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Label-free multiple reaction monitoring, a promising method for quantification analyses of specific proteins in bacteria

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Abstract

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. Bacillus subtilis produces eight industrially important exo-proteases. For the detection of proteases, the activity-and antibody-based assays are normally used. Current activity-based assays require expensive multiplex chemical substrates which allow specificity determination of each enzyme. In this study, we provide evidences pertaining to the usefulness of the label-free multiple reaction monitoring (MRM) assay for a rapid identification and quantitation of specific proteins in bacteria. We used wild-type B. pumilus cells producing at least two serine proteases, subtilisin-like protease (AprBp) and glutamyl endopeptidase (GseBp), as well as optimized recombinant B. subtilis cells containing the same protease genes under control of the LIKE expression system. The Skyline software was used for the selection of three specific proteotypic peptides and their fragment ions for quantification and confirmation of AprBp and GseBp in complex mixtures. MRM indicated that the production of AprBp and GseBp exo-enzymes were respectively 0.9-and 26.6-fold higher in the culture medium of B. pumilus strain in comparison to the recombinant B. subtilis strains carrying optimized LIKE expression systems under identical conditions. The developed procedure in this study is fast, easy to perform and dependable. Additionally, it achieves accurate proteins identification and quantification in complex mixture.

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Keywords

Bacillus pumilus, Glutamyl endopeptidase (GseBp), Mass spectrometry, Multiple reaction monitoring (MRM), Protein quantification, Subtilisin-like protease (AprBp)

References

[1] Wolfe, M.S. Intramembrane Proteolysis. Chem. Rev. 2009, 109, 1599–1612. [CrossRef] [PubMed]

[2] Mahajan, R.T.; Badgujar, S.B. Biological aspects of proteolytic enzymes: A Review. J. Pharm. Res. 2010, 3, 2048–2068.

[3] Li, Q.; Yi, L.; Marek, P.; Iverson, B.L. Commercial proteases: Present and future. FEBS Lett. 2013, 587, 1155–1163. [CrossRef] [PubMed]

[4] Bongers, R.S.; Veening, J.W.; Wieringen, V.M.; Kuipers, O.P.; Kleerebezem, M. Development and characterization of a subtilin-regulated expression system in Bacillus subtilis: Strict control of gene expression by addition of subtilin. Appl. Environ. Microbiol. 2005, 71, 8818–8824. [CrossRef]

[5] Vavrova, L.; Muchova, K.; Barak, I. Comparison of different Bacillus subtilis expression systems. Res. Microbiol. 2010, 161, 791–797. [CrossRef] [PubMed]

[6] Zukowski, M.M.; Miller, L. Hyperproduction of an intracellular heterologous protein in a sacUh mutant of Bacillus subtilis. Gene 1986, 46, 247–255. [CrossRef]

[7] Conrad, B.; Savchenko, R.S.; Breves, R.; Hofemeister, J. A T7 promoter-specific, inducible protein expression system for Bacillus subtilis. Mol. Gen. Genet. 1996, 250, 230–236.

[8] Bhavsar, A.P.; Zhao, X.; Brown, E.D. Development and characterization of a xylose-dependent system for expression of cloned genes in Bacillus subtilis: Conditional complementation of a teichoic acid mutant. Appl. Environ. Microbiol. 2001, 67, 403–410. [CrossRef]

[9] Fukushima, T.; Yamamoto, H.; Atrih, A.; Foster, S.J.; Sekiguchi, J. A polysaccharide deacetylase gene (pdaA) is required for germination and for production of muramic delta-lactam residues in the spore cortex of Bacillus subtilis. J. Bacteriol. 2002, 184, 6007–6015. [CrossRef]

[10] Ho, K.M.; Lim, B.L. Co-expression of a prophage system and a plasmid system in Bacillus subtilis. Protein. Expr. Purif. 2003, 32, 293–301. [CrossRef]

[11] Phan, T.T.P.; Schumann, W. Development of glycine-inducible expression system for Bacillus subtilis. J. Biotechnol. 2007, 128, 486–499. [CrossRef] [PubMed]

[12] Toymentseva, A.A.; Schrecke, K.; Sharipova, M.R.; Mascher, T. The LIKE system, a novel protein expression toolbox for based on the lil promoter. Microb. Cell Fact. 2012, 11, 143. [CrossRef] [PubMed]

[13] Vidova, V.; Spacil, Z. A review on mass spectrometry-based quantitative proteomics: Targeted and data independent acquisition. Anal. Chim. Acta. 2017, 964, 7–23. [CrossRef]

[14] Resta, D.; Brambilla, F.; Arnoldi, A. HPLC-Chip-Multiple Reaction Monitoring (MRM) method for the label-free absolute quantification of γ-conglutin in lupin: Proteotypic peptides and standard addition method. Food Chem. 2012, 131, 126–133. [CrossRef]

[15] Blankley, R.T.; Fisher, C.; Westwood, M.; North, R.; Baker, P.N.; Walker, M.J.; Williamson, A.; Whetton, A.D.; Lin, W.; McCowan, L.; et al. A label-free selected reaction monitoring workflow identifies a subset of pregnancy specific glycoproteins as potential predictive markers of early-onset pre-eclampsia. Mol. Cell Proteomics. 2013, 12, 3148–3159. [CrossRef]

[16] Al Ali, A.; Touboul, D.; Le Caër, J.P.; Schmitz-Afonso, I.; Flinois, J.P.; Marchetti, C.; De Waziers, I.; Brunelle, A.; Laprévote, O.; Beaune, P. Optimization and validation of a label-free MRM method for the quantification of cytochrome P450 isoforms in biological samples. Anal. Bioanal. Chem. 2014, 406, 4861–4874. [CrossRef] [PubMed]

[17] Sharipova, M.R. Late stages of protein secretion in bacilli. Biochemistry 2002, 67, 1207–1216.

[18] Sharipova, M.; Balaban, N.; Kayumov, A. The expression of the serine proteinase gene of B. intermedius in B. subtilis. Microbiol. Res. 2008, 163, 39–50. [CrossRef]

[19] Lange, V.; Picotti, P.; Domon, B.; Aebersold, R. Selected reaction monitoring for quantitative proteomics: A tutorial. Mol. Syst. Biol. 2008, 4, 222. [CrossRef]

[20] MacLean, B.; Tomazela, D.M.; Shulman, N.; Chambers, M.; Finney, G.L.; Frewen, B.; Kern, R.; Tabb, D.L.; Liebler, D.C.; MacCoss, M.J. Skyline: An open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics. 2010, 26, 966–968. [CrossRef]

[21] Sharipova, M.R.; Shagimardanova, E.I.; Chastukhina, I.B.; Shamsutdinov, T.R.; Balaban, N.P.; Mardanova, A.M.; Rudenskaya, G.N.; Demidyuk, I.V.; Kostrov, S.V. The expression of Bacillus intermedius glutamyl endopeptidase gene in Bacillus subtilis recombinant strains. Mol. Biol. Rep. 2007, 34, 79–87. [CrossRef] [PubMed]

[22] Mikhailova, E.O.; Mardanova, A.M.; Balaban, N.P.; Rudenskaya, G.N.; Ilyinskaya, O.N.; Sharipova, M.R. Biochemical properties of Bacillus intermedius subtilisin-like proteinase secreted by a Bacillus subtilis recombinant strain in its stationary phase of growth. Biochemistry 2009, 74, 308–315. [CrossRef] [PubMed]

[23] Tikhonova, A.O.; Toymentseva, A.A.; Sharipova, M.R. Screening of heterologous signal peptides for optimization of the LIKE-expression system. BioNanoScience 2017, 7, 408–414. [CrossRef]

[24] Kuzyk, M.A.; Smith, D.; Yang, J.; Cross, T.J.; Jackson, A.M.; Hardie, D.B.; Anderson, N.L.; Borchers, G.H. CH Multiple reaction monitoring-based, multiplexed, absolute quantitation of 45 proteins in human plasma. Mol. Cell Proteomics. 2009, 8, 1860–1877. [CrossRef]

[25] Rezeli, M.; Végvári, Á.; Silajdžić, E.; Björkqvist, M.; Tabrizi, J.S.; Laurell, T.; Marko-Varga, G. Inflammatory markers in Huntington's disease plasma—A robust nanoLC–MRM-MS assay development. EuPA Open Proteom. 2014, 3, 68–75. [CrossRef]

[26] Park, S.L.; Carmella, S.G.; Chen, M.; Patel, Y.; Stram, D.O.; Haiman, C.A.; Le Marchand, L.; Hecht, S.S. Mercapturic acids derived from the toxicants acrolein and crotonaldehyde in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. PLoS ONE 2015, 10, e0124841. [CrossRef] [PubMed]

[27] Romanova, Y.; Laikov, A.; Markelova, M.; Khadiullna, R.; Makseev, A.; Hasanova, M.; Rizvanov, A.; Khaiboullina, S.; Salafutdinov, I. Proteomic analysis of human serum from patients with chronic kidney disease. Biomolecules 2020, 10, 257. [CrossRef]

[28] Schaafsma, A.; Limay-Rios, V.; Baute, T.; Smith, J.; Xue, Y. Neonicotinoid insecticide residues in surface water and soil associated with commercial maize (corn) fields in southwestern Ontario. PLoS ONE 2015, 10, e0118139. [CrossRef]

[29] Kumar, A.; Gangadharan, B.; Zitzmann, N. Multiple reaction monitoring and multiple reaction monitoring cubed based assays for the quantitation of apolipoprotein F. J. Chromatogr B Analyt. Technol. Biomed. Life Sci. 2016, 1033, 278–286. [CrossRef]

[30] Zheng, J.; Mandal, R.; Wishart, D.S. A sensitive, high-throughput LC-MS/MS method for measuring catecholamines in low volume serum. Anal. Chim. Acta. 2018, 1037, 159–167. [CrossRef]

[31] Chenau, J.; Fenaille, F.; Ezan, E.; Morel, N.; Lamourette, P.; Goossens, P.L.; Becher, F. Sensitive detection of Bacillus anthracis spores by immunocapture and liquid chromatography-tandem mass spectrometry. Anal. Chem. 2011, 83, 8675–8682. [CrossRef] [PubMed]

[32] Mullins, E.A.; Rubinson, E.H.; Pereira, K.N.; Calcutt, M.W.; Christov, P.P.; Eichman, B.F. An HPLC-tandem mass spectrometry method for simultaneous detection of alkylated base excision repair products. Methods 2013, 64, 59–66. [CrossRef]

[33] Domanski, D.; Percy, A.J.; Yang, J.; Chambers, A.G.; Hill, J.S.; Freue, G.V.; Borchers, G.H. MRM-based multiplexed quantitation of 67 putative cardiovascular disease biomarkers in human plasma. Proteomics 2012, 12, 1222–1243. [CrossRef]

[34] Keshishian, H.; Addona, T.; Burgess, M.; Kuhn, E.; Carr, S.A. Quantitative, multiplexed assays for low abundance proteins in plasma by targeted mass spectrometry and stable isotope dilution. Mol. Cell Proteomics 2007, 6, 2212–2229. [CrossRef] [PubMed]

[35] Onisko, B.; Dynin, I.; Requena, J.R.; Silva, C.J.; Erickson, M.; Carter, J.M. Mass spectrometric detection of attomole amounts of the prion protein by nanoLC/MS/MS. J. Am. Soc. Mass Spectr. 2007, 18, 1070–1079. [CrossRef] [PubMed]

[36] Gillette, M.A.; Carr, S.A. Quantitative analysis of peptides and proteins in biomedicine by targeted mass spectrometry. Nat. Methods. 2013, 10, 28–34. [CrossRef]

[37] Stammen, S.; Müller, K.; Korneli, C.; Biedendieck, R.; Franco-Lara, E.; Jahn, D. High-yield intra-and extracellular protein production using Bacillus megaterium. Appl. Environ. Microbiol. 2010, 76, 4037–4046. [CrossRef]

[38] Speicher, K.; Kolbas, O.; Harper, S.; Speicher, D. Systematic analysis of peptide recoveries from in-gel digestions for protein identifications in proteome studies. J. Biomol. Tech. 2000, 11, 74–86.

[39] Yang, J.K.; Shihb, I.L.; Tzengc, Y.M.; Wang, S.L. Production and purification of protease from a Bacillus subtilis that can deproteinize crustacean wastes. Enzyme Microb. Technol. 2000, 26, 406–413. [CrossRef]

[40] Lyublinskaya, L.; Belyaev, S.; Strongin, A.; Matyash, L.; Levin, E.; Stepanov, V. A new chromogenic substrate for subtilisin. Anal. Biochem. 1974, 62, 371–376. [CrossRef]

[41] Lyublinskaya, L.; Voyushina, T.; Stepanov, V. p-Nitroanilides of pyroglutamyl peptides as chromogenic substrates of serine proteinases. Bioorg. Khim. 1974, 13, 748–753.

[42] Mikhailova, E.O.; Balaban, N.P.; Mardanova, A.M.; Rudakova, N.L.; Ilyinskaya, O.N.; Rudenskaya, G.N.; Rizvanov, A.A.; Sharipova, M.R. Purification of a subtilisin-like serine proteinase from recombinant Bacillus subtilis during different phases of growth. Ann. Microbiol. 2009, 59, 1–8. [CrossRef]

[43] Balaban, N.P.; Mardanova, A.M.; Sharipova, M.R.; Gabdrakhmanova, L.A.; Sokolova, E.A.; Garusov, A.V.; Milgotina, E.I.; Rudenskaya, G.N.; Leshchinskaya, I.B. Isolation and characterization of glutamyl endopeptidase from Bacillus intermedius 3-19. Biochemistry 2003, 68, 1514–1521. [CrossRef] [PubMed]