Reviews

http://dx.doi.org/10.7124/bc.000B24
UDC: 579.25-575.8

O.Y. Skorobogatov, N.S. Melnichuk, Z.Yu. Tkachuk
Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143
skorobogatov.alx@gmail.com

CHALLENGES IN PCR DETECTION OF *BACILLUS ANTHRACIS*

Bacillus anthracis is large Gram-positive bacteria which causes anthrax — one of the deadliest known diseases. Different approaches have been developed to detect it, from more precise, time-consuming culture-based methods to more rapid PCR-based methods. The latter rely on the identification of specific regions in the B. anthracis genome, which is composed of two plasmids, pXO1 and pXO2, and the chromosome. Targeting only plasmid markers may provide unreliable results due to high similarities between B. anthracis and other genetically related species of Bacillus cereus sensu lato group, which necessitates the use of additional chromosomal targets. This article aims to provide a brief non-exhaustive overview on the relevant genetic markers used as targets for PCR-based detection of B. anthracis.

Keywords: *Bacillus anthracis, genetic markers, PCR.*

Introduction

Anthrax has been known to human civilization since ancient times. Its name originated from the Greek word for coal, "anthrakis", which is probably related to the coal-black skin lesions caused by the cutaneous form of the anthrax. According to the Book of Genesis, a disease, which resembles anthrax, killed Egyptian cattle in 1491 BC [1]. A similar illness, affecting domestic and wild animals, was described by the roman poet Virgil in his third Georgic [2] in 29 BC.

Anthrax dealt major damage to humans and livestock throughout the Middle Ages, and is believed to be the "Black Bane" — a deadly illness which marched through Europe causing numerous deaths among humans and animals [3].

In the middle of the 19-th century, Pierre-François Olive Rayer discovered small bodies in sheep samples, that were initially disregarded, however, later described as organisms, responsible for causing the disease [4].

The first published scientific article in the PubMed database mentioning *Bacillus anthracis* dates

Citation: Skorobogatov O.Y., Melnichuk N.S., Tkachuk Z.Yu. (2025) Challenges in PCR detection of *Bacillus anthracis*. *Biopolymers & Cell*, 3(41), 171—180. http://dx.doi.org/10.7124/bc.000B24

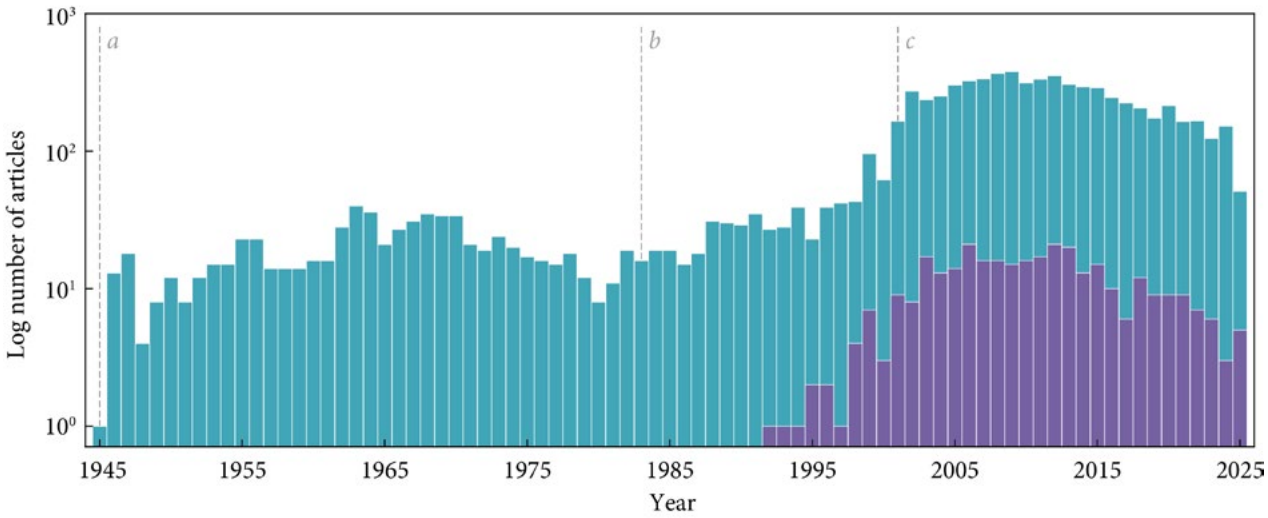

Fig. 1. Log of number of scientific articles published in the period from 1945 to 2025 mentioning *Bacillus anthracis*. Cyan bars correspond to the results of the search query "*Bacillus anthracis*", and violet bars correspond to the results of the search query "(*bacillus anthracis*) AND (detection) AND (PCR)". The annotated vertical dashed lines correspond to the following historical events: *a* — the end of World War 2, *b* — the invention of PCR, *c* — the Amerithrax. The data was obtained from the PubMed database. Seven articles published in the period from 1886 to 1931 are hidden for the sake of visual clarity

back to 1886 [5] which, in its essence, is a clinical report on a patient infected by the cutaneous form of Anthrax provided by a surgeon Arthur E. Barker. In the next 45 years, six more articles were published, focusing on various aspects of *B. anthracis,* including but not limited to cultivation, resistance to chemicals and heat [6—10].

After the end of World War 2, the number of published articles increased to 10 in 1946 (Fig. 1*a*), probably due to the potential of Anthrax as a bioweapon. Surprisingly, perhaps, the invention of the Polymerase chain reaction(PCR) in 1983 (Fig. 1*b*), did not lead to an immediate increase in the number of publications on *B. anthracis*. However, the method was soon to become a fast and sensitive means of detecting *B. anthracis* as an alternative, or even a substitution, to biochemical or culture methods.

Brief introduction to the genetics of *B. anthracis*

Like other members of *Bacillus cereus sensu lato* (Bcsl) group, *B. anthracis* is characterized by elongated shape, is Gram-positive and can form spores. Its genetic material is arranged in a triad — a chromosome (≈5.2 mb) [11], and two plasmids — pXO1 (≈181 kb) [12] and pXO2 (≈94 kb) [13]. Certain plasmid genes mediate the virulence of the bacillus — toxin-encoding genes *pag*, *lef*, *cya* on pXO1 and capsule-encoding *cap* genes (*capA*, *capB*, *capC*) on pXO2. Chromosomal genes, on the other hand, are mainly responsible for the core cellular functions, e.g. metabolism and cell division.

Phenotypical and morphological similarities within Bcsl group are naturally accompanied by genetic sameness, further complicating the task of detection and/or identification of *B. anthracis*. Among the detection methods known today, e.g. microbiological, antigen-based, biosensor-based, MALDI-TOF MS-based, PCR-based assays stand out due their relative speed, safety and efficiency [14—16].

B. anthracis detection methods

The conventional detection methods, though considered the gold standard for identification of

bacteria in general, struggle in the case of *B. anthracis* — certain *B. cereus* isolates are known to possess the same phenotypic features, e.g., susceptibility to penicillin [17]. Furthermore, the need for early detection is complicated by the relative slowness of conventional assays which may require up to 48 hours to complete [18].

Immunoassays, targeting poly-D-glutamic capsule [19], glycoprotein BclA [20] and other antigens have been developed for *B. anthracis* diagnostics. On average, the antigen-based assays may require up to 6 hours to complete, making them relatively fast. However, a preprocessing chromatography step, aimed at removing serum proteins, makes the approach slower and more cumbersome.

Biosensors seem to be a promising technology, offering rapid, sensitive, selective, and cost-effective means of *B. anthracis* detection [21]. However, it is in an early stage of development and requires further research and improvement.

The DNA amplification-based methods do not require preprocessing steps, e.g. chromatography, which makes them faster. Furthermore, the methods utilize inactivated material, ensuring the overall safety when working with hazardous pathogens [22].

Early plasmid markers

The first scientific paper on detection of *B. anthracis* spores with PCR was published in 1992 [23]. As a marker, the authors of the study successfully amplified a fragment of the *cya* gene, responsible for encoding the edema factor. The authors reported that their approach was sensitive enough to detect as few as 2×10^4 spores, and its sensitivity was demonstrated on various *B. anthracis* strains. However, the spore processing step, stimulating the release of DNA, as well as multiple DNA runs are required for the success of the proposed assay.

Later, in 1993, Makino *et al.* managed to amplify 288-bp *capA* gene fragment in both spores and purified bacterial DNA [24]. The assay was sensitive enough to detect 10^3 SFU (spore-forming units). Importantly, the authors stated that both the capsule and the exotoxin genes, located on pXO2 and pXO1 respectively, should be targeted at the same time in the future PCR detection approaches.

Multiplex PCR approaches

In February 1994, Reif *et al.* supported the hypothesis mentioned earlier by stating that in order to identify virulent *B. anthracis*, both pXO1 and pXO2 genes must be targeted simultaneously [25]. As a continuation of their previous work, the authors targeted a 622-bp DNA fragment within the *capB* gene. This time, due to the dual-probe hybridization format, the nested PCR was not necessary, and the sensitivity was higher.

Later in 1994, a pair of primers targeting a 350-bp fragment of *capC* and 536-bp fragment of the *cya* genes was used by Johns *et al.* for *B. anthracis* spores detection [26]. An important assumption regarding the exogenous DNA present in the spore preparation contributing to the sensitivity of the proposed PCR assay was made.

In 1995, a nested PCR assay for the detection of *B. anthracis* from soil samples was developed by Beyer *et al.*, targeting *cap* genes, as well as *pag* gene, which encodes the protective antigen [27]. Despite the increased sensitivity of the assay due to the additional PCR run, the approach remained time-consuming and cumbersome.

Addition of chromosomal markers to the "mix"

The targeting multiple markers on both pXO1 and pXO2 seemed to have become an efficient approach to detect *B. anthracis*. However, considering the fact that avirulent strains, lacking the plasmids, had also been found, the need for an additional specific markers appeared. A promising candidate with a potential to fulfill that role was the chromosomal sequence *BA813*, identified in 1996 [28]. Ramisse *et al.* developed multiplex PCR assays capable of characterizing *B. anthracis* isolates, as well as confirming a species identity

regardless of the plasmid content [29]. This became possible by targeting the *BA813* sequence in addition to pXO1 and pXO2 targets. However, its specificity was questioned when some of the *B. cereus* and *B. thuringiensis* isolates, harboring the *BA813*, were identified [30].

Another chromosomal gene, *vrrA*, was targeted by Jackson *et al.* to perform the PCR analysis of tissue samples from the 1979 Sverdlovsk anthrax victims [31]. The assay was shown to be sensitive, yet required post-PCR steps to further distinguish between several groups of isolates. Furthermore, targeting *vrrA* and *Ba813* yielded false-positive results from various other *Bacillus* elsewhere [32].

Targeting the chromosomal *16S rRNA* gene was demonstrated to be inefficient for distinguishing between a variety of *B. anthracis* strains [24, 25], as well as members of the Bcsl group [35]. However, after the Amerithrax in 2001 (Fig. 1*c*), Sacchi *et al.* showed the potential of *16S rRNA* sequencing for rapid identification of *B. anthracis* in culture, as well as direct detection in clinical specimens [36].

The *rpoB* gene, coding for the RNA-polymerase β-subunit, was shown to be a specific PCR target to identify and distinguish *B. anthracis* from other closely related bacilli [37]. Nonetheless, this approach produced a false positive result when tested on the Zimbabwe *B. anthracis* strain [38]. Ko *et al.* targeted both *rpo B*and *cap* for identification of *B. anthracis*, which turned out to be a more useful application [35]. Oggioni *et al.* targeted *rpoB* and *lef* (encoding the lethal factor) to detect *B. anthracis* spores in nasal swabs [39]. A highly sensitive assay, capable of detecting even a few or single cells on a background of other cells, targeting *rpoB* as well as 2 plasmid markers, *pag* and *capC*, was developed elsewhere [40].

Shin *et al.* evaluated another chromosomal gene *gyrB* (encoding DNA gyrase subunit B) as a potential PCR marker [41]. The authors concluded, that the *gyrB* gene could be used as a chromosomal marker for the rapid screening of *B. anthracis* by PCR or differentiation of *B. anthracis* from other related species by multiplex PCR with other plasmid markers. However, characterization of *gyrB* performed poorly when differentiating *B. anthracis* from *B. cereus*, because of the sequence homology [42]. *gyrA*, encoding the DNA gyrase subunit A, was used as a target by Hurtle *et al.* [43]; however, it was shown to produce false-positive results in *B. anthracis*-specific PCR assays.

The idea that both a chromosomal marker and plasmid markers should be involved for a complete identification of *B. anthracis* had gained a lot of support [20, 35—37].

In search for the gold standard

Despite the abundance of discovered and probed chromosomal markers, none of them had been proven to be 100% specific for *B. anthracis* detection [47]. However, a promising target, *BA_5345*, which was identified with the use of subtractive hybridization earlier [48], was shown to be 100% specific when tested on 328 *Bacillus* strains belonging to 20 different *Bacillus* species [47]. This marker was used by Cieślik *et al.* in a multiplex PCR assay with the plasmid markers *cap* and *pag* to efficiently identify *B. anthracis* among 42 *B. anthracis* and 53 *Bacillus* species [49].

Wielinga *et al.* developed a multiplex PCR assay targeting, along with the plasmid markers, the chromosomal marker *PL3* (prophage lambda-Ba03) [50]. This methodology provided an opportunity to specifically identify *B. anthracis*, as well as discriminate the different virulent types. Another study by Aminu *et al.* described a qPCR assay targeting the *PL3* marker as well as two plasmid genes, *cap* and *lef*, aimed at detecting *B. anthracis* in smear and skin tissue samples with high sensitivity and specificity [51].

In an effort to establish the gold standard, Ågren *et al.* carried out an extensive study evaluating the PCR-based assays, capable of detecting *B. anthracis* chromosomal marker sequences [52]. After the *in silico* analysis of 35 PCR assays, the primers/probes targeting *BA_5345*, *PL3* and *BA_5357* were shown, among other 5 selected for further analysis, to perfectly match all of the studied *B. anthracis* genomes, and poorly match *B. thuringiensis*

and *B. cereus* strains. One of the conclusions of the research was that the *PL3* assay should be recommended as a possible European standard, capable of improving PCR methods for detection of *B. anthracis*.

Lekota *et al.* developed a multiplex PCR assay, targeting 2 plasmid markers, *pag* and *capC*, and a chromosomal *SASP* gene (coding for the small acid soluble protein) [53]. Discussing the results of the study, the authors pointed out that a single diagnostic approach, e.g. multiplex PCR, may not be able to provide sufficient data, potentially leading to false-positive results. Therefore, microbiological tests should accompany molecular tests in order to

A non-exhaustive list of references, describing PCR-based assays, amplifying specific *B. anthracis* target genes/sequences

Location	Target gene or sequence	Reference
pXO1	*cya*	[23], [57], [58], [59], [60], [61], [32], [62], [63], [29], [31]
	pagA	[64], [40], [65], [57], [66], [67], [68], [59], [60], [49], [61], [69], [70], [32], [54], [27], [71], [72], [63], [29], [73], [31]
	lef	[57], [74], [39], [60], [61], [75], [70], [32], [51], [76], [63], [77], [29], [31]
	gerXB	[61]
	atxA	[61]
	ORF53	[63]
pXO2	*capB*	[64], [57], [74], [59], [61], [75], [70], [49], [63], [25], [29], [31]
	capC	[57], [78], [74], [66], [71], [72], [76], [77], [29], [79], [31], [80]
	capA	[57], [74], [58], [54], [24], [51], [63], [29], [31]
	*caps**	[40], [69], [32], [27], [73]
	acpA	[81]
	cerAB	[58]
	ORF7	[63]
Chromosome	*rpoB*	[40], [66], [39], [61], [72], [37]
	alo	[82]
	16S rRNA	[82], [74], [66], [81], [61], [83], [84], [36]
	23S rRNA	[79]
	BA813	[57], [78], [66], [68], [81], [32], [77], [29], [79]
	BA5345	[49], [71]
	spoOA	[61]
	abrB	[61]
	BA-1	[75], [70]
	BA1698	[54]
	BA5354	[54]
	BA5361	[54]
	sasp	[85]
	E4	[86]
	PL3	[51]
	sspE	[76], [63]
	vrrA	[31]

* — *caps* refers to *cap* operon genes.

achieve the highest diagnostic value. Moreover, the authors hypothesized that due to the fact that the PCR analysis was capable of providing only limited information about the genetic basis or virulence of *B. anthracis* isolates, the whole genome sequencing might be required in order to be able to distinguish between the closely related species of *B. cereus/subtilis* group.

In a recent work by Zorigt *et al.*, a pan-genome analysis aimed at finding *B. anthracis*-specific chromosomal marker sequences or genes was carried out [54]. A previously undefined gene region spanning coordinates between 1,596,297 and 1,605,500 was observed. This region includes genes *BA1693-BA1699*, all of which code for hypothetical proteins belonging to the glycosyltransferase family. Additionally, 3 more genes, *BA1701*, *BA1703* and *BA1704*, were identified adjacent to the region, also coding for hypothetical proteins. The genes *BA1698*, *BA5354*, and *BA5361*, identified as *B. anthracis*-specific in this study, as well as two known plasmid markers, *pag* and *capA*, were targeted by multiplex PCR assays, which were shown to be highly specific and sensitive. These findings seem promising for future research.

Commercial PCR detection kits

In 2002, an article describing theMayo-Roche Rapid Anthrax Test, targeting *pagA* and *capB* genes as a part of the kit from Roche Applied Science [55]. The authors reported the detection sensitivity of 100% on all *B. anthracis* strains, probed in the study. Moreover, the assay managed to differentiate all 3 avirulent strains lacking either pXO1 and pXO2 plasmid.

One year later, in 2003, King *et al.* evaluated the performance of 3 commercially available assays for direct detection of *B. anthracis* spores: Anthrax BTA, BADD, and SMART II, priced $19.80, $42.50, and $52.10 respectively [56]. These assays held promise for field detection by first responders (firemen, police, and hospital personnel); however, the overall sensitivity turned out to be less than 40% for Anthrax BTA and BADD and 41.67% for SMART II.

As of year 2025, multiple companies are offering *B. anthracis* PCR detection kits, e.g., NZYtech (prod. num. MD06631), Fisher Scientific (prod. num. 15840373), Labotaq (prod. num. IP21036), and others. For the aforementioned products, the price for one reaction varies from €3.99 (NZYtech) to €17.1 (Fisher Scientific). While, according to the specifications, the assays are highly sensitive, there is no available information about the genes targeted by the primers within the PCR kits.

Final remarks

At the moment, the gold standard for PCR-based detection of *B. anthracis* appears elusive. The recent findings by Zorigt *et al.* [54], although holding a lot of potential, require further testing by other researchers.

As the number of Bcsl group strains being isolated and described grows, it is possible that more target genes/sequences, described here, will prove to be not *B. anthracis*-specific, therefore excluding them from the list of the reliable targets for PCR-based identification assays. On the contrary, newly discovered targets may turn out to be absolutely reliable and, consequently, eliminate the need for further search.

Multiple plasmid and chromosomal markers have been touched on in this overview. Table provides an extended list of targets described in the literature, some of which have not been mentioned in the article to maintain the integrity of the historical flow of events.

Funding. This work was supported by "Development of a PCR-based test system for detection of the anthrax pathogen" project (0125U001098).

REFERENCES

1. *Bradford H.* Anthrax: blains upon man. *Antimicrob Infect Dis Newsl.* 2000; **18**(9):65—71.
2. *Dirckx JH.* Virgil on anthrax. *Am J Dermatopathol.* 1981; **3**(2):191—5.

3. *Cieslak TJ, Eitzen EM Jr.* Clinical and Epidemiologic Principles of Anthrax. *Emerg Infect Dis.* 1999; **5**(4):552—5.
4. *Berry D.* Pierre-François Olive Rayer: Biography. *Med Hist Suppl.* 2005;(24):7—13.
5. *Barker AE.* On Some Points regarding the Distribution of Bacillus Anthracis in the Human Skin in Malignant Pustule. *Med Chir Trans.* 1886; **69**:127—134.1.
6. *Tomkins H.* Note on the Cultivation of Bacillus Anthracis. *Br Med J.* 1887; **1**(1363):328—9.
7. *Hardy WB, Keng LB.* On the Changes in the Number and Character of the Wandering Cells of the Frog induced by the presence of Urari or of Bacillus Anthracis. *J Physiol.* 1893; **15**(4):361—400.1.
8. *Burke V, Skinner H.* Resistance of bacterial spores to the triphenylmethane dyes. *J Exp Med.* 1925; **41**(4):471—7.
9. *Zozaya J.* A serological study of the polysaccharides of meningococcus, B. anthracis, B. proteus, B. subtilis and B. mesentericus. *J Exp Med.* 1931; **54**(5):725—32.
10. *Stein CD, Rogers H.* Observations on the resistance of anthrax spores to heat. *Vet Med.* 1945; **40**:406—10.
11. *Ravel J, Jiang L, Stanley ST, et al., and Rasko DA.* The complete genome sequence of Bacillus anthracis Ames "Ancestor". *J Bacteriol.* 2009; **191**(1):445—6.
12. *Okinaka RT, Cloud K, Hampton O, et al., and Jackson PJ.* Sequence and organization of pXO1, the large Bacillus anthracis plasmid harboring the anthrax toxin genes. *J Bacteriol.* 1999; **181**(20):6509—15.
13. *Okinaka RT, Cloud K, Hampton O, et al., and Jackson P.* Sequence, assembly and analysis of pX01 and pX02. *J Appl Microbiol.* 1999; **87**(2):261—2.
14. *Zasada AA.* Detection and Identification of Bacillus anthracis: From Conventional to Molecular Microbiology Methods. *Microorganisms.* 2020; **8**(1):125.
15. *Zhu H, Zhang H, Xu Y, et al., and Neužil P.* PCR past, present and future. *Biotechniques.* 2020; **69**(4):317—25.
16. *Metzker M, Caskey C.* Polymerase Chain Reaction (PCR). *eLS, John Wiley & Sons, Ltd (Ed).* 2009.
17. *Turnbull PC.* Definitive identification of Bacillus anthracis--a review. *J Appl Microbiol.* 1999; **87**(2):237—40.
18. *Marston CK, Beesley C, Helsel L, Hoffmaster AR.* Evaluation of two selective media for the isolation of Bacillus anthracis. *Lett Appl Microbiol.* 2008; **47**(1):25—30.
19. *Mechaly A, Vitner E, Levy H, et al., and Fisher M.* Simultaneous Immunodetection of Anthrax, Plague, and Tularemia from Blood Cultures by Use of Multiplexed Suspension Arrays. *J Clin Microbiol.* 2018; **56**(4):e01479—17.
20. *Kuehn A, Kovác P, Saksena R, et al., and Grunow R.* Development of antibodies against anthrose tetrasaccharide for specific detection of Bacillus anthracis spores. *Clin Vaccine Immunol.* 2009; **16**(12):1728—37.
21. *Morawska K, Sikora T, Nakonieczna A, et al., and Witkiewicz Z.* Electrochemical Immunodetection of Bacillus anthracis Spores. *Sensors.* 2025; **25**(19):5948.
22. *Brukner I, Oughton M.* Direct PCR for Rapid and Safe Pathogen Detection: Laboratory Evaluation Supporting Field Use in Infectious Disease Outbreak. *LabMed.* 2025; **2**(3):12.
23. *Carl M, Hawkins R, Coulson N, et al., and Woody JN.* Detection of spores of Bacillus anthracis using the polymerase chain reaction. *J Infect Dis.* 1992; **165**(6):1145—8.
24. *Makino SI, Iinuma-Okada Y, Maruyama T, et al., and Yoshikawa M.* Direct detection of Bacillus anthracis DNA in animals by polymerase chain reaction. *J Clin Microbiol.* 1993; **31**(3):547—51.
25. *Reif TC, Johns M, Pillai SD, Carl M.* Identification of capsule-forming Bacillus anthracis spores with the PCR and a novel dual-probe hybridization format. *Appl Environ Microbiol.* 1994; **60**(5):1622—5.
26. *Johns M, Harrington L, Titball RW, Leslie DL.* Improved methods for the detection of Bacillus anthracis spores by the polymerase chain reaction. *Lett Appl Microbiol.* 1994; **18**(4):236—8.
27. *Beyer W, Glöckner P, Otto J, Böhm R.* A nested PCR method for the detection of Bacillus anthracis in environmental samples collected from former tannery sites. *Microbiol Res.* 1995; **150**(2):179—86.
28. *Patra G, Sylvestre P, Ramisse V, et al., and Guesdon JL.* Isolation of a specific chromosomic DNA sequence of Bacillus anthracis and its possible use in diagnosis. *FEMS Immunol Med Microbiol.* 1996; **15**(4):223—31.
29. *Ramisse V, Patra G, Garrigue H, et al., and Mock M.* Identification and characterization of Bacillus anthracis by multiplex PCR analysis of sequences on plasmids pXO1 and pXO2 and chromosomal DNA. *FEMS Microbiol Lett.* 1996; **145**(1):9—16.
30. *Ramisse V, Patra G, Vaissaire J, Mock M.* The Ba813 chromosomal DNA sequence effectively traces the whole Bacillus anthracis community. *J Appl Microbiol.* 1999; **87**(2):224—8.

31. *Jackson PJ, Hugh-Jones ME, Adair DM, et al., and Keim P.* PCR analysis of tissue samples from the 1979 Sverdlovsk anthrax victims: The presence of multiple Bacillus anthracis strains in different victims. *Proc Natl Acad Sci U S A.* 1998; **95**(3):1224—9.
32. *Patra G, Vaissaire J, Weber-Levy M, et al., and Mock M.* Molecular Characterization of BacillusStrains Involved in Outbreaks of Anthrax in France in 1997. *J Clin Microbiol.* 1998; **36**(11):3412—4.
33. *Ash C, Farrow JA, Dorsch M, et al., and Collins MD.* Comparative Analysis of Bacillus anthracis, Bacillus cereus, and Related Species on the Basis of Reverse Transcriptase Sequencing of 16S rRNA. *Int J Syst Bacteriol.* 1991; **41**(3):343—6.
34. *Jackson PJ, Walthers EA, Kalif AS, et al., and Keim P.* Characterization of the variable-number tandem repeats in vrrA from different Bacillus anthracis isolates. *Appl Environ Microbiol.* 1997; **63**(4):1400—5.
35. *Ko KS, Kim JM, Kim JW, et al., and Kook YH.* Identification of Bacillus anthracis by rpoB Sequence Analysis and Multiplex PCR. *J Clin Microbiol.* 2003; **41**(7):2908—14.
36. *Sacchi CT, Whitney AM, Mayer LW, et al., and Popovic T.* Sequencing of 16S rRNA Gene: A Rapid Tool for Identification of Bacillus anthracis. *Emerg Infect Dis.* 2002; **8**(10):1117—23.
37. *Qi Y, Patra G, Liang X, et al., and DelVecchio VG.* Utilization of the rpoB Gene as a Specific Chromosomal Marker for Real-Time PCR Detection of Bacillus anthracis. *Appl Environ Microbiol.* 2001; **67**(8):3720—7.
38. *Brumlik MJ, Szymajda U, Zakowska D, et al., and Del Vecchio VG.* Use of Long-Range Repetitive Element Polymorphism-PCR To Differentiate Bacillus anthracis Strains. *Appl Environ Microbiol.* 2001; **67**(7):3021—8.
39. *Oggioni MR, Meacci F, Carattoli A, et al., and Pozzi G.* Protocol for Real-Time PCR Identification of Anthrax Spores from Nasal Swabs after Broth Enrichment. *J Clin Microbiol.* 2002; **40**(11):3956—63.
40. *Ellerbrok H, Nattermann H,* Özel *M, et al., and Pauli G.* Rapid and sensitive identification of pathogenic and apathogenic Bacillus anthracis by real-time PCR. *FEMS Microbiol Lett.* 2002; **214**(1):51—9.
41. *Shin S, Ryu C, Oh H, et al., and Seong K.* Evaluation of gyrB as Chromosomal Marker in Bacillus anthracis. *J Bacteriol Virol.* 2004; **34**(3):191—200.
42. *La Duc T, Satomi M, Agata N, Venkateswaran K.* gyrB as a phylogenetic discriminator for members of the Bacillus anthracis-cereus-thuringiensis group. *J Microbiol Methods.* 2004; **56**(3):383—94.
43. *Hurtle W, Bode E, Kulesh DA, et al., and Norwood D.* Detection of the Bacillus anthracis gyrA Gene by Using a Minor Groove Binder Probe. *J Clin Microbiol.* 2004; **42**(1):179—85.
44. *Turnbull PC, Hutson RA, Ward MJ, et al., and Melling J.* Bacillus anthracis but not always anthrax. *J Appl Bacteriol.* 1992; **72**(1):21—8.
45. *Koehler TM.* Bacillus anthracis genetics and virulence gene regulation. *Curr Top Microbiol Immunol.* 2002; **271**:143—64.
46. *Bode E, Hurtle W, Norwood D.* Real-Time PCR Assay for a Unique Chromosomal Sequence of Bacillus anthracis. *J Clin Microbiol.* 2004; **42**(12):5825—31.
47. *Antwerpen MH, Zimmermann P, Bewley K, et al., and Meyer H.* Real-time PCR system targeting a chromosomal marker specific for Bacillus anthracis. *Mol Cell Probes.* 2008; **22**(5—6):313—5.
48. *Radnedge L, Agron PG, Hill KK, et al., and Andersen GL.* Genome Differences That Distinguish Bacillus anthracis from Bacillus cereus and Bacillus thuringiensis. *Appl Environ Microbiol.* 2003; **69**(5):2755—64.
49. *Cieślik P, Knap J, Kolodziej M, et al., and Bielawska-Drózd A.* Real-Time PCR Identification of Unique Bacillus anthracis Sequences. *Folia Biol (Praha).* 2015; **61**(5):178—83.
50. *Wielinga PR, Hamidjaja RA,* Ågren *J, et al., and van Rotterdam B.* A multiplex real-time PCR for identifying and differentiating B. anthracis virulent types. *Int J Food Microbiol.* 2011; **145**(Suppl 1):137—44.
51. *Aminu OR, Lembo T, Zadoks RN, et al., and Forde TL.* Practical and effective diagnosis of animal anthrax in endemic low-resource settings. *PLoS Negl Trop Dis.* 2020; **14**(9):e0008655.
52. Ågren *J, Hamidjaja RA, Hansen T, et al., and Derzelle S.* In silico and in vitro evaluation of PCR-based assays for the detection of Bacillus anthracis chromosomal signature sequences. *Virulence.* 2013; **4**(8):671—85.
53. *Lekota KE, Hassim A, Mafofo J, et al., and Madoroba E.* Polyphasic characterization of Bacillus species from anthrax outbreaks in animals from South Africa and Lesotho. *J Infect Dev Ctries.* 2016; **10**(8):814—23.
54. *Zorigt T, Furuta Y, Paudel A, et al., and Higashi H.* Pan-genome analysis reveals novel chromosomal markers for multiplex PCR-based specific detection of Bacillus anthracis. *BMC Infect Dis.* 2024; **24**(1):942.

55. *Uhl JR, Bell CA, Sloan LM, et al., and Cockerill FR 3rd.* Application of rapid-cycle real-time polymerase chain reaction for the detection of microbial pathogens: the Mayo-Roche Rapid Anthrax Test. *Mayo Clin Proc.* 2002; **77**(7):673—80.
56. *King D, Luna V, Cannons A, et al., and Amuso P.* Performance assessment of three commercial assays for direct detection of Bacillus anthracis spores. *J Clin Microbiol.* 2003; **41**(7):3454—5.
57. *Fasanella A, Losito S, Trotta T, et al., and Chiocco D.* Detection of anthrax vaccine virulence factors by polymerase chain reaction. *Vaccine.* 2001; **19**(30):4214—8.
58. *Shangkuan YH, Chang YH, Yang JF, et al., and Shaio MF.* Molecular characterization of Bacillus anthracis using multiplex PCR, ERIC-PCR and RAPD. *Lett Appl Microbiol.* 2001; **32**(3):139—45.
59. *Moser MJ, Christensen DR, Norwood D, Prudent JR.* Multiplexed detection of anthrax-related toxin genes. *J Mol Diagn.* 2006; **8**(1):89—96.
60. *Kumar S, Tuteja U.* Detection of virulence-associated genes in clinical isolates of bacillus anthracis by multiplex PCR and DNA probes. *J Microbiol Biotechnol.* 2009; **19**(11):1475—81.
61. *Liang X, Zhang H, Zhang E, et al., and Zhu J.* Identification of the pXO1 plasmid in attenuated Bacillus anthracis vaccine strains. *Virulence.* 2016; **7**(5):578—86.
62. *Banger S, Pal V, Tripathi NK, Goel AK.* Development of a PCR Lateral Flow Assay for Rapid Detection of Bacillus anthracis, the Causative Agent of Anthrax. *Mol Biotechnol.* 2021; **63**(8):702—9.
63. *Riojas MA, Kiss K, McKee ML, Hazbón MH.* Multiplex PCR for species-level identification of Bacillus anthracis and detection of pXO1, pXO2, and related plasmids. *Health Secur.* 2015; **13**(2):122—9.
64. *Bell CA, Uhl JR, Hadfield TL, et al., and Cockerill FR 3rd.* Detection of Bacillus anthracis DNA by LightCycler PCR. *J Clin Microbiol.* 2002; **40**(8):2897—902.
65. *Cote CK, Rossi CA, Kang AS, et al., and Welkos SL.* The detection of protective antigen (PA) associated with spores of Bacillus anthracis and the effects of anti-PA antibodies on spore germination and macrophage interactions. *Microb Pathog.* 2005; **38**(5—6):209—25.
66. *Elzi MV, Mallard K, Droz S, Bodmer T.* Polyphasic Approach for Identifying Bacillus spp. *J Clin Microbiol.* 2005; **43**(2):1010.
67. *Makino S, Cheun HI.* Application of the real-time PCR for the detection of airborne microbial pathogens in reference to the anthrax spores. *J Microbiol Methods.* 2003; **53**(2):141—7.
68. *Wang SH, Wen JK, Zhou YF, et al., and Zhang XE.* Identification and characterization of Bacillus anthracis by multiplex PCR on DNA chip. *Biosens Bioelectron.* 2004; **20**(4):807—13.
69. *Olani A, Galante D, Lakew M, et al., and Eguale T.* Identification of Bacillus anthracis Strains from Animal Cases in Ethiopia and Genetic Characterization by Whole-Genome Sequencing. *Pathogens.* 2025; **14**(1):39.
70. *Ochai SO, Hassim A, Dekker EH, et al., and van Heerden H.* Comparing microbiological and molecular diagnostic tools for the surveillance of anthrax. *PLoS Negl Trop Dis.* 2024; **18**(11):e0012122.
71. *Wang S, Suluku R, Jalloh MB, et al., and Mi Z.* Molecular characterization of an outbreak-involved Bacillus anthracis strain confirms the spillover of anthrax from West Africa. *Infect Dis Poverty.* 2024; **13**(1):6.
72. *Kędrak-Jabłońska A, Budniak S, Szczawińska A, et al., and Szulowski K.* Evaluation of Real-time PCR Based on SYBR Green I Fluorescent Dye for Detection of Bacillus Anthracis Strains in Biological Samples. *J Vet Res.* 2018; **62**(4):549—54.
73. *Makino SI, Cheun HI, Watarai M, et al., and Takeshi K.* Detection of anthrax spores from the air by real-time PCR. *Lett Appl Microbiol.* 2001; **33**(3):237—40.
74. *Sjöstedt A, Eriksson U, Ramisse V, Garrigue H.* Detection of Bacillus anthracis spores in soil by PCR. *FEMS Microbiol Ecol.* 1997; **23**(2):159—68.
75. *Zincke D, Norris MH, Cruz O, et al., and Blackburn JK.* TaqMan Assays for Simultaneous Detection of Bacillus anthracis and Bacillus cereus biovar anthracis. *Pathogens.* 2020; **9**(12):1074.
76. *Kim K, Seo J, Wheeler K, et al., and Leighton T.* Rapid genotypic detection of Bacillus anthracis and the Bacillus cereus group by multiplex real-time PCR melting curve analysis. *FEMS Immunol Med Microbiol.* 2005; **43**(2):301—10.
77. *Berg T, Suddes H, Morrice G, Hornitzky M.* Comparison of PCR, culture and microscopy of blood smears for the diagnosis of anthrax in sheep and cattle. *Lett Appl Microbiol.* 2006; **43**(2):181—6.
78. *Levine SM, Perez-Perez G, Olivares A, et al., and Blaser MJ.* PCR-Based Detection of Bacillus anthracis in Formalin-Fixed Tissue from a Patient Receiving Ciprofloxacin. *J Clin Microbiol.* 2002; **40**(11):4360—2.

79. *Brightwell G, Pearce M, Leslie D.* Development of internal controls for PCR detection of Bacillus anthracis. *Mol Cell Probes.* 1998; **12**(6):367—77.
80. *Lee MA, Brightwell G, Leslie D, et al., and Hamilton A.* Fluorescent detection techniques for real-time multiplex strand specific detection of Bacillus anthracis using rapid PCR. *J Appl Microbiol.* 1999; **87**(2):218—23.
81. *Levi K, Higham JL, Coates D, Hamlyn PF.* Molecular detection of anthrax spores on animal fibres. *Lett Appl Microbiol.* 2003; **36**(6):418—22.
82. *Shannon JG, Ross CL, Koehler TM, Rest RF.* Characterization of anthrolysin O, the Bacillus anthracis cholesterol-dependent cytolysin. *Infect Immun.* 2003; **71**(6):3183—9.
83. *Braun P, Nguyen MD, Walter MC, Grass G.* Ultrasensitive Detection of Bacillus anthracis by Real-Time PCR Targeting a Polymorphism in Multi-Copy 16S rRNA Genes and Their Transcripts. *Int J Mol Sci.* 2021; **22**(22):12224.
84. *Wang Y, Qian PY.* Conservative Fragments in Bacterial 16S rRNA Genes and Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies. *PLoS One.* 2009; **4**(10):e7401.
85. *Turnbull P.* Guidelines for the Surveillance and Control of Anthrax in Human and Animals, 3rd ed.; World Health Organization: Geneva, Switzerland, 2015.
86. *Bassy O, Jiménez-Mateo O, Ortega MV, et al., and Cabria JC.* Rapid identification of Bacillus anthracis by real-time PCR with dual hybridization probes in environmental swabs. *Mol Cell Probes.* 2018; **37**:22—7.

Received 01.09.2025

О.Ю. Скоробогатов, Н.С. Мельнічук, З.Ю. Ткачук
Інститут молекулярної біології і генетики НАН України
вул. Академіка Заболотного, 150, Київ, Україна, 03143
skorobogatov.alx@gmail.com

ГЕНЕТИЧНІ МАРКЕРИ ДЛЯ ПЛР-ДЕТЕКЦІЇ *BACILLUS ANTHRACIS*

Bacillus anthracis — це велика грампозитивна бактерія, яка спричиняє сибірську виразку — одне з найсмертоносніших захворювань, відомих людству. Для її виявлення було розроблено різні підходи, від більш точних, трудомістких методів на основі культивування до більш швидких методів на основі ПЛР. Останні базуються на ідентифікації певних ділянок геному *B. anthracis*, який складається з двох плазмід, pXO1 та pXO2, та хромосоми. Використання лише плазмідних маркерів може призвести до отримання ненадійних результатів через високу схожість між *B. anthracis* та іншими генетично спорідненими видами групи *Bacillus cereus sensu lato*, що актуалізує використання додаткових хромосомних мішеней. Ця стаття має на меті надати короткий огляд актуальних генетичних маркерів, що використовуються як мішені для виявлення *B. anthracis* за допомогою ПЛР.

***Ключові слова*:** *Bacillus anthracis*, генетичні маркери, ПЛР.