

# Supplementary Material

Table S1: List of plasmids.

Plasmid	Description	Origin
p3STOP1623hp	Shuttle vector for cloning in <i>E. coli</i> (Carb <sup>R</sup> ) and recombinant protein production in <i>P. megaterium</i> (Tet <sup>R</sup> ) using an optimized xylose-inducible promoter; <i>P<sub>xyIA<sup>opt</sup></sub></i> -mcs-stop	[1]
pTKSP0	p3STOP1623hp-derivative; shuttle vector for cloning in <i>E. coli</i> (Carb <sup>R</sup> ) and recombinant protein production in <i>P. megaterium</i> (Tet <sup>R</sup> ) with an optimized xylose-inducible promoter, an extended multiple cloning site and His <sub>6</sub> -tag; <i>P<sub>xyIA<sup>opt</sup></sub></i> -emcs-his <sub>6</sub> -stop	This work
pTKSP <sub>amyE0</sub>	pTKSP0-derivative harboring the <i>amyE</i> gene from <i>B. subtilis</i> 168 without signal peptide inserted via NgoMIV and AgeI	This work
pEJBmD1.3scFv	Shuttle vector for the xylose-inducible recombinant production and secretion of the antibody fragment D1.3 scFv guided by the signal peptide of the lipase A (SP <sub>LipA</sub> ) from <i>P. megaterium</i> ATCC 9885 (CAD23620.1); <i>P<sub>xyIA</sub></i> -SP <sub>LipA</sub> -d1.3scFv	[2]
pTKSP <sub>amyElipA</sub>	pTKSP <sub>amyE0</sub> -derivative containing the signal peptide from lipase A (SP <sub>LipA</sub> ) from <i>P. megaterium</i> ATCC 9885 (CAD23620.1) amplified via PCR from pEJBmD1.3scFv [2] and inserted via the restriction sites BsrGI and NgoMIV	This work
pALBm1	p3STOP1623hp-derivative for recombinant production and secretion of PGA from <i>P. megaterium</i> (BmPGA); <i>P<sub>xyIA<sup>opt</sup></sub></i> -bmpga-stop	[3]
pRBBm311	p3STOP1623hp-derivative for recombinant production and secretion of PGA from <i>Bacillus thermotolerans</i> (BtPGA); <i>P<sub>xyIA<sup>opt</sup></sub></i> -btpga-stop	[4]
pRBBm316	p3STOP1623hp-derivative for recombinant production and secretion of PGA from <i>Bacillus sp. FJAT-27231</i> (FJATPGA); <i>P<sub>xyIA<sup>opt</sup></sub></i> -fjatpga-stop	[4]
pRBBm317	p3STOP1623hp-derivative for recombinant production and secretion of PGA from <i>Bacillus massiliogorillae</i> (BmasPGA); <i>P<sub>xyIA<sup>opt</sup></sub></i> -bmaspga-stop	[4]
pJMBm75	pTKSP0-derivative harboring the <i>bmaspga</i> gene from pRBBm317 without signal peptide inserted via NgoMIV and NheI	This work
pMSP1 - pMSP9	pUC57-derivative encoding 18 signal peptides of <i>P. megaterium</i> DSM 319 separated by the restriction sites BsrGI and NgoMIV, Carb <sup>R</sup> , <i>ori</i> pBR322	This work, General Biosystems
pMSP10	pUC57-derivative encoding 20 signal peptides of <i>P. megaterium</i> DSM 319 separated by the restriction sites BsrGI and NgoMIV, Carb <sup>R</sup> , <i>ori</i> pBR322	This work, General Biosystems

Table S2: List of oligos.

Name of oligo	Description	Sequence
TKMCSHis_fw	Oligo for insertion of MCS extension, coding sequence of His <sub>6</sub> -tag and stop codon into p3STOP1623hp; the restriction sites are depicted in italics, the AgeI overhang in bold, the His <sub>6</sub> -tag in gray and the stop codon in capital letters	<b>ccggcgcgcgtacgcgtac</b> <i>taggagcggccgcgctagcaaccgg</i> catcaccatcaccatcacTA At
TKMCSHis_rv	Oligo for insertion of MCS extension, coding sequence of His <sub>6</sub> -tag and stop codon into p3STOP1623hp; the restriction sites are depicted in italics, the AgeI overhang in bold, the His <sub>6</sub> -tag in gray and the stop codon in capital letters	<b>ccggaTTA</b> gtgatggatggatggatgaccggttgctagcgcggccgctcctaggtacgcgtacg gcg
TKAmyE_fw	Primer for amplification and insertion of <i>B. subtilis amyE</i> gene in pTKSP0 resulting in pTKSPamyE0, the NgoMIV site is shown in italics	atcatagccggcgaaacggcgacaacatcg
TKAmyE_rv	Primer for amplification and insertion of <i>B. subtilis amyE</i> gene in pTKSP0 resulting in pTKSPamyE0, the AgeI site is shown in italics	atcataaccgggtcaatggggaagagaacc
TK83fw_LipA_SP	Primer for amplification of lipase A-SP from pEJBmD1.3scFv [2] and insertion into pTKSPamyE0 yielding pTKSPamyElipA, the BsrGI restriction site is depicted in italics	atcatgtacaatgaaaaaagtattaatggcttc
TK84rev_LipA_SP	Primer for amplification of lipase A-SP from pEJBmD1.3scFv [2] and insertion into pTKSPamyE0 yielding pTKSPamyElipA, the NgoMIV restriction site is depicted in italics	atcatagccggcggcgctagcacctgatg
JMSPBmas_fw	Primer for amplification and insertion of <i>bmaspga</i> gene from pRRBm317 into pTKSP0 resulting in pJMBm75, the NgoMIV restriction site is depicted in italics	tgatctgccggccaaaataacagcggagtgaag ag
JMSPBmas_rv	Primer for amplification and insertion of <i>bmaspga</i> gene from pRRBm317 into pTKSP0 resulting in pJMBm75, the NheI restriction site is depicted in italics	tgatctgctagcttatttttttcgtttctttgtaa atg
SeqpXylA_fw	Primer for colony PCR and sequencing	aagttgggtgtttttgaagc
SeqpMM1520_rv	Primer for colony PCR	gtttgcgcattcacagtctcc

Table S3: List of signal peptides available at pMSP1

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF38846.1	pullulanase, type I	MITLSKKVIVKNVYVFYFMGGFSLKCSW TKYAMVLTALLLVSSFFSPFMQSFASA
SP 2	ADF41409.1	protein of unknown function (DUF1510)	MNNNRSSRHENKEKKVNRLYNLLIAVV AVLIVFVGGTMILGNKDKSDNQA
SP 3	ADF37872.1	conserved hypothetical protein BMD_1011	MYRKKTLLLKRSLGVAVFLSVIALGVW MFQFTFFSPSHQAKS
SP 4	ADF37126.1	SH3 domain protein	MEGKQLKRIMVGMALTATLATAVTPGF GSIGGNQEKAYA
SP 5	ADF38520.1	transporter, MmpL family	MKESPLATFSRLMTGKVSRLVAVWIL ATAFLTIAWP
SP 6	ADF40203.1	beta-lactamase penicillinase	MKTWKKMFRASKLQKMLPVFALSCAA VVGFSSEVHA
SP 7	ADF39316.1	alpha amylase	MQTIAKKGDETMKGKKWTALALTPL AASLSTGVHA
SP 8	ADF37367.1	conserved hypothetical protein BMD_0481	MKTITKSISVLALAAGFTFSSLSGTLPTH EQTASA
SP 9	ADF38797.1	polysaccharide deacetylase	MNQRFKPNKRFYAFSLVLVIVASLFFIT EAKGSES
SP 10	ADF39301.1	methyl-accepting chemotaxis protein	MERVRRKRSLNNFKIKLGLAFAAILLVP SVTIGVSA
SP 11	ADF40620.1	conserved hypothetical protein BMD_3787	MKMKQRLSKAAAAATTGIAAFGFAPV AQFDTPAHA
SP 12	ADF41198.1	protein of unknown function (DUF1002)	MGLKEEKGLKLTIKAIMVTGLLLLFLTA PLKSFADA
SP 13	ADF41350.1	cytochrome c-550	MKRNPLIPFLIAVIGIALMLTSLFNGLN NAKEIAA
SP 14	ADF37697.1	two-component sensor histidine kinase DctS	MRLSMQTKMTGLIFFIVAFSLFLASIVVI NNFVQS
SP 15	ADF40228.1	hypothetical protein BMD_3388	MDFFKNSKILYTIVALVIALIAVFTNNSPI SLIQQ
SP 16	ADF41000.1	flagellar basal body-associated protein FliL	MFKNKLISKMIIMLSLTLIGSIAVIVTLKY TEQA
SP 17	ADF42034.1	putative peptidase	MIGKVLAKAVVAKAVGGATIGTAIAL GSMTSAFA
SP 18	ADF38992.1	hypothetical protein BMD_2139	MNISFKKFRFLWFILLIFVLIFVKNTFFSSS QNA

Table S4: List of signal peptides available at pMSP2

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF38011.1	membrane-bound protein, putative transcription regulator	MSSKAKKEWIGGLVFLLVSVFSI TFAGYHTVKA
SP 2	ADF39126.1	immune inhibitor A metalloprotease	MKSWKKILGTSFLATTVTLSGVG PVLMMNGSTASA
SP 3	ADF39213.1	putative membrane protein	MKFIVKFKWVIAAFILALTVILM LTSPNLTKLA

SP 4	ADF39764.1	conserved hypothetical protein BMD_2923	MKLGNQKLIYFSPHVVAVIFIFIL TLIPSASS
SP 5	ADF37892.1	hypothetical protein BMD_1031	MDRKKVASVAGALVIAVGIWLF QSGKFKSEAQA
SP 6	ADF38238.1	conserved hypothetical protein BMD_1377	MKKLIIVAVAAVLLIIGGISFSIYH SSVKDVQS
SP 7	ADF39097.1	conserved hypothetical protein BMD_2249	MVKKLLAAFIALAVVFSPVGGSL IHDGSNVASA
SP 8	ADF39532.1	2',3'-cyclic-nucleotide 2'-phosphodiesterase	MKKNTRSKTKKILSTSLALSLLS MPLLTQAGA
SP 9	ADF38212.1	cytochrome aa3 oxidase assembly protein	MRLGLKFLSVVTTMAMLLILLG GALVTKTGSA
SP 10	ADF39833.1	Endopeptidase	MKKEKLLVPFIAASVLVGASVTT PVFHSAYA
SP 11	ADF40159.1	putative hydrolase	MKKVKAALPLAVSLALGLQVLP ISTPIVQAEA
SP 12	ADF41059.1	phosphopantothienoylcysteine decarboxylase/phosphopantothenate-cysteine ligase	MMKGKRILLCVSGGIIVYKAAA LTSKLVQAGA
SP 13	ADF41166.1	spore cortex-lytic enzyme	MLSFRCFKKYACLICISFIAASV VCPKAAHA
SP 14	ADF40390.1	putative protease, NlpC/P60 family	MLKKANRTVMNMGLAALLVP AALTFSTTSASA
SP 15	ADF37926.1	endopeptidase LytE	MKKAIIGSITAAAVLFSGASPFIA PAQTVEA
SP 16	ADF38355.1	alkaline phosphatase	MKKRAIGAAVLSAVTVAALAFG TNSPLDAKA
SP 17	ADF39787.1	conserved hypothetical protein BMD_2947	MKKAIVLTYCILSLVLLVSGGLYF KEQMVEA
SP 18	ADF40655.1	polysaccharide deacetylase	MKKYVFFLLIAAALVGFIFFKVV NWEDKAKA

Table S5: List of signal peptides available at pMSP3

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF37265.1	conserved hypothetical protein BMD_0354	MMKPIVLNKKIGSIVLIFSLLYFPI AAAAEA
SP 2	ADF37268.1	Beta-amylase	MKQLCKKGLAFVLMFIFVNAFIL SPINGAAA
SP 3	ADF36976.1	D-alanyl-D-alanine carboxypeptidase	MRNAVQKQLICVIAVMLMASVF FVSTQASA
SP 4	ADF39725.1	hypothetical protein BMD_2884	MSLSAKKILLVLVVICLVLVVM QFINRSA
SP 5	ADF37267.1	Extracellular ribonuclease	MKLYALCKKTWASLLVFVLLVS LTPVSQA
SP 6	ADF37446.1	conserved hypothetical protein BMD_0560	MGDLPLNQMMILVVLLMLSFAF SSAETAFA
SP 7	ADF38066.1	alkaline phosphatase	MRKKLAGITLAGAVALASFGVG NSLIQTEA

SP 8	ADF38542.1	cytochrome C oxidase subunit II, periplasmic domain protein	MSVKNWLMGLVVLFTMVISVM TSGSLGVLA
SP 9	ADF38755.1	putative peptidoglycan binding domain protein	MLKKKTLVTLSTLTLATTCVAFS SPTSEAA
SP 10	ADF39852.1	conserved hypothetical protein BMD_3012	MKSYRLVSFSTLCICLLVLFISPFQ DTSSA
SP 11	ADF39992.1	hypothetical protein BMD_3152	MKMKVRTLFLVLFFAGILVVTSY VDNNSQQ
SP 12	ADF40714.1	putative cell wall endopeptidase	MIWGGNEMKKMLSALLVGGAL FASPAMGHA
SP 13	ADF41182.1	peptidase, M23/M37 family	MIDVLRRILVVLVMGLCIGLLFL GGQMVKA
SP 14	ADF38396.1	levanase	MKKKGAYKWITLAVLLVIIAGLII TAVSK
SP 15	ADF38880.1	hypothetical protein BMD_2027	MEKRHKSKKKRMIISISLVFLVG GGATG
SP 16	ADF39133.1	Bacillolysin precursor (neutral protease)	MKKKKQALKVLLSVGILSSSAF AHTSSA
SP 17	ADF39282.1	VanW family protein	MKKVLGALLLGATIVSAFIAYSM YTNHSA
SP 18	ADF39902.1	N-acetylmuramoyl-L-alanine amidase cw1B (Cell wall hydrolase) (Autolysin)	MNKKHVMYTA VSSVLLSSSLLL HSIDADA

Table S6: List of signal peptides available at pMSP4

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF40267.1	hypothetical protein BMD 3427	MKWKYKHALISLAIGLLSFVCFVI YSKVAA
SP 2	ADF40379.1	conserved hypothetical protein BMD_3539	MKRFRVSAVVLGLALLFSLAP QTVAEA
SP 3	ADF40719.1	polysaccharide deacetylase	MKTMGYKRILTLLGCCILFLGTA GYIVKA
SP 4	ADF41608.1	signal peptide peptidase SppA, 36K type	MNKKRWIALLIAACLFIFSAIVNI GFSVK
SP 5	ADF41874.1	peptidase M23 family protein	MTRKTLVMTATILVGLSAAWIG NEKIAYA
SP 6	ADF38395.1	levansucrase	MKMKRVAKHTTAATLAAALLV GGGYQTFA
SP 7	ADF37374.1	phospholipase/carboxylesterase family protein	MYQRLKKSVELLAILSLAIGIVLS YEKA
SP 8	ADF37703.1	conserved hypothetical protein BMD_0841	MAANKWTKWMVGLSSVAAFTT FIGISQK
SP 9	ADF37131.1	ErfK/YbiS/YcfS/YnhG family protein	MRKIILQAAALFLAAIISISYTASA ANA
SP 10	ADF37220.1	conserved hypothetical protein BMD_0309	MKKKLFGYIMASGLTLGVMAA ANSTAF
SP 11	ADF37360.1	conserved hypothetical protein BMD_0470	MNIKRWLLTATTVILATFGVSAV SSVHA

SP 12	ADF37807.1	hypothetical protein BMD_0946	MATKRTFFSFFLISALLCLLVVPN SVMA
SP 13	ADF38126.1	spore cortex-lytic enzyme	MKHSYMKSMVVACLATLSFIGF NATATA
SP 14	ADF38532.1	bacterial SH3 domain protein	MKKRIKMLISIGIAASLIHSSQSKV HA
SP 15	ADF39018.1	protease	MKKVSIRSVLSTVAFSVALSSFA MGASA
SP 16	ADF39390.1	AhpC/TSA family protein	MVKKIIAVLLLVGFLSYALWQGL SPNGA
SP 17	ADF40060.1	spore cortex-lytic enzyme	MSKKRVKKWAVASAASVAFLA SQGTASA
SP 18	ADF38774.1	hypothetical protein BMD_1921	MKKVLVGMALFTLVAGVASV GTSVATG

Table S7: List of signal peptides available at pMSP5

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF39387.1	hypothetical protein BMD_2542	MTKRMITLLSVIFIAVLAVFFM SQTSLA
SP 2	ADF40202.1	conserved hypothetical protein BMD_3362	MKKIFPIVLSFGLTTSVSFSTAS NVDA
SP 3	ADF40323.1	conserved hypothetical protein BMD_3483	MKKVFNIGVLFFLIFGLFLGVG PTGKA
SP 4	ADF37209.1	subtilisin like serine protease	MKKSLSALLGVSLLVFGLTLG AESSYA
SP 5	ADF38432.1	levanase	MKRTVKKLLMIGVACSISLFS ACPVLA
SP 6	ADF39385.1	conserved hypothetical protein BMD_2540	MKKHVKRKVVRLVSLLVVLII VGSYQ
SP 7	ADF39492.1	conserved hypothetical protein BMD_2648	MEMKKFGIILASLALFFSLSTG VTAQA
SP 8	ADF40338.1	zinc carboxypeptidase family protein	MRKTVKRTAVPALLSIGLLLS PLAVQA
SP 9	ADF40566.1	L-asparaginase II	MKLKRIVSITFLSSVILFSGPLSL VNA
SP 10	ADF40777.1	conserved hypothetical protein BMD_3946	MNRLFKWSASTLLCLMYCFLI PLPSYA
SP 11	ADF41191.1	D-alanyl-D-alanine carboxypeptidase	MKRSKKSIIYLFIVLLCLSGWP SISSA
SP 12	ADF41282.1	stage III sporulation protein AE	MKRWTFKVNILALLFLFFFFP ENVQA
SP 13	ADF41455.1	bypass-of-forespore protein C	MKKVIHQAAFLMLVSIVLCIM PYRADA
SP 14	ADF42031.1	conserved hypothetical protein BMD_5232	MIKWAKVLSTSALGTAIACSSI TTSHA
SP 15	ADF38000.1	conserved hypothetical protein BMD_1139	MKAMVGVNVAAILCGAVM YAGTQHW

SP 16	ADF41919.1	endopeptidase LytF	MKKRQLALSLGAIVGGTFLYQ ASAEA
SP 17	ADF37280.1	conserved hypothetical protein BMD_0369	MKKRFRIATVAGAVSLGLLTA GQANA
SP 18	ADF37788.1	conserved hypothetical protein BMD_0927	MKIFKRMTLAAVTGIFMTGI TETHA

Table S8: List of signal peptides available at pMSP6

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF37802.1	two-component sensor histidine kinase/response regulator	MNNKLKRKSISTQFILLASIL LLAFA
SP 2	ADF37928.1	modifier protein of major autolysin LytC	MRKLTALALVLSVLLFQFTP LASVKA
SP 3	ADF38462.1	cyanophycinase domain protein	MKKKLVSLISFTLLSSLSTP LLAKA
SP 4	ADF38750.1	hypothetical protein BMD_1897	MLKIRFALSSLLLVFSLVMG APFASA
SP 5	ADF39236.1	conserved hypothetical protein BMD_2389	MKKFLAMIFVLIFSLSFTFY STASA
SP 6	ADF39288.1	cell wall-associated protease	MKKKGIIISLLSVLLVSSLSV STTQA
SP 7	ADF39306.1	secreted cell wall DL-endopeptidase	MKKKMLLNNTTVLLGLGGI LSTPAYA
SP 8	ADF39980.1	Lipase/Acylhydrolase (GDSE)	MSSTKKVVFISIIILNVFLG VFAFY
SP 9	ADF39988.1	transcription regulator domain protein	MKKYKIGILAACILLGTA FGIAYQ
SP 10	ADF40077.1	cell wall-associated protease	MNKKGIISLVSAGLLASTLS VQSVQA
SP 11	ADF40397.1	conserved hypothetical protein BMD_3564	MKKFFKIGCLGFIIILIVVI AVVA
SP 12	ADF40722.1	hypothetical protein BMD_3889	MMKKKLAVISTACLLILASD PIPSYA
SP 13	ADF40824.1	conserved hypothetical protein BMD_3993	MNRVKIIAASLLAVLLAFS DNFTSA
SP 14	ADF40826.1	glycerophosphoryl diester phosphodiesterase	MRRKWWFVSSLTLCLCGIF MFIHTQA
SP 15	ADF41380.1	conserved hypothetical protein BMD_4556	MIKFTLKSIVLTIFLLCIVFL GMQTA
SP 16	ADF41930.1	integral membrane protein (DUF1779)	MFKKISVFLAIFITGLFVYHG SYVSN
SP 17	ADF40613.1	conserved hypothetical protein BMD_3780	MRKKWSVLLCTLALAMMT FILPVNA
SP 18	ADF37876.1	conserved hypothetical protein BMD_1015	MYIKKICIGSILFLLFCSSALP AKA

Table S9: List of signal peptides available at pMSP7

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF38398.1	transporter, major facilitator family	MRKRIMTALLLVTVLAAMEGTIVST
SP 2	ADF38764.1	hypothetical protein BMD_1911	MKRKMIALTFSAALCFSASGSLAEA
SP 3	ADF38861.1	foldase protein PrsA	MSKNKIIMIIAAVVIIAAVAIGAVA
SP 4	ADF39025.1	integral membrane protein (DUF6)	MNTSLSKILILCAAVLWGTGTGTAQA
SP 5	ADF39788.1	conserved hypothetical protein BMD_2948	MMKLSKKLMIPVLGASLLMPTIANA
SP 6	ADF40634.1	cell wall hydrolase	MKVMKPIMVTVLGLSLAAFYEGAFP
SP 7	ADF41279.1	stage III sporulation protein AH	MLLKKQTVWLLTMLSLVVVLSVYYM
SP 8	ADF37364.1	putative exported cell wall-binding protein	MKKFVFTLGTTAILSAGFVGAASA
SP 9	ADF40809.1	Endopeptidase	MKKVIAALTLAGVIVSTSPIVSQA
SP 10	ADF41873.1	C-terminal processing peptidase	MNRKIVALLMALSLVVGAAAGTYFG
SP 11	ADF37363.1	poly(3-hydroxybutyrate) depolymerase	MKRLFIAGIIFILFLSLGAVSSSA
SP 12	ADF37632.1	phosphotransferase system (PTS) lichenan-specific enzyme IIB component	MNILLCCAAGMSTSLLVTKMEKSA
SP 13	ADF37780.1	conserved hypothetical protein BMD_0919	MLGILLIVVAVILVVLITMNAKA
SP 14	ADF38785.1	polysaccharide deacetylase	MIRRISILLFTLCFLFNGALPALC
SP 15	ADF38838.1	hypothetical protein BMD_1985	MRKFYLAFLLMISTTMVILPSVKA
SP 16	ADF38954.1	spore cortex-lytic enzyme	MFKKLAIIIGALAVPMFGMSHGANA
SP 17	ADF39086.1	peptidoglycan-binding protein	MKKHIFTLGATALVSI GIADAASA
SP 18	ADF39204.1	spore cortex-lytic enzyme	MIKKLVIAIVMLIPSFGAGYSAFA

Table S10: List of signal peptides available at pMSP8

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF39501.1	glycerophosphodiester phosphodiesterase	MKKYLAGLSAVALCCSIASSAFA
SP 2	ADF39554.1	conserved hypothetical protein BMD_2710	MKKTMITFSLVLSLFGVASGASA
SP 3	ADF39834.1	LysM domain protein	MKKFIFTLGTTAILSAGFVGAASA
SP 4	ADF40014.1	LPXTG-motif cell wall anchor domain protein	MKKLGAILLSFLLFVGVFPTSSFA
SP 5	ADF40015.1	sortase family protein	MEPRKTRRTINRKKRLIVLSLLAA
SP 6	ADF40996.1	flagellar biosynthetic protein FliZ	MLRKVFIAALLISVLGASNQIVYA
SP 7	ADF41210.1	D-alanine-D-alanine carboxypeptidase	MKRTIVKLLVVIFLPLMTQPVLA
SP 8	ADF41368.1	putative membrane protein	MKTLFSFVCFLILATGLFPTISTA
SP 9	ADF39879.1	cell wall endopeptidase	MKKLIYSLALCGSLTVVPTISEA
SP 10	ADF37299.1	delta-lactam-biosynthetic de-N-acetylase	MRRFTFFLVFLFVMSTGIASVEA



SP 11	ADF37407.1	conserved hypothetical protein BMD_0521	MKKIGLVLSIAIFFTLSTGVTA
SP 12	ADF37687.1	conserved hypothetical protein BMD_0825	MKKILAFILVIGGTAVLFSTAF
SP 13	ADF38481.1	endopeptidase LytF (cell wall hydrolase)	MKKISSLALCSSLIVLPTISQA
SP 14	ADF38842.1	Aminopeptidase	MKKTVISLAVATSLTSLFTSVQA
SP 15	ADF39052.1	conserved hypothetical protein BMD_2204	MTKLMILVFMLISFFVHPQKMKA
SP 16	ADF39606.1	hypothetical protein BMD_2765	MVGFILLFPLNMLAGPISSIAEA
SP 17	ADF39900.1	conserved hypothetical protein BMD_3060	MKRLFVMVAVIAFLALSRMLVFA
SP 18	ADF39990.1	conserved hypothetical protein BMD_3150	MIRKWVAVCVVFAGFIFPQAVSA

Table S11: List of signal peptides available at pMSP9

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF40218.1	domain of unknown function (DUF477)	MKFLASFALLSLLCNMVSVAF
SP 2	ADF40725.1	gamma-glutamyltransferase	MKVMKMLVIALFSLVVTPTAAA
SP 3	ADF40822.1	conserved hypothetical protein BMD_3991	MKKTLKIVGVGLAVLVTVTVAFF
SP 4	ADF41772.1	L-Ala--D-Glu endopeptidase	MRKTIILLFMFMSIFTGWHHVT
SP 5	ADF42035.1	conserved hypothetical protein BMD_5236	MKKRVSLGLSLTLASSAASAF
SP 6	ADF39900.1	conserved hypothetical protein BMD_3060	MKRLFVMVAVIAFLALSRMLVFA
SP 7	ADF37524.1	alpha amylase	MKWKRTSMLLILLLFGSSASA
SP 8	ADF37686.1	conserved hypothetical protein BMD_0824	MKLALLLVIIIIVAAALAGTISA
SP 9	ADF38166.1	conserved hypothetical protein BMD_1305	MKKICLLVFLIVLYSGKSVHA
SP 10	ADF38222.1	putative sporulation protein YpjB	MKGRIVGILLVLFAYTTTVHA
SP 11	ADF38236.1	conserved hypothetical protein BMD_1375	MIKILISAVLFFSFMNCSVSAA
SP 12	ADF38239.1	aspartate aminotransferase	MKLAKRVSALTPSSTLAITAKA
SP 13	ADF39160.1	conserved hypothetical protein BMD_2312	MRKIMISAAAFLFMSATPVLA
SP 14	ADF39756.1	polysaccharide deacetylase	MRKISIFVILVLTIVFPSPAFS
SP 15	ADF40784.1	hypothetical protein BMD_3953	MKRYMLSAFLGSLLLLSIAFS
SP 16	ADF40037.1	hypothetical protein BMD_3197	MKKATSILLIVLALAGLGGVCA
SP 17	ADF37869.1	capsule biosynthesis protein	MNKKLLLFNIIAFLLLICVIA
SP 18	ADF40825.1	conserved hypothetical protein BMD_3994	MKWIGGVFTFSFILFMSSWFA

Table S12: List of signal peptides available at pMSP10

Signal peptide	GenBank ID	Native protein	Sequence
SP 1	ADF40830.1	conserved hypothetical protein BMD_3999	MVKKIALCLLFLMLFPMQIN A
SP 2	ADF37228.1	conserved hypothetical protein BMD_0317	MKVITTVLASAVLVGGLGAS A
SP 3	ADF38580.1	conserved hypothetical protein BMD_1725	MKKAVGIILLILLCCINQRVEA
SP 4	ADF39850.1	Copper resistance protein	MKKIIGLLLACFLAVPTVSFA
SP 5	ADF40036.1	hypothetical protein BMD_3196	MRKWSFLFFVLAFMHPASAL A
SP 6	ADF40101.1	competence-specific nuclease	MKKIVSGILILVLAVIVMIEG
SP 7	ADF37283.1	Low molecular weight phosphotyrosine protein phosphatase	MINVLFVCLGNICRSPMAEA
SP 8	ADF40489.1	malate dehydrogenase, NAD-dependent	MAFRRKKIAVIGAGFTGATA
SP 9	ADF40755.1	hypothetical protein BMD_3923	MKRMRAFAIMTFLVVGSGYA
SP 10	ADF40786.1	conserved hypothetical protein BMD_3955	MKRLFYTVSTFCFVWLAF
SP 11	ADF37472.1	conserved hypothetical protein BMD_0586	MSRMKKLAAGAAIGSTVAA
SP 12	ADF37968.1	hypothetical protein BMD_1107	MLKKIAVLLMMLGIAHF
SP 13	ADF38163.1	Ser/Thr protein phosphatase family protein	MKKTYTRRSFLKG
SP 14	ADF39761.1	two-component sensor histidine kinase PhoR	MKISLKAKMGLV
SP 15	ADF41187.1	cytochrome c biogenesis protein	MKKKRLIIRTVI
SP 16	ADF41329.1	penicillin-binding protein	MKKKKRRKSHV
SP 17	ADF41793.1	conserved hypothetical protein BMD_4993	MSKLKDPSSA
SP 18	ADF37949.1	diguanylate cyclase/phosphodiesterase	MSIKKKLSVF
SP 19	ADF39130.1	conserved hypothetical protein BMD_2282	MRKRKQKALI
SP 20	ADF39284.1	conserved hypothetical protein BMD_2438	MTVRKRKRKK

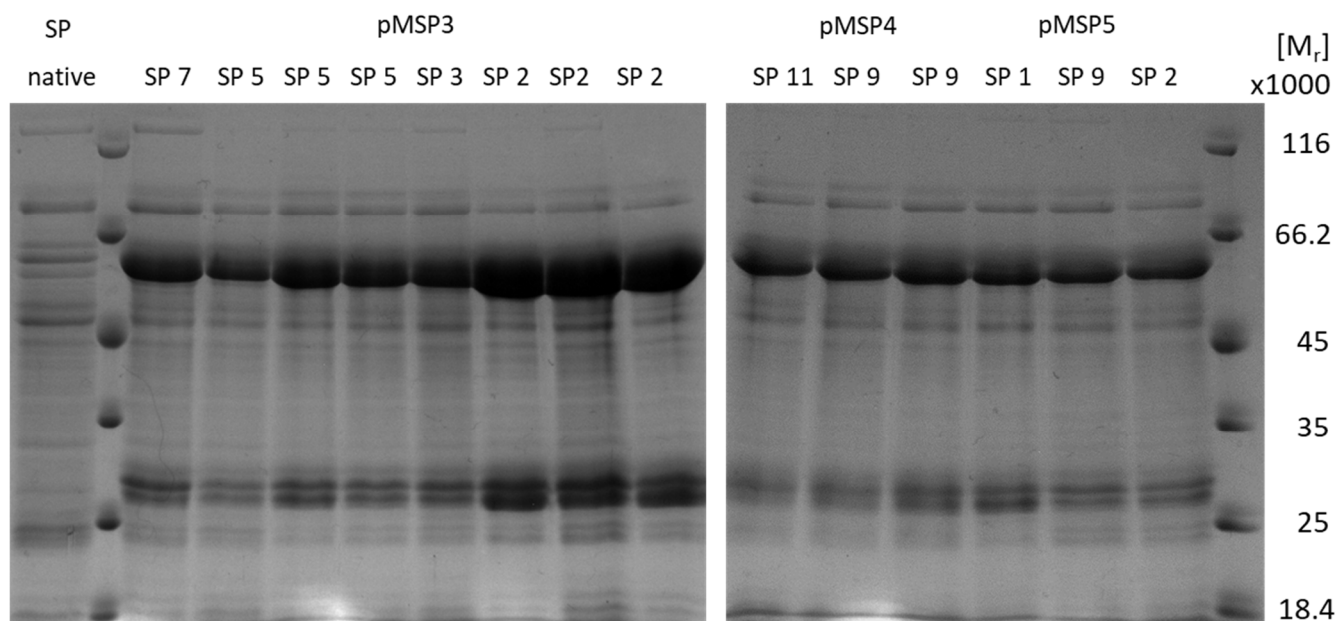


Figure S1: Comparison of extracellular proteins of BmasPGA-secreting selected clones of recombinant *P. megaterium* in upscaling. The clones with the native SP as control or with the alternative SPs were cultured at 37 °C in shake flasks (50 mL culture volume). Twenty-two hours after induction the extracellular proteins were precipitated by ammonium sulfate. pMSP3 to 5 are indicating the corresponding multi SP plasmids, SP X indicates the corresponding SP of the given plasmid as presented in Table 2.

## References

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