Supplemental data

Structural determinant of functionality in acyl lipid desaturases

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Figure S1

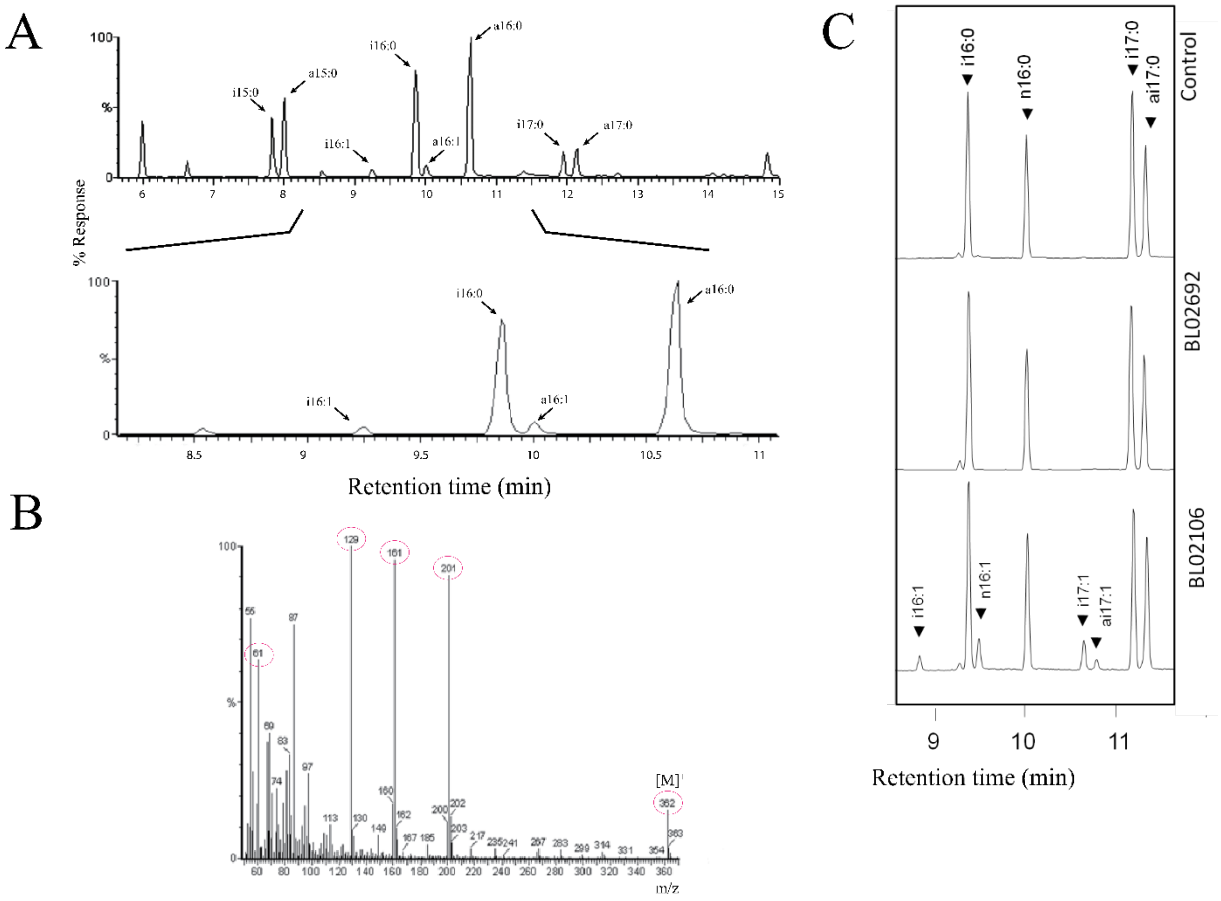

Figure S1: Fatty acids synthesized by *Bacillus* strains. (A) GC-MS of FAME of *B. liqueniformis* ATCC14580, (B) GC-MS of FAME of *B. subtilis* LC5 strains expressing ORF BL02692 and ORF BL02106. Fatty acids of *Bacillus* strains were extracted, converted in methyl esters and analyzed by GC-MS. The peaks corresponding to the identified fatty acids are indicated by arrows in the chromatograms. The numbers on the *x* axis represent times (in minutes). i, iso-branched chain fatty acids (BCFAs); a, anteiso-BCFAs; n, normal. Control represent the *B. subtilis* LC5 strain carrying the empty vector (EV) while BLO 02692 and BLO 02106 represent *B. subtilis* LC5 strains expressing the corresponding ORFs of *B. licheniformis*ATCC14580. The region between 8 and 11 min of the chromatogram shown in panel A is twofold magnified. (C) Mass spectrum of dimethyl disulfide derivatives separated by GC of iso-$C_{16:1}$. [M]+, molecular ion. The mass spectrum of the adducts shows a weak ion at *m/z* 362 corresponding to the theoretical mass of [M]+ from DMDS adducts of C_{16} monosaturated fatty acid. Two prominent ions are formed by cleavage between the methylthio-

substituted (CH_3S) carbons located at the original site of the unsaturation. The strong ions at *m/z* 161 and *m/z* 201 indicate the position of the double bond at Δ5 in the iso-C16:1. A major ion at *m/z* 129 is due to loss of methanol (CH_3OH) from ion *m/z* 161. Ion *m/z* 61 is distinctive in DMDS adducts.

Figure S2

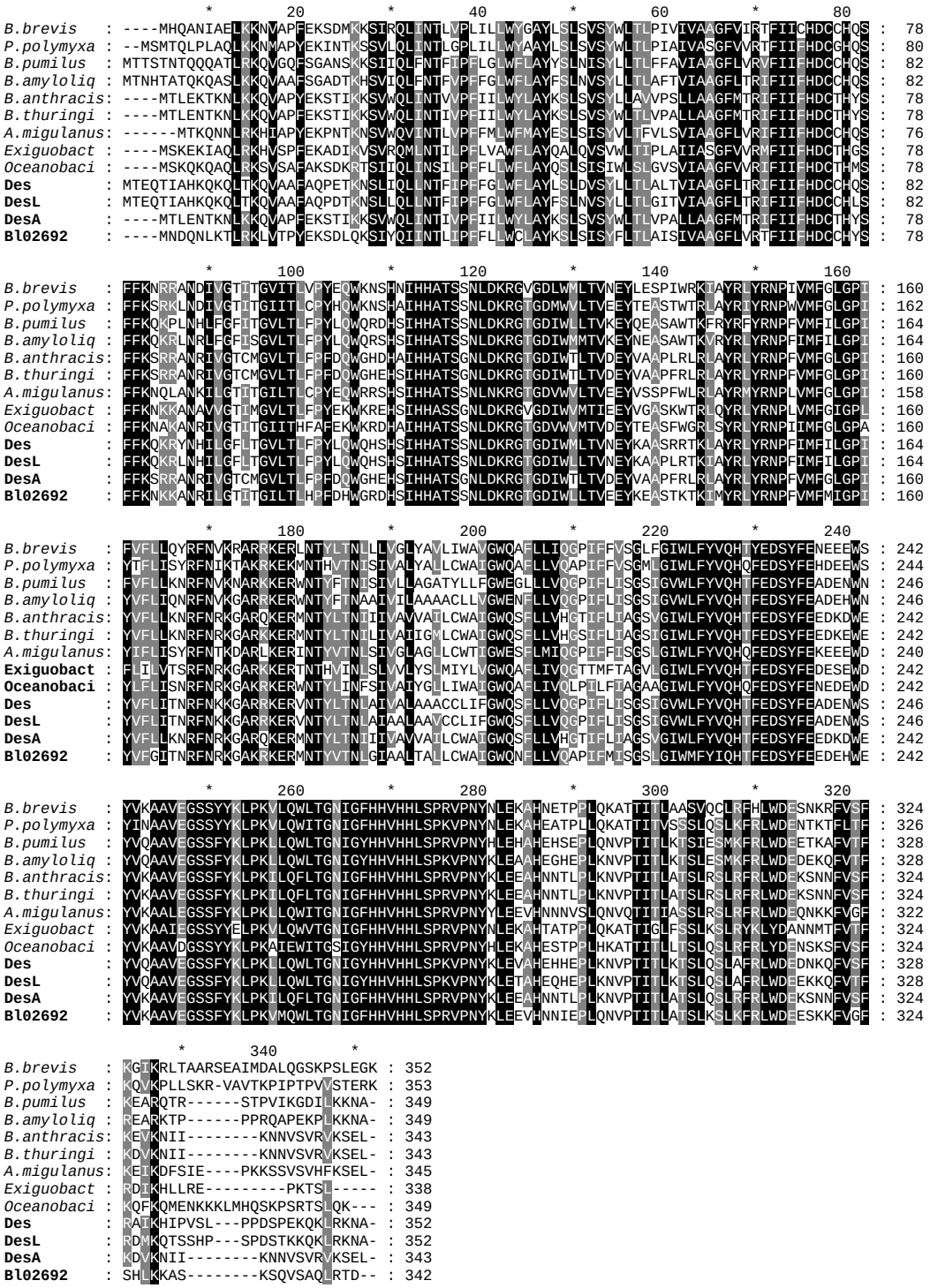

Figure S2. Multiple sequence alignment of full-length Δ5 acyl-lipid desaturases from *Firmicutes*.

The amino acid sequences of these desaturases were aligned using Clustal Omega. Completely conserved residues are shaded in black and partially conserved are shaded in grey.

Figure S3

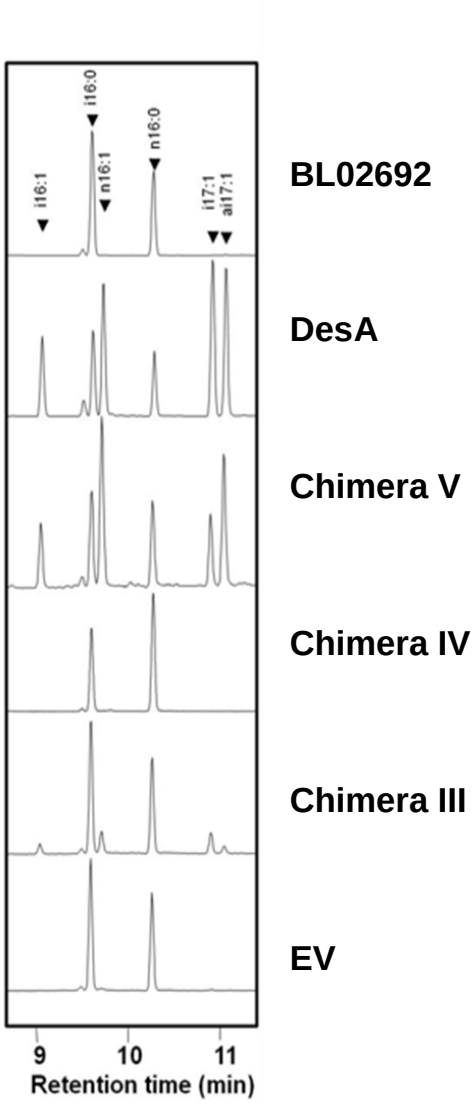

Figure S3. GC-MS analysis of FAME of *B. subtilis* strains. Gas chromatography of the fatty acid methyl esters prepared from *Bacillus subtilis* LC5 transformed with the wild type (ORF BL02692 from *B. licheniformis* and DesA from *B. cereus*) and chimeric desaturases as indicated in each panel. EV represents the *B. subtilis* LC5 strain carrying the empty vector. The peaks corresponding to identified FA are indicated by arrows. The numbers on the *x* axis represent times (in minutes). i, iso-BCFAs; a, anteiso-BCFAs; n, normal fatty acids.

Figure S4

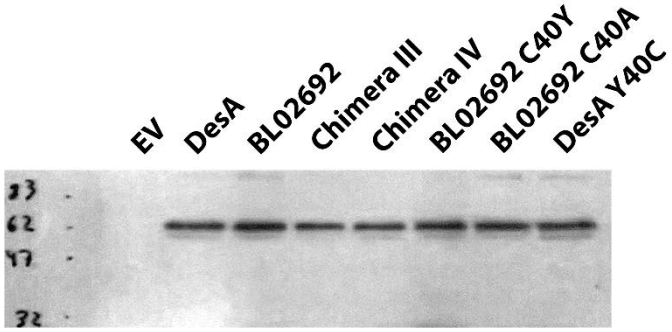

Figure S4. Western blot analysis of proteins heterologous expressed in *Bacillus subtilis* LC5. Expression of protein was induced with 0,5% xylose and harvested after 4-5 hours. Cleared cell lysates containing the chimeric and wild type proteins were resolved on 12% gel electrophoresis and probed by Western blot using anti-GFP tag monoclonal antibody in 1:1000 dilution. As a control we used the extract obtained from *B. subtilis* LC5 strain carrying the empty vector (EV) strain. DesA, BL02692, Chimera III, IV, BL02692C40Y BL02692C40A and DesAY40C are extracts obtained from *B. subtilis* LC5 transformed with the wild type (ORF BL02692 from *B. licheniformis* and DesA from *B. cereus*) and the constructions described in Table 1. Positions of molecular weight markers are indicated at left.

Figure S5

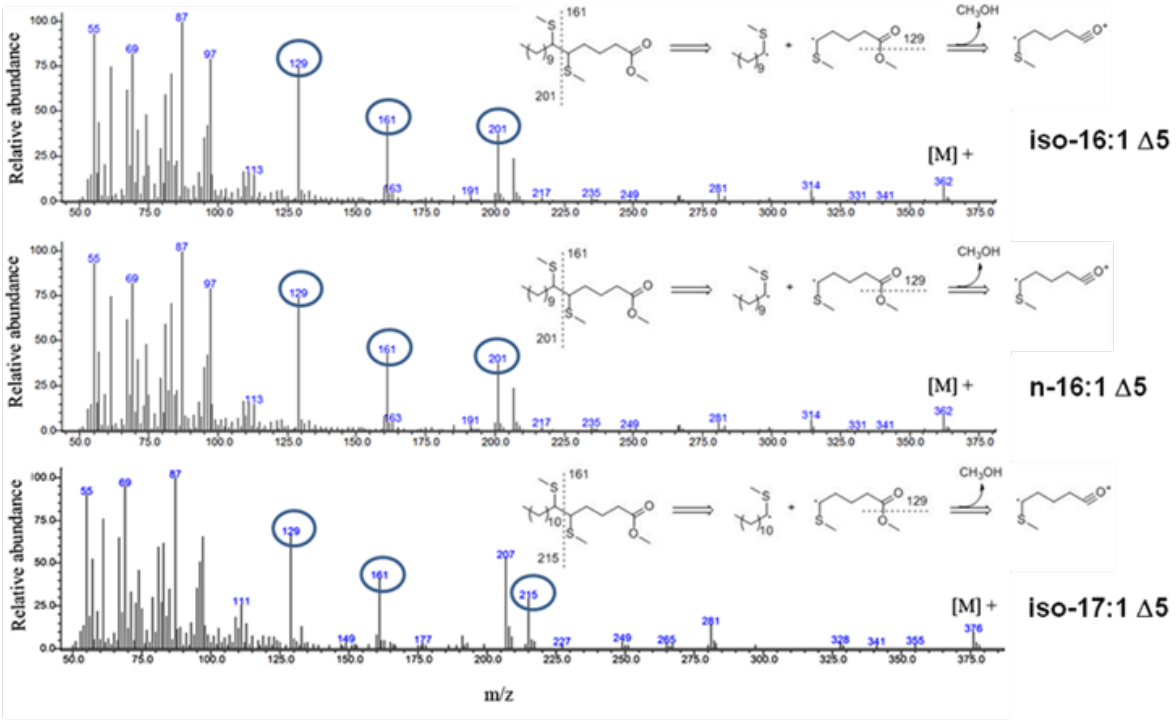

Figure S5. Mass spectra of DMDS derivatives of the fatty acids separated by GC of iso-C16:1, n-16:1 and iso-C17:1. Figure illustrates examples of the electron impact mass spectrum of the DMDS adducts of iso-C16:1, n-16:1 and iso-C17:1 fatty acids produced by desaturases wild type and chimeras. [M]+, molecular ion. The mass spectrum of the adducts shows weak ions at *m/z* 362 and *m/z* 376 corresponding to the theoretical mass of [M]+ from DMDS adducts of C_{16} and C_{17} monosaturated fatty acids, respectively. Two prominent ions are formed by cleavage between the methylthio-substituted (CH_3S) carbons located at the original site of the unsaturation. The strong ions at *m/z* 161 and *m/z* 201 indicate the position of the double bond at Δ5 in the iso-C16:1 and n-C16:1 fatty acids. The ions observed at *m/z* 161 and *m/z* 215 indicate a Δ5 double bond in the iso-C17:1 fatty acid. A major ion at *m/z* 129 is due to loss of methanol (CH_3OH) from ion *m/z* 161.

Figure S6

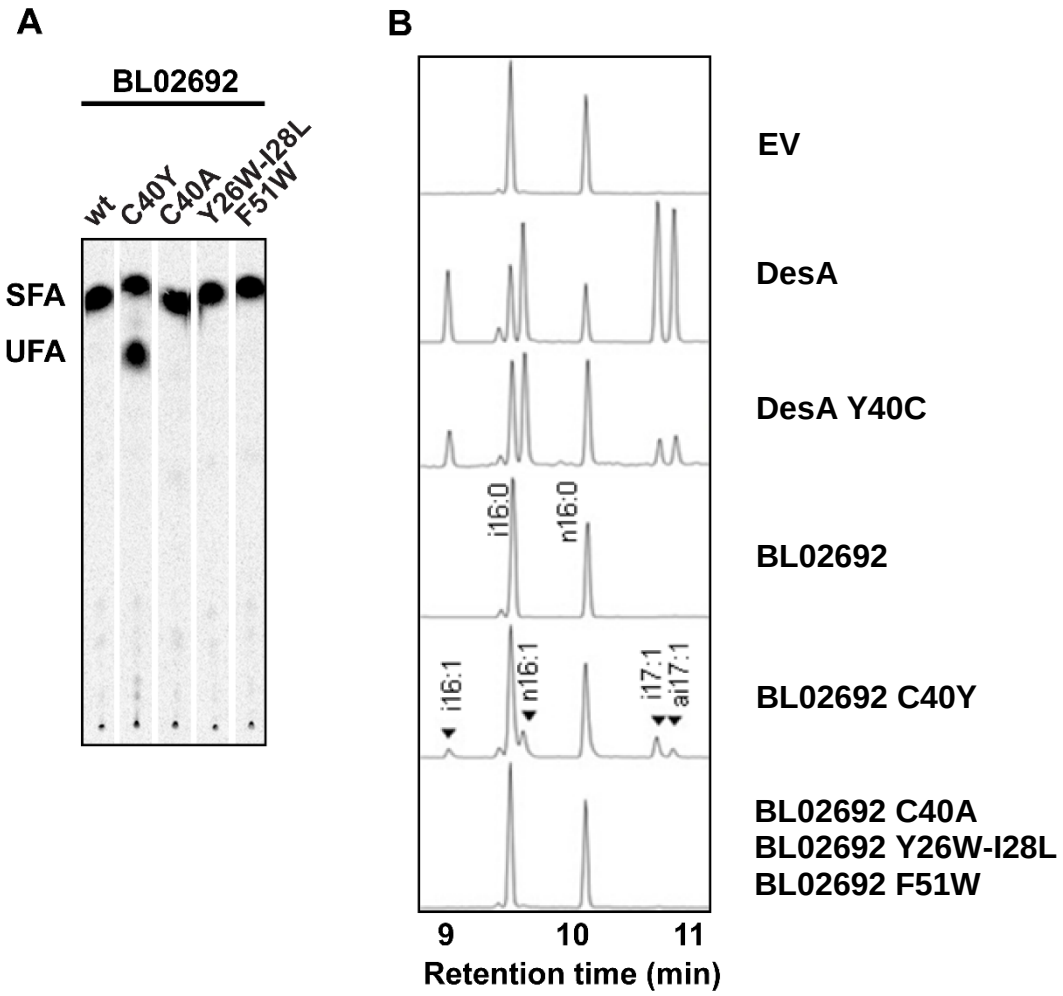

Figure S6. Fatty acids synthesized by *B. subtilis* strains expressing wild type and mutant desaturases. A) Autoradiogram of the products of [^{14}C] -palmitate labeling of *B. subtilis* LC5 expressing ORFBL02692 wild type (wt) and derivative strains described in Table I. Cells were grown overnight at 37°C in MM, washed, resuspended in fresh MM with or without 0,5% xylose, and labeled with 1 μCi ml-1-[^{14}C] palmitate for 4-5 hs at 37°C. Fatty acids were converted to FAME and analyzed using 10% silver nitrate-impregnated Silica Gel G plates and developed in toluene solvent at -20°C. The radioactivity on the plates was visualized using a PhosphorImager screen. UFA, unsaturated fatty acids, SFA, saturated fatty acids. B) Gas chromatography of the FAME prepared from *Bacillus subtilis* LC5 expressing the wild type (ORF BL02692 from *B. licheniformis* and DesA from *B. cereus*) and derivatives mutants desaturases (DesA Y40C, BL02692 C40Y, BL02692 C40A, BL02692 Y26W-I28L and BL0269 F51W) described in Table I, are indicated in each panel. EV represents the *B. subtilis* LC5

strain carrying the empty vector used as a control. The peaks corresponding to the identified FA are indicated by arrows. The numbers on the *x* axis represent times (in minutes). i, iso-BCFAs; a, anteiso-BCFAs; n, normal.

Figure S7

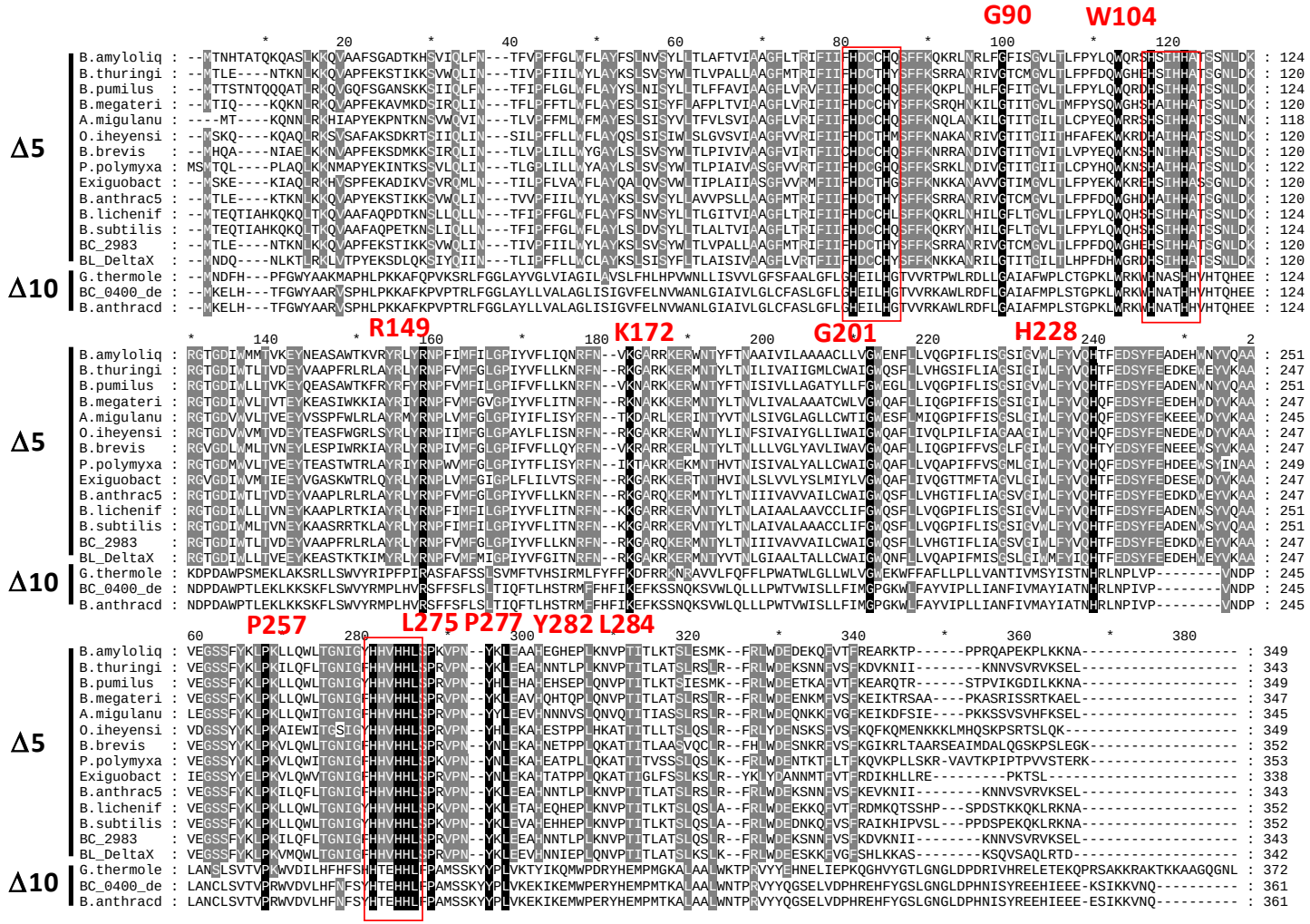
G90
W104
R149
K172
G201
H228
P257
L275
P277
Y282
L284
Δ5
Δ10
Δ5
Δ10
Δ5
Δ10

Figure S7. Multiple sequence alignment of Δ5 and Δ10-acyl-lipid desaturases from *Firmicutes*. The deduced amino acid sequences of these desaturases were aligned by using ClustalW. The eleven amino acid residues conserved in all the proteins are shaded in black and indicated in red. Histidine cluster are denoted by hollow red boxes.

Figure S8

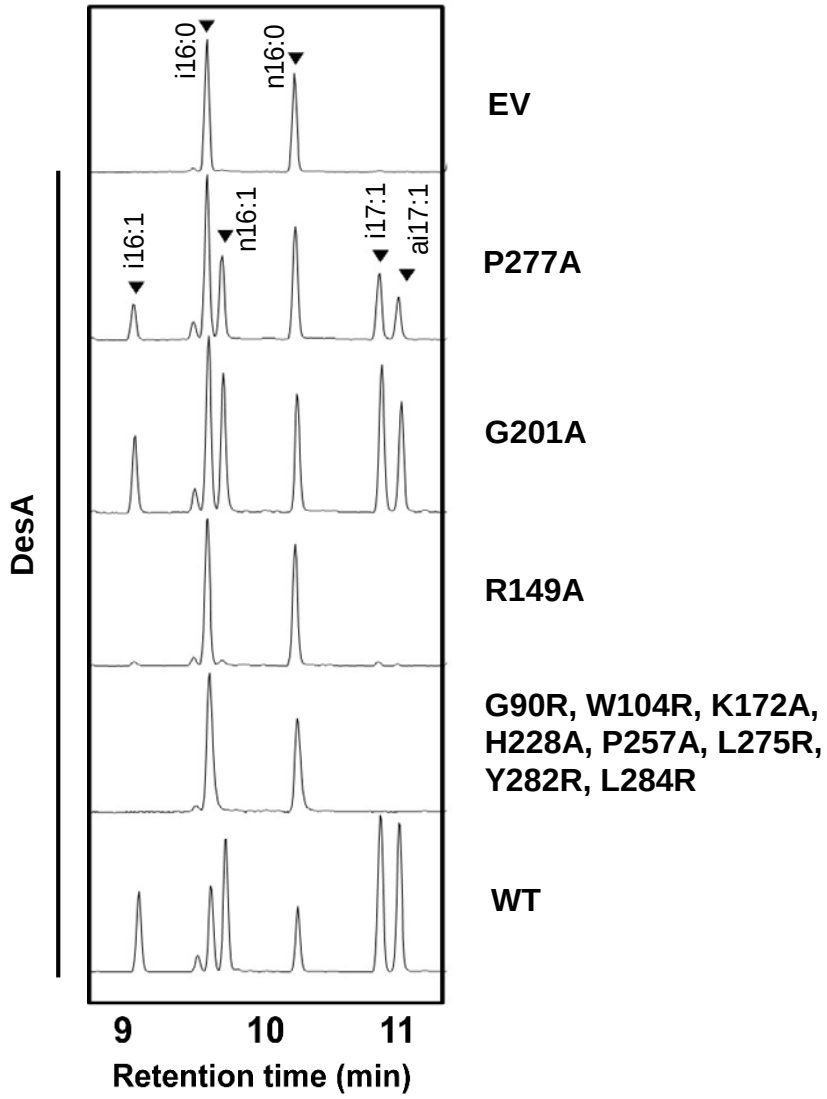

Figure S8. **Fatty acids synthesized by *B. subtilis* LC5 strain expressing *B. cereus* DesA derivatives desaturases.** Gas chromatography of the FAME prepared from *B. subtilis* LC5 expressing the *B. cereus* wild type DesA (WT) and derivatives mutants of DesA (P277A, G201A, R149A, G90R, W104R, K172A, H228A, P257A, L275R, Y282R, and L284R) are indicated in each panel. EV represents the *B. subtilis* LC5 strain carrying the empty vector. The peaks corresponding to the identified FA are indicated by arrows. The numbers on the *x* axis represent times (in minutes). i, iso-BCFAs; a, anteiso-BCFAs; n, normal.

Transmembrane predictor	N°TMs	N-ter	C-ter	Transmembrane segments ubication							His boxes		
											1	2	3
HMMTOP	7	in	out	24-41	46-63	88-105	148-165	184-201	206-225	250-267	in	out	out
MEMSAT(PSIPRED)	6	in	in	26-45	49-73	87-102	145-165	181-202	206-227		in	out	in
SOSUI	6	in	in	24-46	51-73	87-109	142-164	182-204	208-230		in	out	in
PHOBIUS	6	in	in	25-42	48-72	84-101	147-164	184-201	207-226		in	out	in
OCTOPUS	4	in	in	28-44	51-71			182-202	206-226		in	in	in
TOPPRED2	6	in	in	23-43	52-72	84-104	149-169	182-202	206-226		in	out	in
TMHMM	5	out	in	24-43	48-70		147-164	183-202	207-226		out	out	in
DAS	4	in	in	28-43	50-70			185-201	205-224		in	in	in
SPLIT 4.0	3	in	out		46-74			182-201	205-227		out	out	out
TMMOD	6	in	in	24-43	50-70	85-105	147-165	182-202	206-226		in	out	in
TOPCONS	4	in	in	24-44	51-71			182-202	206-226		in	in	in
consensus	4	in	in	25-43	50-70			183-202	206-226		in	in	in

Table S1. Topology prediction servers. The predicted transmembrane domains of the acyl-lipid desaturase DesA from *B. cereus* are indicated.

Table S2. Fatty acid composition of *B. subtilis* strains[a].

	DesA		DesAY40C		BL02692 C40Y		EV	
	SFA	UFA	SFA	UFA	SFA	UFA	SFA	UFA
<C16	64.9±1.6	nd	67.4± 3.7	nd	71.2±3.9	nd	70±3	nd
i-C16	3.0±0.5	3.6±0.6	4.1±0.7	2.3±0.6	6.3±0.7	0.4±0.1	7.4±0.8	nd
n-C16	2.6±0.1	5.5±0.3	4.9±0.8	4.6±0.4	4.6±0.4	1.4±0.2	6.6±0.3	nd
BCFA- C17	6.9±1.3	11.8±1.1	12.4±0.6	3.3±0.5	13.5±2.3	1.3±0.1	14.9±0.5	nd
i-C18	0.9±0.2	0.4±0.1	0.3±0.1	tr	0.4±0.1	tr	0.8±0.1	nd
Total FA	78.3±3.5	21.3±2.1	89.1±5.9	10.2±1.5	96.0±7.4	3.1±0.4	99.7±0.3	nd

[a]Cells were grown at 37ºC in Spizizen salts MM supplemented with glucose to exponential phase and then shifted to 25ºC. Total lipids of *B. subtilis* LC5 expressing the wild type DesA from *B. cereus*, and DesAY40C, BL02692C40Y mutants, were extracted and transesterified to yield FAME. EV represents the *B. subtilis* LC5 strain carrying the empty vector used as a control. The products were identified by GC-MS. Values are the means of the results of three experiments and are expressed as the percentage of total fatty acids (FA). UFA: unsaturated fatty acids, SFA: saturated fatty acids, BCFA: branched chain fatty acids, i: iso-BCFA, n: normal FA, nd: not detected, tr: traces.

Table S3. Oligonucleotides used in this study

Primers	Sequence (5´-3´)	Source/Reference
Bc2983-XhoI up	GGCTCGAGGAAAGGAATTAGAACAATG	This study
Bc2983-low ns	CCGAATTCGCAATTTTAGTTCAGATTTCAC	This study
BL02692-XhoI up	CTCGAGATGAATGACCAAAATCTAAAGAC	This study
BL02692-low ns	CTCGAGTTTATCCGTTCTTAGTTGCGC	This study
BsDes XhoI-up	TGGCACTCGAGTATGACTGAACAAACCATTG	This study
BsDes EcoRI-low	GCTCGGAATTCTCAGGCATTCTTCCGCAGCTTC	This study
Des-Bc2983-Up	CATGAACATTCTATTCACCAGGCAACTAGCAGC AATCTGGAT	This study
Bc2983-Des-Low	ATCCAGATTGCTGCTAGTTGCGTGGTGAATAGA ATGTTCATG	This study
Bc2983-Des-Up	CACAGCCATTCGATTCATCATGCTACGAGTGGT AATTTGGAT	This study
Des-Bc2983-Low	ATCCAAATTACCACTCGTAGCATGATGAATCGA ATGGCTGTG	This study
DesA(N)-Bl02692-up	GAACATTCTATTCACCACGCGACAAGCAGCAAC CTGG	This study
DesA(N)-Bl02692-low	CCAGGTTGCTGCTTGTCGCGTGGTGAATAGAAT GTTC	This study
Bl02692(His2)-DesA-up	CGACCATTCTATCCACCATGCTACGAGTGGTAA TTTGG	This study
Bl02692(His2)-DesA-low	CCAAATTACCACTCGTAGCATGGTGGATAGAAT GGTCG	This study
DesA (N_1)-BL02692-up	CAAGAATTTTCATTATTTTTCATGATTGCTGCCA CTATTC	This study
Bc(N_1)-Bl02692-low	GAATAGTGGCAGCAATCATGAAAAATAATGAA AATTCTTG	This study
DesA-L90R-up	GAATAGTTaGAACGTGTATGGGTGTTTTAAC	This study
DesA-L90R-low	CCCATACACGTTCtAACTATTCTATTTGCACGTC G	This study
DesA-W104R-up	TTGATCAGaGGGGGCATGAACATTCTATTC	This study
DesA-W104R-low	GTTCATGCCCCCtCTGATCAAATGGGAATAATG	This study
DesA-R149A-up	GCTTATATgcCAATCCATTCGTTATGTTTGG	This study
DesA-R149A-low	ACGAATGGATTGgcATATAAGCGATATGCTAAA CG	This study
DesA-K172A-up	CTTAAAAATAGATTTAACCGAgcAGGTGCAAGG CAGAAAG	This study
DesA-K172A-low	CTTTCTGCCTTGCACCTgcTCGGTTAAATCTATTT TTAAG	This study
DesA-G201A-up	GGGCAATTGcGTGGCAATCGTTTCTGTTAG	This study
DesA-G201A-low	ACGATTGCCACgCAATTGCCCAGCAAAGTATAG C	This study
DesA-H228A-up	ACGTACAGgcCACATTTGAAGATTCTTATTTTG	This study
DesA-H228A-low	AATCTTCAAATGTGgcCTGTACGTAAAACAGCC AAATC	This study
DesA-P257A-up	AAGCTTgCTAAAATTTTGCAATTCTTAACTGG	This study

DesA-P257A-low	TTGCAAAATTTTAGcAAGCTTATAAAAAGAACTTCC	This study
DesA-L275R-up	TTCACCATcgAAGCCCAAGGGTACCTAAC	This study
DesA-L275R-low	CCCTTGGGCTTcgATGGTGAACATGATGGAATC	This study
DesA-P277A-up	ATTTAAGCgCAAGGGTACCTAACTATAAAC	This study
DesA-P277A-low	TTAGGTACCCTTGcGCTTAAATGGTGAACATGATG	This study
DesA-Y282R-up	TACCTAACcgTAAACTAGAAGAGGCACAC	This study
DesA-Y282R-low	TCTAGTTTAcgGTTAGGTACCCTTGGGCTTAAATG	This study
DesA-L284R-up	CTATAAACgAGAAGAGGCACACAATAATACGC	This study
DesA-L284R-low	TGTGCCTCTTCTcGTTTATAGTTAGGTACCCTTGG	This study
Bl02692 Y26W-I28L-up	AAAAGCATTTGGCAACTCATTAACACATTGATAC	This study
Bl02692 Y26W-I28L-low	GTGTTAATGAGTTGCCAAATGCTTTTTTGTAAATCGG	This study
Bl02692 C40Y-up	CTGTTATGGTATTTAGCATATAAGAGCTTGTC	This study
Bl02692 C40Y-low	CTTATATGCTAAATACCATAACAGGAAAAATGG	This study
Bl02692 F51W-up	GATTTCTTATTGGCTTACATTAGCGATTTC	This study
Bl02692 F51W-low	CTAATGTAAGCCAATAAGAAATCGACAAGCTC	This study
DesA Y40C-up	ATTTTATGGTGCCTTGCTTATAAAAGTTTGTC	This study
DesA Y40C-low	TTTATAAGCAAGGCACCATAAAATAATAAATGG	This study
Bl02692 C40A-up	CTGTTATGGGCTTTAGCATATAAGAGCTTGTC	This study
Bl02692 C40A-low	CTTATATGCTAAATACCCGAACAGGAAAAATGG	This study