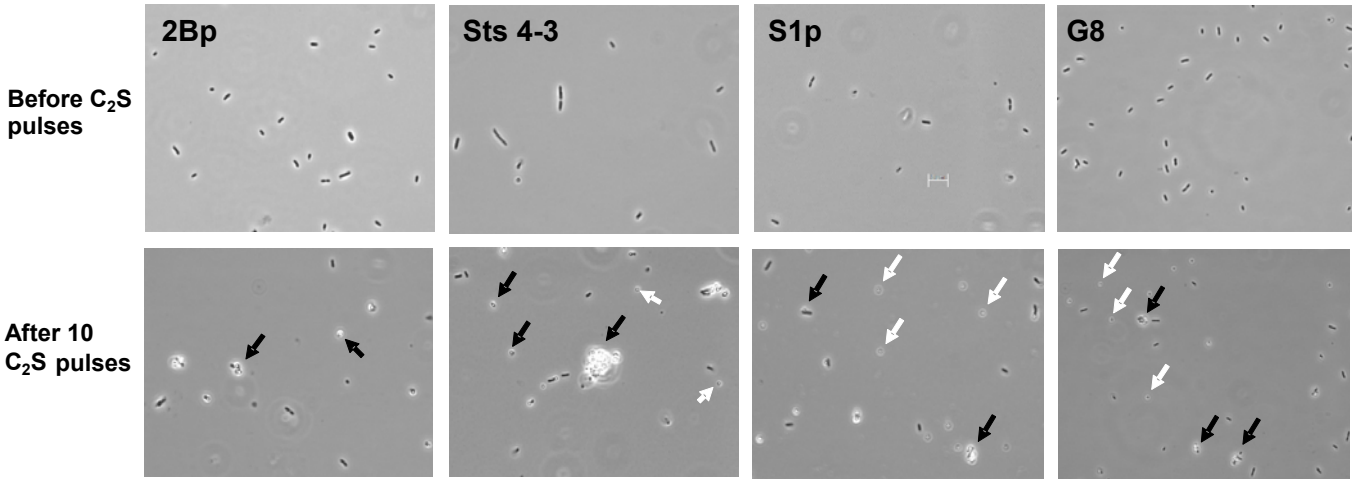

FIG S4 Microscopic examination of S^0 accumulation in *A. thiooxidans* strains 2Bp, Sts 4-3, S1p and G8 before (top row) and after 10 CS_2 pulses (bottom row). Examples of detached S^0 globules are indicated with white arrows and cell-attached S^0 globules with black arrows.