Electronic Supplementary Information

Unassisted stimulation of autotrophic ethanol bioproduction by visible light

Authors

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Text S1. Differential gene expression for stress response mechanisms

The transcriptomic analysis of autotrophic *C. autoethanogenum* under visible light also indicates changes in DNA metabolism, cell wall and glycerophospholipid dynamics, exopolysaccharide (EPS) synthesis, and vitamin B12 metabolism. All of the above have been shown in previous studies to be common bacterial responses to stresses such as atmospheric pollution, solvents, radiation, and antibiotics.[1-3] Genes involved in membrane maintenance are possibly participating in the reparation of cell wall structures damaged by visible light. EPS has been shown to protect microbial cells against desiccation, temperature variations, and acid shift.[4,5] Here, transcriptomic data suggest a higher quantity of EPS that could diminish the fraction of visible light reaching the surface of *C. autoethanogenum*'s cell wall. Vitamin B12, an essential cofactor for acetogen metabolism, and its derivatives are light-sensitive molecules susceptible to photodegradation.[6,7] By upregulating vitamin B12-related synthesis genes, the bacterium possibly increases its capacity to replace damaged cofactors.

Text S2. Protein abbreviations for Figure 5

Ack: acetate kinase, ACS: acetyl-CoA synthase, ADH: alcohol dehydrogenase, AdhE: bifunctional acetaldehyde-CoA/alcohol dehydrogenase, Ald: acetaldehyde dehydrogenase, AlsS: acetolactate synthase, AOR: aldehyde:Fd oxidoreductase, Bdh: 2,3-butanediol dehydrogenase, BudA: acetolactate decarboxylase, CODH: carbon monoxide dehydrogenase, Fdh: formate dehydrogenase, FolD: methenyl-THF dehydrogenase, MetF: methenyl-THF reductase, Nfn: NADH-dependent reduced Fd:$NADP^+$ oxidoreductase,

LDH: lactate dehydrogenase, PFOR: pyruvate:Fd oxidoreductase, Pta: phosphotransacetylase, HytA-E: electron bifurcating FeFe-hydrogenase cluster, Rnf: Fd-NAD:oxidoreductase complex.

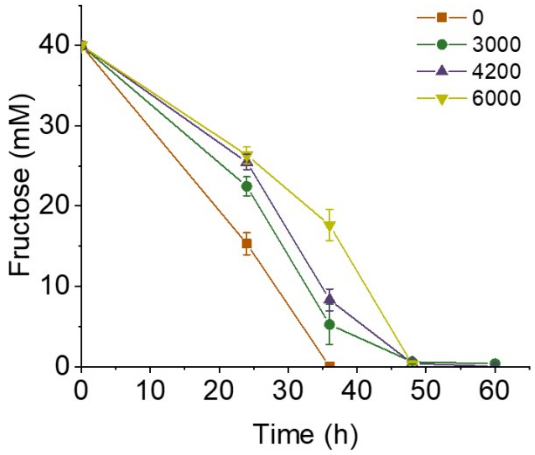

Fig. S1 Fructose consumption by *C. autoethanogenum* during heterotrophic growth under visible light at different intensities. Each curve is the average of at least triplicate with standard deviation. Light intensity is in lux.

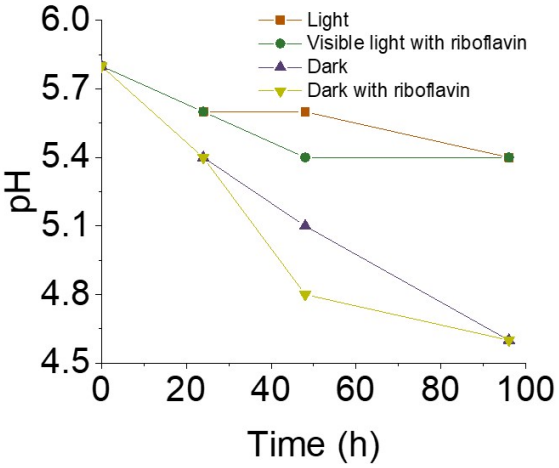

Fig. S2 Extracellular pH when *C. autoethanogenum* was grown with H_2:CO:CO_2 in the dark or under visible at 4200 lux with 1 μM exogenous riboflavin.

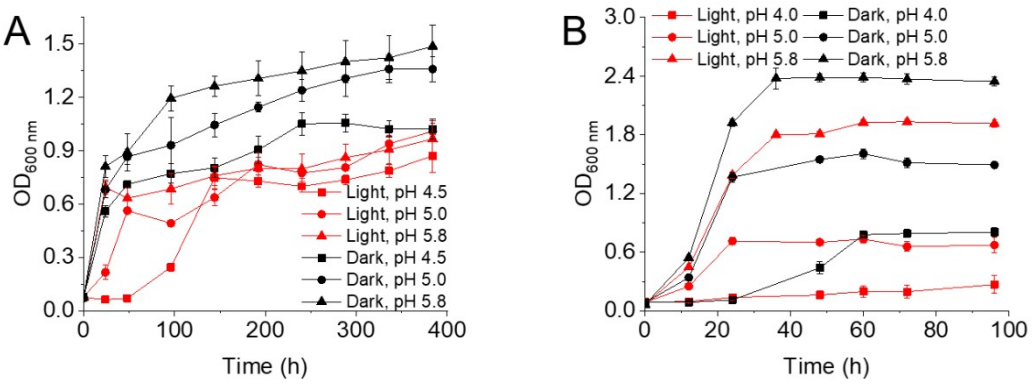

Fig. S3 Synergistic impact of visible light and initial acidic pH on the growth of *C. autoethanogenum*. $OD600_{nm}$ over time of (E) autotrophic and (F) heterotrophic growth of *C. autoethanogenum* at different initial pH values under white light at 4200 lux. In panels BD, * indicates p-values ≤ 0.05. Each curve and bar are the averages of at least triplicate with standard deviation.

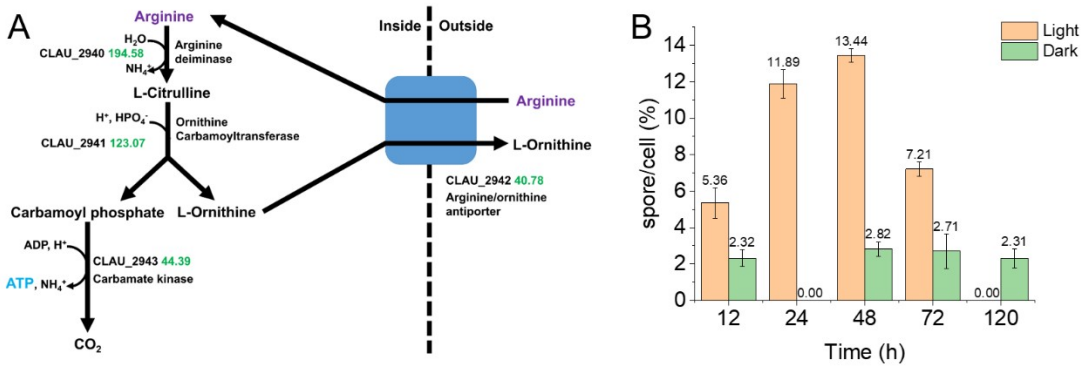

Fig. S4 Visible light stress response mechanisms in *C. autoethanogenum*. (A) Differential gene expression in the arginine deiminase pathway. Values in green indicate upregulated genes when autotrophic *C. autoethanogenum* is exposed to light at 4200 lux. (B) Sporulation in the dark and under visible light. Each bar is the average of at least triplicate with standard deviation.

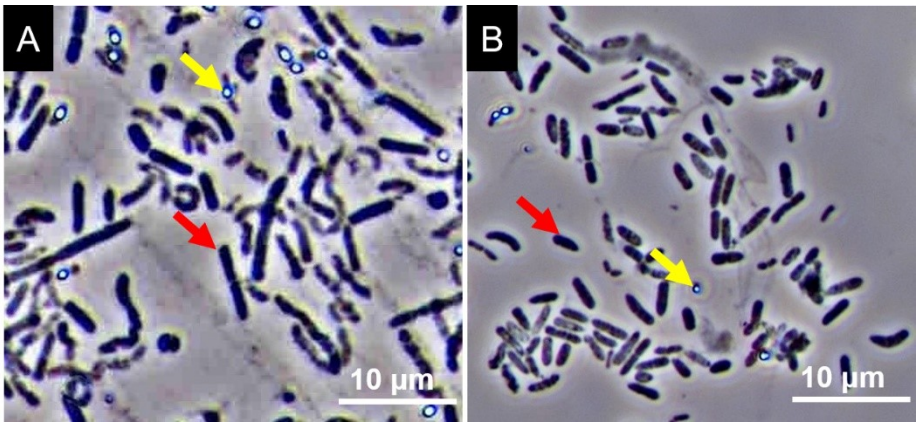

Fig. S5 Micrographs of sporulating *C. autoethanogenum* grown by gas fermentation with H_2:CO:CO_2. (A) Visible light (4200 lux) and (B) dark growth conditions. The micrographs were taken after 48 hours of cultivation. Red arrows indicate vegetative cells and yellow arrows indicate spores.

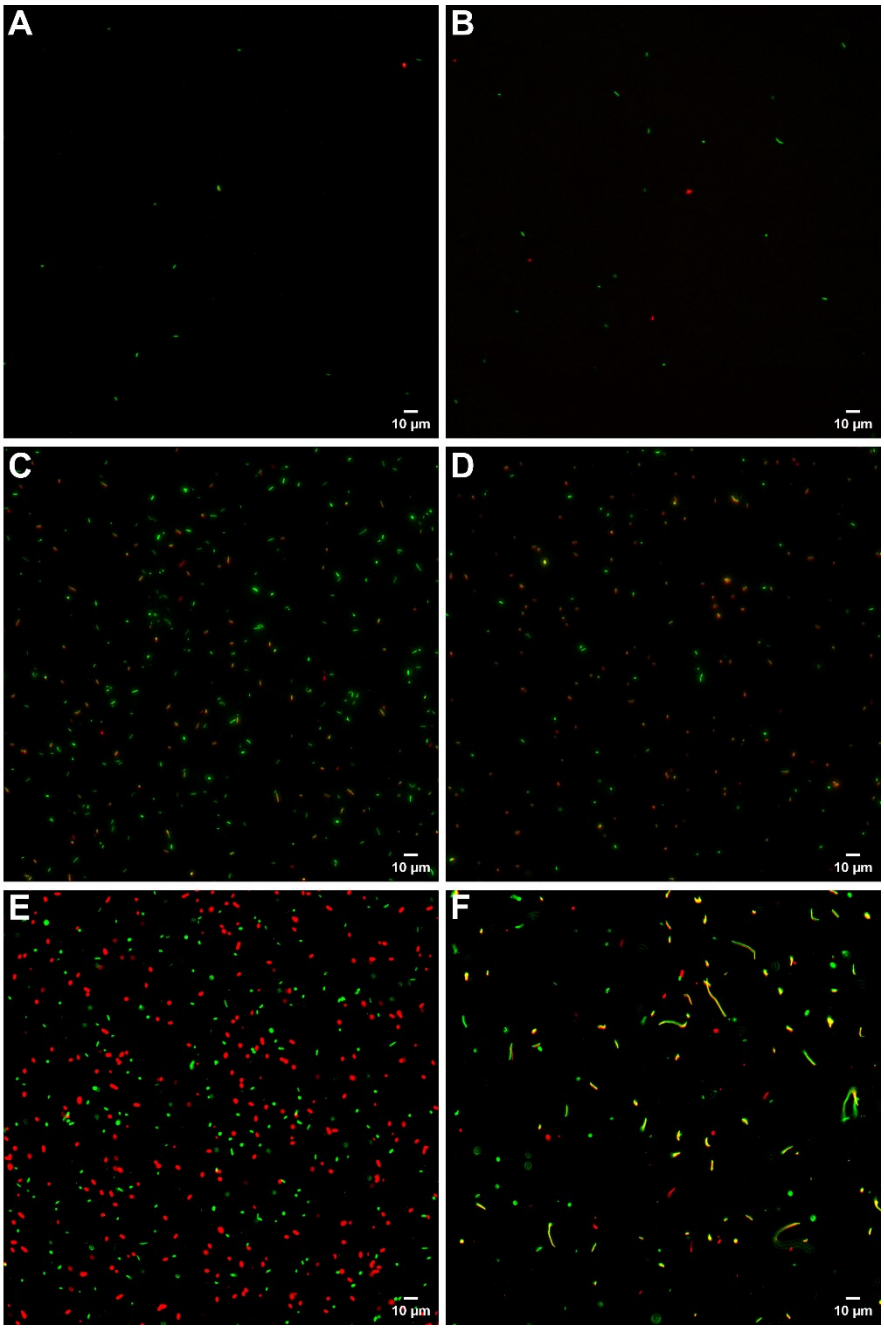

Fig. S6 Live/dead staining of *C. autoethanogenum* grown autotrophically in the dark or under visible light. Micrographs at (AB) 0 h, (CD) 96 h, and (EF) 200 h of growth. Micrographs of stained bacterial cultures (ADE) in the dark or (BDF) under visible light (4200 lux).

Table S1. Composition of the Tanner modified medium per liter.[ab]

Component	Quantity
MES[c]	20 g
Yeast extract	0.5 g
Mineral solution	25 mL
Trace elements solution	10 mL
Vitamins solution	10 mL

[a]Where indicated, 40 mM fructose, 1 mM cysteine, and/or 2 μM resazurin were added. [b]The initial pH of the medium was 5.8. [c]MES: 2-(*N*-morpholino)ethanesulfonic acid

Table S2. Composition of the mineral solution, trace elements solution, and vitamins solution per liter.

Component	Quantity
Mineral solution	
NaCl	80 g
NH_4Cl	100 g
KCl	10 g
$MgSO_4 \cdot 7H_2O$	20 g
$CaCl_2 \cdot 2H_2O$	4 g
KH_2PO_4	10 g
Trace elements solution[a]	
Nitriloacetic acid	2 g
Na_2WO_4	0.2 g
$Na_2SeO_3 \cdot 5H_2O$	0.1 g
$MnSO_4 \cdot H_2O$	1 g
$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$	0.8 g
$ZnSO_4 \cdot 7H_2O$	1 g
$CoCl_2$	0.2 g
$NiCl_2 \cdot 6H_2O$	0.2 g
$CuCl_2$	0.02 g
$Na_2MoO_4 \cdot 2H_2O$	0.02 g
Vitamins solution	
Pyridoxine	10 mg
Thiamine	5 mg
Riboflavin	5 mg
Pantothenic acid	5 mg
Lipoic acid	5 mg
Para-aminobenzoic acid	5 mg
Nicotinic acid	5 mg
B12 vitamin	5 mg
Biotin	2 mg
Na-2-mercaptoethane sulfonate	10 mg
Folic acid	2mg

[a]The pH of the trace elements solution was adjusted to 5.8 with KOH.

References

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