

Table S1. Results of pairwise sequence alignments of SMc02162 (*S. meliloti* FadD) with *E. coli* FadD or with different acyl-CoA synthetases of the *S. meliloti* Rm1021 genome^a.

Locus_tag (ID)	Query coverage	Score	% identity	% similarity	E-value	Annotation
FadD <i>E. coli</i>	15–565	557.9	55.7	81.2	3.5e-163	long-chain fatty acid-CoA ligase FadD
SMA0150 (MatB)	54–565	200.2	31.1	59.2	1.6e-65	malonyl-CoA synthetase
SMb20650	39–562	190.7	29.6	57.1	1.1e-52	long-chain fatty acid-CoA ligase
SMc00261	29–565	182.4	27.5	56.1	3.8e-50	Putative fatty acid-CoA ligase
SMc00741 (AcsA3)	60–565	133.9	27.4	52.2	1.6e-35	Putative acetyl-CoA synthetase
SMc04095	61–565	108.7	23.8	53.5	6.7e-28	Putative acyl-CoA synthetase
SMc04093 (AcsA1)	50–565	103.1	21.5	51.0	3.4e-26	Probable acetyl-coenzyme A synthetase
SMc00965	52–565	87.1	23.7	51.5	1.7e-21	Putative acyl-CoA synthase
SMc00592	213–565	78.5	25.8	54.6	1.2e-18	Hypothetical, transmembrane protein
SMc00774 (AcsA2)	61–565	49.6	21.0	50.1	4.5e-10	Acetoacetyl-coenzyme A synthetase

^a*S. meliloti* Rm1021 protein sequences and their annotation were obtained from

<https://iant.toulouse.inra.fr/bacteria/annotation/cgi/rhime.cgi> (accessed on March 18, 2020). Data of pairwise alignment of SMc02162 with the different ORFs were obtained at the server <https://www.ebi.ac.uk/Tools/psa/lalign/> (accessed on March 18, 2020).

Smc02162 1 -----MAEASTQQAGSSTAKIWLGSYPGPVPAEIGPLT-----
 FadDec 1 -----MKKVWLNRYPADVPTEINPDR-----
 Sma0150 1 -----
 Smb20650 1 -----
 Smc00261 1 -----MTNNEPKGGVT--PVSTR-----
 Smc00741 1 -----MLPKIEDYDLYREFRWIRPERFNIGVAVSDAWAARDPERIC-----
 Smc04095 1 -----MASRYSEVYAANK--TDPHGFWADAASAIWFKRPERIFE--PAGGT--YGHWFDPDGV
 Smc04093 1 MD-----VKYTPV--LEAAKNRTLDDNATYLEWYRESV--ADPEKFWGEHGKRIEWFEPYTKVKNTSTEGDV--SIKWFEQDGL
 Smc00965 1 -----
 Smc00592 1 -----MGGS
 Smc00774 1 MQAERPLWVPDREIVERSPMAEFIDWCGERFGRSFADY--DAFDHWSVSRGAFWTAWEHCKVIGESGEKA--LVDGDRMLDARFFPEAR

Smc02162 34 ----YRSIEGFF--DHAVAQYSWRP-AFTCMGKALTFSIDNTHSAKIGAWL--QSLGLAKGDRVAAMME--NILQNFVIMYGLIRAGTIV
 FadDec 22 ----YQSIIVDMF--EQSVARYADQP-AFVNMGEVITRRKEERSRAFAIY--QQGLGILKKGDRVAAMME--NLQYFPVAFSILIRAGMIV
 Sma0150 1 ----MS-NHLFDA-IRRAARPDSAF-I LTADGFWMTGDLLEHSGHISVL--DALGRFSDRVAQME--KSPREALYLCILIRGAVY
 Smb20650 1 ----MRFEQFL--IRNAAANGAKT-ALVTDRRRLGSAEITDDLSTRLAAAF--AENGAKNDORVLVEMD--NCWEAAAAFAILIRAGATF
 Smc00261 17 ----VMNINANFL--SQAARNPDEI-ALVHGDRRWRWSENEARVDAMAYAV--VHEFGVRKGRDLVHSA--NCNQMFESLFAAFRAGAVW
 Smc00741 43 ----IQ-----HF-SPDGAHLALTGDFAARSSAFGGG--AAHDVSGSERVALLIF--QGFEAAIAHARIYRIGAI
 Smc04095 53 TMTCHNCIDRHVEAGRGEQLAFIYDS-PVTGRIERISVADILADVKAMAIY--RKLGVKGDGRILLYMP--MIPQAAIAIAAARIRGAVH
 Smc04093 73 TNVSYNCIDRHLKT-HGKERTAIWEG-DNPYLDKKITNNIYDVCRLANVL--KEQGVKKGDRVITYMP--MIPEAAYAIAACARISAIH
 Smc00965 1 ----MPDAEAV--ALHASERPHSP-AFRMEGRVITPGLTAAGAKHILPAW--QRSAAEATERSISGE--VRLV-----ALE
 Smc00592 5 GELSVSPFG-----VASLAARGDRPALLLRGGRIVSTRITAEERIDILLSRW-----RGDRCLTYTEADLSHAIVAYLALAEAGHA
 Smc00774 88 LNFAENILR--KTGSGDA--LIFRG--EDKVSYRLTDEIRALVSLQQA--RAQGVGAGDRVAAMME--NMPETIALATASVGAIW

Smc02162 113 VMNPLTPRELEHQ--LVDASAKAIFVLENFAHTVEQVLAR-----TEV-----KHVVVASMGDMLG-AKGAIVNLVVRVKKLV
 FadDec 102 VMNPLTPRELEHQ--LNDSGASAIIVSNFAHTLEKVVDD-----TAV-----QHVLTLMGQQLSTAGTKVNVFVKYIKRIV
 Sma0150 80 LPLNTATLAEIDYF-FGDAEPRILVCPAGAKGIAKHAAD-----CG-----AEVE-----TLD
 Smb20650 79 SPINASTKADKLAYI--VADCEFAAILTQAKLMPVVAEALAL-----APGHA--FFV--ASTAA
 Smc00261 97 VPTNFRQLPEEVAYL-AESSARLIFQAAFEAHAEACRAA-----GEQIG--SCI-----
 Smc00741 107 LPLALLGVEADAYR-LKDAASAAVVTNRFGRYERLAAIRGE-----LPEL-----RMV-----VLA
 Smc04095 139 SVVFGGAANLEAMR-IDDCCAKIVSASCGLPEGRTVAY--PLLQIAITASHKPA--RCLTYQRDLMAAEMVSG-----
 Smc04093 158 SVVFGGSPEALAGR-IYDCESTFVITCDEGVRGKPVALLNENTDAIDIAARQHVTVSKVLVVRRTGKGVGWAPG-----
 Smc00965 67 TGNHPLPAAFIAATAAGHCLIDPHLPEIVRRMRKERL-----PEVVRREGDILLRI-----
 Smc00592 81 ----VALCSTASLRDREVF--R-----PEYCYRRF-----
 Smc00774 168 SSCSPDGEQGVLDLR-FGQILPKLFIIVCDGYWNGRKQDVDSKV-----RAVAKSLGAPTIVVPYAGDSAAALAPTVE-----

Smc02162 186 PAWSIP--GHISFKTV-----AKGATLGFKKRPVAPGDVAFIQYTGTTGVSKGATLTHANLLSNMAQOMELWLNTAFLRKPRPESLTFMCA
 FadDec 176 PKYHLP-DAISERSAL--HNGYRMQYVKPEVPEDAFIQYTGTTGVSAGKAMLTNRNLANLEQVNATY--PLLHPGKELVVTA
 Sma0150 129 EK-----GS-GSLIDI--ARGKAPDFPADRGDDPDAALYISGTTGSRKAGMLTHDNLSNATTLREYWRFT-----ADDLIHA
 Smb20650 132 PGGRIPGAASEECC-----TAA--PAPVRHGGIDVDDEMIIYTSSTGTRPKGVMTNRNIDAASISITYLRNT-----P-DDIILNV
 Smc00261 145 PIGSSR-VGEDDAIV--ARNLGRSVSPVARDDDPCMYFYTSSTGTRPKAVLTHGOMAFVNN--HIGDL--FPATTHDRSIVV
 Smc00741 157 EDEKXP--GTRFRDIA--AGQ--GRFDPATKPKDDPALIYTSSTGTPPKGALHCHRVLLGHIPGFQFHHH-----FLPQPGDRMWT-P
 Smc04095 211 ----RDDEFAEAAARADAGEEASCTP--VASTDELYLYTSSTGTPQPKGVVRDNGGHHVALRWSME-----HFFGVNA-GDVFWAA
 Smc04093 233 ----RDLMWHQET--AAAEHPCHCEKQNAEDPLFLYTSSTGTPKPGVLHTGGYLYVYASMTHQ-----YFVDQD--GVYWC
 Smc00965 122 ----DVPS--GQSRIL--QGSDAPPAIPAGDGGEPFLIVTSSTGTEPPFIIRNRSSVRSVKTGGQF-----FGLGP-ETTTYAP
 Smc00592 106 ----DGRWRMER--ESDAI--APHETAVLLSSTGTHGKVVRLSEMNLQSNARSIAEYLGTL-----AADRGCLI
 Smc00774 239 ----GGTLADFI--AGFQAGPLVFER--PFGHPLYLFSSTGTPVPCIVHSGAGTLLQHLKEHR-----FHCLGRDGERLFY-F

Smc02162 271 LPLYHIALTVNSLMGLATGGNNLLIPNPR-D--IPA-FVKELGRYRTNIFPG-----LNLFNALMNN-SH--FRKLFSSLLITF--
 FadDec 257 LPLYHIALTINCLLFELIGGONLLITNPR-D--IPG-LVLELAKYFFTAITG-----VNLFNALNN-KH--FQQLDFSSLHSA--
 Sma0150 202 LPLFTHLELVASNVILLAGSMFFLPKKD-A--NE--VLRU-MPOSTSMMG-----VPTFYVRLQNP-G--LTHEATAGVRFV--
 Smb20650 209 LPLAFDYGLY-QLLMARLIGATLL-LEKSF-A--FPQAIFFRTRTEFVTGFP--VPTMAAMLQMR-RD--LEPGFLSLRLYS--
 Smc00261 225 APLSHGALHQ--LCQVARGATTLLPSEKLD--IPQ-FWALVEKRVNVLFA--VPTIKVLLD-PG--VDRYDHSRLRYVI--
 Smc00741 235 ADWAWAGGLNALLPSLFFGVPVSSP-----AQKFAHTAFRIIEEMEVRNARIPPTALRLKS-VH--RPRRCALRRTVG
 Smc04095 286 SDIGWVVCHSYIYVGBLLNGCTSILFEGKPVGTTPDGPTVYVVSERGVAMFT--APTALRAKEDPAAHAGRYLISFRALY--
 Smc04093 305 ADGWVTVCHSYIYVGBLLNATTTLMFEGVP-NFPDAGRFVWVDKHLVNIFFY--APTALRSIMAGDIFV--KRSSRSSLRLIG--
 Smc00965 176 GLAHLGLTLTA-LAETLTAGAEFFGARHFE--ADQVLTAATGAKRRLV--VPTMLRRICERAAG--SPLTSSGQIT--
 Smc00592 191 LPLHYSYGLSV-LNAHLAVGASVYVPGCSIL--DEGFLDELAQASSNFAG--VPYSYDLLEKA--G--FRARFPALRMT--
 Smc00774 312 TTCGWM--WNWLASGLAVGTLCLDYDGP-FCPDGNVLFDYAAAEFAVFGT--SAKYIDAIRKG--GFTPARTHLLSSLRMT--

Smc02162 346 GGGMAV--QRPVAERWLE--LFGCIHBSYGLSETSPVATAN-----RLDTDDFTSTIGIPLPSTEYIRDE-DGRTLE-VSEIGE
 FadDec 332 GGGMBV--QQVVAERWVK--LTGQYHDEYGLLECAPVSVN-----PYDLYHSGSIGLPPSTEARLVDD-EDNEVP-PQPGGE
 Sma0150 274 SSSAPL--LAETHRTFAQ--MTGHAIFERYGMETN--MNTSN-----PYDGERIAGTVEPFLGVSIVADAPESGGRPL-KGEVGM
 Smb20650 283 NTAAMP--PAHIALRLREL--FPEARVYSMYGLDECKRCTYLP-----PEELDRRPGSYGIAENTEAFVDD--EGNRVP-PGVGGE
 Smc00261 299 YGCAFP--YRADQKKALE--KLCAYVQYELGEBVTGATVLPFAFHSSDGGFARIGTCEERTGMQLIQODE-DCNEVP-ACATGE
 Smc00741 311 SAGEAL--GRETFEWARA--ALVEVSEFTGQTECNIVISSA-----TGLGVAKAGSMKAAEGHQVALIDG--EGRVLE-PSTVGG
 Smc04095 369 LAGERA--DPDTIRWAER--ALKVPVICHWQETGTGWPVAGNPLGL--GLLPVKYGSPPVPLDEYDVQVVD--AGHPVE-TGTEN
 Smc04093 385 TVGEPT--NPEAWEWYHVVGDERCPVMTWQIETGGILITPLP--GATDLKPSATREFFGVQPOIVDS-DGRVVD-GRADGN
 Smc00965 267 VAGAKLTADRDAARR--A--FPEAQVTEYVGASELGFVSVSR-----AKDRHTPTAVKASEGVRITRDE-RGERLE-AGETGT
 Smc00592 244 VAGGRJ--APELVRRYNEHLSARNARFVMYGQTEATARMAYMFP-----DRLRGREDHICATPEGSTIEN-DGRHSSADQPGGE
 Smc00774 390 STGSPL--SPEGFSFVYEGI-KPDVQLASISGNDIVSCFVLGN-----PLKPVWRSEIQGFLGLAVDWN--EGNVR--SEKGE

Smc02162 421 LCLRGP--QVMAGYQRPDETARAI SPD-----GEFTGDSYGMNAEELTRIVDRKXDMILVSGFNVSSENEIEVAATHHGLLECAALG
 FadDec 407 LCLVGP--QVMAGYQRPDETDE-I IKN-----GMLFTGDELYMDRESEFLRIVDRKXDMILVSGFNVSNELEDDVVMQHGQVQVAAAG
 Sma0150 349 LCLVGP--NVERGYRMPEKTQGEFRAD-----GEFTIGDLRLDERSEYVHIVGRKDDIISGGNIVPREVETEIDQMPGVVETAVIG
 Smb20650 359 LVRGP--HVMOGYVRNDAAETERMLRPGNPWEKVLITGDLRTDEEGFLYFVGRKDDIKTRGKGVAKRDEFTVLHAHPGSAFAVAVIG
 Smc00261 381 LCLVGP--AVFAGYVRNEANAKAFRNG--MERTGDLGHVDAQFLYITGRASDMYISGGSNVPERIEKLLMHDPDSEAAVIG
 Smc00741 386 VAIKRP-DPVFIYFYVRNEATEAKFIGD-----MMTGTQGVMDRESEYFTFFGRDDDVITSSGYRICESGDCLAGHDDQLAAAG
 Smc04095 447 VVNLPLPPGCPPTLNNADHRFHAAY--LEEYPCGKTAIDACTDEDSYIFINARTDDIINVACHRLSTGAEVGCASHDDAPGAVIG
 Smc04093 464 ICITDS-WPGQMRVVGDDHRIQTY--FSTYKKGKRTGDCGRDEDSYVITGRVDDVNVSGHRTGAEVSAVLSNLSSEAVVIG
 Smc00965 342 LVESE--LISDGYIAGDGAG--FRQCKLATVVDLGLTDEDETLLHIGRAGGMVSGGNNITYSEVETVQQAGGNAAFVVG
 Smc00592 324 VYRGFP--NVNMGYASSRADLARGAEL-----SELRTGLVVRDADVFRIYGRTRKRSKIAGLRIGHDALAALQGVGA--AAVVG
 Smc00774 466 VCTRA-FPSVPMTEVNDPQAKYRA-AYDFRFDNVVCHGDFEWTPEHSIYHGRSDATNPGGVRIGTATYVQVEQMDLADALOTG

Smc02162 503 VADHSSEAKVLFVVRKDP-N-LTE--EYVKRHCAASLTNYKRPVVRRTLEPKSNVGKILRKDLRG-----
 FadDec 488 VPSGSSSEAKVLFVVRKDP-S-LTE--ESLVTFCRRLTGYKVPILVFRDELPKSNVGKILRLRLDEARGKVDNKA-----
 Sma0150 431 LPHDFEGVTAVVVRKPGAA-LDE--RALDGLGRRLARYKQERFVILVDELPTNMGVQVQNVRLRTYARLYAGAEARV-----

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SMb20650  446  VPDEVLGAAIGALVVLSDP-T-TE---KDIIHCSRHEEDMVEHIVDFRTELPKTDGKVSRLAAETLEPAE-----
SMc00261  462  VPDEVWCEVGIAVGVARGGAT-VGA---AALREWLDGKIAPIKLPKIVFWSEMPKSAYGKITHKLREELERRGELDIGSAGIGERR--
SMc00741  469  KPDPRLRTEIVKAVVVLKPGVA-AGDETAAGIRDWVKNRISMHEYPRELAFVDSLELITSGKIVIRNLLREKAAAEARAASGG-----
SMc04095  534  IADPLKSGQVPAGFIVINANVSRETEIEKEVVGVLVRERISGPVAAERTAVCVKRLPKTRSGKILNSTHQKIIDRQPWT--MPATIDDPAIL
SMc04093  550  YPHPIKGGGTYCYVSLMAGEV-GDELRQAIVKHVRSEIGPIATFDKIQFAPGLPKTRSGKINRRIILRKIAEDDFGSLGDTSTLADPGVV
SMc00965  423  IHHFDLSSELAAYTEPGDG--FDH---AALERHLAADPPKRPKRRLWLCRKMEMTASGKVAAGELRQWIATENSALERLV-----
SMc00592  403  NDTE---IHAYVVGSGDP-----DEVRRLLVEASGLTLMHV GASRLDKLERLTSGKIDYPALRMRPAAATCG-----SSEEGDV
SMc00774  554  QDWEDDVR-VVLFVRLARGVE-ITTEALTREIKNIRISGASPRHVEAKIIAVADIERKSGKIVELAVRDVVHGPVKKE--ALANPEAL

SMc02162  -----
FadDEc    -----
SMa0150    -----
SMb20650  -----
SMc00261  546  -----SAAP-----
SMc00741  -----
SMc04095  622  DEITELLR-----SKGIGV-----
SMc04093  639  DDLIANRQ-----NRA-----
SMc00965  -----
SMc00592  474  EALFAQLFYPPKVRPEDSFSLGGDSLRFYQLSIGLEKRLGELPEGWEKMPVGALAAHNGRERKSRRIAGDFLI
SMc00774  640  DL-FAGLEELKS-----

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Figure S1. Multiple sequence alignments of *S. meliloti* Rm1021 FadD (SMc02162) with *E. coli* FadD (FadDEc) and different ORFs of *S. meliloti* Rm1021 with homology to SMc02162

(<https://iant.toulouse.inra.fr/bacteria/annotation/cgi/rhime.cgi>, accessed on March 18 th, 2020). Details of the different ORFs are given on Table S1. Alignments were performed using Clustal Omega at EMBL-EBI

(<https://www.ebi.ac.uk/Tools/msa/clustalo/>, accessed on March 18th, 2020) [1] and the figure was created at the BoxShade server using the option of consensus to SMc02162 sequence

(https://embnet.vital-it.ch/software/BOX_form.html, accessed on March 18th, 2020). SMc00592 is a multidomain protein with a total of 885 aminoacids. Only the first 547 amino acids have been used in the alignment. Amino acids identical to those in SMc02162 are shaded in black while amino acids similar to those present in SMc02162 are shaded in grey.

SMa0150	1	-----MSNLFDAIRR---ARPDSAFILTADGRVIT
R1 MatB	1	-----MSNLFDAIRA---APCNAPFIRIDNTRITWT
Bd MatB	1	-----MNRAANANFSRLFDGID---DPKRLAETHDGARIS
Sc MatB	1	-----MSSLFPALSPAPTAPADRPALRFGERSLT
At AAE3	1	-----MEVFKAAFSEA--SNSCDRIA--KADGKSYS
Hs ACSF3	1	MPPHVVLTFRRLLGCALASCRLAPARHRGSGLLHTAPVARSDRSAPVFIARA--LAFCDRIA--LVDQHGRHT
SMa0150	30	YGDMLEISG-----RIASVLDALGVPGDRVAVQVEKSPFALMLYLACLRIGAVYPLNT
R1 MatB	30	YDDAFALSG-----RIASAMDALGIRPGDRVAVQVEKSAEALILYLACLRSGAVYPLNT
Bd MatB	35	YGDLIATAG-----QMANVLVARGVPGDRVAVQVEKSVANVLYLATVRAGAVYPLNT
Sc MatB	31	YAEIAAA-----AGATAGRIG-----AGRVAVWATPAMETGVAVVAALLAGVAAVPLNP
At AAE3	29	YGQILTSSALRISKFLKDDTTNGQETKKYEGFGLKCARIGIVAKPSAEFVAGVLGTFWFSGVAVPLAL
Hs ACSF3	68	YRELYSISLRLSQEICRLCGCV-----GDLREIRVSFLCANDASYVVAQWAWMSGVAVPIYR
SMa0150	85	AYTLAELDYFFGDAEPRLLVCAEGAKEGIAKHAADCGAEVETLDEKGGSLIDLARG--KAPDFPDADRG
R1 MatB	85	AYTLAELDYFIGDAEPRLLVVASSARAGVETIAKPRGATVETLDAAGSGSLIDLARD--EPADFVDASRS
Bd MatB	90	AYTINELDYFIGDAEPSLVVCDSEKAEGLAPIAAKVKAGVETIGEDCKGSLTAAADK--ASSATTVPRE
Sc MatB	81	KSGDKELAHISDSAPSLVIAPDAE-----TPPALGL--LERVDVVRARGAVPEDGAD
At AAE3	99	SYPEAEELHVMNDSISLILISTEHSETMKTIAAKSGARFHLPEFVNSTSETACNQFQDDSEAEGLF
Hs ACSF3	128	KHPAAQLLYVTCDSQSSVVLVSQYLELSPVVRKLVPLLPITPAIYTG---AVPEPAEVPVE--QGW
SMa0150	153	PDDLAAIlyTSgTTGRSGGAMLTHDNLISNATILREYWRFTADRLIHALPIFHTHGLEFVASNVILLAGA
R1 MatB	153	ADDLAAIlyTSgTTGRSGGAMLTHGNLISNALILRDFWRVTAGRLIHALPIFHTHGLEFVATNVTLAGA
Bd MatB	158	NDDLAAIlyTSgTTGRSGGAMLTHDNLISNSLSLVGWRFTRDQVLIHALPIFHTHGLEFVATNVTLSPA
Sc MatB	134	DGDPALVlyTSgTTGPKGAVIPRRALATIDALADAWQNTGEDVLQQLPFHVHGLVILGILGPLRRCG
At AAE3	169	IDDPALVlyTSgTTGPKGVVTHNSINSQVRMLTEAEMTSADHFLHCLPFHHVHGLFNALFAPLYARS
Hs ACSF3	193	RNKGAMILlyTSgTTGTRPKGVISTHONRAVVTGLVHKWATKLDVILHVLPLFHHVHGIVNALLOCLPLVGA
SMa0150	223	SMFELPKFDANEVLR-----LMFQSTSYMGVPTFYVRLVQNPL-----HEATAGV
R1 MatB	223	SMFLLSKFDPEBILS-----LMFQATLMGVPTFYVRLQSPRL-----DKQAVAN
Bd MatB	228	SMIFLPKLDPLIIR-----LMARATVLMGVPTFYTRLQNAAL-----SRETTTHM
Sc MatB	204	SVRHLGFFSTGAAR-----ELNDGATMLFGVPTMYHRIAETLPADPE-----IAKALAGA
At AAE3	239	LVEFLPKFSVSGIWRRWRESYPNDEXTNDSITVFTGVPTMYTRLIQGYEAMDKE---MQDSAFAPARKL
Hs ACSF3	263	ICVMVPEFSPQQVWEKFLSS-----ETERINVMFVPTIYTKLMEYYDRHFTQPHAQDFLRVCEEKT
SMa0150	270	RLFVSGSAPLLAETHRTAQMTGHAILERYGMTETNMNTSNPYDGE-RNAGTVGFPLPGVSLRVADPESG
R1 MatB	270	RLFISGSAPLLAETHTEQARTGHAILERYGMTETNMNTSNPYDCK-RNAGTVGFPLPDVITRVITDPANG
Bd MatB	275	RLFISGSAPLLAETHREWSARTGHAVILERYGMTETNMNTSNPYDGE-RMPGAVGFPLPGVSLRVITDPEAG
Sc MatB	255	RLIVSGSAALPVHDHERIAAATGRRVILERYGMTETLMNTSVRADGE-PRAGTVGVPLPGVELRVVEEGT
At AAE3	306	RLMVSGSALPRPVMQWESITGHRILERYGMTETFMAMSNPLRGA-RNAGTVGKPLPGVEAKIKEDEN-
Hs ACSF3	326	RLMVSGSAALPLPVLEKWKNTTGHTILERYGMTETIGMALSGLTTAMRPGSVGTPLPGVQVRVISENPQ
SMa0150	339	-----RPLHKGETCMIEVKGPNVFKCYWRMPEKTQCEFRADGFFITGDLGKIDBERG
R1 MatB	339	-----LALPEPEQTCMIEIKGPNVFKCYWRMPEKTAAEFTADGFFISGDLGKIDRDG
Bd MatB	344	-----KELPREECMIEVKGPNVFKCYWRMPEKTKAEFRDGGFFITGDLGKIDGKG
Sc MatB	324	P-----IAALDGESVGETIQVGGPNLETEYLNRPDAIAAAFTEDGGFFRTGDMVVRDPDG
At AAE3	374	-----DANGVGETIOVKSESLFKEYWNPEVTKESFTEDGMEKTTGDAGRVDEBG
Hs ACSF3	396	REACSYTIAEGDERGKTVPGFEEKDGEILLVNGPSVFEYWNKPEETKSAFTLDGMEKTTGDTVVF-RDG

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SMa0150  390  YVHIIVGRG-KDLVISGGYNIPKEVEFEIDQMPGVVEIAVIGLPHPDFGEGVTAVVVRP GAA-----
Rl MatB  390  YVHIIVGRG-KDLVISGGYNIPKEVEFEIDQIEGVVEIAVIGVPHPDFGEGVTAVVVRP GAA-----
Bd MatB  395  YVHIIVGRG-KDLVISGGYNIPKEVEFEIDAMPGVVEIAVIGVPHADFGEGVTAVLV CNKGAE-----
Sc MatB  377  YVRIIVGRKATDLIKSGGYKIGAGEIENALEHEVREAAVTGEEDPDLGERIVAMIVPADPAA P-----
At AAE3  422  YVVIIVGRNSADIMKVGGYKISALETEETLEHETVAECCVIGITDNDVGEIVTATIT AESA AKKRREDES
Hs ACSF3  465  QYWIIRGRTSVDLIKIGGYKVSALIEVEWHLAHPSITVAVIGVVDMTNGQRVTAVVTLIEGHS-----

SMa0150  452  ---IDENAILDQLEGRRLARYKQPKRVIFVDDLPRNTMGKVQKNVLRREIYARLYAGAEARV
Rl MatB  452  ---IDENAIIVSAILQDRLARYKQPKRIIFAEDLPRNTMGKVQKNVLRQQYADLYTRT----
Bd MatB  457  ---VSEASVILKALDGRRLAKFKIPKRVFVVDLPRNTMGKVQKNVLRDLYKDIYAKK----
Sc MatB  441  ---PALGTIAHVAARLAPHKRPFRVVRYLDAVPRNDMGKIMKRALNRD-----
At AAE3  492  KPVITLEEICGWAKDMLAPYKLETRLIWESLPRNAMGKVNKKELKKSLENQE-----
Hs ACSF3  528  ---LSHRELKFWARNVLAHYAVSELVVVEEIPRNQMGKIDKKALIRHSHPS-----

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Figure S2. Multiple sequence alignments of SMa0150 with characterized malonyl-CoA synthetases from *Rhizobium leguminosarum* (Rl MatB), *Bradyrhizobium diazoefficiens* (Bd MatB), *Streptomyces coelicolor* (Sc MatB), *Arabidopsis thaliana* (At AAE13) and *Homo sapiens* (Hs ACSF3). Alignments were performed using Clustal Omega at EMBL-EBI (<https://www.ebi.ac.uk/Tools/msa/clustalo/> accessed on February 1, 2020 [1]) and the figure was created at the BoxShade server (https://embnet.vital-it.ch/software/BOX_form.html accessed on 1st February 2020). The conserved motif ERYGMTE found in malonyl-CoA synthetases is shown with a line of red stars (*). Amino acids identical in at least three of the sequences are shaded in black and similar amino acids are shaded in grey.

Table S2. Quantification of free fatty acids in different *S. meliloti* strains. Spot intensities corresponding to free fatty acids observed on TLCs plate autoradiograms of *S. meliloti* ^{14}C -acetate-labeled cultures. Intensity is expressed as arbitrary units ($\times 10^6$). Average and SD are from three independent experiments.

<i>S. meliloti</i> strain	Cell associated	Spent media	Ratio cell associated/spent media
GR4 (pNG28)	2.57 ± 0.42	2.39 ± 0.64	1.07
QS77 (pNG28)	94.1 ± 26.07	15.2 ± 8.81	6.19
QS77 (pRCanul2) <i>smc02162</i>	3.26 ± 1.60	2.74 ± 0.25	1.19
QS77 (pRCanul1) <i>sma0150</i>	118 ± 3.51	12.4 ± 4.62	9.52
QS77 (pRCanul3) <i>smb20650</i>	114 ± 3.0	13.2 ± 3.33	8.64
QS77 (pRCanul4) <i>fadD_{Ecoli}</i>	2.37 ± 0.09	2.41 ± 0.38	0.98

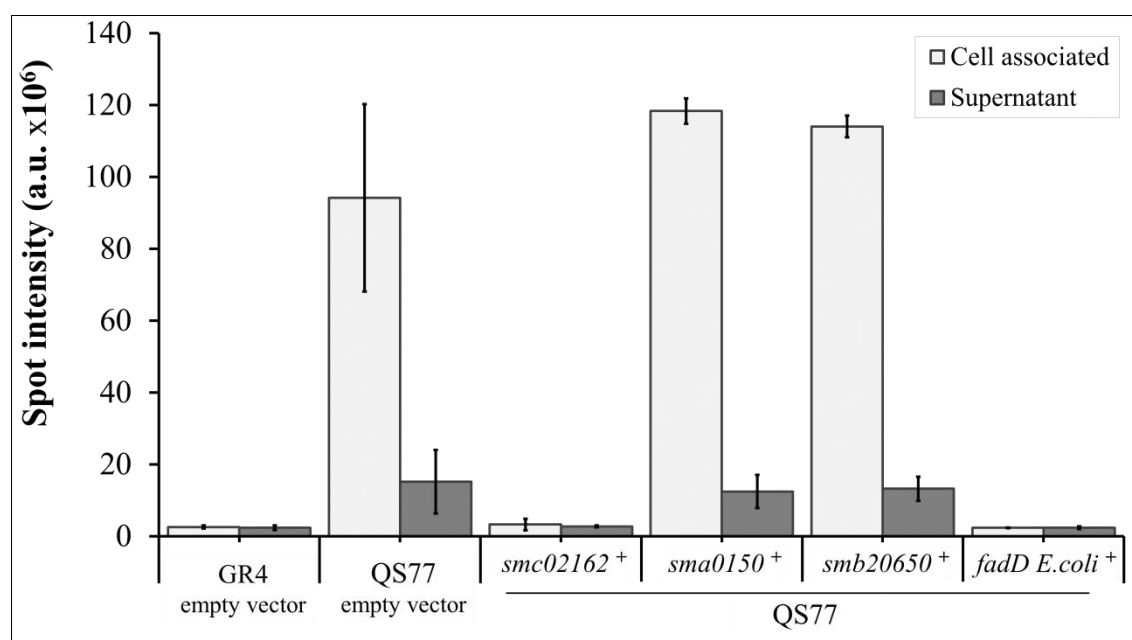


Figure S3: Graphical representation of spot intensities of free fatty acids formed in different *S. meliloti* strains. Bars correspond to spot intensities observed on TLCs plate autoradiograms in the indicated *S. meliloti* strains as shown in Table S1. Error bars represent SD calculated from three independent experiments.

Table S3: Quantification of free fatty acids in different *E. coli* strains. Spot intensities corresponding to free fatty acids observed on TLCs plate autoradiograms of *E. coli* ^{14}C -acetate-labeled cultures. Intensity is expressed as arbitrary units ($\times 10^5$). Average and SD are from three independent experiments.

<i>E. coli</i> strain	Cell associated	Spent media	Ratio cell associated/spent media
BL21(DE3) pLysS pET17b	3.43 ± 1.78	6.54 ± 2.20	0.52
BfadD1 pLysS pET17b	39.4 ± 10.96	59.4 ± 18.40	0.66
BfadD1 pLysS pECH8	2.78 ± 1.42	2.69 ± 1.16	1.03
<i>fadD_{Ecoli}</i> BfadD1 pLysS pECH1	38.1 ± 4.74	73.0 ± 32.33	0.52
<i>sma0150</i> BfadD1 pLysS pECH7	33.0 ± 7.15	80.3 ± 28.56	0.41
<i>smb20650</i> BfadD1 pLysS pECH3	3.47 ± 1.98	3.71 ± 2.20	0.93
<i>smc02162</i>			

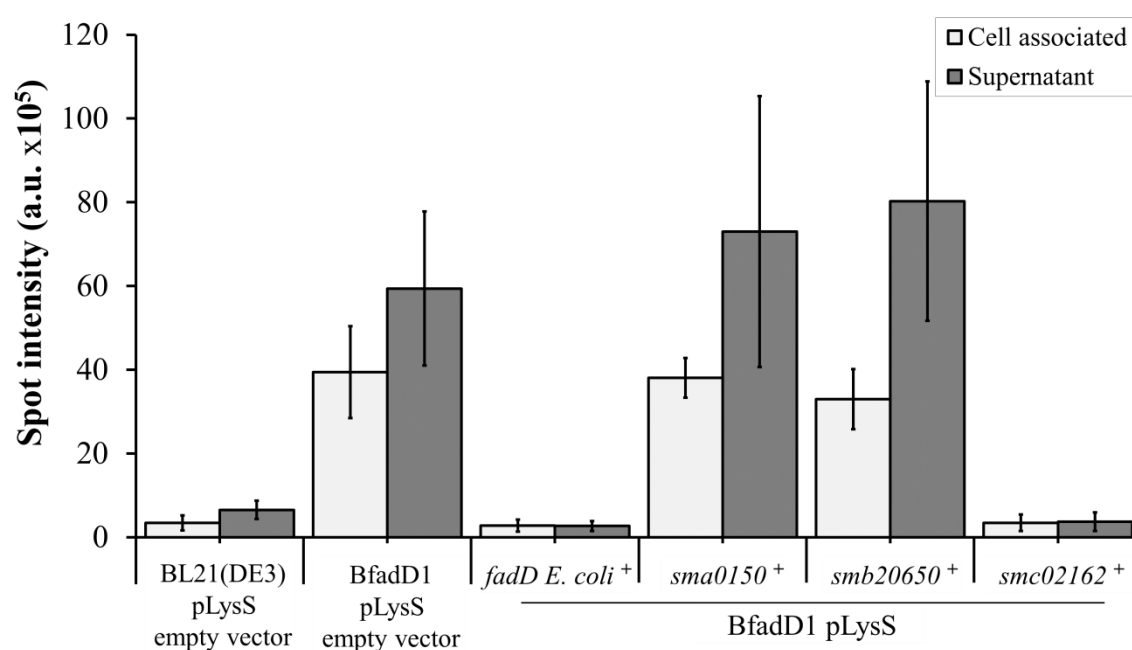


Figure S4: Graphical representation of spot intensities of free fatty acids formed in different *E. coli* strains. Bars correspond to spot intensities observed on TLCs plate autoradiograms in the indicated *E. coli* strains as shown in Table S2. Error bars represent SD calculated from three independent experiments.

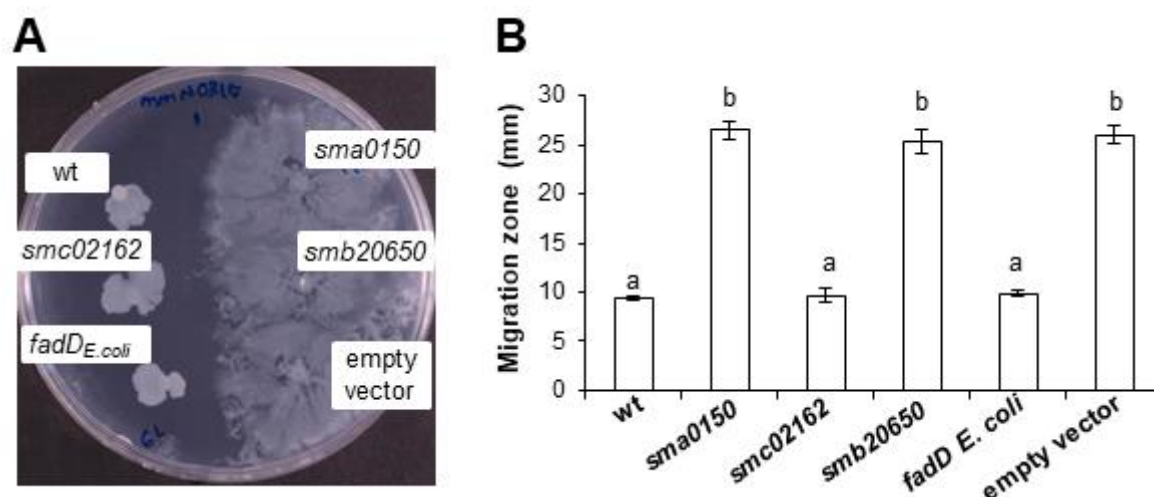


Figure S5. Effect of different *fadD* homologues on the surface motility of *S. meliloti* QS77 (*smc02162*⁻). **(A)** Representative picture of surface motility on Robertsen semisolid MM (0.6% Noble agar) shown by the wild-type strain (wt) and QS77 harbouring either pRCanul1 (*sma0150*), pRCanul2 (*smc02162*), pRCanul3 (*smb20650*), pNG28 (empty vector), or pRCanul 4 (*fadD_{E.coli}*). **(B)** Surface expansion shown by wt and QS77 derivatives shown in **(A)**. Bars and error bars represent the mean and standard error of the migration zones obtained for each strain from two independent biological experiments with at least four technical replicates. Different letters indicate significant differences according to an analysis-of-variance test ($p \leq 0.05$).

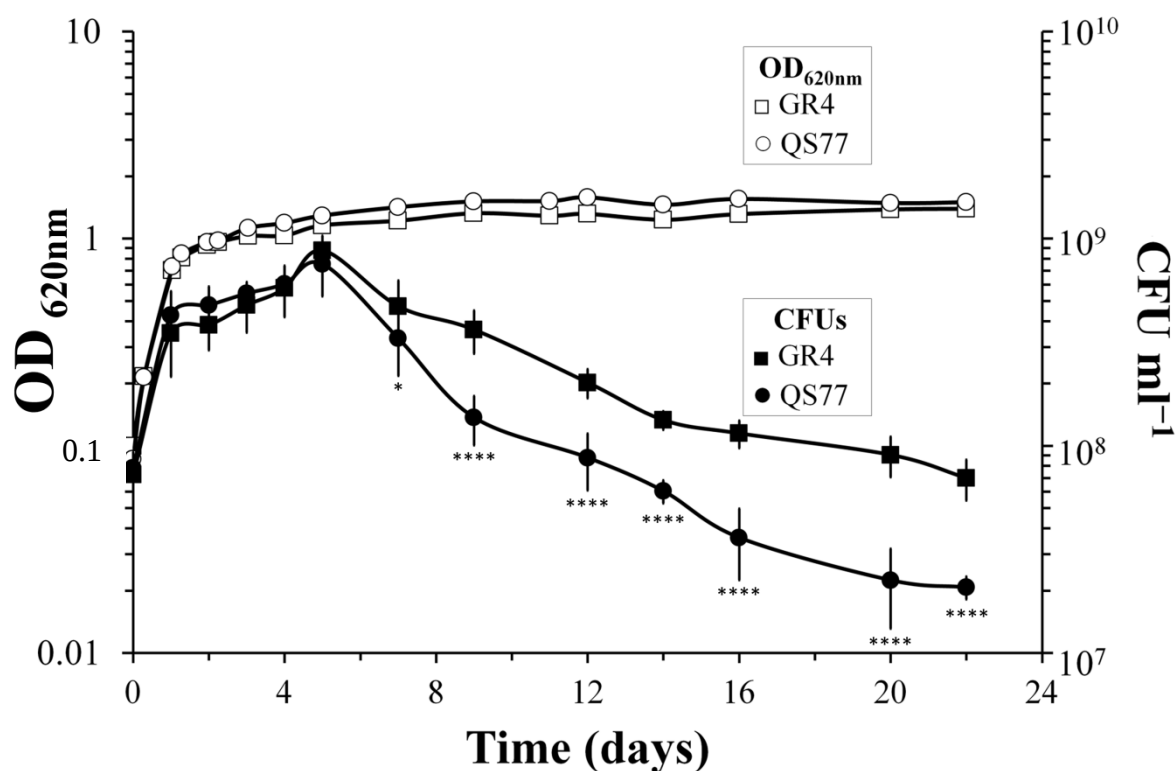


Figure S6. Absence of *fadD* reduces survival in stationary phase of *S. meliloti*. Growth curves and viable cells (CFU ml⁻¹) of *S. meliloti* GR4 and its *fadD* mutant QS77 grown on Robertsen MM. Open symbols represent optical density (OD) whereas CFU are represented with filled symbols. Statistical significance was calculated in Prism 8.4 using an unpaired two-tailed *t*-test in which the *fadD* mutant was compared to the parental strain. Statistical significance is shown (* *p* < 0.05; **** *p* < 0.0001). For each strain three independent cultures were analyzed. The error bars represent the SD.

Reference

1. Madeira, F.; Park, Y.M.; Lee, J.; Buso, N.; Gur, T.; Madhusoodanan, N.; Basutkar, P.; Tivey, A.R.N.; Potter, S.C.; Finn, R.D.; et al. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* **2019**, *47*, W636–W641, doi:10.1093/nar/gkz268.