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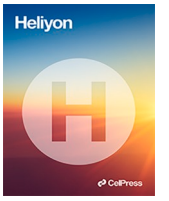

Research article

The use of antigens derived from *Bacillus thuringiensis* bacteria for further differentiation

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ABSTRACT

This study is devoted to studying *Bacillus thuringiensis* antigens and their insecticide activity as critical features in bacterial differentiation. Indeed, 190 samples were examined for flagellar antigenicity as well as the insecticidal activity exhibited. From a serological perspective, 122 isolates (64.2 %) were attributed to 8 H-serogroups, including 3 non-typeable and 65 unverified. The dominant serotype was H3abc (82 % frequency); H6 was less frequent (8.5 %). The other 6 serotypes accounted for a low frequency of occurrence (up to 1.5 %). Of the 190 isolates tested, 125 (65.8 %) formed bipyramidal, and 63 (33.2 %) represented spherical inclusions. All H3abc isolates contained bipyramidal inclusions. The same applied to H8ab and H7 isolates. Insecticide activity was noted in 70.1 % of the population. In general, 128 samples were toxic to both species (*Bombyx mori, Aedes* sp.). Another 3 samples were toxic only to *B. mori*, and 2 for *Aedes* sp. Among the samples exhibiting toxicity to both species, 97.6 % belonged to bipyramidal paraspore inclusions (H3abc). All H7 samples were toxic to two insect species. Monotoxic *B. thuringiensis* against *Aedes* sp. were found only among organisms producing spherical parasporal inclusions in the cell. Examples of such microorganisms include an isolate of the H4ab/43 serotype.

1. Introduction

In recent findings, certain strains of Bacillus thuringiensis have exhibited toxic activity against various insect species [1–4]. Despite the initial assumption that these strains were only effective against lepidopterans, the larvicidal activities of these strains appear to be broader, raising the potential for economic significance in insect pest control [5].

The heightened interest in B. thuringiensis stems from the urgent need for alternative pest control methods, as many insects are developing resistance to traditional insecticides. Additionally, the increasing prevalence of resistance to B. thuringiensis strains underscores the importance of developing reliable classification methods for these strains [6].

This study focuses on the classification of B. thuringiensis strains, exploring the effectiveness of serotyping and biochemical classification methods. Serotyping, which examines flagellar antigens, has been a well-established approach [7]. However, questions persist about whether serotyping fully captures the diversity of B. thuringiensis and its relevance to genetic exchanges with Bacillus cereus [8,9]. Given the similarities in biochemical traits and genetic properties between B. thuringiensis and B. cereus, the need for more precise classification methods becomes evident [10].

Recent studies have shown that conserved genes, core genomes, and pangenomes in related Bacillus species overlap significantly.

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Despite the genetic and phenotypic similarities between B. thuringiensis and B. cereus, detailed investigations are required to identify morphological and molecular distinctions, especially in parasporin synthesis [11].

Differentiating between B. cereus and B. thuringiensis solely based on standard biotyping has proven unreliable [12]. Instead, the focus has shifted to using insecticidal crystalline proteins encoded by cry genes as a distinguishing feature [13].

This study investigates the effectiveness of a developed biomarker, based on transcription regulator genes and Cry protein genes, in differentiating between B. thuringiensis and B. cereus strains. The prevalence of Cry2 protein in B. thuringiensis, known for its abundance, is a key focus in this comparison [14].

Given the common association of B. thuringiensis with the feces of various animals, particularly herbivores, this research delves into fecal animal isolates. The aim is to determine flagellar (H) antigenic serotypes and assess the insect pathogenic activity of B. thuringiensis strains for differentiation purposes. These findings may have practical applications, such as studying the toxicity of B. thuringiensis serotypes against human cancer cells, contributing valuable insights for tumor treatment [15].

In summary, the study addresses the research question of effective classification methods for B. thuringiensis strains, with specific objectives related to serotyping, biochemical classification, and biomarker evaluation. The significance lies in advancing our understanding of the diversity and differentiation of these strains, offering potential applications in pest control and cancer treatment.

2. Methods and materials

2.1. Bacterial isolates and cultivation conditions

A total of 190 samples of *B. thuringiensis* were studied. All samples were obtained from the feces of animals and birds, which, in turn, were sourced from the local zoo. The study included samples obtained from various species of animals in a zoo environment. Samples were collected from 14 species of mammals, including lions, tigers, elephants, giraffes, zebras, llamas, monkeys, kangaroos, hippos, antelopes, rhinoceroses, manatees, koalas, and badgers. Additionally, samples were obtained from 5 species of reptiles, such as crocodiles, snakes, turtles, iguanas, and geckos, as well as from 4 species of birds, including flamingos, parrots, ostriches, and falcons. The specimens were collected during the animals' active period, throughout the spring and summer seasons, to account for seasonal aspects of their physiology.

Samples were collected through regular fecal sampling procedures, adhering to health standards and ethical norms regarding animal welfare. Careful selection aimed to cover a wide spectrum of potential hosts, ensuring a comprehensive representation of B. thuringiensis isolates associated with various animal species. Criteria for sample selection included consideration of the physiological characteristics of animals, their age groups, gender, as well as the circumstances of sample collection. The collection process took into account seasonal changes and specific characteristics of the animals, ensuring diversity and representativeness in the sample for our analysis.

The isolation of bacteria was performed according to the methodology [16]. To perform serological studies, the bacteria were grown at a temperature of 37 °C for 5 h. Nutrient broth with a pH value of 7.5 was used for this purpose. The broth consisted of 15 g of meat extract, the same amount of polypeptone, as well as 1000 ml of distilled water, and 3 g of sodium chloride. Bacterial growth was carried out on a nutrient medium (nutrient agar consisting of 1 L of broth, 30 g of agar). The temperature regime was 28 °C, and the cultivation time was 5–6 days. This was necessary for conducting tests on pathogenic activity for insects and for observing sporulated cultures using a microscope.

2.2. H-serotyping

The study of flagellate H serotypes obtained from fecal samples was used. For this purpose, an agglutination method was used; the study was performed on a slide according to the generally accepted recommendations [7]. The test was performed by referring the H antiserum against *B. thuringiensis* H-serotypes 1–58.

For the serotyping of B. thuringiensis, we employed a method well-recognized for its effectiveness and widespread use in this context [7]. This method allows precise identification of bacterial species based on their flagellar H-antigens, ensuring a high degree of differentiation between various serotypes. The selection of H-antisera against B. thuringiensis H-serotypes 1–58 is justified as it represents a broad spectrum of antibodies specific to flagellar H-antigens, encompassing diverse bacterial species and subspecies. The use of such H-antisera provides comprehensive coverage of many known H-serotypes of B. thuringiensis, making this method more versatile and applicable to different isolates.

Therefore, motile isolates were classified as non-typeable due to their lack of reactivity with the reference H antiserum. Additionally, isolates lacking flagellation were identified, and the presence of a substantial degree of autoagglutination was also taken into consideration. Bacterial serotypes were identified according to the nature of their flagellate H-antigens. However, antisera prepared for the H antigens may share several common antibodies. Monospecific antisera containing one particular antibody were prepared by saturating aliquots of basal antisera with selected antigens one at a time and removing undesirable antibodies by centrifugation after completing the precipitation reaction. Such monospecific antisera allow the detailed typing of bacteria with common antigenic subfactors as serotypes.

2.3. DNA isolation and conventional PCR assay

Cultures of isolates were incubated in LB overnight at 30 °C, and DNA was extracted according to Carozzi et al. [17].

The oligonucleotide primers used in the study were as follows (5'→3'):
GTTATTCTTAATGCAGATGAATGGG, CGGATAAAATAATCTGGGAAATAGT.

The test tube for PCR was filled with 1 ml of a solution containing 10 pmol of a primer for cry2 and 2 ml of DNA. The PCR conditions.

- denaturation (95 °C, 5 min, 1 cycle);
- denaturation (95 °C, 30 s, 35 cycles);
- annealing (55 °C, 30 s, 1 cycle);
- elongation (72 °C, 6 min, 1 cycle).

After amplification, an agar gel solution (1.5 %) was used for gel electrophoresis and identification.

2.4. Morphology of parasporal inclusions

Cultures sporulated from 5 to 6 days were observed using a phase-contrast microscope AmScope T490A-PCT to morphologically identify paraspore inclusions [18]. Biochemical and phenotypic characterization and identification of 190 *B. thuringiensis* isolates were performed based on esculin hydrolysis, lecithinase, hemolytic activity, and motility activity [19]. After the bacterial cultures had sporulated for 5–6 days, samples were processed to detect parasporal inclusions. To achieve this, a small volume of sporulated culture was gently transferred onto a microscope slide. A cover slip was carefully placed on top of the sample, applying minimal pressure to avoid damaging the inclusions. The prepared microscope slide was then examined using a phase-contrast microscope for morphological identification of the parasporal inclusions. Multiple fields of view were observed to ensure a representative assessment of the inclusions.

2.5. Tests for insecticidal activity

Eggs of *Bombyx mori* were kindly provided by employees of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv, Ukraine). Spore-forming samples obtained from fecal samples were tested for insecticidal activity against *Bombyx mori* and *Aedes* mosquitoes. The *Aedes* larvae instar IV were withdrawn from the population maintained in the laboratory. The testing was conducted using the previously described methodology [20]. Each experiment was conducted once for each of the 190 samples of B. thuringiensis. Therefore, the number of repetitions for assessing insecticidal activity in this study was 1 time for each isolate.

2.6. Statistical data processing

The mean and standard deviation (SD) were estimated for statistical analysis of the obtained data. Analysis of Variance (ANOVA) was employed to assess the difference in the mean values of the analyzed criteria using Microsoft Excel and Statistica 10 software [21]. Differences in the obtained results were considered significant at $P \leq 0.05$ based on a Student's test.

3. Results

The results of experiments with pre-extracted populations of *B. thuringiensis* have demonstrated the importance of applying H-serotyping to elucidate the properties of serotypes. After more than 30 years, the H-serotype classification of *B. thuringiensis* remains the most effective. This classification allows for a more precise differentiation among the numerous samples of the strain that are accessible globally.

3.1. H serotyping

The work presents the results of analyzing the H-serotypes of *B. thuringiensis* isolates secluded from the feces of 20 animal species.

Table 1
The distribution of *B. thuringiensis* serotypes by morphological characteristics of the studied parasporal inclusions.

Serotypes *B. thuringiensis*	Quantity of isolates tested	Morphology of parasporal inclusions
H3abc	122	Bipyramidal
H6	48	Spherical
H7	1	Bipyramidal
H4ab/43	4	Spherical
H5ab/21	1	Bipyramidal
H8ab	1	Bipyramidal
H9	6	Spherical
H31	5	Spherical
Atypical isolates	2	Misshapen

Of the 190 isolates, 166 were from 14 mammalian species, and 20 were obtained from 5 reptile species and 4 bird species. The obtained bacterial populations were assigned to 8 serotypes: H31, H6, H3abc, H7, H4ab/43, H5ab/21, H8ab, and H9. There were 3 atypical samples in the analyzed populations, as well as 65 that were not verified. Serotype analysis showed that H3abc dominated, accounting for 82.0 % of the 122 serotyped isolates, H6 serotype was much less common (8.5 %). The remaining 6 serotypes accounted for only up to 1.5 % (Table 1). The resulting isolates could be multiples of the same organism.

Serotype H7 exhibited particularly high toxicity levels, with LC50 values of 2.7 μg/mL and LC90 values of 5.4 μg/mL for both B. mori and Aedes sp. larvae. It displayed the lowest LC50 value, underscoring its high insecticidal activity. Serotype H4ab/43 also demonstrated significant toxicity, with LC50 values of 3.2 μg/mL against both insect species and LC90 values of 6.5 μg/mL. This suggests its effectiveness in combating both B. mori and Aedes sp. Other serotypes, such as H6, H9, H31, and H5ab/21, exhibited moderate toxicity with LC50 values ranging from 3.0 to 6.4 μg/mL against both types of larvae. These serotypes have potential for insect control but require slightly higher concentrations. Conversely, atypical isolates showed lower toxicity, with LC50 values of 6.4 μg/mL and LC90 values of 13.1 μg/mL against both B. mori and Aedes sp. larvae. Although they may be less potent, they still possess insecticidal activity. Furthermore, when considering the specific impact on individual insect species, H7 and H4ab/43 remained highly toxic to both B. mori and Aedes sp. larvae, emphasizing their broad-spectrum effectiveness. In contrast, atypical isolates exhibited relatively lower toxicity to both species. These results underscore the varying levels of insecticidal activity among different serotypes of B. thuringiensis and atypical isolates. The choice of serotype may be influenced by the target pest and the desired level of insect control in specific applications, such as pest management and biotechnology. The PCR images of *B. thuringiensis* serotypes are given in Fig. 1.

In the feces of several herbivores, isolates belonging to H3abc were ubiquitous, accounting for 89 % of *B. thuringiensis* populations. Examples included the feces of anthropoids and bears.

Arginine hydrolase (ADH) was used as a factor for serotype identification. Urease-reducing enzymes, nitrate-reducing enzymes, and enzymes involved in the degradation of sucrose, mannose, cellobiose, and salicin were also used. This also includes enzymes involved in the production of acetyl methyl carbinol (AMC). The latter reaction may be negative only in some cases. All of the above features were used to analyze different antigenic subgroups belonging to different serotypes as well as to analyze several isolates that belonged to certain serotypes. Isolates belonging to the same serotype may have certain differences. In particular, six isolates of serotype H8ab were similar in the main characteristics. At the same time, to confirm the hypothesis of similar main characteristics in one serotype, it is necessary to test a larger number of samples.

3.2. Morphology of parasporal inclusions

The morphology of parasporal inclusions produced by true fecal isolates can be divided into four different groups. These include bipyramidal, spherical, irregularly shaped, or irregularly pointed. Of the 190 isolates tested, 125 (65.8 %) formed bipyramidal inclusions, and 63 (33.2 %) were spherical. All H3abc isolates demonstrated bipyramidal inclusions. The same was true for H8ab and H7

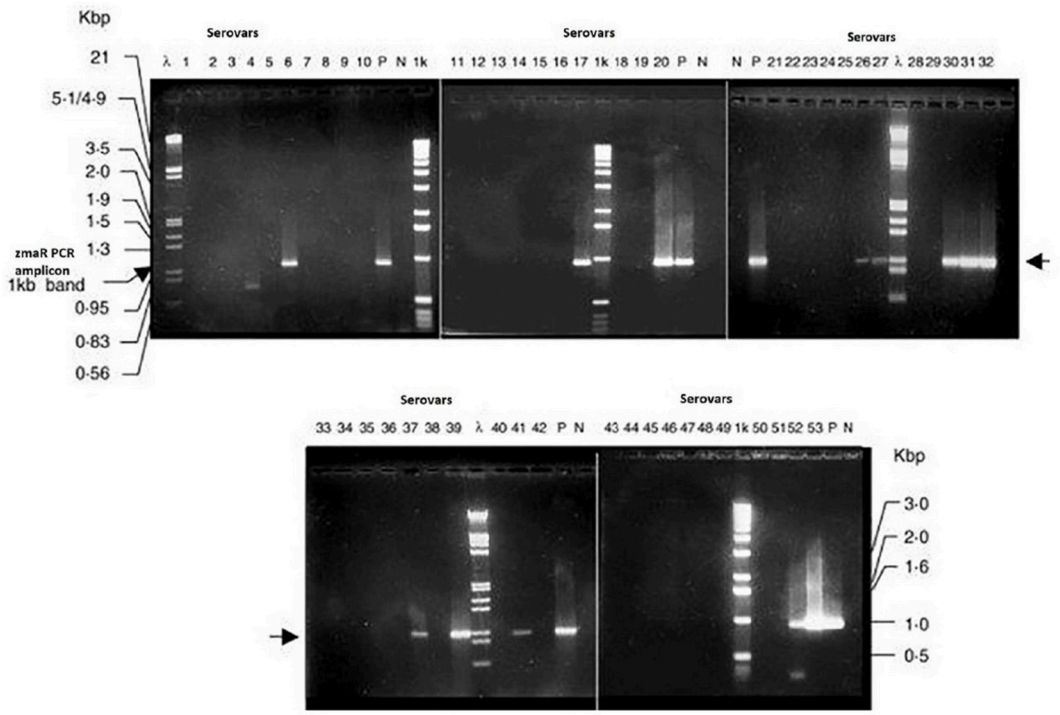

Fig. 1. PCR screening of B. thuringiensis strains (indicated by numbers) from different serovars. The arrow indicates the zmaR PCR amplicon.

isolates (Figs. 2 and 3a-f).

Fig. 3A depicts sporulating vegetative cells, also exhibiting free spores and bipyramidal crystals. Fig. 3B highlights the presence of free spores and rhomboidal crystals. Fig. 3C displays free spores and spherical crystals, which may be associated with spores. In Fig. 3D, free spores and spherical crystals are detected. Fig. 3E reveals free spores as well as bipyramidal crystals. Fig. 3F notes the exclusive presence of free spores.

Analyzing the captions of the figures, it can be inferred that B. thuringiensis possesses various types of crystals and spores, which may be significant in studying its biological properties and potential applications in biotechnology or agriculture. For instance, the diversity of crystals may influence its toxicity to pests or be utilized as markers for identifying this organism in natural ecosystems.

3.3. Insecticidal activity

Fecal isolates *of B. thuringiensis* were analyzed in terms of the oral toxicity of the resulting sporulated bacterial cultures against insect larvae to identify the properties of the obtained serotypes. Of the 190 isolates tested, 133 (70.1 %) showed insecticidal activity, 128 killed *B. mori* and *Aedes* sp. larvae, 3 isolates were monotoxic to *B. mori,* and 2 more were capable of destroying only *Aedes* sp., proving their biological selectivity against laboratory test objects. The results are detailed in Table 2.

All H3abc samples obtained with bipyramid inclusions were toxic to both insect species, i.e., to both *B. mori* and *Aedes* mosquitoes. The obtained H5ab/21 isolates exhibited double toxicity. Monotoxic *B. thuringiensis* against *Aedes* sp. were found only among organisms forming spherical parasporal inclusions. Examples included an H4ab/43 serotype isolate.

The insecticidal activity of the bacterial serotypes is an integral biochemical component when characterizing different serotypes. Research conducted primarily on newly identified serotypes suggests that while biochemical traits are significant, they may not be suitable for analysis within or between serotypes. This approach, when coupled with H serotyping, could prove effective in cases where specific traits remain ambiguous. In addition, there are suggestions as to how reliable the microscopy methods are. These methods have proven effective for many bacteria, in addition, their results are comparable with those obtained by traditional methods. In this regard, in some cases, it is possible to use not only traditional methods but also microscopic methods.

4. Discussion

Our data revealed significant serological diversity within the fecal populations of B. thuringiensis, encompassing at least 9 distinct H-serotypes from undefined serogroups or H-serogroups. Notably, serotype H3abc emerged as the predominant serotype in fecal samples, particularly among 12 different herbivore species. This serotype exhibited particularly high prevalence in the feces of chimpanzees, gorillas, tapirs, two species of bears (polar and black), and rabbits. For example, all 39 isolates from chimpanzees were serologically identified as belonging to serotype H3abc, and 25 isolates from polar bears shared the same H serotype. Interestingly, in some cases, a single fecal sample contained 2–3 different serotypes. For instance, in a rabbit fecal sample, the presence of 3 serotypes was detected, including one H3abc serotype, one H6 serotype, and one untyped serotype.

According to numerous studies, strains of B. thuringiensis are frequently detected in the feces of animals residing in national parks or held in captivity in zoos [22–24]. Remarkably, this bacterium is often associated with herbivorous animals. In our analysis, we investigated the pathogenic activity and distribution of antigenic H serotypes among B. thuringiensis strains isolated from fecal samples.

Researchers have previously reported that *B. thuringiensis* bacteria can be found in the phyllosphere of different plants [25–27]. Serotype H3abc has also been identified as a typical natural flora member of the H serotype on phylloplane [28]. Hence, the obtained H3abc isolates may originate from natural populations of phylloplane. These insecticides can be applied to various plant crops (vegetables, crops). Thus, insecticides based on the H3abc serotype *of B. thuringiensis* are popular in controlling agricultural insect pests [29]. Most *B. thuringiensis* toxins identify their specific target through the bounding of specific cell membrane receptors. Cry proteins are the best-known toxins representing *B. thuringiensis,* with numerous related studies having been published. The cry is cytotoxic to insect larvae, affecting important crops by recognizing certain types of plant membranes using specific receptors such as cadherin,

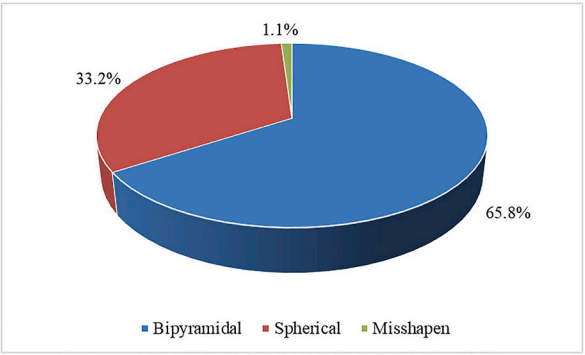

Fig. 2. Shares of *B. thuringiensis* serotypes detected.

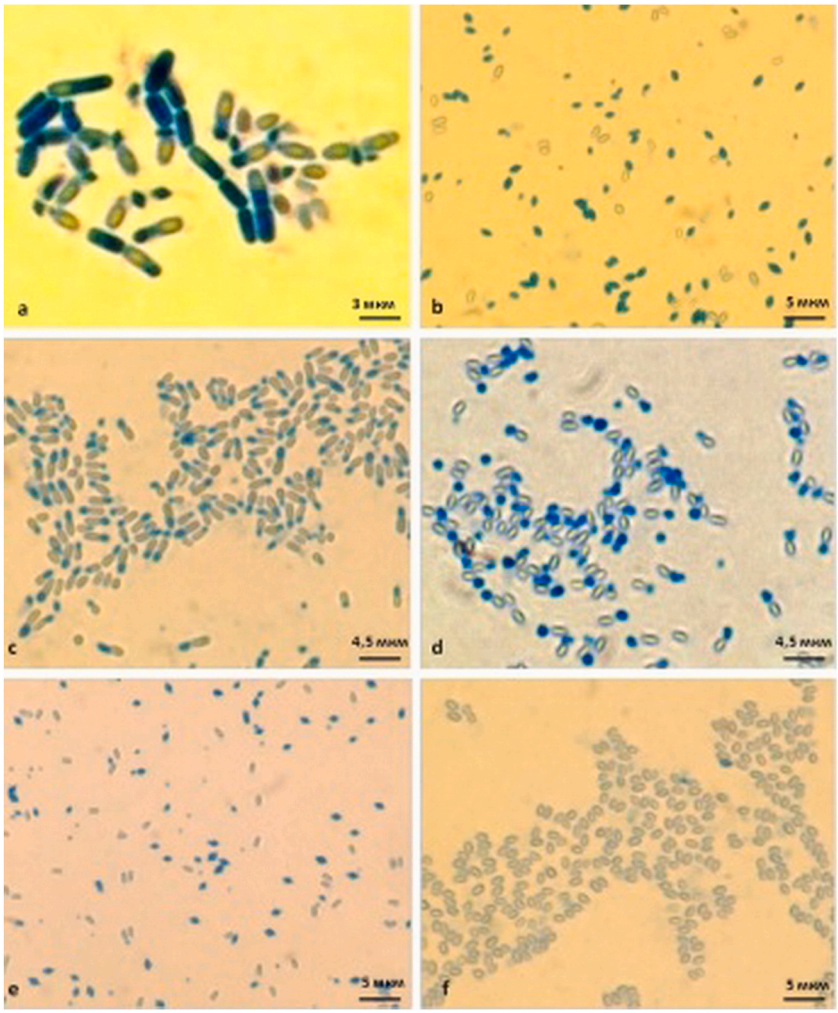

Fig. 3. Photographs of B. thuringiensis. A. Sporulating vegetative cells, also showing free spores and bipyramidal crystals; B. Presence of free spores and rhomboidal crystals noted; C. Presence of free spores and spherical crystals, which may be associated with spores; D. Detection of free spores and spherical crystals; E. Detection of free spores, as well as bipyramidal crystals; F. Presence of exclusively free spores.

aminopeptidase-N, and alkaline phosphatase. These toxins mainly affect mosquitoes that are vectors of human diseases such as *Anopheles* spp (malaria), *Aedes* spp (dengue, Zika, and chikungunya) and *Culex* spp (Nile fever and Rift Valley fever), respectively [29].

Previous research reported that *B. thuringiensis* strains without a pronounced insecticidal activity outperformed insecticidal isolates in natural environments in several countries [30]. Other laboratory study reports have concluded that *B. thuringiensis* isolates with non-insecticidal Cry proteins overperform insecticides in natural ecological niches, comprising over 90 % of natural populations in soils and phylloplane [31]. These data contrast with the present findings that insect pathogenic activity was detected in 70.1 % of fecal samples. Another important result is that the majority of pathogenic isolates belonged to serotype H3abc.

Global efforts are currently focused on discovering local *B. thuringiensis* isolates with unique anticancer properties. Thus, parasporins are a group of non-insecticidal crystalline proteins with potential and specific antitumor activity *in vitro* [31]. However, despite the significant therapeutic potential of PS-producing *B. thuringiensis* strains, knowledge of the effects of these proteins remains limited. *Thuringiensis* has been found to have unique biological activities. Among them are cytotoxicity specific to certain human cancer cells [29,31,32], lectin activity against mammalian red blood cells [33,34], and activity against trichomonads [35].

The results obtained have significant implications for understanding the distribution and pathogenic activity of various serotypes of B. thuringiensis in different ecosystems, especially among herbivorous animals. The detection of serotype H3abc in fecal samples from various species, such as chimpanzees, gorillas, and others, indicates its wide prevalence in nature.

Our results contradict previous studies, indicating the presence of insecticidal activity in the isolates of B. thuringiensis obtained from fecal samples. This underscores not only the role of this microorganism in natural environments but also provides new perspectives for the use of these isolates in combating insect pests in agriculture. Furthermore, the isolation of different H serotypes, such as H3abc, may serve as a basis for developing effective insecticides to target specific insect species, such as mosquitoes, which are vectors for dangerous diseases.

Table 2
Data on insecticidal activity of *B. thuringiensis* obtained via tests.

Activity	LC50 ± SD (μg/ml)	LC90 ± SD (μg/ml)
Toxicity to both *B. mori* and *Aedes* sp. Larvae		
H3abc	8.2 ± 0.8	15.3 ± 2.3[a]
H6	4.3 ± 0.2	8.6 ± 1.0[a]
H7	2.7 ± 0.5	5.4 ± 1.4
H4ab/43	3.2 ± 0.8	6.5 ± 2.0
H5ab/21	6.3 ± 0.9	12.8 ± 2.7[a]
H8ab	5.7 ± 1.1	11.7 ± 3.1[a]
H9	5.9 ± 0.9	11.9 ± 2.7[a]
H31	3.0 ± 0.8	6.2 ± 2.1
Atypical isolates	6.4 ± 0.9	13.1 ± 2.6[a]
Toxicity to *B. mori*		
H3abc	4.4 ± 1.0	34.7 ± 18.6[b]
H6	3.7 ± 0.4	28.6 ± 12.7[b]
H7	2.3 ± 0.6	18.3 ± 10.3[b]
H4ab/43	2.8 ± 0.9	22.1 ± 13.6[b]
H5ab/21	5.4 ± 1.2	42.9 ± 22.7[b]
H8ab	5.0 ± 1.3	39.5 ± 22.7[b]
H9	5.1 ± 1.1	40.3 ± 21.7[b]
H31	2.6 ± 0.9	21.1 ± 13.6[b]
Atypical isolates	5.6 ± 1.1	43.8 ± 22.7[b]
Toxicity to *Aedes* sp.		
H3abc	2.5 ± 0.5	16.3 ± 8.5[b]
H6	2.1 ± 0.2	13.4 ± 5.8[b]
H7	1.3 ± 0.3	8.6 ± 4.7[a]
H4ab/43	1.5 ± 0.4	20.1 ± 10.4[b]
H5ab/21	3.1 ± 0.6	10.6 ± 4.7
H8ab	2.8 ± 0.7	18.5 ± 10.4[a]
H9	2.9 ± 0.6	18.9 ± 9.9[b]
H31	1.5 ± 0.5	9.9 ± 6.3[a]
Atypical isolates	3.1 ± 0.5	20.6 ± 10.4[a]

Note: LC – lethal concentration.
[a] $p \leq 0.05$.
[b] $p \leq 0.01$.

Thus, our findings make a significant contribution to understanding the microbiology of B. thuringiensis and its potential applications in agriculture and insect control. They also provide new insights into the distribution and activity of different serotypes in natural populations. Despite the significance of our results, our study has some limitations that should be considered when interpreting the data. Firstly, there is a limited number of samples included in our research, which may reduce the overall representativeness of the data and necessitate further investigations with a more extensive sample size. Secondly, the serotyping methods described in the study may have their limitations in terms of accuracy and specificity. More comprehensive investigations could involve additional methods to confirm serotypes and ensure higher precision in the results.

For future research, it is recommended to expand the sample size by including more diverse ecosystems and biomaterials to obtain a more comprehensive understanding of the distribution and diversity of B. thuringiensis serotypes. Additionally, conducting a more in-depth molecular analysis, including genetic markers, could refine our conclusions and provide a more detailed understanding of the genetic variability among isolates. It is also worth noting the potential interactions of B. thuringiensis with other microorganisms in the natural environment, which may also influence its distribution and pathogenic properties. Additional research in this direction could shed light on the complex ecological interactions between B. thuringiensis and the surrounding environment. Overall, despite the mentioned limitations, our results constitute a significant contribution to understanding the ecology and pathogenic properties of B. thuringiensis. Future research, considering the proposed enhancements and additional research directions, may further expand our knowledge of this microorganism and its role in nature.

Future research will include tests of parasporal proteins, e.g., animal feces, which may affect indicators of biological activity unrelated to pathogenicity.

5. Conclusions

Among the 8 identified serotypes, H3abc was the most prevalent (82.0 %), followed by H6 (8.5 %), while each of the remaining serotypes constituted less than 1.5 %. Morphological analysis of parasporal inclusions in fecal isolates revealed four distinct groups, including bipyramidal, spherical, and irregular forms. It was observed that all isolates of H3abc, H8ab, and H7 exhibited bipyramidal inclusions.

The oral toxicity of these isolates against insect larvae was also evaluated. A significant portion (70.1 %) of the isolates exhibited insecticidal activity, with 128 strains showing effectiveness against B. mori and Aedes sp. Particularly noteworthy was the dual toxicity of all H3abc isolates with bipyramidal inclusions and all H5ab/21 isolates. High monotoxicity against Aedes sp. was exclusively

observed in the spherical group, primarily associated with the serotype H4ab/43. These results highlight the diversity and biological selectivity of B. thuringiensis isolates, underscoring their potential application in pest control and biotechnology.

Our study provides valuable information about the diversity and characteristics of B. thuringiensis isolated from animal feces, emphasizing their potential significance in various areas, such as plant protection and biomedical technology. The obtained data on insecticidal activity and H-serotype diversity can serve as a basis for further research in the field of biocontrol and understanding the ecological interactions of B. thuringiensis.

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Additional information

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Data statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Ekaterina Savelyeva: Writing – original draft, Validation, Software, Project administration, Investigation, Formal analysis, Conceptualization. **Aleksei Avdeenko:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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