

Current Microbiology 2017 vol.74 N5, pages 550-559

Regulatory Characteristics of *Bacillus pumilus* Protease Promoters

Toymentseva A., Mascher T., Sharipova M.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2017, Springer Science+Business Media New York. Expression of extracellular protease genes of *Bacilli* is subject to regulation by many positive and negative regulators. Here we analyzed 5' regulatory regions of genes encoding proteolytic proteases AprBp, GseBp, and MprBp from *Bacillus pumilus* strain 3-19. Gfp fusion constructs with upstream genomic regions of different lengths were created for all three genes to identify their natural promoters (regulatory regions). Our results suggest that the aprBp gene, encoding the major subtilisin-like protease, has the most extensive promoter region of approximately 445 bp, while the minor protease genes encoding glutamyl endopeptidase (gseBp) and metalloproteinase (mprBp) are preceded by promoters of 150 and 250 bp in length, respectively. Promoter analysis of *P*_{aprBp}-gfp_{mu3} and *P*_{gseBp}-gfp_{mu3} reporter fusion constructs in *degU* and *spo0A* mutants indicates a positive regulatory effect of DegU and Spo0A on protease expression, while the disruption of *abrB*, *sinR*, and *scoC* repressor genes did not significantly affect promoter activities of all protease genes. On the other hand, the expression of *P*_{aprBp}-gfp_{mu3} and *P*_{gseBp}-gfp_{mu3} reporters increased 1.6- and 3.0-fold, respectively, in *sigD*-deficient cells, indicating that the prevention of motility gene expression promotes protease expression. Our results indicate that all examined regulators regulated serine proteases production in *B. subtilis*.

<http://dx.doi.org/10.1007/s00284-017-1212-3>

References

- [1] Balaban NP, Mardanov AM, Sharipova MR, Gabdrakhmanova LA, Sokolova EA, Rudenskaya GN, Leshchinskaya IB (2004) Purification and characterization of serine proteinase 2 from *Bacillus intermedius* 3-19. *Biochemistry* 69:420-426. doi:10.1023/B:BIRY.0000026198.81752.f4
- [2] Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* 7:1513-1523
- [3] Bisicchia P, Botella E, Devine KM (2010) Suite of novel vectors for ectopic insertion of GFP, CFP and IYFP transcriptional fusions in single copy at the *amyE* and *bglIS* loci in *Bacillus subtilis*. *Plasmid* 64:143-149. doi:10.1016/j.plasmid.2010.06.002
- [4] Botella E, Fogg M, Jules M, Piersma S, Doherty G, Hansen A, Denham EL, Le Chat L, Veiga P, Bailey K, Lewis PJ, van Dijk JM, Aymerich S, Wilkinson AJ, Devine KM (2010) pBaSysBioII: an integrative plasmid generating GFP transcriptional fusions for high-throughput analysis of gene expression in *Bacillus subtilis*. *Microbiology* 156:1600-1608. doi:10.1099/mic.0.035758-0
- [5] Chai Y, Kolter R, Losick R (2010) Reversal of an epigenetic switch governing cell chaining in *Bacillus subtilis* by protein instability. *Mol Microbiol* 78:218-229. doi:10.1111/j.1365-2958.2010.07335.x
- [6] Chai Y, Norman T, Kolter R, Losick R (2010) An epigenetic switch governing daughter cell separation in *Bacillus subtilis*. *Genes Dev* 24:754-765. doi:10.1101/gad.1915010

- [7] Chumsakul O, Takahashi H, Oshima T, Hishimoto T, Kanaya S, Ogasawara N, Ishikawa S (2011) Genome-wide binding profiles of the *Bacillus subtilis* transition state regulator AbrB and its homolog Abh reveals their interactive role in transcriptional regulation. *Nucleic Acids Res* 39:414-428. doi:10.1093/nar/gkq780
- [8] Cormack BP, Valdivia RH, Falkow S (1996) FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* 173:33-38
- [9] Danilova YV, Shagimardanova EI, Margulis AB, Toymentseva AA, Balaban NP, Rudakova NL, Rizvanov AA, Sharipova MR, Palotás A (2014) Bacterial enzymes effectively digest Alzheimer's β -amyloid peptide. *Brain Res Bull* 108:113-117. doi:10.1016/j.brainresbull.2014.08.009
- [10] Ferrari E, Jarnagin AS, Schmidt BF (1993) Commercial production of extracellular enzymes. In: Sonenshein AL, Hoch JA, Losick R (eds) *Bacillus subtilis* and other Gram-positive bacteria. American Society for Microbiology, Washington, DC, pp 917-937
- [11] Fujita M, Losick R (2005) Evidence that entry into sporulation in *Bacillus subtilis* is governed by a gradual increase in the level and activity of the master regulator Spo0A. *Genes Dev* 19:2236-2244. doi:10.1101/gad.1335705
- [12] Gabdrakhmanova L, Vishniakov I, Sharipova M, Balaban N, Kostrov S, Leshchinskaya I (2005) Salt stress induction of glutamyl endopeptidase biosynthesis in *Bacillus intermedius*. *Microbiol Res* 160:233-242. doi:10.1016/j.micres.2004.05.005
- [13] Gupta R, Beg QK, Lorenz P (2002) Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl Microbiol Biotechnol* 59:15-32. doi:10.1007/s00253-002-0975-y
- [14] Harwood CR, Cutting SM (1990) *Molecular Biological Methods for Bacillus*. Wiley, Chichester
- [15] Itskovich EL, Liutova LI, Balaban NP, Mardanov AM, Shakirov EV, Sharipova MR, Leshchinskaia IB, Rudenskaia GN (1998) Thrombolytic and anticoagulant properties of thiol-dependent proteinase from *Bacillus intermedius* 3-19. *Vopr Med Khim* 44:288-291
- [16] Jordan S, Rietkötter E, Strauch MA, Kalamorz F, Butcher BG, Helmann JD, Mascher T (2007) LiaRS-dependent gene expression is embedded in transition state regulation in *Bacillus subtilis*. *Microbiology* 153:2530-2540. doi:10.1099/mic.0.2007/006817-0
- [17] Kaiumov AR, Sabirova AR, Balaban NP, Mardanov AM, Il'inskaia ON, Kostrov SV, Sharipova MR (2008) Start codon in the serine proteinase gene from *Bacillus intermedius*. *Mol Biol (Mosk)* 42:117-122. doi:10.1007/s11008-008-1015-5.
- [18] Kallio PT, Fagelson JE, Hoch JA, Strauch MA (1991) The transition state regulator Hpr of *Bacillus subtilis* is a DNA-binding protein. *J Biol Chem* 266:13411-13417
- [19] Kayumov AR, Kirillova JM, Mikhailova EO, Balaban NP, Sharipova MR (2006) The prediction of regulation of subtilisin-like proteinase gene from *Bacillus intermedius* through its regulatory sequence analysis. BGRS-2006: Proceedings of the fifth international conference on Bioinformatics of Genome Regulation and Structure, Novosibirsk, July 16-22, pp 65-68.
- [20] Kearns DB, Losick R (2005) Cell population heterogeneity during growth of *Bacillus subtilis*. *Genes Dev* 19:3083-3094. doi:10.1101/gad.1373905
- [21] Kobayashi K (2007) Gradual activation of the response regulator DegU controls serial expression of genes for flagellum formation and biofilm formation in *Bacillus subtilis*. *Mol Microbiol* 66:395-409. doi:10.1111/j.1365-2958.2007.05923.x
- [22] Kodgire P, Dixit M, Rao KK (2006) ScoC and SinR negatively regulate epr by corepression in *Bacillus subtilis*. *J Bacteriol* 188:6425-6428. doi:10.1128/JB.00427-06
- [23] Kunst F, Rapoport G (1995) Salt stress is an environmental signal affecting degradative enzyme synthesis in *Bacillus subtilis*. *J Bacteriol* 177:2403-2407
- [24] Mäder U, Antelmann H, Buder T, Dahl MK, Hecker M, Homuth G (2002) *Bacillus subtilis* functional genomics: genome-wide analysis of the DegS-DegU regulon by transcriptomics and proteomics. *Mol Genet Genom* 268:455-467. doi:10.1007/s00438-002-0774-2
- [25] Mikhailova EO, Mardanov AM, Balaban NP, Rudenskaya GN, Ilyinskaya ON, Sharipova MR (2009) Biochemical properties of *Bacillus intermedius* subtilisin-like proteinase secreted by a *Bacillus subtilis* recombinant strain in its stationary phase of growth. *BioChemistry* 74(3):308-315. doi:10.1134/S0006297909030109
- [26] Mirouze N, Prepiak P, Dubnau D (2011) Fluctuations in spo0A transcription control rare developmental transitions in *Bacillus subtilis*. *PLoS Genet* 7(4):e1002048. doi:10.1371/journal.pgen.1002048
- [27] Msadek T (1999) When the going gets tough: survival strategies and environmental signaling networks in *Bacillus subtilis*. *Trends Microbiol* 7:201-207
- [28] Msadek T, Kunst F, Klier A, Rapoport G (1991) DegS-DegU and ComP-ComA modulator-effector pairs control expression of the *Bacillus subtilis* pleiotropic regulatory gene degQ. *J Bacteriol* 173:2366-2377
- [29] Ogura M, Matsuzawa A, Yoshikawa H, Tanaka T (2004) *Bacillus subtilis* SalA (YbaL) negatively regulates expression of scoC, which encodes the repressor for the alkaline exoprotease gene, aprE. *J Bacteriol* 186:3056-3064. doi:10.1128/JB.186.10.3056-3064.2004

- [30] Ogura M, Shimane K, Asai K, Ogasawara N, Tanaka T (2003) Binding of response regulator DegU to the *aprE* promoter is inhibited by RapG, which is counteracted by extracellular PhrG in *Bacillus subtilis*. *Mol Microbiol* 49:1685-1697. doi:10.1046/j.1365-2958.2003.03665.x
- [31] Ohsawa T, Tsukahara K, Ogura M (2009) *Bacillus subtilis* response regulator DegU is a direct activator of *pgsB* transcription involved in gamma-poly-glutamic acid synthesis. *Biosci Biotechnol Biochem* 73:2096-2102. doi:10.1271/bbb.9034
- [32] Sabirova AR, Rudakova NL, Balaban NP, Ilyinskaya ON, Demidyuk IV, Kostrov SV, Rudenskaya GN, Sharipova MR (2010) A novel secreted metzincin metalloproteinase from *Bacillus intermedius*. *FEBS Lett* 584:4419-4425. doi:10.1016/j.febslet.2010.09.049
- [33] Sánchez A, Olmos J (2004) *Bacillus subtilis* transcriptional regulators interaction. *Biotechnol Lett* 26:403-407
- [34] Shafikhani SH, Mandic-Mulec I, Strauch MA, Smith I, Leighton T (2002) Postexponential regulation of *sin* operon expression in *Bacillus subtilis*. *J Bacteriol* 184:564-571. doi:10.1128/JB.184.2.564-571.2002
- [35] Sharipova M, Balaban N, Kayumov A, Kirillova Y, Mardanova A, Gabdrakhmanova L, Leshchinskaya I, Rudenskaya G, Akimkina T, Safina D, Demidyuk I, Kostrov S (2008) The expression of the serine proteinase gene of *Bacillus intermedius* in *Bacillus subtilis*. *Microbiol Res* 163:39-50. doi:10.1016/j.micres.2006.03.003
- [36] Sharipova MR, Shagimardanova EI, Chastukhina IB, Shamsutdinov TR, Balaban NP, Mardanova AM, Rudenskaya GN, Demidyuk IV, Kostrov SV (2007) The expression of *Bacillus intermedius* glutamyl endopeptidase gene in *Bacillus subtilis* recombinant strains. *Mol Biol Rep* 34:79-87. doi:10.1007/s11033-006-9017-7
- [37] Sharipova MR, Toymentseva AA, Sabirova AR, Mukhametzianova AD, Akhmetova AI, Mardanova AM, Balaban NP (2011) New phylogenetic position Of *Bacillus Intermedius* 3-19 strain. *Mikrobiologiya* 80:424-426. doi:10.1134/S0026261711030192
- [38] Stepanov VG, Tirumalai MR, Montazari S, Checinska A, Venkateswaran K, Fox GE (2016) *Bacillus pumilus* SAFR-032 genome revisited: sequence update and re-annotation. *PLoS One* 11(6):e0157331. doi:10.1371/journal.pone.0157331
- [39] Strauch MA, Hoch JA (1993) Transition-state regulators: sentinels of *Bacillus subtilis* post-exponential gene expression. *Mol Microbiol* 7:337-342
- [40] Tsukahara K, Ogura M (2008) Characterization of DegU-dependent expression of *bpr* in *Bacillus subtilis*. *FEMS Microbiol Lett* 280:8-13. doi:10.1111/j.1574-6968.2007.01019.x
- [41] Zheng XL (2013) Structure-function and regulation of ADAMTS-13 protease. *J Thromb Haemost* 1:11-23. doi:10.1111/jth.12221