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Development and Validation of SYBR Green-Based qPCR Technique of Detection and Quantification of *Salmonella enterica*

Rodica Sturza[1*], Valentin Mitin[2], Irina Mitina[2], Alexandra Buga[1], Dan Zgardan[1], Emilia Behta[1]

[1]Technical University of Moldova, Chisinau, Republic of Moldova
[2]Institute of Genetics, Physiology and Plant Protection, Chisinau, Republic of Moldova
Email: *rodica.sturza@chim.utm.md

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Abstract

Salmonella enterica serovars is a leading cause of human gastroenteritis, and the incidence of salmonellosis is constantly increasing, causing millions of infections and many deaths annually. The detection of the pathogen in optimal terms is an essential factor for reducing the impact on the human body. In this work, SYBR Green I-based qPCR method of detection and quantification of *Salmonella enterica* was developed and validated. For detection of *Salmonella enterica* subsp. *enterica*, two pairs of primers were designed using publically available Primer-BLAST software. Primer efficiency was calculated by establishing a standard curve. The specificity, sensitivity, accuracy, and precision of PCR results were tested. Both primer pairs showed an acceptable performance, proving the developed techniques were sensitive, reliable and precise. The validated qPCR technology has a good potential to replace the traditional culture method in microbial diagnosis.

Keywords

Dilution, DNA, Primer, Real-Time PCR, *Salmonella enterica*

1. Introduction

In recent decades, according to World Health Organization (WHO), the incidence of *Salmonella* infection (salmonellosis) has been increasing worldwide. Contamination of food with *Salmonella* spp. and, as a consequence, the development of salmonellosis is a serious threat that requires great attention to the control of the microbiological purity of food, the development of quick and rapid, accurate methods for its detection.

According to the Bergey's Manual of Systematic Bacteriology, *Salmonella* belongs to the Enterobacteriaceae family, genus—*Salmonella*, which includes 2 species*: Salmonella bongori* and *Salmonella enterica* [1]. *S. enterica* subdivided into six subspecies with about 2500 serovars (serotypes) [2]. These serovars can cause illnesses in human ranging from gastroenteritis to typhoid fever which kills around 500,000 people worldwide per year [3].

The source of salmonellosis, most often, are eggs (up to 90% of cases of salmonellosis are associated with the consumption of raw or insufficiently cooked eggs), meat and dairy products, and, to a lesser extent, fish and fish products, as well as plant products. *Salmonella* is relatively stable in the external environment and is well preserved in room dust (almost up to three months), in open water (11 to 120 days), in meat and sausages (2 - 6 months), in frozen meat (more than six months), in milk at room temperature for up to 10 days and in the refrigerator for up to 20 days; in butter (52 - 128 days); in eggs (over a year), on eggshells (24 days). At 70˚C, salmonella die within 5 - 10 minutes, in the thickness of meat they withstand boiling for several hours. In meat stored in a refrigerator at a low above zero temperature, salmonella not only survive, but are also able to reproduce [4].

Recently, salmonella serotypes have become widespread, characterized by resistance to many modern common antibiotics and disinfectants, as well as increased heat resistance [5].

It has been extensively reported that classical microbiological methods, even though reliable, are lengthy and tedious. In this sense molecular applications, relies on the detection of specific *Salmonella* genes by PCR techniques, such as Real-Time PCR [6] [7], which have greatly allowed the reduction in the time needed to achieve the final result and to help control the spread of disease [8].

For designing a reliable PCR assay, it is necessary to pick a good target gene for PCR amplification. Several target genes of Salmonella have been reported, such as *invA* [6] [7] [9], *sdiA*, *fliC*, *fliB*, *sefB*, *hilA* [10] [11] [12] [13] [14].

The *invA* gene lies at centisome 63 region of the Salmonella chromosome and is widely used as a target in PCR assays for Salmonella detection [10].

The *invA* gene usually codes for a protein of the inner bacterial membrane, which is responsible for the invasion of the intestinal cells of the host [4] and is promising for primer design since it contains conservative and well characterized sequences. In order to validate the PCR assays of *Salmonella* molecular detection it is necessary to test specificity, sensitivity, accuracy, and precision of results [15].

References

[1] Boone, D., Castenholz, R. and Garrity, G. (2001) Bergey's Manual of Systematic Bacteriology. 2nd Edition, Part 2, Springer, New York, 305-1106. https://doi.org/10.1007/978-0-387-21609-6

[2] Bahrani-Mougeot, F., Scobey, M. and Sansonetti, P. (2009) Enteropathogenic Infections. In: Schaechter, M., Ed., *Encyclopedia of Microbiology*, Academic Press, Oxford, 329-343. https://doi.org/10.1016/B978-012373944-5.00224-8

[3] Primerdesign Ltd. (2018) *Salmonella enterica*. Invasine A Gene (InvA) Genesig Advanced Kit. https://www.genesig.com/assets/files/s_enterica.pdf

[4] Mąka, L. and Popowska, M. (2016) Antimicrobial Resistance of *Salmonella* spp. Isolated from Food. *Annals of the National Institute of Hygiene*, **67**, 343-358.

[5] Popa, G. and Papa, M.I. (2021) *Salmonella* spp. Infection—A Continuous Threat Worldwide. *GERMS*, **11**, 88-96. https://doi.org/10.18683/germs.2021.1244

[6] Anderson, A., Pietsch, K., Zucker, R., Mayr, A., Müller-Hohe, E., Messelhäusser, U., Sing, A., Busch, U. and Huber, I. (2011) Validation of a Duplex Real-Time PCR for the Detection of *Salmonella* spp. in Different Food Products. *Food Analytical Methods*, **4**, 259-267. https://doi.org/10.1007/s12161-010-9142-8

[7] Bohaychuk, V., Gensler, G., McFall, M., King, R. and Renter, D. (2007) A Real-Time PCR Assay for the Detection of *Salmonella* in a Wide Variety of Food and Food-Animal Matrices. *Journal of Food Protection*, **70**, 1080-1087. https://doi.org/10.4315/0362-028X-70.5.1080

[8] Azinheiro, S.A., Carvalho, J., Prado, M. and Garrido-Maestu, A. (2018) Evaluation of Different Genetic Targets for *Salmonella enterica* Serovar Enteriditis and *Typhimurium*, Using Loop-Mediated Isothermal AMPlification for Detection in Food Samples. *Frontiers in Sustainable Food Systems*, **2**, Article No. 5. https://doi.org/10.3389/fsufs.2018.00005

[9] Sunar, N., Stentiford, E., Stewart, D. and Fletcher, L. (2014) Molecular Techniques to Characterize the invA Genes of *Salmonella* spp. for Pathogen Inactivation Study in Composting. https://arxiv.org/abs/1404.5208v1

[10] Halatsi, K., Oikonomou, I., Lambiri, M., Mandilara, G., Vatopoulos, A., Kyriacou, A. (2006) PCR Detection of *Salmonella* spp. Using Primers Targeting the Quorum Sensing Gene *sdiA*. *FEMS Microbiology Letters*, **259**, 201-207. https://doi.org/10.1111/j.1574-6968.2006.00266.x

[11] Chiu, T.H., Pang J.C., Hwang, W.Z. and Tsen, H.Y. (2005) Development of PCR Primers for the Detection of *Salmonella enterica* Serovar Choleraesuis Based on the *fliC* Gene. *Journal of Food Protection*, **68**, 1575-1580. https://doi.org/10.4315/0362-028X-68.8.1575

[12] Silva, C., Betancor, L., García, C., Astocondor, L., Hinostroza, N., *et al.* (2017) Characterization of *Salmonella enterica* Isolates Causing Bacteremia in Lima, Peru, Using Multiple Typing Methods. *PLoS ONE*, **12**, Article ID: e0189946. https://doi.org/10.1371/journal.pone.0189946

[13] Wang, S.J., Yeh, D.B. and Wei, C.I. (2009) Specific PCR Primers for the Identification of *Salmonella enterica* Serovar Enteritidis in Chicken-Related Samples. *Journal of Food and Drug Analysis*, **17**, Article No. 9. https://doi.org/10.38212/2224-6614.2612

[14] McCabe, E., Burgess, C., Walsh, D., O'Regan, E., McGuinness, S., Barry, T., Fanning, S. and Duffy, G. (2011) Validation of DNA and RNA Real-Time Assays for Food Analysis Using the *hilA* Gene of *Salmonella enterica* Serovars. *Journal of Microbiological Methods*, **84**, 19-26. https://doi.org/10.1016/j.mimet.2010.10.004

[15] Zheng, W., Jiang, L., Lei, Q., Yang, J., Gao, X., Wang, W., *et al.* (2019) Development and Validation of Quantitative Real-Time PCR for the Detection of Residual Cho Host Cell DNA and Optimization of Sample Pretreatment Method in Biopharmaceutical Products. *Biological Procedures Online*, **21**, Article No. 17. https://doi.org/10.1186/s12575-019-0105-1

[16] ISO (International Organization for Standardization) (2003) ISO 16140:2003 Microbiology of Food and Animal Feeding stuffs—Protocol for the Validation of Alternative Methods. https://www.iso.org/standard/30158.html

[17] Kralik, P. and Ricchi, M. (2017) A Basic Guide to Real-Time PCR in Microbial Diagnostics: Definitions, Parameters, and Everything. *Frontiers in Microbiology*, **8**, Article No. 108. https://doi.org/10.3389/fmicb.2017.00108

[18] Nutz, S., Döll, K. and Karlovsky, P. (2011) Determination of the LOQ in Real-Time PCR by Receiver Operating Characteristic Curve Analysis: Application to qPCR Assays for *Fusarium verticillioides* and *Fusarium proliferatum*. *Analytical and Bioanalytical Chemistry*, **401**, 717-726. https://doi.org/10.1007/s00216-011-5089-x

[19] U.S. Food and Drug Administration (2019) Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds Edition 3.0 U.S. Food and Drug Administration Foods Program, U.S. Food and Drug Administration, Silver Spring. https://www.fda.gov/media/83812/download