Supplementary Material

Supplementary Figure Legends

Fig. S1

The elution pattern of apo GPPMT from size exclusion chromatography using S200 10/300 column (GE healthcare, Fairfield, USA) is shown in red. Retention volumes of standard proteins are shown in blue with their molecular weights.

Fig. S2

Hydrophobic interaction and hydrogen bond formation between monomers of hexameric GPPMT related with point group 32. From (a) to (e), the apo- structures are shown. In (f), the SFG/GPP complex structure is shown. In order to clarify, it is defined that monomers A-B and monomers A-C are related with two-fold rotation, thus monomers B-C is related with three-fold rotation. (a) Hydrogen bonds between crystallographically two-fold related monomer A (green) and monomer B (magenta) are shown with dotted lines. Numbers indicate distance between two atoms. Residues from monomer B is indicated with a prime sign next to residue numbers. (b) Residues making hydrophobic interactions between monomer A (green stick with green surface) and monomer A (magenta stick) are shown. Residues from monomer B is indicated with a prime sign next to residue numbers. (c) Hydrogen bonds between crystallographically two-fold related monomer A (green) and monomer C (light blue) are shown with dotted lines. Numbers indicate distance between two atoms. Residues from monomer C is indicated with a prime sign next to residue numbers. (d) Residues making hydrophobic interactions between monomer A (green stick with green surface) and monomer C (light blue stick) are shown. (e) The monomer B-C interface related with three-fold rotation axis. Two residues at the interface which are hydrogen bonded are described with distance. Residue from monomer C is indicated with a prime sign next to residue number. (f) Hydrogen bonds and hydrophobic interaction only observed in SFG/GPP complex structure. Monomer A (green) - C (light blue) is related with NCS two-fold axis. Residues from monomer C is indicated with a prime sign next to residue numbers.

Fig. S3

Amino acid sequence alignment of GPPMT and structurally homologous proteins by CLUSTALW (Thompson *et al.*, 1994) and ESPRIPT (Gouet *et al.*, 2003). The secondary structure of GPPMT (substrate-bound form) is also shown with typically conserved motifs. Identical residues are highlighted in red, and similar residues are framed in black with red letters. Putative catalytic base of GPPMT is marked with a blue star. Residues recognizes SAM are marked with blue circles.

Fig. S4

Enzymatic activity monitored by capillary GC-MS (Shimadzu GC-MS-QP2010). (a) Reaction scheme catalyzed by GPPMT. (b) Retention time (horizontal axis) of substrate (GPP and SAM) enzyme mixture from gas chromatography of each sample as labeled in the inset. The vertical axis indicates intensity. The signals observed around 5 min are from hydrolyzed product of GPP (geraniol), the signals around 5.8 min are from hydrolyzed product of 2-methyl GPP (2-methyl geraniol). (c) GC-MS spectra of geraniol

with m/z along the horizontal axis and relative intensity of the signal for each component along the vertical axis. (d) GC-MS spectra of 2-methyl geraniol.

Fig. S5

Amino acid sequence alignment of GPPMT, IPPMT, and representative of sterol 24-C-methyltransferase (EC. 2.1.1.41), cycloartenol 24-C-methyltransferase (EC. 2.1.1.142), and 24-methylenesterol C-methyltransferase (EC. 2.1.1.143). The protein designations are as follows; GPPMT, GPPMT from *Streptomyces lasaliensis*; Lon23, IPPMT from *Streptomyces argenteolus*; EC.2.1.1.41_Pichia , sterol 24-C-methyltransferase from *Pichia pastoris*; EC.2.1.1.42_Candida, 24-C-methyltransferase from *Cryptococcus gattii*; EC.2.1.1.42_Candida, 24-C-methyltransferase from *Candida albicans*; EC.2.1.1.42_Saccharomyces, sterol 24-C-methyltransferase from *Saccharomyces cerevisiae*; EC.2.1.1.43_Arabidopsis, 24-methylenesterol C-methyltransferase from *Arabidopsis thaliana*; EC.2.1.1.43_Glycine, sterol 24-C methyltransferase from *Glycine max*.

Fig. S6

Proposed biosynthesis pathway of KS-505a (longestin). IPP methylation with IPPMT (Lon23) to produce Z-3-methyl IPP (mIPP) was experimentally confirmed. Methyl groups introduced are shown in pink circle.

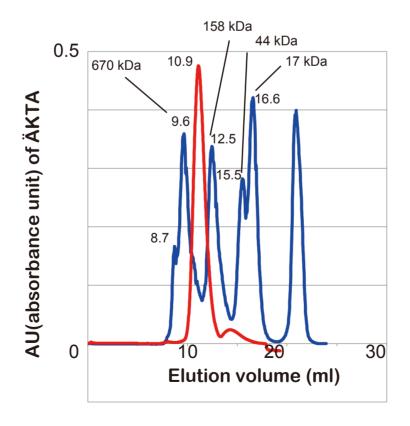
Supplementary Table S1

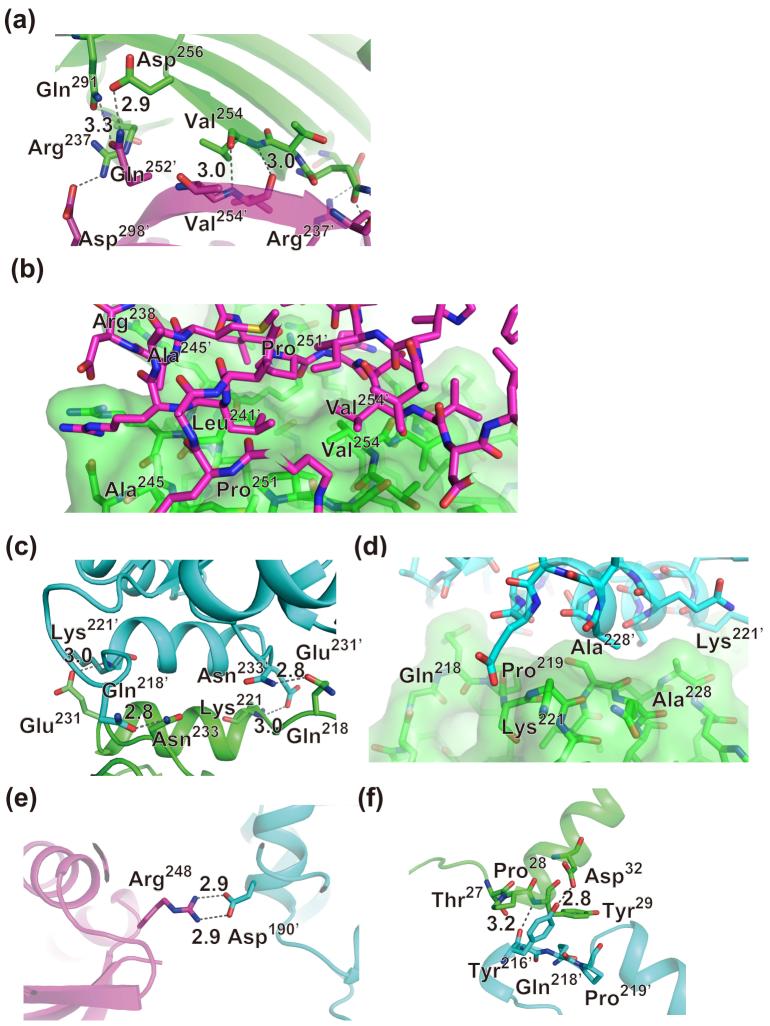
Primer sequences used for generating mutants are listed on the table below. Bases changed for mutagenesis are underlined.

mutants	Forward primer	Reverse primer
Y59F	CTCTACCACCACCAC <u>TT</u> CGGCATCGGT	CACGGCACCGATGCCG <u>AA</u> GTGGTGGT
	GCCGTG	GGTAGAG
E181D	CCTCGTGGAACAACGA <u>T</u> TCGAGCATGT	CGACGTACATGCTCGA <u>A</u> TCGTTGTTCC
	ACGTCG	ACGAGG
E181A	CTCGTGGAACAACG <u>CG</u> TCGAGCATGT	GTCGACGTACATGCTCGA <u>CG</u> CGTTGTT
	ACGTCGAC	CCACGAG

References

Gouet, P., Robert, X. & Courcelle, E. (2003). Nucleic Acids Res. 31, 3320-3323.
Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). Nucleic Acids Res. 22, 4673-4680.





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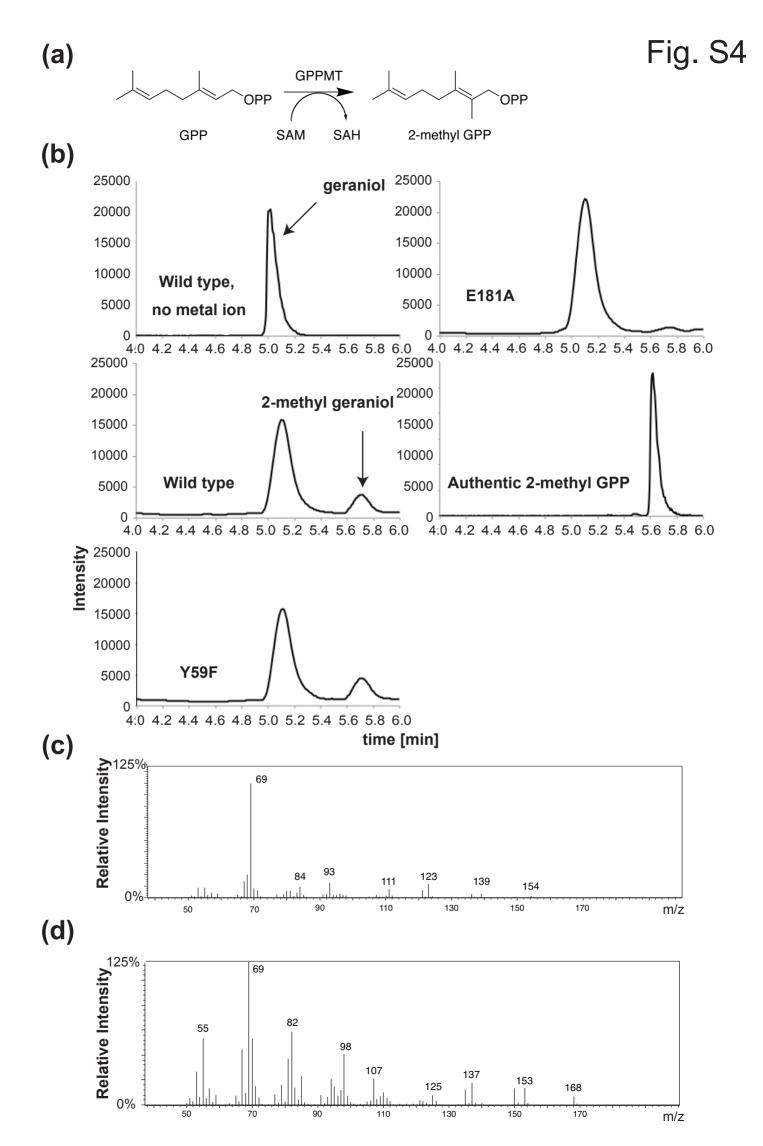
GPPMT	ļ	ıņ	20	α1 <u>2000000</u> 30	2000 A.O.	α2 200000000 50	6 Q	тт
GPPMT RebM Hma CmaA1 CmaA2 PcaA	MGAQPPVTI	 DTIQENMT 	MTESKSEGTA RMAEKPISPT MPD SQGD.TTSGT	VAAPTPEEVI KTRTRFEDIÇ ELKPHFANVÇ QLKPPVEAVI	R Q M Y D D F T D F Q A H Y D V S D D F Q A H Y D L S D D F R S H Y D K S N E F	VNLRLGDVDGL FARIWGEN FALFQDPT FRLFLDPT FRLFLDPT	LHFGYWED RTYSCAYF QTYSCAYF MTYSCAYF	AGAD EPPE ERDD ERPD

			αZ		β1	αΑ	β2
GPPMT		22222222	2222222222	ll	тт ———————————————————————————————————	LLLLLLLLL	2 2
	70	80	90	100	110	120	130
GPPMT	LGDPGDGG	GYEARLIAE <mark>I</mark>	HRLESAQAEF			GRGGSMVMAHQRE	
RebM	VS	<mark>V</mark>	D <mark>DATDRLT</mark> DE			GIGKPAVRLATAF	
Hma	LT	<mark>I</mark>				GWGTTMRRAVER	
CmaA1	MT	<mark>I</mark>	QEAQIAKIDL	ALGK <mark>L</mark> G.LÇ		G W <mark>G</mark> ATMMRAVEKY	
CmaA2	MT	<mark>I</mark>	EEAQYAKRKL	ALDK <mark>L</mark> N.LH	E P <mark>G</mark> M T L L D I G C	GWGSTMRHAVAEY	DVNVIGLTLSE
PcaA	MT	<mark>L</mark>	QEAQIAKIDL	ALGKLN.LH	EP <mark>G</mark> MTLL DIGC	GWGATMRRAIEKY	DVNVGLTLSE

GPPMT	αB 20000000 140	2222 150	β3 160	TT . 170	ΓT	n3	αD 222222222 190
GPPMT RebM Hma CmaA1 CmaA2 PcaA	PQVNQANA NQHARCEQ NQANHVQQ NQYAHDKA	RAT <mark>A</mark> AGLAN VLA <mark>S</mark> IDTNR LVANSENLR MFD <mark>E</mark> VDSPR	RVTFSYAD SRQVLLQG SKRVLLAG RKEVRIQG	DAMDLPFEI GWEDFAE. GWEQFDE. GWEEFDE.	DASFDAVWAL PVDRIVSI PVDRIVSI PVDRIVSI PVDRIVSL	ESLHHM EAFEHFGHE. GAFEHFGHE. GAFEHFADGAC	DLHDVFAEHSRF PDRGRALREMARV NYDDFFKRCFNI RYDAFFSLAHRL JDAGFERYDTFFKKFYNL RYHHFFEVPHRT
	β5				α4		αE

		33		α4		αE
GPPMT		>	222222	ll	· · · · · l	QQQQQQQQ
	200	210	220		230	240
GPPMT	LRVG <mark>G</mark> R	Y <mark>VT</mark> VTGCWN.P	RYGQP <mark>S</mark> KWVSQI <mark>N</mark>	АН	<mark>F</mark> . ECNI	IHS <mark>RRE</mark> YL <mark>RA</mark> MADNR
RebM				AGGGVLSLO		
Hma				TARFIKFIVTEIFPGO		
CmaA1	LPADGVN	M <mark>LL</mark> HTITGLHP	KEIHE <mark>R</mark> GLPMSF T	FARFLKFIVTEIFPGO	GRLPS <mark>I</mark> PMVQH	ECA <mark>SAN</mark> GF <mark>TV</mark> TRVQS
CmaA2				LLRFIKFILTEIFPGO		
PcaA	LPAD <mark>GK</mark> N	MLLHTIVRPTF	KEGRE <mark>K</mark> GLTLTH <mark>E</mark>	LVHFTKFILAEIFPGC	GWLPS <mark>I</mark> PTVHB	EYA <mark>EKV</mark> GF <mark>RV</mark> TAVQS

	β6	(x5	α6	β7	
GPPMT		\blacktriangleright lelle	llll	22222222	—— —	>
	250	260	270	280	290	300
GPPMT	LVPQTV	DLTPETLP	YWELRATSSLV	TGIEEAFIESYRD	G <mark>S</mark> F <mark>Q</mark> Y <mark>V</mark> I	IAADRV
RebM	ISAQARE	SLVKTAEA	FENARSQVEPF	MGAEGLD <mark>R</mark> MIATF <mark>R</mark> G	LAEVPEAGYVI	IG <mark>A</mark> R <mark>K</mark> P
Hma			~	T S E E V Y N <mark>R</mark> Y M K Y L <mark>R</mark> G		
CmaA1	~		~ ~ ~	QSEEVYE <mark>R</mark> YMKYL <mark>T</mark> G		~
CmaA2			~	KGQETYD <mark>I</mark> YMHYL <mark>R</mark> G		~
PcaA	LQLHYAF	TIDMWATA	LEANKDQAIAI	Q S Q T V Y D <mark>R</mark> Y M K Y L <mark>T</mark> G	CAKLFRQGYTC	VDQFTLEK



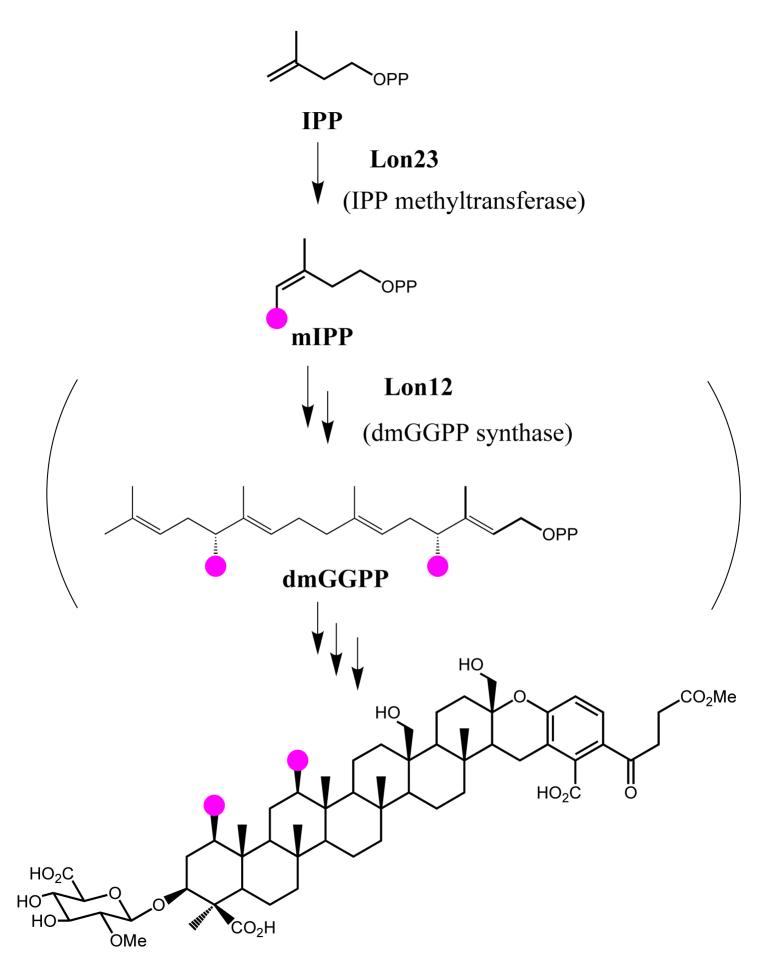
GPPMT Streptomyces Lon23 Streptomyces EC.2.1.1.41 Pichia EC.2.1.1.41 Cryptococcus EC.2.1.1.42 Candida EC.2.1.1.42 Saccharomyces EC.2.1.1.43 Arabidopsis EC.2.1.1.43 Glycine	110203040MAAASAPVPGPGGASSTARGRIPAPATPYQEDIARYWNNEAR MSLETVRTNEIIRDDFEKDLSTYWETKQNMTTSTTEQESQPLAPKNYEQDREFAKALHGKDAAKKTGLGAWVTKDASAQEVAVDGYFKHWNGKTDQEAE MBAETRSADRVSMYNKFWEKKSAHDNDMSPQLAEKNYERDEQFTKALHG.ESYKKTGLSALIAKSKDAASVAAEGYFKHWDGGISKDDE MSETELRKRQ.AQFTRELHGDDIGKKTGLSALMSKNNSAQKEAVQKYLRNWDGRTDKDAE MDSVALYCTAGLIAGAVYWFICVLGPAERKGKRASDLSGGSISAEKVK.DNYNQYWSFFRRPKEI MDPLSLFCTGALLAGGLYWFVCVLGPAEQKGKRATDLSGGSISAEKVQ.DNYKQYWSFFRRPKEI
GPPMT_Streptomyces Lon23_Streptomyces EC.2.1.1.41_Pichia EC.2.1.1.41_Cryptococcus EC.2.1.1.42_Candida EC.2.1.1.42_Saccharomyces EC.2.1.1.43_Arabidopsis EC.2.1.1.43_Glycine	5060708090100110.PVNLRLGDVDGLYHHHYGIGAVDHAALGDPGDGGYEARLIAELHRLESAQAEFLLDHLGPVGPGDTLVD DQINLLLGEEDGLYHHHFGIGDFDRSVADLPPEE.RESRVLEEMHSLENTQVETLIGALGDVPRDARLD HSRKSDYSELTKHYYNL.VTDFYEYGWGSSFHFSRYYRGEAFRQATARHEHYLALKMGITENMKVLD THRANRLDQYTEVVNGYYDG.ATELYEYGWSSSFHFSRYYRGEAFRQATARHEHYLALKMGITENMKVLD EKRLDYNEATHSYYNL.VTDFYEYGWGSSFHFSRYYKGEAFRQATARHEHFLAHKMNLNENMKVLD ERRLEDYNEATHSYYNU.VTDFYEYGWGSSFHFSRYKGEAFRQATARHEHFLAHKMNLNENMKVLD ERRLEDYNEATHSYYNU.VTDFYEYGWGSSFHFSRYKGEAFRQATARHEHFLAHKMLNENMKVLD ESAEKVPDFVDTFYNL.VTDIYEWGWGQSFHFSPNVPGKSDKDATRIHEEMAVDLIKVKPGQKILD ETADKVPDFVDTFYNL.VTDIYEWGWGQSFHFSPSIPGKSHRDATRLHEEMAVDLIEAKPGNRILD
GPPMT_Streptomyces Lon23_Streptomyces EC.2.1.1.41 Pichia EC.2.1.1.42_Cryptococcus EC.2.1.1.42_Candida EC.2.1.1.42_Saccharomyces EC.2.1.1.43_Arabidopsis EC.2.1.1.43_Glycine	120130140150160170AGCGRGGSMVMAHQRFGCKVEGVTLSAAQAEFGNRRARELGIDDHVRSRVCNMLDTP.FEKGTVAASWNMGSGRGGTSFMIYDRFGCTDGVTFAQYQVDFSNRLAETRGCADRVRFHYRNMVKTG.FPEAGAFQYVVTVGCGVGGPAREIARFTGCSVVGLNNNDYQVERAEFYSKKYNMTKQLSYVKGDFMQM.DFEPETFDAVYAVGCGVGGPAREISRFSDANIVGVNNDF0IGRATAKTKKAGLSDKVSFVKGDFMKLSEQFGENSFDAIYAVGCGVGGPAREISRFSCNIVGUNNDYQIERAEFYSKKYNMTYALSYVKGDFMQM.DFEPETFDAVYAVGCGVGGPAREIARFTGCNVIGLNNNDYQIERANHYAKYHLDHKLSYVKGDFMQM.DFEPESFDAVYAVGCGVGGPAREIARFTGCNVIGLNNNDYQIERANHYAKYHLDHKLSYVKGDFMKLSDFDGAYSDFEPENFFDRYAVGCGVGGPMRAIAAHSKAQVTGITINEYQVNRARMHNKKAGLESLCEVVCGNFLKMP.FPDNSFDGAYS
1 GPPMT_Streptomyces Lon23_Streptomyces EC.2.1.1.41_Pichia EC.2.1.1.42_Candida EC.2.1.1.42_Candida EC.2.1.1.42_Saccharomyces EC.2.1.1.43_Arabidopsis EC.2.1.1.43_Glycine	80 190 200 210 220 230 240 NESSMYV.DLHDVFAEHSRFLRVGGRYVTVTGCWNPRYGQPSKWVSQINAHFECNIHSRREY NETTPYV.KLDEVFELSRVLAPGGRYVSLTWCRNDAVASQCDEVLEIDRHYICRTHRRSSY IEATVHAPVLEGVYSEIYKVLKPGGAFGVYEWVMTDAYDETNPEHRAIAYGIEVGDGIPKMYKRQVAEDA IEATCHAPNFEGIYGEIFKCLKPGGVFGVYEWCMTDAWDPSNPEHKEIAHGIEIGDGIPEMRDLTAARNA IEATVHAPVLEGVYSEIYKVLKPGGVFGVYEWVMTDKYDETNEEHRKIAYGIEVGDGIPKMYKRQVAEDA IEATCHAPKLEGVYSEIYKVLKPGGFFAVYEWVMTDKYDETNEEHRKIAYGIEUGDGIPKMYKRQVAEDA IEATCHAPKLEGVYSEIYKVLKPGGFFAVYEWVMTDKYDETNEEHRKIAYGIEUGDGIPKMYKRQVAEDA IEATCHAPKLEGVYSEIFKVMKPGSLFVSYEWVTTEKYRDDDEEHKKIAYGIEGGALPGLRSYADIAVT IEATCHAPKLEEVYSEIFRVMKPGSLFVSYEWVTTEKYRDDDEEHKKDVIQGIERGDALPGLRSYADIAVT IEATCHAPKLEEVYAEIFRVLKPGALYVSYEWVTTDKYRGDDPEHVEVIQGIERGDALPGLRNYTDIAET
GPPMT Streptomyces Lon23_Streptomyces EC.2.1.1.41_Pichia EC.2.1.1.41_Cryptococcus EC.2.1.1.42_Candida EC.2.1.1.42_Saccharomyces EC.2.1.1.43_Arabidopsis EC.2.1.1.43_Glycine	250260270280LRAMADNRLVPOTVVDLTPETLPYWELR
GPPMT_Streptomyces Lon23_Streptomyces EC.2.1.1.41_Pichia EC.2.1.1.42_Candida EC.2.1.1.42_Candida	290 300 DGSFQYVLIAADRVL SDRINYLLIVAERV. SRQVTNALEDAAVNLVKGGELKLFTPMMLYVARKPLDAK TYSVGQSLIVAAKALVAGGKTKLFTPMALWVARKPAN SKQVTHALEDAAVNLVEGGRQKLFTPMMLYVVRKPLEKKD SKQVTHALEDAAVNLVEGGRQKLFTPMMLYVVRKPLEKAFTDSOTSOFATO

 EC.2.1.1.42_Candida
 SkQVTHALEDAAVNLVEGGROKLFTPMMLYVRKPLEKKD......

 EC.2.1.1.42_Saccharomyces
 SkEVTAALEDAAVNLVEGGROKLFTPMMLYVRKPLEKKD.....

 EC.2.1.1.43_Arabidopsis
 TVDVHKMLFKTADYLTRGGETGIFSPMHMILCRKPEKASE.....

 EC.2.1.1.43_Glycine
 TVDVHEMLFKTADYLTRGGDSGIFSPMHMILCRKPEKASE.....



KS-505a (longestin)