Supplemental data

Structural determinant of functionality in acyl lipid desaturases

Diego E. Sastre, Emilio Saita, Antonio D. Uttaro, Diego de Mendoza and Silvia G. Altabe*.

Instituto de Biología Molecular y Celular de Rosario (IBR), and Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Esmeralda y Ocampo, S2002LRKRosario, Argentina.

*Corresponding author: altabe@ibr-conicet.gov.ar



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 the adducts shows a weak ion at m/z 362 corresponding to the theoretical mass of [M]+ from DMDS adducts of C₁₆ monosaturated fatty acid. Two prominent ions are formed by cleavage between the methylthio-

substituted (CH₃S) 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 $\Delta 5$ in the iso-C16:1. A major ion at m/z 129 is due to loss of methanol (CH₃OH) from ion m/z 161. Ion m/z 61 is distinctive in DMDS adducts.

	*	20 *	4	10	*	60	* 80	
B.brevis : P.polymyxa : B.pumilus : B.amyloliq : B.anthracis: B.thuringi : A.migulanus: Exiguobact : Oceanobaci : Des : Des : Blo2692 :	MHQANIAEIKKN MSMTQLPLAQLKKN MTTSTNTQQQATLRKQ MTNHTATQKQASLKKQ MTLEKTKNLKKQ MTLENTKNLKKQ MTLENTKNLKKQ MSKEKIAQLRKS MTEQTIAHKQKQLTKQ MTEQTIAHKQKQLTKQ MTEQTIAHKQKQLTKQ MTLENTKNLKKQ MTLENTKNLKKQ	APERSDAKKSI APYEKINT'SSVI GOSSANS'KSI AAPSGADT'HSVI APEKSTI'KSVV APPEKSTI'KSVV APYEKPNT'NSVV SPEKADI'VSVV SABAKSDK'TSI AABAQPET'NSI AABAQPET'NSI AABAQPET'NSI AABAQPET'NSI APEKSTI'KSVV TPYEKSDLQKST	QLINTLOP QLINTLOP QLFNTFIP QLINTVPP QLINTVPP QVINTLVP QLINTLPP QLINSILPP QLINTFIP QLINTFIP QLINTFIP QLINTFIP	ILLWYGAYLS JILWYGAYLS ILLWYGAYLS FGLWELAYFS IILWYLAYKS FMLWENAYES IVANTAYOA FFLWELAYCS FGLWELAYES IILWYLAYKS FILWCLAYKS	LSVSYWLTL LSVSYWLTL LNVSYLITL LSVSYLTL LSVSYLTL LSVSYWLTL LSVSYWLTL LSVSYWLTL LSVSYWLTL LSVSYWLTL LSVSYWLTL LSVSYWLTL	PIJIVAAGEVI PIAIVASGEVI AFTVIAAGELI AFTVIAAGELI VUSULAAGEVI VUSVIAAGELI VUSVIAAGELI VUSVIAAGELI GITVIAAGELI GITVIAAGELI AISIVAAGELI	RTFIIGHDCH RTFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH RIFIIFHDCH	2S : 78 2S : 80 2S : 82 2S : 78 2S : 76 2S : 78 2S : 82 2S : 82 2S : 78 2S : 78 2S : 82 2S : 82 XS : 78 XS : 78
B.brevis : P.polymyxa : B.pumilus : B.anthracis: B.thuringi : A.migulanus: Exiguobact : Oceanobaci : Des : DesL : Bl02692 :	PFKNREANT VGTITG FFKSREINDIVGTITG FFKSREINRIGGFIGV FFKSREANRIVGTCMGV FFKSREANRIVGTCMGV FFKSREANRIVGTCMGV FFKNLEANRIVGTITG FFKNLEANRIVGTITG FFKNLEANRIVGTITG FFKSREANRIVGTCMGV FFKNKEANRIVGTCMGV FFKNKEANRIVGTCMGV	100 TILVEYEOWKNS ITICFYLOWORD LTIFEYLOWORD LTIFEFDOWGHB LTIFEFDOWGHB LTIFEFDOWGHB LTIFEFEWKRD LTIFEFDUWGHS LTIFEFDUWGHS LTIFEFDOWGHB LTIFEFDOWGHB LTIFEFDOWGHB	IZ NIHHATSSN SIHHATSSN SIHHATSSN SIHHATSGN SIHHATSGN SIHHATSSN SIHHATSSN SIHHATSSN SIHHATSSN SIHHATSSN SIHHATSSN SIHHATSSN	10 ILDKRGVGDLW ILDKRGTGDIW ILDKRGTGDIW ILDKRGTGDIW ILDKRGTGDIW ILDKRGTGDVW ILDKRGTGDVW ILDKRGTGDIW ILDKRGTGDIW ILDKRGTGDIW ILDKRGTGDIW ILDKRGTGDIW	* 1: MLTVMBYLE; VLTVESYTE! LLTVKSYQE MMTVKSYNE; TLTVDSYVA; VLTVBSYVS; VLTVBSYVS; VMTVDSYTE; MLTVMSYKA; LLTVNSYKA; LLTVBSYKE;	40 SPIMRKI AYRI ASTWTRI AYRI ASAWTKPRYR ASAWTKVRYRI ADIRLRI AYRI APFRLRI AYRI ASFWRI AYRI ASFWRI SYRI ASFRTKI AYRI APFRLRI AYRI ASFKTKI AYRI	* 160 YRNEI VMFGLGI YRNEWMFGLG YRNEF VMFILGI YRNEF VMFGLG YRNEF VMFGLG YRNELVMFGLG YRNELVMFGLG YRNEF IMFILG YRNEF IMFILG YRNEF IMFILG YRNEF MFILG YRNEF VMFGLG YRNEF VMFGLG	PI : 160 PI : 162 PI : 164 PI : 164 PI : 160 PI : 160 PI : 160 PI : 164 PI : 164 PI : 164 PI : 164 PI : 160 PI : 160
B.brevis : P.polymyxa : B.pumilus : B.amyloliq : B.anthracis: B.thuringi : A.migulanus: Exiguobact : Oceanobaci : Des : Des : DesA : Bl02692 :	* TJELI QYRFNUK RARR TJELI SYRFNUK RARR YVFLI CNRFNUK GARR YVFLI CNRFNUK GARR YVFLI KNRFNRK GARR YVFLI KNRFNRK GARR YLFLI SYRFNR GARR YVFLI INRFNRK GARR YVFLI INRFNRK GARR YVFLI INRFNRK GARR YVFLI KNRFNRK GARR	180 * ERLNTYITNISI ERMNTYFINISI ERMNTYFINISI ERMNTYFINISI ERMNTYINISI ERTNTYFINISI ERTNTYFINISI ERTNTYFINISI ERNTYFINISI ERWNTYFINISI ERWNTYFINISI ERMNTYFINISI ERMNTYFINISI	20 GLYAVLIWA ALYAL LCWA LLAGATYLL ILAAACLI AVVA LCWA GLAG LCWI VLYS MIYI ALYACLI ALAACCLI ALAACCLI ALAACCLI ALAACCLI ALAALCWA	10 VGWQA = LLT Q FGWBG LLVV VGWBN = LLVQ VGWBN = LLVQ UGWGS = LLVH VGWGA = LTVQ VGWQA = LTVQ FGWQS = LLVQ I GWQS = LLVQ I GWQS = LLVQ	* 2. CPIFPVSCH CPIFLSCS CPIFLSCS CTIFLACS CSIFLACS CSIFLACS CTMFTACV LPIFTACA CPIFLSCS CTIFLSCS CTIFLSCS CTIFLSCS	20 FGIWLFYVQH GGWLFYVQH IGVWLFYVQH IGVWLFYVQH IGWLFYVQH IGWLFYVQH LGWLFYVQH GGWLFYVQH IGVWLFYVQH IGVWLFYVQH UGIWLFYVQH LGIWMFYVQH	* 240 YEDSYFBNEEE FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN FEDSYFBADEN	NS : 242 NS : 244 NN : 246 NE : 242 NE : 242 ND : 240 ND : 242 ND : 242 NS : 246 NS : 246 NS : 246 NS : 242 NE : 242 NE : 242
B.brevis : P.polymyxa : B.pumilus : B.amyloliq : B.anthracis: B.thuringi : A.migulanus: Exiguobact : Oceanobaci : Des : Des : DesA : Bl02692 :	* YVKAAVEGSSYYKLPK YVQAAVEGSSFYKLPK YVQAAVEGSSFYKLPK YVKAAVEGSSFYKLPK YVKAALEGSSFYKLPK YVKAALEGSSFYKLPK YVKAALEGSSFYKLPK YVQAAVEGSSFYKLPK YVQAAVEGSSFYKLPK YVKAAVEGSSFYKLPK YVKAAVEGSSFYKLPK	260 LQWLTGNIGFHHV LQWLTGNIGFHHV LQWLTGNIGYHHV LQFLTGNIGFHHV LQFLTGNIGFHHV LQWTTGNIGFHHV IEWITGSIGFHHV LQWLTGNIGFHHV LQWLTGNIGFHHV LQFLTGNIGFHHV	28 HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN HHLSPRVPN	10 INNERALEAT IVNERALEAT IVNERALEAT IVNERALEA IVNERALEA IVNERALEA IVNERALEAT IVNERALEST IVNERALEST IVNERALEST IVNERALEAT IVNERALING	* 3 PELQKATTI PLQKATTI EPLQNVPTI EPLKNVPTI LPLKNVPTI LPLKNVPTI PPLQKATTI PPLHKATTI EPLKNVPTI LPLKNVPTI EPLQNVPTI	00 TLAASVOCLAR TVKSSLOSLAR TVKTSLOSMAR TVKTSLOSMAR TTKTSLOSMAR TTATSLRSLAR GUFSSLKSLAV TTLTSSOSLAR TUKTSUOSLAR TUKTSUOSLAR TUKTSUOSLAR TUATSLOSLAR	* 320 HLWD SNKRFV RLWD STKKFV RLWD DEKQFV RLWD DEKQFV RLWD KSNNFV RLWD KSNNFV RLWD SNNFV RLWD SNNFV RLWD SKKFV RLWD DKKFV RLWD EKKQFV RLWD EKKQFV RLWD SKKFV	SF : 324 IF : 328 IF : 328 SF : 324 SF : 324 SF : 324 SF : 324 SF : 324 SF : 328 SF : 328 SF : 328 SF : 328 SF : 328
B.brevis : P.polymyxa : B.pumilus : B.amyloliq : B.anthracis: B.thuringi : A.migulanus: Exiguobact : Oceanobaci : Des : DesL : BesA : Bl02692 :	* 340 GTKRLTAARSEAIMDA QVKPLLSKR-VAVTKE KEARKTPSPRC KEVKNIIKN KVKNIIKN KETKDFSIEPKKS DIKHLLRE	* LLQGSKPSLEGK : VIFDDVSTERK : VIKGDI KKNA- : NVSVRVKSEL- : SVSVHFKSEL- : SVSVHFKSEL- : -PKTSI : PERCKIRKNA- : VERKKIRKNA- : TKKQKIRKNA- : NVSVRVKSEL- : QVSAQLRTD :	352 353 349 343 343 343 343 345 338 349 352 352 352 343 342					

Figure S2. Multiple sequence alignment of full-length $\Delta 5$ 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. 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. 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 35. Mass spectra of DMDS derivatives of the farty actus separated by GC of iso-C10.1, in-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 C₁₆ and C₁₇ monosaturated fatty acids, respectively. Two prominent ions are formed by cleavage between the methylthio-substituted (CH₃S) 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 $\Delta 5$ in the iso-C16:1 and n-C16:1 fatty acids. The ions observed at m/z 161 and m/z 215 indicate a $\Delta 5$ double bond in the iso-C17:1 fatty acid. A major ion at m/z 129 is due to loss of methanol (CH₃OH) 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 [¹⁴C] -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 μ Ci ml-1-[¹⁴C] 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.

					G90	W104
	* 20	* 40	* 60	* 80	* 100	* 120
	B.amyloliq :WTNHTATQKQASLKKQVAAFS	GADTKHSVIQLFNTFVPFF0	GLWFLAYFSLNVSYLLTLAF	VIAAGFLTRIFII <mark>FH</mark> DC <mark>CHQ</mark> S	FFKQKRLNRLF <mark>G</mark> FISGVLTLI	FPYLQWQR <mark>SHSIHHAT</mark> SSNLDK : 124
	B.thuringi : TLENTKN KKQVAPFE	KSTIKKSVWOLINTIVPFI	ILWYLAYKSLSVSYWLTLVP	LLAAGFMTRIFIIFHDOTHYS	FFKSRRANRIVGTCMGVLTL	FPFDQWGHEISIHHATSGNLDK : 120
	B.pumilus :WTISTNTQQQATLRKQVGQFS	GANSKKSIIGLFNTFIPFL KAVMKDSIROLINTFIPFF	ELATISTICTURES STOLETING	VIAAGHLVRVFIIFHDOCHVS	FFKQKPLNHLFGFIIGVLILI	PYLOWORDEST HEATSSNLDK : 124
	A.migulanu :MTKQNNLRKHIAPYE	KPNTKNSVWQVINTLVPFF	1LWFMAYESLSISYVLTFVLS	SVIAAGFLVRIFIIFHDCCHQS	FFKNQLANKILCTITGILTL	CPYEQWRRSHSIHHATSSNLNK : 118
	0.iheyensi :MSKQKQAQURKSVSAFA	KSDKRTSIIQLINSILPFFI	LWFLAYQSLSISIWLSLGVS	SVIAAGFVVRIFII <mark>FH</mark> DCTHMS	FFK <mark>NAKANRIVE</mark> TITGIITH	FAFEKWKR <mark>DE</mark> AIHEA <mark>T</mark> SSNLDK : 120
Δs	B.brevis :HQANIAE KKNVAPFE	KSDMKKSIROLINTLVPLII	LWYGAYLSLSVSYWLTLPIV	VIVAAGEVIRTEII <mark>CHDCCHQ</mark> S	FFKNRRANDIVGTITGVITL	/PYEQWKNS:NIH:ATSSNLDK : 120
	Exiguobact : SKEKIAO RKHWSPFE	KADIKVSVROMINTILPFIA	JEWYAAYLSISVSYWITEPIA ZAWFLAYOALOVSVWITTPLA	ATTASGEVVRIFITEHDCGHQS	FFKNKKANAVVETINGVITI	PYHOWKNSHALLHALSSNIDK · 122
	B.anthrac5 :MTLEKTKNLKKQVAPYE	KSTIKKSVWQLINTVVPFI	ILWYLAYKSLSVSYLLAVVPS	SLLAAGFMTRIFIIFHDCTHYS	FFK <mark>SRRANRIVG</mark> TCMGVLTL	PFDQWGHDHAIHHATSGNLDK : 120
	B.lichenif :MTEQTIAHKQKQLTKQVAAFA	QPDTKNSLLQLLNTFIPFF0	SLWFLAYFSLNVSYLLTLGI	TVIAAGFLTRIFII <mark>FH</mark> DCC <mark>H</mark> LS	FFKQKRLNHIL <mark>C</mark> FLTGVLTLI	PYLQWQHSHSIHHATSSNLDK : 124
	B.subtilis : TEQTIAHKQKQ TKQVAAFA	QPETKNSLICLLNTFIPFFC	SLWFLAYLSLDVSYLLTLALT	VIAAGFLTRIFIIFHDCCHQS	FFKQKRYNHILCFLTGVLTL	FPYLQWQHSESIHEATSSNLDK : 124
	BL DeltaX : NDONLKT RKLVTPYE	KSDLOKSTYOTINTLIPFT	LWCLAYKST STSYFT TLATS	STVAAGELVRTEITEHDCCHYS	FFKNKKANRIUGTITGILTL	HPFDHWGRD STHEADSSNIDK : 120
	G.thermole :MNDFHPFGWYAAKMAPHL	PKKAFQPVKSRLFGGLAYVGLV	AGILAVSLFHLHPVWNLLIS	SVVLGFSFAALGFL <mark>GH</mark> EILHGT	VVRTPWLRDLLCAIAFWPLC	GPKLWRKNENASEHVHTQHEE : 124
$\Delta 10$	BC_0400_de :MKELHTFGWYAARVSPHL	PKKAFKPVPTRLFGGLAYLLVAI	AGLISIGVFELNVWANLGI	IVLGLCFASLGFL <mark>GH</mark> EIL <mark>D</mark> GT	'VVRKAWLRDFL <mark>C</mark> AIAFMPLS'	IGPKLWRKWENATEHVHTQHEE : 124
	B.anthracd : KELHTFGWYAAR SPHL	PKKAFKPVPTRLFGGLAYLLVAI	AGLISIGVFELNVWANLGI	AIVLGLCFASLGFL <mark>G</mark> EIL <mark>I</mark> GT	VVRKAWLRDFLCAIAFMPLS	IGPKLORKNINATEHVHTQHEE : 124
	* 140 *	(149	180 K172 *	200 G201	220 H228	240 * 2
	B.amyloliq : RGTGDIWMMTVKEYNEASAWTKV	RYRLYRNPFIMFILGPIYVFLI	NRFNVKGARRKERWNTYI	TNAAIVILAAAACLLVGWENF	LLVQGPIFLISGSIGVWLFY	/Q <mark>H</mark> TFEDSYFE <mark>ADEH</mark> WNYV <mark>Q</mark> AA : 251
	B.thuringi : RGTGDIWTLTVDEYVAAPFRLRL	AYRLYRNPFVMFGLGPIYVFLL	INRFNR <mark>K</mark> GARKKERMNTYI	JTNILIVAIIGMLCWAICWQSF	LLVHGSIFLIAGSIGIWLFY	QHTFEDSYFEEDKEWEYVKAA : 247
	B.pumilus : RefediwLLIVKEYQEASAWTKF	RYRFYRNPFVMF1LGP1FVFLL	INRFNVKNARKKERWNTYF	TNISIVLLAGATYLLFGWEGL	LLVQGPIFLISGSIGVWLFY	OFFEDSYFEADENWNYVQAA : 251
	A.migulanu : RETEDYWVLUVEDWVSSPFWLRL	AYRMYRNPLVMFGLGPIYIFLIS	SYRFNTKDARLKERINTY	TNLSIVGLAGLLCWTIC WESF	LMIOGPIFFISGSLGIWLFY	OFEDSTFELEEEWDYVKAA : 245
ΛE	O.iheyensi : RGTGDVWVMTVDEYTEASFWGRL	SYRLY <mark>R</mark> NPIIMF <mark>G</mark> LGP <mark>AYLFLIS</mark>	SNRFNR <mark>K</mark> GAKRKERWNTYI	JINFSIVAIYGLLIWAI <mark>G</mark> WQAF	LIVQLPILFIAGAAGIWLFY	/Q <mark>H</mark> QFEDSYFE <mark>NEDE</mark> WDYVKAA : 247
Δэ	B.brevis : RGVGDLWMLTVNEYLESPIWRKI	AYRLYRNPIVMFGLGPIFVFLLQ	YRFNV <mark>K</mark> RARRKERLNTYI	JTNLLLVGLYAVLIWAVCWQAF	LLIQGPIFFVSGLFGIWLFY	QHTYEDSYFENEEEWSYVKAA : 247
	P.polymyxa : RGTGDMWVLTVEEYTEASTWTRL Exiguobact : RCVGDIWVMTIFEYVGASKWTRL	AYRIYRNPWVMFGLGPIYTFLIS	SYRFNIKTAKRKEKMNTHU	TNISIVALYALLCWAICNQAF	LLVQAPIFFVSGMLGIWLFYV I IVOGTTMETAGVLGIWLFYV	QHQFEDSYFEHDEEWSYINAA : 249
	B.anthrac5 : RGTGDIWTLTVDEYVAAPLRLRL	AYRLYRNPFVMFGLGPIYVFLL	INRFNRKGAROKERMNTYI	JTNIIIVAVVAILCWAIGWOSF	LLVHGTIFLIAGSVGIWLFY	OHTFEDSYFEEDKDWEYVKAA : 247
	B.lichenif : RGTGDIWLLTVNEYKAAPLRTKI	AYRLY <mark>R</mark> NPFIMFILGPIYVFLIT	INRFNK <mark>K</mark> GARRKERVNTYI	JTNLAIAALAAVCCLIF <mark>C</mark> WQSF	LLVQGPIFLISGSIGVWLFY	/Q <mark>H</mark> TFEDSYFEADENWSYV <mark>Q</mark> AA : 251
	B.subtilis : RGTGDIMLIVNEYKAASRRTKL	AYRLYRNPFIMFILGPIYVFLI	INRFNKKGARRKERVNTYI	JTNLAIVALAAACCLIFCWQSF	LLVQGPIFLISGSIGVWLFY	QHTFEDSYFEADENWSYVQAA : 251
	BC_2983 : RETEDIWITTVDEYVAAPFRLRL BL DeltaX : RETEDIWLITVEEYKEASTKTKI	AYRLYRNPFVMFGLGPIYVFLL MYRLYRNPFVMFMIGDIYVFGI	INRFNRKGAROKERMNTYI	TNIIIVAVVAILCWAIGNQSF	LLVHGTIFLIAGSVGIWLFY LLVOAPIFMISCSLCIWMFY	OHTFEDSYFEEDEHWEYVKAA : 247
A 1 O	G.thermole : KDPDAWPSMEKLAKSRLLSWVYR	IPFPIRASFAFSS SVMFTVHS	RMLFYFF <mark>K</mark> DFRRKNRAVVLF	QFFLPWATWLGLLWLVGWEKW	FFAFLLPLLVANTIVMSYIS	INHRLNPLVPVNDP : 245
$\Delta 10$	BC_0400_de : NDPDAWPTLEKLKKSKFLSWVYR	MPLHV <mark>R</mark> SFFSFLSLTIQFTLHST	RMFFHFI <mark>K</mark> EFKSSNQKSVWI	QLLLPWTVWISLLFIM <mark>C</mark> PGKW	LFAYVIPLLIANFIVMAYIA	INHRLNPIVPVNDP : 245
	B.anthracd : NDPDAWPTLEKLKKSKFLSWVYR	MPLHVRSFFSFLSTIQFTLHS	TRMEFHFIREFKSSNQKSVWI	QLLLPWTVWISLLFIM <mark>C</mark> PGKW	FAYVIPLLIANFIVMAYIA	INERLNPIVPWNDP : 245
	₆₀ PZ57 ₂₈₉		82 L284 320	* 340	* 360	* 380
	B.amyloliq : VEGSSFYKLEKLLQWLTGNIGYH	hvhhilsekven – – ykleaahegi	HEPLKNVPTITLKTSLESMK-	-FRLWDEDEKQFVTFREARKT	PPPRQAPEKPLKKN	A : 349
	B.thuringi : VEGSSFYKLEKILQFLIGNIGF	HVHHLSPRVPNYKLEEAHNN	LPLKNVPTITLATSIRSER-	-FRLWDEKSNNFVSFKDVKNI	IKNNVSVRVKSEI	
	B.megateri : VEGSSFIKLEKLLOWLIGNIGFH	HVHHISERVEN - YKLEAVHOH	OPLONVPTITLATSIRSIR-	-FRIWDEEIKASVISKEIKTR	SAAPKASRISSRTKAE	L: 349
	A.migulanu : LEGSSFYKLPKLLQWITGNIGFH	hvhhlsprvpnYyleevhnni	WSLQNVQTITIASSLRSLR-	FRLWDEQNKKFVGFKEIKDF	SIEPKKSSVSVHFKSEI	: 345
ΛE	0.iheyensi : VDGSSYYKLPKAIEWITG <mark>S</mark> IG <mark>Y</mark> H	HVHHLSPRVPNYHLEKAHES	PPLHKATTITLLTSLQSLR-	-FRLYDENSKSFVSFKQFKQM	ENKKKLMHQSKPSRTSLQK	: 349
ΔJ	B.brevis : VEGSSYYKLEKVLOWLIGNIGH	HVHHLSPRVPN YNLBKAHNE' HVHHLSPRVDN YNLBKAHEA'	PPLQKATI TLAASVQCLR-	-FHLWDESNKRFVSFKGIKRL	TAARSEAIMDALQGSKPSLE(352 28:: 352
	Exiguobact : IEGSSYYELPKVLQWVTGNIGTH	HVHHLSPRVPNYNLEKAHTA	PPLQKATTIGLFSSLKSLR-	-YKLYDANNMTFVTFRDIKHL	LREPKTSL	: 338
	B.anthrac5 : VEGSSFYKLEKILQFLTGNIGFH	hvhhlsprvpnykleeahnn:	LPLKNVPTITLATSLRSLR-	-FRLWDEKSNNFVSFKEVKNI	IKNNVSVRVKSEI	: 343
	B.lichenif : VEGSSFYKLPKLLQWLTGNIG	HVHHLSPKVPNYKLETAHEQI	HEPLKNVPTITLKTSLQSLA-	-FRIWDEEKKQFVTFRDMKQT	SSHPSPDSTKKQKLRKNA	A : 352
	B. SUDCIIIS : VEGSSEMKIENLLQWLTGNIG	HVHHISPRVPNMKIDDVAHEHI HVHHISPRVPNMKIDEAHNN	1EPEKNVPTTTLKTSLQSLA- TLPLKNVPTTTLATSLOSLR-	-FRIWDEDNKOFVSFRAIKHI	TKNNVSVRVKSRI	: 352 : 343
	BL_DeltaX : VEGSSFYKLPKVMQWLTGNIGFH	HVHHLSPRVPNYKLEEVHNNI	IEPLQNVPTITLATSLKSLK-	-FRLWDEESKKFVGFSHLKKA	SKSQVSAQLRTD	: 342
A 1 O	G.thermole : LANSLSVTVEKWVDILHFHFSH	TEHHLFPAMSSKYYPIVKTYIK(MWPDRYHEMPMGKALAALWF	TPRVYYEHNELIEPKQGHVYG	TLGNGLDPDRIVHRELETEK	PRSAKKRAKTKKAAGQGNL : 372
$\nabla 10$	BC_0400_de : LANCLSVTVPRWVDVLHFNFS	TEHHI PAMSSKYNPIVKEKIKI	MWPERYHEMPMTKALAALWI	ITPRVYYQGSELVDPHREHFYG	SLGNGLDPHNISYREEHIEEH	E-KSIKKVNQ : 361
	B.anthracd : LANCLSVIVERWVDVLHFMFSY	TEMPTORESKYNPHYKEKIKI	SMWPERTHEMPMIKALAALWI	ITEN I YQGSELVDPHREHFYG	SLGNGLDPHNISYREEHIEE	S-ESIKKVNQ : 361

Figure S7. Multiple sequence alignment of $\Delta 5$ and $\Delta 10$ -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. 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	N°T	N-ter	C-ter	Transmembrane segments ubication						His box			
predictor	Ms										1	2	3
НММТОР	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
ТМНММ	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.

	DesA		DesAY	40C	BL0269	02 C40Y	EV	
	SFA	UFA	SFA	UFA	SFA	UFA	SFA	UFA
<c16< td=""><td>64.9±1.6</td><td>nd</td><td>67.4± 3.7</td><td>nd</td><td>71.2±3.9</td><td>nd</td><td>70±3</td><td>nd</td></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

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

^aCells were grown at 37°C in Spizizen salts MM supplemented with glucose to exponential phase and then shifted to 25°C. 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	GG <u>CTCGAG</u> GAAAGGAATTAGAACAATG	This study
Bc2983-low ns	CCGAATTCGCAATTTTAGTTCAGATTTCAC	This study
BL02692-XhoLup	CTCGAGATGAATGACCAAAATCTAAAGAC	This study
BL02692-low ns	CTCGAGTTTATCCGTTCTTAGTTGCGC	This study
Belozoji iowins BsDes XhoI-un		This study
BsDes EcoRLlow	GCTCGGA ATTCTCAGGCATTCTTCCGCAGCTTC	This study
DSDCS LCORI-10W		This study
Des-Bc2983-Up	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)-B102692-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 (N1)-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	TTGATCAGaGGGGGGCATGAACATTCTATTC	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

TTGCAAAATTTTAGcAAGCTTATAAAAAGAACT TCC	This study
TTCACCATcgAAGCCCAAGGGTACCTAAC	This study
CCCTTGGGCTTcgATGGTGAACATGATGGAATC	This study
ATTTAAGCgCAAGGGTACCTAACTATAAAC	This study
TTAGGTACCCTTGcGCTTAAATGGTGAACATGA TG	This study
TACCTAACcgTAAACTAGAAGAGGCACAC	This study
TCTAGTTTAcgGTTAGGTACCCTTGGGCTTAAAT G	This study
CTATAAACgAGAAGAGGCACACAATAATACGC	This study
TGTGCCTCTTCTcGTTTATAGTTAGGTACCCTTG G	This study
AAAAGCATTTGGCAACTCATTAACACATTGATA C	This study
GTGTTAATGAGTTGCCAAATGCTTTTTTGTAAAT CGG	This study
CTGTTATGGTATTTAGCATATAAGAGCTTGTC	This study
CTTATATGCTAAATACCATAACAGGAAAAATGG	This study
GATTTCTTATTGGCTTACATTAGCGATTTC	This study
CTAATGTAAGCCAATAAGAAATCGACAAGCTC	This study
ATTTTATGGTGCCTTGCTTATAAAAGTTTGTC	This study
TTTATAAGCAAGGCACCATAAAATAATAAATGG	This study
CTGTTATGGGCTTTAGCATATAAGAGCTTGTC	This study
CTTATATGCTAAATACCCGAACAGGAAAAATGG	This study
	TTGCAAAATTTTAGCAAGCTTATAAAAAGAACT TCC TTCACCATcgAAGCCCAAGGGTACCTAAC CCCTTGGGCTTcgATGGTGAACATGATGGAATC ATTTAAGCgCAAGGGTACCTAACTATAAAC TTAGGTACCCTTGcGCTTAAATGGTGAACATGA TG TACCTAACcgTAAACTAGAAGAGGGCACAC TCTAGTTTAcgGTTAGGTACCCTTGGGCTTAAAT G CTATAAACgAGAAGAGGCACACAATAATACGC TGTGCCTCTTCTcGTTTATAGTTAGGTACCCTTG G AAAAGCATTTGGCAACTCATTAACACATTGATA C GTGTTAATGAGTTGCCAAATGCTTTTTTGTAAAT CGG CTGTTATGGTATTTAGCATATAAGAGCTTGTC CTTATATGCTAAATACCATAACAGGAAAAATGG GATTTCTTATTGGCTTACATTAGCGATTC CTAATGTAAGCCAATAAGAAATCGACAAGCTC ATTTTATGGTGCCTTGCTTATAAAATCGACAAGCTC TTTATAGGCAAGGCA