Isolation of butanol- and isobutanol-tolerant bacteria and physiological characterization of their butanol tolerance

SUPPLEMENTAL MATERIAL

Supplemental TABLE S1. Primers used for the amplification, direct sequencing, and cloning of the *cfa* gene of strain CM4A.

Primer name	Use	Primer sequence (5' to 3')
cfa-F [a]	Amplification	GAGGGAATGCAATGTTAG
cfa-R [a]	Amplification	TCTATTAACCAATCCGG
cfa362R [a]	Sequencing	CCTAGATCGTAATGGCTGTG
cfa871F [a]	Sequencing	GGTGGCTATATTCCTGGTG
cfa-5EF [b]	Cloning	CCGAATTCGAATGCAATGTTAG
cfa-5XR [b]	Cloning	CCCTCGAGATTAACCAATCCG

[a]The primers were designed from the *cfa* gene sequence in the *Enterococcus faecalis* V583 genome (NC004668). [b]Primers containing *Eco*RI and *Xho*I sites, as underlined, were used to amplify the *cfa* gene for cloning into the pET-28b expression vector (Novagen) to produce an N-terminal His 6-tagged fusion protein.

Supplemental TABLE S2. Cell surface hydrophobicity of strain CM4A grown with or without 2.0% butanol.

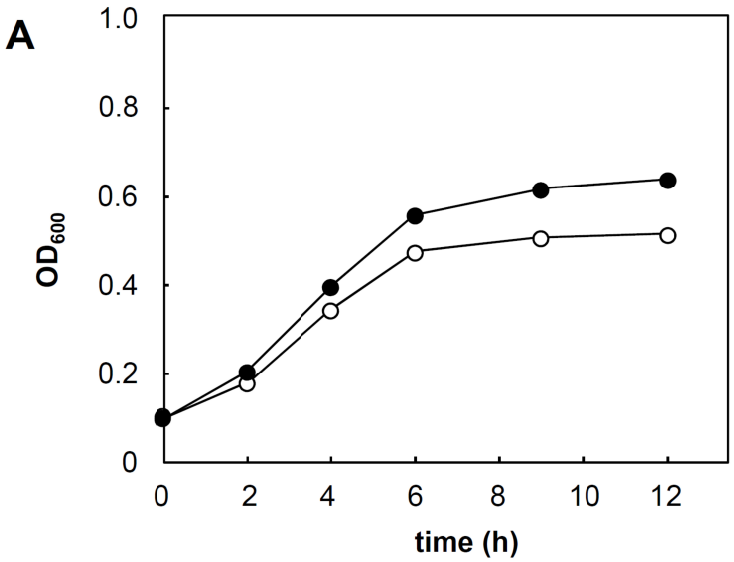
CM4A cells	BATH (%) [a]				
	Butanol	*n*-Hexane	*n*-Tetradecane	Toluene	Xylene
without butanol	11.6 ± 2.94	22.3 ± 0.26	15.9 ± 0.45	30.5 ± 0.45	21.9 ± 1.58
with 2.0% butanol	0.8 ± 4.11	4.3 ± 1.62	6.5 ± 1.39	5.3 ± 1.39	2.6 ± 1.87

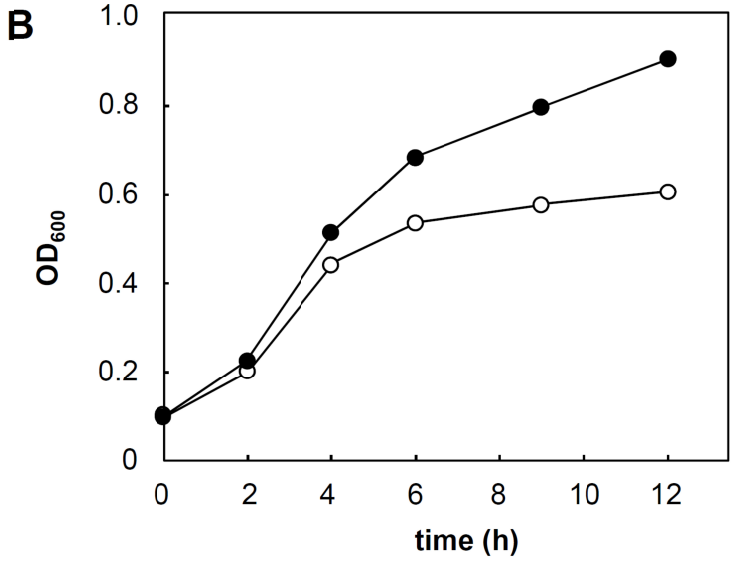
[a]The value represents the percentage of cells adhering to a given solvent in three independent measurements. [1-(OD_{600} of aqueous phase after mixing)/(OD_{600} of initial suspension)]×100.

Supplemental TABLE S3. Changes in the membrane fatty acid compositions of the strain *E. coli*/pCFA and the control strain *E. coli*/pET28 in response to the presence of 0.1 mM IPTG[a].

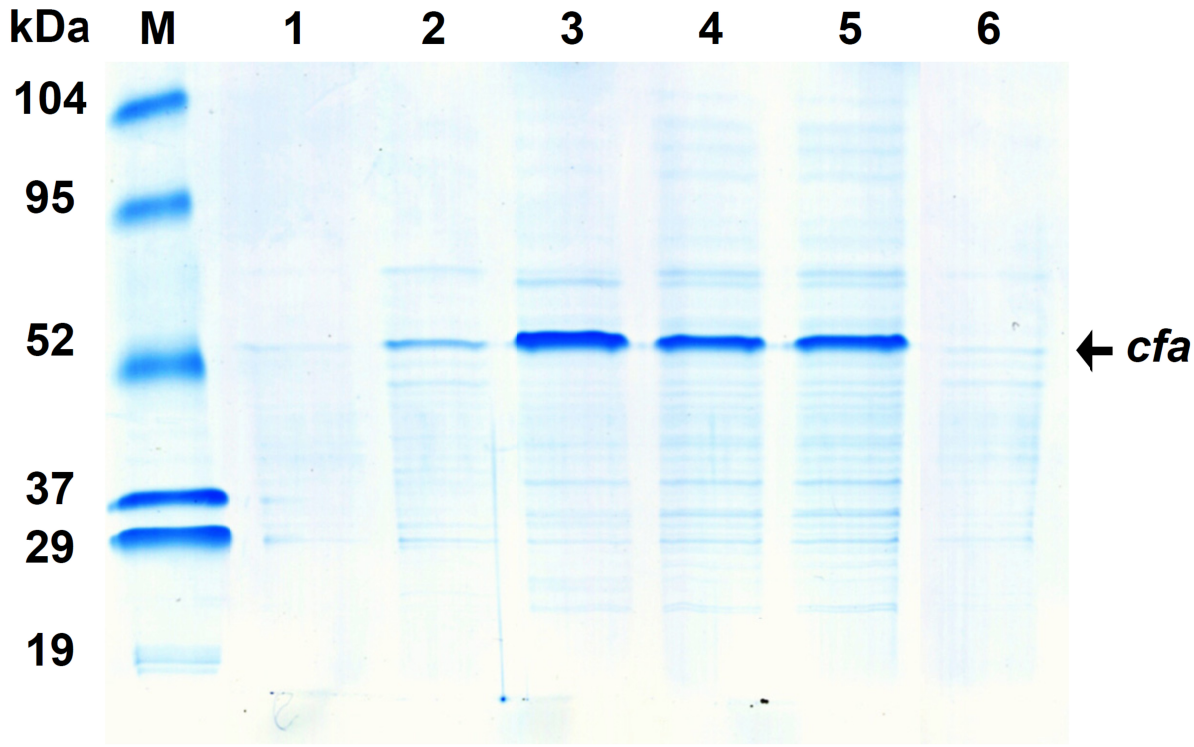
	E. coli/pET28	*E. coli*/pCFA
C12:0	4.1 ± 0.1	4.3 ± 0.3
C14:0	8.8 ± 0.5	11.1 ± 0.1
C14:0-3OH	3.4 ± 0.3	3.4 ± 1.4
C14:1ω7c	ND	0.1± 0.1
C15:0	0.2 ± 0.0	0.1 ± 0.1
C16:0	43.2 ± 0.2	45.2 ± 0.6
C16:1ω7c	16.3 ± 0.1	17.6 ± 0.6
cyclo-C17:0	3.9 ± 0.1	5.6 ± 0.7
C18:0	0.5 ± 0.1	0.2 ± 0.2
C18:1ω7c	19.4 ± 0.7	6.1 ± 0.6
cyclo-C19:0	0.3 ± 0.0	6.5 ± 1.3

[a]Each fatty acid composition is described as a percentage of the total fatty acids. The values are the means ± standard deviations of three independent measurements. Abbreviations: X:YωZc, fatty acid containing X carbon atoms with Y double bonds at position Z, counted from the methyl terminus in the *cis* configuration; C14:0-3OH, 3-hydroxy tetradecanoic acid; cyclo-C17:0, *cis*-9,10-methylene hexadecanoic acid; cyclo-C19:0, *cis*-11,12-methylene octadecanoic acid; ND, not detected.

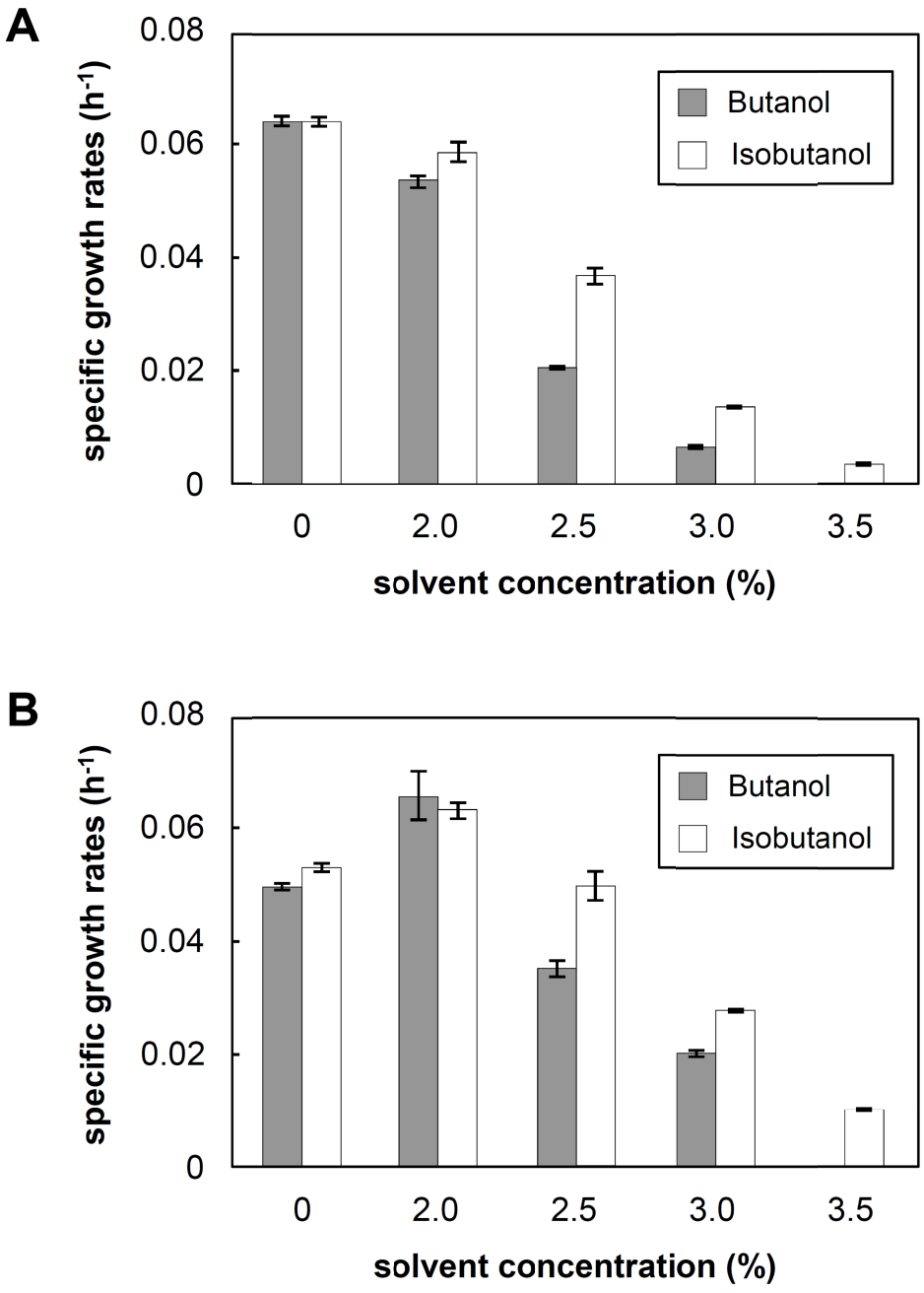




Supplemental FIG. S1. Growth of *E. coli*/pCFA (●) and the control strain *E. coli*/pET28 (○) in the presence of 0.8% butanol (A) or 0.8% isobutanol (B). The values represent the mean of triplicate experiments.



Supplemental FIG. S2. SDS-PAGE analysis of *cfa* gene expression in *E. coli*/pCFA (lane 1-5) and *E. coli*/pET28 (lane 6) at different concentration of IPTG. In total, 60 μg of protein in the sonicated supernatant was purified by His-selective nickel affinity gel chromatography and analyzed by SDS-PAGE. Lane M, molecular weight marker; lane 1, 10 mM glucose without IPTG (negative control); lane 2, no IPTG; lane 3, 0.01 mM IPTG; lane 4, 0.1 mM IPTG; lane 5, 1.0 mM IPTG; lane 6, *E. coli*/pET28 grown without IPTG (negative control).



Supplemental FIG. S3. Butanol and isobutanol tolerance of strain GK12. The specific growth rates of non-adapted (A) and butanol-adapted (B) cells following different butanol or isobutanol challenges. Cell adaptation was previously achieved by 15 consecutive passages with 2.0% butanol. The values and error bars represent the mean and SD of triplicate experiments.