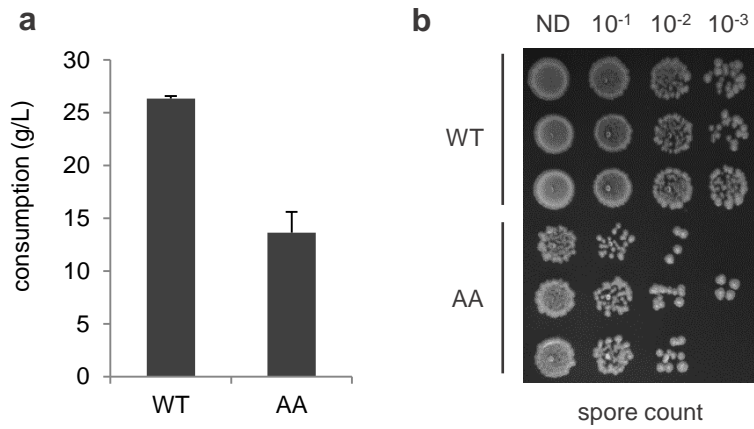


**σ^{54} (σ^L) plays a central role in carbon metabolism in the industrially relevant
*Clostridium beijerinckii***

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Supplementary files



S1 : Comparative phenotypic analysis of *C. beijerinckii* DSM 6423 wild-type and AA strains, supplementary data. a. Final glucose consumption measured after 48h of fermentation in Gapes medium for wild-type *C. beijerinckii* DSM 6423 and the AA mutant. Biomass is given as a measure of optical density at 600 nm (OD_{600}). Error bars indicate the standard deviation of triplicate experiments. b. Sporulation assay on 2YTG plates comparing triplicate heat-shocked cultures of the wild-type and AA strains.

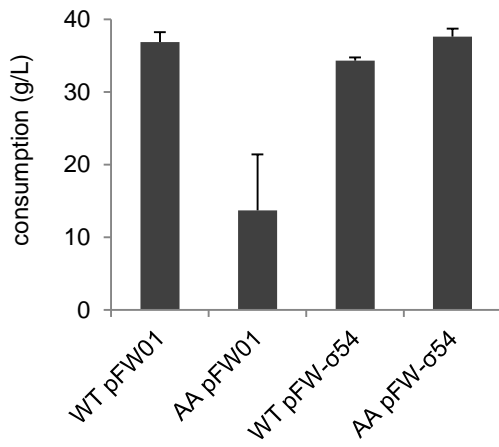
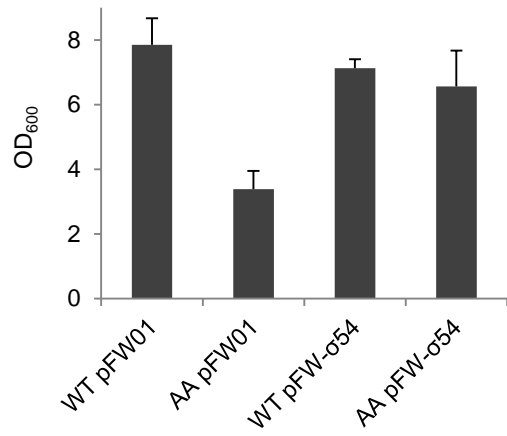
Supplementary file S1
(Wasels)

Locus tag	Automatic Annotation	Comment
CIBE_0239	Sigma-54 factor, interaction domain-containing protein	putative operon involved in PP pathway
CIBE_0709	PTS sugar transporter	putative operon involved in fructose/galactitol transport
CIBE_0715	Iditol 2-dehydrogenase	putative operon involved in PP pathway
CIBE_0882	phosphotransferase system (PTS) lichenan-specific enzyme IIA component	putative operon involved in lactose metabolism/transport
CIBE_1109	Phage-rlike protein	
CIBE_1140	chitosanase	
CIBE_1144	PTS lactose transporter subunit IIC	putative PTS transport encoding operon
CIBE_1149	PTS system fructose subfamily IIA component	putative sugar transport/metabolism encoding operon
CIBE_1157	PTS mannose transporter subunit IIA	putative operon involved in mannose metabolism/transport
CIBE_1696	acetoin reductase/2,3-butanediol dehydrogenase	
CIBE_1707	phosphotransferase system (PTS) lichenan-specific enzyme IIA component	putative operon involved in cellobiose metabolism/transport
CIBE_2050	NADPH-dependent butanol dehydrogenase	butanol metabolism
CIBE_2524	Acyl carrier protein	putative operon involved in fatty acid metabolism
CIBE_2531	4-hydroxybutyryl-CoA dehydratase/vinylacetyl-CoA-Delta-isomerase	putative operon involved in 4-hydroxybutyrate conversion to crotonyl-CoA
CIBE_2581	dihydroxyacetone kinase, N-terminal domain	putative operon yielding dihydroxyacetone kinase
CIBE_2622	NADPH-dependent butanol dehydrogenase	butanol metabolism
CIBE_2943	PTS fructose transporter subunit IIA	
CIBE_3081	ketopantoate hydroxymethyltransferase	putative operon involved in coenzyme A metabolism
CIBE_3134	conserved protein of unknown function	
CIBE_3200	cytidine deaminase	
CIBE_3470	NADP-dependent isopropanol dehydrogenase	putative operon, isopropanol metabolism
CIBE_3479	Methyl-accepting chemotaxis protein McpA	
CIBE_3483	ABC transporter substrate-binding protein	putative operon with CoA transferase
CIBE_3485	Citrate transporter	
CIBE_4059	Acetate CoA-transferase YdiF	putative operon
CIBE_4264	putative PTS system fructose subfamily IIA component	putative operon involved in glycosaminoglycan metabolism
CIBE_4670	fragment of fused mannose-specific PTS enzymes: IIA component ; IIB component (part 1)	mannose metabolism
CIBE_4671	putative Transcriptional regulatory protein LevR	
CIBE_5166	Flagellar hook-associated protein FlgK	
CIBE_5246	conserved protein of unknown function	
CIBE_5470	PTS system fructose subfamily IIA component	putative operon involved in fructosamine metabolism
CIBE_5548	phosphotransferase system (PTS) lichenan-specific enzyme IIA component	putative operon involved in sugar transport
CIBE_5598	phosphotransferase system (PTS) lichenan-specific enzyme IIA component	putative operon involved in sugar transport
CIBE_5606	Membrane spanning protein	

PP, pentose phosphate

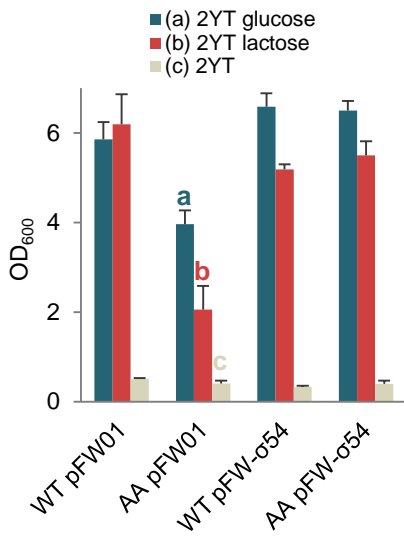
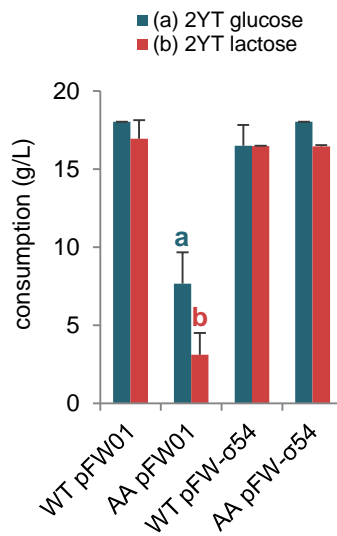
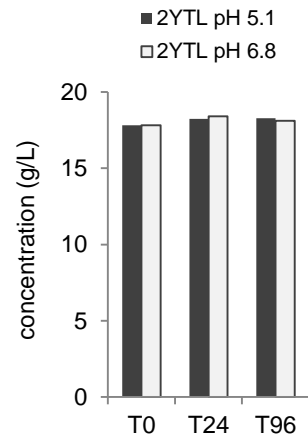
S2 : List of *C. beijerinckii* DSM 6423 genes predicted to be controlled by σ^{54} . Predictions are based on the presence of the σ^{54} consensus motif (TGGCANNNNNTTGCW) in the correct orientation up to 500 bp upstream of a given coding sequence.

Supplementary file S2
(Wasels)

a**b**

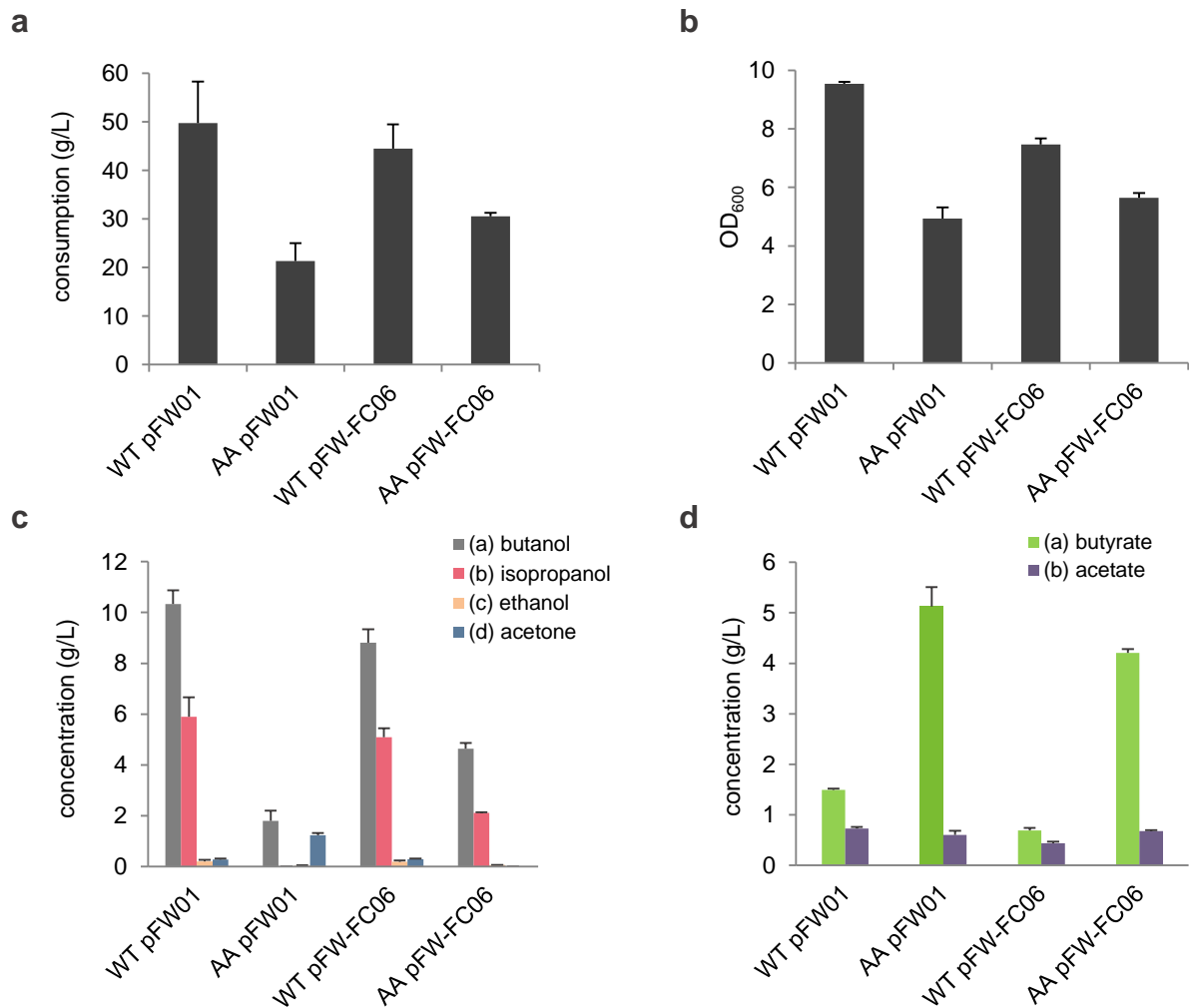
S3 : Phenotypic analysis of *C. beijerinckii* DSM 6423 σ^{54} complementation in the AA strain, supplementary data 1. a,b, Final (a) glucose consumption and (b) biomass measured after 48h of fermentation in Gapes medium for wild-type *C. beijerinckii* DSM 6423 (WT) and the AA mutant containing an empty plasmid (pFW01) or the complementation plasmid (pFW01- σ^{54}). Biomass is given as a measure of optical density at 600 nm (OD₆₀₀). Error bars indicate the standard deviation of triplicate experiments.

Supplementary file S3
(Wasels)

a**b****c**

S4: Phenotypic analysis of *C. beijerinckii* DSM 6423 σ^{54} complementation in the AA strain, supplementary data 2. a,b, Sugar utilization assay in liquid 2YT with 20 g/L glucose, lactose or without added carbon source. Biomass (optical density at 600 nm, OD₆₀₀) (a) and carbon consumption (b) are measured 96h after the beginning of the fermentation. c. Lactose does not degrade during a 96-hour period in a 2YT-based medium. Error bars indicate the standard deviation of triplicate experiments.

Supplementary file S4
(Wasels)



S5: Comparative fermentation assays in *C. beijerinckii* DSM 6423 wild-type and AA strains overexpressing *s-adh* and *ctfAB*. a,b,c,d Final (a) glucose consumption, (b) biomass, (c) solvent and (d) acid concentrations measured after 72h of fermentation in Gapes medium for wild-type *C. beijerinckii* DSM 6423 (WT) and the AA mutant containing an empty plasmid (pFW01) or the pFW-FC06 plasmid. Biomass is given as a measure of optical density at 600 nm (OD₆₀₀). Error bars indicate the standard deviation of triplicate experiments.

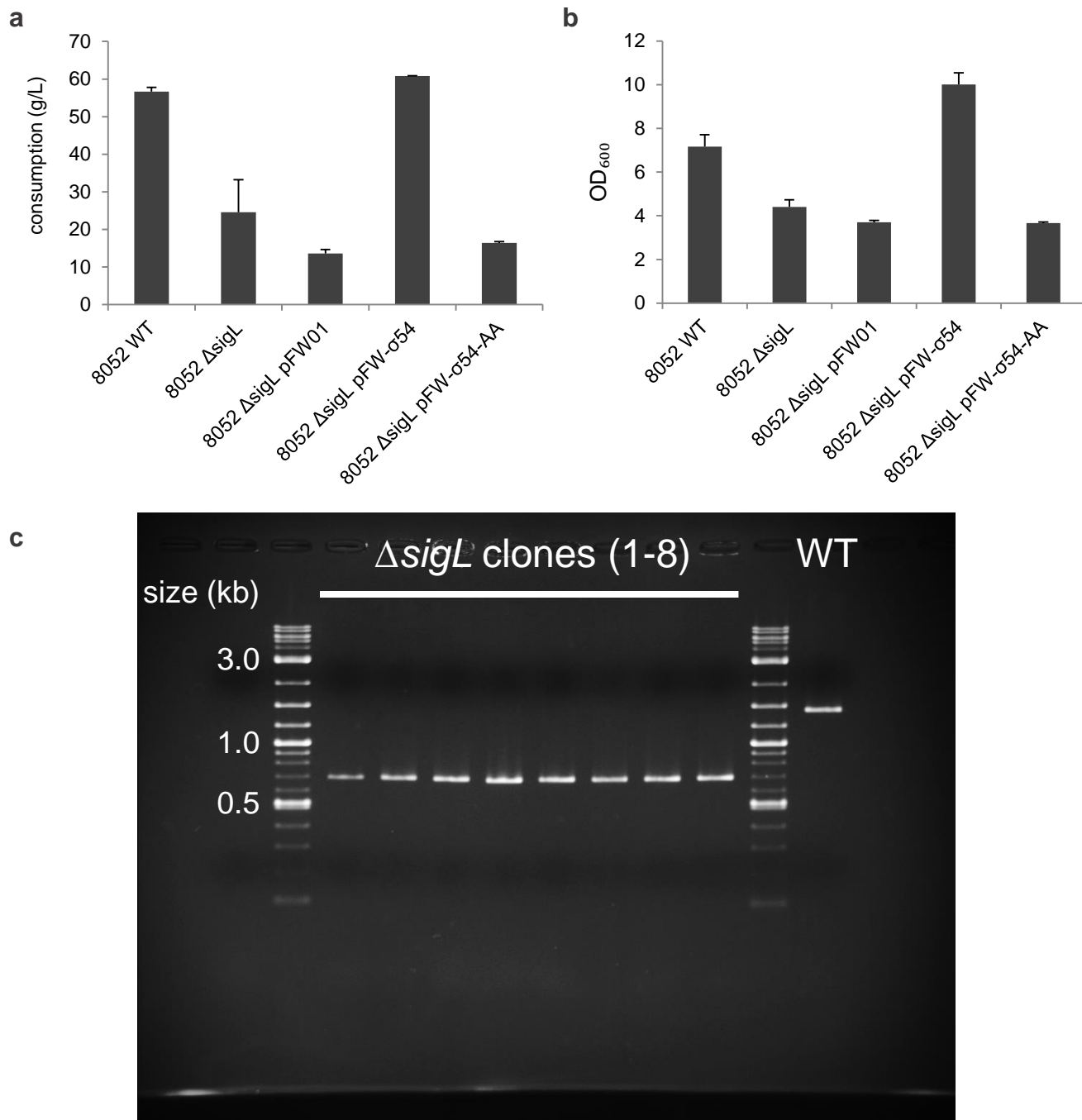
Supplementary file S5
(Wasels)

Name	Sequence (5' → 3')
Construction of pFW-σ54	
RH077	ACTGTTCTCGAGGACCTGGATGCTGTGGATTAG
RH078	TGACACGTCGACTTATAATCTTTCCTTGCTGAAGATG
RH086	CAGCAAGGAAGAGATTATAAGTCGACAAAGTATTGTAAAAATAACTCTG
RH087	AAAAACTAATCCACAGCATCCAGGTCCTCGAGCCGCTTATAATCCATAAC
Construction of pFW-σ54-AA	
RH136	TCAATCTTCATGAATTTACCGTAAGTAGAGC
RH137	GCTCTACTTACGGTAAATTCATGAAGATTGA
Construction of pΔsigL	
RH125	TTGTTATGGATTATAAGCGGCTCGAG
RH126	CAATCTATCACTGATAGGGACTCGAG
guide_sigL_fwd	TCATATTATATTCTGATAATTACA
guide_sigL_rev	AAACTGTAATTATCAGAATATAAT
Verification of sigL deletion	
RH129	GGTGTTAACTTTATTGAATGTCC
RH130	GCAACTTCTTTAATGTCAAAGG
Construction of pFW-FC06	
Mkz01	AAAAAAGGTACCCAAGTATTTTTTATTACATTAATATAGTTAAA
Mkz02	AAAAAAGTCGACTGTTTCATAGTATTCTTTCT

Target	Name	Sequence (5' → 3')	qPCR efficiency (%)
<i>sigL</i>	0767_F	AGGGTATTTGGAGATATCAACACGA	106,2
	0767_R	GCAATCAACGACTTAACAGATGCT	
<i>gapC</i>	0769_F	AGGGTATTTGGAGATATCAACACGA	104,6
	0769_R	TGGTTCCAATGCCTGTACCA	
<i>adh-1</i>	2050_F	GGAGTAGAACCCAGATCCATCAGT	105,2
	2050_R	GTGATCCTCCACCCATAGCAA	
<i>EBP-A</i>	2621_F	GGATGGGACCTTTACAGGAGG	105,3
	2621_R	CGGCATTTCCCAATTCGT	
<i>adh-2</i>	2622_F	ACAGTAATGAAAGGCGCAGAAG	106,3
	2622_R	TCCACATAGCCTTTGCAGCA	
<i>s-adh</i>	3470_F	AATTGGCATTGGAGCTGTTGG	105,7
	3470_R	CTCAACACAAATCGGCCTGC	
<i>EBP-B</i>	3471_F	TTGTGGCAATAAACTGTGGCG	105,2
	3471_R	CACCCTCTCTTTAGCTCCC	
<i>ald</i>	4606_F	CTCCATATGGCGTTATAGGTGCA	108,1
	4606_R	ACTACAGCATTCCAGCAGCT	

S6 : List of primers used in this study.

Supplementary file S6
(Wasels)



S7: CRISPR/Cas9-mediated deletion of σ^{54} in *C. beijerinckii* NCIMB 8052, complementation and comparative fermentation assays, supplementary data. a,b, Final (a) glucose consumption and (b) biomass measured after 72h of fermentation in Gapes medium for wild-type (WT) *C. beijerinckii* NCIMB 8052 and the Δ sigL mutant containing an empty plasmid (pFW01), the pFW- σ 54 plasmid or the pFW- σ 54-AA plasmid. Biomass is given as a measure of optical density at 600 nm (OD₆₀₀). Error bars indicate the standard deviation of triplicate experiments. c. Verification by colony PCR of sigL deletion with primers RH129 and RH130 encompassing the deleted region, full agarose gel corresponding to figure 4b. Expected band sizes are 1406 (wild-type) and 670 (Δ sigL) bp.

Supplementary file S7
(Wasels)