Supplementary Table 1: Protein production and secretion in *B. subtilis* protease-deficient strains

Product (origin) / Naturally secreted via Sec (Y/N*)[1,2]	Producing vector/Promoter/SP[1]	Strain	Secreted yield	Biological active/ Degradation*	Ref.**
L-asparaginase (ASN) (*B. subtilis*) / Y (original SP mentioned[2])	pP43NMK/P43 variant (B2, −28:A→G, 13:A→G)/WapA	WB600	After deletion of the N-terminal 25-residues of ASN 407.6 U/mL (2.5 g/L)	Y/N	[1]
Pullulanase (*Bacillus naganoensis*) / N	pMA0911/P*hpaII* or P43/LipA	WB600, WB800	Best yield obtained using WB600 in combination with SP-*lipA* and P43 (24.5 ± 0.3 U/ml, 6.28x better than original (WB800 SP-*lipA* and P-*hpaII*)	Y/n.d.	[2]
Recombinant alkaline α-amylase (Unknown origin) / Y (mentioned in text[2])	pMA0911-*amy*/P*hpaII*/n.d.	WB600 mut-12#	Yield increased with 35.0% and productivity with 8.8%, the extracellular protein concentration increased 37.9% when compared to WB600	Y/N	[3]
CotA-laccase (*Bacillus pumilus*) / N	pMA0911/ P*hpaII*/WapA	WB600	373.1 U/mL	Y/n.d.	[4]
γ-Polyglutamic acid synthetase (*B. subtilis)* / N	pWB980/P43/n.d.	WB600	1.74 g/L	n.d./N	[5]
Cellobiohydrolases (*cbhA*, *celK*, *celO*, *cel48Y*, *cel48S*) and endoglucanase (*celA*) (*Clostridium thermocellum)* / n.d.	pP43JM2/P43/NprB	WB600, WB800	Efficient secretion for all but CelO. No quantification	Y/I	[6]
Keratinase (*Bacillus licheniformis*) / Y (JQ894491[2])	pMA0911/P*hpaII*/native	WB600	323 units/mL (non-induced)	Y/N	[7]
Xylanase (*Thermoanaerobacterium sp*) / Y (mentioned in text[2])	pJX33/P*bj31*/native	WB800	(8.4U/ml)	Y/Y	[8]

Bacillopeptidase F (*B. subtilis*) / Y (KT259045[2])	pMD18-T/native/+Proseq, native	WB700	n.d.	Y/Y	[9]
Synthesized cecropin A-melittin mutant (U) / n.d.	pDM030/P*glv*(maltose-inducible)/SacB	WB700	159 mg/L	Y/N	[10]
α-Amylase (*Bacillus amyloliquefaciens*) /Y (mentioned in text[2])	pP43X/P43/32aa (non-specified)	WB800	5566 U/mg, 1.48x increased production in WB800, when compared to wildtype	Y/I	[11]
Lysozyme (*Bacillus licheniformis*) / Y (mentioned in text)	pGJ203/P43 fused to P*sacB/*SacB	WB800	78 U/ml	Y/?	[12] ***
Protease (*Halobacillus* sp) / n.d.	pSaltExSePR5/P*opuAA* (NaCl induction)/SubE	WB800	9.1 U/ml	Y/I	[13]
β-1,3-1,4-glucanase (LicB) (*C. thermocellum*) / Y (mentioned in text[2])	pP43JM2/P43/NprB	WB800	1.18 U/g cell mass	Y/Y	[14]
Nattokinase (*B. subtilis* VTCC-DVN-12-01) / Y (EF061457[2])	n.d./P*acoA*/n.d.	WB800	600 mg/L	Y/N	[15]
Cellobiose 2-epimerase from (*Caldicellulosiruptor saccharolyticus*) / n.d.	pMA09/n.d./n.d.	WB800	5.3 U/mL	Y/Y	[16]
PA (component of the anthrax toxin)(*Bacillus anthracis*) / n.d.	pHT28*pagA*/P*grac*/AmyL	BRB07, BRB08, BRB11, BRB12, BRB14	BRB07: Approximately 1 g/L, BRB08: Slightly more than 1 g/L, BRB11-14: Slightly less than 1 g/L	n.d./Y in BRB07-12, hardly any degradation in BRB14	[17]
1,3-1,4-β-glucanase(U) / n.d.		WB600	2493.8 U/mL	Y/n.d.	[18]

Paramyosin (*Clororchis sinensis*) / N (cyst wall)	pEB03-CotC-CsPmy/PcotC/spore located	WB600	n.d.	Y/n.d.	[19]
Pullulanase (*Bacillus naganoensis*) / N	pMA0911/ Psac/LipA	WB600, WB800	Simultaneous DegQ production let to 60% increased enzyme activity in WB800. 26.5 U/ml	Y/n.d.	[20]
β-Mannanase *(Bacillus clausii*) / Y (WP_041823500.1[2])	Pma5/P43/LipA	WB600	1050 U/ml	Y/n.d.	[21]
Phospholipase D (*Streptomyces racemochromogenes*) / Y (AB573232[2])	pMA0911/HpaII/AmyE RBS optimized	WB600	24.2 U/ml	Y/n.d.	[22]
L-Asparaginase (*Bacillus cereus*) / N	pP43NMK/P43/AmyE	WB600	374.9 U/ml	Y/n.d.	[23]
GH30-8 endoxylanase (*B. subtilis*) / Y (mentioned in text[2])	pMA05/n.d./n.d.	WB800	55 U/mL	Y/N	[24]
Cel8A endoglucanase fused to LysM cell wall binding module (*C. thermocellum)* / n.d.	pBL113/P IPTG inducible/ PhrC Integration vector	WB800, BRB07, BRB08, BRB14	n.d. BRB08 highest production without causing secretion stress	Y/Y	[25]
Endo-inulinase (*Pseudomonas mucidolens*) / n.d.	PHY300PLK/P43/NprB Integration vector	WB800-R	67.84 ± 0.72 g/L	Y/n.d.	[26]
Nattokinase / Y (mentioned in text[2])	pBSG03/P08 (CodY biding sequence deleted)/WapA	WB800	292 FU/ml	Y/N	[27]
β-galactosidase (*Bacillus megaterium*) / n.d.	pMA05/n.d./n.d..	WB800	17.55 U/ml	Y/n.d.	[28]
Chitinase (*Bacillus* sp. DAU101) / Y (mentioned in text[2])	pP43NMK/P43/NprB also RBS optimized	WB600	51.67 U/mL	Y/reduced when compared to previous results	[29]

Pullulanase (*Bacillus naganoensis*) / N	Chromosomally inserted/PHpaII/LipA	WB800 WB600	30.32 U/ml 18.83 U/ml	Y/N Y/Y	[30]
Alkaline serine protease (BcaPRO, *Bacillus clausii*) / Y (FJ940727.1[2])	pWPROn/PBsamy-PBaamy/DacB	WB600	27,860 U/ml	Y/n.d.	[31]
Cyclomaltodextrin glucanotransferase (*Bacillus firmus*) / Y (KF270899)	pWB980/P43/SacB	WB800	1.33 µmol β-CD/min/mL	Y/Y	[32]
β-mannanase (*Bacillus* sp. MK-2) / Y (CAB12407.2[2])	pP43NMK/P43/NprB	WB800	2802 U/mg	Y/n.d.	[33]
Nattokinase (*B. subtilis* natto) / Y (mentioned in text[2])	pMA0911-*wapA-pro*-NK/PHpaII-PHpaII-PP43/WapA	WB800	816.7 ± 30.0 FU/mL	Y/N	[34]
CIPS/SCIN/IsaA/Nuc (*Stapylococcus aureus*) / Y (mentioned in text[2])	pRAG3/PspaS/AmyQ	PG10 (mini *Bacillus*)	Production in mini-*Bacillus* when this was not possible in the wt *Bacillus* strain (168)	Y/Y	[35]
Diacetylchitobiose deacetylase R157T (*Pyrococcus horikoshii*) / N	pMA0911/P43/ YncM	WB600	3,112.2 U/mg	Y/Y	[36]
α-amylase (AmySA K82E/S405R, *Bacillus stearothermophilus*) / Y (mentioned in text[2])	pBE/PaprE/YojL	WS11 (Δ*hrcA*)****	9201.1 U mL-1	Y/n.d.	[37]
Recombinant keratinase, KerZ1 (*Bacillus licheniformis*) / Y (mentioned in text[2])	pP43NMK/p43/NprB	WB600	426.60 KU/mL	Y/N	[38]
Polyethylene terephthalate hydrolase (PETase, *Ideonella sakaiensis*) / Y (mentioned in text[2])	pUBC19/P43/Amy	WB600	Increased 8 fold when compared to wt SP	Y/n.d.	[39]
Adenylate deaminase (AMPF*, Aspergillus oryzae* GX-08) / Y (mentioned in text[2])	pMA5/PHpaII/n.d..	WB600	2540 U/mL	Y/n.d.	[40]
Chlorothalonil hydrolytic dehalogenase (Chd, n.d.) / n.d.	Integrated in chromosome/PaprE	WB800	2622 U/L	Y/n.d.	[41]
OUC-Lipase 6 (*Streptomyces violascens*) / N	pP43NMK/P43/NprB	WB800	Increased activity 3.24 fold	Y/n.d.	[42]

Lantibiotics (*B. subtilis*) / Y (mentioned in text[2])	Integration in chromosome/Pspank-hy/leader peptide SpaS	PG10 (mini *Bacillus)*	n.d., clean background to simplify downstream processing	Y/n.d.	[43]

[1]Verified with SignalP4.1 if a Genbank accession was available
[2]Where appropriate the Genbank accession code or a reference to the main manuscript is indicated
[*]Y, yes; N, no; n.d., not described; I, inconclusive
[**]References cover the period between 2012 and 2020
[***]Only the abstract is publicly available.
[****]Mutation results in overexpression of chaperones GroEL-GroES and DnaK-DnaJ-GrpE

Supplementary Table 2: **Protein production and secretion in *L. lactis,* using various strains and expression systems**

Product / Naturally secreted via Sec (Y/N*)[1,2]	**Producing vector/Promoter/SP[1]**	**Strain**	**Secreted yield**	**Biologically active/ recognized***	**Ref****
Virulence factors (SA0620, FtsL, ClfB, SA2100, Pro-Atl, IsdB) (*Staphylococcus aureus*) / Y (mentioned in text[2])	pNG4110/P*nisA*/Usp45 pNG4111/P*nisA*/Usp45 pNG4210/P*nisA*/Usp45	PA1001	mg/L range, post-translational phosphorylation obtained	Y (partially done)/Y	[44]
Virulence factors (HtrA1, HtrA2)(*S. aureus*) / Y (KF322112 and KF322111[2])	pLB145/P*ZitR*/Exp4	MG1363	2.5 and 2.2 mg/L (HtrA1 and HtrA2 resp.) Max 7 mg	Y/N	[45]
S. aureus virulence factors (LytM, Nuc, Aly, SA0620, SA2097) / Y (mentioned in text[2])	pNG400/P*nisA*/Usp45	PA1001	22; 18.8; 1.4; 4.2 mg/L (LytM, Nuc, SA2097, Aly resp.)	Y/Y	[46]
β-Cyclodextrin Glucanotransferase / Y (mentioned in text[2])	pNZ8048/P*nisA*/native-SP (NSP), SPK1 or Usp45	NZ9000	SPK1 secretion efficiency higher than Usp45. Total protein production higher for Usp45-fusion proteins	Y/Y	[47]
Designed Ankyrin Repeat Proteins (DARPins) fused to LysM-domain / n.d.	pNZ8148/P*nisA*/Usp45	NZ9000	n.d.	Y/N	[48]
Murine IL-10, murine TGF-β1, human Elafin, murine SLP-1 / Y (mentioned in text[2])	pSEC/P*nisA*/Usp45	NZ9000 (wt) and NZ9000Δ*htrA* (Δ*htrA*)	IL-10: 40 ng/ml (wt) TGF-β1:50 ng/ml (wt) Elafin: 37 ng/ml (wt) *vs* 55 ng/ml (Δ*htrA*) SLP-1: 100% secreted, yield not quantified (wt)	Y/I	[49]
Kisspeptin / Y (BC022819.1[2])	pNZ401/P*nisA*/Usp45 + LEISSTCDA pro-peptide	NZ9000	27.9 µg/ml	Y/I	[50]
B. subtilis originated Subtilisin QK-2 / Y (AJ579472.2[2])	pRF01 (pNZ8149 derivative)/P*nisA*/Usp45	NZ9000 NZ3900	Nd	Y/I	[51]

Mouse heme oxygenase-1 / n.d.	pNZ8148#2:SEC/P*nisA*/Usp45	NZ9000	Intracellular production, 5.37 ug/ml	Y/N	[52]
Pancreatitis-associated protein I (PAP) / n.d.	pSEC/P*nisA*/Usp45	NZ9000	n.d.	Y/n.d.	[53]
Human interleukin 22 (IL-22) / Y (mentioned in text[2])	pSEC/P*nisA*/Usp45	NZ9000	10 ng/ml	Y/Y	[54]
HSP65-6IA2P2 as autoantigens against T1 diabetes / n.d.	pCYT/P*nisA* and pHJ/P32/Usp45	NZ9000	n.d.	Y/n.d.	[55]
Carcinoembryonic antigen / n.d.	pSEC:LEISS/P*nisA*/Usp45 + LEISSTCDA pro-peptide	NZ9000	Surface display on *L. lactis* cells using LcsB anchor	Y/Y	[56]
Infectious bronchitis virus (IBV) multi-epitope EpiC fused to protein A anchor *S. aureus* / n.d.	pNZ8149/P*nisA*/Usp45	NZ3900	27 mg/L	Y/Y	[57]
Campylobacter jejuni originated CjaA antigen presenting CjaD peptide epitopes fused to the C-terminus of the *L. lactis* YndF containing the LPTXG motif / Y (mentioned in text[2])	pUWM1000/P*usp45*/Usp45	IL1403	n.d., however protein was detected on the surface of the L. lactis IL1403 producing cells	Y/I	[58]
Bacteriocin Pediocin PA-1 / Y (mentioned in text)	pSEC/P*nisA*/Usp45 + LEISSTCDA or SD pro-peptide	NZ9000	~2-4 µmol pediocin PA-1 equivalents/g of dry weight bacteria.	Y/n.d.	[59]
Leuconostoc mesenteroides originated glucansucrase (DsrI)*** / Y (mentioned in text[2])	pMSP3535H3/P*nisA*/Usp45,	LM0230	380 mg/L (at pH 6.0, in optimized fermentation set-up, high copy number plasmid (45–85 copies per cell))	Y/I	[60]
Thymic stromal lymphopoietin / n.d.	pLB333/P*groESL*/Exp4	MG1363	Maximum of 2500 pg/ml/OD bacteria, SICE system	Y/n.d.	[61]
Mature sakacin A (SakA) and its cognate immunity protein (SakI), two chimeras	pNZ8048/P*nisA*/Usp45 pMG36c/P32/Usp45	NZ9000	3.2 and 4.9 ug/mg dry weight bacteria producing EntP/SakA or SakA, resp.	Y/N	[62]

mimicking the N-terminal end of mature enterocin P (EntP/SakA) and mature enterocin A (EntA/SakA) together with SakI / mentioned in text; SakA contains 18 aa leader peptide, SakI not secreted					
Panel of 31 recombinant proteins (*Plasmodium falciparum*) / mentioned in text; diverse sub-cellular locations[2]	pSS1/n.d./n.d	MG1363	n.d.	Y/N	[63]
Phytase (*Escherichia coli*) / n.d.	pFUM003/PlacA/Usp45	NZ9000	19 U/ml	Y/N	[64]
E6 oncoprotein (Human papillomavirus) / n.d.	pNZ8123/PnisA/Usp45	NZ9000	n.d.	Y/N	[65]
Tumor necrosis factor-related apoptosis-inducing ligand (synthetic human) / N (transmembrane)	pNZ8124/PnisA/Usp45	NZ9000	97.4 ng/ml	Y/Y	[66]
Plantaricin E (*Lactobacillus plantarum*) / Y (mentioned in text)	pNZ8148/PnisA/PlnA	NZ3900	n.d.	Y/n.d.	[67]
Apical membrane antigen 1 (*Eimeria tenella*) / N (transmembrane)	pTX8048/PnisA/Usp45	NZ9000	n.d.	Y/Y	[68]
Nuclease (*S. aureus*) / Y (mentioned in text)	pS (integrative)/P170/Usp45	NZ9000	n.d.	Y/N	[69]
Microbial transglutaminase (*Streptomyces mobaraensis*) / Y (DQ132977)	pNZ8048/Pp5 or PnisA/Usp45 or Usp45(K2A)	NZ9000	43.5 ± 0.4 mg/L	Y/N	[70]
MPB70 (*Mycobacterium bovis*) / Y (mentioned in text[2])	pNZ8048e/PnisA/ Usp45TM8	NZ9000	n.d.	Y/n.d.	[71]
VP1 (enterovirus 71) / n.d. (located on virion surface)	pMG36e/P32/Usp45	MG1363	n.d.	Y/n.d.	[72]

β-1,3-1,4-glucanase (*Bacillus* sp. SJ-10) / n.d.	pNG8149/PnisA/Usp45	NZ3900	n.d.	Y/N	[73]
cyclodextrin glucanotransferase/ Y (mentioned in text[2])	pNG8048/PnisA/G1	NZ9000	16.89 U/ml	Y/n.d.	[74]
Tumour necrosis factor-related apoptosis-inducing ligand (Human) / N (transmembrane protein)	pNG7021/PpepN/Usp45	MG1363	n.d.	Y/Y	[75]
E7 oncoprotein (optiE7; human papillomavirus type 16) / Y (mentioned in text[2])	pNZ8123/PnisA/Usp45	NZ9000	35.49 µg/mL	Y/N	[76]
IMP1 (*Eimeria tenella*) / N (membrane protein)	pTX8048/PnisA/Usp45	NZ9000	n.d.	Y/n.d.	[77]
Circumsporozoite protein (*Plasmodium falciparum*) / Y (mentioned in text[2]; sporozoite surface located)	pSS1/n.d./n.d.	MG1363	25 mg/L	Y/n.d.	[78]

[1]Verified with SignalP4.1 if a Genbank accession was available

[2]Where appropriate the Genbank accession code or a reference to the main manuscript is indicated

[*]Y, yes; N, no; n.d., not described; I, inconclusive

[**]References cover the period between 2012 and 2020

[***]First publication describing industrial application of *L. lactis* for secreted protein production

REFERENCES

[1] Feng, Y., Liu, S., Jiao, Y., Gao, H., Wang, M., Du, G. et al. (2017) Enhanced extracellular production of L-asparaginase from *Bacillus subtilis* 168 by *B. subtilis* WB600 through a combined strategy. *Appl.Microbiol.Biotechnol.* **101**: 1509-1520, https://doi.org/10.1007/s00253-016-7816-x

[2] Song, W., Nie, Y., Mu, X.Q., Xu, Y. (2016) Enhancement of extracellular expression of *Bacillus naganoensis pullulanase* from recombinant *Bacillus subtilis*: Effects of promoter and host. *Protein Expr.Purif.* **124**: 23-31, https://doi.org/10.1016/j.pep.2016.04.008

[3] Ma, Y., Yang, H., Chen, X., Sun, B., Du, G., Zhou, Z., et al. (2015) Significantly improving the yield of recombinant proteins in *Bacillus subtilis* by a novel powerful mutagenesis tool (ARTP): Alkaline alpha-amylase as a case study. *Protein Expr.Purif.* **114**: 82-88, https://doi.org/10.1016/j.pep.2015.06.016

[4] Guan, Z.B., Shui, Y., Song, C.M., Zhang, N., Cai, Y.J., Liao, X.R. (2015) Efficient secretory production of CotA-laccase and its application in the decolorization and detoxification of industrial textile wastewater**.** *Environ.Sci.Pollut.Res.Int.* **22**: 9515-9523, https://doi.org/10.1007/s11356-015-4426-6

[5] Lin, B., Li, Z., Zhang, H., Wu, J., Luo, M. (2016) Cloning and Expression of the gamma-Polyglutamic Acid Synthetase Gene *pgsBCA* in *Bacillus subtilis* WB600. *Biomed.Res.Int.* **2016**: 3073949, https://doi.org/10.1155/2016/3073949

[6] Liu, J.M., Xin, X.J., Li, C.X., Xu, J.H., Bao, J. (2012) Cloning of thermostable cellulase genes of *Clostridium thermocellum* and their secretive expression in *Bacillus subtilis*. *Appl.Biochem.Biotechnol.* **166**: 652-662, https://doi.org/10.1007/s12010-011-9456-z

[7] Liu, B., Zhang, J., Li, B., Liao, X., Du, G., Chen, J. (2013) Expression and characterization of extreme alkaline, oxidation-resistant keratinase from *Bacillus licheniformis* in recombinant *Bacillus subtilis* WB600 expression system and its application in wool fiber processing. *World J.Microbiol.Biotechnol.* **29**: 825-832, https://doi.org/10.1007/s11274-012-1237-5

[8] Phuong, N.D., Jeong, Y.S., Selvaraj, T., Kim, S.K., Kim, Y.H., Jung, K.H., et al. (2012) Production of XynX, a large multimodular protein of *Thermoanaerobacterium* sp., by protease-deficient *Bacillus subtilis* strains [corrected]. *Appl.Biochem.Biotechnol.* **168**: 375-382, https://doi.org/10.1007/s12010-012-9781-x

[9] Meng, D., Dai, M., Xu, B.L., Zhao, Z.S., Liang, X., Wang, M., et al. (2015) Maturation of Fibrinolytic Bacillopeptidase F Involves both Hetero- and Autocatalytic Processes. *Appl.Environ.Microbiol.* **82**: 318-327, https://doi.org/10.1128/AEM.02673-15.

[10] Ji, S., Li, W., Baloch, A.R., Wang, M., Li, H., Cao, B., et al. (2017) Efficient biosynthesis of a Cecropin A-melittin mutant in *Bacillus subtilis* WB700. *Sci.Rep.* **7**: 40587, https://doi.org/10.1038/srep40587

[11] Chen, J., Chen, X., Dai, J., Xie, G., Yan, L., et al. (2015) Cloning, enhanced expression and characterization of an alpha-amylase gene from a wild strain in *B. subtilis* WB800. *Int.J.Biol.Macromol.* **80**: 200-207, https://doi.org/10.1016/j.ijbiomac.2015.06.018

[12] Zhang, H., Fu, G., Zhang, D. (2014) Cloning, characterization, and production of a novel lysozyme by different expression hosts. *J.Microbiol.Biotechnol.* **24**: 1405-1412, https://doi.org/10.4014/jmb.1404.04039.

[13] Promchai, R., Promdonkoy, B., Tanapongpipat, S., Visessanguan, W., Eurwilaichitr, L., Luxananil, P. (2016) A novel salt-inducible vector for efficient expression and secretion of heterologous proteins in *Bacillus subtilis*. *J.Biotechnol.* **222**: 86-93, https://doi.org/10.1016/j.jbiotec.2016.02.019

[14] Luo, Z., Gao, Q., Li, X., Bao, J. (2014) Cloning of LicB from *Clostridium thermocellum* and its efficient secretive expression of thermostable beta-1,3-1,4-glucanase. *Appl.Biochem.Biotechnol.* **173**: 562-570, https://doi.org/10.1007/s12010-014-0863-9

[15] Nguyen, T.T., Quyen, T.D., Le, H.T. (2013) Cloning and enhancing production of a detergent- and organic-solvent-resistant nattokinase from *Bacillus subtilis* VTCC-DVN-12-01 by using an eight-protease-gene-deficient *Bacillus subtilis* WB800. *Microb.Cell.Fact.* **12**: 79-2859, https://doi.org/10.1186/1475-2859-12-79

[16] Wu, L., Xu, C., Li, S., Liang, J., Xu, H., Xu, Z. (2017) Efficient production of lactulose from whey powder by cellobiose 2-epimerase in an enzymatic membrane reactor. *Bioresour.Technol.* **233**: 305-312, https://doi.org/10.1016/j.biortech.2017.02.089

[17] Pohl, S., Bhavsar, G., Hulme, J., Bloor, A.E., Misirli, G., Leckenby, M.W., et al. (2013) Proteomic analysis of Bacillus subtilis strains engineered for improved production of heterologous proteins. *Proteomics* **13**: 3298-3308, https://doi.org/10.1002/pmic.201300183

[18] Niu, C., Liu, C., Li, Y., Zheng, F., Wang, J., Li, Q. (2018) Production of a thermostable 1,3-1,4-beta-glucanase mutant in *Bacillus subtilis* WB600 at a high fermentation capacity and its potential application in the brewing industry. *Int.J.Biol.Macromol.* **107**: 28-34, https://doi.org/10.1016/j.ijbiomac.2017.08.139.

[19] Sun, H., Lin, Z., Zhao, L., Chen, T., Shang, M., Jiang, H. et al. (2018) *Bacillus subtilis* spore with surface display of paramyosin from *Clonorchis sinensis* potentializes a promising oral vaccine candidate. *Parasit.Vectors* 2018, **11**: 156-018, https://doi.org/10.1186/s13071-018-2757-0.

[20] Deng, Y., Nie, Y., Zhang, Y., Wang, Y., Xu, Y. (2018) Improved inducible expression of *Bacillus naganoensis* pullulanase from recombinant *Bacillus subtilis* by enhancer regulation. *Protein Expr.Purif.* **148**: 9-15, https://doi.org/10.1016/j.pep.2018.03.012

[21] Zhou, C., Xue, Y., Ma, Y. (2018) Characterization and high-efficiency secreted expression in *Bacillus subtilis* of a thermo-alkaline beta-mannanase from an alkaliphilic *Bacillus clausii* strain S10. *Microb.Cell.Fact.* **17**: 124-018, https://doi.org/10.1186/s12934-018-0973-0

[22] Huang, T., Lv, X., Li, J., Shin, H.D., Du, G., Liu, L. (2018) Combinatorial Fine-Tuning of Phospholipase D Expression by *Bacillus subtilis* WB600 for the Production of Phosphatidylserine. *J.Microbiol.Biotechnol.* **28**: 2046-2056, https://doi.org/10.4014/jmb.1806.06048

[23] Feng, Y., Liu, S., Jiao, Y., Wang, Y., Wang, M., Du, G. (2019) Gene cloning and expression of the l-asparaginase from *Bacillus cereus* BDRD-ST26 in *Bacillus subtilis* WB600. *J.Biosci.Bioeng.* **127**: 418-424, https://doi.org/10.1016/j.jbiosc.2018.09.007

[24] Guo, Y., Gao, Z., Xu, J., Chang, S., Wu, B., He, B. (2018) A family 30 glucurono-xylanase from *Bacillus subtilis* LC9: Expression, characterization and its application in Chinese bread making. *Int.J.Biol.Macromol.* **117**: 377-384, https://doi.org/10.1016/j.ijbiomac.2018.05.143

[25] Huang, G.L., Gosschalk, J.E., Kim, Y.S., Ogorzalek Loo, R.R., Clubb, R.T. (2018) Stabilizing displayed proteins on vegetative *Bacillus subtilis* cells. *Appl.Microbiol.Biotechnol.* **102**: 6547-6565, https://doi.org/10.1007/s00253-018-9062-x

[26] Jiang, R., Qiu, Y., Huang, W., Zhang, L., Xue, F., Ni, H., et al. (2018) One-Step Bioprocess of Inulin to Product Inulo-Oligosaccharides Using *Bacillus subtilis* Secreting an Extracellular Endo-Inulinase. *Appl.Biochem.Biotechnol.* **187**:116-128, https://doi.org/10.1007/s12010-018-2806-3

[27] Cui, W., Suo, F., Cheng, J., Han, L., Hao, W., Guo, J., et al. (2018) Stepwise modifications of genetic parts reinforce the secretory production of nattokinase in *Bacillus subtilis*. *Microb.Biotechnol.* **11**: 930-942, https://doi.org/10.1111/1751-7915.13298

[28] Zhao, L., Zhou, Y., Qin, S., Qin, P., Chu, J., He, B. (2018) beta-Galactosidase BMG without galactose and glucose inhibition: Secretory expression in *Bacillus subtilis* and for synthesis of oligosaccharide. *Int.J.Biol.Macromol.* **120**: 274-278, https://doi.org/10.1016/j.ijbiomac.2018.07.148

[29] Pan, M., Li, J., Lv, X., Du, G., Liu, L. (2019) Molecular engineering of chitinase from Bacillus sp. DAU101 for enzymatic production of chitooligosaccharides. *Enzyme Microb.Technol.* **124**: 54-62, https://doi.org/10.1016/j.enzmictec.2019.01.012

[30] Wang, Y., Chen, S., Zhao, X., Zhang, Y., Wang, X., Nie, Y., et al. (2019) Enhancement of the production of *Bacillus naganoensis* pullulanase in recombinant *Bacillus subtilis* by integrative expression. *Protein Expr.Purif.* **159**: 42-48, https://doi.org/10.1016/j.pep.2019.03.006

[31] Liu, Y., Shi, C., Li, D., Chen, X., Li, J., Zhang, Y., et al. (2019) Engineering a highly efficient expression system to produce BcaPRO protease in *Bacillus subtilis* by an optimized promoter and signal peptide. *Int.J.Biol.Macromol.* **138**: 903-911, https://doi.org/10.1016/j.ijbiomac.2019.07.175

[32] Gimenez, G.G., Costa, H., de Lima Neto, Q.A., Fernandez, M.A., Ferrarotti, S.A., Matioli, G. (2019) Sequencing, cloning, and heterologous expression of cyclomaltodextrin glucanotransferase of *Bacillus firmus* strain 37 in *Bacillus subtilis* WB800. *Bioprocess Biosyst Eng.* **42**: 621-629, https://doi.org/10.1007/s00449-018-02068-4

[33] Zhang, W., Liu, Z., Zhou, S., Mou, H., Zhang, R. (2019) Cloning and expression of a beta-mannanase gene from *Bacillus* sp. MK-2 and its directed evolution by random mutagenesis. *Enzyme Microb.Technol.* **124**: 70-78, https://doi.org/10.1016/j.enzmictec.2019.02.003

[34] Liu, Z., Zheng, W., Ge, C., Cui, W., Zhou, L., Zhou, Z. (2019) High-level extracellular production of recombinant nattokinase in Bacillus subtilis WB800 by multiple tandem promoters. *BMC Microbiol.* **19**: 89-019, https://doi.org/10.1186/s12866-019-1461-3

[35] Aguilar Suarez, R., Stulke, J., van Dijl, J.M. (2019) Less is more: towards a genome-reduced *Bacillus* cell factory for 'difficult proteins'. *ACS Synth.Biol.* **8**: 99-108, https://doi.org/10.1021/acssynbio.8b00342

[36] Jiang, Z., Niu, T., Lv, X., Liu, Y., Li, J., Lu, W., et al. (2019) Secretory Expression Fine-Tuning and Directed Evolution of Diacetylchitobiose Deacetylase by *Bacillus subtilis*. *Appl.Environ.Microbiol.* 2019, **85**: e01076-19, https://doi.org/10.1128/AEM.01076-19

[37] Yao, D., Su, L., Li, N., Wu, J. (2019) Enhanced extracellular expression of *Bacillus stearothermophilus* alpha-amylase in *Bacillus subtilis* through signal peptide optimization, chaperone overexpression and alpha-amylase mutant selection. *Microb.Cell.Fact.* **18**: 69-019, https://doi.org/10.1186/s12934-019-1119-8

[38] Peng, Z., Mao, X., Zhang, J., Du, G., Chen, J. (2020) Biotransformation of keratin waste to amino acids and active peptides based on cell-free catalysis. *Biotechnol.Biofuels* **13**: 61-020, https://doi.org/10.1186/s13068-020-01700-4

[39] Wang, N., Guan, F., Lv, X., Han, D., Zhang, Y., Wu, N., et al. (2020) Enhancing secretion of polyethylene terephthalate hydrolase PETase in *Bacillus subtilis* WB600 mediated by the SPamy signal peptide. *Lett.Appl.Microbiol.* **71**: 235-241, https://doi.org/10.1007/s00449-020-02288-7

[40] Ke, C., Li, S., Cui, Y., Qiu, H., Pang, Z. (2020) Effects of different expression systems on characterization of adenylate deaminase from *Aspergillus oryzae*. *Bioprocess Biosyst Eng.* **43**: 919-926, https://doi.org/10.1007/s00449-020-02288-7.

[41] Ye, B., Luo, Y., Zhong, B., Zhu, J., Huang, J., Gu, J., et al. (2020) High-level stable expression of gene for preparation of chlorothalonil hydrolytic dehalogenase and its application in elimination of chlorothalonil inhibition on bioconversion of lignocellulosic biomass. *J.Biosci.Bioeng.* **130**: 630-636, https://doi.org/10.1016/j.jbiosc.2020.08.003

[42] Gao, K., Chu, W., Sun, J., Mao, X. (2020) Identification of an alkaline lipase capable of better enrichment of EPA than DHA due to fatty acids selectivity and regioselectivity. *Food Chem.* **330**: 127225, https://doi.org/10.1016/j.foodchem.2020.127225

[43] van Tilburg, A.Y., van Heel, A.J., Stulke, J., de Kok, N.A.W., Rueff, A.S., Kuipers, O.P. (2020) Mini*Bacillus* PG10 as a Convenient and Effective Production Host for Lantibiotics. *ACS Synth.Biol.* **9**: 1833-1842, https://doi.org/10.1021/acssynbio.0c00194

[44] Neef, J., Milder, F.J., Koedijk, D.G., Klaassens, M., Heezius, E.C., van Strijp, J.A., et al. (2015) Versatile vector suite for the extracytoplasmic production and purification of heterologous His-tagged proteins in *Lactococcus lactis*. *Appl.Microbiol.Biotechnol.* **99:** 9037-48, https://doi.org/10.1007/s00253-015-6778-8

[45] Samazan, F., Rokbi, B., Seguin, D., Telles, F., Gautier, V., Richarme, G., et al. (2015) Production, secretion and purification of a correctly folded staphylococcal antigen in *Lactococcus lactis*. *Microb.Cell.Fact.* **14**: 104-015, https://doi.org/10.1186/s12934-015-0271-z

[46] Neef, J., Koedijk, D.G., Bosma, T., van Dijl, J.M., Buist, G. (2014) Efficient production of secreted staphylococcal antigens in a non-lysing and proteolytically reduced *Lactococcus lactis* strain. *Appl.Microbiol.Biotechnol.* **98:** 10131-41, https://doi.org/10.1007/s00253-014-6030-y

[47] Subramaniam, M., Baradaran, A., Rosli, M.I., Rosfarizan, M., Khatijah, Y., Raha, A.R. (2012) Effect of signal peptides on the secretion of beta-cyclodextrin glucanotransferase in *Lactococcus lactis* NZ9000. *J.Mol.Microbiol.Biotechnol.* **22**: 361-372, https://doi.org/10.1159/000343921

[48] Zadravec, P., Strukelj, B., Berlec, A. (2015) Improvement of LysM-mediated surface display of designed ankyrin repeat proteins (DARPins) in recombinant and nonrecombinant strains of *Lactococcus lactis* and *Lactobacillus* Species. *Appl.Environ.Microbiol.* **81**: 2098-2106, https://doi.org/10.1128/AEM.03694-14

[49] Bermudez-Humaran, L.G., Motta, J.P., Aubry, C., Kharrat, P., Rous-Martin, L., Sallenave J.M., et al. (2015) Serine protease inhibitors protect better than IL-10 and TGF-beta anti-inflammatory cytokines against mouse colitis when delivered by recombinant lactococci. *Microb.Cell.Fact.* **14**: 26-015, https://doi.org/10.1186/s12934-015-0198-4

[50] Zhang, B., Li, A., Zuo, F., Yu, R., Zeng, Z., Ma, H., et al. (2016) Recombinant *Lactococcus lactis* NZ9000 secretes a bioactive kisspeptin that inhibits proliferation and migration of human colon carcinoma HT-29 cells. *Microb.Cell.Fact.* **15**: 102-016, https://doi.org/10.1186/s12934-016-0506-7

[51] Mao, R., Zhou, K., Han, Z., Wang, Y. (2016) Subtilisin QK-2: secretory expression in Lactococcus lactis and surface display onto gram-positive enhancer matrix (GEM) particles. *Microb.Cell.Fact.* **15**: 80-016, https://doi.org/10.1186/s12934-016-0478-7

[52] Shigemori, S., Watanabe, T., Kudoh, K., Ihara, M., Nigar, S., Yamamoto, Y., et al. (2015) Oral delivery of *Lactococcus lactis* that secretes bioactive heme oxygenase-1 alleviates development of acute colitis in mice. *Microb.Cell.Fact.* **14**: 189-015, https://doi.org/10.1186/s12934-015-0378-2

[53] Carvalho, R.D., Breyner, N., Menezes-Garcia, Z., Rodrigues, N.M., Lemos, L., Maioli, T.U., et al. (2017) Secretion of biologically active pancreatitis-associated protein I (PAP) by genetically modified dairy *Lactococcus lactis* NZ9000 in the prevention of intestinal mucositis. *Microb.Cell.Fact.* **16**: 27-017, https://doi.org/10.1186/s12934-017-0624-x

[54] Loera-Arias, M.J., Villatoro-Hernandez, J., Parga-Castillo, M.A., Salcido-Montenegro, A., Barboza-Quintana, O., Munoz-Maldonado, G.E., et al. (2014) Secretion of biologically active human interleukin 22 (IL-22) by *Lactococcus lactis*. *Biotechnol.Lett.* **36**: 2489-2494, https://doi.org/10.1007/s10529-014-1626-y

[55] Liu, K.F., Liu, X.R., Li, G.L., Lu, S.P., Jin, L., Wu, J. (2016) Oral administration of *Lactococcus lactis*-expressing heat shock protein 65 and tandemly repeated IA2P2 prevents type 1 diabetes in NOD mice. *Immunol.Lett.* **174**: 28-36, https://doi.org/10.1016/j.imlet.2016.04.008

[56] Zhang, X., Hu, S., Du, X., Li, T., Han, L., Kong, J. (2016) Heterologous expression of carcinoembryonic antigen in *Lactococcus lactis* via LcsB-mediated surface displaying system for oral vaccine development. *J.Microbiol.Immunol.Infect.* **49**: 851-858.

[57] Cao, H.P., Wang, H.N., Yang, X., Zhang, A.Y., Li, X., Ding, M.D., et al. (2013) *Lactococcus lactis* anchoring avian infectious bronchitis virus multi-epitope peptide EpiC induced specific immune responses in chickens. *Biosci.Biotechnol.Biochem.* **77**: 1499-1504, https://doi.org/10.1271/bbb.130157

[58] Kobierecka, P.A., Olech, B., Ksiazek, M., Derlatka, K., Adamska, I., Majewski, P.M., et al. (2016) Cell Wall Anchoring of the Campylobacter Antigens to *Lactococcus lactis***.** *Front.Microbiol.* **7**: 165, https://doi.org/10.3389/fmicb.2016.00165

[59] Back, A., Borges, F., Mangavel, C., Paris, C., Rondags, E., Kapel, R., et al. (2016) Recombinant pediocin in *Lactococcus lactis*: increased production by propeptide fusion and improved potency by co-production with PedC. *Microb.Biotechnol.* **9**: 466-477, https://doi.org/10.1111/1751-7915.12285

[60] Skory, C.D., Cote, G.L. (2015) Secreted expression of *Leuconostoc mesenteroides* glucansucrase in *Lactococcus lactis* for the production of insoluble glucans. *Appl.Microbiol.Biotechnol.* 2015, **99**: 10001-10010, https://doi.org/10.1007/s00253-015-6854-0

[61] Aubry, C., Michon, C., Chain, F., Chvatchenko, Y., Goffin, L., Zimmerli, S.C., et al. (2015) Protective effect of TSLP delivered at the gut mucosa level by recombinant lactic acid bacteria in DSS-induced colitis mouse model. *Microb.Cell.Fact.* **14**: 176-015, https://doi.org/10.1186/s12934-015-0367-5

[62] Jimenez, J.J., Borrero, J., Diep, D.B., Gutiez, L., Nes, I.F., Herranz, C., et al. (2013) Cloning, production, and functional expression of the bacteriocin sakacin A (SakA) and two SakA-derived chimeras in lactic acid bacteria (LAB) and the yeasts Pichia pastoris and *Kluyveromyces lactis*. *J.Ind.Microbiol.Biotechnol.* **40**: 977-993, https://doi.org/10.1007/s10295-013-1302-6

[63] Singh, S.K., Tiendrebeogo, R.W., Chourasia, B.K., Kana, I.H., Singh, S., Theisen, M. (2018) *Lactococcus lactis* provides an efficient platform for production of disulfide-rich recombinant proteins from *Plasmodium falciparum*. *Microb.Cell.Fact.* **17**: 55-018, https://doi.org/10.1186/s12934-018-0902-2

[64] Pakbaten, B., Majidzadeh Heravi, R., Kermanshahi, H., Sekhavati, M.H., Javadmanesh, A., Mohammadi Ziarat, M., et al. (2019) Production of Phytase Enzyme by a Bioengineered Probiotic for Degrading of Phytate Phosphorus in the Digestive Tract of Poultry**.** *Probiotics Antimicrob.Proteins* **11:** 580-587, https://doi.org/10.1007/s12602-018-9423-x

[65] Taghinezhad, S.S., Mohseni, A.H., Keyvani, H., Razavilar, V. (2018) Protection against human papillomavirus type 16-induced tumors in C57BL/6 mice by mucosal vaccination with *Lactococcus lactis* NZ9000 expressing E6 oncoprotein. *Microb.Pathog.* **126**: 149-156, https://doi.org/10.1016/j.micpath.2018.10.043

[66] Ciacma, K., Wieckiewicz, J., Kedracka-Krok, S., Kurtyka, M., Stec, M., Siedlar, M., et al. (2018) Secretion of tumoricidal human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by recombinant *Lactococcus lactis*: optimization of in vitro synthesis conditions. *Microb.Cell.Fact.* **17**: 177-018, https://doi.org/10.1186/s12934-018-1028-2

[67] Mustopa, A.Z., Mariyah, S., Fatimah, Budiarti, S., Murtiyaningsih, H., Alfisyahrin, W.N. (2018) Construction, heterologous expression, partial purification, and in vitro cytotoxicity of the recombinant plantaricin E produced by *Lactococcus lactis* against Enteropathogenic *Escherichia coli* K.1.1 and human cervical carcinoma (HeLa) cells. *Mol.Biol.Rep.* **45**: 1235-1244, https://doi.org/10.1007/s11033-018-4277-6

[68] Li, J., Wang, F., Ma, C., Huang, Y., Wang, D., Ma, D. (2018) Recombinant *Lactococcus lactis* expressing *Eimeria tenella* AMA1 protein and its immunological effects against homologous challenge. *Exp.Parasitol.* **191**: 1-8, https://doi.org/10.1016/j.exppara.2018.05.003

[69] Koko, I., Song, A.A., Masarudin, M.J., Abdul Rahim, R. (2019) Engineering integrative vectors based on phage site-specific recombination mechanism for *Lactococcus lactis*. *BMC Biotechnol.* **19**: 82-019, https://doi.org/10.1186/s12896-019-0575-x

[70] Ma, T., Lu, J., Zhu, J., Li, X., Gu, H., Montalban-Lopez, M., et al. (2019) The Secretion of *Streptomyces monbaraensis* Transglutaminase From *Lactococcus lactis* and Immobilization on Porous Magnetic Nanoparticles. *Front.Microbiol.* **10**: 1675, https://doi.org/10.3389/fmicb.2019.01675

[71] Stedman, A., Chambers, M.A., Gutierrez-Merino, J. (2019) Secretion and functional expression of Mycobacterium bovis antigens MPB70 and MPB83 in lactic acid bacteria**.** *Tuberculosis* **117**: 24-30, https://doi.org/10.1016/j.tube.2019.05.007

[72] Xu, P., Wang, Y., Tao, L., Wu, X., Wu, W. (2019) Recombinant *Lactococcus lactis* secreting viral protein 1 of enterovirus 71 and its immunogenicity in mice. *Biotechnol.Lett.* 2019, **41**: 867-872, https://doi.org/10.1007/s10529-019-02695-1

[73] Tak, J.Y., Jang, W.J., Lee, J.M., Suraiya, S., Kong, I.S. (2019) Expression in *Lactococcus lactis* of a beta-1,3-1,4-glucanase gene from *Bacillus* sp. SJ-10 isolated from fermented fish. *Protein Expr.Purif.* **162**: 18-23, https://doi.org/10.1016/j.pep.2019.05.006

[74] Mahmud, H., Ismail, A., Abdul Rahim, R., Low, K.O., Md Illias, R. (2019) Enhanced secretion of cyclodextrin glucanotransferase (CGTase) by *Lactococcus lactis* using heterologous signal peptides and optimization of cultivation conditions. *J.Biotechnol.* 2019, **296**: 22-31, https://doi.org/10.1016/j.jbiotec.2019.02.013

[75] Bohlul, E., Hasanlou, F., Taromchi, A.H., Nadri, S. (2019) TRAIL-expressing recombinant *Lactococcus lactis* induces apoptosis in human colon adenocarcinoma SW480 and HCT116 cells. *J.Appl.Microbiol.* **126**: 1558-1567, https://doi.org/10.1111/jam.14237

[76] Mohseni, A.H., Taghinezhad-S, S., Keyvani, H., Razavilar, V. (2019) Extracellular overproduction of E7 oncoprotein of Iranian human papillomavirus type 16 by genetically engineered *Lactococcus lactis*. *BMC Biotechnol.* **19**: 8-019, https://doi.org/10.1186/s12896-019-0499-5

[77] Ma, C., Li, G., Chen, W., Jia, Z., Yang, X., Pan, X., et al. (2021) *Eimeria tenella*: IMP1 protein delivered by *Lactococcus lactis* induces immune responses against homologous challenge in chickens. *Vet.Parasitol.* 2021, **289**: 109320, https://doi.org/10.1016/j.vetpar.2020.109320

[78] Singh, S.K., Plieskatt, J., Chourasia, B.K., Singh, V., Bolscher, J.M., Dechering, K.J., et al. (2020) The *Plasmodium falciparum* circumsporozoite protein produced in *Lactococcus lactis* is pure and stable. *J.Biol.Chem.* **295**: 403-414, https://doi.org/10.1074/jbc.RA119.011268