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PCR-BASED SCREENING OF INSECTICIDAL CRY2-TYPE GENES IN NATIVE *BACILLUS SPP.* STRAINS FROM THE REPUBLIC OF MOLDOVA

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Abstract
Agriculture is the fundamental sector of the Republic of Moldova's economy. Despite the availability of advanced technologies, the productivity of agricultural and horticultural crops remains significantly limited by biotic and abiotic stresses, with insect pests representing a major biotic constraint. Although chemical insecticides are widely used in pest control, their environmental impact and non-target effects have spurred interest in sustainable alternatives, such as biopesticides based on entomopathogenic bacteria Bacillus thuringiensis (Bt). The insecticidal properties of Bt strains are attributed to the production of a wide variety of crystalline proteins (Cry toxins), which exhibit specific activity against certain insect taxa. Using native entomopathogenic strains for biological plant protection offers several benefits over commercial or imported strains, owing to their better adaptation and high bioactivity against regionally important pests. Despite the extensive global research focused on the discovery of novel Bt isolates, data regarding the genetic diversity, distribution, and insecticidal potential of native strains in Eastern Europe, and the Republic of Moldova, particularly, remain limited. Thus, as a result of the present study, molecular characterization of novel Bacillus spp. strains isolated from the Republic of Moldova was performed by screening for insecticidal genes via PCR using universal primer pair targeting Cry2-type genes.

Keywords: Bacillus thuringiensis, Cry genes, native strains, Republic of Moldova, screening.

1. INTRODUCTION

The Republic of Moldova is a country with a strong agricultural tradition and fertile lands, particularly black soils that cover approximately 75% of the agricultural area. However, several factors continue to pose a growing threat with significant implications for agricultural productivity and food security, preventing the country from realizing its full agricultural potential (Stratan, 2025). Biotic stress, including pests, pathogens, and weeds, is a major constraint on agricultural productivity. These factors interfere with plant physiological processes, inducing metabolic changes that ultimately decrease both yield and crop quality (Verma et al., 2025). Insects are among the most impactful agricultural pests, with Lepidoptera constituting one of the most numerous insect pest orders (Fig. 1) (Moldovan et al., 2017; Tuneu-Corral et al., 2023). Together with other harmful organisms, insects are responsible for at least a 40% reduction in global crop yields, resulting in food losses equivalent to the amount required to feed nearly one billion people. As a result, chemical control strategies have become widely adopted because of their relatively low cost and ability to effectively control a broad range of pest species (Deka et al., 2021; Angon et al., 2023).

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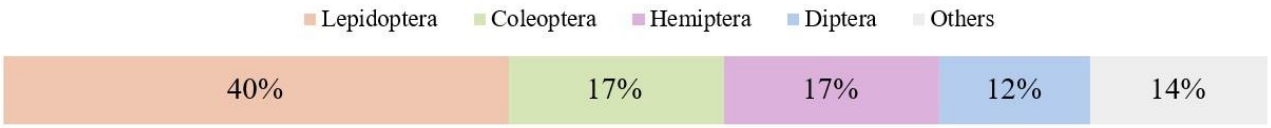


Figure 1. Agricultural insect pests damaging crops worldwide (Based on Tuneu-Corral et al., 2023)

The excessive and uncontrolled use of chemical pesticides has posed major risks to environmental sustainability because these substances interact with the environment through various pathways (Ahmad et al., 2024). For example, after field application, pesticides can volatilize and disperse through the air, affecting non-target organisms such as pollinators. Part of the pesticide is absorbed by plants, generating residues in crops. The bioaccumulation and biomagnification of pesticide residues throughout food chains can disrupt ecosystem stability and reduce biodiversity. In addition, the persistence of pesticides in soil for extended periods contributes to environmental contamination by facilitating the transfer of residues into groundwater and surface water systems (Muñoz-Bautista et al., 2025). Environmental contamination, in turn, creates multiple pathways for human exposure to pesticides through skin contact, inhalation, and the consumption of contaminated food and water. Prolonged exposure to pesticide residues has been associated with increased risks of cancer, infertility, endocrine disruption, and respiratory, neurological, and reproductive disorders (Ahmad et al., 2024).

Due to growing concerns regarding the environmental and human health impacts associated with intensive pesticide use, agricultural research has increasingly focused on sustainable crop management strategies (Moldovan et al., 2023). Among the most promising approaches are Precision Agriculture (PA) and Integrated Pest Management (IPM), both of which aim to improve agricultural productivity while reducing negative environmental effects. PA relies on digitalization, automation, and smart technologies to improve productivity, resource management, and sustainability, enabling site-specific management of irrigation, fertilization, and pest control. IPM, in turn, promotes the integration of combined biological, physical, and chemical measures to maintain pest populations below economically damaging thresholds (Kebe et al., 2023).

The implementation of smart agriculture technologies in the Republic of Moldova is currently underway, supported by several favorable conditions, including a growing IT sector, expanding digital infrastructure, and increasing governmental support for digital innovation in agriculture (Stratan, 2025). However, the successful transition toward sustainable agriculture cannot rely solely on Precision Agriculture (PA) and equal attention must be given to Integrated Pest Management (IPM). As biological control constitutes a cornerstone of IPM, identifying and developing effective biocontrol agents remains a key research priority in order to minimize reliance on synthetic pesticides.

In this context, the entomopathogenic bacterium *Bacillus thuringiensis* (*Bt*) has attracted considerable attention. *Bt* is a ubiquitous, Gram-positive, rod-shaped, sporulating bacterium that has been isolated worldwide from a wide variety of ecosystems, including soil, leaves, and dead insects (Palma et al., 2014). *Bt* belongs to the *Bacillus cereus* group and is distinguished from other related species phenotypically and genotypically by the presence of parasporal crystalline inclusions formed during the stationary phase of growth. These inclusions contain insecticidal crystal proteins, known as δ-endotoxins or Crystal (Cry) toxins, which are considered the major factors for the entomopathogenic properties of *Bt*. Cry toxins present interest as an effective biological control due to the specific insecticidal activity against different orders of insects (Fig. 2) (Moldovan et al., 2023; García-Cruz et al., 2026).

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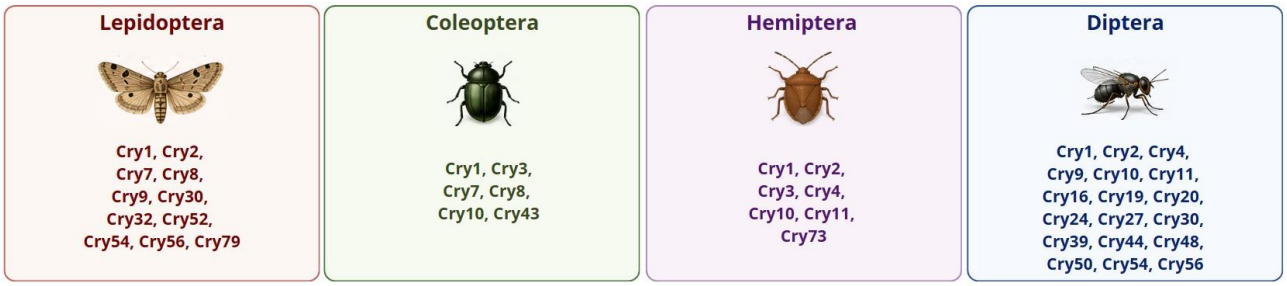


Figure 2. Insecticidal activity of Cry proteins (Adapted from García-Cruz et al., 2026)

Due to the ability of Cry proteins to act selectively on harmful insects without affecting non-target organisms, products containing *Bt* spores and crystal proteins are widely used for the control of insect larvae belonging to different orders. At present *Bt*-based biopesticides account for more than 90% of biopesticides available on the international market (Jallouli et al., 2020). According to the State Register of Plant Protection Products and Fertilizers, four imported *Bt*-based products are currently registered and authorized for use in the Republic of Moldova (ANSA, 2026).

However, continuous exposure of insect populations to a single type of Cry toxin can lead to the development of resistance, reducing field efficacy over time. This challenge highlights the need for alternative and complementary strategies, including the exploration and identification of new *Bt* species and strains capable of producing novel toxins with different modes of action (Hassan et al., 2021).

In this context, the use of native *Bacillus thuringiensis* strains offers several important advantages over commercial or introduced ones. First, *Bt* strains isolated directly from local insect pest populations often exhibit high host specificity and may produce novel Cry toxins that have not been previously characterized, making them especially valuable for managing resistant pest populations. Second, native strains are typically better adapted to local environmental conditions, including climate, soil characteristics, and indigenous microbial communities, which enhances their survival, persistence, and overall field efficacy (Tursunova, 2026). Finally, the use of indigenous strains reduces ecological risks associated with the introduction of non-native microorganisms, thereby contributing to the preservation of ecosystem stability and natural biological interactions (Moldovan et al., 2017).

A wide range of methods is used to characterize *Bt* strains to evaluate their toxicity against different insect species. A key approach is the identification of genes encoding insecticidal protein toxins through Polymerase Chain Reaction (PCR) as it is highly accurate, requires only small amounts of DNA, and enables rapid detection of specific DNA sequences in an organism. This method allows efficient screening of large numbers of *Bt* isolates to identify novel Cry genes and determine their distribution. The use of universal primers is commonly applied for detecting the presence of Cry genes (Hassan et al., 2021).

Research groups worldwide are continually testing different geographic regions to discover highly active novel isolates, because *Bt* collections represent an important source of new strains with potential insecticidal properties as well as a source of novel genes encoding insecticidal proteins (Sahin et al., 2018). The substantial volume of publications underscores global interest in this topic. A search conducted in an open-access database Lens.org, using the keywords "*Bacillus thuringiensis*", "Cry gene", and "molecular characterization" retrieved more than 1100 scholarly works including book chapters, dissertations and journal articles, highlighting the extensive research efforts devoted to this topic over the past decades (Fig. 3, a) (The Lens, 2026).

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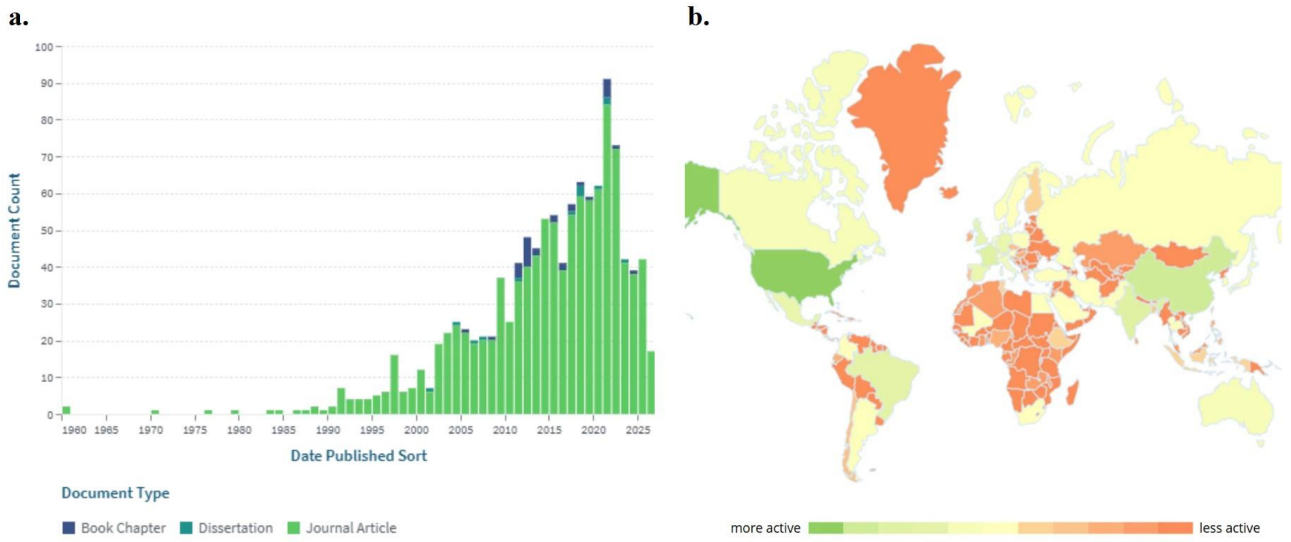


Figure 3. Bibliometric analysis of records obtained from the database search (Adapted from The Lens, 2026), ***a.*** *Annual distribution of scholarly works by document type;* ***b.*** *Countries/regions of institutions represented in the result set by their scholarly works*

Among the most active contributors to this research field are the United States, China, France, India, and Brazil. Notably, Eastern European countries remain relatively underrepresented in this research area (Fig. 3, b) (The Lens, 2026).

However, significant contributions to the study of the genetic diversity of *Bacillus thuringiensis* have been made by Polish researchers. Święcicka et al., 2005 investigated the genetic diversity of *B. thuringiensis* strains isolated from the intestines of insectivores based on Cry gene content and DNA profiling. The authors identified strains harboring Cry1, Cry2, and Cry9 genes encoding toxins active against Lepidoptera, as well as Diptera-specific strains carrying the Cry4 gene.

Later, Konecka et al., 2012 isolated *B. thuringiensis* strains from soil and water samples in search of novel isolates synthesizing crystals with high and broad insecticidal activity. Soil isolates were found to contain Cry1, Cry2, and Cry9 genes, whereas the strain isolated from water possessed Cry1 and Cry15 genes. Furthermore, Konecka et al., 2013 analyzed *B. thuringiensis* isolates obtained from the intestinal tracts of dead larvae and caterpillars of *Spodoptera exigua* and *Agrotis segetum*. The authors reported limited diversity of Cry genes, as only Cry1 and Cry2 genes were detected in the DNA of the studied strains.

In Bulgaria, researchers investigated the diversity of crystal toxin-producing *Bacillus thuringiensis* strains by analyzing isolates recovered from soil samples collected in remote agricultural areas and mountainous ecosystems. PCR-based analysis revealed the presence of multiple Cry gene types, including Cry1, Cry7, Cry8, and Cry11 (Arsov et al., 2023).

In Russia, Kryzhko A., 2024 analyzed *Bacillus thuringiensis* strains from the Crimean Collection of Microorganisms of the Research Institute of Agriculture of Crimea. PCR analysis based on the detection of specific amplicons using Cry1, Cry2, Cry4, Cry5, Cry7–8, Cry9, and Cry11 universal primers showed that the majority of the collection carried exotoxin genes, indicating strong insecticidal potential against pests belonging to the orders Hemiptera and Lepidoptera.

In the Republic of Moldova, Moldovan et al., 2023 isolated *Bacillus spp.* strains exhibiting morphological and cultural characteristics similar to *Bacillus thuringiensis* from the microflora of the insects *Helicoverpa armigera* and *Spodoptera exigua*. Molecular characterization was

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performed to identify genes encoding Cry1 proteins. The results showed that the majority of the investigated strains carried Cry1 genes, confirming their insecticidal potential against representatives of the orders Lepidoptera, Diptera, and Coleoptera.
Since insect pests belonging to the order Lepidoptera cause major damage in crop production, and it is known that Cry2-type proteins are toxic to this order of insects, the aim of the study was the molecular characterization of novel *Bacillus spp*. strains isolated in the Republic of Moldova, performed by screening for insecticidal genes via PCR using universal primer pairs targeting Cry2-type genes in order to expand the current knowledge of valuable strains from Eastern Europe, Republic of Moldova particularly.

2. MATERIALS AND METHODS

For the Cry2-type gene screening, 20 novel *Bacillus spp.* strains from the collection of the Laboratory of Systematics and Molecular Phylogeny, Institute of Zoology, Moldova State University were used. The strains selected for the study had been previously isolated from the Lepidopteran insect species *Helicoverpa armigera* and *Spodoptera exigua* and characterized by the institute's researchers as Gram-positive, spore-forming bacilli exhibiting morphological properties similar to those of *Bacillus thuringiensis*. Two strains of *Bacillus thuringiensis* were used as reference (Munteanu et al., 2013; Munteanu et al., 2014). A Gram-negative *Bacillus spp.* strain was included in the study as a negative control alongside the experimental samples.
Entomopathogenic bacterial strains isolated in pure culture were cultivated for routine maintenance on Nutrient Agar medium (per liter: 12 g tryptone, 12 g tryptose, 6 g sodium chloride, 10 g microbiological agar). For the DNA extraction bacterial cultures were grown for 24 hours at 30 °C on sterile Nutrient Agar. Genomic DNA was extracted from the vegetative biomass of bacterial cells according to the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific) protocol for Gram positive bacteria.
Quantity of DNA was visually assessed by electrophoresis in 1% agarose gel (1 g agarose, 100 ml 1X Tris-Acetat-EDTA buffer, 5 µL ethidium bromide) in a horizontal electrophoresis chamber for 70 min at 5V/cm in TAE 1X buffer solution (40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA). Gene Ruler DNA Ladder Mix (Thermo Fisher Scientific) was used as a molecular marker. A pair of universal primers was used to explore presence of Cry2-type encoding genes (Table 1) (Ben-Dov et al., 1997).

Table 1. Characteristics of universal primers

Primer pair	Sequence of primer (5'→3')	Amplicon expected size (bp)
Un2(d)	GTTATTCTTAATGCAGATGAATGGG	689-701
Un2(r)	CGGATAAAATAATCTGGGAAATAGT	

The reaction mixture in a total volume of 25 µL contained 10X Dream Taq Green Buffer (Thermo Fisher Scientific) in a final concentration of 1X, 0.5 µL (10 mM) deoxyribonucleoside triphosphate (dNTP), 0.5 µM of each primer (forward and reverse), 1 U/µL Dream Taq Hot Start Polymerase (Thermo Fisher Scientific), 1 µL of DNA as template and sterile distilled water. Conventional PCR was performed in a SimpliAmp™ thermal cycler (Applied Biosystems). The amplification steps were carried out as follows: an initial denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at the optimal temperature of 52°C for 45 s, and extension at 72°C for 1.40 min. A final extension step was performed at 72°C for 5 min. After PCR,

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all experimental samples were resolved by electrophoresis on a 2% agarose gel (2 g agarose, 100 ml 1X Tris-Acetat-EDTA buffer, 5 µL ethidium bromide) in a horizontal electrophoresis chamber for 80 min at 5V/cm in TAE 1X buffer solution (40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA). Gene Ruler DNA Ladder Mix (Thermo Fisher Scientific) was used as a molecular marker to access the PCR product band sizes. The amplified products were visualized and documented under the Ultraviolet Transilluminator TFX-20M (Enprotech).

3. RESULTS AND DISCUSSIONS

Genomic DNA extraction from the bacterial cells was successfully performed for all analyzed strains. Agarose gel electrophoresis demonstrated the presence of distinct high-molecular-weight bands in all samples, confirming the integrity and sufficient quality of the extracted genomic DNA for downstream PCR analysis (Fig. 4).

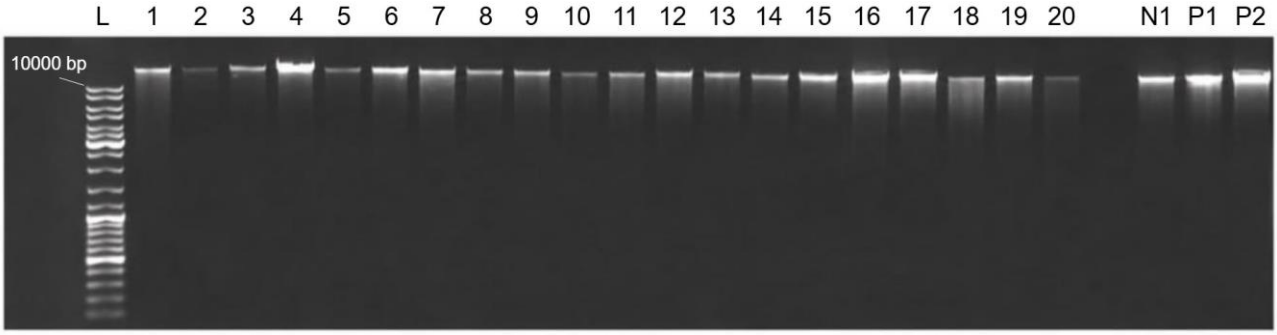


Figure 4. Agarose gel electrophoresis of genomic DNA,
lanes: L DNA molecular marker; 1-20 Bacillus spp. strains.;
N1 Gram-negative Bacillus spp. strain; P1-P2 B. thuringiensis strains

Since its introduction the PCR-based approach has been widely used for the rapid and reproducible identification of both known and novel Cry genes, as well as for the prediction of insecticidal activity through the correlation between coded protein toxicity and gene amplification profiles (Carozzi et al., 1991). This study aimed to investigate the distribution and diversity of Cry2-type genes, which expression is known to produce the proteins with the specificity toward lepidopteran species. The PCR strategy for the detection of Cry2 genes employs a universal primer set comprising a common forward primer and a specific reverse primer for the amplification of the Cry2 family genes based on C-terminal variable regions with a product size of approximately 700 bp (Ben-Dov et al., 1997). However, the currently known diversity of the Cry2 protein family is substantially broader than at the time when the universal detection primers were originally designed. According to the Pesticidal Protein Database a total of 103 Cry2 protein entries are currently registered, including 101 Cry2A and 2 Cry2B family members (BPPRC, 2026).
To assess the suitability of universal primers for comprehensive detection of Cry2-family genes, prior the experiment, primer alignment analysis was performed to evaluate binding sites and predicted amplicon lengths across the existing Cry2 variants. To avoid redundancy and overrepresentation of highly similar sequences, genes coding the representative proteins was selected. As a result, it was found that the primers align with all currently existing Cry2 classes with 0–5 nucleotide substitutions. The resulting amplicon size ranged from 698 to 704 base pairs, which is consistent with the originally reported expected product length (Table 2).

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Table 2. Universal primer binding in representative Cry2-type gene sequences

Name	Year	Protein GenBank accession no.	Nucleotide GenBank accession no.	Gene length (bp)	Nucleotide positions hybridized to primers	Mismatch of primers	Product size (bp)
Cry2Aa1	1987	AAA22335	M31738	2269	726-751, 1402-1427	0(d) 0(r)	701
Cry2Ab1	1989	AAA22342	M23724	1917	571-596, 1247-1272	9(d) 3(r)	701
Cry2Ac1	1991	CAA40536	X57252	4315	2695-2720, 3359-3384	3,6(d) 22(r)	689
Cry2Ad1	1999	AAF09583	AF200816	3121	1236-1261, 1912-1937	0(d) 13,15,19(r)	701
Cry2Ae1	2003	AAQ52362	AX098631	1899	571-596, 1244-1269	4,9,18(d) 0(r)	698
Cry2Af1	2007	ABO30519	EF439818	1881	571-596, 1244-1269	0(d) 2,3,6,15,24(r)	698
Cry2Ag1	2008	ACH91610	FJ032364	1905	574-599, 1250-1275	9(d) 3,17,19(r)	701
Cry2Ah1	2008	ACL13555	EU939453	1899	571-596, 1244-1269	18(d) 0(r)	698
Cry2Ai1	2009	ACV97158	FJ788388	1902	571-596, 1247-1272	3,7(d) 0(r)	701
Cry2Ak1	2012	AGU13825	KC156660	1905	571-596, 1250-1275	0(d) 0(r)	704
Cry2Al1	2014	AHH35153	KJ149819	1902	571-596, 1247-1272	0(d) 0(r)	701
Cry2Am1	2014	AIT70915	KJ634087	1902	571-596, 1247-1272	0(d) 0(r)	701
Cry2Ba1	2012	AGU13823	KC156658	1914	610-635, 1277-1302	0(d) 3,6,15,18,24(r)	692

Cry2-type gene screening was performed by conventional PCR with the universal primers Un2(d) and Un2(r). Primers for the detection of Cry2-type genes showed successful amplification as indicated by their specific product size of 701 bp on agarose gel electrophoresis. The target fragment of the Cry2-type gene was detected in a total of eleven (55%) *Bacillus spp.* strains and in both reference *Bacillus thuringiensis* isolates (Fig. 5).

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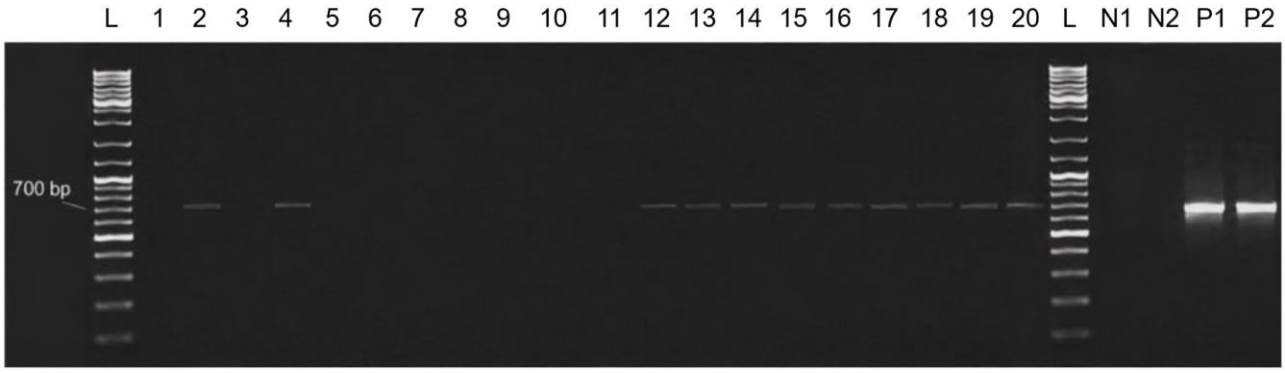


Figure 5. Agarose gel electrophoresis of PCR products amplified with universal primers,
lanes: L DNA molecular marker; 1-20 Bacillus spp. strains.;
N1 Gram-negative Bacillus spp. strain, N2 Distilled water; P1-P2 B. thuringiensis strains

The prevalence of Cry2-type genes observed in the present study agrees with data reported for *Bacillus thuringiensis* isolates from different geographical regions worldwide (Fig. 6). For example, Lone et al., 2017 observed the frequency of Cry-type genes in 44 native *B. thuringiensis* strains using universal primer pairs. PCR analysis resulted in the amplification of Cry2 in 14 isolates (31,8%). Salama et al., 2015 selected *Bt* isolates based on their potential activity against the two target lepidopteran insects *Spodoptera littoralis* and *Helicoverpa armigera*. Among the tested Cry-type genes, the frequency of the Cry2 gene was 38,88%. In Hassan et al., 2021 study of based on the PCR products obtained with universal primers, the frequency of Cry2 gene distribution among the 12 potential *Bt* isolates from cultivated and non-cultivated soils was 54%. Jain et al., 2012 observed the frequency of cry-type genes in 8 *Bt* strains through PCR and found that 75% of the studied strains amplified with the Cry2 universal primer pair. Arietta et al., 2004 reported that Cry2 was the predominant gene detected among the analyzed *B. thuringiensis* isolates collected from infested coffee plantations, being identified in 82 out of 105 strains (71,8%).

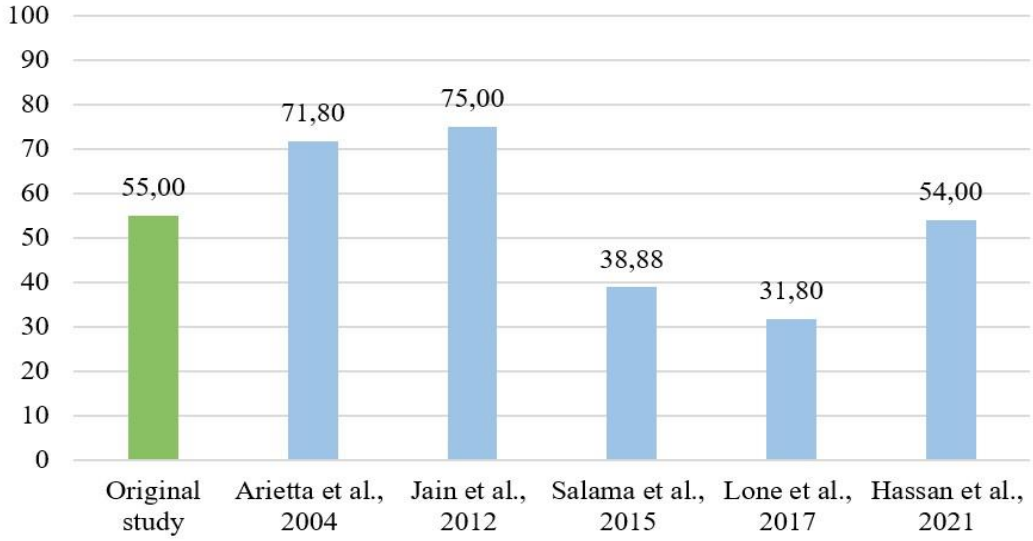


Figure 6. Prevalence of Cry2-type genes in strains across different countries

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4. CONCLUSIONS

PCR screening represents a rapid approach for the detection and differentiation of *B. thuringiensis* strains based on their amplification profiles. The identification of known Cry genes in the *Bacillus spp.* strains is important, since the specificity of action is known for many of the Cry toxins. This fact allows the possibility of selecting native strains based on their potential insecticidal activity for further bioassays against specific insect taxa, which in consequence could lead to their use in biological control of local target insect pests.

In this study, 20 novel strains of *Bacillus spp*. were screened for the presence of Cry2-type genes. Analysis of the frequency of occurrence of toxin-producing genes revealed that more than a half of the studied *Bacillus spp*. strains (55%) harbor Cry2-type specific amplicons, indicating the potential entomopathogenic activity of these strains against insect pests belonging to the order Lepidoptera, which represent one of the most widespread groups of insect pests. Moreover, target amplicons were detected in all reference *Bacillus thuringiensis* strains. On the other hand, isolates that haven't produced amplicons with universal Cry2 PCR primers are potential candidates for harboring other types of Cry genes. Further research is required to screen studied novel strains with a broader range of universal and specific primers to detect genes belonging to other Cry gene families. Additionally, the insecticidal potential of isolates harboring Cry2-type toxin gene will be investigated in target host insect bioassays.

In conclusion, this study expands current knowledge of the *B. thuringiensis* gene pool by introducing potentially valuable *Bacillus spp.* strains from Eastern Europe, Republic of Moldova particularly, a region that remains relatively underrepresented in worldwide collections.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

Ahmad, M. F., Ahmad, F. A., Alsayegh, A. A., Zeyaullah, M., AlShahrani, A. M., Muzammil, K., Saati, A. A., Wahab, S., Elbendary, E. Y., Kambal, N., Abdelrahman, M. H., Hussain, S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. *Heliyon, 10*(7), e29128.

Angon, P. B., Mondal, S., Jahan, I., Datto, M., Antu, U. B., Ayshi, F. J., Islam, M. S. (2023). Integrated pest management (IPM) in agriculture and its role in maintaining ecological balance and biodiversity. *Advances in Agriculture*, 5546373.

ANSA, Agenția Națională pentru Siguranța Alimentelor [National Food Safety Agency]. Registrul de stat al produselor de uz fitosanitar și al fertilizanților [State Register of Plant Protection Products and Fertilizers]. Retrieved June 03, 2026, from https://cnsapsa.md/

Arrieta, G., Hernández, A., Espinoza, A. M. (2004). Diversity of *Bacillus thuringiensis* strains isolated from coffee plantations infested with the coffee berry borer *Hypothenemus hampei*. *Revista de Biología Tropical, 52*(3), 757–764.

Arsov, A., Gerginova, M., Paunova-Krasteva, T., Petrov, K., Petrova, P. (2023). Multiple cry genes in *Bacillus thuringiensis* strain BTG suggest a broad-spectrum insecticidal activity. *International Journal of Molecular Sciences, 24*(13), 11137.

Ben-Dov, E., Zaritsky, A., Dahan, E., Barak, Z., Sinai, R., Manasherob, R., Khamraev, A., Troitskaya, E., Dubitsky, A., Berezina, N., Margalith, Y. (1997). Extended screening by PCR for seven cry-group genes from field-collected strains of *Bacillus thuringiensis*. *Applied and Environmental Microbiology, 63*(12), 4883–4890.

*Corresponding author, E-mail address: n.colodrivscaia@yahoo.com

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BPPRC, Bacterial Pesticidal Protein Database. Crickmore, N., Berry, C., Panneerselvam, S., Mishra, R., Connor, T.R. and Bonning, B.C. Bacterial Pesticidal Protein Resource Center. Retrieved May 15, 2026, from https://www.bpprc.org

Carozzi, N. B., Kramer, V. C., Warren, G. W., Evola, S., Koziel, M. G. (1991). Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product profiles. *Applied and Environmental Microbiology, 57*(11), 3057–3061.

Deka, B., Baruah, C., Babu, A. (2021). Entomopathogenic microorganisms: Their role in insect pest management. *Egyptian Journal of Biological Pest Control*, *31*, 121.

García-Cruz, K. V., Arce-Vázquez, M. B., Cruz-Monterrosa, R. G., Aguilar-Toalá, J. E., Rosas-Espejel, M., Hernández-Rodríguez, M., Pérez-Ruiz, R. V. (2026). Cry proteins from *Bacillus thuringiensis*: From natural insecticide to biotechnological tool for agricultural pest control. *Agro Productividad, 19*(1), 23–37.

Hassan, A. A., Youssef, M. A., Elashtokhy, M. M. A., Ismail, I. M., Aldayel, M., Afkar, E. (2021). Isolation and identification of *Bacillus thuringiensis* strains native of the Eastern Province of Saudi Arabia. *Egyptian Journal of Biological Pest Control, 31*, 6.

Jain, D., Kachhwaha, S., Jain, R., Kothari, S. L. (2012). PCR-based detection of cry genes in indigenous strains of *Bacillus thuringiensis* isolated from the soils of Rajasthan. *Indian Journal of Biotechnology, 11*(4), 491–494.

Jallouli, W., Driss, F., Fillaudeau, L., Rouis, S. (2020). Review on biopesticide production by *Bacillus thuringiensis* subsp. *kurstaki* since 1990: Focus on bioprocess parameters. *Process Biochemistry, 98*, 224–232.

Kebe, A. A., Hameed, S., Farooq, M. S., Sufyan, A., Malook, M. B., Awais, S., Riaz, M., Waseem, M., Amjad, U., & Abbas, N. (2023). Enhancing crop protection and yield through precision agriculture and integrated pest management: A comprehensive review. *Asian Journal of Research in Crop Science*, *8*(4), 443–453.

Konecka, E., Baranek, J., Hrycak, A., Kaznowski, A. (2012). Insecticidal activity of *Bacillus thuringiensis* strains isolated from soil and water. *The Scientific World Journal*, 710501.

Konecka, E., Baranek, J., Kaznowski, A. (2013). Genetic similarity and distribution of cry genes of *Bacillus thuringiensis* pathogenic for *Spodoptera exigua* (Lepidoptera: Noctuidae) and *Agrotis segetum* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology, 23*(4), 474–479.

Kryzhko, A. V. (2024). Frequency of cry-like genes in *Bacillus thuringiensis* strains of the Crimean microorganism collection. *Proceedings of Universities. Applied Chemistry and Biotechnology, 14*(4), 567–577.

Lone, S. A., Malik, A., Padaria, J. C. (2017). Characterization of lepidopteran-specific cry1 and cry2 gene harbouring native *Bacillus thuringiensis* isolates toxic against *Helicoverpa armigera*. *Biotechnology Reports, 15*, 27–32.

Moldovan, A., Munteanu-Molotievskiy, N., & Toderaș, I. (2023). *Bacteriile entomopatogene* [Entomopathogenic bacteria]. Chișinău: Tipografia Centrală, Chișinău.

Moldovan, A., Toderaș, I., Munteanu-Molotievskiy, N. (2017). Noi agenți bacterieni de control biologic al insectelor dăunătoare în Republica Moldova [New bacterial agents for biological control of harmful insects in the Republic of Moldova]. *Actual problems of zoology and parasitology: achievements and prospects*, 303–309.

Munteanu, N., Toderaș, I., Moldovan, A., Malevanciuc, N., Toderaș, L., Bacal, S., Railean, N. Tulpină de bacterii *Bacillus thuringiensis* subsp. *kurstaki* – bioinsecticid pentru combaterea lepidopterelor din genul *Lymantria* [*Bacillus thuringiensis* subsp. *kurstaki* bacterial strain – a bioinsecticide for the control of *Lymantria* lepidopteran pests]. Patent No. 4304 (13) B1, C1, C12N 1/20; C12R 1/07; A01N 63/00; A01P 7/04. Institutul de Zoologie al Academiei de Științe a Moldovei [Institute of Zoology of the Academy of Sciences of Moldova]. Application No. a 2013 0057. Filed: August 13, 2013. Published: September 30, 2014. In: *BOPI*, 2014, No. 9; 2015, No. 4.

Munteanu, N., Toderaș, I., Moldovan, A., Malevanciuc, N., Toderaș, L., Railean, N. Tulpină de bacterii *Bacillus thuringiensis* subsp. *kurstaki* – bioinsecticid pentru combaterea coleopterelor curculionoide [*Bacillus thuringiensis* subsp. *kurstaki* bacterial strain – a bioinsecticide for the control of curculionid beetles]. Patent No. 4196 (13) B1, C1, A01N 63/00; C12N 1/20; C12R 1/07; A01P 7/04. Institutul de Zoologie al Academiei de Științe a Moldovei [Institute of Zoology of the Academy of Sciences of Moldova]. Application No. a 2012 0040. Filed: April 27, 2012. Published: February 28, 2013. In: *BOPI*. 2013, No. 2; No. 9.

Muñoz-Bautista, J. M., Bernal-Mercado, A. T., Martínez-Cruz, O., Burgos-Hernández, A., López-Zavala, A. A., Ruiz-Cruz, S., Ornelas-Paz, J. d. J., Borboa-Flores, J., Ramos-Enríquez, J. R., Del-Toro-Sánchez, C. L. (2025). Environmental and health impacts of pesticides and nanotechnology as an alternative in agriculture. *Agronomy, 15*(8), 1878.

Palma, L., Muñoz, D., Berry, C., Murillo, J., Caballero, P. (2014). *Bacillus thuringiensis* toxins: An overview of their biocidal activity. *Toxins, 6*(12), 3296–3325.

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ISSN-L: 2284-9521

Şahin, B., Gomis-Cebolla, J., Güneş, H., Ferré, J. (2018). Characterization of *Bacillus thuringiensis* isolates by their insecticidal activity and their production of Cry and Vip3 proteins. *PLoS ONE, 13*(11), e0206813.

Salama, H. S., Abd El-Ghany, N. M., Saker, M. M. (2015). Diversity of *Bacillus thuringiensis* isolates from Egyptian soils as shown by molecular characterization. *Journal of Genetic Engineering and Biotechnology, 13*(2), 101–109.

Stratan, D. (2025). Smart transformation in Moldova's agriculture. *Journal of Financial and Monetary Economics, 13*(1), 83–90.

Święcicka, I., Mahillon, J. (2005). The clonal structure of *Bacillus thuringiensis* isolates from north-east Poland does not correlate with their cry gene diversity. *Environmental Microbiology, 7*(1), 34–39.

The Lens, The Lens - Patent and Scholarly Search and Analysis. Jefferson, O. A., Koellhofer, D., Warren, B., and Jefferson, R. (2019). The Lens MetaRecord and LensID: An open identifier system for aggregated metadata and versioning of knowledge artefacts. (https://doi.org/10.31229/osf.io/t56yh). Retrieved June 03, 2026, from https://www.lens.org

Tuneu-Corral, C., Puig-Montserrat, X., Riba-Bertolín, D., Russo, D., Rebelo, H., Cabeza, M., López-Baucells, A. (2023). Pest suppression by bats and management strategies to favour it: A global review. *Biological Reviews of the Cambridge Philosophical Society, 98*(5), 1564–1582.

Tursunova, A., Adilkhankyzy, A., Turbekova, S., Abylayeva, U., Balabek, A., Uspanov, A., Duisembekov, B. (2026). Molecular characterisation and screening for cry genes of native *Bacillus thuringiensis* strains from Kazakhstan. *Plant Protection Science, 62*(1), 27–35.

Verma, A., Sindhu, S. R., Boora, N., Rani, R., Aarti, Devi, R., Yashveer, S. (2025). Stress biology in agriculture: A comprehensive review of abiotic and biotic challenges. *Plant Cell Biotechnology and Molecular Biology, 26*(9–10), 128–147.

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